STUDY NUMBER: CASE 9813

STUDY TITLE: Sentinel Lymph Node Mapping for Endometrial Cancer

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TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Endometrial Cancer and Treatment

The incidence of endometrial cancer in the United States in 2008 was 40,100, with 7,470 deaths from this disease [1]. The majority of patients diagnosed with uterine cancer are diagnosed at an early stage (FIGO stage I), when surgical resection often leads to a good prognosis [2]. While hysterectomy and bilateral salpingo-oophorectomy are the standard of care for stage I tumors, the role for lymphadenectomy is still highly debated. This is due to the fact that there are significant risks associated with this procedure, including increased operative time and surgical complications, lymphedema, and lymphocyst formation [3]. Furthermore, evidence suggests that lymphadenectomy for stage I disease affords no improvement in overall or recurrence free survival [4]. Nonetheless, approximately 10% of women with stage I disease will have metastases to the pelvic lymph nodes, a finding which changes both the treatment recommendations and prognosis for the patient [5].

For this reason, SLN sampling is a surgical procedure that has gained enthusiasm as a means of identifying those patients who should be upstaged. This technique has been shown to be more accurate than MRI or CT scan [6], but without the risks associated with complete lymphadenectomy [7].

In higher risk disease (both higher stage as well as for tumors with intermediate risk factors), the role of SLN biopsy also remains unclear. While lymphadenectomy remains the standard of care in this patient population, SLN biopsy may be useful in patients who either refuse lymphadenectomy or for those patients who are unable to undergo this procedure due to surgical risk factors.

1.2 Sentinel Lymph Node Biopsy

The theory behind SLN biopsy is that the sentinel node is the first node in the lymphatic drainage pattern from a tumor; therefore, if this node is negative for metastasis, the remaining nodes should also be negative [8]. Techniques to identify the sentinel lymph node include injection of dye or radioisotope (most often into the tumor site), which then travels through the lymphatic system to the sentinel node. This node is then detected either by visualization (if dye is used) and/or by a gamma probe and specialized imaging (for radioisotope localization). Once the sentinel node has been identified, the node is removed and sent to pathology to be examined for evidence of metastases. Pathologists are now using advanced techniques such as ultrastaging and immunohistochemistry to help reveal micrometastases [9,10]. If metastases are noted, then a complete lymphadenectomy is carried out. If no metastasis is evident in the sentinel node, then further dissection is not necessary [11]. While the literature suggests that there is a learning curve for surgeons utilizing this technique, after approximately 30 cases [12,13], SLN biopsy can be performed with good and reliable results [11].

This concept was first described by Cabanas in 1977 [14]. Subsequently, significant work was done in both melanoma [15] and breast cancers [8], where sentinel lymph node biopsy, rather than complete lymphadenectomy, has become accepted as routine practice [16]. The approval of this technique in these cancers is based on significant evidence showing that the correct sentinel node from a tumor could be determined with high sensitivity and low false

negative rate [11]. This means that there must be few cases in which the sentinel node is negative, while a positive node went unidentified.

1.3 **Sentinel Lymph Node Biopsy in Endometrial Cancer**

Burke et al. first introduced SLN biopsy for endometrial cancer in 1996[17]; however this technique has only more recently begun further evaluation as of the 21st century. Kang et al performed a recent meta-analysis in 2011, which identified the current body of evidence available. Through an extensive literature search, this manuscript identified 26 studies on sentinel lymph node biopsy in endometrial cancer (see Table 1) [18].

