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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Frumovitz M, Plante M, Lee PS, et al. Near-infrared fluorescence for detection of sentinel lymph nodes in women with cervical and uterine cancers (FILM): a randomised, phase 3, multicentre, non-inferiority trial. *Lancet Oncol* 2018; published online Aug 21. http://dx.doi.org/10.1016/S1470-2045(18)30448-0.

List of Participating Centers

Site	**Principle Investigator**	**Number of Patients Recruited**
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Centre Hospitalier de l'Universite Laval	Marie Plante	34
Duke Cancer Institute	Paula Lee	24
Lee Memorial Hospital	James Orr	24
Memorial Sloan Kettering Cancer Center	Nadeem Abu Rustum	22
O'Connor Hospital	James Lilja	14
HIMA San Pablo	Pedro Escobar	12
Sunnybrook Health Science Center	Lilian Gien	8

FILM

Clinical Study Protocol

PROTOCOL NUMBER
PP LNM 01

TITLE
A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

SHORT TITLE
FILM

PROTOCOL VERSION
June 24, 2015

SPONSOR
Novadaq Technologies Inc.
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Canada, L4W 4T9

FILM

PROTOCOL APPROVAL

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Version: June 24, 2015

Written by:

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Yohan D'Souza, PhD
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PROTOCOL APPROVAL

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PROTOCOL SUMMARY

Study Number and Title:

PP LNM 01: A Randomized, Prospective, Open Label Multicenter Study Assessing the Safety and Utility of PINPOINT® Endoscopic Fluorescence Imaging to Identify Lymph Nodes in Patients with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping.

Clinical Phase: Pivotal / Investigational Device Study

Study Objectives:

Primary:

To assess the effectiveness of intraoperative PINPOINT Near-Infrared Fluorescence in the identification of lymph nodes in patients with uterine and cervical malignancies who are undergoing lymph node mapping.

Secondary:

- To evaluate the effectiveness of PINPOINT and Blue dye in the identification of at least one lymph node (confirmed to be lymphoid tissue) per subject.
- To evaluate the effectiveness of PINPOINT and Blue dye in the intraoperative identification of lymphatic channels in uterine and cervical malignancies.
- To assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

Study Design:

This is a randomized prospective, open label, multicenter study to assess the safety and effectiveness of PINPOINT® Near Infrared Fluorescence Imaging (PINPOINT) in identification of lymph nodes (LN) and lymphatic channels (LC) in subjects with uterine and cervical malignancies who are undergoing LN mapping. This is a non-inferiority within-patient comparison study to determine the effectiveness of PINPOINT in the identification of LNs compared to LNs identified with Blue dye (1% Isosulfan blue). Approximately 150 subjects will be enrolled at up to 10 centers in North America. Prior to enrolling study subjects, participating surgeons at each center will be trained to perform intraoperative identification and mapping of lymph nodes with ICG and Blue dye. Participating surgeons will be required to have completed at least 10 LN mapping procedures with a minimum of 3 LN mapping cases performed with PINPOINT prior to the initiation of enrollment.

Screening:
Subjects diagnosed with International Federation of Gynecology and Obstetrics (FIGO) clinical stage I endometrial or cervical cancer scheduled for surgery that includes clinically indicated LN mapping will be evaluated at baseline to determine if they meet the inclusion/exclusion criteria of the protocol. Subjects will be assessed to determine overall health status including demographics, vital signs, diagnosis and relevant medical history/underlying conditions. Eligible subjects who provide informed consent will be considered for inclusion into the study.

Day 0:
During anesthesia, subjects will be randomized (1:1) to either the Blue-PINPOINT (B-P) Arm or the PINPOINT-BLUE (P-B) Arm. Subjects in each arm will be randomized according to an independently generated randomization scheme to undergo lymphatic mapping with Blue dye first followed by mapping with PINPOINT (B-P Arm) or to undergo lymphatic mapping with PINPOINT first followed by mapping with Blue dye (P-B Arm).

Minimally invasive surgery will be performed according to the surgeon’s standard practice.

The cervix will be injected four (4) times with a 1 ml solution Blue dye and four (4) times with 1 ml of a 1.25 mg/ml solution of ICG. The injection of Blue dye and ICG will occur while the subject is under anesthesia in the operating room. Subjects who do not receive either Blue Dye or ICG for any reason will be considered screen failures.

In order to minimize the spillage of Blue dye or ICG interfering with the mapping procedure when LNs are excised, mapping will be performed on a hemi-pelvis and mapping with both Blue dye and PINPOINT will be performed prior to the excision of any LNs.

Subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of ICG and undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'ICG' nodes are identified or the investigator determines that 'ICG' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered ICG first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping with PINPOINT will be performed until the investigator identifies all 'ICG' nodes or determines that 'ICG' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

LN mapping for Clinical Stage I endometrial cancer will be performed according to the NCCN Guidelines for Uterine Neoplasms, SLN Algorithm for Surgical Staging of Endometrial Cancer; and SLN mapping for Clinical Stage I cervical cancer will be performed according to the NCCN Guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-Stage Cervical Cancer[1,2].

The surgeon will identify LN and lymphatic vessels based on visualization with white light for blue dye or PINPOINT for ICG.

LN Mapping with PINPOINT:
Intraoperative identification of LNs with ICG will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with ICG using PINPOINT.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible fluorescence.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel by NIR fluorescence leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area around the injection site. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised.

LN Mapping with Blue dye:
Intraoperative identification of LNs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible blue dye.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel stained with blue dye leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area around the injection site. Blue ducts should be followed in both directions in order to identify LNs to be excised.

Classification of LNs and LCs:

All LNs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard
5. Non-stained node found at origin or termination of fluorescent duct
6. Non-stained node found at origin or termination of blue duct
7. Non-stained node found at origin or termination of fluorescent and blue duct

Effectiveness of intraoperative Blue dye in identifying LNs will be based on the proportion of LNs identified by visualization under white light and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Effectiveness of intraoperative PINPOINT in identifying LNs will be based on the proportion of LNs identified by PINPOINT and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

All LCs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)

Effectiveness of intraoperative Blue dye in identifying LCs will be based on the proportion of LCs identified by visualization under white light divided by the total number of LCs identified.

Effectiveness of intraoperative PINPOINT in identifying LCs will be based on the proportion of LCs identified by PINPOINT divided by the total number of LCs identified.

Details of the surgery, including intraoperative findings will be documented. Surgical data will be collected including the ability to identify LNs and LCs, the number of LNs and LCs identified and removed, the ability to unilaterally and bilaterally map LNs and LCs, and the anatomical location and distribution of identified LNs. Failed mapping is defined as no LNs detected.

Follow-up and Post-operative Complications:
Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits to monitor occurrence of any adverse events/ adverse device effects on postoperative Day 1, the date of discharge and Day 30 (±7 days).

Study Population

To be eligible for the study, subjects must meet the following main inclusion criteria:
- 18 years of age or older
- Subjects with FIGO Clinical Stage I endometrial cancer undergoing minimally invasive hysterectomy with lymph node mapping.
- Subjects with FIGO Clinical Stage I cervical cancer < 2 cm in size undergoing minimally invasive hysterectomy, trachelectomy, or conization with lymph node mapping.
- Subjects with negative nodal status (N0)
- Subjects with negative metastatic involvement (M0).

Subjects meeting any of the following criteria will be *excluded* from the study:
- Have had prior dissection and/or radiation in pelvis.
- Advanced cervical or endometrial cancer, T3/T4 lesions
- Diagnosis of cervical cancer with a tumor size greater than 2 cm.
- Locally advanced or inflammatory cervical or uterine cancer

- Metastatic cervical or uterine cancer.
- Known allergy or history of adverse reaction to Indocyanine green, iodine or iodine dyes.
- Known allergy or history of adverse reaction to Blue dye (Isosulfan blue) or triphenylmethane.
- Hepatic dysfunction defined as MELD Score > 10.
- Renal dysfunction defined as serum creatinine ≥ 2.0 mg/dl.
- Subjects who have participated in another investigational study within 30 days prior to surgery.
- Pregnant or lactating subjects.
- Subjects who, in the Investigator's opinion, have any medical condition that makes the subject a poor candidate for the investigational procedure, or interferes with the interpretation of study results.

Study Devices and Imaging Agents:

PINPOINT® Endoscopic Fluorescence Imaging System

An endoscopic fluorescence imaging system for high definition (HD) visible (VIS) light and near infrared (NIR) fluorescence imaging that includes the following components:

- A surgical endoscope optimized for VIS/NIR illumination and imaging.
- A camera head that is also optimized for VIS/NIR imaging and mounts to the endoscope eyepiece
- A flexible light guide cable.
- An endoscopic Video Processor/Illuminator for VIS/NIR illumination to the surgical endoscope via a flexible light guide cable, and the image processing required to generate simultaneous, real-time HD video color and NIR fluorescence images.
- A high-definition medical video recorder that allows the capture of still images and video.
- The PINPOINT kit containing the imaging agent and aqueous solvent.

The imaging agent used with PINPOINT is indocyanine green (IC2000), which is a sterile, water-soluble tricarbocyanine dye with a peak spectral absorption at 800-810 nm in blood plasma or blood. IC2000 contains not more than 5.0% sodium iodide.

Lymphazurin (1% Isosulfan blue) is a water-soluble contrast dye and is administered as a 1% solution for the purposes of lymphography.

Study Variables:

Primary Variables

Effectiveness of intraoperative PINPOINT and Blue dye in identifying LNs defined as the proportion of LNs identified by PINPOINT and Blue dye respectively divided by the total number of LNs identified.

Secondary Variables

LN detection rate with PINPOINT or Blue dye, defined as the proportion of cases in which at least one LN is identified with PINPOINT or Blue dye.

Number of LCs identified with PINPOINT and Blue dye

Incidence of adverse events and adverse device events/effects of PINPOINT and Blue dye.

Other Variables

- Bilateral LN detection rate defined as the proportion of cases in which at least one LN is identified on right and left side of the pelvis.
- Unilateral LC detection rate defined as the proportion of cases in which at least one LC is identified on one side of the pelvis.
- Bilateral LC detection rate defined as the proportion of cases in which at least one LC is identified on right and left side of the pelvis.
- Number of LNs identified from following LCs.
- Anatomic distribution of LNs.
- Rate of positive LN detection defined as the proportion of cases with at least one LN diagnosed as pathologically positive for metastasis when at least one LN was identified during surgery.
- Rate of negative LN defined as the proportion of cases with no positive LN when at least one LN was identified during surgery.

- Unilateral SLN detection rate defined as the identification of the primary LN draining from the tumor on one side of the pelvis.
- Bilateral SLN detection rate defined as the identification of the primary LNs draining from the tumor on the right and left side of the tumor.

Study Procedures and Assessments:

The following tests and procedures will be performed:

- Vital signs, height, weight, demographics, surgical predictive factors.
- Relevant medical history and underlying conditions.
- Assessment of eligibility criteria.
- Randomization to the B-P Arm or P-B Arm.
- Imaging agent administration
- LN identification with PINPOINT and Blue dye followed by excision of all LNs identified.
- Documentation of LN mapping procedure.
- Histological assessment of all excised lymph nodes
- Concomitant medications
- Assessment of surgical complications
- Adverse events and adverse device effects
- Follow-up visits on Day 1, date of discharge, and Day 30. Subjects with a discharge date later than Day 30 will have their last study visit on Day 30.

Sample Size and Statistical Analysis:

The FILM trial is a non-inferiority study comparing LN detection rates between Blue dye and PINPOINT. A power calculation was performed and revealed that a sample size of 125 subjects (including 8% subject fallout rate) is required to obtain a LN detection rate for PINPOINT that is non-inferior to blue dye with 95% power and 5% margin of error. The power calculation was obtained using a weighted average of published LN detection rates for blue dye and ICG in gynecological cancer.

Both a modified Intent-to-Treat (mITT) and Per-Protocol (PP) analysis population will be utilized for the primary analysis. Analysis of the primary objective will be conducted using a z-test for proportions (i.e. normal approximation to binomial distribution with a continuity correction) with a one-sided significance level of 0.025. A superiority test will be conducted using the mITT analysis population and a non-inferiority test will be conducted using the PP analysis population according to the method of Moyé et al[3].

The secondary objectives will be tested using a one-sided significance level of 0.025 with a step-down multiplicity adjustment.

Study Duration:

The study is expected to begin in 2015 and complete enrollment in 12 months. Therefore, the study is expected to complete in 2016.

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS

ADE	Adverse device effect
AE	Adverse event
AT	As-treated
B-P	BLUE-PINPOINT
CFR	Code of Federal Regulations
CRF	Case Report Form
CSF	Color Segmented Fluorescence
CT	Computerized Tomography
DMC	Data Monitoring Committee
EC	Ethics Committee
FIGO	International Federation of Gynecology and Obstetrics
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
H&E	Hematoxylin and eosin
HD	High Definition
HAS	Human serum albumin
IA	Imaging agent
ICH	International Conference on Harmonization
ICG	Indocyanine Green
IRB	Institutional Review Board
IRC	Image Review Committee
IV	Intravenous
LC	Lymphatic channel
LN	Lymph node
MELD	Model for end-stage liver disease
mITT	Modified intent-to-treat
NCCN	National Comprehensive Cancer Network
NDA	New drug application
NIR	Near-Infrared
P-B	PINPOINT-BLUE
PINPOINT	PINPOINT Endoscopic Fluorescence Imaging System
PP	Per-Protocol
REDCap	Research Electronic Data Capture
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SLN	Sentinel lymph node
US	United States
UADE	Unanticipated Adverse Device Effect
VIS	Visible

1 INTRODUCTION AND BACKGROUND

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol, Good Clinical Practice (GCP) and all applicable regulations.

1.1 Background

The purpose of this study is to assess the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in identification of lymph nodes (LN) and lymphatic channels (LC) in subjects with uterine and cervical malignancies who are undergoing lymph node mapping and to investigate the safety of interstitial injection of indocyanine green (ICG) dye for intraoperative lymphatic mapping using PINPOINT Near Infrared Fluorescence Imaging.

1.1.1 Sentinel Lymph Node Identification and Mapping

Identification of the first tumor draining lymph node (LN), defined as the sentinel lymph node (SLN), has become an important step for staging cancers that spread through the lymphatic system. SLN mapping involves the use of dyes and/or radiotracers to identify the sentinel nodes either for biopsy or resection and subsequent pathological assessment for metastasis. The procedure is based on the concept that lymph drains in an orderly pattern away from the tumor, and therefore if the SLN is negative for metastasis, the nodes farther down the system should be negative as well[4]. As a result, the tumor bearing nodes are accurately identified and only these are removed, avoiding the morbidity associated with a full lymphadenectomy. Over the past 20 years, SLN mapping has become an established procedure in the staging of breast and melanoma cancers[5].

SLN identification can be accomplished using several different methods, with the basic technique involving the injection of a tracer that identifies the lymphatic drainage pathway from the primary tumor[6]. The tracers used may be colored dyes (e.g. Isosulfan blue), or radioisotopes (e.g. technetium-99m) for intraoperative localization with a gamma probe; or, a combination of both. Recently, ICG using near-infrared (NIR) fluorescence imaging for visualization has emerged as a potentially effective method for several cancers including breast, skin (melanoma), cervical, endometrial, lung and gastrointestinal[7].

The PINPOINT system is an endoscopic NIR fluorescence imaging system used during minimally invasive surgical procedures. PINPOINT acquires NIR fluorescence images of an imaging agent (ICG) to allow for direct real time visual identification of a lymph node and/or the afferent lymphatic channel intraoperatively. ICG is a commonly used water soluble intravascular dye approved for human use in the United States and Canada. It has a peak spectral absorption at approximately 800 nm and can be used as a lymphatic NIR fluorophore. ICG binds primarily to globulins and to a lesser extent to lipoproteins and albumin[8]. In general, when injected interstitially, the protein binding properties of ICG cause it to be rapidly taken up by the lymph and moved through the conducting vessels to the SLN[9]. The NIR fluorescent positive lymph nodes are represented on the PINPOINT screen in PINPOINT (green pseudo-color superimposed on white light image), SPY (black and white) or SPY CSF (color segmented fluorescence) mode.

1.1.2 Endometrial and Cervical Cancer

Endometrial cancer is the most common gynecological cancer, and it is estimated that 52,630 new cases will be diagnosed in the United States (US)[10] and 6,000 new cases will be diagnosed in Canada in 2014[11]. Cervical cancer affects a lower number of women with 12,360[12] and 1,450[11] new cases estimated in the US and Canada respectively for 2014. The most important prognostic factor for subjects is accurate surgical staging, as lymph node status is a predictor of outcome and may influence treatment following surgery.

Identification of SLN and SLN mapping for endometrial cancer is being evaluated as a method for surgical staging to be used in subjects with uterine confined tumors when there is no metastasis demonstrated by imaging studies and no obvious extrauterine disease[13]. The current standard of care is to perform a complete or selective para-aortic lymphadenectomy for staging which can lead to morbidities such as lower extremity lymphedema and lymphocyst formation[4]. As most subjects present with early-stage disease and the rate of metastasis is low, the ability to utilize SLN identification and mapping as an accurate method for staging and thus avoidance of a complete or selective para-aortic lymphadenectomy may be an acceptable approach.

1.2 Summary of Clinical Data for NIR Fluorescence Imaging with ICG for SLN Mapping

The imaging agent (IA), ICG (indocyanine green for injection, USP) 25 mg for Injection in the form of a sterile lyophilized powder containing indocyanine green with no more than 5% sodium iodide is approved by the FDA for determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography via intravascular administration[14,15]. ICG is also approved under a 510(k) by the FDA for assessing blood flow and tissue perfusion in a variety of surgical and non-surgical procedures[16]. ICG can be administered intravenously or intra-arterially. It absorbs light in the near-infrared region at 806 nm, and emits fluorescence (light) at a slightly longer wavelength, 830 nm. When injected intravenously, ICG rapidly and extensively binds to plasma proteins and is confined to the intravascular compartment with minimal leakage into the interstitium. This property makes ICG an ideal agent for the acquisition of high quality images of lymph nodes and lymphatic vessels (i.e., for NIR fluorescence lymphography).

1.2.1 Clinical Studies

The Sponsor has not conducted any clinical trials on the use of intraoperative PINPOINT Near Infrared Fluorescence Imaging with ICG in identification and mapping of SLN to date. However, 12 studies with performance and/or safety data using the Sponsor's fluorescence imaging devices or those with equivalent characteristics have been reported in the literature on the use of ICG in SLN detection/mapping of gynecological cancers (Table 1).

TABLE 1. Sentinel Lymph Node Detection/Mapping Studies Gynecological cancers

Author	Indication	Device	Summary
Furukawa et al., 2010[17]	Cervical	PDE	ICG fluorescence imaging was used to identify SLN during sentinel node navigation surgery in cervical cancer patients. No allergic reactions to ICG were observed.
Crane et al., 2011[18]	Cervical	Prototype multispectral fluorescence camera system	Intraoperative lymphatic mapping and SLN detection was carried out in cervical cancer patients using ICG fluorescence imaging and patent blue dye. No side effects were noted after injection of ICG or patent blue dye.

Author	Indication	Device	Summary
Crane et al., 2011[19]	Vulvar	Prototype multispectral fluorescence camera system	Intraoperative transcutaneous lymphatic mapping was carried out in vulvar cancer patients in a comparison using ICG fluorescence imaging, ^{99m}-Technetium-nanocolloid and patent blue dye. No side effects related to ICG injection were noted.
Holloway et al., 2012[20]	Endometrial	da Vinci Surgical System	Retrospective comparison of results from lymphatic mapping of pelvic SLN using fluorescence NIR imaging of ICG and colorimetric imaging of Isosulfan blue dyes in women with endometrial cancer undergoing robotic-assisted lymphadenectomy.
Rossi et al 2012[21]	Endometrial	da Vinci Surgical System	Sentinel lymph node (SLN) mapping with indocyanine green (ICG) detected by robotic near infrared (NIR) imaging is a feasible technique.
Schaafsma et al 2012[22]	Cervical	Mini-FLARE	Eighteen consecutive early-stage cervical cancer patients scheduled to undergo pelvic lymphadenectomy were included. Prior to surgery, 1.6 mL of 500 µM ICG:HSA or 500 µM ICG alone was injected transvaginally in 4 quadrants around the tumor. The Mini-FLARE imaging system was used for intraoperative NIR fluorescence detection and quantitation.
Rossi et al 2013[23]	Cervical or endometrial, Stage 1	SPY Scope (7 subjects); da Vinci (13 subjects)	Robotically assisted endoscopic NIR imaging after injection of ICG was used for LN mapping in patients with clinical stage 1 cervical or endometrial cancer. No AEs occurred.
Schaafsma et al 2013[24]	Vulvar	Mini-FLARE	NIR fluorescence imaging for SLN biopsy was investigated and ICG vs ICG:HSA compared in a double-blind, randomized, non-inferiority trial of vulvar cancer patients. The study confirmed the feasibility of NIR fluorescence imaging for SLN mapping in vulvar cancer and found no advantage in using ICG:HSA over ICG alone. No AEs were associated with the use of ICG or ICG:HSA.
Jewell et al., 2014[25]	Endometrial and cervical	da Vinci Surgical System	Robotically assisted endoscopic NIR imaging after injection of ICG was used for SLN mapping in patients with uterine and cervical malignancies
Sinno et al. 2014[26]	Endometrial or CAH	da Vinci Surgical System	Comparison of NIR fluorometric imaging vs colorimetric imaging for SLN mapping in endometrial cancer
Plante et. al. 2015[27]	Endometrial and cervical	PINPOINT Endoscopic System	Initial experience with PINPOINT Endoscopic NIR imaging for LN identification was reported. Study determined PINPOINT/ICG is an excellent and safe modality for SLN mapping with a very high (96%) detection rate.
How et. al. 2015[28]	Endometrial	da Vinci Surgical System	Comparison of ICG, blue dye and 99mTc-SC mapping in patients undergoing robotic-assisted surgery. A mixture of all three modalities is feasible and provides good mapping. ICG was found to be superior to blue dye and comparable to 99mTc-SC and thus blue dye may not be essential for SLN detection.

A total of 550 subjects with early stage gynecological cancers (cervical, endometrial and vulvar) have been reported in the literature as having undergone intraoperative SLN mapping with ICG fluorescence imaging. Table 2 lists the published studies including the number of subjects receiving ICG, the ICG dose, route of administration, SLN detection rate and safety results (when reported). All subjects receiving an injection of ICG are listed for studies that included co-administration of a colorimetric dye or radiotracer. Of the 12 studies, 7 specifically reported that there were no adverse reactions to the procedure. In 4 studies (Crane et al 2011; Furukawa et al 2010; Rossi et al, 2011, Plante et al. 2015), ICG fluorescence was used to identify SLNs in subjects with cervical or endometrial cancer[17,19,21]. Detection rates ranged from 60% to 96%. In a study comparing ICG fluorescence and blue dye, Crane et al (2011) found that 89.7% of radioactively labelled SLNs in subjects with vulvar cancer could be detected by ICG fluorescence compared to 72.4% detected by blue dye[18].

Schaafsma et al. compared the use of ICG and ICG conjugated to HSA for NIR fluorescence detection of SNs in subjects with early cervical cancer[22] and vulvar cancer[24]. In both studies, no difference in SLN detection rates was found between the ICG (6/9; 67% for cervical cancer and 9/12; 75% for vulvar cancer) and ICG:HSA groups (8/9; 89% for cervical cancer; 10/12; 83% for vulvar cancer) (p=0.13, cervical cancer; p=0.27, vulvar cancer).

Jewell et al. completed a retrospective, open label, single center study using NIR fluorescence imaging on the robotic platform with intracervical ICG injection for SLN detection in uterine and cervical malignancies[25]. A total of two hundred and twenty-seven cases were performed. When ICG alone was used to map cases, 188/197 subjects mapped for a detection rate of 95% compared to 93% in cases in which both blue dye and ICG were used. Bilateral mapping was seen in 79% of ICG alone cases compared to 77% of cases using ICG and blue dye. The authors concluded that NIR fluorescence imaging on the robotic platform has a high bilateral detection rate and appears to be favorable to ICG dye alone, with blue dye combined with ICG unnecessary for SLN mapping.

How et al. recently completed a prospective study comparing NIR fluorescence using ICG, blue dye and technetium for SLN mapping in endometrial cancer[28]. A total of 100 subjects underwent SLN mapping with a mixture of ICG, blue dye and 99mTc-SC injected directly into the cervix. A total of 286 LN were mapped (2.9 per subject). ICG had a significantly higher detection rate than blue dye both in unilateral (87% vs 71%) and bilateral (65% vs 43%) detection rates. The authors concluded that mapping with ICG is superior to blue dye and comparable to mapping with 99mTc-SC.

TABLE 2. Sentinel Lymph Node Detection/Mapping Studies Gynecological cancers

Author	Type of Cancer	Number of Patients	ICG Concentration (mg/ml)	Total Dose of ICG (mg)	Route of Administration	Detection Rate	Safety
Furukawa et al., 2010	Cervical	10	5.00	1.0	Cervical injection[a]	83%	No allergic reactions
Crane et al., 2011	Cervical (1A1, 1B1, IIA)	10	0.50	0.50	Cervical injection	60%	No side effects reported
Crane et al., 2011	Vulvar	10	0.50	0.50	Vulva (peritumoral injection)	89.7%	No side effects related to intraoperative injection of ICG
Holloway et al., 2012[b]	Endometrial	35	1.25	2.50	Cervical injection[a]	100%	Not reported
Rossi et al., 2012	Cervical or endometrial Stage 1	3 17	0.50 0.50	0.50, 0.75 & 1.5 1.00	Cervical injection[d]	85% 88%	No adverse events reported
Rossi et al., 2012	Endometrial	12	0.50	0.50	Hysteroscopic endometrial	33%	Not reported
		17	0.50	1.00	Cervical Injection	82%	Not reported
Schaafsma et al., 2012	Cervical (1B1)	9	0.38	0.62	Cervical[a]	67%[f]	Not reported

Author	Type of Cancer	Number of Patients	ICG Concentration (mg/ml)	Total Dose of ICG (mg)	Route of Administration	Detection Rate	Safety
Schaafsma et al., 2013	Vulvar	12	0.38	0.62	Vulva (peritumorally or around excision scar)	75%	No adverse reactions reported
Jewell et al. 2014	Endometrial and cervical	227	1.25	2.50	Cervical Injection	100%	Not reported
Sinno et al., 2014	Endometrial or CAH[e]	38	1.25	5.00	Cervical injection[c]	78.9%	Not reported
Plante et al. 2015	Cervical or endometrial Stage 1	50	1.25	5.00	Cervical Injection	96%	No adverse events reported
How et al. 2015	Endometrial	100	0.25	0.1	Cervical Injection	96%	No adverse events reported

a Injection into 4 quadrants of the cervix (3, 6, 9 and 12 o'clock positions)
b Retrospective study
c Injection into cervix at 3 and 9 o'clock positions with 1 cc deep in the stroma and 1 cc submucosally on the right and left of cervix for a total volume of 4 ml
d Injection 1 cm into cervical stroma at 3 and 9 o'clock positions
e Complex Atypical Hyperplasia
f Intraoperative bilateral detection rate

1.3 Potential Risks and Benefits to Human Subjects

Currently the PINPOINT is classified by the United States Food and Drug Administration (FDA) as a Class II medical device with a Product Code of GCJ. PINPOINT for the purposes of fluorescence angiography is not identified as a significant risk device on the FDA Information Sheet titled "Significant Risk and Non-significant Risk Medical Device Studies". PINPOINT is a commercially available product in the United States (US). PINPOINT has a 510(k) clearance from the FDA for the following indication:

"The PINPOINT system is intended to provide real-time endoscopic visible and endoscopic NIR fluorescence imaging. PINPOINT enables surgeons to perform routine visible light endoscopic procedures as well as further visually assess vessels, blood flow and related tissue perfusion with near infrared imaging during minimally invasive surgery".

Note: PINPOINT has not yet been classified by the FDA or Health Canada for the purposes of lymphatic mapping.

The imaging agent, ICG, is approved for human use by the FDA and Health Canada. In this study, investigational ICG will be administered interstitially for the visualization of LN and LC. The safety of interstitial administration of investigational ICG for NIR fluorescence imaging to identify LN has not been studied. However, safety data from published studies reported above combined with the well-established safety profile for ICG after intravenous or intra-arterial injection support an acceptable risk/benefit ratio for interstitial administration of ICG. . The most serious risk of ICG when administered intravenously in humans, is anaphylactic death, which has been reported following ICG administration during cardiac catheterization.

These and other risks of the PINPOINT system in humans are described further in Section 7, Risks/Precautions. For additional information, please refer to the PINPOINT Operator's Manual[16].

2 STUDY OBJECTIVES

2.1 Objectives

2.1.1 Primary Objectives

The primary objective of this study is to assess the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in identification of lymph nodes in subjects with uterine and cervical malignancies who are undergoing lymph node mapping.

2.1.2 Secondary Objectives

To evaluate the effectiveness of PINPOINT and Blue dye in the identification of at least one lymph node (confirmed to be lymphoid tissue) per subject.

To evaluate the effectiveness of PINPOINT and Blue dye in the intraoperative identification of lymphatic channels in uterine and cervical malignancies.

To assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

3 INVESTIGATIONAL PLAN

3.1 Study Design Overview

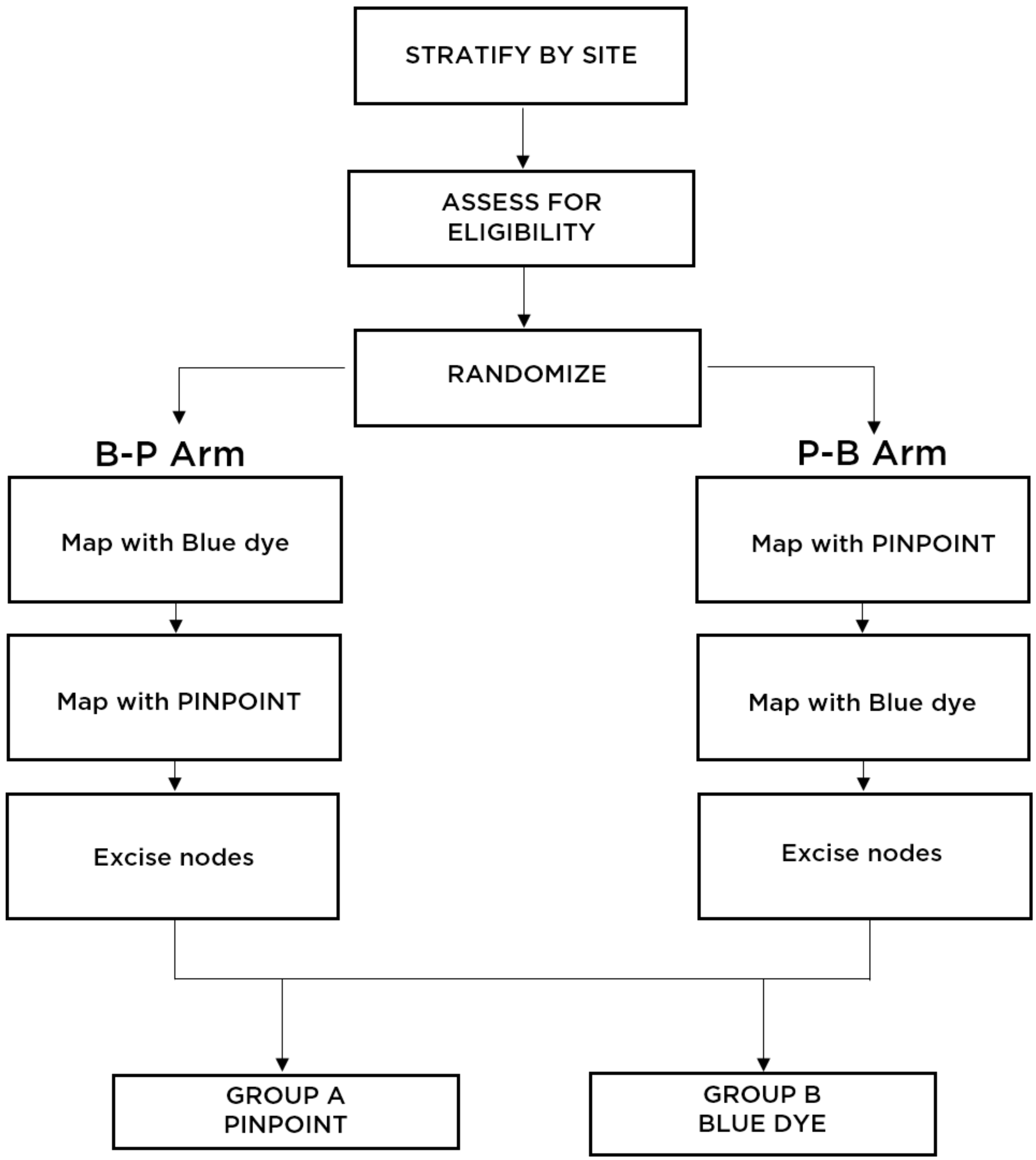
This is a randomized prospective, open label, multicenter study to assess the safety and effectiveness of PINPOINT Near Infrared Fluorescence Imaging (PINPOINT) in identification of LN and LC in subjects with uterine and cervical malignancies who are undergoing LN mapping. This is a non-inferiority within-patient comparison study to determine the effectiveness of PINPOINT in the identification of LNs compared to LNs identified with Blue dye. Approximately 150 subjects will be enrolled at up to 10 centers in North America. Prior to enrolling study subjects, participating surgeons at each center will be trained to perform intraoperative identification and mapping of lymph nodes with ICG and Blue dye. Participating surgeons will be required to have completed at least 10 LN mapping procedures with a minimum of 3 LN mapping cases performed with PINPOINT prior to the initiation of enrollment.

Screening:
Subjects diagnosed with International Federation of Gynecology and Obstetrics (FIGO) clinical stage I endometrial or cervical cancer scheduled for surgery that includes clinically indicated LN mapping will be evaluated at baseline to determine if they meet the inclusion/exclusion criteria of the protocol. Subjects will be assessed to determine overall health status including demographics, vital signs, diagnosis and relevant medical history/underlying conditions. Eligible subjects who provide informed consent will be considered for inclusion into the study.

Day 0:

During anaesthesia, subjects will be randomized (1:1) to either the BLUE-PINPOINT (B-P) Arm or the PINPOINT-BLUE (P-B) Arm. Subjects in each arm will be randomized according to an independently generated randomization scheme to undergo lymphatic mapping with Blue dye first followed by mapping with PINPOINT (B-P Arm) or to undergo lymphatic mapping with PINPOINT first followed by mapping with Blue dye (P-B Arm).

FIGURE 1. Group Assignment and Randomization



Eligible subjects who have provided informed consent will be enrolled in the study upon randomization on the day of surgery (Day 0). Minimally invasive surgery will be performed according to the surgeon's standard practice.

The cervix will be injected four (4) times with a 1 ml solution of Blue Dye (1% Isosulfan blue) and four (4) times with 1 ml of a 1.25 mg/ml solution of ICG. The injection of Blue dye and ICG will occur while the subject is under anaesthesia in the operating room. Subjects who do not receive either Blue Dye or ICG for any reason will be considered screen failures.

In order to minimize the spillage of blue dye or ICG interfering with the mapping procedure when LNs are excised, mapping will be performed on a hemi-pelvis and mapping with both Blue dye and PINPOINT will be performed prior to the excision of any LNs.

Subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of ICG and undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'ICG' nodes are identified or the investigator determines that 'ICG' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered ICG first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping with PINPOINT will be performed until the investigator identifies all 'ICG' nodes or determines that 'ICG' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both PINPOINT and Blue dye have been completed and documented, all LNs identified with PINPOINT or Blue dye will be excised.

LN mapping for Clinical Stage I endometrial cancer will be performed according to the NCCN Guidelines for Uterine Neoplasms, SLN Algorithm for Surgical Staging of Endometrial Cancer; and SLN mapping for Clinical Stage I cervical cancer will be performed according to the NCCN Guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-Stage Cervical Cancer[1,2].

The surgeon will identify LN and LC based on direct visualization with white light for blue dye or PINPOINT for ICG.

LN Mapping with PINPOINT:
Intraoperative identification of LNs with ICG will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with ICG using PINPOINT.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible fluorescence.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel by NIR fluorescence leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area around the injection site. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised.

LN Mapping with Blue dye:

Intraoperative identification of LNs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible blue dye.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel stained with blue dye leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area around the injection site. Blue ducts should be followed in both directions in order to identify LNs to be excised.

Classification of LNs and LCs:

All LNs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard
5. Non-stained node found at origin or termination of fluorescent duct
6. Non-stained node found at origin or termination of blue duct
7. Non-stained node found at origin or termination of fluorescent and blue duct

Effectiveness of intraoperative Blue dye in identifying of LN will be based on the proportion of LNs identified by visualization of blue dye under white light and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Effectiveness of intraoperative PINPOINT in identifying of LN will be based on the proportion of LNs identified by PINPOINT and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

All LCs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)

Effectiveness of intraoperative Blue dye in identifying of LC will be based on the proportion of LCs identified by visualization of blue dye under white light divided by the total number of LCs identified.

Effectiveness of intraoperative PINPOINT in identifying of LC will be based on the proportion of LCs identified by PINPOINT divided by the total number of LCs identified.

Details of the surgery, including intraoperative findings will be documented. Surgical data will be collected including the ability to identify LN and LC, the number of LNs and LCs identified and removed, the ability to unilaterally and bilaterally map LN and LCs, and the anatomical location and distribution of identified LN. Failed mapping is defined as no LNs detected.

Follow-up and Post-operative Complications:

All Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits to monitor occurrence of any adverse events/adverse device effects on postoperative Day 1, the date of discharge and Day 30 (±7 days). All subjects will be followed to monitor occurrence of adverse events up to Day 30 (±7 days) post-surgery. All adverse events will be followed up to Day 30 (±7 days). Adverse events thought to be related to the use of PINPOINT or to the LN mapping procedure will be followed until resolution or deemed chronic.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Number of Subjects

Approximately 150 subjects will be enrolled in the study (See Section 10.4).

4.2 Inclusion Criteria

To be eligible for the study, a subject must fulfill all of the following criteria:

1. Be 18 years of age or older
2. Have either of the following diagnoses and surgical plan:
    a. FIGO Clinical stage I endometrial cancer undergoing minimally invasive hysterectomy with lymphatic mapping
    b. FIGO Clinical stage I cervical cancer < 2 cm undergoing minimally invasive hysterectomy, trachelectomy or conization with lymphatic mapping
3. Negative nodal status (N0)
4. Subjects with negative metastatic involvement (M0)
5. Subjects of child-bearing potential must not be pregnant or lactating and must have a negative pregnancy test at Day 0
6. Have signed an approved informed consent form for the study
7. Be willing to comply with the protocol

4.3 Exclusion Criteria

A subject meeting any of the following criteria will be excluded from the study:

1. Have had prior dissection and/or radiation in pelvis
2. Advanced cervical or endometrial cancer, T3/T4 lesions
3. Diagnosis of cervical cancer with a tumor size greater than 2 cm
4. Locally advanced or inflammatory cervical or uterine cancer
5. Metastatic cervical or uterine cancer
6. Known allergy or history of adverse reaction to ICG, iodine or iodine dyes
7. Known allergy or history of adverse reaction to Blue dye (Isosulfan blue) or

triphenylmethane

8. Hepatic dysfunction defined as MELD Score > 10
9. Renal dysfunction defined as serum creatinine $\geq$ 2.0 mg/dl
10. Subjects who have participated in another investigational study within 30 days prior to surgery
11. Pregnant or lactating subjects
12. Subjects who, in the Investigator's opinion, have any medical condition that makes the subject a poor candidate for the investigational procedure, or interferes with the interpretation of study results

4.4 Withdrawal of Subjects

Subjects can voluntarily withdraw (or be withdrawn) at any time during the study.

Investigators may withdraw a subject from the study because:

- A new health condition, diagnosis or finding appears that is suspected to require care or medication prohibited by the protocol.
  - E.g., the planned surgical procedure is modified to a procedure prohibited by the protocol.
- The subject has unacceptable adverse events.
- It is in the subject's best interest according to the Investigator's clinical judgment.

If a subject is prematurely withdrawn from the study, the reason(s) for withdrawal must be recorded on the relevant page of the subject's Study Completion case report form (CRF).

Subjects who discontinue the study prematurely will not be replaced.

The Sponsor may stop the study at any time.

5 RANDOMIZATION, BLINDING AND SUBJECT IDENTIFICATION PROCEDURES

5.1 Randomization

Subjects will be prospectively randomized into the FILM Clinical Trial. Randomization will occur at the time of surgery, just after induction of anesthesia. Prior to surgery, the subject will have provided written informed consent, completed all baseline procedures and met the requirements of inclusion and exclusion criteria. Randomization should be performed as closely as possible to the mapping procedure to minimize the incidence of dropout.

Subjects will be randomly assigned on a one to one (1:1) basis to either the B-P Arm (LN mapping with Blue dye followed by LN mapping with PINPOINT) or the P-B Arm (LN mapping with PINPOINT followed by LN mapping with Blue dye). Randomization will be stratified by study site. Permuted block randomization will be performed within strata. To minimize the opportunity for the sequence to be predicted, the block size will be variable and randomly chosen from small multiples of 2 (i.e. 2, 4 or 6). The randomization schedules will be generated in advance using a computerized random number generator. Investigational sites will not have access to the randomization schedules.

Randomization will be accomplished using a secure web-based software (REDCap) supported by the Data Coordinating Center. Treatment assignment is made only after verification of proper informed consent execution and study eligibility.

5.1.1 Randomization Procedure

The study coordinator or a designee will verify that the patient is eligible and that informed consent has been obtained prior to initiating the randomization process. Once the subject is under anesthesia, the study coordinator will log onto the REDCap system and, after confirming the subject's eligibility, will enroll a subject and obtain the randomization assignment. The study coordinator will then disclose the randomization assignment to the Investigator. As this is a within-subject comparison study, the investigator cannot be blinded to the use of Blue dye or PINPOINT device. All subjects will be blinded to their randomization assignment until after the procedure.

A subject is not randomized until randomization has been assigned by REDCap. The randomization procedure should not be initiated unless the study coordinator confirms that a subject is eligible and verifies baseline information. If any deviations occur (errors such as misplacing envelopes), or if the randomization assignment is made incorrectly, the clinical site will be required to contact the Sponsor and await guidance on how to proceed.

If, at any time after randomization, the subject becomes ineligible or withdraws, the subject is still considered randomized. If an intraoperative decision is made to perform a procedure other than what was intended, the subject will be categorized with respect to the definitions outlined for the analysis populations (see the Statistical Methods Section 10.5).

5.2 Subject Identification

Screening ID Number: All subjects screened for the study shall be assigned a 5-digit "screening" number on the Screening Log and if they are randomized, will subsequently be assigned a Subject Enrollment Number in the electronic data capture system. The Screening Identification Number shall be unique and categorize a subject in sequence of screening by an "S" followed by a 5-digit number. The first 2 digits identify the site and the last three digits identify the subject. For example, the first subject screened at site 01 is identified as screening number S-01-001. The screening log shall be maintained by the site to identify those subjects that have failed screening with the reason why they did not qualify for enrollment. The screening number will be assigned sequentially within each study center in order of subject presentation for screening.

Enrolled Subject ID Number: Once a subject is randomized, they will be assigned a Subject Enrollment Number which is also a 5-digit number. The first two digits of the Subject Enrollment Number identify the site and the last three digits identify the subject. Each site will be given a Site Identification Number. For example, the first subject randomized at site 03 is identified as subject 03-001, the next subject as 03-002, etc.

Subjects who sign an informed consent form but do not receive either Blue dye or ICG, are considered screen failures. These subjects must be entered on the Screening Log but do not receive a subject number. The reason for non-enrollment must be documented on the log.

6 STUDY TREATMENTS

6.1 Device Description

PINPOINT is an endoscopic fluorescence imaging system for high definition (HD) visible (VIS) light and near-infrared (NIR) fluorescence imaging. PINPOINT includes the following components:

- A surgical endoscope optimized for VIS/NIR illumination and imaging, which is available in different diameters, lengths and directions of view (Model: SC9104, SC9134).
- A camera head that is also optimized for VIS/NIR imaging and mounts to the endoscope eyepiece (Model: PC9002).
- A flexible light guide cable (Model: PC9004).
- An endoscopic Video Processor / Illuminator (VPI) capable of providing VIS/NIR illumination to the surgical endoscope via a flexible light guide cable, and the image processing required to generate simultaneous, real-time HD video color and NIR fluorescence images (Model: PC9001).

  PINPOINT is designed to be connected to a medical-grade HD color monitor, such as those normally used in surgical endoscopy.

PINPOINT is connected to a high-definition medical video recorder (Sony HVO-1000) that allows the capture of still images and video during operation.

PINPOINT acquires NIR fluorescence images of an imaging agent (ICG) to allow for visual assessment of vessels, blood flow and related tissue perfusion during minimally invasive surgery. For the purpose of this study, PINPOINT will be used for visual identification of a lymph node and/or the afferent lymphatic channel during lymph node mapping procedures.

The PINPOINT Operator's Manual[16] describes the contents, use and storage of the PINPOINT PAQ's. Instructions for preparation, handling and administration of ICG are provided in the Clinical trial protocol (Section 8.3.1.2).

PINPOINT allows simultaneous display of multiple images. Real time NIR fluorescence video images are acquired by using the imaging agent and may be viewed in two ways:

PINPOINT image:	NIR fluorescence is superimposed in pseudo-color (green) on a white light image
SPY image:	A black and white NIR fluorescence image is displayed
CSF:	A high-definition, white light image is displayed in grayscale with NIR fluorescence overlaid on a color scale. Increasing fluorescence levels transition from blue through yellow to red.

PINPOINT also operates as a conventional endoscopic imaging system and provides illumination for real-time color (white light) HD video imaging in the area of interest.

PINPOINT is a commercially available product in the United States (US). PINPOINT has a 510(k) clearance from the FDA for the following indication:

"The PINPOINT system is intended to provide real-time endoscopic visible and endoscopic NIR fluorescence imaging. PINPOINT enables surgeons to perform routine visible light endoscopic procedures as well as further visually assess vessels, blood flow and related tissue perfusion with near infrared imaging during minimally invasive surgery".

PINPOINT is manufactured by Novadaq Technologies Inc. (Novadaq). Please refer to the current version of the PINPOINT Operator's Manual[16] for a full description and specifications of the system.

In this study, the use of PINPOINT for visual identification of lymph nodes via interstitial injection of ICG is investigational.

6.2 Imaging Agent Description

6.2.1 Indocyanine Green IC2000

6.2.1.1 Overview and Pharmacokinetics of Investigational Product

ICG was originally approved by the FDA for human medical use in 1959 for use in determining cardiac output, hepatic function and liver blood flow. In 1975, a NDA Supplement was approved for ICG for use in ophthalmic angiography. In 2005, ICG was approved under a 510K for assessing blood flow and tissue perfusion in a variety of surgical and non-surgical procedures. Over the past 50 years ICG has been marketed in the United States and has demonstrated an excellent safety profile. ICG has received approval from Health Canada (DIN 02014793) in 1994 for human medical use as a diagnostic agent, and is classified as an "Ethical" drug.

ICG absorbs light in the near-infrared (NIR) region at 806 nm and emits fluorescence (light) at a slightly longer wavelength, 830 nm. After injection, ICG rapidly binds to blood proteins primarily lipoproteins and to a lesser extent globulins and albumin and drains into the lymphatic system without extravasation enabling sensitive non-invasive visualization of SLN and lymphatic architecture. Within minutes after intravenous injection, ICG is cleared by the liver and excreted into the bile[24]. Since lymph fluid flows into the venous blood stream through the subclavien veins[9], it is assumed that, after interstitial injection, ICG drains with lymph fluid into the circulatory system where it is then cleared by the liver and excreted in the bile. Although formal pharmacokinetic studies have not been performed with non-intravenous routes of administration, after intradermal injection in mice, ICG was shown to be taken up by lymphatic vessels and cleared within 48 hours[29,30]. Another study using ICG conjugated to human serum albumin (HSA) in rabbits found ICG is efficiently taken up by the lymphatic vessels and cleared within 24 hours after peritumoral injection[31]. Chi et al. investigated the use of ICG for LN mapping in 5 mice, 10 rabbits and 22 breast cancer subjects and found LNs are identifiable 3-5 minutes after injection with the occurrence of peak fluorescence intensities varying with the dose of ICG and occurs between 10 and 90 minutes[32].

6.2.1.2 Description of Investigational Product (IC2000)

The chemical name of the investigational drug, indocyanine green is 1 *H*-Benz[e]indolium, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[e]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-,hydroxide, inner salt, sodium salt. ICG has a molecular weight of 774.96 daltons and will be administered with an interstitial injection into the cervix.

The imaging agent (IA), IC2000 (indocyanine green, USP) is provided in the form of a sterile lyophilized powder containing 25 mg indocyanine green with no more than 5% sodium iodide. IC2000 is packaged with aqueous solvent consisting of sterile Water for Injection, which is used to reconstitute the indocyanine green. When injected, indocyanine green rapidly and extensively binds to plasma proteins (primarily lipoproteins and globulins) with minimal leakage through vessel walls. This property makes indocyanine green an ideal agent for the acquisition of high quality images of lymph nodes and lymphatic vessels (i.e., for NIR fluorescence lymphography). Note: The use of IC2000 in this study is investigational.

6.2.1.3 Chemistry and Manufacturing

Investigational IC2000 is supplied as a 25 mg sterile lyophilized green powder in a glass vial with a grey stopper and grey overseal. The product is stored at 20°C to 25°C (68° to 77° F). IC2000 is manufactured by Patheon (Patheon Italia S.p.A., 2° Trav. SX Via Morolense, 503013 Ferentino- Italy) and, like commercially available indocyanine green, IC2000 is tested according to the USP monograph for Indocyanine green for Injection.

IC2000 was manufactured in full compliance with the FDA's current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals-standards at 21 CFR Part 211 and meets all GMP requirements for Health Canada.

6.2.1.4 Labelling of ICG for Injection

IC2000 is labeled according to US and Canadian regulatory requirements with the following information:

- IC2000 (indocyanine green for injection, USP), 25 mg lyophilized ICG
- Sterile
- Protocol No.: PP LMN 01
- Directions for use: Refer to Clinical Protocol
- Store at: 20–25°C (68-77°F)
- Lot: 14GRF03
- EXP.: May 2016
- CAUTION—New drug - Limited by Federal (or United States) law to investigational use.
- Investigational drug to be used only by a qualified investigator.
- Sponsor: Novadaq Technologies Inc. 13155 Delf Place, Unit 250, Richmond, BC, Canada, V6V 2A2 1.800.665.2236

6.2.1.5 Packaging and Distribution of IC2000

IC2000 will be packaged into investigational PINPOINT LN Mapping Kits as described below and distributed by the study Sponsor (Novadaq). Each LN-mapping procedure kit is indicated for use exclusively with PINPOINT and should only be used for the purposes of this study.

Each PINPOINT LN Mapping kit contains:

- One single use 25 mg vial of sterile IC2000, USP
- Two single use 10 ml ampules of sterile Aqueous solvent
- Two 3 ml syringes, sterile
- Two 10 ml syringes, sterile
- Two Spinal needles, 22G, 1.5 inch, sterile
- Labels for syringes

All information included on the investigational ICG for Injection vial labels will appear on the kit labels along with a description of the contents of the kit. Each kit will bear a unique serial number and will meet regulatory requirements necessary for each participating country.

6.2.1.6 Storage and administration of ICG for Injection

ICG will be stored at 20°C to 25°C (68° to 77° F) in a secure locked area, accessible to authorized personnel only. ICG will be stored, handled, prepared and administered by qualified, trained personnel only as described in section 8.3.1.2.

6.2.2 Blue Dye (Isosulfan Blue)

The Blue dye (Isosulfan Blue Injection, 1% aqueous solution) a sterile and pyrogen-free 5 ml single-use vial[33]. Isosulfan blue is supplied as a 1% aqueous solution in a phosphate buffer and is approved under an ANDA by the US FDA for the identification of lymphatic vessels draining the region of injection, lymphography, and identification of lymph node involvement by primary and secondary neoplasms. Isosulfan blue will be obtained from commercially available sources and is approved for subcutaneous injection.

6.2.2.1 Storage and administration

Blue dye will be stored at 20°C to 25°C (68° to 77° F) in a secure locked area, accessible to authorized personnel only. Blue dye will be stored, handled, prepared and administered by qualified, trained personnel only as described in section 8.3.1.1. Installation, Training and Storage

PINPOINT will be installed by Novadaq representatives. The Investigator(s) and study staff shall be required to participate in training on the operational and procedural use of PINPOINT as it relates to the conduct of this study. Training will be provided by Novadaq.

Prior to enrollment, participating Investigator surgeons must be experienced with LN mapping procedures (i.e. performed a minimum of 10 LN mapping procedures) with a minimum of 3 LN mapping cases performed with PINPOINT prior to initiation of enrollment.

Investigator surgeons new to this technique will receive training consisting of the following:

- Didactic training on product and procedure.
- Hands on training session with clinical specialists on site.
- Guidance on use of the system and interpretation of the images with a clinical educator during a minimum of 10 cases prior to study enrollment.

Supporting personnel operating and cleaning PINPOINT will also receive training and be familiar with all applicable aspects of the operation and cleaning of the system.

PINPOINT shall be stored at room temperature in a secure and limited access area available to study staff.

6.3 Concomitant Treatment

Any concomitant medications the subject is receiving at the start of the study (Day 0) or given for any reason during the study must be recorded in the CRF and source documents with the exception of routine medications given for preparation of surgery, during surgery and post-operative care. These include but are not limited to the following:

- Sedatives and anesthetics
- Anticoagulants
- Prophylactic antibiotics
- Anti-emetics
- Routine post-operative pain medications

The drug name, start and stop dates, indication, dose, frequency and route information will be recorded for concomitant medications.

Other surgical and diagnostic procedures concomitant to the laparoscopic hysterectomy, trachelectomy, conization and lymphatic mapping procedure that take place during the study must be recorded in the CRF and in the source document, including start and stop dates.

7 RISKS/PRECAUTIONS

Refer to the PINPOINT Operator's Manual for a full description of the risks and precautions associated with all components of PINPOINT. The entire PINPOINT Operator's Manual should be read before using PINPOINT[16]. Failure to follow the instructions and warnings in the manual may result in unsafe operation of the system and/or injury to the patient or operator.

Refer to the Isosulfan Blue prescribing information for a full description of risks and precautions associated with the use of Isosulfan Blue[33]. The entire package insert should be read before using Isosulfan blue. Failure to follow instructions and warnings may result in injury to the subject.

7.1 PINPOINT System – Endoscopes, Camera and Video Processor Illuminator Unit

Currently, PINPOINT is classified by the FDA as a Class II medical device with a Product Code of GCJ. PINPOINT for the purposes of fluorescence angography is not identified as a significant risk device on the FDA Information Sheet titled "Significant Risk and Non-significant Risk Medical Device Studies".

The use of PINPOINT for LN mapping is investigational. PINPOINT should only be used in accordance to its approved Indication for Use or in accordance to the study procedures described in this protocol.

7.2 Indocyanine Green (IC2000)

IC2000 will be injected directly into the cervical submucosa and stroma; and will not be administered intravenously. Additionally, the subject is anesthetized and the cervix and uterus are removed as part of the surgical procedure. Therefore the risks associated with injection into the cervix are expected to be lower than those associated with intravenous administration. Based on the clinical information for systemic indocyanine green administration, the risks and precautions are discussed below.

Severe allergic reaction (affects fewer than one in every 10,000 subjects) with symptoms that could include the following: tightness in the throat, itchy skin, blotchy skin, rash, coronary artery spasm, facial edema, breathing difficulties, tightness and/or pain in the chest, faster heartbeat, a fall in blood pressure and shortness of breath, cardiac arrest, restlessness, nausea, feeling of warmth, flushes, hypereosinophilia[15].

The possibility of an allergic reaction is greater in subjects with extremely serious kidney failure. To minimize the risk of an allergic reaction, all subjects with renal dysfunction are excluded as well as subjects with a known allergy to iodine, ICG or iodine dyes[15].

ICG contains sodium iodide and should be used with caution in subjects who have a history of allergy to iodides or iodinated imaging agents. Anaphylactic or urticarial reactions have been reported in subjects with and without history of allergy to iodides. Anaphylactic deaths have been reported following ICG administration during cardiac catheterization[14].

Radioactive iodine uptake studies should not be performed for at least 1 week following ICG administration[14,15].

Animal reproduction studies have not been conducted with ICG. It is not known whether ICG can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. It is not known whether ICG is secreted in human milk. Women who are pregnant or lactating are excluded from this study.

7.3 Isosulfan Blue (Blue Dye)

Similar to the administration of IC2000 in this study, Isosulfan blue will be directly injected into the cervical submucosa and stroma. The subject will be anaesthetized and the cervix and uterus are removed as part of the surgical procedure.

Severe anaphylactic reactions have occurred after injection of Isosulfan blue (approximately 2% of subjects). Manifestations include respiratory distress, shock, angioedema, urticarial and pruritus. Reactions are more likely to occur in subjects with a history of bronchial asthma, or subjects with allergies or drug reactions to triphenylmethane dyes.

Isosulfan blue interferes with measurements of oxygen saturation by pulse oximetry and methemolobin by gas analyzer.

The use of Isosulfan blue may result in transient of long-term (tattooing) blue coloration.

It is not known whether Isosulfan blue can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. It is not known whether Isosulfan blue is secreted in human milk. Women who are pregnant or lactating are excluded from this study.

8 STUDY PROCEDURES

8.1 Schedule of Events

Table 3 presents the schedule of events for this study. TABLE 3 Schedule of Events

Procedure	Baseline (Day -30 to Day 0)	Day 0 (Operative Phase)			Follow-up Visits		
		Pre-OP	Intra-Op	Post-Op	Day 1 (24±12 hrs.)	Date of Discharge[a]	Day 30±7 d
Informed consent	X						
Demography, Surgical risk factors	X						
Vital signs, height, weight	X						
Pre-operative diagnosis	X						
Pregnancy test	X						
Inclusion/exclusion criteria	X						
Hemoglobin		X	X[b]		X		
Randomization (in OR during anesthesia)			X				
LN mapping with Blue dye			X				
LN mapping with PINPOINT			X				
Surgical Procedure			X				
Documentation of LN mapping and surgical procedure			X	X			
Concomitant medications and procedures	X	X	X	X	X	X	X
Adverse events/adverse device effects		X	X	X	X	X	X[c]
Histopathological evaluation of Lymph Nodes			X	X			

[a] Subjects with a discharge date later than Day 30, will have the last study visit on Day 30
[b] Hemoglobin measurement at the time of mapping if the subject had any hypotensive events or blood loss over 500 ml at the time of mapping.
[c] All subjects with adverse events thought to be related to the LN mapping procedure or the PINPOINT system will be followed until resolution or deemed chronic

8.2 Baseline/Screening Procedures (Day -30 to Day 0)

After signing the informed consent form, subjects will be assigned a screening number (see Section 5.2) and be evaluated for eligibility into the study.

The following procedures will be conducted during the baseline assessment:

- Collection of demographics, surgical risk factors, pre-operative diagnosis
- Vital signs (heart rate and blood pressure), height and weight
- Serum tests (bilirubin, creatinine and INR) for MELD Score
    - The following calculator from the OPTN website should be used to calculate the MELD Score:

      http://optn.transplant.hrsa.gov/resources/MeldPeldCalculator.asp?index=98
- Pregnancy test (urine test or institution standard of care) for subjects of childbearing potential on Day 0

8.3 Day 0 Procedures

On Day 0, a serum hemoglobin measurement will be obtained prior to randomization, LN mapping and surgery.

During anesthesia, subjects will be randomized as described in Section 5.1. Subjects are considered enrolled upon randomization.

According to the randomization assignment, subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of ICG. Subjects in the B-P arm will undergo LN mapping with Blue dye first followed by mapping with PINPOINT.

Subjects randomized into the P-B Arm will be administered ICG first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye.

All subjects will undergo the appropriate surgical procedure (minimally invasive hysterectomy, trachelectomy or conization) according to the surgeon's standard practice.

All subjects will receive the hospital/institution and surgeon's standard pre-operative and post-operative care with the addition of any study specific requirements.

An intra-operative measurement of hemoglobin should be performed at the time of mapping if the subject experiences a hypotensive event requiring intraoperative management (e.g. use of vasopressors) or blood loss greater than 500 ml at the time of mapping.

Either during or immediately post-operatively, the details of the LN mapping procedure and surgical procedure will be documented. LN mapping data will be collected including the ability to identify LN and LC, the ability to unilaterally and bilaterally map LN and LC, and anatomical location and distribution of identified LN. Failed mapping is defined as no LN detected.

The following elements must be included and recorded in the case report form (CRF):

- Surgical time and estimated blood loss
- Dose of Blue dye administered
- Dose of ICG administered
- Details on injection technique
- Total number of LNs identified with PINPOINT
- Total number of LNs identified with Blue dye
- Total number of LCs identified with PINPOINT
- Total number of LCs identified with Blue dye
- Total number of abnormal LNs identified (suspicious nodes)
- Total number of LNs excised
- Classification of LNs identified
- Classification of LCs identified
- LNs anatomical location and distribution
- Pathological status of LNs excised
- Concomitant medications and procedures as described in Section 6.3
- Adverse events according to section 9

8.3.1 Lymph Node Mapping Procedure

LN mapping for FIOG Clinical Stage I endometrial cancer will be performed according to the NCCN Guidelines for Uterine Neoplasms, SLN Algorithm for Surgical Staging of Endometrial Cancer.

LN mapping for FIGO Clinical Stage I cervical cancer will be performed according to the NCCN Guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-Stage Cervical Cancer[1,2].

In order to minimize the spillage of blue dye or ICG interfering with the mapping procedure when LNs are excised, mapping will be performed on a hemi-pelvis, and LN mapping with both Blue dye and PINPOINT will be completed prior to the excision of any LNs.

