Concurrent Use of Lymphoseek and Indocyanine Green for Sentinel Lymph Node Detection in Endometrial Cancer - a Prospective Study

Study Protocol and Statistical Analysis Plan

NCT04511026

March 9, 2021

Concurrent use of Lymphoseek and Indocyanine Green for sentinel lymph node detection in endometrial cancer – a prospective study

Coordinating Center

Stanford Cancer Institute
875 Blake Wilbur Drive
Stanford, CA 94305

Protocol Director

Amer Karam, MD
Gynecologic Oncology Division
Stanford Cancer Center
650-724-0456
650-736-0902
amkaram@stanford.edu

Co-Investigators

Oliver Dorigo, MD, PhD
Gynecologic Oncology Division
Stanford Cancer Center
650-724-0456
650-736-0902
odorigo@stanford.edu

Nelson Teng, MD, PhD
Gynecologic Oncology Division
Stanford Cancer Center
650-723-7334
650-723-7737
teng@stanford.edu

Elisabeth Diver, MD
Gynecologic Oncology Division
Stanford Cancer Center
650-724-0456
650-736-0902
elisabeth.diver@stanford.edu

Version -2.0/ Version Date: (03/09/2021)

Babak Litkouhi, MD
Gynecologic Oncology Division
Stanford Cancer Center
650-724-0456
650-736-0902
blitkouhi@stanford.edu

Andrei Iagaru, MD
Nuclear Medicine and Molecular Imaging Division
Stanford University School of Medicine
650-725-4711
650-498-5047
aiagaru@stanford.edu

Malte Renz, MD, PhD
Gynecologic Oncology Division
Stanford University School of Medicine
650-724-0456
650-736-0902
renzmalt@stanford.edu

Biostatistician
Stephanie A.Leonard, PhD
Maternal and Fetal Medicine Division
Department of Obstetrics and Gynecology
Stanford University School of Medicine
650-721-4329
stephanie.leonard@stanford.edu

Study Coordinator

Neha Aidasani
Cancer Clinical Trial Office
Stanford Cancer Center
650-723-0622
aidasani@stanford.edu

SRC Approval Date: 02/11/2020

TABLE OF CONTENTS

PROTOCOL SYNOPSIS **5**
SCHEMA **7**
LIST OF ABBREVIATIONS AND DEFINITION OF TERMS **8**
1. OBJECTIVES **9**
1.1 PRIMARY OBJECTIVE 9
1.2 SECONDARY OBJECTIVES 9
2. BACKGROUND **9**
2.1 STUDY DISEASE 9
2.1.1 TRACERS FOR SENTINEL LYMPH NODE DETECTION 10
2.2 STUDY AGENT - LYMPHOSEEK 11
2.3 RATIONALE 11
2.4 STUDY DESIGN 12
3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES **12**
3.1 INCLUSION CRITERIA 12
3.2 EXCLUSION CRITERIA 12
3.3 INFORMED CONSENT PROCESS 12
3.4 STUDY TIMELINE 13
4. TREATMENT PLAN **13**
4.1 CRITERIA FOR REMOVAL FROM STUDY 14
4.2 ALTERNATIVES 14
5. INVESTIGATIONAL AGENT/ PROCEDURE INFORMATION **15**
5.1 LYMPHOSEEK 15
5.1.1 HANDLING AND DISPENSING OF LYMPHOSEEK 15
5.1.2 PREPARATION AND ADMINISTRATION OF LYMPHOSEEK 15
5.2 INDOCYANINE GREEN 16
5.2.1 HANDLING AND DISPENSING OF INDOCYANINE GREEN 16
5.2.2 PREPARATION AND ADMINISTRATION OF INDOCYANINE GREEN 16
5.3 AVAILABILITY 16
6. DOSE MODIFICATIONS **16**
7. ADVERSE EVENTS AND REPORTING PROCEDURES **16**
7.1 POTENTIAL ADVERSE EVENTS 16
7.1.1 POTENTIAL ADVERSE EVENTS ASSOCIATED WITH LYMPHOSEEK 16
7.1.1.1 POTENTIAL RADIATION RISKS WITH LYMPHOSEEK 17
7.1.2 POTENTIAL ADVERSE EVENTS ASSOCIATED WITH INDOCYANINE GREEN 17
7.2 ADVERSE EVENT REPORTING 17
8. STUDY CALENDAR **18**
9. MEASUREMENTS **19**
9.1 PRIMARY AND SECONDARY OUTCOME MEASURES 19
9.2 SECONDARY OUTCOME 20