Table 1. (from Kang et al.) [18] Meta-analysis of 26 studies

Authors	Year	N	Injection site	Route of surgery	Detection method	Pathology Assessment	Study
Burke et al.	1996	15	SM	Laparotomy	Dye only	HE	Unknown
Gargiulo et al.	2003	11	C	Laparoscopy	Both	HE/IHC	Unknown
Pelosi et al.	2003	16	C	Laparoscopy	Both	HE/IHC	Unknown
Petynski et al.	2003	33	SM/C	N/S	Both	N/A	Unknown
Fersis et al	2004	10	HS	Laparotomy	Isotope only	HE	Unknown
Houb et al.	2004	25	SM/C	Laparoscopy	Dye only	HE	Unknown
Lelievre et al.	2004	12	C	Laparotomy/scopy	Both	HE/IHC	Prospective
Niikura et al.	2004	28	HS	Laparotomy	Isotope only	HE/IHC	Unknown
Gien et al.	2005	16	HS	Laparotomy	Dye only	HE	Unknown
Maccauro et al.	2005	26	HS	Laparotomy	Both	HE/IHC	Unknown
Dzvincuk et al.	2006	33	C	Laparotomy/scopy	Isotope only	N/A	Prospective
Altgassen et al.	2007	23	SM	Laparotomy	Dye only	HE/IHC	Unknown
Dealoye et al.	2007	60	HS	Laparotomy/scopy	Both	HE/IHC	Unknown
Frumovitz et al.	2007	18	SM	Laparotomy	Both	HE	Prospective
Li et al.	2007	20	SM	Laparotomy	Dye only	HE	Unknown
Lopes et al.	2007	40	SM	Laparotomy	Dye only	HE/IHC	Unknown
Ballester e al.	2008	46	C	Laparotomy/scopy	Both	HE/IHC	Unknown
Bats et al.	2008	43	C	Laparotomy/scopy	Both	HE/IHC	Prospective
Perrone et al.	2008	40	HS, C	Laparoscopy	Isotope only	HE/IHC	Unknown
Robova et al.	2009	91	SM/H	Laparotomy	Both	HE	Prospective
Vidal-Sicart et al.	2009	35	N/A	N/A	Isotope only	N/A	Unknown
Zenzola et al.	2009	14	C	Laparotomy	Both	N/A	Unknown
Feranec et al.	2010	21	HS	Laparotomy	Both	N/A	Unknown
Mais et al.	2010	34	C	Laparotomy/scopy	Dye only	HE/IHC	Prospective
Ballester et al.	2011	125	C	Laparotomy/scopy	Both	HE/IHC	Prospective
Khoury-Collado et al.	2011	266	C, C/SM	Laparotomy/scopy	Dye only, both	HE/IHC	Prospective

N/A = not available; SM = subserosal myometrium; C = cervix; HS = hysteroscopic; HE = hematoxylin-eosin staining; IHC = immunohistochemistry.

This meta-analysis reported a detection rate of 78% and a sensitivity of 93% for sentinel lymph node biopsy. From this study, assuming a 10% metastasis risk, a false negative rate of 1% was calculated [18].

Since the publication of this meta-analysis, there have been four additional prospective studies on SLN biopsy in endometrial cancer that these authors were able to identify. Examining the results from these 4 prospective trials, sensitivity of SLN biopsy is high, and negative predictive value (NPV) is also relatively high (Table 2.).

Table 2. Results from recent prospective studies

Author	Year	Type	Pts	Detection	Inj	Procedure	Detection	Sens	NPV
How	2012	Prospective	100	Isotope	C	Robotic	92%	89%	99%
Solima	2012	Prospectve	59	Isotope	H	Ex-lap or LSC	95%	90%	98%
Buda	2012	Prospective	25	Isotope/ISB	C	Ex-lap or LSC	91%	100%	Unk
Ballester	2011	Prospective	125	Isotope/ISB	C	Ex-lap or LSC	89%	84%	97%

While the evidence that exists is encouraging, it is important to recognize that we are still in the early stages of analysis of SLN biopsy in endometrial cancer. The number of patients studied thus far does not yet allow us to get an accurate sense of the true false-negative rate [3], and large, prospective, randomized controlled trials will be necessary to validate these findings.

1.4 **Clinical Data-Techniques for Sentinel Lymph Node biopsy in Endometrial Cancer**

While SLN biopsy appears to be a promising development in the treatment of endometrial cancer, several details regarding the techniques utilized must also be clarified. Two areas that remain controversial are the ideal site of injection, and the detection method utilized (dye versus isotope versus both isotope and dye, versus new techniques using ICG). To date, the studies that have been done have utilized a variety of these techniques, and it remains unclear which provide the most optimal results (Table 1).

1.4.1 Injection Site

Anatomically, the lymphatic drainage from the uterus is complex, with the lower uterine segment draining to the pelvic lymph nodes, and the upper segment draining to the para-aortic nodes. For this reason, injection site for SLN biopsy in endometrial cancer has been brought into question. Three sites of injection have been explored and reported: 1. uterine subserosa (7 studies with detection rates ranging from 0-92%), 2. cervix (7 studies [with or without additional injection into the myometrium] with detection rates ranging from 83-100%), and 3. endometrium, via hysteroscopy (5 studies with detection rates from 0-100%) [3]. While all 3 sites have shown high detection rates, there are several advantages to performing the injection into the cervix.

Although cervical and endometrial lymphatic spread patterns are different, deep injection into the cervix has demonstrated that the proper areas of drainage are reached [3], with good

penetration to the uterine vessels, parametria, lower uterine segment, and to the cornua [19], Accordingly, a recent meta-analysis showed that peri-cervical injection was associated with a statistically significant increase in detection rate of sentinel node when compared with other sites [18]. Additionally, injection into the cervix is technically advantageous as this is the easiest site to reach pre-operatively [3]. The trend in the literature is also toward cervical injection, with the 4 most recent prospective studies utilizing this technique (see Table 1).

