A Safety and Efficacy Trial of Circumferential Anal Canal Radiofrequency Ablation for High-Grade Anal Intraepithelial Neoplasia Using The BARRX™ Anorectal Wand

(Clinical Protocol 01-2017)

AUGUST 31, 2017

PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site _____, agree to conduct and follow this protocol: **PROTOCOL 01-2017: A Safety and Efficacy Trial of Circumferential Anal Canal Radiofrequency Ablation for Anal Intraepithelial Neoplasia using the Barrx™ Anorectal Wand** as written according to FDA guidelines. I understand that no deviations from the above protocol may be made without written permission from the Protocol Chair(s).

_____ _____

Signature Date (mm/dd/yyyy)

PROTOCOL SUMMARY

Title	A Safety and Efficacy Trial of Circumferential Anal Canal Radiofrequency Ablation for Anal Intraepithelial Neoplasia using the Barrx™ Anorectal Wand
Design	Multi-center prospective trial involving up to 70 subjects
Subject Population	HIV-positive and HIV-negative subjects with intra-anal intraepithelial neoplasia (AIN) containing at least one high-grade squamous intraepithelial lesions (HSIL) involving the squamocolumnar junction (SCJ).
Objective	Assess the safety, and efficacy of circumferential radiofrequency ablation (RFA) to the anal canal using the FDA cleared Barrx™ Anorectal Wand to eradicate anal HSIL lesions
Hypothesis	The study device RFA procedure will have an acceptable safety profile and demonstrate superior disease free survival at 12 months over standard ablative techniques
Primary Endpoint	• Histologic clearance of HSIL at the SCJ at 12 months
Secondary Endpoints	• Persistence of treated HSIL index lesions at 3 months post RFA • Safety of circumferential ablation of anal canal HSIL using the Barrx™ Anorectal Wand • Histologic clearance of low-grade squamous intraepithelial lesions (LSIL) at 12 months
Principal Investigator Institution	Laser Surgery Care 420 W 23rd St, New York, NY 10011
Investigator	Stephen E. Goldstone, MD FACS (Laser Surgery Care) (212) 242-6500
Institutional Review Board	Solutions IRB (Little Rock, AR)
Sponsor Information	Stephen E. Goldstone, MD FACS (SEGMD PC) Laser Surgery Care 420 West 23rd St New York, NY 10011 (212) 242-6500

GLOSSARY

AE: adverse event

AIN: anal intraepithelial neoplasia

ASCH: atypical squamous cells-cannot exclude high-grade lesions (ASCH),

ASCUS: atypical squamous cells of undetermined significance

ANC: Absolute neutrophil count

BQE: baseline qualifying exam

CO_2: carbon dioxide

CD4: T-cell

CRF: case report form

CR-HSIL: complete response high-grade squamous intraepithelial lesion

CR-HSIL/LSIL: complete response high-grade squamous intraepithelial lesion and low-grade squamous intraepithelial lesion

ELISA: enzyme-linked immunosorbent assay

ETZ: eligible treatment zone

FDA: Food & Drug Administration

HIV: human immunodeficiency virus

HPV: Human Papillomavirus

HRA: high-resolution anoscopy

HSIL: high-grade squamous intraepithelial lesion

ICF: informed consent form

IDSA: Infectious Disease Society of America

IRB: institutional review board

IRC: infrared coagulation

J/cm^2: Joules per square centimeter

LAT: lower anogenital tract

LSIL: low-grade squamous intraepithelial lesion

MSM: men who have sex with men

Nd:YAG: neodymium-doped yttrium aluminum garnet

NSAID: non-steroidal anti-inflammatory drug

PTT: Partial Thromboplastin Time

QOL: Quality of Life

RFA: radiofrequency ablation

SAE: serious adverse event

SCC: squamous cell carcinoma

SCJ: squamocolumnar junction

W: Watts

5-FU: 5-Fluorouracil

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1. INTRODUCTION

Anal Intraepithelial Neoplasia and Ablative Therapy

Anal squamous cell carcinoma (SCC) is a relatively uncommon cancer, however, it is on the rise in men and women worldwide. In the US, it is estimated that approximately 8,080 new cases predicted in the United States in 2016.[1] Over the past decade, the rate of anal cancer cases has increased by approximately 2.2% each year while mortality has increased 3.8% each year.[2]

Like cervical cancer, anal SCC is thought to be preceded by a spectrum of intra-epithelial changes of varying cytological and histological abnormality, referred collectively to as anal intraepithelial neoplasia (AIN).[1,3,4] AIN involves both the perianal skin and the anal canal including the anal transition zone.[5]

AIN is a multifocal disease process strongly associated with human papillomavirus (HPV; usually HPV types 6, 11, 16 and 18). A large majority of anal cancers are attributable to HPV-16 (~72%).[6] Oncogenic HPV infection persistence is thought to be necessary in the development of anal SCC and high-grade squamous intraepithelial lesions (HSIL), the precursor to anal SCC.[7]

The exact prevalence of AIN in the general population is unknown, but is thought to range from < 1% to 5.25%; notably, the incidence is increasing.[8-10] Several higher-risk groups have been identified and include subjects with HIV, those who are systemically immunocompromised such as transplant recipients and those on long-term steroids (e.g. for connective tissue disorders), women with a history of genital intraepithelial neoplasia and those with extensive anogenital condylomata.[11] In men and women, common risk factors include receptive anal sex, increased lifetime number of sexual partners, a history of genital warts and cigarette smoking.[4] HSIL is common in HIV-positive men who have sex with men (MSM), with a prevalence of 43% to 52% and an incidence of 49% over 4 years.[12,13] Though less pervasive, HSIL among HIV-negative MSM is still substantial, with a prevalence of 25% and incidence of 17% over 4 years.[13,14]

Subjects may undergo screening for anal dysplasia due to their inclusion in populations with increased risk (e.g. MSM, subjects with HIV or cervical dysplasia), the presence of symptoms such as pruritus and anal discharge, or signs of HPV infection such as anal condylomata. Anal Pap cytology was proposed as a potential screening methodology based on observations by Palefsky et al. in 1997 and Goldstone et al. in 2001, with the investigators

noting physical and cytological similarities between anal and cervical carcinomas and intraepithelial neoplasia.[15,16] In this procedure, a Dacron swab is inserted to a depth of ~3 to 4 cm and then slowly removed in a circular motion to sample the cells from all areas of the anal canal. Samples are immediately preserved on slides or in a liquid medium to prevent drying.[10]