According to the randomization assignment, subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of ICG. Blue dye and ICG will be prepared in separate syringes and administered into the cervix at the three and nine o'clock position with a superficial and deep injection. Subjects in the B-P Arm, will undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'ICG' nodes are identified or the investigator determines that 'ICG' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered ICG first followed by the administration of Blue dye. Similar to the B-P Arm, ICG and Blue dye will be prepared in separate syringes and administered into the cervix at the three and nine o'clock position with a superficial and deep injection. Subjects in the P-B Arm, will undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping will be performed until the investigator identifies all 'ICG' nodes or determines that 'ICG' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

The Investigator will identify 'Blue' nodes and 'ICG' nodes based on direct visualization with white light for Blue dye and PINPOINT for ICG according to Section 8.3.1.3 and 8.3.1.4 respectively. All nodes identified during the mapping procedure will be classified according to section 8.3.1.5 and recorded in the operative CRFs.

8.3.1.1 Imaging Agent Dosing and Administration of Blue dye

The Blue dye solution will be prepared and administered by the appropriate qualified study and/or operating room personnel according to the method of Abu Rustum[4]. The cervix will be injected with Blue dye while the subject is under anesthesia in the operating room, approximately 15 minutes prior to planned mapping.

- One vial of Isosulfan Blue (1% solution) will be utilized
- A 22G spinal needle will be used to inject Blue dye at the 3 and 9 o'clock positions.
- At each position, a superficial (1-3 mm) and a deep (1-3 cm) injection of 1 mL of Blue dye will be made into the cervical submucosa and stroma.
- Blue dye should be injected at a rate of 5 to 10 seconds per injection.

8.3.1.2 Imaging Agent Dosing and Administration of ICG (IC2000)

Similar to administration of Blue dye solution, the ICG solution will be prepared and administered by the appropriate qualified study and/or operating room personnel according to the method of Abu Rustum[4]. The cervix will be injected with ICG while the subject is under anesthesia in the operating room, approximately 15 minutes prior to the planned time of imaging.

- One 25 mg vial of ICG will be reconstituted with 20 ml sterile water for injection to yield a 1.25 mg/ml solution immediately prior to ICG administration.
- A 22G spinal needle will be used to inject ICG at the 3 and 9 o'clock positions.
- At each position, a superficial (1-3 mm) and a deep (1-3 cm) injection of 1 mL of ICG will be made into the cervical submucosa and stroma.
- ICG should be injected at a rate of 5 to 10 seconds per injection.

The total dose of ICG administered will be 5 mg.

8.3.1.3 Identification of Lymph Nodes and Lymphatic Channels with Blue Dye

Intraoperative identification of LNs and LCs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visible or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible blue dye.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel stained with blue dye leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified, classified and documented and the surgeon has scanned the full 360-degree area around the injection site. Blue ducts should be followed in both directions in order to identify LNs to be excised. Once all 'Blue' nodes have been identified, all remaining nodes in the basin are designated as negative based on the criteria.

8.3.1.4 Identification of Lymph Nodes and Lymphatic Channels with PINPOINT

Intraoperative identification of LNs and LCs with PINPOINT will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with ICG using PINPOINT.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible fluorescence.

Intraoperative identification of lymphatic channels will be determined by direct visualization of a lymphatic channel by NIR fluorescence leading to or emerging from a lymph node.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified, classified and documented and the surgeon has scanned the full 360-degree area around the injection site. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised. Once all 'ICG' nodes have been identified, all remaining nodes in the basin are designated as negative based on the criteria.

8.3.1.5 Classification of Lymph Nodes

All LNs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard
5. Non-stained node found at termination of fluorescent duct
6. Non-stained node found at termination of blue duct
7. Non-stained node found at termination of fluorescent and blue duct

All LN identified and subsequently removed will be histologically examined to confirm the presence of lymphoid tissue according to section 8.5.

8.3.1.6 Classification of Lymphatic Channels

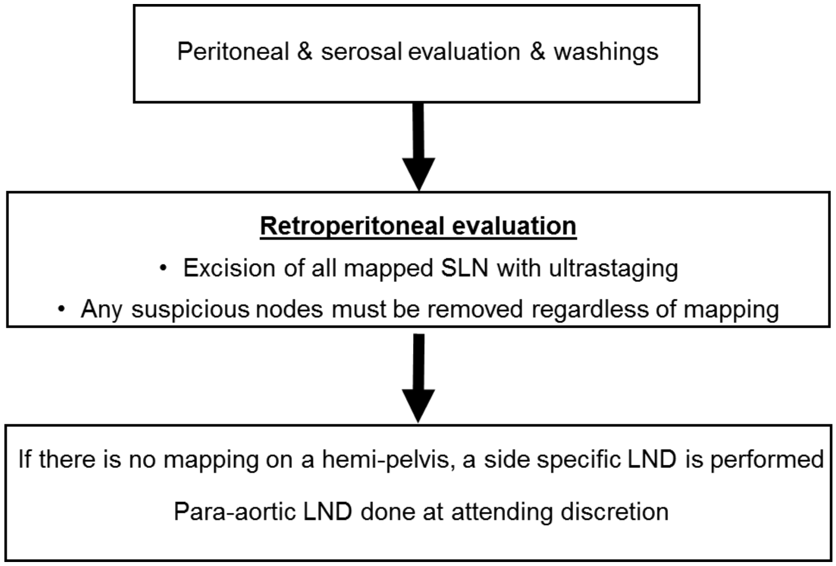
All LCs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)

8.3.1.6.1 National Comprehensive Cancer Network Guidelines

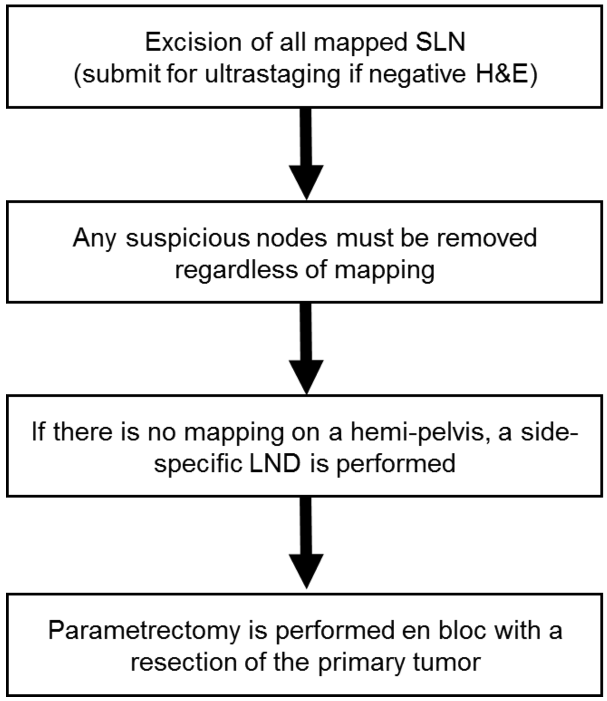
The LN mapping procedure for subjects with uterine neoplasms will be performed according to the guidelines from the NCCN for Uterine Neoplasms, SLN Algorithm for Surgical staging of Endometrial Cancer[1] (Figure 2).

FIGURE 2. SLN Algorithm for Surgical Staging of Endometrial Cancer



The LN mapping procedure for subjects with cervical neoplasms will be performed according to the guidelines from the NCCN for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-stage Cervical Cancer[2] (Figure 3).

FIGURE 3. SLN Algorithm for Surgical Staging of Cervical Cancer



8.4 Post-operative Follow-up Visits (Day 1 to Day 30)

Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits on postoperative Day 1, the date of discharge and Day 30 (±7 days). Subjects will be assessed for the following throughout the study:

- Serum hemoglobin measurement (Day 1)
- Concomitant medications and procedures as described in Section 6.2
- Adverse events and adverse device effects according to section 9

Although the patient may be seen by various individuals post-surgery, the study-specific visits must be conducted by a surgeon who is part of the study and has signed the Signature and Delegation Log. Any complications and outcomes of study required assessments that occur between visits will be documented at the next study required visit.

8.5 Histopathology of Excised LN

The presence of lymphoid tissue for each excised LN will be confirmed by tissue analysis. All lymph nodes will be routinely sectioned and stained with hematoxylin and eosin (H&E). If this initial H&E assessment is negative, LN ultra-staging will be performed according to the method of Kim et al.[34]. Briefly, two adjacent 5-µm sections will be cut and sectioned at each of the two levels, 50-µm apart from each paraffin block. Sections at each level will be stained with H&E and with immunohistochemistry using anti-cytokeratin AE1:AE3.

8.6 Image Acquisition and Transmission

Videos of the image sequences acquired using PINPOINT will be recorded to a medical grade video recorder. All required images will be provided to the Sponsor by the Investigative Center. Image files will be identified using the subject number and initials only (no information identifying the subject shall be included in the files).

9 EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

All untoward medical occurrences either observed by the Investigator or one of his/her medical collaborators, or reported by the subject spontaneously, or in response to the direct question below, will be reported as follows:

- All events occurring before randomization should be recorded in the source documents and will be considered part of the subject's case history.
- All adverse events occurring during the study will be recorded as adverse events on the adverse event CRF.
- Events occurring as a result of the surgery will be reported as adverse events related to the surgical procedure in the adverse event CRF.
- Events related to PINPOINT will be recorded as adverse device effects in the CRF.
    - Events that are not related to PINPOINT shall be recorded as adverse events.
- Events related to PINPOINT that affect a user of the device (non-patient) are recorded as adverse device effects.
- Events related to Blue dye will be recorded as adverse events related to blue dye in the adverse event CRF.

In an attempt to optimize consistency of AE reporting across centers, the subjects must be asked a standard question to elicit events. At each clinic or telephone evaluation of the subject, study personnel will ask the following questions: "Have you had any problems since your last visit?"

AEs reported on the CRF will include the date of onset, severity, relationship to PINPOINT or Blue dye, relationship to surgical procedure, date of resolution (or the fact that it is ongoing or has become chronic), action taken, and whether the AE is serious or not.

9.1 Definitions

9.1.1 Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory findings) in subjects whether or not related to the investigational medical device.

- Includes events related to the PINPOINT or Blue dye.
- Includes events related to procedures involved (any procedure in the clinical investigation plan).

9.1.2 Adverse Device Effect (ADE)

Any adverse event related to the use of PINPOINT (includes imaging agent and all hardware components).

- Includes any event that is a result of a use error or intentional misuse.

ADEs that affect subjects will be recorded as ADEs in the AE CRF. ADEs that only affect a user (non-subject) are recorded in the technical complaint form only.

9.1.3 Serious Adverse Event (SAE)

Any adverse event that:

a. Led to a death.

b. Led to a serious deterioration in health that either:

   i. Resulted in a life-threatening illness or injury,

   ii. Resulted in a permanent impairment of a body structure or a body function,

   iii. Required in-patient hospitalization or prolongation of existing hospitalization,

   iv. Resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function.

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

This includes device deficiencies that might have led to a serious adverse event if suitable action had not been taken; intervention had not been made, or if circumstances had been less fortunate. These are handled as SAEs. A planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in heath, is not considered to be a serious adverse event.

9.1.4 Unanticipated Adverse Device Effect (UADE)

Any ADE that meets the following:

- By its nature, incidence, severity or outcome has not been identified in the current version of the PINPOINT risk analysis report.
- On health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.2 Adverse Event Descriptions

9.2.1 Intensity

The intensity of AEs, including ADEs and surgical complications will be characterized as mild, moderate, or severe, as follows:

Mild	Usually transient, requiring no special treatment, and does not interfere with the subject's daily activities.
Moderate	Introduces a low level of inconvenience or concern to the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
Severe	Significantly interferes with a subject's usual daily activities and requires systemic drug therapy or other treatment, if available.

9.2.2 Relationship

Suspected	There is a reasonable possibility that the AE is associated with use of the study device or treatment (ICG or Blue dye), such as temporal relationship of the event to use.
Not suspected	A relationship between the AE and the study device or treatment (ICG or Blue dye) can reasonably be ruled out based on lack of any temporal relationship of the event to use, or when the subject's underlying condition, medical history, or other therapy provide sufficient explanation for the observed event.

9.3 Reporting and Evaluation of Serious Adverse Events and Unanticipated Adverse Device Effects

Any SAE or UADE occurring in this study must be reported immediately (within 24 hours of discovery) by email to the Novadaq contact listed below:

Attention: Alicia Wilton

Mobile: 905-629-3822 x209

Email: Film@novadaq.com

SAEs and UADEs will be reported to the Institutional Review Board (IRB)/Ethics Committee (EC) according to the institution's policies, but within 10 days of occurrence.

The Sponsor will be responsible for reporting SAEs/UADEs to the FDA or Health Canada in accordance with federal regulatory requirements.

The Sponsor will provide documentation of reportable events to the Investigator, as specified in Section 13.1.4.

The Investigator will ensure that the subject receives appropriate medical treatment and that the subject is followed up until the SAE or UADE resolves or becomes chronic, as defined in Section 9.4.

9.4 Follow-up for Adverse Device Effects and Adverse Events

Throughout the study to the final study visit contact, ADEs will be followed until they resolve or become chronic. All AEs will be followed throughout the study until the Day 30 visit. All AEs related to the PINPOINT, ICG, Blue dye or the mapping procedures, as determined by the Investigator, will be followed until resolution or deemed chronic

At the final study visit, new AEs, as well as follow-up information for continuing AEs, will be recorded in the CRF and source document. If an SAE or UADE is unresolved at the final study visit, it will be followed by the Investigator until it resolves or becomes chronic (as judged by the Investigator). Follow-up data for such SAEs will be recorded in the source document and reported to the safety contacts. Non-serious ongoing AEs will be followed beyond the final study visit if they are related to the study procedures at the discretion of the Investigator.

9.5 Reporting of Technical Complaints/Device Deficiencies

9.5.1 Definitions

Device Complaint: A quality complaint received in writing, electronically, or orally that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a device product. (In this definition, "effectiveness" refers to the actual function of the device, not to how the subject responds to the action of the device. Also in this definition, "device product" refers only to devices provided by the Sponsor for clinical studies and to investigational devices.)

In this definition, safety includes the safety of a subject, user, or other person associated with the use of a medical device.

Device Deficiency: Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling.

9.5.2 Reporting Procedures

Any technical complaint/device deficiency should be reported to the Sponsor. Technical Complaints occurring in this study must be reported immediately (within 24 hours) by fax or email to the appropriate Novadaq representative or to Novadaq Quality (quality@novadaq.com)

Any complaint about a device must be reported regardless of whether the defect or deficiency had any effect on a subject or on study personnel.

9.6 ADEs Technical Complaints/Device Deficiencies that are UADEs

Novadaq will evaluate all ADE reports and technical complaints/device deficiencies to determine if the report meets the definition of an unanticipated adverse device effect. If Novadaq determines that it does meet the definition, an investigation will be begun immediately. Novadaq will inform the Investigator of any additional reporting requirements beyond those stated in Sections 9.4 and 9.5 as applicable.

Novadaq will report the UADE and the results of any investigations to the FDA and Health Canada according to the applicable regulatory guidelines. Novadaq will also report to the Investigator(s), who will submit the required reports to their IRB/ECs within 10 working days after Novadaq first received notice of the effect.

10 STATISTICAL CONSIDERATIONS

10.1 Primary Objective

To assess the effectiveness of intraoperative PINPOINT in identification of lymph nodes in subjects with uterine and cervical malignancies who are undergoing lymph node mapping.

10.2 Hypotheses

To assess the effectiveness of PINPOINT in the identification of LNs, a non-inferiority test will be performed using the per-protocol (PP) analysis population according to the method of Moyé et al[3].

$$H_{01}: \quad p_t \leq p_c - 0.05$$
$$H_{11}: \quad p_t > p_c - 0.05$$

Here p_t and p_c represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively. A non-inferiority margin of 0.05 was determined to be clinically significant based on feedback from Investigators. Within their respective groups, p_t and p_c represent the proportion of LNs identified (and confirmed to be lymphoid tissue, See Section 3) with PINPOINT and Blue dye respectively divided by the total number of LNs excised, across all patients.

The effectiveness of intraoperative PINPOINT in identification of lymph nodes will be determined by a superiority test using the modified intent-to-treat (mITT) analysis population as justified by the method of Moyé et al.

$$H_{02}: \quad p_t = p_c$$
$$H_{12}: \quad p_t > p_c$$

The use of both a superiority test and non-inferiority test for the primary endpoint can be utilized without statistical penalty due to the closed testing principle according to the method of Lesaffre et al[35].

10.3 Statistical Analysis

To test these hypotheses we will estimate the difference $p_t - p_c$ with a 95% one-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority, and if the lower bound of the interval is greater than 0 we will claim superiority.

10.4 Sample Size Considerations

The clinical literature provides an estimate of the expected effectiveness of LN mapping with ICG and Blue Dye[17–28,36]. 12 studies using ICG for the purposes of LN mapping have been published[17–28] (Table 1). Detection rates for LN mapping with ICG and Blue dye are reported to occur in a wide range (33-100% for ICG[17–28] and 40-80% for Blue dye[36]). A number of factors play a role in the identification of LN including tumor size, familiarity with LN mapping technique (learning curve) and obesity[36].

A weighted average of published LN detection rates for Blue dye and ICG (i.e., PINPOINT) in gynecological cancer suggests unilateral detection rates of 91% for ICG and 80% for Blue dye. However, we assumed LN detection rates in gynecological cancer[17–28] of 83% and 80% using ICG and Blue dye, respectively. We also assumed a non-inferiority margin of 5%.

With 300 LNs in each arm we will have 81% power to reject H_{01} above and claim non-inferiority of PINPOINT with respect to Blue dye. This sample size calculation was performed using nQuery Advisor ® 7.0 (Copyright © 1995-2007, Statistical Solutions, Saugus, MA). We will test the hypothesis using a two-sided 95% confidence interval (i.e., two-sided significance level of 0.05). We will enroll patients and identify LNs using both Blue dye and PINPOINT as described above until we have at least 300 LNs in each arm. We expect that we will enroll approximately 150 patients (based on clinical literature suggesting an average of 1.8-2.2 LNs excised per subject[17–28]).

10.5 Data Sets to be Analyzed

10.5.1 Per-protocol (PP)

The Per-Protocol (PP) analysis population includes all subjects that: [1] meet critical eligibility criteria, [2] have no significant protocol deviations; and [3] have evaluable assessment endpoints for the primary endpoint.

10.5.2 Modified Intent to treat (mITT)

The mITT analysis population includes all randomized subjects who received at least one injection of ICG or blue dye. All subjects meeting this criterion are included in the mITT population regardless of whether or not they received the minimally invasive surgical intervention or lymphatic mapping. Subjects who have the mapping procedure aborted due to circumstances such as a higher stage cancer than initially expected will not be included in the mITT. Approximately 8% of subjects are expected to have the mapping procedure aborted.

10.5.3 As-Treated (AT)

The As-Treated (AT) analysis population includes all randomized subjects in whom the intended minimally invasive surgical procedure was performed and received at least one injection of ICG or Blue dye. Subjects in whom the mapping procedure with PINPOINT or Blue dye is not performed are excluded from the AT population. Subjects will be analyzed according to the LN mapping procedure performed. The AT population will be used for a secondary analysis of the primary endpoint.

10.5.4 Safety (S)

The safety analysis population includes all randomized subjects enrolled in the study who received at least one injection of ICG or Blue dye. Secondary safety endpoints, including the summary of adverse events or adverse device effects in the trial, will be analyzed using this analysis population.

10.6 Secondary Objectives

The planned secondary objectives are intended to support product labelling.

The first secondary outcome is the ability of PINPOINT and Blue dye to detect at least one lymph node in a patient.

Let q_t and q_c represent the proportion of subjects with a least one lymph node identified (according to section 3.1 and confirmed by histology) with PINPOINT and Blue dye respectively, divided by the total number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H_{03}: \; q_t \leq q_c - 0.05$$
$$H_{13}: \; q_t > q_c - 0.05$$

To test this hypothesis we will estimate the difference $q_t - q_c$ with a 95% one-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

The second secondary outcome is the ability of PINPOINT and Blue dye to detect LCs intraoperatively.

The following hypotheses will be tested:

$$H_{04}: \; r_t \leq r_c - 0.05$$
$$H_{14}: \; r_t > r_c - 0.05$$

Where r_t and r_c represent the proportion of LCs identified (according to Section 3.1) with PINPOINT and Blue dye divided by the total number of LCs identified. A non-inferiority margin of 0.05 was determined to be clinically significant based on feedback from Investigators.

To test these hypotheses we will estimate the difference $r_t - r_c$ with a 95% one-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

10.6.1 Demographic and Baseline Data

The demographic and baseline analysis will be done for the mITT and PP data set.

The following subject variables will be summarized: age, race, body mass index, histological type (squamous cell carcinoma, adenocarcinoma, endometrioid carcinoma etc.), previous neoadjuvant therapy, FIGO stage and type of surgical intervention. Continuous variables will be summarized as mean, standard deviation, median, minimum and maximum, and categorical variables will be summarized by counts and percentages.

10.6.2 Safety Variables and Analysis

Safety variables will be documented and summarized for the AT population. Safety variables will include all adverse events (AEs) and adverse device effects (ADEs), concomitant medications, and vital signs. Safety analysis will be performed on all subjects who receive ICG regardless of whether LN mapping was initiated or successful.

Adverse events will be coded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE). Treatment-emergent AEs and ADEs will be summarized descriptively. The number and percentage of subjects experiencing AEs and ADEs and the total number of AEs and ADEs will be summarized by system organ class and preferred term. Associated AEs that investigators suspect are related to study treatment will also be summarized. Summary of each type of event will be prepared by severity and for all severities combined.

10.7 Handling of Missing Data

Reasonable efforts will be made to obtain complete data for all patients; however, missing observations will inevitably occur due to patients lost to follow-up or noncompliance with required assessments. The reasons for missing data will be documented and evaluated (e.g. patient is deceased, lost to follow up, missed visit, etc.). In addition, the distribution of prognostic factors between patients with data and those without data will be examined to evaluate any potential sources of bias. Any missing observations will be described in detail and evaluated for assessment of possible bias.

10.8 Pooling of Site Data

The homogeneity of safety and effectiveness results across study sites will be examined and if no significant heterogeneity is found, the results will be pooled. The justification for pooling is that all study sites will follow one Protocol, use the same device system (PINPOINT) follow the same Instructions for Use and perform mapping in accordance with NCCN guidelines. Additionally, frequent contact and monitoring of the sites will be performed to ensure that all Study sites are evaluating participants and recording Study results in a reliable and reproducible manner. It is not anticipated that any individual Study site will dominate the Study results. Therefore, it is believed that these procedures will help to ensure that the data from these Study sites can be combined and analyzed as if generated at a single site.

11 ESTIMATED DURATION OF THE STUDY

The expected study duration is approximately 1 year. The study is expected to start in 2015 and take 12 months to complete enrollment.

12 STUDY ETHICAL CONSIDERATIONS

12.1 Ethical Conduct of the Study

The study will be conducted in accordance with US 21 CFR Parts 50, 54, 56, 312 and 812 as well as ICH E6: Good Clinical Practice: Consolidated Guideline. It will be constituted in keeping with the principles of ICH E8: General Considerations for Clinical Trials and Part C, Division 5 of the Canadian Food and Drug Regulations. Any additional requirements imposed by the local

Institutional Review Board/Ethics Committee/Research Ethics Board or regulatory agency will be followed as necessary.

12.2 Informed Consent

The informed consent forms used for the study must comply with applicable laws and regulations. An Investigator must explain the medical aspects of the study, including the nature of the study and procedure, orally and in writing, in such a manner that the subject is aware of potential benefits and risks. Other elements of the informed consent process may be delegated by the Investigator. Subjects must be informed about all aspects of the clinical study that are necessary to make the decision to participate in the clinical trial. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Documentation of the discussion and the date of informed consent must be recorded in the source documentation. Subjects must give informed consent in writing.

The informed consent process must be conducted, and the form must be signed, before the subject undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

12.3 Institutional Review Board, Ethics Committee, or Research Ethics Board (IRB)

The protocol, protocol amendments (as specified by the IRB), and the informed consent form for the proposed study, along with any other documents required by the center's IRB must be submitted by the Investigator to the center's duly constituted IRB for review and approval. The Investigator must also ensure that the IRB reviews the progress of the study on a regular basis and, if necessary, renews its approval of the study on an annual basis. A copy of each IRB approval letter must be forwarded to the Sponsor before the study is implemented. Documentation of subsequent reviews of the study must also be forwarded to the Sponsor.

12.4 Data and Safety Monitoring Board

The study will be reviewed annually by the University of Texas M.D. Anderson Data and Safety Monitoring Board (DSMB). The study statistician will prepare a report for the DSMB in advance of the scheduled review meeting using the report template provided by the DSMB. The study statistician will also prepare a safety report for the study Principal Investigator (PI) in preparation of the DSMB meeting to review the study. A DSMB charter will outline specific monitoring procedures.

13 ADMINISTRATIVE PROCEDURES

13.1 Sponsor's Responsibilities

13.1.1 Public Disclosure of Clinical Trials

The Sponsor will submit information about this protocol to the appropriate web-based national clinical trial registry and results database in each applicable regulatory region where the study is conducted. This includes but is not limited to US National Institute of Health (www.clinicaltrials.gov).

13.1.2 Study Supplies

The Sponsor will provide the PINPOINT Endoscopic Fluorescence Imaging System along with sufficient quantities of PINPOINT LN Mapping Kits and sufficient quantities of Blue dye (Isosulfan blue).

13.1.3 Investigator Training

13.1.3.1 Study Initiation Visit

Study centers will have a study initiation meeting to ensure the research personnel understand the protocol, study requirements, and data capture processes. This training will take place prior to enrollment of the first subject at each study center.

13.1.3.2 PINPOINT System

Appropriate personnel at the study centers shall be required to participate in training on the procedural use of PINPOINT as it relates to the conduct of this study (refer to Section 6.2.3).

13.1.4 Ongoing Communication of Safety Information During the Study

The Sponsor will provide the Investigator with documentation of UADEs and reportable events/effects, from all study centers, reported to regulatory authorities during the conduct of the study. The Investigator must forward this documentation to the IRB, as described in Section 9.

The Sponsor will also notify the Investigator about any other safety findings that could affect the safety of subjects, affect the conduct of the study, or alter the IRB's opinion about continuation of the study.

13.1.5 Study Monitoring

The conduct of the study will be monitored by representatives of the Sponsor to ensure compliance with the protocol, GCP and applicable regulations. A separate study specific Monitoring Plan will outline the monitoring procedures to be followed, the required access to source data and the extent of source verification planned.

13.1.6 Records Retention

The Sponsor must retain all documentation pertaining to the study according to Novadaq standard operating procedures.

13.2 Investigator's Responsibilities

13.2.1 Reporting and Recording of Study Data

Data will be captured and compiled using procedures developed by the Sponsor or their representatives. All requested study data must be recorded clearly on the CRF and other study forms as required. An explanation should be provided for all missing data. Only individuals who are identified on the Study Signature and Delegation Log may enter or correct data in the CRF. Incomplete or inconsistent data on the CRFs will result in data queries that require resolution by the Investigator.

The protocol, informed consent form, protocol amendments, safety information, and other required documents must be submitted to the IRB in a timely manner, as described in Section 12.3.

13.2.2 Source Documentation

The Investigator must maintain adequate and accurate source documents upon which CRFs for each subject are based. They are to be separate and distinct from CRFs, except for cases in which the Sponsor has predetermined that direct data entry into specified pages of the subject's CRF is appropriate. These records should include detailed notes on:

- The oral and written communication with the subject regarding the study treatment (including the risks and benefits of the study). The date of informed consent must be recorded in the source documentation.
- The subject's basic identifying information, such as demographics, that links the subject's source documents with the CRFs.
- All relevant observations and data on the condition of the subject throughout the study.
- The subject's exposure to PINPOINT.
- All adverse events.

13.2.3 Study Devices and Imaging Agents

The Investigator is responsible for ensuring the PINPOINT system, including imaging agent, and Blue dye are controlled and are used or dispensed only to subjects enrolled in the study. Only Investigators identified on the Signature and Delegation Log may use PINPOINT for the purposes of this study.

The Investigator shall keep records documenting the receipt, use, return and disposal of the study device, drugs and components.

The Investigator will ensure that PINPOINT is returned and that any other study material will be returned to the Sponsor or disposed of according to the Sponsor's instructions on completion of the study.

13.2.4 Records Retention

The Investigator must ensure that clinical study records are retained according to national regulations, as documented in the clinical trial agreement entered into with the Sponsor in connection with this study.

Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution, or private practice. The Investigator must inform the Sponsor immediately if any documents are to be destroyed, to be transferred to a different facility, or to be transferred to a different owner.

14 DATA MANAGEMENT

Study data will be collected using paper and/or or electronic case report forms (CRFs). Data will be entered into a study specific database in one of two ways or a combination of the following:

- Center research personnel will enter study data directly into an electronic case report form which functions as an electronic data capture screen for the study database.
- Center research personnel will enter study data onto paper CRFs, which will be submitted to the Sponsor or assigned designee.

The Sponsor or designee will follow standardized procedures for data review, database cleaning and issuing/resolving queries. Procedures for data verification, validation, security and data retention will be followed in order that the study data reported are complete, accurate and consistent with source data.

For the purpose of data analysis and presentation, the data of the original data set may be manipulated and additional variables calculated when necessary. Once the study data are completely entered, reviewed and checked, the study database will be locked and no further changes will be made.

The electronic CRFs will be created and managed using REDCap[33] (Research Electronic Data Capture) electronic data capture tools hosted at M.D. Anderson. REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (https://redcap.mdanderson.org) is hosted on a secure server by M.D. Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (May 2014) by M.D. Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335.

Those having access to the data include the study PI and research team personnel. Users are authenticated against M.D. Anderson's Active Directory system. External collaborators are given access to the database once approved by the PI, with their access expiring in 6 months but renewable in 6 months increments at the request of the PI. The application is accessed through Secure Socket Layer (SSL). All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each patient will be shifted by a different randomly generated number.

Following publication study data will be archived in REDCap. Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely in REDCap. Since REDCap is a secure electronic database with controlled access, and because patient identifiers may be needed to link study data to data from other sources under future IRB approved protocols, patient identifying information will be retained in the archived database.

15 POLICY FOR PUBLICATION AND PRESENTATION OF DATA

The results of the study will be published by the study group. In addition to the principal investigators, any additional authors listed separately on the manuscript will be selected based on scientific input on the design of the study, interpretation of results and on enrollment numbers. The final number of authors will depend on the journal’s publication guidelines. All participating centers will be acknowledged in the main study manuscript.

The Sponsor also encourages the scientific publication of data from clinical research studies. However, Investigators may not present or publish partial or complete study results individually without participation of the study Principal Investigator as well as the Sponsor. The Principal Investigators and the Sponsor may propose appropriate scientific manuscripts or abstracts from the study data. All proposed publications must be reviewed and commented on by the Sponsor before submission for publication. The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for publication against the validated study database for accuracy. Names of all Investigators and Sponsor representatives responsible for designing the study and analyzing the results will be included in the publication(s).

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FILM

Clinical Study Protocol

PROTOCOL NUMBER
PP LNM 01

TITLE
A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

SHORT TITLE
FILM

PROTOCOL VERSION
Version 6.0, August 25th, 2017

SPONSOR
Novadaq Technologies Inc.
5090 Explorer Dr., Ste.
202, Mississauga, Ontario,
Canada, L4W 4T9

Confidential

FILM

PROTOCOL APPROVAL

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Version: 6.0, August 25th, 2017

Written by: Jen Pendlebury 2017-08-25

Jen Pendlebury on behalf of Yohan D'Souza, PhD Clinical Regulatory Scientist Novadaq Technologies Inc.