1.4.2 Detection Method

Blue dye alone, radioisotope alone, or combinations of both detection methods have all been utilized in the literature (see Table 1), and while the optimal strategy remains unknown, each technique has advantages and disadvantages. The major disadvantages of isotope injection are that it must be performed on either the day before or the morning of surgery. Furthermore, this must be done in the nuclear medicine department, and it therefore requires extensive logistical coordination and preparation in order to optimize results [20]. This technique can therefore be technically challenging as well as costly. The injection of dye is much simpler, as it is injected immediately prior to surgery, and additional dye can be injected if needed [19]. It is additionally less costly. While allergic reactions to dye are rare (<1%) and often mild, severe allergic reactions have been reported [19].

The evidence for the optimal technique is inconsistent, with some evidence suggesting that the detection rates are equivalent and some suggesting that the combination of isotope and blue dye injection is superior [7]. However, due to the logistical challenges and cost associated with isotope injection, the cost-benefit of adding this technique is unclear. Furthermore, evidence from gynecologic oncology literature suggests a similar learning curve for SLN biopsy, with approximately 30 cases as the plateau point [10]. Some evidence suggests that experience of the surgeon rather than detection method is the more critical factor in the detection rate [18].

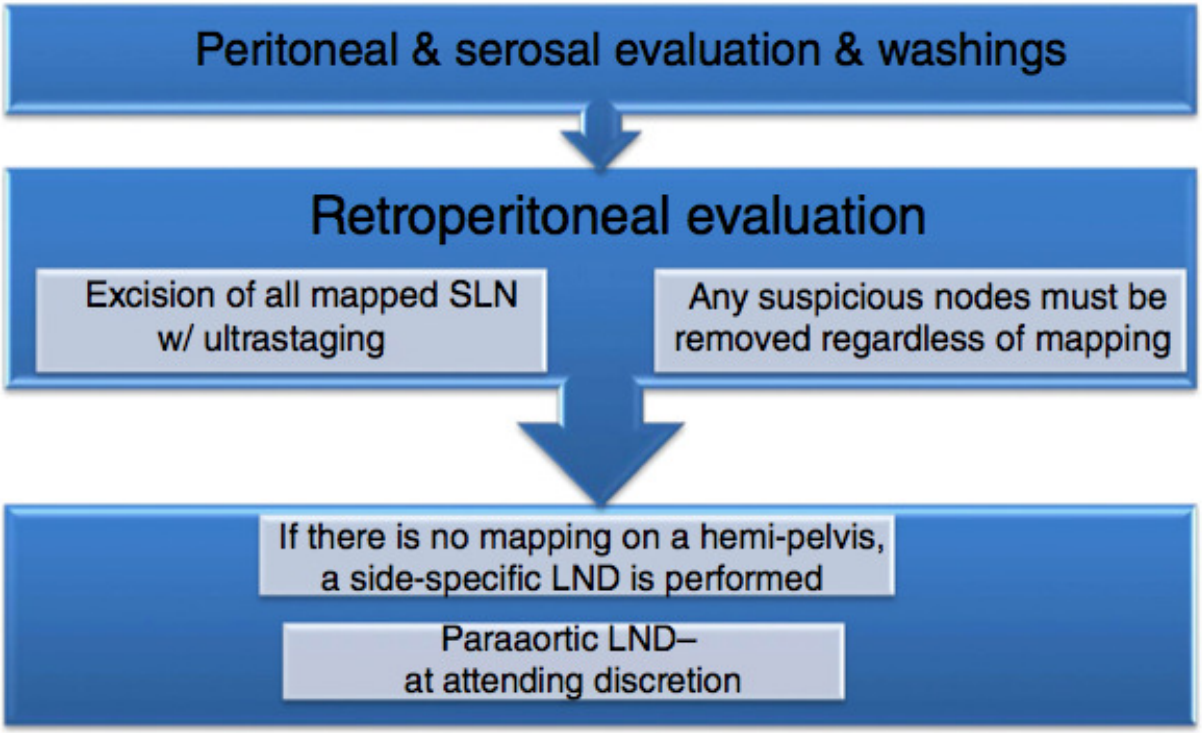
New detection methods and techniques are also currently being investigated, such as the use of fluorescing dye. While further studies are needed to compare these methods to conventional blue dye and isotopes, studies that have utilized ICG in other sites are very promising. ICG is a tricarbocyanine dye that fluoresces in the near infrared spectrum when illuminated with 806nm light. The fluorescent light is then captured with a special video camera device that allows the ICG to be displayed in the visible light spectrum. ICG is highly water-soluble and rapidly binds to plasma proteins, qualities that make it useful for assessment of blood flow [21]. Therefore, it has been used surgically to assess patency of grafts in vascular surgery [22], coronary artery bypass grafting [21], transplant surgery and plastic surgery procedures that require flap reconstruction [23]. Because ICG binds to albumin and therefore has a propensity for lymphatic tissue, in the early 2000's, it was first hypothesized that ICG might be a useful injectant for sentinel node identification. In 2005, Kitai et al investigated the use of ICG for SLN biopsy in breast cancer and were the first to propose that the use of ICG could improve both detection rate and negative predictive value of SLN biopsy in breast cancer [24]. This technique has become more popular, particularly for SLN biopsy in breast cancer [25] and melanoma, and recent data suggests that the lymphatic drainage pattern of ICG is identical to that of radioisotope [26]. There are only 2 studies that have utilized ICG for sentinel node detection in endometrial cancer. The first study found that ICG was superior to isosulfan blue for SLN detection and that by using a