Typically, interpretation of anal Pap smears is based on the 2001 Bethesda System for evaluating cervical cytology. In this system, smears are classified as normal, or inclusive of atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells-cannot exclude high-grade lesions (ASCH), low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL).[17]

Upon detection of ASCUS or worse, further diagnostic work-up includes examination of the anus in a manner similar to cervical colposcopy (referred to as high-resolution anoscopy or HRA). HRA is performed in conjunction with application of 5% aqueous acetic acid ± Lugol's iodine stain. The anoscope is inserted to allow placement of a small swab wrapped in a gauze pad that has been soaked in the acetic acid. The anoscope is removed, leaving the gauze pad in place for one minute to allow absorption of the acetic acid. After removal of the gauze-wrapped swab, the anoscope is reinserted and an examination of the anal canal and perianal skin with magnification is performed. Acetic acid produces an epithelial change known as aceto-whitening, which is seen in regions of squamous cellular abnormality. Vascular abnormalities, ulceration, and friability are signs of dysplasia. During the exam, Lugol's iodine may be applied with a small swab to further identify lesions suspicious for HSIL; such areas do not take up the Lugol's stain and appear yellow or light beige upon application of the stain. Upon identification, lesions suspicious of HSIL are biopsied.[18]

Grading of AIN is performed based on this histopathological examination. AIN 1 is characterized by 20 to 25% of the epithelium being replaced by abnormal basaloid cells and by the presence of koilocytes, which are indicative of actively replicating HPV infection. AIN 2 and 3 are diagnosed when abnormal basaloid cells replace more than 50% of the epithelium.[13]

Because the current understanding of HPV biology does not support a 3-tiered classification system of mild, moderate, and severe dysplasia/carcinoma in situ, recent multi-society consensus advocates a separation of morphologic designations to two categories that reflect transient active HPV replication and persistent HPV-associated pre-cancer. According to this

2013 consensus statement from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology, "The recommended terminology for HPV-associated squamous lesions of the LAT (lower anogenital tract) is low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), which may be further classified by the applicable –IN (intraepithelial neoplasia) subcategorization." Using this two-category system, AIN 1 is categorized as LSIL, and AIN 2 and AIN 3 are categorized as HSIL. While LSIL lesions may regress or progress to HSIL, HSIL lesions rarely regress regardless of the subject's HIV status.[19]

While the natural history of AIN has not been as well established as that of cervical intraepithelial neoplasia (CIN), HSIL is considered to be the immediate precursor to anal cancer.[20-22] Several studies have demonstrated the oncogenic potential of AIN 2–3.[23-25] Stanley et al. report that, "…even with surveillance and treatment, on average 9-13% AIN 3 may progress to invasive disease over 5–10 years, comparable to that observed for progression for CIN 3 over similar time periods."[26] Rates of anal HSIL histology are high in HIV-positive patients of all sexual risk groups with abnormal anal cytology.[27] Compared to HIV-negative subjects, in HIV-positive subjects, squamous intraepithelial lesions appear to be more aggressive; with ~50% of LSIL lesions progressing to HSIL within 2 years for HIV-positive MSM. Risk of progression to invasive cancer may be as high as 50% for lesions in HIV-positive subjects.[24,25,28]

Despite increasing acknowledgement of AIN amongst medical and subject communities and the availability of screening and diagnostic tools, there is no uniform standard management for dysplastic lesions. However, due to the high progression rate to anal SCC, experts recommend treatment for HSIL.[7, 29, 30]

In the cervix, treatment of HSIL focuses on removal of the squamous columnar epithelial transformation zone where most dysplasia occurs by performing the Loop Electrosurgical Excision Procedure (LEEP) or Conization. Unfortunately, this cannot be done in the anal canal and current treatment options are limited, and generally consist of local treatment with clinician- or subject-applied creams or gels, clinician-applied ablative techniques such as electrocautery, carbon dioxide (CO_2) laser, infrared coagulation (IRC; Redfield Corporation, Rochelle Park, NJ), and surgical excision. Reports of these treatments are plagued by a lack of controlled studies and small study sizes. Unfortunately targeted ablation of lesions has been associated with frequent persistent or recurrent disease, reflecting the field nature of

infection.[31-38] Widespread topical treatment with Imiquimod or 5-Fluorouracil (5-FU) has been reported with moderate success but treatment occurs over lengthy periods (4 months), with a lack of control subjects and limited study sizes precluding meaningful conclusions.[36-40]

Park et al. recently further detailed limitations in current AIN therapeutic management, stating, "AIN treatment can be challenging because recurrence after treatment and development of metachronous lesions (concurrent separate lesions) is common. There are no randomized controlled trials of AIN treatments to inform treatment recommendations, thus selection of treatment modality is directed not only by the extent and location of disease but also by available expertise and resources."[7]

Radiofrequency Ablation Study Device

The radiofrequency ablation (RFA) or Barrx™ Ablation System used in this protocol comprises an RFA generator (Barrx™ FLEX) and the Barrx™ Anorectal Wand. The generator and wand are cleared by the FDA for human use and the wand is specifically cleared for treatment of anal neoplasia. More than 100,000 procedures have been performed in the US (mostly RFA of esophageal metaplasia and dysplasia). The present FDA indication for use statement is: "The Barrx™ catheters are indicated for use in the coagulation of bleeding and non-bleeding sites in the gastrointestinal tract including, but not limited to, the esophagus. Indications include esophageal ulcers, Mallory-Weiss tears, arteriovenous malformations, angiomata, Barrett's esophagus, Dieulafoy lesions, angiodysplasia, gastric antral vascular ectasia, and radiation proctitis" (a Dieulafoy lesion is defined as a large tortuous arteriole in the stomach wall that erodes and bleeds). Thus, in this study, the device will be utilized in accordance with the FDA indications for use.