Date

FILM

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Version: 6.0, August 25th, 2017

Approved by: Lori Swalm (signature) 08/25/2017

Lori Swalm
Sr. Vice President, Regulatory,
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Date

Approved by:

Matt McKittrick On behalf of
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Date

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Version: 6.0, August 25th, 2017

Approved by:

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Michael Frumovitz, MD Study Principal Investigator M.D. Anderson Cancer Center	Date	Nadeem Abu-Rustum, MD Study Principal Investigator Memorial Sloan Kettering Cancer Center	Date
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FILM

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Version: 6.0, August 25th, 2017

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PROTOCOL SUMMARY

Study Number and Title:

PP LNM 01: A Randomized, Prospective, Open Label Multicenter Study Assessing the Safety and Utility of PINPOINT® Endoscopic Fluorescence Imaging to Identify Lymph Nodes in Patients with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping.

Clinical Phase: Pivotal / Investigational Device Study

Study Objectives:

Primary:

To assess the effectiveness of intraoperative PINPOINT Near-Infrared Fluorescence imaging in the identification of lymph nodes in subjects with uterine and cervical malignancies who are undergoing lymph node mapping.

Secondary:

- To evaluate the effectiveness of PINPOINT and Blue dye in the identification of at least one lymph node (confirmed to be lymphoid tissue) per subject.
- To assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

Study Design:

This is a randomized prospective, open label, multicenter study to assess the safety and effectiveness of PINPOINT® Near Infrared Fluorescence Imaging (PINPOINT) in identification of lymph nodes (LN) in subjects with uterine and cervical malignancies who are undergoing LN mapping. This is a non-inferiority within-patient comparison study to determine the effectiveness of PINPOINT in the identification of LNs compared to LNs identified with Blue dye (1% Isosulfan blue). Approximately 150 subjects will be enrolled at up to 10 centers in North America. Prior to enrolling study subjects, participating surgeons at each center will be trained to perform intraoperative identification and mapping of LNs with IC2000 and Blue dye. Participating surgeons will be required to have completed at least 10 LN mapping procedures with a minimum of 3 LN mapping cases performed with PINPOINT prior to the initiation of enrollment.

Screening:
Subjects diagnosed with International Federation of Gynecology and Obstetrics (FIGO) clinical stage I endometrial or cervical cancer scheduled for surgery that includes clinically indicated LN mapping will be evaluated at baseline to determine if they meet the inclusion/exclusion criteria of the protocol. Subjects will be assessed to determine overall health status including demographics, vital signs, diagnosis and relevant medical history/underlying conditions. Eligible subjects who provide informed consent will be considered for inclusion into the study.

Day 0:
On the day of surgery subjects will be randomized (1:1) to either the Blue-PINPOINT (B-P) Arm or the PINPOINT-BLUE (P-B) Arm. Subjects in each arm will be randomized according to an independently generated randomization scheme to undergo lymphatic mapping with Blue dye first followed by mapping with PINPOINT (B-P Arm) or to undergo lymphatic mapping with PINPOINT first followed by mapping with Blue dye (P-B Arm).

Minimally invasive surgery will be performed according to the surgeon’s standard practice.

The cervix will be injected four (4) times with a 1 ml solution Blue dye (1% solution: 10mg/ml) for a total dose of 40 mg and four (4) times with 1 ml of a 1.25 mg/ml solution of IC2000 for a total dose of 5 mg. The injection of Blue dye and IC2000 will occur while the subject is under anesthesia in the operating room. .

In order to minimize the spillage of Blue dye or IC2000 interfering with the mapping procedure when LNs are excised, mapping will be performed on one side of the pelvis followed by other side and mapping with both Blue dye and PINPOINT will be performed prior to the excision of any LNs.

Subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of IC2000 and undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'IC2000' nodes are identified or the investigator determines that 'IC2000' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered IC2000 first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping with PINPOINT will be performed until the investigator identifies all 'IC2000' nodes or determines that 'IC2000' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, LNs identified with Blue dye or PINPOINT will be excised.

Bilateral LN mapping for Clinical Stage I endometrial cancer will be performed according to the *NCCN Guidelines for Uterine Neoplasms, SLN Algorithm for Surgical Staging of Endometrial Cancer;* and LN mapping for Clinical Stage I cervical cancer will be performed according to the *NCCN Guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-Stage Cervical Cancer*[1,2].

The surgeon will identify LN and lymphatic vessels based on visualization with white light for blue dye or PINPOINT for IC2000.

LN Mapping with PINPOINT:
Intraoperative identification of LNs with IC2000 will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with IC2000 using PINPOINT.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible fluorescence.

Mapping will be considered complete when all nodes meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area within the abdominal cavity. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised.

LN Mapping with Blue dye:
Intraoperative identification of LNs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visibly or palpably abnormal lymph nodes designated as palpable masses and excised regardless of visible blue dye.

Mapping will be considered complete when all nodes meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area within the abdominal cavity Blue ducts should be followed in both directions in order to identify LNs to be excised.

Classification of LNs:

All LNs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard (non-stained)
5. Non-stained node found at origin or termination of fluorescent duct
6. Non-stained node found at origin or termination of blue duct

7. Non-stained node found at origin or termination of fluorescent and blue duct

Effectiveness of intraoperative Blue dye in identifying LNs will be based on the proportion of LNs identified by visualization under white light and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Effectiveness of intraoperative PINPOINT in identifying LNs will be based on the proportion of LNs identified by PINPOINT and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Details of the surgery, including intraoperative findings will be documented. Surgical data will be collected including the ability to identify LNs the number of LNs identified and removed, the ability to unilaterally and bilaterally map LNs, and the anatomical location and distribution of identified LNs. Failed mapping is defined as no LNs detected.

Follow-up and Post-operative Complications:
Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits to monitor occurrence of any adverse events/ adverse device effects on the date of discharge and Day 30 (±7 days).

Study Population

To be eligible for the study, subjects must meet the following main inclusion criteria:

- 18 years of age or older
- Subjects with FIGO Clinical Stage I endometrial cancer undergoing minimally invasive hysterectomy with lymph node mapping.
- Subjects with FIGO Clinical Stage IA cervical cancer ≤ 2 cm in size undergoing minimally invasive hysterectomy, trachelectomy, or conization with lymph node mapping. Subjects with clinical Stage IA1 cervical cancer without lympho vascular space involvement (LVSI) and negative margins on cone biopsy are not to be included.
- Subjects with negative nodal status (N0)
- Subjects with negative metastatic involvement (M0).

Subjects meeting any of the following criteria will be *excluded* from the study:

- Have had prior dissection and/or radiation in pelvis.
- Advanced cervical or endometrial cancer, T3/T4 lesions
- Diagnosis of cervical cancer with a tumor size greater than 2 cm.
- Locally advanced or inflammatory cervical or uterine cancer
- Metastatic cervical or uterine cancer.
- Known allergy or history of adverse reaction to ICG, iodine or iodine dyes.
- Known allergy or history of adverse reaction to Blue dye (Isosulfan blue) or triphenylmethane.
- Hepatic dysfunction defined as MELD Score > 12.
- Renal dysfunction defined as serum creatinine ≥ 2.0 mg/dl.
- Subjects who have participated in another investigational study within 30 days prior to surgery.
- Pregnant or lactating subjects.
- Subjects who, in the Investigator's opinion, have any medical condition that makes the subject a poor candidate for the investigational procedure, or interferes with the interpretation of study results.

Study Devices and Imaging Agents:

PINPOINT® Endoscopic Fluorescence Imaging System

An endoscopic fluorescence imaging system for high definition (HD) visible (VIS) light and near infrared (NIR) fluorescence imaging that includes the following components:

- A surgical endoscope optimized for VIS/NIR illumination and imaging.
- A camera head that is also optimized for VIS/NIR imaging and mounts to the endoscope eyepiece
- A flexible light guide cable.
- An endoscopic Video Processor/Illuminator for VIS/NIR illumination to the surgical endoscope via a flexible light guide cable, and the image processing required to generate simultaneous, real-time HD video color and NIR fluorescence images.
- A high-definition medical video recorder that allows the capture of still images and video.
- The PINPOINT kit containing the imaging agent and aqueous solvent.

The imaging agent used with PINPOINT is ICG, which is a sterile, water-soluble tricarbocyanine dye with a peak spectral absorption at 800-810 nm in blood plasma or blood. ICG contains not more than 5.0% sodium iodide. In this study, investigational ICG, IC2000, will be administered to subjects.

Isosufan Blue 1% (Mylan) is a water-soluble contrast dye and is administered as a 1% solution for the purposes of lymphography.

Study Variables:

Primary Variables

Effectiveness of intraoperative PINPOINT and Blue dye in identifying LNs defined as the proportion of LNs identified by PINPOINT and Blue dye respectively (confirmed as lymphoid tissue by histology) divided by the total number of LNs identified and excised.

Secondary Variables

LN detection rate with PINPOINT or Blue dye, defined as the proportion of cases in which at least one LN is identified with PINPOINT or Blue dye (confirmed as lymphoid tissue by histology).

Incidence of adverse events and adverse device events/effects of PINPOINT and Blue dye.

Other Variables

- Bilateral LN detection rate defined as the proportion of cases in which at least one LN is identified on right and left side of the pelvis.
- Proportion of LNs identified from following LCs.
- Anatomic distribution of LNs.

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Study Procedures and Assessments:

The following tests and procedures will be performed:

- Vital signs, height, weight, demographics, surgical predictive factors.
- Relevant medical history and underlying conditions.
- Assessment of eligibility criteria.
- Randomization to the B-P Arm or P-B Arm.
- Imaging agent administration
- LN identification with PINPOINT and Blue dye followed by excision of all LNs.
- Documentation of LN mapping procedure.
- Histological assessment of all excised lymph nodes
- Concomitant medications
- Assessment of surgical complications
- Adverse events and adverse device effects
- Follow-up visits on date of discharge, and Day 30. Subjects with a discharge date later than Day 30 will have their last study visit on Day 30.

Sample Size and Statistical Analysis:
The FILM trial is a non-inferiority study comparing lymph node detection rates between Blue dye and PINPOINT. A sample size of approximately 150 evaluable subjects (to identify 525 LNs) is required to show the LN detection rate for PINPOINT is non-inferior to that with Blue dye with 80% power and a 5% 2-sided significance level with a 5% non-inferiority margin. Both a modified Intent-to-Treat (mITT) and Per-Protocol (PP) analysis population will be utilized for the primary analysis. Analysis of the primary objective will be conducted using a 2-sided 95% confidence interval for the difference in proportions. A a non-inferiority test will be conducted using the PP and the mITT analysis populations. If and only if non-inferiority is claimed, a superiority test will be conducted using the mITT analysis population.[3] As a supporting analysis of the primary outcome, the analysis will also be repated using the as-treated (AT) population. The secondary objectives will be tested using a two -sided 5% significance level with a step-down multiplicity adjustment.
Study Duration: The study is expected to begin in 2015 and complete enrollment in 18 months. Therefore, the study is expected to complete in 2017.

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS

ADE	Adverse device effect
AE	Adverse event
AT	As-treated
B-P	BLUE-PINPOINT
CFR	Code of Federal Regulations
CRF	Case Report Form
CSF	Color Segmented Fluorescence
CT	Computerized Tomography
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
FIGO	International Federation of Gynecology and Obstetrics
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
H&E	Hematoxylin and eosin
HD	High Definition
HAS	Human serum albumin
IA	Imaging agent
ICH	International Conference on Harmonization
ICG	Indocyanine Green
IRB	Institutional Review Board
IRC	Image Review Committee
IV	Intravenous
LC	Lymphatic channel
LN	Lymph node
MELD	Model for end-stage liver disease
mITT	Modified intent-to-treat
NCCN	National Comprehensive Cancer Network
NDA	New drug application
NIR	Near-Infrared
P-B	PINPOINT-BLUE
PINPOINT	PINPOINT Endoscopic Fluorescence Imaging System
PP	Per-Protocol
REDCap	Research Electronic Data Capture
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SLN	Sentinel lymph node
US	United States
UADE	Unanticipated Adverse Device Effect
VIS	Visible

1 INTRODUCTION AND BACKGROUND

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol, Good Clinical Practice (GCP) and all applicable regulations.

1.1 Background

The purpose of this study is to assess the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in identification of lymph nodes (LN) in subjects with uterine and cervical malignancies who are undergoing lymph node mapping and to investigate the safety of interstitial injection of indocyanine green (IC2000) dye for intraoperative lymphatic mapping using PINPOINT Near Infrared Fluorescence Imaging.

1.1.1 Lymph Node Identification and Mapping

Identification of the tumor draining lymph nodes (LN has become an important step for staging cancers that spread through the lymphatic system. LN mapping involves the use of dyes and/or radiotracers to identify the LNs either for biopsy or resection and subsequent pathological assessment for metastasis. The goal of lymphadenectomy at the time of surgical staging is to identify and remove the LNs that are at high risk for local spread of the cancer

LN identification can be accomplished using several different methods, with the basic technique involving the injection of a tracer that identifies the lymphatic drainage pathway from the primary tumor[6]. The tracers used may be colored dyes (e.g. Isosulfan blue), or radioisotopes (e.g. technetium-99m) for intraoperative localization with a gamma probe; or, a combination of both. Recently, ICG using near-infrared (NIR) fluorescence imaging for visualization has emerged as a potentially effective method for several cancers including breast, skin (melanoma), cervical, endometrial, lung and gastrointestinal[7].

The PINPOINT system is an endoscopic NIR fluorescence imaging system used during minimally invasive surgical procedures. PINPOINT acquires NIR fluorescence images of an imaging agent (ICG) to allow for direct real time visual identification of a lymph node and/or the afferent lymphatic channel intraoperatively. ICG is a commonly used water soluble intravascular dye approved for human use in the United States and Canada. It has a peak spectral absorption at approximately 800 nm and can be used as a lymphatic NIR fluorophore. ICG binds primarily to globulins and to a lesser extent to lipoproteins and albumin[8]. In general, when injected interstitially, the protein binding properties of ICG cause it to be rapidly taken up by the lymph and moved through the conducting vessels to the LN[9]. The NIR fluorescent positive lymph nodes are represented on the PINPOINT screen in PINPOINT (green pseudo-color superimposed on white light image), SPY (black and white) or SPY CSF (color segmented fluorescence) mode.

1.1.2 Endometrial and Cervical Cancer

Endometrial cancer is the most common gynecological cancer, and it is estimated that 52,630 new cases will be diagnosed in the United States (US)[10] and 6,000 new cases will be diagnosed in Canada in 2014[11]. Cervical cancer affects a lower number of women with 12,360[12] and 1,450[11] new cases estimated in the US and Canada respectively for 2014. The most important prognostic factor for subjects is accurate surgical staging, as lymph node status is a predictor of outcome and may influence treatment following surgery.

Identification of LNs and LN mapping for endometrial cancer is being evaluated as a method for surgical staging to be used in subjects with uterine confined tumors when there is no metastasis demonstrated by imaging studies and no obvious extrauterine disease[13]. The current standard of care is to perform a complete or selective para-aortic lymphadenectomy for staging (FIGO) which can lead to morbidities such as lower extremity lymphedema and lymphocyst formation[4].Although the inclusion of pelvic and para-aortic lymphadenectomy in the surgical management is part of the FIGO staging, it remains controversial. Therefore, the decision about the extent of lymphadenectomy (e.g. pelvic nodes only or both pelvic and para-aortic nodes) done by the surgeon can still be based on the preoperative and intraoperative findings.

1.1.3 Summary of Clinical Data for NIR Fluorescence Imaging with ICG for Lymphatic Mapping

The imaging agent (IA), ICG (indocyanine green for injection, USP) 25 mg for Injection in the form of a sterile lyophilized powder containing indocyanine green with no more than 5% sodium iodide is approved by the FDA for determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography via intravascular administration14,15. ICG is also approved under a 510(k) by the FDA for assessing blood flow and tissue perfusion in a variety of surgical and non-surgical procedures16. ICG can be administered intravenously or intra-arterially. It absorbs light in the near-infrared region at 806 nm, and emits fluorescence (light) at a slightly longer wavelength, 830 nm. When injected intravenously, ICG rapidly and extensively binds to plasma proteins and is confined to the intravascular compartment with minimal leakage into the interstitium. This property makes ICG an ideal agent for the acquisition of high quality images of lymph nodes and lymphatic vessels (i.e., for NIR fluorescence lymphography).

1.1.4 Clinical Studies

The Sponsor has not conducted any clinical trials on the use of intraoperative PINPOINT Near Infrared Fluorescence Imaging with ICG in identification and mapping of LN to date. However, 12 studies with performance and/or safety data using the Sponsor's fluorescence imaging devices or those with equivalent characteristics have been reported in the literature on the use of ICG in lymphatic mapping of gynecological cancers (Table 1).

TABLE 1. Lymphatic Mapping Studies in Gynecological Cancers

Author	Indication	Device	Summary
Furukawa et al., 2010[17]	Cervical	PDE	ICG fluorescence imaging was used to identify SLN during sentinel node navigation surgery in cervical cancer patients. No allergic reactions to ICG were observed.
Crane et al., 2011[18]	Cervical	Prototype multispectral fluorescence camera system	Intraoperative lymphatic mapping and SLN detection was carried out in cervical cancer patients using ICG fluorescence imaging and patent blue dye. No side effects were noted after injection of ICG or patent blue dye.
Crane et al., 2011[19]	Vulvar	Prototype multispectral fluorescence camera system	Intraoperative transcutaneous lymphatic mapping was carried out in vulvar cancer patients in a comparison using ICG fluorescence imaging, ^{99m}-Technetium-nanocolloid and patent blue dye. No side effects related to ICG injection were noted.

Author	Indication	Device	Summary
Holloway et al., 2012[20]	Endometrial	da Vinci Surgical System	Retrospective comparison of results from lymphatic mapping of pelvic SLN using fluorescence NIR imaging of ICG and colorimetric imaging of Isosulfan blue dyes in women with endometrial cancer undergoing robotic-assisted lymphadenectomy.
Rossi et al 2012[21]	Endometrial	da Vinci Surgical System	Sentinel lymph node (SLN) mapping with indocyanine green (ICG) detected by robotic near infrared (NIR) imaging is a feasible technique.
Schaafsma et al 2012[22]	Cervical	Mini-FLARE	Eighteen consecutive early-stage cervical cancer patients scheduled to undergo pelvic lymphadenectomy were included. Prior to surgery, 1.6 mL of 500 µM ICG:HSA or 500 µM ICG alone was injected transvaginally in 4 quadrants around the tumor. The Mini-FLARE imaging system was used for intraoperative NIR fluorescence detection and quantitation.
Rossi et al 2013[23]	Cervical or endometrial, Stage 1	SPY Scope (7 subjects); da Vinci (13 subjects)	Robotically assisted endoscopic NIR imaging after injection of ICG was used for LN mapping in patients with clinical stage 1 cervical or endometrial cancer. No AEs occurred.
Schaafsma et al 2013[24]	Vulvar	Mini-FLARE	NIR fluorescence imaging for SLN biopsy was investigated and ICG vs ICG:HSA compared in a double-blind, randomized, non-inferiority trial of vulvar cancer patients. The study confirmed the feasibility of NIR fluorescence imaging for SLN mapping in vulvar cancer and found no advantage in using ICG:HSA over ICG alone. No AEs were associated with the use of ICG or ICG:HSA.
Jewell et al., 2014[25]	Endometrial and cervical	da Vinci Surgical System	Robotically assisted endoscopic NIR imaging after injection of ICG was used for SLN mapping in patients with uterine and cervical malignancies
Sinno et al. 2014[26]	Endometrial or CAH	da Vinci Surgical System	Comparison of NIR fluorometric imaging vs colorimetric imaging for SLN mapping in endometrial cancer
Plante et. al. 2015[27]	Endometrial and cervical	PINPOINT Endoscopic System	Initial experience with PINPOINT Endoscopic NIR imaging for LN identification was reported. Study determined PINPOINT/ICG is an excellent and safe modality for SLN mapping with a very high (96%) detection rate.
How et. al. 2015[28]	Endometrial	da Vinci Surgical System	Comparison of ICG, blue dye and 99mTc-SC mapping in patients undergoing robotic-assisted surgery. A mixture of all three modalities is feasible and provides good mapping. ICG was found to be superior to blue dye and comparable to 99mTc-SC and thus blue dye may not be essential for SLN detection.

A total of 550 subjects with early stage gynecological cancers (cervical, endometrial and vulvar) have been reported in the literature as having undergone intraoperative SLN mapping with ICG fluorescence imaging. Table 2 lists the published studies including the number of subjects receiving ICG, the ICG dose, route of administration, SLN detection rate and safety results (when reported). All subjects receiving an injection of ICG are listed for studies that included co-administration of a colorimetric dye or radiotracer. Of the 12 studies, 7 specifically reported that there were no adverse reactions to the procedure. In 4 studies (Crane et al 2011; Furukawa et al 2010; Rossi et al, 2011, Plante et al. 2015), ICG fluorescence was used to identify SLNs in subjects with cervical or endometrial cancer [17,19,21] Detection rates ranged from 60% to 96%. In a study comparing ICG fluorescence and blue dye, Crane et al (2011) found that 89.7% of radioactively labelled SLNs in subjects with vulvar cancer could be detected by ICG fluorescence compared to 72.4% detected by blue dye[18].

Schaafsma et al. compared the use of ICG and ICG conjugated to HSA for NIR fluorescence detection of SNs in subjects with early cervical cancer[22] and vulvar cancer[24]. In both studies, no difference in SLN detection rates was found between the ICG (6/9; 67% for cervical cancer and 9/12; 75% for vulvar cancer) and ICG: HSA groups (8/9; 89% for cervical cancer; 10/12; 83% for vulvar cancer) (p=0.13, cervical cancer; p=0.27, vulvar cancer).

Jewell et al. completed a retrospective, open label, single center study using NIR fluorescence imaging on the robotic platform with intracervical ICG injection for SLN detection in uterine and cervical malignancies[25]. A total of two hundred and twenty-seven cases were performed. When

ICG alone was used to map cases, 188/197 subjects mapped for a detection rate of 95% compared to 93% in cases in which both blue dye and ICG were used. Bilateral mapping was seen in 79% of ICG alone cases compared to 77% of cases using ICG and blue dye. The authors concluded that NIR fluorescence imaging on the robotic platform has a high bilateral detection rate and appears to be favorable to ICG dye alone, with blue dye combined with ICG unnecessary for SLN mapping.

How et al. recently completed a prospective study comparing NIR fluorescence using ICG, blue dye and technetium for SLN mapping in endometrial cancer[28]. A total of 100 subjects underwent SLN mapping with a mixture of ICG, blue dye and 99mTc-SC injected directly into the cervix. A total of 286 LNs were mapped (2.9 per subject). ICG had a significantly higher detection rate than blue dye both in unilateral (87% vs 71%) and bilateral (65% vs 43%) detection rates. The authors concluded that mapping with ICG is superior to blue dye and comparable to mapping with 99mTc-SC.

TABLE 2 Lymphatic Mapping Studies in Gynecological Cancers

Author	Type of Cancer	Number of Patients	ICG Concentration (mg/ml)	Total Dose of ICG (mg)	Route of Administration	Detection Rate	Safety
Furukawa et al., 2010	Cervical	10	5.00	1.0	Cervical injection[a]	83%	No allergic reactions
Crane et al., 2011	Cervical (1A1, 1B1, IIA)	10	0.50	0.50	Cervical injection	60%	No side effects reported
Crane et al., 2011	Vulvar	10	0.50	0.50	Vulva (peritumoral injection)	89.7%	No side effects related to intraoperative injection of ICG
Holloway et al., 2012[b]	Endometrial	35	1.25	2.50	Cervical injection[a]	100%	Not reported
Rossi et al., 2012	Cervical or endometrial Stage 1	3 17	0.50 0.50	0.50, 0.75 & 1.5 1.00	Cervical injection[d]	85% 88%	No adverse events reported
Rossi et al., 2012	Endometrial	12	0.50	0.50	Hysteroscopic endometrial	33%	Not reported
		17	0.50	1.00	Cervical Injection	82%	Not reported
Schaafsma et al., 2012	Cervical (1B1)	9	0.38	0.62	Cervical[a]	67%[f]	Not reported
Schaafsma et al., 2013	Vulvar	12	0.38	0.62	Vulva (peritumorally or around excision scar)	75%	No adverse reactions reported
Jewell et al. 2014	Endometrial and cervical	227	1.25	2.50	Cervical Injection	100%	Not reported
Sinno et al., 2014	Endometrial or CAH[e]	38	1.25	5.00	Cervical injection[c]	78.9%	Not reported
Plante et al. 2015	Cervical or endometrial Stage 1	50	1.25	5.00	Cervical Injection	96%	No adverse events reported

Author	Type of Cancer	Number of Patients	ICG Concentration (mg/ml)	Total Dose of ICG (mg)	Route of Administration	Detection Rate	Safety
How et al. 2015	Endometrial	100	0.25	0.1	Cervical Injection	96%	No adverse events reported

a Injection into 4 quadrants of the cervix (3, 6, 9 and 12 o'clock positions)
b Retrospective study
c Injection into cervix at 3 and 9 o'clock positions with 1 cc deep in the stroma and 1 cc submucosally on the right and left of cervix for a total volume of 4 ml
d Injection 1 cm into cervical stroma at 3 and 9 o'clock positions
e Complex Atypical Hyperplasia
f Intraoperative bilateral detection rate

Based on the data available from clinical studies, this Phase III study has been designed to demonstrate the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in lymphatic mapping and to assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

1.2 Potential Risks and Benefits to Human Subjects

Currently the PINPOINT system is classified by the United States Food and Drug Administration (FDA) as a Class II medical device with a Product Code of GCJ. PINPOINT for the purposes of fluorescence angiography is not identified as a significant risk device on the FDA Information Sheet titled "Significant Risk and Non-significant Risk Medical Device Studies". PINPOINT is a commercially available product in the United States (US) and Canada PINPOINT has a 510(k) clearance from the FDA and is licensed in Canada with the following indication:

"The PINPOINT system is intended to provide real-time endoscopic visible and near infrared fluorescence imaging. PINPOINT enables surgeons to perform routine visible light endoscopic procedures as well as further visually assess vessels, blood flow and related tissue perfusion with near infrared imaging during minimally invasive surgery".

Note: PINPOINT has not yet been classified by the FDA or Health Canada for the purposes of lymphatic mapping.

The imaging agent, ICG, is approved for human use by the FDA and Health Canada. In this study, investigational ICG (IC2000) will be administered interstitially for the visualization of LN and LC. The safety of interstitial administration of investigational IC2000 for NIR fluorescence imaging to identify LN has not been studied. However, safety data from published studies reported above combined with the well-established safety profile for ICG after intravenous or intra-arterial injection support an acceptable risk/benefit ratio for interstitial administration of ICG. The most serious risk of ICG when administered intravenously in humans, is anaphylactic death, which has been reported following ICG administration during cardiac catheterization.

These and other risks of the PINPOINT system in humans are described further in Section 7, Risks/Precautions. For additional information, please refer to the PINPOINT Operator's Manual[16].

2 STUDY OBJECTIVES

2.1 Objectives

2.1.1 Primary Objectives

The primary objective of this study is to assess the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in identification of LNs in subjects with uterine and cervical malignancies who are undergoing LN mapping.

2.1.2 Secondary Objectives

- To evaluate the effectiveness of PINPOINT and Blue dye in the identification of at least one LN (confirmed to be lymphoid tissue) per subject.
- To evaluate the effectiveness of PINPOINT and Blue dye in the identification of bilateral LNs (confirmed to be lymphoid tissue).
- To assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.
- To determine the proportion of LNs identified from following LCs.
- To find the anatomic distribution of LNs.

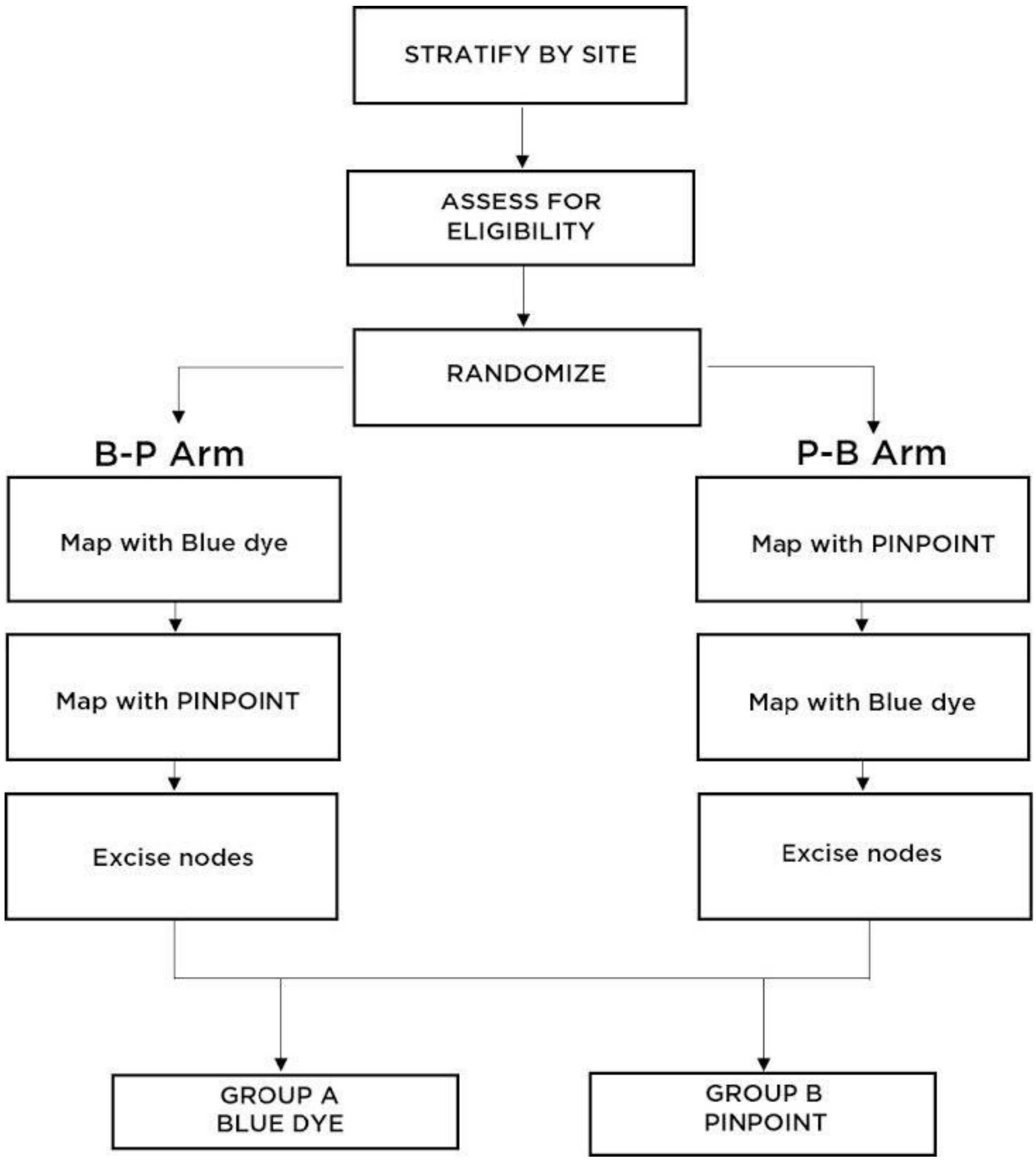
3 INVESTIGATIONAL PLAN

3.1 Study Design Overview

This is a randomized prospective, open label, multicenter study to assess the safety and effectiveness of PINPOINT Near Infrared Fluorescence Imaging (PINPOINT) in identification of LN in subjects with uterine and cervical malignancies who are undergoing LN mapping. This is a non-inferiority within-subject comparison study to determine the effectiveness of PINPOINT in the identification of LNs compared to LNs identified with Blue dye. Approximately 150 subjects will be enrolled at up to 10 centers in North America. Prior to enrolling study subjects, participating surgeons at each center will be trained to perform intraoperative identification and mapping of LNs with ICG and Blue dye. Participating surgeons will be required to have completed at least 10 LN mapping procedures with a minimum of 3 LN mapping cases performed with PINPOINT prior to the initiation of enrollment.

