STATISTICAL ANALYSIS PLAN

FILM Study:

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

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Introduction

The FILM study compares lymph node (LN) detection with IC2000 to node detection with isosulfan blue. In the study, approximately 150 evaluable subjects with clinical stage 1 endometrial cancer or clinical stage 1A cervical cancer who are scheduled to undergo minimally invasive surgery with lymphatic mapping are randomized to one of two groups (arms):

- P-B arm: subjects will have IC2000 injected into the cervix initially followed by isosulfan blue cervical injection; subsequent LN mapping will be performed first for IC2000 mapping followed by isosulfan blue mapping, or
- B-P arm: subjects will have isosulfan blue injected into the cervix initially followed by IC2000 cervical injection; subsequent LN mapping will be performed first for isosulfan blue mapping followed by IC2000 mapping.

Mapping is only considered complete when all nodes meeting the criteria outlined in the protocol (all nodes identified and mapped by isosulfan blue or IC2000 or both and all visibly or palpably abnormal nodes) are documented and the surgeon has performed a full 360-degree scan of the area within the abdominal cavity. Further, fluorescent ducts are to be followed in both directions in order to identify LNs to be excised. Surgeons will then excise all mapped LNs. It is also important to note that, in addition to mapping of LNs in accordance with the guidelines from the NCCN for Uterine Neoplams, SLN Algorithm for Surgical staging of Endometrial Cancer or the NCCN guidelines for Cervical Neoplasms, Surgical/SLN Mapping Algorithm for Early-stage Cervical Cancer, the site investigators are to remove additional LNs according to their standard of care for the patient (refer to page 37 of the FILM protocol), a consideration that is personalized based upon surgical experience, intra-operative observations, patient co-morbidities and other factors.

This document describes the analyses we will perform in support of the primary study outcome, secondary study outcomes, and safety outcomes. The document includes descriptions of planned analysis sets, planned analyses, and mock-ups of planned tables and listings to display data collected during the study to support the primary and secondary outcomes and the safety outcomes.

We define analysis sets as follows:

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 PINPOINT with respect to their ability to identify LNs, should a difference exist. Thus, since we are trying to show non-inferiority of PINPOINT with respect to Blue dye, we believe that using the PP population for testing non-inferiority is the conservative approach.

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 PINPOINT with respect to their ability to identify LNs, 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 LNs identified by gross inspection. Thus, we believe that using the mITT population for testing superiority will be the conservative approach.

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.

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.

Table 1 summarizes the disposition of subjects screened and enrolled in the study.

Table 1. Subject Disposition

	Number of Subjects
Screened	
Screen Failure	
Eligible	
Enrolled (Signed Informed Consent)	
Randomized	
Determined to be Ineligible after Randomization	
Completed Study	

We will use descriptive statistics to summarize demographic and clinical characteristics of subjects enrolled on the study, as shown in Table 2. This summary will be provided for the PP, mITT, and AT analysis sets.

Table 2. Subject Characteristics

	B-P Arm		P-B Arm		Total	
	N	%	N	%	N	%
Total Enrolled						
Study Center:						
CHU de Québec						
Duke Cancer Institute						
HIMA San Pablo						
Lee Memorial						
MD Anderson						
Memorial Sloan Kettering						
Sunnybrook Health Science Centre						
Age:						
30 to 39						
40 to 49						
50 to 59						
60 to 69						
70 to 79						
BMI (kg/m^2)						
< 25						
25 to < 30						
≥ 30						
Race & Ethnicity:						
White						
Black or African American						
Asian						
Hispanic						
Smoking Status						
Current						
Past						
Never						
Alcohol Consumer						
ASA Classification						
I						
II						
III						
Hypertension						
Cardiac Disease						

Table 2. Subject Characteristics

	B-P Arm		P-B Arm		Total	
	N	%	N	%	N	%
COPD						
Diabetes						
Renal Failure						
Liver Disease						
Preop Diagnosis						
Clinical Stage 1 Endometrial Cancer						
Clinical Stage 1 Cervical Cancer						
Endometrial Cancer Histology						
Adenocarcinoma						
Serous Carcinoma						
Clear Cell Carcinoma						
Carcinosarcoma						
Undifferentiated						
Mixed						
Cervical Cancer Histology						
Squamous Cell Carcinoma						
Adenocarcinoma						
Adenosquamous Carcinoma						
Neuroendocrine						
Clear Cell Carcinoma						
Undifferentiated						
Mixed						
Surgical Procedure						
Hysterectomy						
Radical Hysterectomy						
Radical Trachelectomy						
Bilateral Salpingo-Oophorectomy						
Unilateral Salpingo-Oophorectomy						
Ovarian Transposition						
Sentinel Node Mapping						
Pelvic Node Sampling						
Pelvic Node Dissection						
Para-Aortic Node Sampling						
Para-Aortic Node Dissection						
Other						
Length of Surgery (minutes)						
Median (Min – Max)						
Dose of IC2000 (mg)						
Median (Min – Max)						
Dose of Blue Dye (mg)						
Median (Min – Max)						

Primary Efficacy Outcome

The primary objective of this study is to assess the effectiveness of intraoperative PINPOINT Near Infrared Fluorescence Imaging (PINPOINT) in identification of LNs in subjects with uterine and cervical malignancies who are undergoing LN mapping. Table 3 summarizes the study primary outcome.