Radiofrequency Ablation of Gastrointestinal Tissue

RFA is used for the treatment of a number of disease states throughout the gastrointestinal tract, including; Barrett's esophagus, vascular lesions, bleeding, tumor palliation, dysplasia, carcinoma, and others. Historically, RFA has been applied with the assistance of an endoscope inserted via the mouth or anus. Commonly used terms related to the treatment of gastrointestinal tissue with RF energy include "coagulation" which may refer to sealing bleeding tissue and vessels, and "ablation" which may refer to the thermal removal of tissue such as the case for Barrett's esophagus.

Previously available techniques for coagulation and ablation, such as argon plasma coagulation, Nd:YAG (neodymium-doped yttrium aluminum garnet) laser photocoagulation and bipolar electrocauterization, rely on small focal probes (~2 mm width) to deliver energy to tissue. Variability in tissue effect, lack of uniformity, lack of broad surface area coverage, lack of depth control, and occasionally subject toxicity, remain limitations in the application of these tools for treating gastrointestinal tissue.

In response to the need for a more uniform energy delivery system, a predecessor of the present study device was developed in 2000 and subsequently cleared by FDA in 2001 (K013139). This device (Barrx™360 Ablation System) utilizes the same electrode design and energy delivery mechanism as the study device and has been studied extensively for the ablation of Barrett's esophagus in human subjects. To date, more than 100,000 procedures have been performed with the Barrx™ Ablation System.

The present study device (Barrx™ 60) has been used to provide RFA in a controlled programmed manner to any endoscopically accessible portion of the gastrointestinal tract, using treatment settings derived from experience with the predecessor device (Barrx™360).

Supporting Studies involving RFA in the GI Tract

The initial reports assessed the ablation depth and effect in the porcine esophageal model, demonstrating that application energy using the Barrx™ 360 device could be safely performed in the range of 5-12 J/cm^2 (energy density) and at high power (350 W). Such control of energy and power prevented stricture formation and other injury.[44]

Subsequent "treat and resect" studies were performed in subjects having their esophagus removed. These authors reported that 10 and 12 J/cm^2 applied twice (2x) resulted in complete removal of squamous epithelium and that the maximum depth of ablation was limited to the superficial submucosa at even the highest treatment settings tested (14 J/cm^2 delivered 4x). [45, 46]

Since these initial studies, RFA has become an accepted treatment for the management of high-grade dysplasia associated with Barrett's esophagus.[39-41] Preliminary studies in porcine models and humans demonstrated that depth of ablation was linearly related to energy density of treatment and subsequent studies have utilized energy densities to ablate the epithelial mucosa with sparing of the muscularis and submucosa.[44-46] Clinical trials using RFA for the treatment of esophageal dysplasia (Barrett's dysplasia and squamous dysplasia)

have resulted in the complete eradication of dysplasia in ≥ 90% of individuals with a low incidence of side-effects or complications.[41,47,48]

Several additional trials have been conducted in subjects with Barrett's esophagus with and without dysplasia using both the Barrx™ 360 device and the Barrx™ 90 device. Safety has been established for both devices at these treatment setting ranges for use in the esophagus and upper stomach (cardia). Further, effectiveness for complete removal of the pre-malignant Barrett's epithelium has been established within this treatment setting range as well.[41, 51]

The use of RFA outside the esophagus is growing. RFA has been applied to colonic samples at the time of resection. Two applications at a dose of 12 J/cm^2 resulted in changes no deeper than muscularis propria and was recommended as the dose to guide future studies of the lower GI tract.[49] RFA use in the stomach has been reported in two studies in which the bleeding dilated vessels in patients with gastric antral vascular ectasia (GAVE) were successfully treated. [52, 53] In one study comprising 21 patients who received a median of 2 RFA sessions, two adverse events were reported consisting of minor bleeding and superficial ulceration. [52] In the second study, 6 patients underwent 10 RFA sessions with no complications. Baldaque-Silva reported treating 3 patients with gastric dysplasia with 2 RFA sessions per patient and noted mild to moderate epigastric pain treatable with acetaminophen but no major complications.[54] The rectum has also been investigated as a site of RFA to treat bleeding sites in 4 patients suffering from chronic radiation proctitis with no adverse events reported. [55, 56]

RFA in the Anal Canal

Data was published on 19 HSIL subjects who had undergone RFA with the Barrx™90 device in a Phase I safety/tolerability study. Subjects underwent dose escalation from 1 - 3 ablation applications at a single location (n=13) to 2 - 3 applications hemi- circumferentially (n=6). The procedure was well-tolerated with no serious adverse events, and all subjects demonstrated healing of the anal mucosa by 28 days post-procedure.[50]

The Principal Investigator published the results of a single center, 22-patient study assessing this device for hemi-circumferential ablation reviewed and approved by Solutions IRB titled, "A Trial of Radiofrequency Ablation for Anal Intraepitheilial Neoplasia Using the Barrx™ Ablation System" (B-250,, Rev. 3/14/13).[57] The trial included only HIV-negative subjects (3 women) with HSIL involving a maximum or 50% of the SCJ circumference. Mean number

of index lesions was 1.7 (range1-4). The treatment zone was limited to the 50% of the anal canal involving the HSIL. Participants were followed at 3-month intervals with HRA and biopsy of treated lesion sites at any visit following RFA and at the 12-month endpoint. At 12 months Kaplan-Meier estimates of histologic clearance of HSIL within the treated half of the anal canal was 76%. Of 35 HSIL treated 4 lesions persisted at 3 months in 4 subjects. These were retreated with focal RFA to the quadrant involving the lesion and one persisted again at 12 months. One-third of subjects were noted to develop HSIL recurrence in the non-RFA treated half of the anal canal (metachronous recurrence).