combination of ICG and isosulfan blue, a 100% detection rate could be achieved [27]. The other study was a feasibility study for sentinel lymph node biopsy utilizing robotics in both endometrial and cervical cancer patients. This study showed a comparable detection rate to isotope and blue dye in a small sample [28].

1.4.3 An Algorithm for Sentinel Lymph Node Biopsy

In a recent report by Barlin et al., the use of a sentinel lymph node mapping algorithm for endometrial cancer was introduced. These authors propose that by following this algorithm, the false-negative rate for SLN biopsy can be minimized. This algorithm is as follows: evaluation of the peritoneum and serosa via washings, evaluation of the retroperitoneal lymph nodes via mapping for the SLN and removal of any suspicious node. If there is no mapping to a hemi-pelvis, a complete pelvic lymphadenectomy should be performed on that side. Para-aortic lymphadenectomy should be performed at the discretion of the surgeon (please see Figure 1). Utilizing this algorithm may help to further diminish the false-negative rate of SLN biopsy, even in more high risk stage I endometrial cancers [29].

Figure 1. (From Barlin et al. [29]) Surgical algorithm for endometrial cancer



1.4.4 Surgical Modality and Sentinel Lymph Node Biopsy

To our knowledge, thus far, sentinel lymph node biopsy procedures have been performed by laparotomy, conventional laparoscopy, and conventional robotics. Evidence suggests that minimally invasive techniques, including laparoscopy and robotics, should now be the standard of care for endometrial cancer [30], however, at times, open procedures may also be

necessary. Single-port laparoscopy is also now being utilized for endometrial cancer cases [31], yet, to our knowledge, there are no reports of single-port technology utilized for sentinel lymph node biopsy. While there is still limited prospective data on the benefits of single-port techniques, the available data suggests that outcomes for single-port laparoscopy are similar to those of conventional laparoscopy, with the added benefit of improved cosmesis and decreased pain [32].

The most recent advance in single-port technology is the single-port robot, which is now FDA-approved for gynecologic cases [33]. Robotic technology has provided improved optics, improved range of motion, and a shorter learning curve when compared to conventional laparoscopy [34]. A recent study by Escobar et al. compared LESS surgery to conventional LSC and robotic surgery for endometrial cancer and found no difference in outcomes [35]. In a recent study by Rossi et al, the robot was utilized to perform SLN biopsy in both endometrial and cervical cancers. The findings in this study suggest that robotics might have additional advantages specific for SLN biopsy procedure. This study showed a relatively short operating room time (186 min from patient entry into the room until exiting the room), as well as the ability to utilize new and integrated technologies (a fluorescence imager) [28]. These advantages will also translate to single-port robotics as technology develops [31].

1.5. **Rationale**

By performing sentinel lymph node biopsy for endometrial cancer, we may be able to further minimize the risks of lymph node evaluation, while continuing to obtain critical prognostic information for patients. In this study, we examine detection rates, false negative rates, and negative predictive value of SLN biopsy for endometrial cancer in order to further add to the body of evidence on SLN biopsy for endometrial cancer.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the detection rate, sensitivity, and negative predictive value of SLN biopsy in endometrial cancer patients.

2.2 Secondary Objective(s)

To compare different surgical modalities (open procedures, minimally invasive procedures, and single-site technology) and different injectants (isosulfan blue and indocyanine green) for SLN biopsy.

To determine total operating room time (from the time the patient enters the room to the time the patient leaves the room) as well as console time (robotic)/ operating time for minimally invasive procedures.

3.0 STUDY DESIGN

3.1 Study design

This is a prospective cohort analysis. Consecutive, eligible, consenting patients will be enrolled into the study, and data will be collected on their operative procedure and pathology.

3.2 Number of Subjects

For this study, a sample size of 200 patients will initially be included. This sample size was calculated using the Raosoft sample size calculator online (available at http://www.raosoft.com/samplesize.html). A 5% margin of error was used, with a 95% confidence interval, a population size of 20,000 (as suggested), and a 15% response distribution. This 15% response distribution was chosen based on 2 large randomized trials that showed that the rates of positive pelvic lymph nodes in patients with presumed stage I-II disease were 9% and 13% respectively [36, 37]. In order to ensure an adequate sample size a 15% difference was utilized for this calculation.