Table 3. Lymph Nodes Identified

Identification Method	B-P Arm N	B-P Arm %	P-B Arm N	P-B Arm %	Total N	Total %
PINPOINT Only						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Blue Dye Only						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Both PINPOINT and Blue Dye						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Following a Fluorescent Duct (No Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Following a Fluorescent Duct (Blue Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Following a Blue Duct (No Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Following a Blue Duct (Fluorescent Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Following a Blue and Fluorescent Duct (No Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						

Table 3. Lymph Nodes Identified

Identification Method	B-P Arm N	B-P Arm %	P-B Arm N	P-B Arm %	Total N	Total %
Appearance (No Dye Present in Node)						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Confirmed as Lymphatic Tissue (negative for cancer)						
Confirmed as Lymphatic Tissue (positive for cancer)						
Total						
PINPOINT						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Blue Dye						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Neither Dye						
Confirmed as NOT Lymphatic Tissue						
Confirmed as Lymphatic Tissue						
Total						

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

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

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 (i.e., with PINPOINT alone or with both Blue dye and PINPOINT) and confirmed as lymphoid tissue, and we define the numerator for p_c as the number of nodes identified with Blue dye (i.e., with Blue dye alone or with both Blue dye and PINPOINT) 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 (i.e., PINPOINT alone, Blue dye alone, both Blue dye and PINPOINT, abnormal appearance or palpably hard, non-stained node found at termination of Fluorescent duct, non-stained node found at termination of Blue duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye). 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) with PINPOINT and Blue dye respectively divided by the total number of LNs identified and excised, across all subjects.

We will use the Z_O statistic described by Nam and Kwon (Nam J-M and Kwon D. Non-inferiority tests for clustered matched-pair data. *Statistics in Medicine.* 2009(28):1668-1679) 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 hypothesis (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.

Secondary Efficacy Outcomes

The planned secondary outcomes are intended to support product labelling. We will use the step-down method described by Benjamini and Liu (Benjamini Y, Liu W. A step-down multiple hypothesis testing procedure that controls the false discovery rate under independence. *Journal of Statistical Planning and Inference*. 1999(82):163-170) 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 node identified and confirmed as lymphoid tissue 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 (PINPOINT alone, both Blue dye and PINPOINT, non-stained node found at termination of Fluorescent duct, Blue-stained node found at termination of a Fluorescent duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye) 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 (Blue dye alone, both Blue dye and PINPOINT, non-stained node found at termination of Blue duct, Fluorescent-stained node found at termination of Blue duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye) and confirmed as lymphoid tissue. The denominator of both q_t and q_c is the number of subjects where mapping was attempted.

Table 4 summarizes the data to address this secondary objective.

Table 4. Number of Subjects with ≥ 1 Lymph Node Identified

	B-P Arm		P-B Arm		Total	
Identification Method	N	%	N	%	N	%
PINPOINT						
Blue Dye						
Number of Subjects						

We will test the following hypotheses:

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

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 if the lower bound of the interval is > -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 > 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 (PINPOINT alone, both Blue dye and PINPOINT, non-stained node found at termination of Fluorescent duct, Blue-stained node found at termination of Fluorescent duct, non-stained node found at termination of duct stained with both Blue dye

and Fluorescent dye) 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 (Blue dye alone, both Blue dye and PINPOINT, non-stained node found at termination of Blue duct, Fluorescent-stained node found at termination of Blue duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye) 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.

Table 5 summarizes the data to address this secondary objective.

Table 5. Number of Subjects with Bilateral Lymph Nodes Identified

	B-P Arm		P-B Arm		Total	
Identification Method	N	%	N	%	N	%
PINPOINT Blue Dye						
Number of Subjects						

We will test the following hypotheses:

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

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 if the lower bound of the interval is > -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 > 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 (non-stained node found at termination of Fluorescent duct, Blue-stained node found at termination of a Fluorescent duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye) and confirmed as lymphoid tissue. Similarly, the numerator for d_c is the number of nodes identified with Blue dye by following a duct (non-stained node found at termination of Blue duct, Fluorescent-stained node found at termination of a Blue duct, non-stained node found at termination of duct stained with both Blue dye and Fluorescent dye) 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. The denominator will include excised nodes confirmed as lymphoid tissue.

Table 6 summarizes the data to address this secondary objective.

Table 6. Number of Subjects with Lymph Nodes Identified by Following a Duct

	B-P Arm		P-B Arm		Total	
Identification Method	N	%	N	%	N	%
PINPOINT Blue Dye						
Number of Subjects						

We will test the following hypotheses:

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

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 if the lower bound of the interval is > -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 > 0 we will claim superiority.

We will also estimate overall percent agreement, positive percent agreement, and negative percent agreement with exact (Clopper-Pearson) 95% confidence intervals. The data will be summarized as shown in Table 7.