Post treatment patients completed a diary assessing symptoms and tolerability. Overall, patients experience mild discomfort following RFA. The pain was considered tolerable and easily controlled with over the counter medication or mild narcotic. These findings are in keeping with other trials using targeted ablation of individual lesions. Bleeding with bowel movements as reported in the diary was almost universal and resolved within a median of 13 days. 2 subjects noted SAE's that were felt to be unrelated to the treatment (Appendicitis and narcotic addiction unrelated to this procedure). AE's were reported in 4 participants (19%) but only 2 were felt to be possibly device related. One subject developed a low-grade fever the night of the procedure. The other subject developed asymptomatic granulation tissue at 9 months post treatment after he had been judged to be fully healed at prior visits. At 12 months there were a small amount of purulent drainage from the area. It was probed and did not track anywhere. It was treated with silver nitrate coagulation and it resolved.

Given that risk of developing metachronous recurrence in high in studies of focal ablation of HSIL as well as the prior study of hemicircumferential RFA, the Principal Investigator recently published the results of a 10 participant (9 HIV-infected), single center study assessing this device for circumferential ablation reviewed and approved by Solutions IRB titled, "Radiofrequency ablation therapy for anal intraepithelial neoplasia: results from a single-center prospective pilot study in HIV+ participants."[58] The results were recently presented at the Infectious Disease Society of America annual meeting and at HPV 2017 in Cape Town South Africa. While this represented a pilot study with a primary endpoint of safety and tolerability, data was analyzed for a secondary endpoint of efficacy. Participants had to have at least one HSIL involving the SCJ and the entire SCJ was ablated (3 x 12 J/cm^2 per site) to treat baseline and occult HSIL(s). A post-RFA biopsy was taken. Subjects were assessed with HRA at 3, 6, 9, and 12 months. Mandatory lesion site biopsies occurred at month 12. Recurrence was retreated with focal RFA. At baseline, subjects had a mean 2.7 HSIL's (range

2-8). Median time to treat was 6.5 (5-13) minutes. Lesion persistence occurred in 4 subjects (3 at 3 months, 1 at 6 months). Metachronous recurrence occurred in only 1 subject at 3 months. No lesion persisted after retreatment. All subjects were dysplasia free at 12 months. Immediate post RFA biopsy of a treated lesion showed dysplasia in 50% and 2 of these participants developed persistence.

Safety and tolerability analysis showed that eight subjects were healed by 3 months and one each had asymptomatic granulation tissue and superficial erosion. All healed by 9 months. Median post-ablation anal pain was 5 (range 2-9) but resolved to a minimal level (0 or 1) within a median of 20 (0-29) days. During the first week after RFA, seven subjects experienced mild or moderate anal bleeding, not requiring any intervention. Median days to bleeding resolution was 1 (range 0-4) days. Seven subjects experienced mild or moderate incontinence (mucous or stool discharge) following treatment. One subjected developed thin stool and straining to pass bowel movements at 3 months after significant diarrhea several weeks before. He had a soft, circumferential, mid canal scar that was easily dilated. All symptoms resolved. Narcotics usage for pain relief was minimal (N= 3 subjects) and median days of pain reliever usage was 13 (3-22) for non-narcotics and 8 (4-14) for narcotics. All subjects who tried receptive anal sex post-RFA succeeded, including the subject with the dilated stricture. Median subject score for worry about anal canal condition was 4.5 (range 0-8) falling to 0.5 (range 0-5) 9 to 12 months post-RFA. Two device-related mild adverse events (AE) occurred in one subject each (externally thrombosed hemorrhoid and soft anal stricture). No serious AE occurred.

To date RFA has been shown to be effective in treating individual HSIL lesions and has been tolerable without undo complications in treating the hemi-circumference and circumference of the anal canal. Most importantly, it appears that there is a trend to decreased metachronous recurrence when the entire circumference of the anal canal SCJ is ablated without undo complications. Circumferential RFA is hypothesized to reduce metachronous recurrence in that it not only ablates identified HSIL, but also treats occult disease. Moreover, flat LSIL is not usually ablated during focal ablation. Some LSIL could progress to HSIL over time creating metachronous recurrence. With circumferential ablation this disease could be ablated thereby preventing progression. We now propose a larger, multicenter trial to better determine the efficacy and safety of circumferential RFA of the anal canal SCJ for the treatment of anal HSIL to determine if our prior results on a very limited pilot trial hold.

Figure 1. Post treatment tolerability.

Subject 21-day diary: self-reported symptoms after initial RFA			
Variable		**Subjects (N=21)**	**Duration (days)**
Bleeding (with bowel movement)		20 (95%)	13 (0-23)
Bleeding (without bowel movement)		12 (57%)	2 (0-16)
Variable	**At day 1**	**At day 7**	**At day 14**
Pain score [a]	4 (0-8)	3 (0-6)	1.5 (0-6)
Data shown as n (%) or median (range); [a] Based on a visual analog scale of 0-10.			

Non-Significant Risk Device Study

The use of the Barrx™ Ablation System has been determined to present non-significant risk in accordance with 21 CFR 812.3(m) for the intended use in this study. The Barrx™ Ablation System is not an implant and does not present a potential risk for serious risk to the health, safety, or welfare of a subject. Transient side effects that can be expected after treatment with the device include pain, fever, bleeding, and discharge. These are temporary and will subside with healing. Although not observed, additional adverse events may include anal stenosis, incontinence, scarring, disfiguration, infection, abscess, fissure, or fistula formation. Potential serious adverse events, also not been observed to date, may include ablation-related visceral perforation, major bleeding or death. There is potential for the occurrence of adverse events or serious adverse events related to the monitored sedation. The Barrx™ Ablation System will be utilized in accordance with the FDA 510k indications for use.

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2. MATERIALS AND METHODS

Subject Selection

The study will include female and male adult subjects who have biopsy-proven HSIL and are HIV-negative or HIV-positive.