3.3 Expected Duration of Subject Participation

Participation in this study will begin at the time of the patient's initial evaluation and will last through the time of their post-operative visit (approximately 6 weeks after surgery)

3.4.1 Duration of Therapy

There will only be one point of intervention, which will be on the day of the patient's surgery.

3.4.2 Duration of Follow Up

Patients will be followed only until the time of their first post-operative visit.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

4.0 PATIENT SELECTION

The form below has also been included as Appendix A:

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The checklist must be completed for each patient and must be signed and dated by the treating physician.

Patient's Name _____

Medical Record # _____

Research Nurse /
Study Coordinator Signature: _____ **Date** _____

Treating Physician [Print] _____

Treating Physician Signature: _____ **Date** _____

4.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment:

4.1.1 Women must have newly diagnosed histologically or cytologically confirmed Endometrial Cancer**.**

4.1.2 Women should have received no prior therapy for their disease

4.1.3 Women who are planning to undergo hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy for the management of their endometrial cancer

4.1.4 Women must have the ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

The presence of any of the following will exclude a patient from study enrollment.

4.2.1 Women who are receiving any other investigational agents.

4.2.2 Women with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to isosulfan blue or indocyanine green or other agents used in this study.

4.2.3 Women with hypersensitivity to phenylmethane compounds, or a history of allergic reaction to iodides

4.2.4 Patients with uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.5 Women with a history of prior LEEP or Cone procedures performed on their cervix

4.2.6 Women with a history of lymphedema, lymphoma, or lymphatic hyperplasia (Castleman disease)

4.2.7 Women with a history of a prior malignancy

4.2.8 Women may also be excluded at the discretion of their surgeon if he or she feels that the patient is not an appropriate candidate.

4.3 **Inclusion of *Women and* Minorities**

Members of all races and ethnic groups are eligible for this trial.

5.0 REGISTRATION

5.1 Registration

All subjects who have been consented are to be entered into the RedCap Database. There will be no further registration that is necessary for this study.

6.0 TREATMENT PLAN

6.1 Isosulfan Blue and Indocyanine Green

Isosulfan blue (Lymphazurin)1% is an aqueous solution that is available in the operating rooms at Cleveland Clinic Main campus and Fairview and Hillcrest campuses. It is supplied as a 5ml single dose vial, 1% aqueous solution in a phosphate buffer, and it is manufactured as a sterile and pyrogen-free product. No special storage or preparation is needed for this dye as it is stored at room temperature. Adverse effects only include hypersensitivity reactions, which occur in approximately 2% of patients. Blue discoloration of the skin may occur. No drug interactions are known [38].

Indocyanine Green is a water-soluble, tricarbocyanine dye that is comes prepared in a sterile solution containing 25mg of indocyanine green and 5% sodium iodide. No special storage is necessary for this dye. It is dissolved using sterile water and titrated to the appropriate concentration desired. For the purposes of this study, a 500µg dose will be prepared. A 25mg vial of ICG will first be reconstituted in 10cc of saline. Once dissolved,1cc of this ICG solution will be added to1.5cc of additional saline. This new solution will therefore contain 2.5mg of ICG in 2.5cc of saline (or 500 µg/0.5 cc). Adverse effects of ICG include anaphylactic or urticarial reactions in patients with a history of iodine allergy and these patients are therefore excluded from this study. The ICG is available in the operating rooms at both the Cleveland Clinic Main campus and Hillcrest and Fairview campuses.

6.2 Intervention

Treatment must be administered only on an inpatient basis.

Patients who consent to participate in this study will be scheduled for their procedure in the normal fashion. Procedures will not be delayed for the purpose of the study. All procedures will be performed via the surgical modality determined by the surgeon.

On the day of surgery, after the patient has been placed under general anesthesia in the operating room, she will be placed in the dorsal lithotomy position, and she will undergo a routine examination under anesthesia. The patient will be prepped and draped in the usual sterile fashion.

Depending upon the surgical modality utilized, access the intra-abdominal cavity will be obtained (either via laparotomy or minimally invasive techniques). Subsequently, attention will be turned to the cervix, where a spinal needle will be used to inject 2ml of isosulfan blue directly into the cervix (1 ml at the 3 o'clock position and 1 ml at the 9 o'clock position) and/ or ICG with 0.5 ml of ICG will be injected at both the 3 o'clock and 9 o'clock positions on the cervix. Following injection, the SLN will be identified and removed in a manner consistent with the algorithm proposed by Barlin et al (see Figure 1.), with sentinel lymph nodes removed from each hemi-pelvis [29]. After all mapped nodes are removed, they will be sent for pathologic examination via frozen section. Results of this analysis will be delivered to the surgeon via telephone call into the operating room. Subsequently, hysterectomy, bilateral salpingo-oophorectomy and a complete pelvic lymphadenectomy will be performed. All specimens removed will be sent to pathology. Para-aortic lymphadenectomy will be performed if the frozen section of the sentinel lymph node comes

back positive for metastasis. Otherwise, para-aortic lymphadenectomy will be performed at the discretion of the surgeon.