Screening:
Subjects diagnosed with International Federation of Gynecology and Obstetrics (FIGO) clinical stage I endometrial or cervical cancer scheduled for surgery that includes clinically indicated LN mapping will be evaluated at baseline to determine if they meet the inclusion/exclusion criteria of the protocol. Subjects will be assessed to determine overall health status including demographics, vital signs, diagnosis and relevant medical history/underlying conditions. Eligible subjects who provide informed consent will be considered for inclusion into the study.

FIGURE 1. Group Assignment and Randomization



Day 0:

On the day of surgery, subjects will be randomized (1:1) to either the BLUE-PINPOINT (B-P) Arm or the PINPOINT-BLUE (P-B) Arm. Subjects in each arm will be randomized according to an independently generated randomization scheme to undergo lymphatic mapping with Blue dye first followed by mapping with PINPOINT (B-P Arm) or to undergo lymphatic mapping with PINPOINT first followed by mapping with Blue dye (P-B Arm).

Eligible subjects who have provided informed consent will be enrolled in the study upon randomization on the day of surgery (Day 0). Minimally invasive surgery will be performed according to the surgeon's standard practice.

The cervix will be injected four (4) times with a 1 ml solution of Blue Dye (1% Isosulfan blue; 10 mg/ml) for a total dose of 40 mg Blue Dye) and four (4) times with 1 ml of a 1.25 mg/ml solution of IC2000 for a total dose of 5 mg IC2000 . The injection of Blue dye and IC2000 will occur while the subject is under anesthesia in the operating room.

In order to minimize the spillage of blue dye or ICG interfering with the mapping procedure when LNs are excised, mapping will be performed on one side of the pelvis followed by the other side and mapping with both Blue dye and PINPOINT will be performed prior to the excision of any LNs.

Subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of IC2000 and undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'IC2000' nodes are identified or the investigator determines that 'ICG' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered IC2000 first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping with PINPOINT will be performed until the investigator identifies all 'IC2000' nodes or determines that 'IC2000' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both PINPOINT and Blue dye have been completed and documented, all LNs identified with PINPOINT or Blue dye will be excised.

LN mapping for Clinical Stage I endometrial cancer will be performed according to the NCCN Guidelines for Uterine Neoplasms, LN Algorithm for Surgical Staging of Endometrial Cancer; and LN mapping for Clinical Stage I cervical cancer will be performed according to the NCCN Guidelines for Cervical Neoplasms, Surgical/LN Mapping Algorithm for Early-Stage Cervical Cancer[1, 2].

The surgeon will identify LN based on direct visualization with white light for blue dye or PINPOINT for IC2000.

LN Mapping with PINPOINT:
Intraoperative identification of LNs with IC2000 will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with IC2000 using PINPOINT.
- Visibly or palpably abnormal LNs designated as palpable masses and excised regardless of visible fluorescence.

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Mapping will be considered complete when all nodes meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area within the abdominal cavity. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised.

LN Mapping with Blue dye:

Intraoperative identification of LNs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visibly or palpably abnormal LNs designated as palpable masses and excised regardless of visible blue dye.

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area within the abdominal cavity. Blue ducts should be followed in both directions in order to identify LNs to be excised.

Classification of LNs:

All LNs identified will be classified as:

1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard
5. Non-stained node found at origin or termination of fluorescent duct
6. Non-stained node found at origin or termination of blue duct
7. Non-stained node found at origin or termination of fluorescent and blue duct

Effectiveness of intraoperative Blue dye in identifying of LN will be based on the proportion of LNs identified by visualization of blue dye under white light and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Effectiveness of intraoperative PINPOINT in identifying of LN will be based on the proportion of LNs identified by PINPOINT and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised.

Details of the surgery, including intraoperative findings will be documented. Surgical data will be collected including the ability to identify LNs, the number of LNs identified and removed, the ability to unilaterally and bilaterally map LNs, and the anatomical location and distribution of identified LNs. Failed mapping is defined as no LNs detected.

Follow-up and Post-operative Complications:

All Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits to monitor occurrence of any adverse events/adverse device effects on the date of discharge and Day 30 (±7 days). All subjects will be followed to monitor occurrence of adverse events up to Day 30 (±7 days) post-surgery. All adverse events will be followed up to Day 30 (±7 days). Adverse events thought to be related to the use of PINPOINT or to the LN mapping procedure will be followed until resolution or deemed chronic.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Number of Subjects

Approximately 150 subjects will be enrolled in the study (See Section 10.4).

4.2 Inclusion Criteria

To be eligible for the study, a subject must fulfill all of the following criteria:

1. Be 18 years of age or older
2. Have either of the following diagnoses and surgical plan:
    a. FIGO Clinical stage I endometrial cancer undergoing minimally invasive hysterectomy with lymphatic mapping
    b. FIGO Clinical stage IA cervical cancer ≤ 2 cm undergoing minimally invasive hysterectomy, trachelectomy or conization with lymphatic mapping. Note: Subjects with clinical Stage IA1 cervical cancer without lympho vascular space involvement (LVSI) and negative margins on cone biopsy are not to be included.
3. Negative nodal status (N0)
4. Subjects with negative metastatic involvement (M0)
5. Subjects of child-bearing potential must not be pregnant or lactating and must have a negative pregnancy test at Day 0
6. Have signed an approved informed consent form for the study
7. Be willing to comply with the protocol

4.3 Exclusion Criteria

A subject meeting any of the following criteria will be excluded from the study:

1. Have had prior dissection and/or radiation in pelvis
2. Advanced cervical or endometrial cancer, T3/T4 lesions
3. Diagnosis of cervical cancer with a tumor size greater than 2 cm
4. Locally advanced cervical or uterine cancer
5. Metastatic cervical or uterine cancer
6. Known allergy or history of adverse reaction to ICG, iodine or iodine dyes
7. Known allergy or history of adverse reaction to Blue dye (Isosulfan blue) or triphenylmethane
8. Hepatic dysfunction defined as MELD Score > 12
9. Renal dysfunction defined as serum creatinine ≥ 2.0 mg/dl
10. Subjects who have participated in another investigational study within 30 days prior to surgery
11. Pregnant or lactating subjects
12. Subjects who, in the Investigator's opinion, have any medical condition that makes the subject a poor candidate for the investigational procedure, or interferes with the interpretation of study results

4.4 Withdrawal of Subjects

Subjects can voluntarily withdraw (or be withdrawn) at any time during the study.

Investigators may withdraw a subject from the study because:

- A new health condition, diagnosis or finding appears that is suspected to require care or medication prohibited by the protocol.
  - e.g., the planned surgical procedure is modified to a procedure prohibited by the protocol.
- The subject has unacceptable adverse events.
- It is in the subject's best interest according to the Investigator's clinical judgment.

If a subject is prematurely withdrawn from the study, the reason(s) for withdrawal must be recorded on the relevant page of the subject's Study Completion case report form (CRF).

Subjects who discontinue the study prematurely will not be replaced.

The Sponsor may stop the study at any time.

5 RANDOMIZATION, BLINDING AND SUBJECT IDENTIFICATION PROCEDURES

5.1 Randomization

Subjects will be prospectively randomized into the FILM Clinical Trial. Randomization will occur on the day of surgery. Prior to surgery, the subject will have provided written informed consent, completed all baseline procedures and met the requirements of inclusion and exclusion criteria. Randomization should be performed as closely as possible to the mapping procedure to minimize the incidence of dropout.

Subjects will be randomly assigned on a one to one (1:1) basis to either the B-P Arm (LN mapping with Blue dye followed by LN mapping with PINPOINT) or the P-B Arm (LN mapping with PINPOINT followed by LN mapping with Blue dye). Randomization will be stratified by study site. Permuted block randomization will be performed within strata. To minimize the opportunity for the sequence to be predicted, the block size will be variable and randomly chosen from small multiples of 2 (i.e. 2, 4 or 6). The randomization schedules will be generated in advance using a computerized random number generator. Investigational sites will not have access to the randomization schedules.

Randomization will be accomplished using a secure web-based software (REDCap) supported by the Data Coordinating Center. Treatment assignment is made only after verification of proper informed consent execution and study eligibility.

5.1.1 Randomization Procedure

The study coordinator or a designee will verify that the subject is eligible and that informed consent has been obtained prior to initiating the randomization process. On the day of surgery the study coordinator will log onto the REDCap system and, after confirming the subject's eligibility, will enroll a subject and obtain the randomization assignment. The study coordinator will then disclose the randomization assignment to the Investigator. As this is a within-subject comparison study, the investigator cannot be blinded to the use of Blue dye or PINPOINT device. All subjects will be blinded to their randomization assignment until after the procedure.

A subject is not randomized until randomization has been assigned by REDCap. The randomization procedure should not be initiated unless the study coordinator confirms that a subject is eligible and verifies baseline information. If any deviations occur (errors such as assigning incorrect randomization), the clinical site will be required to contact the Sponsor and await guidance on how to proceed.

If, at any time after randomization, the subject becomes ineligible or withdraws, the subject is still considered randomized. If an intraoperative decision is made to perform a procedure other than what was intended, the subject will be categorized with respect to the definitions outlined for the analysis populations (see the Statistical Methods Section 10.5).

5.2 Subject Identification

Screening ID Number: All subjects screened for the study shall be assigned a 5-digit “screening” number on the Screening Log and if they are randomized, will subsequently be assigned a Subject Enrollment Number in the electronic data capture system. The Screening Identification Number shall be unique and categorize a subject in sequence of screening by an “S” followed by a 5-digit number. The first 2 digits identify the site and the last three digits identify the subject. For example, the first subject screened at site 01 is identified as screening number S- 01001. The screening log shall be maintained by the site to identify those subjects that have failed screening with the reason why they did not qualify for enrollment. The screening number will be assigned sequentially within each study center in order of subject presentation for screening.

Enrolled Subject ID Number: Once a subject is randomized, they will be assigned a Subject Enrollment Number which is also a 5-digit number. The first two digits of the Subject Enrollment Number identify the site and the last three digits identify the subject. Each site will be given a Site Identification Number. For example, the first subject randomized at site 03 is identified as subject 03-001, the next subject as 03-002, etc.

Subjects who sign an informed consent form but are not randomized are considered screen failures. These subjects must be entered on the Screening Log but do not receive a subject number. The reason for non-enrollment must be documented on the log.

6 STUDY TREATMENTS

6.1 Device Description

PINPOINT is an endoscopic fluorescence imaging system for high definition (HD) visible (VIS) light and near-infrared (NIR) fluorescence imaging. PINPOINT includes the following components:

- A surgical endoscope optimized for VIS/NIR illumination and imaging, which is available in different diameters, lengths and directions of view (Model: SC9104, SC9134).
- A camera head that is also optimized for VIS/NIR imaging and mounts to the endoscope eyepiece (Model: PC9002).
- A flexible light guide cable (Model: PC9004).

- An endoscopic Video Processor / Illuminator (VPI) capable of providing VIS/NIR illumination to the surgical endoscope via a flexible light guide cable, and the image processing required to generate simultaneous, real-time HD video color and NIR fluorescence images (Model: PC9001).

  PINPOINT is designed to be connected to a medical-grade HD color monitor, such as those normally used in surgical endoscopy.

PINPOINT is connected to a high-definition medical video recorder (Sony HVO-1000) that allows the capture of still images and video during operation.

PINPOINT acquires NIR fluorescence images of an imaging agent (IC2000) to allow for visual assessment of vessels, blood flow and related tissue perfusion during minimally invasive surgery. For the purpose of this study, PINPOINT will be used for visual identification of LNs during LN mapping procedures.

The PINPOINT Operator's Manual[16] describes the contents, use and storage of the PINPOINT PAQ's. Instructions for preparation, handling and administration of Investigational ICG (IC 2000) are provided in Section 8.3.1.2.

PINPOINT allows simultaneous display of multiple images. Real time NIR fluorescence video images are acquired by using the imaging agent and may be viewed in two ways:

PINPOINT image:	NIR fluorescence is superimposed in pseudo-color (green) on a white light image
SPY image:	A black and white NIR fluorescence image is displayed
CSF:	A high-definition, white light image is displayed in grayscale with NIR fluorescence overlaid on a color scale. Increasing fluorescence levels transition from blue through yellow to red.

PINPOINT also operates as a conventional endoscopic imaging system and provides illumination for real-time color (white light) HD video imaging in the area of interest.

PINPOINT is a commercially available product in the United States (US) and Canada. PINPOINT has a 510(k) clearance from the FDA and a Health Canada license with the following indication:

"The PINPOINT system is intended to provide real-time endoscopic visible and near infrared fluorescence imaging. PINPOINT enables surgeons to perform routine visible light endoscopic procedures as well as further visually assess vessels, blood flow and related tissue perfusion with near infrared imaging during minimally invasive surgery".

PINPOINT is manufactured by Novadaq Technologies Inc. (Novadaq). Please refer to the current version of the PINPOINT Operator's Manual[16] for ~~a~~ full description and specifications of the system.

In this study, the use of PINPOINT for visual identification of lymph nodes via interstitial injection of ICG is investigational.

6.2 Imaging Agent Description

6.2.1 Indocyanine Green IC2000

6.2.1.1 Overview and Pharmacokinetics of Investigational Product

ICG was originally approved by the FDA for human medical use in 1959 for use in determining cardiac output, hepatic function and liver blood flow. In 1975, a NDA Supplement was approved for ICG for use in ophthalmic angiography. In 2005, ICG was approved under a 510K for assessing blood flow and tissue perfusion in a variety of surgical and non-surgical procedures. Over the past 50 years ICG has been marketed in the United States and has demonstrated an excellent safety profile. ICG has received approval from Health Canada (DIN 02014793) in 1994 for human medical use as a diagnostic agent, and is classified as an "Ethical" drug.

ICG absorbs light in the near-infrared (NIR) region at 806 nm and emits fluorescence (light) at a slightly longer wavelength, 830 nm. After injection, ICG rapidly binds to blood proteins primarily lipoproteins and to a lesser extent globulins and albumin and drains into the lymphatic system without extravasation enabling sensitive non-invasive visualization of LN and lymphatic architecture. Within minutes after intravenous injection, ICG is cleared by the liver and excreted into the bile[24]. Since lymph fluid flows into the venous blood stream through the subclavien veins[9], it is assumed that, after interstitial injection, ICG drains with lymph fluid into the circulatory system where it is then cleared by the liver and excreted in the bile. Although formal pharmacokinetic studies have not been performed with non-intravenous routes of administration, after intradermal injection in mice, ICG was shown to be taken up by lymphatic vessels and cleared within 48 hours[29,30]. Another study using ICG conjugated to human serum albumin (HSA) in rabbits found ICG is efficiently taken up by the lymphatic vessels and cleared within 24 hours after peritumoral injection[31]. Chi et al. investigated the use of ICG for LN mapping in 5 mice, 10 rabbits and 22 breast cancer subjects and found LNs are identifiable 3-5 minutes after injection with the occurrence of peak fluorescence intensities varying with the dose of ICG and occurs between 10 and 90 minutes[32].

6.2.1.2 Description of Investigational Product (IC2000)

The chemical name of the investigational drug, indocyanine green is 1 *H*-Benz[e]indolium, 2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benz[e]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-,hydroxide, inner salt, sodium salt. ICG has a molecular weight of 774.96 daltons and will be administered with an interstitial injection into the cervix.

The imaging agent , IC2000 (indocyanine green, USP) is provided in the form of a sterile lyophilized powder containing 25 mg ICG with no more than 5% sodium iodide. IC2000 is packaged with aqueous solvent consisting of sterile Water for Injection, which is used to reconstitute the ICG. When injected, ICG rapidly and extensively binds to plasma proteins (primarily lipoproteins and globulins) with minimal leakage through vessel walls. This property makes ICG an ideal agent for the acquisition of high quality images of LNs and s (i.e., for NIR fluorescence lymphography). Note: The use of IC2000 in this study is investigational.

6.2.1.3 Chemistry and Manufacturing

Investigational IC2000 is supplied as a 25 mg sterile lyophilized green powder in a glass vial with a grey stopper and grey over seal. The product is stored at 20°C to 25°C (68° to 77° F). IC2000 is manufactured by Patheon (Patheon Italia S.p.A., 2° Trav. SX Via Morolense, 503013 Ferentino- Italy) and, like commercially available ICG, IC2000 is tested according to the USP monograph for Indocyanine green for Injection.

IC2000 was manufactured in full compliance with the FDA's current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals-standards at 21 CFR Part 211 and meets all GMP requirements for Health Canada.

6.2.1.4 Labelling of IC2000 for Injection

IC2000 is labeled according to US and Canadian regulatory requirements with the following information:

- IC2000 (indocyanine green for injection, USP), 25 mg lyophilized ICG
- Sterile
- Protocol No.: PP LMN 01
- Directions for use: Refer to Clinical Protocol
- Store at: 20–25°C (68-77°F)
- Lot: 14GRF03
- EXP.: May 2016
- CAUTION—New drug - Limited by Federal (or United States) law to investigational use.
- Investigational drug to be used only by a qualified investigator.
- Sponsor: Novadaq Technologies Inc. 13155 Delf Place, Unit 250, Richmond, BC, Canada, V6V 2A2 1.844 6682327

6.2.1.5 Packaging and Distribution of IC2000

IC2000 will be packaged into investigational PINPOINT LN Mapping Kits as described below and distributed by the study Sponsor (Novadaq). Each LN-mapping procedure kit is indicated for use exclusively with PINPOINT and should only be used for the purposes of this study.

Each PINPOINT LN Mapping kit contains:

- One single use 25 mg vial of sterile IC2000, USP
- Two single use 10 ml ampules of sterile Aqueous solvent
- Four 3 ml syringes, sterile
- Two 10 ml syringes, sterile
- Two Lure-lock 10 ml syringes with controlled handle
- Four Spinal needles, 22G, 3.5 inch, sterile
- Labels for syringes

All information included on the investigational IC2000 for Injection vial labels will appear on the kit labels along with a description of the contents of the kit. Each kit will bear a unique serial number and will meet regulatory requirements necessary for each participating country.

6.2.1.6 Storage and administration of IC2000 for Injection

IC2000 will be stored at 20°C to 25°C (68° to 77° F) in a secure locked area, accessible to authorized personnel only. IC2000 will be stored, handled, prepared and administered by qualified, trained personnel only as described in Section 8.3.1.2.

6.2.2 Blue Dye (Isosulfan Blue)

The Blue dye (Isosulfan Blue Injection, 1% aqueous solution) a sterile and pyrogen-free 5 ml single-use vial[33]. Isosulfan blue is supplied as a 1% aqueous solution in a phosphate buffer and is approved under an ANDA by the US FDA for the identification of lymphatic vessels draining the region of injection, lymphography, and identification of lymph node involvement by primary and secondary neoplasms. Isosulfan blue will be obtained from commercially available sources and is approved for subcutaneous injection.

6.2.2.1 Storage and administration

Blue dye will be stored at 20°C to 25°C (68° to 77° F) in a secure locked area, accessible to authorized personnel only. Blue dye will be stored, handled, prepared and administered by qualified, trained personnel only as described in Section 8.3.1.1.

Installation, Training and Storage

PINPOINT will be installed by Novadaq representatives. The Investigator(s) and study staff shall be required to participate in training on the operational and procedural use of PINPOINT as it relates to the conduct of this study. Training will be provided by Novadaq.

Prior to enrollment, participating Investigator surgeons must be experienced with LN mapping procedures (i.e. performed a minimum of 10 LN mapping procedures) with a minimum of 3 LN mapping cases performed with PINPOINT prior to initiation of enrollment.

Investigator surgeons new to this technique will receive training consisting of the following:

- Didactic training on product and procedure.
- Hands on training session with clinical specialists on site.
- Guidance on use of the system and interpretation of the images with a clinical educator during a minimum of 10 cases prior to study enrollment.

Supporting personnel operating and cleaning PINPOINT will also receive training and be familiar with all applicable aspects of the operation and cleaning of the system.

PINPOINT shall be stored at room temperature in a secure and limited access area available to study staff.

6.3 Concomitant Treatment

Any concomitant medications the subject is receiving at the start of the study (Day 0) or given for any reason during the study must be recorded in the CRF and source documents with the exception of routine medications given for preparation of surgery, during surgery and post-operative care. These include but are not limited to the following:

- Sedatives and anesthetics
- Anticoagulants
- Prophylactic antibiotics
- Anti-emetics
- Routine post-operative pain medications

The drug name, start and stop dates, indication, dose, frequency and route information will be recorded for concomitant medications.

Other surgical and diagnostic procedures concomitant to the laparoscopic hysterectomy, trachelectomy, conization and lymphatic mapping procedure that take place during the study must be recorded in the CRF and in the source document, including start and stop dates.

7 RISKS/PRECAUTIONS

Refer to the PINPOINT Operator's Manual for a full description of the risks and precautions associated with all components of PINPOINT. The entire PINPOINT Operator's Manual should be read before using PINPOINT[16]. Failure to follow the instructions and warnings in the manual may result in unsafe operation of the system and/or injury to the subject or operator.

Refer to the Isosulfan Blue prescribing information for a full description of risks and precautions associated with the use of Isosulfan Blue[33]. The entire package insert should be read before using Isosulfan blue. Failure to follow instructions and warnings may result in injury to the subject.

7.1 PINPOINT System – Endoscopes, Camera and Video Processor Illuminator Unit

Currently, PINPOINT is classified by the FDA as a Class II medical device with a Product Code of GCJ. PINPOINT for the purposes of fluorescence angiography is not identified as a significant risk device on the FDA Information Sheet titled "Significant Risk and Non-significant Risk Medical Device Studies".

The use of PINPOINT for LN mapping is investigational. PINPOINT should only be used in accordance to its approved Indication for Use or in accordance to the study procedures described in this protocol.

7.2 Indocyanine Green (IC2000)

IC2000 will be injected directly into the cervical submucosa and stroma; and will not be administered intravenously. Additionally, the subject is anesthetized and the cervix and uterus are removed as part of the surgical procedure. Therefore the risks associated with injection into the cervix are expected to be lower than those associated with intravenous administration. Based on the clinical information for systemic ICG administration, the risks and precautions are discussed below.

Severe allergic reaction (affects fewer than one in every 10,000 subjects) with symptoms that could include the following: tightness in the throat, itchy skin, blotchy skin, rash, coronary artery spasm, facial edema, breathing difficulties, tightness and/or pain in the chest, faster heartbeat, a fall in blood pressure and shortness of breath, cardiac arrest, restlessness, nausea, feeling of warmth, flushes, hypereosinophilia[15].

The possibility of an allergic reaction is greater in subjects with extremely serious kidney failure. To minimize the risk of an allergic reaction, all subjects with renal dysfunction are excluded as well as subjects with a known allergy to iodine, ICG or iodine dyes[15].

IC2000 contains sodium iodide and should be used with caution in subjects who have a history of allergy to iodides or iodinated imaging agents. Anaphylactic or urticarial reactions have been reported in subjects with and without history of allergy to iodides. Anaphylactic deaths have been reported following ICG administration during cardiac catheterization[14].

Radioactive iodine uptake studies should not be performed for at least 1 week following IC2000 administration[14,15].

Animal reproduction studies have not been conducted with ICG or IC2000. It is not known whether ICG or IC2000 can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. It is not known whether ICG or IC 2000 is secreted in human milk. Women who are pregnant or lactating are excluded from this study.

7.3 Isosulfan Blue (Blue Dye)

Similar to the administration of IC2000 in this study, Isosulfan blue will be directly injected into the cervical submucosa and stroma. The subject will be anaesthetized and the cervix and uterus are removed as part of the surgical procedure.

Severe anaphylactic reactions have occurred after injection of Isosulfan blue (approximately 2% of subjects). Manifestations include respiratory distress, shock, angioedema, urticarial and pruritus. Reactions are more likely to occur in subjects with a history of bronchial asthma, or subjects with allergies or drug reactions to triphenylmethane dyes.

Isosulfan blue interferes with measurements of oxygen saturation by pulse oximetry and methemoglobin by gas analyzer.

The use of Isosulfan blue may result in transient of long-term (tattooing) blue coloration.

It is not known whether Isosulfan blue can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. It is not known whether Isosulfan blue is secreted in human milk. Women who are pregnant or lactating are excluded from this study.

8 STUDY PROCEDURES

8.1 Schedule of Events

Table 3 Schedule of Events

Procedure	Baseline (Day -30 to Day 0)	Day 0 (Operative Phase)				
		Pre-OP	Intra-Op	Post-Op	Date of Discharge	Day 30 Phone call (± 7 days) [a]
Informed consent	X					
Demography, Surgical risk factors	X					
Vital signs, blood pressure, heart rate, height, weight	X					
Pre-operative diagnosis	X					
Pregnancy test	X					
Inclusion/exclusion criteria	X					
Serum sodium, bilirubin, creatinine and INR for MELD Score	X					
Hemoglobin	X[d]		X[b]			
Randomization		X				
LN mapping with Blue dye			X			
LN mapping with PINPOINT			X			
Surgical Procedure			X			
Documentation of LN mapping and surgical procedure			X	X		
Concomitant medications and procedures	X	X	X	X	X	X
Adverse events/adverse device effects		X	X	X	X	X[c]
Histopathological evaluation of Lymph Nodes			X	X		

[a] Subjects with a discharge date later than Day 30, will have the last study visit on Day 30.
[b] Hemoglobin measurement at the time of mapping if the subject had any hypotensive events or blood loss over 500 ml at the time of mapping.
[c] All subjects with adverse events thought to be related to the LN mapping procedure or the PINPOINT system will be followed until resolution or deemed chronic.
d Hemoglobin measurement must occur within 14 days prior to the day of surgery (Day -14 to day 0)

8.2 Baseline/Screening Procedures (Day -30 to Day 0)

After signing the informed consent form, subjects will be assigned a screening number (see Section 5.2) and be evaluated for eligibility into the study.

The following procedures will be conducted during the baseline assessment:

- Collection of demographics, surgical risk factors, pre-operative diagnosis
- Vital signs (heart rate and blood pressure), height and weight
- Serum tests (sodium, bilirubin, creatinine and INR) for MELD Score
    - The following calculator from the OPTN website should be used to calculate the MELD Score:

      https://optn.transplant.hrsa.gov/resources/allocation-calculators/meld-calculator/

- Pregnancy test (urine test or institution standard of care) for subjects of childbearing potential on Day 0
- Hemoglobin measurement within 14 days prior to the date of surgery.

8.3 Day 0 Procedures

On the day of surgery, subjects will be randomized as described in Section 5.1. Subjects are considered enrolled upon randomization.

According to the randomization assignment, subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of IC2000. Subjects in the B-P arm will undergo LN mapping with Blue dye first followed by mapping with PINPOINT.

Subjects randomized into the P-B Arm will be administered IC2000 first followed by the administration of Blue dye and undergo LN mapping with PINPOINT first followed by mapping with Blue dye.

All subjects will undergo the appropriate surgical procedure (minimally invasive hysterectomy, trachelectomy or conization) according to the surgeon's standard practice. The surgical procedures include the following:

- Hysterectomy, Radical Hysterectomy
- Radical Trachelectomy
- Bilateral Salpingo-ophorectomy, Unilateral Salpingo-ophorectomy
- Ovarian Transposition
- Sentinel Lymph Node Mapping

- Pelvic Lymph Node Sampling
- Pelvic Lymph Node Dissection
- Para-aortic Lymph Node Sampling
- Para-aortic Lymph Node Dissection

All subjects will receive the hospital/institution and surgeon's standard pre-operative and post- operative care with the addition of any study specific requirements.

An intra-operative measurement of hemoglobin should be performed at the time of mapping if the subject experiences a hypotensive event requiring intraoperative management (e.g. use of vasopressors) or blood loss greater than 500 ml at the time of mapping.

Either during or immediately post-operatively, the details of the LN mapping procedure and surgical procedure will be documented. LN mapping data will be collected including the ability to identify LN, the ability to unilaterally and bilaterally map LN, and anatomical location and distribution of identified LN. Failed mapping is defined as no LN detected.

The following elements must be included and recorded in the case report form (CRF):

- Surgical time and estimated blood loss
- Dose of Blue dye administered
- Dose of IC2000 administered
- Details on injection technique
- Total number of LNs identified with PINPOINT
- Total number of LNs identified with Blue dye
- Total number of abnormal LNs identified (suspicious nodes)
- Total number of LNs excised
- Classification of LNs identified
- LNs anatomical location and distribution
- Pathological status of LNs excised
- Concomitant medications and procedures as described in Section 6.3
- Adverse events according to Section 9

8.3.1 Lymph Node Mapping Procedure

LN mapping for FIGO Clinical Stage I endometrial cancer will be performed according to the *NCCN Guidelines for Uterine Neoplasms, SLN Algorithm for Surgical Staging of Endometrial Cancer*[1].

LN mapping for FIGO Clinical Stage I cervical cancer will be performed according to the *NCCN Guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-Stage Cervical Cancer*[2].

In order to minimize the spillage of blue dye or IC2000 interfering with the mapping procedure when LNs are excised, mapping will be performed on one side of the pelvis followed by the other side of the pelvis, and LN mapping with both Blue dye and PINPOINT will be completed prior to the excision of any LNs.

According to the randomization assignment, subjects randomized into the B-P Arm will be administered Blue dye first followed by the administration of IC2000. Blue dye and IC2000 will be prepared in separate syringes and administered into the cervix at the three and nine o'clock position with a superficial and deep injection. Subjects in the B-P Arm, will undergo LN mapping with Blue dye first followed by mapping with PINPOINT. LN mapping with Blue dye will be performed until the investigator identifies all blue nodes or determines that blue nodes cannot be identified. Once complete, the Investigator will begin mapping with PINPOINT until all 'IC2000' nodes are identified or the investigator determines that 'IC2000' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.

Subjects randomized into the P-B Arm will be administered IC2000 first followed by the administration of Blue dye. Similar to the B-P Arm, IC2000 and Blue dye will be prepared in separate syringes and administered into the cervix at the three and nine o'clock position with a superficial and deep injection. Subjects in the P-B Arm, will undergo LN mapping with PINPOINT first followed by mapping with Blue dye. LN mapping will be performed until the investigator identifies all 'IC2000' nodes or determines that 'IC2000' nodes cannot be identified. Once complete, the Investigator will begin mapping with Blue dye until all 'blue' nodes are identified or the investigator determines that 'blue' nodes cannot be identified. Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised.
.