Inclusion Criteria

Candidates for this study must meet **all** of the following criteria:

1. Age 18-75 years
2. HRA 2 to 8 weeks prior to the 0 month RFA visit yielding one or more flat, non-condylomatous biopsy-proven HSILs that are
    - Located entirely within the eligible treatment zone

      AND
    - Contiguous with the squamocolumnar junction
3. Eligible treatment zone (ETZ) is defined as
    - 3 cm above the dentate line to the anocutaneous line

      AND
    - Full anorectal circumference
4. If female of child-bearing age, negative pregnancy test within 8 weeks of the 0 month RFA visit and declared intent to remain on birth control throughout the trial, or declaration of infertility defined as subject report of status as post-menopausal or surgically sterile (status post hysterectomy or tubal ligation).
5. If HIV positive
    - HIV positive on antiretroviral therapy for at least 3 months with laboratory blood work within 12 weeks prior to the 0 month visit demonstrating viral load < 50
    - CD4 count $\geq 250/mm3$
    - ANC $> 750/mm3$
    - Platelet count $\geq 75{,}000/mm3$
    - Hemoglobin ≥ 9.0 g/dl

Exclusion Criteria

Candidates will be **ineligible** for enrollment in the study if **any** of the following conditions apply:

1. Any biopsy-proven HSIL partially outside of the ETZ (for example, an HSIL lesion with extension to the perianal skin)
2. Any condylomas in the eligible treatment zone > 1/2 cm diameter
3. Any anal or rectal pathology requiring treatment including ulcer, fistula, fissure, or proctitis
4. Any anal stricture or stenosis in patient history or upon examination.
5. Symptomatic scarring in anal canal (i.e. not pliable, hyperkeratosis)
6. History of or present anal or rectal cancer
7. History of pelvic radiation therapy
8. History of HPV vaccination or plans to initiate HPV vaccination during the trial
9. History of ablation or resection therapy within the ETZ within 3 months prior to the 0 month RFA visit (other than cauterization or excision of condylomata)
10. History of topical therapy (e.g. Imiquimod, 5-FU, Trichloroacetic acid) within the ETZ within 3 months prior to the 0 month RFA visit
11. Hemorrhoids > grade III
12. Fecal incontinence
13. Concurrent disease requiring systemic immunosuppression therapy
14. Concurrent malignancy requiring systemic therapy
15. Life expectancy < 2 years

16. Subject is on a platelet inhibiting agent (i.e. Clopidogrel) and/or anti-thrombotic agent (Heparin, Warfarin) and unable to discontinue 7 days before and after treatment for 14 days total.

    - Exception: Aspirin 81 mg PO daily does not need to be discontinued

Subject Enrollment

Upon approval of the protocol and informed consent form (ICF) by the institutional review board (IRB), subject enrollment may begin. The goal is to enroll a total of 70 subjects.

Informed Consent

Once the subject's eligibility has been determined, the Investigator or a member of the Research Team will discuss the background of the proposed study and explain the benefits and risks of the procedures and follow-up. If this is of interest to the subject, the ICF will be discussed and presented. The subject must sign the consent form prior to enrollment. This form must have prior approval of the study site's IRB. Failure to obtain a signed ICF renders the subject ineligible for the study.

Eligibility Process and Informed Consent

All potential subjects are screened for study eligibility and interest in participation. The study is described in detail, all questions answered, and an IRB approved ICF is provided to the subject for review and signature.

Study Procedure

The subject is required to attend a minimum of 5 visits to complete the study. This will consist of three HRAs with possible biopsy, and one HRAs with RFA with possibility of biopsy. If HSIL is found during follow-up then the subject will be asked to return for additional visits with HRA and RFA with possibility of biopsy. See Figure 2 for study flow diagram.

Preparatory instructions are given consistent with the Investigator's standard practice for HRA. Topical anesthesia is often sufficient for HRA with or without biopsy. Occasionally subjects might require addition of local anesthetic for biopsy. Conscious sedation or monitored anesthesia care, along with the administration of topical and local anesthesia, are typically adequate for performance of HRA with RFA. In certain cases due to airway issues, sleep apnea, body habitus, or co-morbidities, general anesthesia may be required.

HRA entails specific subject positioning, such as jackknife prone or lateral decubitus, and the use of a high-resolution video camera to assess the anal canal with the aid of an anoscope or tissue retractor. Tissue staining with acetic acid ± Lugol's solution is used to better identify lesions consistent with AIN.

At the baseline qualifying exam, lesion(s) within the ETZ suspicious for HSIL are biopsied using standard forceps (one lesion per jar). At the initial RFA visit and prior to RFA, any new lesions suspicious for HSIL are biopsied. If a quadrant(s) is void of a lesion and has not been biopsied at the BQE a random biopsy will be collected.

At 3, 6, and 9 months, an HRA will be performed and any lesions suspicious for HSIL will be biopsied using standard forceps. Patients positive for HSIL at 3, 6 or 9 months will be treated with focal RFA.

At 12 months an HRA will be performed and any lesions suspicious for HSIL will be biopsied. In the absence of lesions suspicious for HSIL at the site of a prior HSIL lesion, a biopsy will be taken from that site. Additionally at 12 months, any quadrant in the anal canal without a prior HSIL or new lesion suspicious for HSIL will be biopsied randomly at the SCJ.

Figure 2. Study Procedure Flow Diagram

RFA Procedure

At visit 1, anal canal RFA is performed to the entire ETZ (the ETZ comprises full anal canal, 3 cm above the dentate line to the anocutaneous line proximal to the verge). The procedure entails Barrx Anorectal Wand, introducing the device into the anus through the anoscope, placing the electrode surface in contact with the target tissue and delivering 3 bursts of energy in rapid succession at an energy density setting of 12 J/cm^2/burst.

Biopsy proven HSIL identified at the 3, 6, or 9 month visits are subsequently treated with focal RFA (3 ablations per site at 12 J/cm^2/burst). One cm of normal tissue is ablated on all sides of the lesion (the width of the RFA device), unless this would cross the anocutaneous line. Field ablation is applied only to the ETZ quadrant(s) from which HSIL-positive

biopsies were obtained at the previous visit without visible lesions identified at treatment. HSIL lesions that appear following the 0-month RFA visit outside of the ETZ are ablated in a manner consistent with the Investigator's standard technique.

Subjects are discharged to home using instructions consistent with the Investigator's standard practice for HRA-directed HSIL ablation, which includes pain medication, stool softeners and dietary suggestions.