If, at any time, the surgeon determines that the above protocol is inappropriate, the procedure will be varied in the best interest of the patient.

The data that will be collected falls into three categories: demographic variables, procedural variables, SLN variables. The demographic variables will be collected at the time of the patient's initial clinic visit. These variables will include: age, race, number of prior abdominal surgeries, and medical comorbidities.

The procedural and SLN variables will be collected at the time of the procedure. The procedural variables that will be collected will include: surgical modality, total operating room time, console time (robotic)/SILS operating time, time from cervical injection to SLN inspection/detection, estimated blood loss, intra-operative complications, conversion to laparotomy (for MIS) (Figure 2.)

Figure 2. Data Collection Sheet: Procedural Variables

Patient ID	Surgical modality	Total OR time	Console/ SILS operating time	Injection – SLN detection	EBL	Complications	Conversion

The console time/ operating time (for MIS procedures) will be documented from the time that the sentinel lymph node dissection begins and include the SLN biopsy, the hysterectomy, bilateral salpingo-oophorectomy, and complete lymph node dissection. All procedural variables collected will be documented at the time of surgery by either the Gynecologic Oncology fellow or the attending surgeon. Pathologic variables will include: the number of SLN detected on each hemipelvis (both by isosulfan blue and by ICG), location of mapped SLNs, number of positive lymph nodes (both SLN and in full lymphadenectomy), total number of pelvic lymph nodes dissected, total number of para-aortic lymph nodes dissected, histologic subtype, depth of myometrial invasion, grade, tumor size, lymphovascular space invasion, final stage of disease.

Reported adverse events and potential risks of Isosulfan Blue and Indocyanine Green are described in Section 7.0.

6.3 **Duration of Therapy**

There will only be one point of intervention, which will be on the day of the patient's surgery.

6.4 **Duration of Follow Up**

Patients will be followed only until the time of their first post-operative visit.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

7.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

The following is a list of AEs (Section 7.1) and the reporting requirements associated with observed AEs (Sections 7.3 and 7.4).

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

7.1 Adverse Events and Potential Risks

7.1.1 Isosulfan Blue (Lymphazurin) 1%

- hypersensitivity reactions - approximately 2% of patients
- blue discoloration of the skin
- No drug interactions are known [38].

7.1.2 Indocyanine Green

- anaphylactic or urticarial reactions in patients with a history of iodine allergy

7.2 Definitions

7.2.1 Adverse Events

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject. In general, adverse events that are at least partially the result of (a) or (b) would be considered related to the research, whereas adverse events solely related to (c) or (d) would be considered unrelated to the research.

External adverse events are adverse events experienced by subjects enrolled in multicenter clinical trials at sites other than the site(s) over which the Institutional Review Board has jurisdiction.

Internal adverse events are adverse events experienced by subjects enrolled at the site(s) under the IRB's jurisdiction for either multicenter or single-center research projects.

7.2.2 The significance of an adverse event is used to describe the patient/event outcome or action criteria associated with events that pose a threat to a patient's life or functioning (i.e., moderate, severe or life threatening). Based on the National Cancer Institute Guidelines for the Cancer Therapy Evaluation Program, severity can be defined by the following grades of events:

Grades 1 are mild adverse events. (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance)

Grades 2 are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 3 are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 4 are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life–threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

Grades 5 are fatal adverse event resulting in death.

7.2.3 Serious Adverse Events

A **serious adverse event** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 12 hours OR

- The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
- The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.2.4 Expectedness

Adverse Events can be Expected or Unexpected.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

7.2.5 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

7.3 Reporting Procedures for All Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. Subjects will be followed for toxicity for 30 days after treatment has been discontinued or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

7.4 Serious Adverse Event Reporting Procedures

Serious adverse events that occur beginning with the signing of the informed consent form, during treatment, or within 30 days of the last dose of treatment must be reported to The Cleveland Clinic Principal Investigator.

Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

7.5 Data Safety Toxicity Committee

It is the Case Comprehensive Cancer Center's Principal Investigator's responsibility to ensure that ALL serious adverse events are reported to the Case Comprehensive Cancer Center's Data Safety Toxicity Committee. This submission is simultaneous with their submission to the Sponsor or other Regulatory body.

8.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 8.0.

8.1 Lymphazurin

Chemical Name: N-[4- [[4-(diethylamino)phenyl] (2,5-disulfophenyl) methylene]-2,5- cyclohexadien-1-ylidene]-N-ethylethanaminium hydroxide

Other Names: Isosulfan Blue

Mode of Action: binds to serum proteins and is picked up by lymphatic vessesls

Metabolism: 10% is excreted unchanged in the urine in 24 hrs

Product description: a sterile aqueous solution for subcutaneous administration. The solution contains no preservative.