The Investigator will identify 'Blue' nodes and 'IC2000' nodes based on direct visualization with white light for Blue dye and PINPOINT for IC2000 according to Section 8.3.1.3 and 8.3.1.4 respectively. All nodes identified during the mapping procedure will be classified according to Section 8.3.1.5 and recorded in the operative CRFs.

8.3.1.1 Imaging Agent Dosing and Administration of Blue dye

The Blue dye solution will be prepared and administered by the appropriate qualified study and/or operating room personnel according to the method of Abu Rustum[4]. The cervix will be injected with Blue dye while the subject is under anesthesia in the operating room, approximately 15 minutes prior to planned mapping.

- One vial of Isosulfan Blue (1% solution) will be utilized A 22G spinal needle will be used to inject Blue dye at the 3 and 9 o'clock positions.
- At each position, a superficial (1-3 mm) and a deep (1-3 cm) injection of 1 mL of Blue dye will be made into the cervical submucosa and stroma. Blue dye should be injected at a rate of 5 to 10 seconds per injection.
- Total dose of Isosulfan blue administered will be 40 mg (10 mg/ml Isosulfan Blue, 4 x 1 ml injections/subject)

8.3.1.2 Imaging Agent Dosing and Administration of IC2000

Similar to administration of Blue dye solution, the IC2000 solution will be prepared and administered by the appropriate qualified study and/or operating room personnel according to the method of Abu Rustum[4]. The cervix will be injected with IC2000 while the subject is under anesthesia in the operating room, approximately15 minutes prior to the planned time of imaging.

- One 25 mg vial of IC2000 will be reconstituted with 20 ml sterile water for injection to yield a 1.25 mg/ml solution immediately prior to IC2000 administration.
- A 22G spinal needle will be used to inject IC2000 at the 3 and 9 o'clock positions.
- At each position, a superficial (1-3 mm) and a deep (1-3 cm) injection of 1 mL of IC2000 will be made into the cervical submucosa and stroma.
- IC2000 should be injected at a rate of 5 to 10 seconds per injection.

The total dose of IC2000 administered will be 5 mg (4 x 1 ml injections/subject).

8.3.1.3 Identification of Lymph Nodes with Blue Dye

Intraoperative identification of LNs with Blue dye will be based on the following criteria:

- Direct visual identification of a node stained with Blue dye.
- Visible or palpably abnormal LNs designated as palpable masses and excised regardless of visible blue dye.

Mapping will be considered complete when all nodes meeting any of the criteria above are identified, classified and documented and the surgeon has scanned the full 360-degree area within the abdominal cavity. Blue ducts should be followed in both directions in order to identify LNs to be excised.

8.3.1.4 Identification of Lymph Nodes with PINPOINT

Intraoperative identification of LNs with PINPOINT will be based on the following criteria:

- Direct visual identification of a node by NIR fluorescence with IC2000 using PINPOINT.
- Visibly or palpably abnormal LNs designated as palpable masses and excised regardless of visible fluorescence.

Mapping will be considered complete when all nodes meeting any of the criteria above are identified, classified and documented and the surgeon has scanned the full 360-degree area around the injection site. Fluorescent ducts should be followed in both directions in order to identify LNs to be excised. Once all 'IC2000' nodes have been identified

8.3.1.5 Classification of Lymph Nodes

All LNs identified will be classified as:

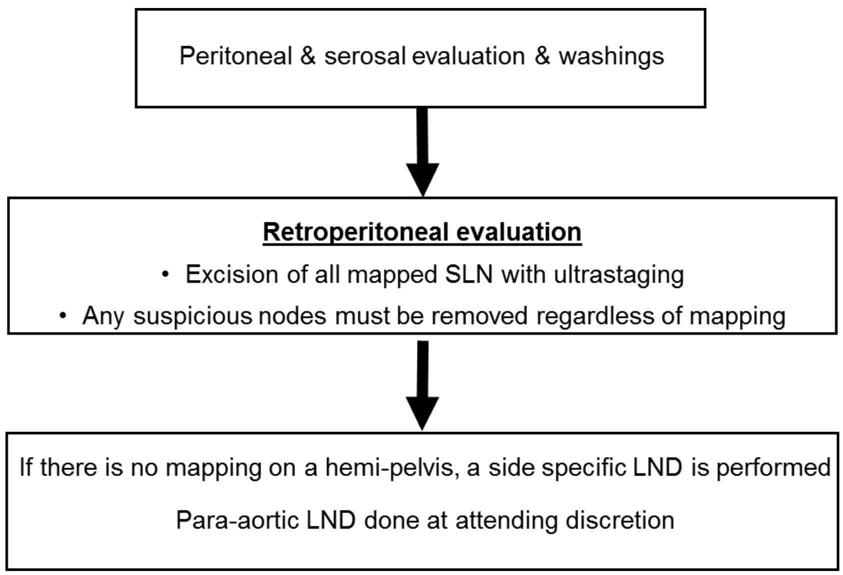
1. Fluorescent only
2. Blue only
3. Both (Fluorescent and blue)
4. Abnormal or palpably hard
5. Non-stained node found at termination of fluorescent duct
6. Non-stained node found at termination of blue duct
7. Non-stained node found at termination of fluorescent and blue duct

All LN identified and subsequently removed will be histologically examined to confirm the presence of lymphoid tissue according to section 8.5.

8.3.1.4.1 National Comprehensive Cancer Network Guidelines

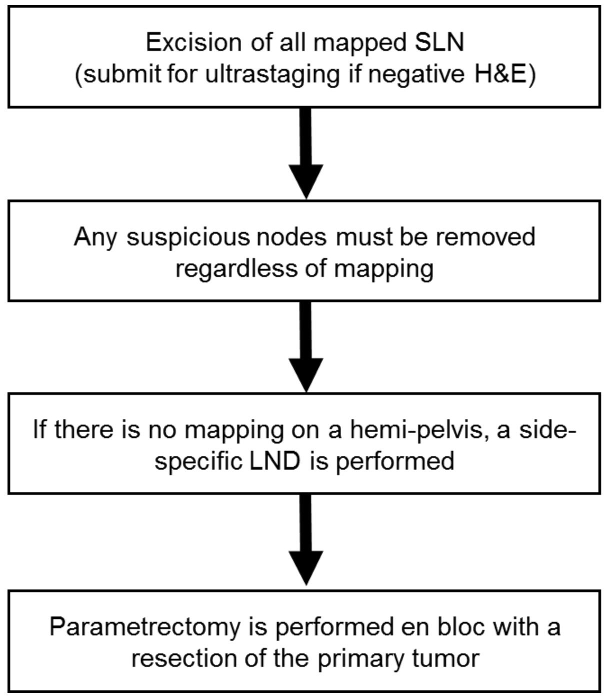
The LN mapping procedure for subjects with uterine neoplasms will be performed according to the guidelines from the *NCCN for Uterine Neoplasms, SLN Algorithm for Surgical staging of Endometrial Cancer*[1] (Figure 2) in addition to the removal of other LNs according to standard of care.

FIGURE 2. SLN Algorithm for Surgical Staging of Endometrial Cancer



The LN mapping procedure for subjects with cervical neoplasms will be performed according to the guidelines from the *NCCN for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-stage Cervical Cancer*[2] (Figure 3) in addition to the removal of other LNs according to standard of care.

FIGURE 3. SLN Algorithm for Surgical Staging of Cervical Cancer



8.4 Post-operative Follow-up Visits (Day of discharge to Day 30)

Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits on the date of discharge and Day 30 (±7 days). Day 30 visit will be a telephone call and subjects will be assessed for the following throughout the study:

- Concomitant medications and procedures as described in section 6.3
- Adverse events and adverse device effects according to section 9

Although the subject may be seen by various individuals post-surgery, the study-specific visits must be conducted by a surgeon who is part of the study and has signed the Signature and Delegation Log. Any adverse events/adverse device effects, complications and outcomes of the study that occur between visits will be documented at the next study required visit.

8.5 Histopathology of Excised LN

The presence of lymphoid tissue for each excised LN will be confirmed by tissue analysis. All LNs will be routinely sectioned and stained with hematoxylin and eosin (H&E). LN ultra-staging will be performed according to the method of Kim et al.[34]. Briefly, two adjacent 5-µm sections will be cut and sectioned at each of the two levels, 50-µm apart from each paraffin block. Sections at each level will be stained with H&E and with immunohistochemistry using anti-cytokeratin AE1:AE3. Image Acquisition and Transmission

Videos of the image sequences acquired using PINPOINT will be recorded to a medical grade video recorder. All required images will be provided to the Sponsor by the Investigative Center.

Image files will be identified using the subject number and initials only (no information identifying the subject shall be included in the files).

9 EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

All untoward medical occurrences either observed by the Investigator or one of his/her medical collaborators, or reported by the subject spontaneously, or in response to the direct question below, will be reported as follows:

- All events occurring before randomization should be recorded in the source documents and will be considered part of the subject's case history.
- All adverse events occurring during the study will be recorded as adverse events on the adverse event CRF.
- Events occurring as a result of the surgery will be reported as adverse events related to the surgical procedure in the adverse event CRF.
- Events related to PINPOINT will be recorded as adverse device effects in the CRF.
  - Events that are not related to PINPOINT shall be recorded as adverse events.
- Events related to PINPOINT that affect a user of the device (non-subject) are recorded as technical complaints.
- Events related to IC2000 and/or Blue dye will be recorded as adverse events related to IC2000 and/or blue dye in the adverse event CRF.

In an attempt to optimize consistency of AE reporting across centers, the subjects must be asked a standard question to elicit events. At each clinic or telephone evaluation of the subject, study personnel will ask the following questions: "Have you had any problems since your last visit?"

AEs reported on the CRF will include the date of onset, severity, relationship to PINPOINT, IC2000 and/or Blue dye, relationship to surgical procedure, date of resolution (or the fact that it is ongoing or has become chronic), action taken, and whether the AE is serious or not.

9.1 Definitions

9.1.1 Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory findings) in subjects whether or not related to the investigational medical device.

- Includes events related to the IC2000 or Blue dye.
- Includes events related to procedures involved (any procedure in the clinical investigation plan).
- Postoperative nausea or vomiting occurring during the first 24 to 48 hours, and post-operative pain related to surgical procedure is not considered an adverse event.

9.1.2 Adverse Device Effect (ADE)

Any adverse event related to the use of PINPOINT (includes all hardware components).

- Includes any event that is a result of a use error or intentional misuse.

ADEs that affect subjects will be recorded as ADEs in the AE CRF. ADEs that only affect a user (non-subject) are recorded in the technical complaint form only.

9.1.3 Serious Adverse Event

(SAE) Any adverse event that:

a. Led to a death.

b. Led to a serious deterioration in health that either:

    i. Resulted in a life-threatening illness or injury,

    ii. Resulted in a permanent impairment of a body structure or a body function,

    iii. Required in-patient hospitalization or prolongation of existing hospitalization,

    iv. Resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function.

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

This includes device deficiencies that might have led to a serious adverse event if suitable action had not been taken; intervention had not been made, or if circumstances had been less fortunate. These are handled as SAEs. A planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in heath, is not considered to be a serious adverse event.

9.1.4 Unanticipated Adverse Device Effect

(UADE) Any ADE that meets the following:

- By its nature, incidence, severity or outcome has not been identified in the current version of the PINPOINT risk analysis report.
- On health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.2 Adverse Event Descriptions

9.2.1 Intensity

The intensity of AEs, including ADEs will be characterized as mild, moderate, or severe, as follows:

Mild	Usually transient, requiring no special treatment, and does not interfere with the subject's daily activities.
Moderate	Introduces a low level of inconvenience or concern to the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
Severe	Significantly interferes with a subject's usual daily activities and requires systemic drug therapy or other treatment, if available.

9.2.2 Relationship

Suspected	There is a reasonable possibility that the AE is associated with use of the study device or treatment (IC2000 or Blue dye), such as temporal relationship of the event to use.
Not suspected	A relationship between the AE and the study device or treatment (IC2000 or Blue dye) can reasonably be ruled out based on lack of any temporal relationship of the event to use, or when the subject's underlying condition, medical history, or other therapy provide sufficient explanation for the observed event.

9.3 Reporting and Evaluation of Serious Adverse Events and Unanticipated Adverse Device Effects

Any SAE or UADE occurring in this study must be reported immediately (within 24 hours of discovery) by email to the Novadaq contact listed below:

Attention: Alicia Wilton

Mobile: 905-629-3822 x209

Email: Film@novadaq.com

SAEs and UADEs will be reported to the Institutional Review Board (IRB)/Ethics Committee (EC) according to the institution's policies, but within 10 days of occurrence.

The Sponsor will be responsible for reporting SAEs/UADEs to the FDA or Health Canada in accordance with federal regulatory requirements.

The Sponsor will provide documentation of reportable events to the Investigator, as specified in Section 13.1.4.

The Investigator will ensure that the subject receives appropriate medical treatment and that the subject is followed up until the SAE or UADE resolves or becomes chronic, as defined in Section 9.4.

9.4 Follow-up for Adverse Device Effects and Adverse Events

Throughout the study to the final study visit contact, ADEs will be followed until they resolve or become chronic. All AEs will be followed throughout the study until the Day 30 visit. All AEs related to the PINPOINT, IC2000, Blue dye or the mapping procedures, as determined by the Investigator, will be followed until resolution or deemed chronic

At the final study visit, new AEs, as well as follow-up information for continuing AEs, will be recorded in the CRF and source document. If an SAE or UADE is unresolved at the final study visit, it will be followed by the Investigator until it resolves or becomes chronic (as judged by the Investigator). Follow-up data for such SAEs will be recorded in the source document and reported to the safety contacts. Non-serious ongoing AEs will be followed beyond the final study visit if they are related to the study procedures at the discretion of the Investigator.

9.5 Reporting of Technical Complaints/Device Deficiencies

9.5.1 Definitions

Device Complaint: A quality complaint received in writing, electronically, or orally that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a device product. (In this definition, "effectiveness" refers to the actual function of the device, not to how the subject responds to the action of the device. Also in this definition, "device product" refers only to devices provided by the Sponsor for clinical studies and to investigational devices.)

In this definition, safety includes the safety of a subject, user, or other person associated with the use of a medical device.

Device Deficiency: Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling.

9.5.2 Reporting Procedures

Any technical complaint/device deficiency should be reported to the Sponsor. Technical Complaints occurring in this study must be reported immediately (within 24 hours) by fax or email to the appropriate Novadaq representative or to Novadaq Quality (quality@novadaq.com)

Any complaint about a device must be reported regardless of whether the defect or deficiency had any effect on a subject or on study personnel.

9.6 ADEs Technical Complaints/Device Deficiencies that are UADEs

Novadaq will evaluate all ADE reports and technical complaints/device deficiencies to determine if the report meets the definition of an unanticipated adverse device effect. If Novadaq determines that it does meet the definition, an investigation will be begun immediately. Novadaq will inform the Investigator of any additional reporting requirements beyond those stated in Sections 9.4 and 9.5 as applicable.

Novadaq will report the UADE and the results of any investigations to the FDA and Health Canada according to the applicable regulatory guidelines. Novadaq will also report to the Investigator(s), who will submit the required reports to their IRB/ECs within 10 working days after Novadaq first received notice of the effect.

10 STATISTICAL CONSIDERATIONS

10.1 Primary Objective

To assess the effectiveness of intraoperative PINPOINT in identification of LNs in subjects with uterine and cervical malignancies who are undergoing LN mapping.

10.2 Hypotheses

To assess the effectiveness of PINPOINT in the identification of LNs, a non-inferiority test will be performed using the per-protocol (PP) analysis population.

$$H_{01}: \quad p_t \leq p_c - 0.05$$
$$H_{11}: \quad p_t > p_c - 0.05$$

Here p_t and p_c represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively. We define the numerator for p_t as the number of nodes identified with PINPOINT (classifications 1, 3, 5, 7 as defined in section 8.3.1.5) and confirmed as lymphoid tissue, and we define the numerator for p_c as the number of nodes identified with Blue dye (classifications 2, 3, 6, 7 as defined in section 8.3.1.5) and confirmed as lymphoid tissue. We define the denominator for both p_t and p_c as the number of nodes identified by ANY method (classifications 1-7 as defined in section 8.3.1.5). The denominator will include excised nodes confirmed as lymphoid tissue.

A non-inferiority margin of 0.05 was determined to be clinically significant based on feedback from Investigators. Within their respective groups, p_t and p_c represent the proportion of LNs identified (and confirmed to be lymphoid tissue, See Section 8.5) with PINPOINT and Blue dye respectively divided by the total number of LNs identified and excised, across all subjects.

We will repeat the analysis described above using the as-treated (AT) analysis set as a supporting analysis of the primary outcome.

As a sensitivity analysis we will also perform the non-inferiority test using the mITT analysis set. We will also perform sensitivity analyses of the primary endpoint using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology to be lymphoid tissue for PINPOINT and non-lymphoid tissue for Blue dye. The worst case scenario will consider nodes with missing histology to be non-lymphoid tissue for PINPOINT and lymphoid tissue for Blue dye.

10.3 Statistical Analysis

We will use the Z_O statistic described by Nam and Kwon () in formulae (6) to derive the estimates of p_t, p_c, and the variance of the difference between these estimates to construct the 95% 2-sided confidence interval for $p_t - p_c$ as:

$$\left((\hat{p_t} - \hat{p_c}) - 1.96 \times \sqrt{\widehat{var}(\hat{p}_t - \hat{p_c})}\ ,\ (\hat{p_t} - \hat{p_c}) + 1.96 \times \sqrt{\widehat{var}(\hat{p}_t - \hat{p_c})} \right)$$

We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{01}) stated above, and if the lower bound of the interval is > -0.05 we will claim non-inferiority. If and only if we reject the null (H_{01}) hypothesis of inferiority and claim non-inferiority we will use the mITT analysis set to test the null (H_{02}) stated below, and if the lower bound of the interval is > 0 we will claim superiority.

$$H_{02}: \quad p_t = p_c$$
$$H_{12}: \quad p_t > p_c$$

We will repeat the analysis described above using the as-treated (AT) analysis set as a supporting analysis of the primary outcome.

As a sensitivity analysis we will also perform the non-inferiority test using the mITT analysis set. We will also perform sensitivity analyses of the primary endpoint using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology to be lymphoid tissue for PINPOINT and non-lymphoid tissue for Blue dye. The worst case scenario will consider nodes with missing histology to be non-lymphoid tissue for PINPOINT and lymphoid tissue for Blue dye.

10.4 Sample Size Considerations

The clinical literature provides an estimate of the expected effectiveness of LN mapping with ICG and Blue Dye[17–28,36]. 12 studies using ICG for the purposes of LN mapping have been published[17–28] (Table 1). Detection rates for LN mapping with ICG and Blue dye are reported to occur in a wide range (33-100% for ICG[17–28] and 40-80% for Blue dye[36]). A number of factors play a role in the identification of LN including tumor size, familiarity with LN mapping technique (learning curve) and obesity[36].

A weighted average of published LN detection rates for Blue dye and ICG (i.e., PINPOINT) in gynecological cancer suggests unilateral detection rates of 91% for ICG and 80% for Blue dye. However, we assumed LN detection rates in gynecological cancer[17–28] of 84% and 80% using ICG and Blue dye, respectively. We used a lower LN detection rate of 84% for ICG and a LN detection rate at the upper end of the range for Blue dye as a conservative approach to the sample size calculation. We also assumed a non-inferiority margin of 5%.

The power calculation was performed for a 2-level hierarchical model in which nodes are nested within subject. Power was calculated as a function of the effective sample size (ESS), which is a function of the total number of nodes excised, the number of nodes within subjects (clusters), and the intra-cluster correlation (ICC). Specifically, ESS = n / VIF, where n is the total number of nodes excised across all patients, and VIF is the variance inflation factor, calculated as VIF = 1 + (m - 1) × ICC, where m is the number of nodes per subject. Because we expect to excise 3 to 4 nodes per subject, with 150 subjects we expect to excise 3.5 × 150 = 525 nodes. With 3.5 nodes per subject on average and an ICC at most 0.125 we will have VIF at most 1 + (2 × 3.5 – 1) × 0.125 = 1.75 and an ESS at least 525 / 1.75 = 300 nodes. Note that here m is twice the number of expected nodes per subject, since nodes will be identified using 2 different methods within the same subject. We expect the ICC to be at most 0.125 based on our estimate of the ICC = 0.07 from Crane et al.[18].

With an ESS of at least 300 Ns we will have at least 82% power to reject H_{01} above and claim non-inferiority of PINPOINT with respect to Blue dye. This sample size calculation was performed using nQuery Advisor ® 7.0 (Copyright © 1995-2007, Statistical Solutions, Saugus, MA). We will test the hypothesis using a two-sided 95% confidence interval (i.e., two-sided significance level of 0.05). We will enroll subjects and identify Ns using both Blue dye and PINPOINT as described above in section 8.3.1.5 until we have at least 525 LNs identified, regardless of the method used to identify them. We expect that we will enroll approximately 150 subjects to obtain 525 nodes (based on clinical literature suggesting an average of 3-4 Ns excised per subject[17–28]).

10.5 Data Sets to be Analyzed

10.5.1 Per-protocol (PP)

The Per-Protocol (PP) analysis population includes all subjects that: [1] meet critical eligibility criteria, [2] have no significant protocol deviations; and [3] have evaluable assessment endpoints for the primary endpoint.

We believe that the PP population will be the population that will most likely demonstrate a difference between Blue dye and IC2000 with respect to their ability to identify lymph nodes, should a difference exist. Thus, since we are trying to show non-inferiority of IC2000 with respect to Blue dye, we believe that using the PP population for testing non-inferiority is the conservative approach

10.5.2 Modified Intent to treat (mITT)

The mITT analysis population includes all randomized subjects who received at least one injection of IC2000 or Blue dye. All subjects meeting this criterion are included in the mITT population regardless of whether or not they received the minimally invasive surgical intervention or lymphatic mapping. Subjects who have the mapping procedure aborted due to circumstances such as a higher stage cancer than initially expected will not be included in the mITT. Approximately 8% of subjects are expected to have the mapping procedure aborted.

We believe the mITT population will be the population that will most likely demonstrate no difference between the Blue dye and IC2000 with respect to their ability to identify lymph nodes, because the mITT population includes subjects who may not have received the full dose of dye, and it includes subjects who may have not received lymphatic mapping, but who may have had lymph nodes identified by gross inspection. Thus, we believe that using the mITT population for testing superiority will be the conservative approach.

10.5.3 As-Treated (AT)

The As-Treated (AT) analysis population includes all randomized subjects in whom the intended minimally invasive surgical procedure was performed and received at least one injection of IC2000 or Blue dye. Subjects in whom the mapping procedure with PINPOINT or Blue dye is not performed are excluded from the AT population. Subjects will be analyzed according to the LN mapping procedure performed. The AT population will be used for a secondary analysis of the primary endpoint.

10.5.4 Safety (S)

The safety analysis population includes all randomized subjects enrolled in the study who received at least one injection of IC2000 or Blue dye. Secondary safety endpoints, including the summary of adverse events or adverse device effects in the trial, will be analyzed using this analysis population.

10.6 Secondary Objectives

The planned secondary out comes are intended to support product labelling. We will use the step-down method described by Benjamini and Liu[37] to control the false discovery rate at 0.05 (2-sided) while testing our secondary objectives.

The first secondary outcome is the ability of PINPOINT and Blue dye to detect at least one lymph node in a subject.

Let q_t and q_c represent the proportion of subjects with a least one lymph node identified (according to section 8.3.1.5 and confirmed by histology) with PINPOINT and Blue dye respectively, divided by the total number of subjects where mapping was attempted. That is, the numerator for q_t is the number of subjects with at least 1 node identified with PINPOINT (classifications 1, 3, 5, 7) and confirmed as lymphoid tissue, and the numerator for q_c is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) and confirmed as lymphoid tissue. The denominator of both q_t and q_c is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H_{03}: q_t \le q_c - 0.05$$
$$H_{13}: q_t > q_c - 0.05$$

To test this hypothesis we will estimate the difference $q_t - q_c$ with a 95% two-sided confidence interval. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{03}) stated above, and if the lower bound of the interval is greater than -0.05 we will claim non-inferiority. If and only if we reject the inferiority hypothesis (H_{03}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority.

Another secondary outcome is the bilateral LN detection rate. Let b_t and b_c represent the proportion of subjects with at least one node identified on the right side and on the left side of the pelvis and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for b_t is the number of subjects with at least 1 node identified with PINPOINT () on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with PINPOINT on the left side of the pelvis and confirmed as lymphoid tissue. Similarly, the numerator for b_c is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with Blue dye on the left side of the pelvis and confirmed as lymphoid tissue. The denominator of both b_t and b_c is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H_{04}: b_t \le b_c - 0.05$$
$$H_{14}: b_t > b_c - 0.05$$

To test this hypothesis we will estimate the difference $b_t - b_c$ with a 95% two-sided confidence interval. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H04) stated above, and if the lower bound of the interval is greater than -0.05 we will claim non-inferiority. If and only if we reject the inferiority hypothesis (H_{04}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority.

A third secondary outcome is the proportion of LNs identified by following lymphatic channels (ducts). Let d_t and d_c represent the proportion of nodes identified by following a duct and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for d_t is the number of nodes identified with PINPOINT by following a duct (classifications 5, 7) and confirmed as lymphoid tissue. Similarly, the numerator for d_c is the number nodes identified with Blue dye by following a duct (classifications 6, 7) and confirmed as lymphoid tissue. We define the denominator for both d_t and d_c as the number of nodes identified by ANY method (classifications 1-7). The denominator will include excised nodes confirmed as lymphoid tissue.

The following hypotheses will be tested:

$$H_{05}: d_t \leq d_c - 0.05$$
$$H_{15}: d_t > d_c - 0.05$$

To test this hypothesis we will estimate the difference $d_t - d_c$ with a 95% two-sided confidence interval. We will estimate this confidence interval in a manner similar to that described for the primary outcome. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H05) stated above, and if the lower bound of the interval is greater than -0.05 we will claim non-inferiority. If and only if we reject the inferiority hypothesis (H_{05}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority.

We will also tabulate the anatomic distribution of LNs identified by each dye.

10.6.1 Demographic and Baseline Data

The demographic and baseline analysis will be done for the mITT and PP data set.

The following subject variables will be summarized: age, race, body mass index, histological type (squamous cell carcinoma, adenocarcinoma, endometrioid carcinoma etc.), previous neoadjuvant therapy, FIGO stage and type of surgical intervention. Continuous variables will be summarized as mean, standard deviation, median, minimum and maximum, and categorical variables will be summarized by counts and percentages.

10.6.2 Safety Variables and Analysis

Safety variables will be documented and summarized for all randomized subjects enrolled in the study who received at least one injection of IC2000 or Blue dye. Safety variables will include all adverse events (AEs) and adverse device effects (ADEs), concomitant medications, and vital signs. Safety analysis will be performed on all subjects who receive IC2000 regardless of whether LN mapping was initiated or successful.

Adverse events will be coded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE). Treatment-emergent AEs and ADEs will be summarized descriptively. The number and percentage of subjects experiencing AEs and ADEs and the total number of AEs and ADEs will be summarized by system organ class and preferred term.

Associated AEs that investigators suspect are related to study treatment will also be summarized. Summary of each type of event will be prepared by severity and for all severities combined.

10.7 Handling of Missing Data

Reasonable efforts will be made to obtain complete data for all subjects; however, missing observations will inevitably occur due to subjects lost to follow-up or noncompliance with required assessments. The reasons for missing data will be documented and evaluated (e.g. subject is deceased, lost to follow up, missed visit, etc.). In addition, the distribution of prognostic factors between subjects with data and those without data will be examined to evaluate any potential sources of bias. Any missing observations will be described in detail and evaluated for assessment of possible bias.

A sensitivity analyses of the primary and secondary endpoints will be conducted using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology as lymphoid tissue for PINPOINT and as non-lymphoid tissue for Blue dye. The worst-case scenario will consider nodes with missing histology as non-lymphoid tissue for PINPOINT and as lymphoid tissue for Blue dye.

10.8 Pooling of Site Data

The homogeneity of safety and effectiveness results across study sites will be examined and if no significant heterogeneity is found, the results will be pooled. The justification for pooling is that all study sites will follow one Protocol, use the same device system (PINPOINT) follow the same Instructions for Use and perform mapping in accordance with NCCN guidelines. Additionally, frequent contact and monitoring of the sites will be performed to ensure that all Study sites are evaluating participants and recording Study results in a reliable and reproducible manner. It is not anticipated that any individual Study site will dominate the Study results. Therefore, it is believed that these procedures will help to ensure that the data from these Study sites can be combined and analyzed as if generated at a single site.

11 ESTIMATED DURATION OF THE STUDY

The expected study duration is approximately 1 year. The study is expected to start in 2015 and take 18 months to complete enrollment.

12 STUDY ETHICAL CONSIDERATIONS

12.1 Ethical Conduct of the Study

The study will be conducted in accordance with US 21 CFR Parts 50, 54, 56, 312 and 812 as well as ICH E6: Good Clinical Practice: Consolidated Guideline. It will be constituted in keeping with the principles of ICH E8: General Considerations for Clinical Trials and Part C, Division 5 of the Canadian Food and Drug Regulations. Any additional requirements imposed by the local Institutional Review Board/Ethics Committee/Research Ethics Board or regulatory agency will be followed as necessary.

12.2 Informed Consent

The informed consent forms used for the study must comply with applicable laws and regulations. An Investigator must explain the medical aspects of the study, including the nature of the study and procedure, orally and in writing, in such a manner that the subject is aware of potential benefits and risks. Other elements of the informed consent process may be delegated by the

Investigator. Subjects must be informed about all aspects of the clinical study that are necessary to make the decision to participate in the clinical trial. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Documentation of the discussion and the date of informed consent must be recorded in the source documentation. Subjects must give informed consent in writing.

The informed consent process must be conducted, and the form must be signed, before the subject undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

12.3 Institutional Review Board, Ethics Committee, or Research Ethics Board (IRB)

The protocol, protocol amendments (as specified by the IRB), and the informed consent form for the proposed study, along with any other documents required by the center's IRB must be submitted by the Investigator to the center's duly constituted IRB for review and approval. The Investigator must also ensure that the IRB reviews the progress of the study on a regular basis and, if necessary, renews its approval of the study on an annual basis. A copy of each IRB approval letter must be forwarded to the Sponsor before the study is implemented. Documentation of subsequent reviews of the study must also be forwarded to the Sponsor.

12.4 Data and Safety Monitoring Board

The study will be reviewed annually by an independent Data and Safety Monitoring Board (DSMB). The independent statistician will prepare a report for the DSMB in advance of the scheduled review meeting using the report template provided by the DSMB. The independent statistician will also prepare a safety report for the study Principal Investigator (PI) in preparation of the DSMB meeting to review the study. A DSMB charter will outline specific safety and data monitoring procedures.

13 ADMINISTRATIVE PROCEDURES

13.1 Sponsor's Responsibilities

13.1.1 Public Disclosure of Clinical Trials

The Sponsor will submit information about this protocol to the appropriate web-based national clinical trial registry and results database in each applicable regulatory region where the study is conducted. This includes but is not limited to US National Institute of Health (www.clinicaltrials.gov).

13.1.2 Study Supplies

The Sponsor will provide the PINPOINT Endoscopic Fluorescence Imaging System along with sufficient quantities of PINPOINT LN Mapping Kits and sufficient quantities of Blue dye (Isosulfan blue).