Discharge Instructions

Discharge instructions are given consistent with the Investigator's standard practice for HRA with and without ablation, depending on the procedure(s) performed during the visit. After undergoing RFA, these instructions may include:

- Pain medication [for example, Vicodin® (hydrocodone bitatrate/acetaminophen/hydrocodone 5 mg-300mg), 1 tablet by mouth every 6 hours as needed for pain]. OTC pain medication such as ibuprofen or acetaminophen are acceptable when pain is mild to moderate.
- Metamucil or Citrucel (over the counter) by mouth, taken twice daily
- Colace 100 mg (docusate sodium) by mouth, take three times a day
- High fiber diet
- Drink 6 to 10 glasses of water every day
- Sitz baths as needed for pain
- Subject instructed to contact treating physician immediately for fever, anal bleeding, or other warning signs provided by the physician

Study Pathology

Each individual biopsy specimen is placed in its own jar (i.e. one specimen per jar). If more than one specimen is obtained from a single lesion, those specimens may go into the same jar (i.e.one lesion per jar). The location from which each specimen was obtained is recorded on a CRF with inclusion of octant descriptors. Biopsy specimens are fixed with formalin and processed in accordance with the local laboratory protocol for histology. The slides are examined by a local pathologist for diagnosis.

Pathology Adjudication

There is no pathology adjudication.

Baseline Qualifying Exam (BQE)

HRA and assessment of eligibility criteria will largely be completed with the BQE. After signing the informed consent approved by the IRB, subjects undergo a medical history and a physical exam. Inclusion and exclusion criteria are reviewed to ensure eligibility (a breast and pelvic exam will not be done). HRA does not need to be repeated if one has been done at the study site within 8 weeks prior to enrollment and biopsy reports and lesion documentation are available for study records.

Mapping of the anal canal will be complete based on the BQE and will be used throughout the study. The mapping consists of 8 octants starting with the octant pointing to the subject's posterior. Moving clockwise, the octants are labeled Posterior, Right Posterior (RP), Right Lateral (RL), Right Anterior (RA), Anterior (A), Left Anterior (LA), Left Lateral (LL), and Left Posterior (LP) (See Figure 3). In addition, the anal canal should be divided into quadrants to help with random collection of biopsies at final visit. (See Figure 4); these designated quadrants will be kept consistent through the duration of the trial as depicted in Figure 3. Lesions will be identified and followed throughout the study. Should a lesion be eradicated, the location will still be followed to monitor recurrence.

If female of child bearing age, patient will undergo a pregnancy test (urine test or equivalent).

Figure 3. Mapping of the anal canal by fixed octant designation

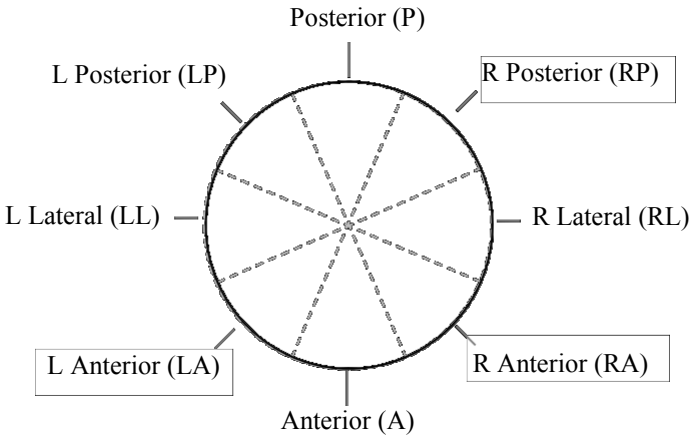
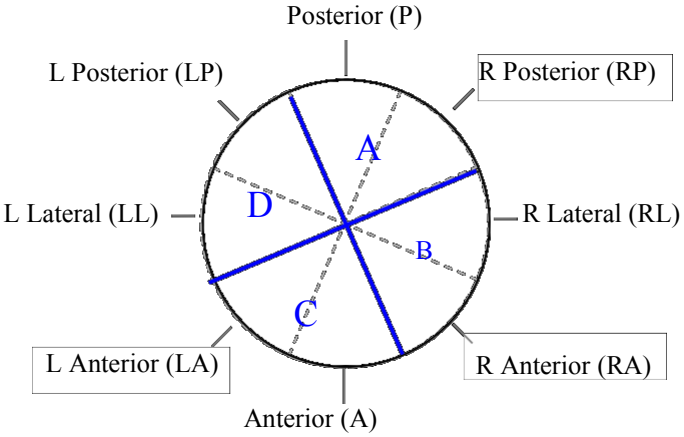


Figure 4. Mapping of the anal canal by a possible quadrant designation



Visit 1 Month 0

Subjects who have had an HRA and are deemed to meet eligibility requirements return for enrollment within 2-8 weeks. All subjects must have at least one lesion containing HSIL. The index lesion(s) are first identified. If a quadrant does not have a lesion and has not been biopsied at the BQE, a biopsy will be collected in this quadrant immediately prior to RFA. The anal canal is surveyed for lesions.

- Any new lesions suspicious for HSIL are biopsied using standard forceps (one lesion per jar).
- If any quadrant does not have any visible abnormalities and has not already been biopsied, one biopsy is taken randomly from that quadrant to define baseline histologic status prior to treatment.

RFA

- Circumferential RFA the entire anal canal as described RFA Procedure section.

Condyloma in the ETZ < 1/2 cm in diameter judged not to be sufficiently treated by RFA are excised or cauterized immediately after application of RFA. The anal canal is surveyed for lesions.

Visit 2 Month 3

The subject returns 3 months ± 2 weeks after Visit 1 for HRA (referred to as Visit 2) and the anal canal is surveyed to assess healing (epithelialization, granulation tissue, fissure, scar formation) and for possible recurrent dysplasia. Patients are asked about potential AE's and SAE's.

HRA and Biopsy – Quadrant

- Biopsy only lesion suspicious for persistent/recurrent HSIL.
- Biopsy any new lesion suspicious for HSIL.

Visit 2.1 (within 4 weeks of Visit 2)

If any ETZ biopsy is positive for HSIL, the patient returns 4 ± 2 weeks later for RFA.

- Any new lesions suspicious for HSIL are biopsied using standard forceps (one lesion per jar).