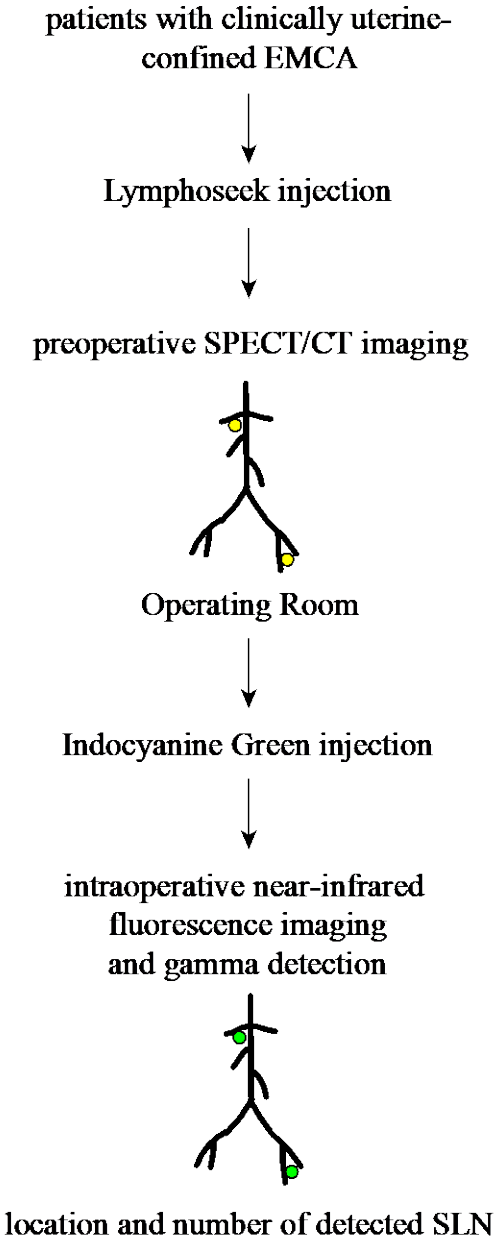
10. REGULATORY CONSIDERATIONS 20
10.1 INSTITUTIONAL REVIEW OF PROTOCOL 20
10.2 DATA AND SAFETY MONITORING PLAN 20
10.3 DATA MANAGEMENT PLAN 21
11. STATISTICAL CONSIDERATIONS 21
11.1 STATISTICAL DESIGN 21
11.2 INTERIM ANALYSES 21
11.3 DESCRIPTIVE STATISTICS AND EXPLORATORY DATA ANALYSIS 21
11.4 PRIMARY ANALYSIS 20
11.4.1 ANALYSIS POPULATION 20
11.4.2 ANALYSIS PLAN 21
11.5 SECONDARY ANALYSIS 22
11.5.1 ANALYSIS POPULATION 21
11.5.2 ANALYSIS PLAN 21
11.6 SAMPLE SIZE 22
11.6 .1 ACCRUAL ESTIMATES 22
11.6 .2 SAMPLE SIZE JUSTIFICATION 22
11.6 .3 EFFECT SIZE JUSTIFICATION 22
12. REFERENCES 24
APPENDICES 25
APPENDIX A: PARTICIPANT ELIGIBILITY CHECKLIST 25

PROTOCOL SYNOPSIS

TITLE	Concurrent use of Lymphoseek and Indocyanine Green for sentinel lymph node detection in endometrial cancer – a prospective study
STUDY PHASE	Feasibility
INDICATION	Sentinel lymph node sampling in clinically uterine confined early-stage endometrial cancer
INVESTIGATIONAL PRODUCT OR PROCEDURE	Concurrent use of Lymphoseek and Indocyanine Green in minimally invasive surgery
PRIMARY OBJECTIVE(S)	To determine the detection rate of bilateral sentinel lymph nodes with the concurrent use of Lymphoseek and Indocyanine Green as compared to each agent alon.
SECONDARY OBJECTIVE(S)	(1) To determine the overall detection rate of sentinel lymph nodes with concurrent use of Lymphoseek and Indocyanine Green (2) To determine location and number of Lymphoseek-positive sentinel lymph nodes preoperatively by single-photon emission computed tomography (SPECT/CT imaging) and intraoperatively by a laparoscopic handheld gamma detection (3) To determine the location and number of Indocyanine Green-positive sentinel lymph nodes by intraoperative infrared fluorescent imaging (4) To determine the concordance of Lymphoseek-positive sentinel lymph nodes with intraoperatively detected Indocyanine Green-positive sentinel lymph nodes, and vice versa, the concordance of Indocyanine Green-positive sentinel lymph nodes with Lymphoseek-positive sentinel lymph nodes. (5) To assess the safety of the concurrent use of Lymphoseek and Indocyanine Green
TREATMENT SUMMARY	Sentinel lymph node assessment - Preoperatively, within 24 hours prior to surgery, the subject will receive in the nuclear medicine suite of Stanford Hospital injections of 0.125 mCi or 0.5 mCi each Lymphoseek into the uterine cervix at 3, 6, 9, and 12 o'clock. Immediate and delayed static planar images will be obtained with and without a transmission source. SPECT-CT of the abdomen and pelvis will be obtained for further characterization. - Subjects will then undergo minimally invasive endometrial cancer surgery. The SPECT/ CT images will be available for review to the surgeon prior to the start of the surgery. - In the operating room, following anesthesia induction, the uterine cervix will be injected with 0.5 mL each of Indocyanine Green at positions of 3, 6, 9, and 12 o'clock. - The surgery will start with the identification and dissection of