13.1.3 Investigator Training

13.1.3.1 Study Initiation Visit

Study centers will have a study initiation meeting to ensure the research personnel understand the protocol, study requirements, and data capture processes. This training will take place prior to enrollment of the first subject at each study center.

13.1.3.2 PINPOINT System

Appropriate personnel at the study centers shall be required to participate in training on the procedural use of PINPOINT as it relates to the conduct of this study (refer to Section 6.2.3).

13.1.4 Ongoing Communication of Safety Information During the Study

The Sponsor will provide the Investigator with documentation of UADEs and reportable events/effects, from all study centers, reported to regulatory authorities during the conduct of the study. The Investigator must forward this documentation to the IRB, as described in Section 9.

The Sponsor will also notify the Investigator about any other safety findings that could affect the safety of subjects, affect the conduct of the study, or alter the IRB's opinion about continuation of the study.

13.1.5 Study Monitoring

The conduct of the study will be monitored by representatives of the Sponsor to ensure compliance with the protocol, GCP and applicable regulations. A separate study specific Monitoring Plan will outline the monitoring procedures to be followed, the required access to source data and the extent of source verification planned.

13.1.6 Records Retention

The Sponsor must retain all documentation pertaining to the study according to Novadaq standard operating procedures.

13.2 Investigator's Responsibilities

13.2.1 Reporting and Recording of Study Data

Data will be captured and compiled using procedures developed by the Sponsor or their representatives. All requested study data must be recorded clearly on the CRF and other study forms as required. An explanation should be provided for all missing data. Only individuals who are identified on the Study Signature and Delegation Log may enter or correct data in the CRF. Incomplete or inconsistent data on the CRFs will result in data queries that require resolution by the Investigator.

The protocol, informed consent form, protocol amendments, safety information, and other required documents must be submitted to the IRB in a timely manner, as described in Section 12.3.

13.2.2 Source Documentation

The Investigator must maintain adequate and accurate source documents upon which CRFs for each subject are based. They are to be separate and distinct from CRFs, except for cases in which the Sponsor has predetermined that direct data entry into specified pages of the subject's CRF is appropriate. These records should include detailed notes on:

- The oral and written communication with the subject regarding the study treatment (including the risks and benefits of the study). The date of informed consent must be recorded in the source documentation.
- The subject's basic identifying information, such as demographics, that links the subject's source documents with the CRFs.
- All relevant observations and data on the condition of the subject throughout the study.
- The subject's exposure to PINPOINT.
- All adverse events.

13.2.3 Study Devices and Imaging Agents

The Investigator is responsible for ensuring the PINPOINT system, including imaging agent, and Blue dye are controlled and are used or dispensed only to subjects enrolled in the study. Only Investigators identified on the Signature and Delegation Log may use PINPOINT for the purposes of this study.

The Investigator shall keep records documenting the receipt, use, return and disposal of the study device, drugs and components.

The Investigator will ensure that PINPOINT is returned and that any other study material will be returned to the Sponsor or disposed of according to the Sponsor’s instructions on completion of the study.

13.2.4 Records Retention

The Investigator must ensure that clinical study records are retained according to national regulations, as documented in the clinical trial agreement entered into with the Sponsor in connection with this study.

Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution, or private practice. The Investigator must inform the Sponsor immediately if any documents are to be destroyed, to be transferred to a different facility, or to be transferred to a different owner.

14 DATA MANAGEMENT

Study data will be collected using paper and/or or electronic case report forms (CRFs). Data will be entered into a study specific database in one of two ways or a combination of the following:

- Center research personnel will enter study data directly into an electronic case report form which functions as an electronic data capture screen for the study database.
- Center research personnel will enter study data onto paper CRFs, which will be submitted to the Sponsor or assigned designee.

The Sponsor or designee will follow standardized procedures for data review, database cleaning and issuing/resolving queries. Procedures for data verification, validation, security and data retention will be followed in order that the study data reported are complete, accurate and consistent with source data.

For the purpose of data analysis and presentation, the data of the original data set may be manipulated and additional variables calculated when necessary. Once the study data are completely entered, reviewed and checked, the study database will be locked and no further changes will be made.

The electronic CRFs will be created and managed using REDCap[33] (Research Electronic Data Capture) electronic data capture tools hosted at M.D. Anderson. REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (https://redcap.mdanderson.org) is hosted on a secure server by M.D. Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (May 2014) by M.D. Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335.

Those having access to the data include the study PI and research team personnel. Users are authenticated against M.D. Anderson's Active Directory system. External collaborators are given access to the database once approved by the PI, with their access expiring in 6 months but renewable in 6 months increments at the request of the PI. The application is accessed through Secure Socket Layer (SSL). All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given subject will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each subject will be shifted by a different randomly generated number.

Following publication study data will be archived in REDCap. Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely by the sponsor. Since REDCap is a secure electronic database with controlled access, and because subject identifiers may be needed to link study data to data from other sources under future IRB approved protocols, subject identifying information will be retained in the archived database.

15 POLICY FOR PUBLICATION AND PRESENTATION OF DATA

The results of the study will be published by the study group. In addition to the principal investigators, any additional authors listed separately on the manuscript will be selected based on scientific input on the design of the study, interpretation of results and on enrollment numbers. The final number of authors will depend on the journal's publication guidelines. All participating centers will be acknowledged in the main study manuscript.

The Sponsor also encourages the scientific publication of data from clinical research studies. However, Investigators may not present or publish partial or complete study results individually without participation of the study Principal Investigator as well as the Sponsor. The Principal Investigators and the Sponsor may propose appropriate scientific manuscripts or abstracts from the study data. All proposed publications must be reviewed and commented on by the Sponsor before submission for publication. The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for publication against the validated study database for accuracy. Names of all Investigators and Sponsor representatives responsible for designing the study and analyzing the results will be included in the publication(s).

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Protocol Revision History:

Version		
Date	Number	Summary of Revisions Made:
October 15, 2014		**Initial Version**
June 24, 2015	**1.0**	**Final Version**
January 18, 2016	**2.0**	**Amendment 1**

Summary of Protocol Changes

Protocol Number: Protocol PP LNM 01 (FILM study)

Protocol Title: A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

	Version Number	Version Date
Current Approved Protocol	1.0	June 24, 2015
Amended Protocol	2.0	January 18, 2016

A summary of changes made to Protocol PP LNM 01 (FILM protocol; FILM trial) from the version dated June 24, 2015 to the version dated January 18, 2016 is provided below. For ease of review, all revisions to the text are highlighted with ~~strikethrough~~ for deleted text and <u>underline</u> for new text.

<u>General Changes to the FILM Protocol (PP LNM 01)</u>

The following general changes have been made throughout the protocol:

1. For clarity, reference to investigational ICG was changed to IC2000 to reflect the company's code name for the investigational drug product.

2. The following sections have been updated to reflect the various changes throughout the protocol:

   a. Protocol Summary
   b. Table of Contents
   c. Protocol section numbers
   d. References

3. The following changes have been made to the protocol approval pages:

- The author of the protocol amendment, Vasanthi Govindaraju, MD, PhD Clinical Operations was added to the author of the original protocol, Yohan D'Souza, PhD Clinical Regulatory Scientist.
- One of the approvers of the protocol was changed:
  From: John Fengler, Vice President Technology, R&D
  To: Lori Swalm, Vice President, Marketing
- The following investigator was added to the list of Principal Investigators section:
  Fidel Valea, MD
  Study Principal Investigator
  Duke University Medical Center, Gynecology/Oncology

4. The secondary objective, intra-operative identification lymphatic channels and related references were removed from the protocol (see also Detailed Change #2 below).

5. Additional, minor editorial changes have been made for clarity and are not detailed below.

FILM

Detailed Summary of Changes to the FILM Protocol (PP LNM 01)

Section and page numbers referenced below correspond to the amended protocol PP LNM 01, V 2.0, dated January 18, 2016.

Revisions to the text:

1. **Section 1.3: Potential Risks and Benefits to Human Subjects (page 18)**

   PINPOINT is a commercially available product in the United States (US) and Canada. PINPOINT has a 510(k) clearance from the FDA and is licensed in Canada with the following indication:

   "The PINPOINT system is intended to provide real-time endoscopic visible and ~~endoscopic NIR~~ near infrared fluorescence imaging. PINPOINT enables surgeons to perform routine visible light endoscopic procedures as well as further visually assess vessels, blood flow and related tissue perfusion with near infrared imaging during minimally invasive surgery".

   Rationale for Change: This section has been revised to include reference to approval of PINPOINT in Canada. Also, the indication for use has been revised to be identical to the approved wording in the US and Canada.

2. **Section 2.1.2 Secondary Objectives (page 19)**

   ~~To evaluate the effectiveness of PINPOINT and Blue dye in the intraoperative identification of lymphatic channels in uterine and cervical malignancies~~.

   Rationale for Change: This section has been revised to remove the secondary end point of intra-operative identification of lymphatic channels. The method for identification of lymphatic channels has not been shown to be reproducible and quantifying the number of lymphatic channels is not clinically relevant for this protocol.

3. **Section 3.1: Study Design Overview (page 19)**

   Day 0:

   ~~During anesthesia~~, On the day of surgery, subjects will be randomized (1:1) to either the BLUE-PINPOINT (B-P) Arm or the PINPOINT-BLUE (P-B) Arm.

   Rationale for Change: For logistical reasons the timing of randomization has been revised to allow for randomization on the day of surgery instead of after anesthesia. This change still allows patients to receive both dyes without compromising the order of administration of the dyes.

   Section 3.1: Study Design Overview (page 20)

Group A ~~PINPOINT~~ BLUE DYE	GROUP B ~~BLUE DYE~~ to PINPOINT

Rationale for Change: Changes to Figure 1 has been made to correct the groupings based on the flow of the diagram.

4. Section 3.1: Study Design Overview (page 21)

The cervix will be injected four (4) times with a 1 ml solution of Blue Dye (1% Isosulfan blue; 10 mg/ml) for a total dose of 40 mg Blue Dye) and four (4) times with 1 ml of a 1.25 mg/ml solution of IC2000 for a total dose of 5 mg IC2000 .

Rationale for Change: The concentration (10 mg/ml) and total dose (40 mg) of Isosulfan Blue and the total dose of IC2000 (5 mg; 1.25 mg/ml x 4 ml) have been added to this section for clarity.

5. Section 3.1: Study Design Overview (page 21)

Once mapping with both Blue dye and PINPOINT have been completed and documented, ~~all~~ LNs identified with Blue dye or PINPOINT will be excised according to surgeon's clinical judgement.

Rationale for Change: This section has been revised to remove the word 'all' as only lymph nodes identified with Blue dye and/or IC2000 will be excised and to allow for the surgeon to excise the lymph nodes according to their clinical judgement.

6. Section 3.1: Study Design Overview (page 22)

Mapping will be considered complete when all nodes and channels meeting any of the criteria above are identified and documented and the surgeon has scanned the full 360-degree area ~~around the injection site~~ within the abdominal cavity.

Rationale for Change: This section has been revised to clarify that the area scanned after identification of lymph nodes is within the abdominal cavity, not just the area surrounding the injection site.

7. Section 3.1: Study Design Overview (page 23)

Study specific visits to monitor occurrence of any adverse events/adverse device effects on ~~postoperative Day 1~~, the date of discharge and Day 30 (±7 days).

Rationale for Change: As per standard hospital practice at the clinical study sites, patients are discharged on the day of surgery. To reflect this process, the postoperative day 1 visit was removed.

8. Section 4.3: Exclusion Criteria (page 24)

1. Locally advanced ~~or inflammatory~~ cervical or uterine cancer

Rationale for Change: This section has been revised to remove the word inflammatory as it pertains to breast cancer not cervical or endometrial cancer.

9. Section 4.2: Exclusion Criteria (page 24)

8. Hepatic dysfunction defined as MELD Score > ~~10~~ to 12

Rationale for Change: The value for the MELD score used as exclusion criteria in the study has been increased from greater than 10 to greater than 12 to allow for additional patient enrolment.

Since one of the approved indications for commercially available ICG is in determining hepatic function, the inclusion of patients with MELD scores up to 12 does not adversely affect patient safety.

10. Section 5.1.1 : Randomization Procedure (page 25)

If any deviations occur (errors such as ~~misplacing envelopes), or if the~~ assigning incorrect randomization ~~assignment is made incorrectly~~), the clinical site will be required to contact the Sponsor and await guidance on how to proceed.

Rationale for Change: This section has been revised to correct the randomization procedure for the trial as it will occur using the REDCap electronic system.

11. Section 6.2.1.5 Packaging and Distribution of IC2000 (Page 29)

Each PINPOINT LN Mapping kit contains:

- One single use 25 mg vial of sterile IC2000, USP
- Two single use 10 ml ampules of sterile Aqueous solvent
- ~~Two~~ Four 3 ml syringes, sterile
- Two 10 ml syringes, sterile
- Two Lure-lock 10 ml syringes with controlled handle
- ~~Two~~ Four Spinal needles, 22G, 3.5 inch, sterile
- Labels for syringes

Rationale for Change: This section was revised to clearly identify the components of the PINPOINT LN Mapping kit and include the addition of two Lure-lock 10 ml syringes with controlled handles to be used for the mapping procedure and a change in the size of 22G spinal needle from 1 inch to 3.5 inch to facilitate the deep injections.

12. Section 8.1 Schedule of Events (Page 33)

Table 3 ~~presents the schedule of events for this study.~~ Schedule of Events

Procedure	Baseline (Day -30 to Day 0)	Day 0 (Operative Phase)			Date of Discharge[a]	Day 30+7 d Phone Call[a]
		Pre-OP	Intra-Op	Post-Op		
Informed consent	X					
Demography, Surgical risk factors	X					
Vital signs, blood pressure, heart rate, height, weight	X					
Pre-operative diagnosis	X					
Pregnancy test	X					
Inclusion/exclusion criteria	X					
Serum sodium, bilirubin, creatinine and INR for MELD Score	X					
Hemoglobin	X	~~X~~	X[b]			
Randomization ~~(in OR during anesthesia)~~		X	~~X~~			
LN mapping with Blue dye			X			
LN mapping with PINPOINT			X			
Surgical Procedure			X			
Documentation of LN mapping and surgical procedure			X	X		
Concomitant medications and procedures	X	X	X	X	X	X
Adverse events/adverse device effects		X	X	X	X	X[c]
Histopathological evaluation of Lymph Nodes			X	X		

a Subjects with a discharge date later than Day 30, will have the last study visit on Day 30.
b Hemoglobin measurement at the time of mapping if the subject had any hypotensive events or blood loss over 500 ml at the time of mapping.
c All subjects with adverse events thought to be related to the LN mapping procedure or the PINPOINT system will be followed until resolution or deemed chronic
d Hemoglobin measurement must occur within 14 days prior to the day of surgery (Day -14 to day 0)

Rationale for Change: The Scheduled of Events table was revised to include the procedures used to determine the MELD score (blood pressure, heart rate, serum bilirubin, creatinine, INR and sodium) which were originally not included in the table.

Also the Post-Op Day 1 visit has been removed from the table (refer to Detailed Change # 7 above) and the Day 30 visit has been changed to phone call (refer to Detailed Change # 18 below).

13. Section 8.2 Baseline/Screening Procedures (Day -30 to Day 0) (Page 34)

Serum tests (sodium, bilirubin, creatinine and INR) for MELD Score

The following calculator from the OPTN website should be used to calculate the MELD Score:

~~http://optn.transplant.hrsa.gov/resources/MeldPeldCalculator.asp?index=98~~

https://optn.transplant.hrsa.gov/resources/allocation-calculators/meld-calculator/

Rationale for Change: This section was changed to include serum sodium in the MELD score calculations. Recently the US department of Health and Home services changed the formula for calculating the MELD score to include serum sodium in addition to serum bilirubin, creatinine and INR. The new link to the MELD score calculation replaces the old link.

14. Section 8.2 Baseline/Screening Procedures (Day -30 to Day 0) (Page 34)

- Hemoglobin measurement within 14 days prior to the date of surgery.

Rationale for Change: This section was revised to reflect hospital standard of care which is to measure hemoglobin during the pre-operative procedure not on the day of surgery.

15. Section 8.3 Day 0 Procedures (Page 34)

All subjects will undergo the appropriate surgical procedure ~~(minimally invasive hysterectomy, trachelectomy or conization)~~ according to the surgeon's standard practice. The surgical procedures include the following:

- Hysterectomy, Radical Hysterectomy
- Radical Trachelectomy
- Bilateral Salpingo-ophorectomy, Unilateral Salpingo-ophorectomy
- Ovarian Transposition
- Sentinel Lymph Node Mapping
- Pelvic Lymph Node Sampling-
- Pelvic Lymph Node Dissection
- Para-aortic Lymph Node Sampling
- Para-aortic Lymph Node Dissection

Rationale for Change: All procedures that could be performed during the study, including the level of nodal dissection that would be performed for each procedure, have been listed in the protocol in order to clearly describe the standard of care provided to the patients.

16. Section 8.4 Post-operative Follow-up Visits (Day of discharge ~~1~~ to Day 30) (Page 40)

Subjects will have standard of care assessments throughout the study according to the hospital/institution's standard procedures as well as study specific visits on ~~postoperative day 1~~, the date of discharge and Day 30 (±7 days). Day 30 visit will be a telephone call and subjects will be assessed for the following throughout the study:

Rationale for Change: This section was changed to remove reference to the post-operative day 1 visit as it will no longer be performed (refer to Detailed Change #7). According to the participating clinical study sites' standard of the care, patients will be discharged on the same day of surgery. Also, for logistical reasons, the day 30 visit was changed to phone call instead of patient visit to the hospital. The site will call patients to collect adverse events/adverse device effects and concomitant medications and procedures.

17. Section 9.1.1 Adverse Event (AE) (Page 42)

Postoperative nausea or vomiting occurring during the first 24 to 48 hours, and post-operative pain related to surgical procedure is not considered an adverse event.

Rationale for Change: This sentence was included to provide clarity in defining the adverse events following surgery.

18. Section 10.3 Statistical Analysis (Page 45)

To test these hypotheses we will estimate the difference pt – pc with a 95% ~~one~~two-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority, and if the lower bound of the interval is greater than 0 we will claim superiority.

As a secondary analysis we will model the logit of the probability of identifying a lymph node as a function of treatment (Blue dye, ICG), order of randomization, and patient, with patient as a random effect, using a generalized linear mixed model. We may also consider other potential prognostic factors in the model, such as BMI and study center.

Section 10.4 Sample Size Considerations (Page 46)

A weighted average of published LN detection rates for Blue dye and ICG (i.e., PINPOINT) in gynecological cancer suggests unilateral detection rates of 91% for ICG and 80% for Blue dye. However, we assumed LN detection rates in gynecological cancer17–28 of 8~~3~~4% and 80% using ICG and Blue dye, respectively. We also assumed a non-inferiority margin of 5%.

With 300 LNs ~~in each arm~~ we will have 82~~1~~% power to reject H01 above and claim non-inferiority of PINPOINT with respect to Blue dye. This sample size calculation was performed using nQuery Advisor ® 7.0 (Copyright © 1995-2007, Statistical Solutions, Saugus, MA). We will test the hypothesis using a two-sided 95% confidence interval (i.e., two-sided significance level of 0.05). We will enroll patients and identify LNs using both Blue dye and PINPOINT as described above until we have at least 300 LNs identified, regardless of the method used to identify them ~~LNs in each arm~~. We expect that we will enroll approximately 150 patients (based on clinical literature suggesting an average of 1.8-2.2 LNs excised per subject17–28).

Rationale for Change: This statistical analysis section was revised to clarify terms and values.

19. Section 10.6 Secondary Objectives (Page 47)

~~The second secondary outcome is the ability of PINPOINT and Blue dye to detect LCs intraoperatively.~~

~~The following hypotheses will be tested:~~

~~H_{04}: $r_t \leq r_c - 0.05$~~
~~H_{14}: $r_t > r_c - 0.05$~~

~~Where r_t and r_c represent the proportion of LCs identified (according to Section 3.1) with PINPOINT and Blue dye divided by the total number of LCs identified. A non-inferiority margin of 0.05 was determined to be clinically significant based on feedback from Investigators.~~

~~To test these hypotheses we will estimate the difference $r_t - r_c$ with a 95% one-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).~~

Rationale for Change: This section was removed as the secondary objective of intra-operative identification of lymphatic channels has been deleted from the protocol.

20. Section 10.6.2 Safety Variables and Analysis (Page 48)

Safety variables will be documented and summarized for all randomized subjects enrolled in the study who received at least one injection of IC2000 or Blue dye ~~the AT population~~.

Rationale for Change: This section was revised to clarify the safety population that will be used for analyzing safety variables.

21. Section 12.4 Data and Safety Monitoring Board (Page 49)

The study will be reviewed annually by ~~the University of Texas MD Anderson~~ an independent Data and Safety Monitoring Board (DSMB). The ~~study~~ independent statistician will prepare a report for the DSMB in advance of the scheduled review meeting using the report template provided by the DSMB. The ~~study~~ independent statistician will also prepare a safety report for the study Principal Investigator (PI) in preparation of the DSMB meeting to review the study. A DSMB charter will outline specific safety and data monitoring procedures.

Rationale for Change: This section was revised to clearly identify individuals participating in the DSMB for this trial.

Protocol Revision History:

Version		
Date	Number	Summary of Revisions Made:
October 15, 2014		**Initial Version**
June 24, 2015	**1.0**	**Final Version**
January 18, 2016	**2.0**	**Amendment 1**
March 1st, 2016	**3.0**	**Amendment 2**

Summary of Protocol Changes

Protocol Number: Protocol PP LNM 01 (FILM study)

Protocol Title: A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

	Version Number	Version Date
Current Approved Protocol	1.0	June 24, 2015
Amended Protocol	2.0	January 18, 2016
Amended Protocol	3.0	March 1st, 2016

A summary of changes made to Protocol PP LNM 01 (FILM protocol; FILM trial) from version 2.0 dated January 18, 2016 to version 3.0 dated March 1st, 2016 is provided below. For ease of review, the revisions to the text are highlighted with underline for new text.

Detailed Summary of Changes to the FILM Protocol (PP LNM 01)

Section and page numbers referenced below correspond to the amended protocol PP LNM 01, V3.0, dated March 1st, 2016.

Revisions to the text:

1. Protocol Summary: Study Population (page 7)

- Subjects with FIGO Clinical Stage IA cervical cancer < 2 cm in size undergoing minimally invasive hysterectomy, trachelectomy, or conization with lymph node mapping. Subjects with clinical Stage IA1 cervical cancer without lympho vascular space involvement (LVSI) and negative margins on cone biopsy are not to be included.

Rationale for Change: This section has been revised to be consistent with the recommendations outlined in the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for Cervical Cancer. According to the NCCN Guidelines, lymph node dissection is not indicated in patients with stage IA1 cervical cancer without LVSI and with negative margins on cone biopsy. Also, revision of this section provides clarification of a subset of the study population, subjects with cervical cancer stage IA, as requested by FDA.

2. Section 4.2 Inclusion Criteria (page 23)

b) FIGO Clinical stage IA cervical cancer < 2 cm undergoing minimally invasive hysterectomy, trachelectomy or conization with lymphatic mapping. Note: Subjects with clinical Stage IA1 cervical cancer without lympho vascular space involvement (LVSI) and negative margins on cone biopsy are not to be included.

Rationale for Change: Same as the above.

Protocol Revision History:

Version		
Date	Number	Summary of Revisions Made:
October 15, 2014		**Initial Version**
June 24, 2015	**1.0**	**Final Version**
January 18, 2016	**2.0**	**Amendment 1**
March 1st, 2016	**3.0**	**Amendment 2**
April 15th, 2016	**4.0**	**Amendment 3**

Summary of Protocol Changes

Protocol Number: Protocol PP LNM 01 (FILM study)

Protocol Title: A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

	Version Number	Version Date
Current Approved Protocol	1.0	June 24, 2015
Amended Protocol	2.0	January 18, 2016
Amended Protocol	3.0	March 1st, 2016
Amended Protocol	4.0	April 15th, 2016

A summary of changes made to Protocol PP LNM 01 (FILM protocol; FILM trial) from version 3.0 dated March 1st to the version dated April 16th 2016 is provided below. For ease of review, all revisions to the text are highlighted with ~~strikethrough~~ for deleted text and <u>underline</u> for new text.

<u>General Changes to the FILM Protocol (PP LNM 01)</u>

The following general changes have been made in the protocol:

1. A statement of confidentiality has been included on page 1 as per internal requirements.
2. The following change has been made to the protocol approval page (page 4):
   - The following investigator name was removed from the list of Principal Investigators section:
     James Orr, MD
     Study Principal Investigator
     Florida Gynecology Oncology
3. The word ‘patient’ has been replaced with ‘subject’ throughout the protocol.
4. Additional, minor editorial changes have been made for clarity and are not detailed below.

Detailed Summary of Changes to the FILM Protocol (PP LNM 01)

Section and page numbers referenced below correspond to the amended protocol PP LNM 01, V4.0, dated April 15th, 2016.

Revisions to the text:

1. **Protocol Summary: Day 0 (page 6)**

In order to minimize the spillage of Blue dye or IC2000 interfering with the mapping procedure when LNs are excised, mapping will be performed on ~~a hemi~~ one side of the pelvis followed by other side and mapping with both Blue dye and PINPOINT will be performed prior to the excision of any LNs.

Rationale for Change: This section has been revised to clarify that lymph node mapping will be performed on both sides of the pelvis. The surgeons may perform lymphatic mapping on either the right or the left side first followed by other side. The same revision has been made to sections 3.1 Study Design Overview Day 0: and 8.3.1 Day 0 Procedures, Lymph Node Mapping Procedure.

2. **Protocol Summary: Day 0 (page 6)**

Once mapping with both Blue dye and PINPOINT have been completed and documented, all LNs identified with Blue dye or PINPOINT will be excised ~~according to the surgeon's clinical judgement.~~

Rationale for Change: This section has been revised to capture that all lymph nodes identified with blue, green or both dyes as well as the nodes identified from following channels will be removed. This revision ensures the lymph node mapping will be performed according to the standard of care (NCCN guidelines). The same revision has been made to the Study Procedures and Assessments section of the protocol summary as well as sections 3.1 Study Design Overview Day 0: and 8.3.1 Day 0 Procedures, Lymph Node Mapping Procedure.

3. **Protocol Summary: Study Population (page 7)**

Subjects with FIGO Clinical Stage IA cervical cancer ~~<~~≤ 2 cm in size undergoing minimally invasive hysterectomy, trachelectomy, or conization with lymph node mapping.

Rationale for Change: This section has been revised to clarify that subjects with clinical stage 1A cervical cancer and size is equal or less than 2 cm can be included in the study. This revision ensures consistency with exclusion criteria # 3 (diagnosis of cervical cancer with a tumor size greater than 2 cm).

4. **Protocol Summary: Study Variables-Other Variables (page 8)**

- Bilateral LN detection rate defined as the proportion of cases in which at least one LN is identified on right and left side of the pelvis.
- ~~Number~~ Proportion of LNs identified from following LCs.
- Anatomic distribution of LNs.
- ~~Rate of positive LN detection defined as the proportion of cases with at least one LN diagnosed as pathologically positive for lymphoid tissue when at least one LN was identified during surgery.~~
- ~~Rate of negative LN defined as the proportion of cases with no positive LN when at least one LN was identified during surgery~~

Rationale for Change: The secondary variable, the number of the LNs identified, was changed to the proportion LNs identified. Also, since the purpose of the protocol is not to calculate the proportion of LNs positive or negative for lymphoid tissue, these variables were removed from the protocol.

5. Protocol Summary: Sample Size and Statistical Analysis (page 9)

A sample size of approximately 150 evaluable subjects (to identify ~~300~~525 LNs) is required to show the LN detection rate for PINPOINT is non-inferior to that with Blue dye with 8~~2~~0% power and a 5% 2-sided significance level with a 5% non-inferiority margin .

Rationale for Change: Originally, the sample size was 150 subjects to identify 300 nodes based on the average of 2 nodes per subject. The protocol has been clarified to state that all the LNs identified with PINPOINT/IC2000 and Blue dye will be removed. In this case, based on the available literature, the average number of LNs removed will be 3 to 4, resulting in a revised sample size of 150 patients to identify 525 nodes.

6. Section 1.1.1: Sentinel Lymph Node Identification and Mapping (page 14, page 15 and page 18)

Identification of the ~~first~~ tumor draining lymph nodes (LN), ~~defined as the sentinel lymph node (SLN),~~ has become an important step for staging cancers that spread through the lymphatic system. SLN mapping involves the use of dyes and/or radiotracers to identify the sentinel nodes LNs either for biopsy or resection and subsequent pathological assessment for metastasis. The goal of lymphadenectomy at the time of surgical staging is to identify and remove the LNs that are at high risk for local spread of the cancer

~~The procedure is based on the concept that lymph drains in an orderly pattern away from the tumor, and therefore if the SLN is negative for metastasis, the nodes farther down the system should be~~

~~negative as well. As a result, the tumor bearing nodes are accurately identified and only these are removed, avoiding the morbidity associated with a full lymphadenectomy. Over the past 20 years, SLN mapping has become an established procedure in the staging of breast and melanoma cancers.~~

Identification of ~~S~~LNs and LN mapping for endometrial cancer is being evaluated as a method for surgical staging to be used in subjects with uterine confined tumors when there is no metastasis demonstrated by imaging studies and no obvious extrauterine disease[13]. The current standard of care is to perform a complete or selective para-aortic lymphadenectomy for staging (FIGO) which can lead to morbidities such as lower extremity lymphedema and lymphocyst formation[4]. Although the inclusion of pelvic and para-aortic lymphadenectomy in the surgical management is part of the FIGO staging, it remains controversial. The decision about the extent of lymphadenectomy (e.g. pelvic nodes only or both pelvic and para-aortic nodes) done by the surgeon can still be based on the preoperative and intraoperative findings.

~~As most subjects present with early-stage disease and the rate of metastasis is low, the ability to utilize SLN identification and mapping as an accurate method for staging and thus avoidance of a complete or selective para-aortic lymphadenectomy may be an acceptable approach.~~

Based on the data available from clinical studies, this Phase III study has been designed to demonstrate the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging in lymphatic mapping and to assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

Rationale for Change: The purpose of this protocol is to identify and remove all the LNs identified by PINPOINT/IC2000 and Blue dye. In order to reflect this appropriately, the background section on ‘Lymph Node Identification and Mapping’ has been modified.

7. Section 2.1.2: Secondary Objectives (page 19)

To evaluate the effectiveness of PINPOINT and Blue dye in the identification of at least one LN (confirmed to be lymphoid tissue) per subject.

To evaluate the effectiveness of PINPOINT and Blue dye in the identification of bilateral LNs (confirmed to be lymphoid tissue).

To assess the safety of interstitial injection of ICG for intraoperative lymphatic mapping.

Proportion of LNs identified from following LCs.

Anatomic distribution of LNs.

Rationale for Change: This section was revised to address the following recommendation from the Food and Drug Administration (FDA):

- The protocol synopsis differs significantly from the body of the protocol. We note that many of the secondary endpoints listed in the synopsis would provide important information for the public health as well as supportive evidence for the utility of your product.

8. Section 8.3.1.4.1 National Comprehensive Cancer Network Guidelines (page 37)

The LN mapping procedure for subjects with uterine neoplasms will be performed according to the guidelines from the NCCN for Uterine Neoplasms, SLN Algorithm for Surgical staging of Endometrial Cancer[1] (Figure 2) in addition to the removal of other LNs according to standard of care.