Table 7. Agreement of PINPOINT with Blue Dye

		Blue Dye (Reference)		Total
		(+)	(–)	
PINPOINT (Test)	(+)	A	B	A + B
	(–)	C	D	C + D
Total		A + C	B + D	

We define the following quantities:

- overall percent agreement = 100% × (A + D) / (A + B + C + D)
- positive percent agreement = 100% × A / (A + C)
- negative percent agreement = 100% × D / (B + D)

As sensitivity analyses, we will perform the non-inferiority tests of these secondary outcomes using the mITT analysis set. We will also perform sensitivity analyses of these secondary endpoints 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 last secondary objective of the study is to find the anatomic distribution of lymph nodes. Table 8 summarizes the data to address this secondary objective. We will use Fisher's exact test to compare study arms with respect to the distribution of anatomic location of lymph nodes for each identification method. We will also use Fisher's exact test to compare identification methods with respect to the distribution of anatomic location of lymph nodes. We summarize these data using both the PP and mITT analysis sets.

Table 8. Anatomic Location of Lymph Nodes Identified

Identification Method	B-P Arm		P-B Arm		Total	
	N	%	N	%	N	%
PINPOINT						
Left obturator/internal iliac						
Left external iliac						
Left common iliac						
Right obturator/internal iliac						
Right external iliac						
Right common iliac						
Presacral						
Para-aortic node below IMA						
Para-aortic node above IMA						
Other						
Blue Dye						
Left obturator/internal iliac						
Left external iliac						
Left common iliac						
Right obturator/internal iliac						
Right external iliac						
Right common iliac						
Presacral						
Para-aortic node below IMA						
Para-aortic node above IMA						
Other						
Following a Channel (No Dye Present in Node)						
Left obturator/internal iliac						
Left external iliac						
Left common iliac						
Right obturator/internal iliac						
Right external iliac						
Right common iliac						
Presacral						
Para-aortic node below IMA						
Para-aortic node above IMA						
Other						
Appearance (No Dye Present in Node)						
Left obturator/internal iliac						
Left external iliac						
Left common iliac						
Right obturator/internal iliac						
Right external iliac						
Right common iliac						
Presacral						
Para-aortic node below IMA						
Para-aortic node above IMA						
Other						

Safety Outcomes

One secondary objective of the study is to assess the safety of interstitial injection of IC2000 for intraoperative lymphatic mapping. Tables 9, 10, and 11 summarize the data to address this secondary objective. These analyses will be performed on the safety (S) analysis set. Adverse events include Adverse Device Effects.

Table 9. Summary of Adverse Events

Concise Term	Intensity	Relationship to Study Drug / Surgery	Number of Adverse Events B-P Arm	P-B Arm
Term #1	Mild	Not Suspected		
	Moderate	Not Suspected		
	Severe	Not Suspected		
	Mild	Related to Blue Dye		
	Moderate	Related to Blue Dye		
	Severe	Related to Blue Dye		
	Mild	Related to Fluorescent Dye		
	Moderate	Related to Fluorescent Dye		
	Severe	Related to Fluorescent Dye		
	Mild	Related to Surgery		
	Moderate	Related to Surgery		
	Severe	Related to Surgery		
Term #2	Mild	Not Suspected		
	Moderate	Not Suspected		
	Severe	Not Suspected		
	Mild	Related to Blue Dye		
	Moderate	Related to Blue Dye		
	Severe	Related to Blue Dye		
	Mild	Related to Fluorescent Dye		
	Moderate	Related to Fluorescent Dye		
	Severe	Related to Fluorescent Dye		
	Mild	Related to Surgery		
	Moderate	Related to Surgery		
	Severe	Related to Surgery		
Etc.				
Total				

We will use Fisher's exact test to compare study arms with respect to the distribution of intensity and relationship to study drug/surgery for each adverse event. We will estimate the incidence of each adverse event with a 95% exact binomial confidence interval.

Table 10. Number of Subjects with Adverse Events by Maximum Intensity

Maximum Intensity	B-P Arm N	B-P Arm %	P-B Arm N	P-B Arm %	Total N	Total %
Mild						
Moderate						
Severe						
Total						

We will use Fisher's exact test to compare the distribution of maximum intensity of adverse events between study arms. We will estimate the percent of subjects with each maximum intensity level with a 95% exact binomial confidence interval.

We will provide a listing of all adverse events by study arm and subject, as shown in Table 11.

Table 11. Adverse Event Listing

Arm	Subject	Concise Term	Intensity	Date of Onset	Date Resolved	Action Taken	Relationship to Study Drug / Surgery	Outcome

Protocol Deviations

We will list all protocol deviations for patients screened and enrolled on the study.

Table 12. Protocol Deviation Listing

Arm	Subject	Date	Deviation	Other …	Description	Severity

Other Data Summaries

We will provide listings of conditions, illnesses, or surgical procedures from the subjects' baseline medical history by subject and study arm (Table 13).

We will provide listings of concomitant medications by subject and study arm (Table 14).