RFA

If the HSIL-positive biopsy was obtained from a visible lesion, that lesion (and any additional ETZ HSIL) is focally ablated as described in the RFA Procedure section. If there are new lesion(s) suspicious for HSIL in the ETZ that were not seen at Visit 2.0, they should be biopsied and then treated with RFA immediately (same session). If a lesion had been present at V2 and is not visible at the time of RFA, then the quadrant where that lesion occurred is treated. For any new lesion(s) suspicious for HSIL that are outside the ETZ, treatment is at the discretion and timing of the investigator.

Visit 3 Month 6

The subject returns 6 months ± 2 weeks after Visit 1 for HRA (referred to as Visit 3) and the anal canal is surveyed to assess healing (epithelialization, granulation tissue, fissure, scar formation) and for possible recurrent dysplasia. Patients are asked about potential AE's and SAE's.

HRA and Biopsy – Quadrant

- Biopsy only lesion suspicious for persistent/recurrent HSIL.
- Biopsy any new lesion suspicious for HSIL.

Visit 3.1 (within 4 weeks of Visit 3)

If any ETZ biopsy is positive for HSIL, the patient returns 4 ± 2 weeks later for RFA.

RFA

If the HSIL-positive biopsy was obtained, that lesion (and any additional ETZ HSIL) is focally ablated as described in the RFA Procedure section. If there are new lesion(s) suspicious for HSIL in the ETZ that were not seen at Visit 3.0, they should be biopsied and then treated with RFA immediately (same session). If a lesion had been present at V3 and is not visible at the time of RFA, then the quadrant where that lesion occurred is treated. For any new lesion(s) suspicious for HSIL that are outside the ETZ, treatment is at the discretion and timing of the investigator.

Visit 4 Month 9

The subject returns 9 months ± 2 weeks after Visit 1 for HRA (referred to as Visit 4) and the anal canal is surveyed to assess healing (epithelialization, granulation tissue, fissure, scar formation) and for possible recurrent dysplasia. Patients are asked about potential AE's and SAE's.

HRA and Biopsy – Quadrant

- Biopsy only lesions suspicious for persistent/recurrent HSIL.
- Biopsy any new lesion suspicious for HSIL.

Visit 4.1 (within 4 weeks of Visit 4)

If any ETZ biopsy is positive for HSIL, the patient returns 4 ± 2 weeks later for RFA.

- Biopsy any new lesions suspicious for HSIL are biopsied using standard forceps (one lesion per jar).

RFA

If the HSIL-positive biopsy was obtained, that lesion (and any additional ETZ HSIL) is focally ablated as described in the RFA Procedure section. If there are new lesion(s) suspicious for HSIL in the ETZ that were not seen at Visit 4.0, they should be biopsied and then treated with RFA immediately (same session). If a lesion had been present at V4 and is not visible at the time of RFA, then the quadrant where that lesion occurred is treated. For any new lesion(s) suspicious for HSIL that are outside the ETZ, treatment is at the discretion and timing of the investigator.

Visit 5 Month 12

The subject returns 12 months ± 2 weeks after Visit 1 for HRA (referred to as Visit 5), and the anal canal is surveyed to assess healing (epithelialization, granulation tissue, fissure, scar formation) and for possible recurrent dysplasia. Patients are asked about potential AE's and SAE's.

HRA and Biopsy – Quadrant

- Biopsy all locations of HSIL, lesions indexed at BQE and at Visit 1, 2, 3 and 4.

- Biopsy any new lesion suspicious for HSIL.
- Any quadrant without biopsy will undergo random biopsy.

Study Endpoints

Primary:

1. 1. Histologic clearance of all HSIL within the ETZ on a patient and lesion basis at 12 months from first RFA
   Related adverse event (AE) incidence by subject (study device relationship considered related if categorized by Investigator as: definite, probably, possibly, or unknown)

Secondary:

2. Related adverse event (AE) incidence by subject (study device relationship considered related if categorized by Investigator as: definite, probably, possibly or unknown)
3. Persistence of index HSIL at 3 months post RFA.

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Hypothesis

The study device RFA procedure will have an acceptable safety profile and demonstrate superior disease-free survival at 12 months over standard ablative techniques.

3. ADVERSE EVENTS

General

Transient side effects that can be expected after treatment include pain, fever, bleeding, and discharge. Adverse events may include anal stenosis, incontinence (including leakage), scarring, disfiguration, infection, abscess, fissure, or fistula formation. Potential serious adverse events (SAE's) (although they have not been observed to date in Barrx™ Ablation System clinical trials) may include ablation-related visceral perforation, major bleeding or death. There is potential for the occurrence of adverse events or serious adverse events related to the monitored sedation.

Reporting

The Investigator will assess each adverse event with respect to severity and relationship to the

study device and make the determination regarding reportability of any adverse event to the Study Sponsor and GI Solutions, Covidien *formerly* BÂRRX Medical, Inc. (the manufacturer of the device) and the IRB according to the guidelines of the IRB. All reported events will be evaluated by GI Solutions, Covidien for determination of reporting requirements with respect to the FDA under the MDR reporting regulation in 21 C.F.R. Part 803. All SAE's deemed to be possibly, probably or definitely related to the RFA will be reported to the sponsor and IRB within 72 hours of discovery of the event.

Reports relating to the subject's subsequent medical course must be submitted to the GI Solutions, Covidien until the event has subsided or, in case of permanent impairment, until the event stabilizes and the overall clinical outcome has been ascertained.

4. STATISTICAL METHODS

As this is a single arm, open label trial, no power calculations are applicable. Baseline and study outcomes will be summarized using descriptive statistics. Lesion-free survival will be estimated using the Kaplan-Meier method, and multivariate analysis on HSIL persistent recurrence will be performed using Cox's proportional hazards regression model. A p-value <0.05 is considered statistically significant.

Assuming the usual binomial distribution assumptions, a 95% Wald/Laplace confidence interval is formed assuming an 80% expected proportion of healing. To form a confidence interval of width 20% (that is, 10% below and 10% above), and a dropout rate of 10%, we arrive at a sample size needed of 70.