…..

The LN mapping procedure for subjects with cervical neoplasms will be performed according to the guidelines from the NCCN for Cervical Neoplasms, Surgical/SLN Algorithm for Early-stage Cervical Cancer[2] (Figure 3) in addition to the removal of other LNs according to standard of care.

Rationale for Change: Reference for LN mapping from the NCCN guidelines addresses SLN mapping. Therefore, a sentence for LN mapping to meet the requirements according to standard of care has been included.

9. Section 10 .2: Hypotheses (page 44)

To assess the effectiveness of PINPOINT in the identification of LNs, a non-inferiority test will be performed using the per-protocol (PP) analysis population according to the method of Moyé et al[3].

$$H_{01}: \quad p_t \le p_c - 0.05$$
$$H_{11}: \quad p_t > p_c - 0.05$$

Here p_t and p_c represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively. We define the numerator for *pt* as the number of nodes identified with PINPOINT (classifications 1 and 3 as defined in section 8.3.1.5) and confirmed as lymphoid tissue, and we define the numerator for pc as the number of nodes identified with Blue dye (classifications 2 and 3 as defined in section 8.3.1.5) and confirmed as lymphoid tissue. We define the denominator for both *pt* and *pc* as the number of nodes identified by ANY method (classifications 1-7 as defined in section 8.3.1.5). The denominator will include excised nodes confirmed as lymphoid tissue and excised nodes not confirmed as lymphoid tissue.

Rationale for Change: This section was revised to address the following recommendation from the FDA:

- For the primary objective of LNs identification, you use *pt* and pc to represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively, which are based on the proportion of LNs identified by the particular method and confirmed as lymphoid tissue by histology divided by the total number of LNs identified and excised. Please provide fraction expressions for the proportions using your classification categories. For example, *pt* could equal = (number of class 1 confirmed as lymphoid tissue + number of class 3 confirmed as lymphoid tissue) / (total number of class1 + class 2 + class 3 + class 4), please give your expression for *pt* and *pc* in details.

10. Section 10.3: Statistical Analysis and 10.4. Sample Size Considerations (page 44 and 45)

As a secondary analysis to support our primary objective and account for the fact that ~~patients~~ subjects will have multiple nodes and each node will have the opportunity to be identified by each of 2 dyes, we will model the logit of the probability of identifying a ~~lymph~~ node as a function of ~~treatment~~ dye (Blue dye, IC~~G~~2000), order of randomization, and subject, with subject as a random effect, using a generalized linear mixed model. We may also consider other potential prognostic factors in the model, such as BMI and study center. Formally, our model will be defined by

$$logit_{ijk} = \alpha_{ij} + s_{i(k(l))} + \varepsilon_{i(k(l))j}$$

where $\alpha_{ij} = \mu + \tau_{d(i,j)} + \pi_j + \lambda_{d(i,j-1)}$

$s_{i(k(l))}$ = effect due to Node l in Subject k in Sequence i

α_{ij} = effect due to Period j in Sequence i

$\varepsilon_{i(k(l))j}$ = random error $\sim N(0, \sigma^2)$

$\tau_{d(i,j)}$ = effect due to Dye d in Sequence i and Period j

π_j = effect due to Period j

and

$\lambda_{d(i,j-1)}$ = carryover effect of Dye d in Sequence i from Period $j-1$.

10.4. Sample Size Considerations

The clinical literature provides an estimate of the expected effectiveness of LN mapping with ICG and Blue Dye17–[28-36]. 12 studies using ICG for the purposes of LN mapping have been published17–28 (Table 1). Detection rates for LN mapping with ICG and Blue dye are reported to occur in a wide range (33-100% for ICG17–28 and 40-80% for Blue dye36). A number of factors play a role in the identification of LN including tumor size, familiarity with LN mapping technique (learning curve) and obesity36.

A weighted average of published LN detection rates for Blue dye and ICG (i.e., PINPOINT) in gynecological cancer suggests unilateral detection rates of 91% for ICG and 80% for Blue dye. However, we assumed LN detection rates in gynecological cancer17–28 of 84% and 80% using ICG and Blue dye, respectively. We used a lower LN detection rate of 84% for ICG and a LN detection rate at the upper end of the range for Blue dye as a conservative approach to the sample size calculation. We also assumed a non-inferiority margin of 5%.

The power calculation was performed for a 2-level hierarchical model in which nodes are nested within subject. Power was calculated as a function of the effective sample size (ESS), which is a function of the total number of nodes excised, the number of nodes within subjects (clusters), and the intra-cluster correlation (ICC). Specifically, ESS = n / VIF, where n is the total number of nodes excised across all patients, and VIF is the variance inflation factor, calculated as VIF = 1 + (m - 1) × ICC, where m is the number of nodes per subject. Because we expect to excise 3 to 4 nodes per subject, with 150 subjects we expect to excise 3.5 × 150 = 525 nodes. With 3.5 nodes per subject on average and an ICC at most 0.125 we will have VIF at most 1 + (2 × 3.5 – 1) × 0.125 = 1.75 and an ESS at least 525 / 1.75 = 300 nodes. Note that here m is twice the number of expected nodes per subject, since nodes will be identified using 2 different methods within the same subject. We expect the ICC to be at most 0.125 based on our estimate of the ICC = 0.07 from Crane et al .18.

With an ESS of at least 300 ~~L~~Ns we will have at least 82% power to reject H01 above and claim non-inferiority of PINPOINT with respect to Blue dye. This sample size calculation was performed using nQuery Advisor ® 7.0 (Copyright © 1995-2007, Statistical Solutions, Saugus, MA). We will test the hypothesis using a two-sided 95% confidence interval (i.e., two-sided significance level of 0.05). We will enroll subjects ~~patients~~ and identify LNs using both Blue dye and PINPOINT as described above in section 8.3.1.5 until we have at least 300 LNs identified, regardless of the method used to identify them. We expect that we will enroll approximately 150 ~~patients~~subjects to obtain 525 nodes (based on clinical literature suggesting an average of ~~1.8-2.2~~ 3-4 LNs excised per subject [17–28].

Rationale for Change: This section was revised to address the following recommendation from the FDA:

- Please note because there could be multiple LNs, LCs identified in the same subject, the resulting data set is thus cluster-correlated. Furthermore, there is correlation between two methods because both blue dye and PINPOINT methods will be applied on the same subject. Please account for these correlations in your sample size determination and the subsequent statistical analysis. Please pre-specify an appropriate statistical method to account for the correlations.

11. Section 10.5.1: Per-protocol (PP) and 10.5.2 and Modified Intent to treat (mITT) (page 46)

10.5.1 Per–protocol (PP)

The Per-Protocol (PP) analysis population includes all subjects that: [1] meet critical eligibility criteria, [2] have no significant protocol deviations; and [3] have evaluable assessment endpoints for the primary endpoint.

We believe that the PP population will be the population that will most likely demonstrate a difference between Blue dye and IC2000 with respect to their ability to identify lymph nodes, should a difference exist. Thus, since we are trying to show non-inferiority of IC2000 with respect to Blue dye, we believe that using the PP population for testing non-inferiority is the conservative approach

10.5.2 Modified Intent to treat (mITT)

The mITT analysis population includes all randomized subjects who received at least one injection of IC2000 or bBlue dye. All subjects meeting this criterion are included in the mITT population regardless of whether or not they received the minimally invasive surgical intervention or lymphatic mapping. Subjects who have the mapping procedure aborted due to circumstances such as a higher stage cancer than initially expected will not be included in the mITT. Approximately 8% of subjects are expected to have the mapping procedure aborted.

We believe the mITT population will be the population that will most likely demonstrate no difference between the Blue dye and IC2000 with respect to their ability to identify lymph nodes, because the mITT population includes subjects who may not have received the full dose of dye, and it includes subjects who may have not received lymphatic mapping, but who may have had lymph nodes identified by gross inspection. Thus, we believe that using the mITT population for testing superiority will be the conservative approach.

Rationale for Change: This section was revised to address the following recommendations from the FDA:

- Please provide justification for the use of differing populations (PP vs. mITT) for determination of your endpoints.
- For the primary hypothesis testing, you propose to use the per-protocol (PP) population for non-inferiority testing, and the modified intent-to-treat (mITT) for superiority testing. This is also the case for your secondary objective hypotheses testing. Please explain why you use different study populations for non-inferiority and superiority testing.

12. 10.6 Secondary Objectives (page 47 and 48)

The planned secondary objective is out comes are intended to support product labelling. We will use the step-down method described by Benjamini and Liu[37] to control the false discovery rate at 0.05 (2-sided) while testing our secondary objectives.

The first secondary outcome is the ability of PINPOINT and Blue dye to detect at least one lymph node in a patient subject.

Let *qt* and *qc* represent the proportion of subjects with a least one lymph node identified (according to section 3.1 8.3.1.5 and confirmed by histology) with PINPOINT and Blue dye respectively, divided by the total number of subjects where mapping was attempted. That is, the numerator for *qt* is the number of subjects with at least 1 node identified with PINPOINT (classifications 1, 3, 5, 7) and confirmed as lymphoid tissue, and the numerator for qc is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) and confirmed as lymphoid tissue. The denominator of both *qt* and *qc* is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H03: \; qt \le qc - 0.05$$

$$H13: \; qt > qc - 0.05$$

To test this hypothesis we will estimate the difference *qt* – *qc* with a 95% two-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

Another secondary outcome is the bilateral LN detection rate. Let bt and bc represent the proportion of subjects with at least one node identified on the right side and on the left side of the pelvis and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for bt is the number of subjects with at least 1 node identified with PINPOINT (classifications 1, 3, 5, 7) on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with PINPOINT on the left side of the pelvis and confirmed as lymphoid tissue. Similarly, the numerator for bc is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with Blue dye on the left side of the pelvis and confirmed as lymphoid tissue. The denominator of both bt and bc is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H04: \; bt \le bc - 0.05$$

$$H14: \; bt > c - 0.05$$

To test this hypothesis we will estimate the difference bt – bc with a 95% two-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

A third secondary outcome is the proportion of LNs identified by following lymphatic channels (ducts). Let dt and dc represent the proportion of nodes identified by following a duct and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for dt is the number of nodes identified with PINPOINT by following a duct (classifications 5, 7) and confirmed as lymphoid tissue. Similarly, the numerator for dc is the number nodes identified with Blue dye by following a duct (classifications 6, 7) and confirmed as lymphoid tissue. We define the denominator for both dt and dc as the number of nodes identified by ANY method (classifications 1-7). The denominator will include excised nodes confirmed as lymphoid tissue and excised nodes not confirmed as lymphoid tissue.

The following hypotheses will be tested:

$$H05: \; dt \le dc - 0.05$$

$$H15: \; dt > dc - 0.05$$

To test this hypothesis we will estimate the difference dt – dc with a 95% two-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority (using the PP data set), and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

We will also tabulate the anatomic distribution of LNs identified by each dye.

Rationale for Change: This section was revised to address the following recommendation from the FDA:

- In the protocol summary section, you propose to use a step-down multiplicity adjustment for multiple secondary objectives. However, you do not give details of the multiplicity adjustment method in the corresponding section. Please pre-specify the details of the method you propose to use for multiplicity adjustment in the Secondary Objectives section.

The step-down method described by Benjamini and Liu (*Journal of Statistical Planning and Inference 1999;82:163-170*) will be used to control the false discovery rate (FDR) at 0.05 (2-sided) while testing the secondary objectives.

13. Section 10.7 Handling of Missing Data (page 49)

Reasonable efforts will be made to obtain complete data for all patients subjects; however, missing observations will inevitably occur due to patients subjects lost to follow-up or noncompliance with required assessments. The reasons for missing data will be documented and evaluated (e.g. ~~patients~~subject is deceased, lost to follow up, missed visit, etc.). In addition, the distribution of prognostic factors between ~~patients~~subjects with data and those without data will be examined to evaluate any potential sources of bias. Any missing observations will be described in detail and evaluated for assessment of possible bias.

A sensitivity analyses of the primary and secondary endpoints will be conducted using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology as lymphoid tissue for PINPOINT and as non-lymphoid tissue for Blue dye. The worst-case scenario will consider nodes with missing histology as non-lymphoid tissue for PINPOINT and as lymphoid tissue for Blue dye.

Rationale for Change: This section was revised to address the following recommendation from the FDA:

- For missing data handling, you propose to record the reasons of missing and further conduct analysis to evaluate any potential sources of bias. In addition, please conduct a sensitivity analysis including best-case and worst-case scenario analysis for the missing data.

14. Section 14 Data Management (page 49)

Since study data may be useful for future research studies performed under separate IRB approved protocols, study data will be archived indefinitely by the sponsor ~~in REDCap~~ .

Rationale for Change: After the completion of the study, all data will be archived with the sponsor as per Novadaq standard operating procedure requirements.

Protocol Revision History:

Version		
Date	**Number**	**Summary of Revisions Made:**
October 15, 2014		**Initial Version**
June 24, 2015	**1.0**	**Final Version**
January 18, 2016	**2.0**	**Amendment 1**
March 1st, 2016	**3.0**	**Amendment 2**
April 14th, 2016	**4.0**	**Amendment 3**
November 14th, 2016	**5.0**	**Amendment 4**

Summary of Protocol Changes

Protocol Number: Protocol PP LNM 01 (FILM study)

Protocol Title: A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

	Version Number	**Version Date**
Current Approved Protocol	1.0	June 24, 2015
Amended Protocol	2.0	January 18, 2016
Amended Protocol	3.0	March 1st, 2016
Amended Protocol	4.0	April 14th, 2016
Amended Protocol	5.0	November 14th, 2016

A summary of changes made to Protocol PP LNM 01 (FILM protocol; FILM trial) from version 4.0 dated April 14th to version 5.0 dated November 14th 2016 is provided below. For ease of review, all revisions to the text are highlighted with ~~strikethrough~~ for deleted text and <u>underline</u> for new text.

<u>Detailed Summary of Changes to the FILM Protocol (PP LNM 01)</u>

Section and page numbers referenced below correspond to the amended protocol PP LNM 01, V5.0, dated November 14th, 2016.

Revisions to the text:

1. Section 10.2 Hypotheses (page 43)

Here p_t and p_C represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively. We define the numerator for p_t as the number of nodes identified with PINPOINT (<u>classifications 1,3,5,7 as defined in section 8.3.1.5</u>) and confirmed as lymphoid tissue, and we define the numerator for p_C as the number of nodes identified with Blue dye (<u>classifications 2,3,6, and 7 as defined in section 8.3.1.5</u>) and confirmed as lymphoid tissue. We define the denominator for both p_t and p_C as the number of nodes identified by ANY method (classifications 1-7 as defined in section 8.3.1.5). The denominator will include excised nodes confirmed as lymphoid tissue and excised nodes not confirmed as lymphoid tissue. <u>As a sensitivity analysis we will also perform the non-inferiority test using the modified intention-to-treat (mITT) analysis population.</u>

Rationale for Change: This section has been revised to clarify the discrepancy in the definitions of p_t as identified with classifications 1,3,5,7 (as defined in section 8.3.1.5) and p_c as identified with classification 2,3,6 and 7 (as defined in sections 8.3.1.5) in section 10.2.

In addition, it has been clarified that non-inferiority testing will be performed on both the per-protocol (PP) analysis population and the modified intent-to-treat (mITT) analysis population. This will allow for comparison of results between non-inferiority and superiority testing.

2. Section 10.2 Hypotheses (page 43)

~~As a secondary analysis to support our primary objective and~~ To account for the fact that subjects will have multiple nodes and each node will have the opportunity to be identified by each of 2 dyes, we will model the logit of the probability of identifying a node as a function of dye (Blue dye, IC2000), order of randomization, and subject, with subject as a random effect, using a generalized linear mixed model. We may also consider other potential prognostic factors in the model, such as BMI and study center. Formally, our model will be defined by

$$\text{logit}_{ijk}=\alpha_{ij}+s_{i(k(l))}+\varepsilon_{i(k(l))j}$$

$$\text{where } \alpha_{ij}=\mu+\tau_{d(i,j)}+\pi_j+\lambda_{d(i,j-1)}$$

$$s_{i(k(l))}=\text{effect due to node } l \text{ in subject } k \text{ in sequence } i$$

$$\alpha_{ij}=\text{ effect due to period } j \text{ in sequence } i$$

$$\varepsilon_{i(k(l))j}=\text{random error } \sim N(0,\sigma^2)$$

$$\tau_{d(i,j)}=\text{effect due to dye } d \text{ in sequence } i \text{ and period } j$$

$$\pi_j=\text{effect due to period } j$$

and

$$\lambda_{d(i,j-1)}=\text{carryover effect of dye } d \text{ in sequence } i \text{ from period } j-1.$$

We will then estimate pt and pc as well as their variance and covariance from this model, and we will use these estimates to construct our 95% 2-sided confidence interval for pt-pc to test our hypotheses.

Rationale for Change: This section has been revised to address the details of the statistical method that will be used and appropriately account for the within-cluster correlation for primary hypothesis testing.

Protocol Revision History:

Version		
Date	**Number**	**Summary of Revisions Made:**
October 15, 2014		**Initial Version**
June 24, 2015	**1.0**	**Final Version**
January 18, 2016	**2.0**	**Amendment 1**
March 1st, 2016	**3.0**	**Amendment 2**
April 14th, 2016	**4.0**	**Amendment 3**
November 14th, 2016	**5.0**	**Amendment 4**
August 25th, 2017	**6.0**	**Amendment 5**

Summary of Protocol Changes

Protocol Number: Protocol PP LNM 01 (FILM study)

Protocol Title: A Randomized, Prospective, Open Label, Multicenter Study Assessing the Safety and Utility of PINPOINT® Near Infrared Fluorescence Imaging in the Identification of Lymph Nodes in Subjects with Uterine and Cervical Malignancies who are Undergoing Lymph Node Mapping

	Version Number	Version Date
Current Approved Protocol	1.0	June 24, 2015
Amended Protocol	2.0	January 18, 2016
Amended Protocol	3.0	March 1st, 2016
Amended Protocol	4.0	April 14th, 2016
Amended Protocol	5.0	November 14th, 2016
Amended Protocol	6.0	August 25th, 2017

A summary of changes made to Protocol PP LNM 01 (FILM protocol; FILM trial) from version 5.0 dated November 14th 2016 to version 6.0 dated August 25th, 2017 is provided below.

Please note the following:

- At this time study enrollment is closed.
- The changes to protocol PP LNM 01are being implemented prior to study database lock.
- The changes described herein affect the statistical sections of the protocol only and were implemented to address recent comments from the United States Food and Drug Administration (FDA) on the Statistical Analysis Plan (SAP) for protocol PP LNM 01.

The revisions to the SAP, described below, were recommended by the FDA in the April 26, 2017 letter for G150254/S003. The changes do not affect the primary or secondary objectives of the study and consist of using a straightforward method for the primary analysis rather than a linear mixed model, and minor revisions to indicate specifically that testing for superiority will be performed only if non-inferiority is first claimed.

Consequently, the changes described in this document do not have a significant impact on the study design or planned statistical analysis. Further, since study enrollment and participation has been completed, the changes to protocol PP LNM 01 do not affect the rights, safety, or welfare of the subjects.

As such, it is concluded that protocol PP LNM 01 Amendment 5 can be implemented with notice to the FDA since the changes to the protocol do not affect:

- **The validity of the data or information resulting from the completion of the approved protocol, or the relationship of likely patient risk to benefit relied upon to approve the protocol;**
- **The scientific soundness of the investigational plan; or**

- **The rights, safety, or welfare of the human subjects involved in the investigation.**

For ease of review, all revisions to the text are highlighted with ~~strikethrough~~ for deleted text and underline for new text.

Detailed Summary of Changes to the FILM Protocol (PP LNM 01)

Section and page numbers referenced below correspond to the amended protocol PP LNM 01, V6.0, dated August 25th, 2017.

Revisions to the text:

1. Synopsis Sample Size and Statistical Analysis: (page 9)

The FILM trial is a non-inferiority study comparing lymph node detection rates between Blue dye and PINPOINT. A sample size of approximately 150 evaluable subjects (to identify 525 LNs) is required to show the LN detection rate for PINPOINT is non-inferior to that with Blue dye with 80% power and a 5% 2-sided significance level with a 5% non-inferiority margin.

Both a modified Intent-to-Treat (mITT) and Per-Protocol (PP) analysis population will be utilized for the primary analysis. Analysis of the primary objective will be conducted using a 2-sided 95% confidence interval for the difference in proportions. A ~~superiority test will be conducted using the mITT analysis population and a~~ non-inferiority test will be conducted using the PP and the mITT analysis populations. If and only if non-inferiority is claimed, a superiority test will be conducted using the mITT analysis population. ~~according to the method of Moyé et al[3].~~ As a supporting analysis of the primary outcome, the analysis will also be repeated using the as-treated (AT) population.

The secondary objectives will be tested using a two-sided 5% significance level with a step-down multiplicity adjustment.

Rationale for Change: This section has been revised to accurately reflect the planned analysis and to be consistent with revisions to Section 10, Statistical Considerations, of the protocol

2. Section 10.2 Hypotheses (page 43)

To assess the effectiveness of PINPOINT in the identification of LNs, a non-inferiority test will be performed using the per-protocol (PP) analysis population ~~according to the method of Moyé et al[3]~~.

$$H_{01}: \quad p_t \leq p_c - 0.05$$

$$H_{11}: \quad p_t > p_c - 0.05$$

Here p_t and p_c represent the effectiveness of LN mapping with PINPOINT and Blue dye respectively. We define the numerator for p_t as the number of nodes identified with PINPOINT (classifications 1, 3, 5, 7 as defined in section 8.3.1.5) and confirmed as lymphoid tissue, and we define the numerator for p_c as the number of nodes identified with Blue dye (classifications 2, 3, 6, 7 as defined in section 8.3.1.5) and confirmed as lymphoid tissue. We define the denominator for both p_t and p_c as the number of nodes identified by ANY method (classifications 1-7 as defined in section 8.3.1.5). The denominator will include excised nodes confirmed as lymphoid tissue. ~~and excised nodes not confirmed as lymphoid tissue.~~

~~As a sensitivity analysis we will also perform the non-inferiority test using the modified intention-to-treat (mITT) analysis population.~~

A non-inferiority margin of 0.05 was determined to be clinically significant based on feedback from Investigators. Within their respective groups, pt and pc represent the proportion of LNs identified (and confirmed to be lymphoid tissue, See Section 8.5) with PINPOINT and Blue dye respectively divided by the total number of LNs identified and excised, across all subjects.

~~The effectiveness of intraoperative PINPOINT in identification of LNs will be determined by a superiority test using the modified intent-to-treat (mITT) analysis population as justified by the method of Moyé et al.~~

~~H02: $p_t = p_c$~~

~~H12: $p > p_c$~~

We will repeat the analysis described above using the as-treated (AT) analysis set as a supporting analysis of the primary outcome.

As a sensitivity analysis we will also perform the non-inferiority test using the mITT analysis set. We will also perform sensitivity analyses of the primary endpoint using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology to be lymphoid tissue for PINPOINT and non-lymphoid tissue for Blue dye. The worst case scenario will consider nodes with missing histology to be non-lymphoid tissue for PINPOINT and lymphoid tissue for Blue dye.

~~The use of both a superiority test and non-inferiority test for the primary endpoint can be utilized without statistical penalty due to the closed testing principle according to the method of Lesaffre et al[35].~~

Rationale for Change: This section has been revised based on comments received from the FDA in April and May, 2017 to apply a straightforward method to evaluate (p_t-p_c) and its variance, and use that for the primary analysis. After reviewing published papers by Nam J-M and Kwon D (*Non-inferiority tests for clustered matched-pair data*. Statist Med. 2009(28):1668-1679) and Obuchowski NA (*On the comparison of correlated proportions for clustered data*. Statist. Med. 1998; 17:1495-1507), it was decided to revise the statistical analysis accordingly for the analysis of the primary efficacy outcome.

3. Section 10.2 Statistical Analysis (page 43)

We will use the Z_O statistic described by Nam and Kwon[3] in formulae (6) to derive the estimates of p_t, p_c, and the variance of the difference between these estimates to construct the 95% 2-sided confidence interval for $p_t - p_c$ as:

$$\left((\widehat{p_t} - \widehat{p_c}) - 1.96 \times \sqrt{\widehat{var}(\hat{p}_t - \widehat{p_c})} \ , \ (\widehat{p_t} - \widehat{p_c}) + 1.96 \times \sqrt{\widehat{var}(\hat{p}_t - \widehat{p_c})} \right)$$

We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{01}) stated above, and if the lower bound of the interval is > -0.05 we will claim non-inferiority. If and only if we reject the null (H_{01}) hypothesis of inferiority and claim non-inferiority we will use the mITT analysis set to test the null (H~~02~~) stated below, and if the lower bound of the interval is > 0 we will claim superiority.

H_{02}: $p_t = p_c$

H_{12}: $p_t > p_c$

We will repeat the analysis described above using the as-treated (AT) analysis set as a supporting analysis of the primary outcome.

As a sensitivity analysis we will also perform the non-inferiority test using the mITT analysis set. We will also perform sensitivity analyses of the primary endpoint using a best-case and a worst-case scenario. The best-case scenario will consider nodes with missing histology to be lymphoid tissue for PINPOINT and non-lymphoid tissue for Blue dye. The worst case scenario will consider nodes with missing histology to be non-lymphoid tissue for PINPOINT and lymphoid tissue for Blue dye.

~~To test these hypotheses we will estimate the difference pt – pc with a 95% two-sided confidence interval. If the lower bound of the interval is greater than -0.05 we will claim non-inferiority, and if the lower bound of the interval is greater than 0 we will claim superiority.~~

~~To account for the fact that subjects will have multiple nodes and each node will have the opportunity to be identified by each of 2 dyes, we will model the logit of the probability of identifying a node as a function of dye (Blue dye, IC2000), order of randomization, and subject, with subject as a random effect, using a generalized linear mixed model. We may also consider other potential prognostic factors in the model, such as BMI and study center. Formally, our model will be defined by~~

~~〖logit〗_ijk=α_ij+s_(i(k(l)))+ ε_(i(k(l))j)~~

~~"where" α_ij=μ+τ_(d(i,j))+π_j+λ_(d(i,j-1))~~

~~s_(i(k(l)))="effect due to node " l" in subject" k "in sequence" i~~

~~α_ij= "effect due to period" j "in sequence" i~~

~~ε_i(k(l))j="random error" ~N(0,σ^2)~~

~~τ_(d(i,j))="effect due to dye " d "in sequence " i "and period" j~~

~~π_j="effect due to period" j~~

~~and~~

~~λ_(d(i,j-1))=c"arryover effect of dye " d "in sequence " i "from period" j-1.~~

~~We will then estimate pt and pc as well as their variance and covariance from this model, and we will use these estimates to construct our 95% 2-sided confidence interval for pt-pc to test our hypotheses.~~

Rationale for Change: This section has also been revised based on comments received from the FDA in April and May, 2017 to apply a straightforward method to evaluate (p_t-p_c) and its variance, and use that for the primary analysis, due to the potential issues with violation of assumptions and model misspecification using the mixed models approach. The Z_O statistic described by Nam J-M and Kwon D (*Non-inferiority tests for clustered matched-pair data*. Statist Med. 2009(28):1668-1679) will be used for the analysis of the primary efficacy outcome. Therefore, the 95% confidence interval for (pt-p_c) will be estimated using the estimates of pt-p_c, and the variance of the difference between these estimates as defined in formula (6) of the Nam and Kwon paper for the analysis of the primary efficacy outcome.

4. Section 10.6 Secondary Objectives (page 47)

The first secondary outcome is the ability of PINPOINT and Blue dye to detect at least one lymph node in a subject.

Let q_t and q_c represent the proportion of subjects with a least one lymph node identified (according to section 8.3.1.5 and confirmed by histology) with PINPOINT and Blue dye respectively, divided by the total number of subjects where mapping was attempted. That is, the numerator for q_t is the number of subjects with at least 1 node identified with PINPOINT (classifications 1, 3, 5, 7) and confirmed as lymphoid tissue, and the numerator for q_c is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) and confirmed as lymphoid tissue. The denominator of both q_t and q_c is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H_{03}: q_t \leq q_c - 0.05$$

$$H_{13}: q_t > q_c - 0.05$$

To test this hypothesis we will estimate the difference $q_t - q_c$ with a 95% two-sided confidence interval. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{03}) stated above, and ~~I~~if the lower bound of the interval is greater than -0.05 we will claim non-inferiority. ~~(using the PP data set).,~~ If and only if we reject the inferiority hypothesis (H_{03}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority ~~(using the mITT data set)~~.

Another secondary outcome is the bilateral LN detection rate. Let b_t and b_c represent the proportion of subjects with at least one node identified on the right side and on the left side of the pelvis and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for b_t is the number of subjects with at least 1 node identified with PINPOINT () on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with PINPOINT on the left side of the pelvis and confirmed as lymphoid tissue. Similarly, the numerator for b_c is the number of subjects with at least 1 node identified with Blue dye (classifications 2, 3, 6, 7) on the right side of the pelvis and confirmed as lymphoid tissue and at least 1 node identified with Blue dye on the left side of the pelvis and confirmed as lymphoid tissue. The denominator of both b_t and b_c is the number of subjects where mapping was attempted.

The following hypotheses will be tested:

$$H_{04}: b_t \leq b_c - 0.05$$

H_{14}: $b_t > b_c – 0.05$

To test this hypothesis we will estimate the difference $b_t – b_c$ with a 95% two-sided confidence interval. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{04}) stated above, and Iif the lower bound of the interval is greater than -0.05 we will claim non-inferiority. (using the PP data set), If and only if we reject the inferiority hypothesis (H_{04}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

A third secondary outcome is the proportion of LNs identified by following lymphatic channels (ducts). Let d_t and d_c represent the proportion of nodes identified by following a duct and confirmed as lymphoid tissue with PINPOINT and Blue dye, respectively. That is, the numerator for d_t is the number of nodes identified with PINPOINT by following a duct (classifications 5, 7) and confirmed as lymphoid tissue. Similarly, the numerator for d_c is the number nodes identified with Blue dye by following a duct (classifications 6, 7) and confirmed as lymphoid tissue. We define the denominator for both d_t and d_c as the number of nodes identified by ANY method (classifications 1-7). The denominator will include excised nodes confirmed as lymphoid tissue and excised nodes not confirmed as lymphoid tissue.

The following hypotheses will be tested:

H_{05}: $d_t \leq d_c – 0.05$

H_{15}: $d_t > d_c – 0.05$

To test this hypothesis we will estimate the difference $d_t – d_c$ with a 95% two-sided confidence interval. We will estimate this confidence interval in a manner similar to that described for the primary outcome. We will perform this analysis using the PP analysis set to test the inferiority hypothesis (H_{05}) stated above, and Iif the lower bound of the interval is greater than -0.05 we will claim non-inferiority. (using the PP data set), If and only if we reject the inferiority hypothesis (H_{05}) we will use the mITT analysis set to test for superiority, and if the lower bound of the interval is greater than 0 we will claim superiority (using the mITT data set).

Rationale for Change: This section has also been revised based on changes to the primary outcome analysis and to clearly indicate that the tests for superiority for each secondary objective will only be performed if the inferiority hypothesis is rejected. In addition the population datasets used for each analysis has been clarified.