Solution preparation: Phosphate buffer in sterile, pyrogen free water is added in sufficient quantity to yield a final pH of 6.8-7.4. Each ml of solution contains 10mg Isosulfan blue, 6.6 mg sodium monohydrogen phosphate and 2.7mg potassium dihydrogen phosphate.

Storage requirements*:* none

Route of administration*:* cervical

Drug Procurement: Isosulfan Blue must be obtained from commercial sources.

8.2 Indocyanine Gree

Chemical Name: sodium 4-[2-[(1*E*,3*E*,5*E*,7*Z*)-7-[1,1-dimethyl-3-(4-sulfonatobutyl)benzo[*e*]indol-2-ylidene]hepta-1,3,5-trienyl]-1,1-dimethylbenzo[*e*]indol-3-ium-3-yl]butane-1-sulfonate

Other Names: Cardiogreen, Foxgreen, IC-Green

Classification: Cyanine Dye

Mode of Action: Peak spectral absorption at about 800 nm and therefore visible in the near infrared spectrum. Requires special camera device to visualize. Binds tightly to plasma proteins and therefore has a propensity for lymphatic tissue

Metabolism: removed by the liver and bile

Product description: A fluorescent dye used in medicine as an indicator substance

Solution preparation: available in a powder form and reconstituted in sterile water

Storage requirements*:* none

Route of administration*:* cervical

Drug Procurement: indocyanine green must be obtained from commercial sources.

9.0 STUDY PARAMETERS AND CALENDAR

9.1 Study Parameters

9.1.1 Screening Evaluation

Screening evaluations for inclusion and exclusion criteria will be used to determine the eligibility of each subject for the study. All evaluations must be completed ≤ 30 days prior to administration of protocol therapy.

9.1.2 Study Period

The study begins at the time of pre-operative evaluation and ends at the time of the post-operative visit.

9.2 Calendar

Screening evaluation will be conducted within 1 day prior to administration of protocol therapy.

10.0 MEASUREMENT OF EFFECT

10.1 Sentinel Node Biopsy Evaluation

For the purposes of this study, the success of sentinel node biopsy will be evaluated by the detection rate of the sentinel lymph node for each patient, the sensitivity of the sentinel node to determine the true lymph node status (metastatic disease or not), and the false negative rate. Definitions for these are below. The comparator for these values will be the detection rate, sensitivity, and false negative rate for breast and vulvar cancers, in which sentinel lymph node biopsy is standard practice. In 2005, the American Society of Clinical Oncology stated that for breast cancer, sentinel lymph node detection rate should reach 85% with a false negative rate $\leq$ 5% [39]. In the most recent randomized trials examining sentinel lymph node biopsy in breast cancer, detection rates ranged from 93-97%. The false negative rates in the SNAC, ALMANAC, and NSABP B-32 trials ranged from 4.7% (ALMANAC) to 9.8% (NSABP B-32) [40, 41, 42]. In vulvar cancer, where sentinel lymph node biopsy has also become more widely accepted, studies similarly report detection rates ranging from 93-98% while false negative rates are approximately 7.7 -8.3% [43,44].

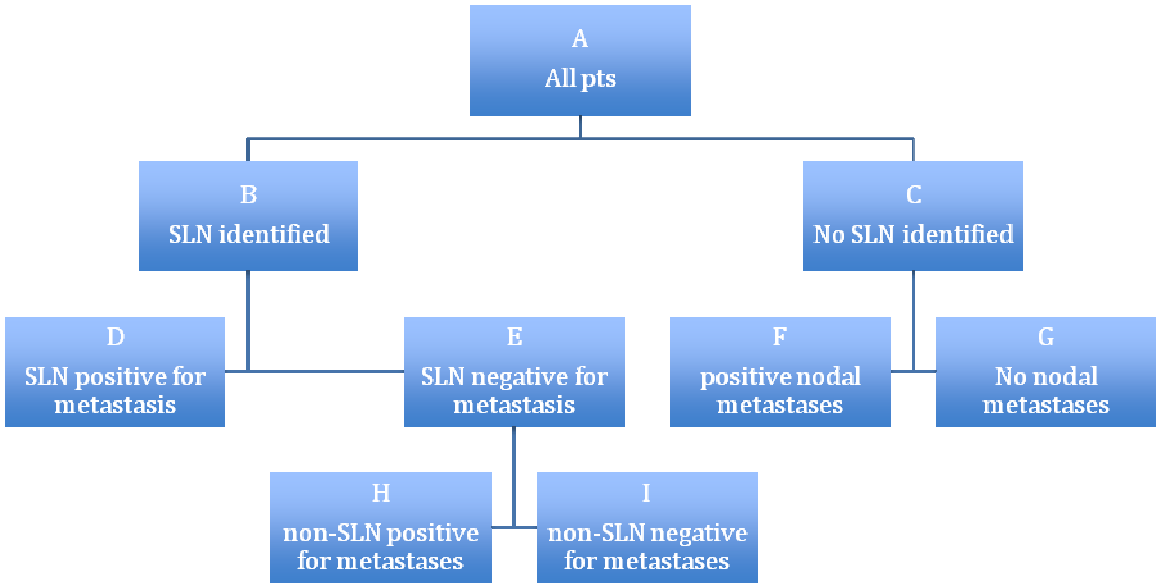
10.1.1 Definitions

Sensitivity, false negative and false negative rate will be limited to those patients in whom a sentinel node is detected.