	sentinel lymph nodes using near infrared imaging and the gamma probe.
SAMPLE SIZE	30 patients over 1 year
STATISTICAL CONSIDERATIONS	The analysis population will include all patients who complete the study procedures specified above. We do not expect missing data or loss-to-follow up since the outcome is ascertained at the time of surgery. Patients who do not adhere to the study treatment plan of both injections, SPECT/CT and surgery with sentinel lymph node assessment will not be included in the analysis population. To address our primary objective, we plan to enroll 30 patients with concurrent use of lymphoseek and indocyanine green over 1 year. The primary analysis is the calculation of the bilateral SLN detection rate which will be calculated as the number of patients with bilateral SLN detection divided by the total number of patients. With 30 participants and assuming a true bilateral detection rate of 80%, the probability of correctly concluding that our approach has an acceptable bilateral detection rate (i.e., 24 patients being detected with bilateral SLNs) is 61%. The probability of incorrectly concluding this (assuming that the true detection rate is 69%) is low at 13%. We will deem the approach used in this study promising if we observe a bilateral detection rate of at least 80%.

SCHEMA


patients with clinically uterine-confined EMCA
Lymphoseek injection
preoperative SPECT/CT imaging
Operating Room
Indocyanine Green injection
intraoperative near-infrared fluorescence imaging and gamma detection
location and number of detected SLN

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADL	Activities of daily living
AE	Adverse event
BSA	Body surface area
CBC	Complete blood count
CD	Cluster of differentiation
CI	Confidence interval
CRF	Case report/Record form
CTCAE	Common Terminology Criteria for Adverse Events
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
EMCA	Endometrial cancer
FDA	Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
GCP	Good Clinical Practice
GI	Gastrointestinal
Hgb	Hemoglobin
IC-GREEN	Indocyanine Green
IRB	Institutional Review Board
IV	Intravenous
OP	Operative
OR	Operation room
PANES	Preoperative anesthesia
PD	Protocol Director
PLT	Platelet
SAE	Serious adverse event
SLN	Sentinel lymph node
SPECT/CT	Single-photon emission computed tomography
SRC	Scientific Review Committee
Tc 99m	Technetium 99m
WBC	White blood cell
WHO	World Health Organization

1. OBJECTIVES

1.1 Primary Objective

To determine the detection rate of bilateral sentinel lymph nodes with the concurrent use of Lymphoseek and Indocyanine Green.

1.2 Secondary Objectives

(1) To determine the overall detection rate of sentinel lymph nodes with concurrent use of Lymphoseek and Indocyanine Green;
(2) To determine the location and number of Lymphoseek-positive sentinel lymph nodes preoperatively by single-photon emission computed tomography (SPECT/CT imaging) and intraoperatively by a laparoscopic handheld gamma detection device and near-infra-red fluorescent imaging;
(3) To determine the concordance of Lymphoseek-positive sentinel lymph nodes detected by SPECT/CT and/or gamma probe with intraoperatively detected Indocyanine Green-positive sentinel lymph nodes, as well as reverse concordance.
(4) To assess the safety of the concurrent use of Lymphoseek and Indocyanine Green.

2. BACKGROUND

2.1 Study Disease

Endometrial cancer accounts for 50% of all gynecological malignancies in the US. The majority are diagnosed at an early stage. Although clinically locally confined, endometrial cancers may have microscopically spread to lymph nodes; therefore, International Federation of Gynecology and Obstetrics (FIGO) revised the endometrial cancer staging in 1988 to make it a surgical staging system and included a complete pelvic lymphadenectomy [1]. Despite the increased accuracy in staging, complete lymphadenectomy has not been shown in randomized trials to increase progression-free or overall survival [2, 3]. Instead, complete lymphadenectomy carries the risk of increased morbidity, including lymph edema and nerve damage. To reduce this morbidity while maintaining prognostic information, the concept of sentinel lymph node (SLN) sampling has been introduced [4, 5].