5. STUDY MANAGEMENT

Case Report Forms

All study data will be recorded onto paper case report forms (CRF) and forwarded to the Principal Investigator as a scan or fax, where it will be entered into an internet-based secured data entry and processing system. Data entered onto CRF pages will be typed or legibly printed using a black ballpoint pen. Although CRF completion may be delegated to other study personnel, the Investigator remains responsible for the accuracy and integrity of all data entered on CRFs.

Protocol Modification

Significant changes to the protocol will require that the protocol be formally amended. Protocol amendments will be agreed upon by the Investigators and approved of by the IRB.

Administrative changes to the protocol such as a change that has no effect on the conduct of the study or risk to the subject will be documented in a memorandum. The IRB must be notified of administrative changes, but formal approval is not required unless it is deemed necessary under the standard IRB operating procedures.

Study Data Collection

Primary data collection based on source-documented chart reviews will be performed by study coordinators at the clinical site. All study data will be recorded on CRFs and sent to the Principal Investigator. As per the ICF, all subject confidentiality will be maintained. Data entered through the internet-based data entry system will reside in a secure password protected database. The database will be backed up periodically, and the backup files will be stored in a secure location. Any issues identified will be communicated back to the site for clarification.

Data Handling and Record Keeping

In order to assure adequate control and provide study data that are consistent and of the highest quality, the methods described in this section will be employed. Data will be handled by qualified personnel at the study site and the Principal Investigator.

Data Receipt

All CRFs and online data will be subjected to initial inspection for omitted data, gross data inconsistencies, and timeliness of reporting. Any deficiencies will be addressed with the clinical site.

Data Entry

Data will be entered into a secured database with limited access.

Data Queries and Resolution

If an issue is discovered at Principal Investigator, a data query will be made to the site. The issue will be stated and response will be requested from the site. Pending the nature of the query, a signature from site personnel or the Investigator may be required.

Data Back-up

Database back-ups will be performed regularly at the Principal Investigator.

Data Analysis

All exported datasets for analyses will be inspected using edit checks so as to minimize errors.

Data Ownership

The Principal Investigator retains ownership of all clinical data generated in this study and controls the use of the data for purposes of regulatory submissions.

Record Retention

All documents related to this study must be retained by the Investigator for a minimum of two (2) years after the investigation has been completed or terminated. Study records that must be retained include, but are not limited to:

- signed subject informed consent forms,
- subject medical records including laboratory and diagnostic reports,
- supporting documentation of all serious adverse events
- study-related correspondence and study reports.

6. ETHICAL AND REGULATORY CONSIDERATIONS

Institutional Review Board (IRB) Approval

The Principal Investigator is responsible for submitting this protocol, the informed consent document, and all relevant supporting data to the IRB. IRB approval of the protocol, informed consent document, and any advertisement used to recruit study subjects must be obtained before the study may be initiated.

The Principal Investigator is responsible for keeping the IRB advised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The Principal Investigator is also responsible for notifying the IRB of any serious adverse events that occur during the study.

Informed Consent

Each subject will have the nature and the purpose of this investigation explained to him or her by the Investigator or another member of the investigative team at the place of care. The study will be explained to the subjects in lay language. Prior to entry into this investigation the subject must give voluntary, written informed consent to participate by signing the ICF. On the same occasion, the Investigator or another staff member responsible for the conduct of

the study (i.e. Sub-investigator) will also sign the ICF. The person responsible for obtaining the informed consent must also sign it if he/she is not the Investigator or Sub- investigator. The subject will receive a copy of the signed informed consent.

Subject Identification

All CRFs are coded with a site code and subject code, no subject identifying information will be contained on study documents. Further, no source documents will be provided to the Principal Investigator so subject confidentiality is completely protected in this study. All subjects are identified with a site code and a subject code. Sites will be assigned a unique 3 digit number upon IRB approval. Each subject is uniquely identified by the site number followed by a 3 digit subject number. For example, subject 001 from site 002 is identified as 002-001.

7. SUBJECT WITHDRAWAL AND STUDY TERMINATION

Subject Withdrawal

Any subject who wishes to withdraw from this investigation on his/her own accord and for whatever reason is entitled to do so without obligation and prejudice to further treatment. In addition, the Investigator may decide for reasons of medical prudence, to withdraw a subject. In either event, the Investigator will clearly document the date and reason(s) for the subject's withdrawal from this investigation in the CRF and should indicate whether or not he considers it related to the device.

Termination of Investigation

The Principal Investigator reserves the right to terminate the study at any time. Should this be necessary, the Investigator and subject will arrange the procedures on an individual basis after review and consultation. In terminating the study, the Investigator will assure that adequate consideration is given to the protection of subject interests.

8. REGULATORY STANDARDS

This protocol was designed using Regulatory Reference: ISO 14155:2011, and all applicable parts of 21CFR.

9. APPENDIX A. SCHEDULE OF VISITS AND TASKS

Task↓ Visit →	BQE	V1 RFA	V2-3Mth	V.2.1	V3-6Mth	V3.1	V4-9Mth	V4.1	V5 Final-12 Mth
Informed Consent Signed	X								
Inclusion/Exclusion Criteria	X	X							
Confirm Baseline HSIL Dx	X								
Med. Hx Obtained from Subject	X								
Pregnancy Test on Female Subjects of Child Bearing Potential	X								
Digital Anorectal Exam	X	X	X	X	X	X	X	X	X
HRA	X	X	X	X	X	X	X	X	X
Map anal canal; identify lesions	X	X			X				X
Designate quadrants (A,B,C,D) to be used throughout study	X								
Biopsy lesions suspicious of HSIL	X		X		X		X		X
Biopsy <u>new</u> HSIL-appearing lesions and send to local pathology		X	X	X	X	X	X	X	X
Random biopsy of any quadrant without a biopsy and send to local pathology	X	X(If not done @ BQE)							X
Radiofrequency Ablation (RFA) Treatment with Barrx™ RFA System		X Circumferential		X Focal		X Focal		X Focal	
Post Treatment Discharge Instructions and "As Needed" Pain Medications Addressed	X	X		X		X		X	
Adverse Event/SAE Inquiry	X	X	X	X	X	X	X	X	X