False negative = a sentinel node that is identified and determined to be negative, however, upon complete lymphadenectomy, a different lymph node is found to be positive

Sensitivity = the number of patients with a positive SLN over those patients with a positive SLN plus those patients with a false negative lymph node

False negative rate = 1-sensitivity or the number of patients with a false negative SLN over the number of patients with a positive SLN plus those with a false negative lymph node



	Our definition	Other definition
FN	H	F+H
FN Rate	H/D+H	F+H/D+F+H
Sensitivity	D/D+H	D/D+F+H
NPV	I/E	-

11.0 RECORDS TO BE KEPT / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

11.1 **Data Reporting**

The RedCap Database will be utilized for data collection for both accrual entry and trial data management. RedCap is a Data Management System housed on secure servers maintained at The Cleveland Clini. Access to data through RedCap is restricted by user accounts and assigned roles. Once logged into the RedCap system with a user ID and password, RedCap defines roles for each user which limits access to appropriate data.

RedCap is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification.

11.2 **Regulatory Considerations**

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

11.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject.

11.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

11.2.3 Retention of records

The Principal Investigator supervises the retention of all documentation of adverse events, case report forms, source documents, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with national and international regulations and

the institution in which the study will be conducted, or for the period specified by the sponsor, whichever is longer. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

11.2.4 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the Center to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

11.2.5 Data Safety and Monitoring Plan

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

12.0 STATISTICAL CONSIDERATIONS

Numerical values will be summarized by mean and standard deviation when the data have an approximately normal distribution; otherwise they will be summarized by median and interquartile range. Normality will be evaluated through visual inspection of histograms, boxplots, and normal QQ plots. Analyses specific to each aim are listed below. Secondary multivariable analysis may included logistic regression using metastasis as the dependent variable with type of dy utilized, surgical modality, surgeon, tumor stage, grade and presence of lymphovascular space invasion as independent variables. Significance will be determined by a p-value less than or equal to 0.05 unless otherwise stated.

Specific Aim 1:

Sensitivity, specificity, and negative predictive value of SLN biopsy for detecting metastasis will be estimated and appropriate 95% confidence intervals for each value will be provided.

Specific Aim 2:

Sensitivity, specificity, and negative predictive value of SLN biopsy for detecting metastasis will be compared between surgical modalities using pairwise comparisons for each surgical modality. Comparisons will be perfomed using two sample tests of proportions based on a normal approximation. Since three such pairwise comparisons are required, significance will be determined by a Bonferonni adjusted significance level of 0.05 / 3 = 0.17.

Specific Aim 3:

Sensitivity, specificity, and negative predictive value of SLN biopsy for detecting metastasis will be compared between injectants utilized using a two sample test of proportions based on a normal approximation.

Specific Aim 4:

Total operating room time will be estimated as a mean with 95% confidence interval if the data have an approximately normal distribution. Otherwise, the median and a bootstrapped 95% confidence interval for the median will be reported. Similar summaries will be provided for the console time (robotic)/ operating time.

Appendix A:

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The checklist must be completed for each patient and must be signed and dated by the treating physician.

Patient's Name _____

Medical Record # _____

Research Nurse /
Study Coordinator Signature: _____ **Date** _____

Treating Physician [Print] _____

Treating Physician Signature: _____ **Date** _____

Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment:

Women must have newly diagnosed histologically or cytologically confirmed Endometrial Cancer**.**

Women should have received no prior therapy for their disease

Women who are planning to undergo hysterectomy, bilateral salpingo-oophorectomy, and possible pelvic lymphadenectomy for the management of their endometrial cancer

Women must have the ability to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

The presence of any of the following will exclude a patient from study enrollment.

Women who are receiving any other investigational agents.

Women with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to isosulfan blue or indocyanine green or other agents used in this study.

Women with hypersensitivity to phenylmethane compounds, or a history of allergic reaction to iodides

Patients with uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

Women with a history of prior LEEP or Cone procedures performed on their cervix

Women with a history of lymphedema, lymphoma, or lymphatic hyperplasia (Castleman disease)

Women with a history of a prior malignancy

Women may also be excluded at the discretion of their surgeon if he or she feels that the patient is not an appropriate candidate.

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