Various tracers have been used to identify the first draining lymph node, the sentinel lymph node, and assess for tumor involvement. In the thus-far largest multicenter prospective trial, detection rates for SLNs in minimally invasive surgery for endometrial cancer using Indocyanine Green alone have been reported as only 52% bilaterally and 86% overall [6]. Our own institutional data shows detection rates using Indocyanine Green of 69% and 88%, bilaterally and overall, respectively [7]. If a sentinel lymph node is detected, surgery is restricted to the removal of the SLN and a complete lymphadenectomy is avoided. If no lymph nodes are mapped, current guidelines recommend a complete lymphadenectomy on the side where no sentinel lymph node was detected. Thus, for 30-50% of patients the morbidity of a complete lymphadenectomy cannot be avoided.

2.1.1 Tracers for sentinel lymph node detection

Historically, sentinel lymph node sampling was first described in the late 70's for penile cancer using the injection of radiocolloids [8]. Subsequently, sentinel lymph node sampling has become standard of care in cutaneous melanoma and breast cancer [9, 10]. Vulva cancer was the first gynecologic malignancy where sentinel lymph node sampling was tested [11, 12]. The role of sentinel lymph node mapping in endometrial carcinoma is under evaluation [13].

In endometrial cancer, as in all other cancer entities, radioactive tracers, or dyes, either non-fluorescent or fluorescent, or combinations of radioactive tracers and dyes have been employed. The radioactive tracers used are filtered or non-filtered sulfur colloids, most commonly radiolabeled with technetium-99m (Tc 99m). The major advantage of radioactive tracers is deep tissue penetration and the ability to detect sentinel lymph node locations preoperatively. Reported overall detection rates are 92-96% with a bilateral detection rate of 73-80% [14, 15]. Concerns regarding the use of radioactive colloids are (i) the unspecific and passive distribution of the tracer colloids throughout the lymphatic system by lymph flow and/or diffusion which diminishes the retention of the tracer in the first draining lymph node and results in the leakage of radiotracers to subsequent lymph nodes and thereby the detection of the second or third lymph node stations instead of the actual sentinel lymph node; (ii) the need for preoperative injection and imaging; and (iii) the handling of radioactivity.

To simplify the intraoperative detection and avoid the use of laparoscopic gamma counters, various dyes have become available, i.e. Isosulfan Blue 1%, Methylene Blue 1%, Patent Blue 2.5% sodium. Isosulfan Blue is the only dye that has been FDA-approved for sentinel lymph node detection. Overall and bilateral detection rates have been reported at 63-78% and 40-46%, respectively [16-18]. Disadvantages of Isosulfan Blue are (i) the costs, (ii) a significant risk of an anaphylactic reaction (1.1%) and (iii) the comparably poor deep tissue penetration, especially of fat tissue. There is evidence from breast cancer on the equivalence of Methylene Blue and Isosulfan Blue [19] and reported overall and bilateral detection rate for Methylene Blue in robotic-assisted laparoscopic surgery in endometrial cancer are 86% and 52% [20]. Methylene Blue is less expensive, and has a lower, but still significant risk of allergic reactions. Furthermore, combinations of blue dyes and radioactive tracer colloids have been used in endometrial cancer, with reported overall and bilateral detection rates of 90-97% and 65-76%, respectively [21-23].

Recently, Indocyanine Green has emerged as useful imaging dye that requires a near-infrared camera for localization. One main advantage of a near-infrared fluorescent dye over a blue dye is the improved visibility through visceral fat. Overall and bilateral detection rates have been reported as 86-96% and 52-88%, respectively [6, 16, 18, 24, 25]. Compared to blue dyes, allergic reactions are very rare. Indocyanine Green is now commonly used in many practices [13]. However, the thus-far largest prospective multi-center study, the FIRES study, reports an overall detection rate of 86% and a 52% bilateral detection rate only. Higher detection rates have mainly been reported by single-center studies. Disadvantages of Indocyanine green are (i) the required special equipment, i.e. near-infrared camera, and (ii) the problem of leakage which is inherent to all unspecific radioactive and colored tracers and thereby the diminished retention in the first draining lymph nodes and the possibility of detecting lymph nodes subsequent in the lymph node chain.

2.2 Study agent - Lymphoseek

(99m)Tc-diethylenetriaminepentaacetic acid (DTPA)--mannosyl-dextran (Lymphoseek) was first synthesized and described in 2001 by Vera et al. [26]. In contrast to all other dyes and tracers used in sentinel lymph node detection, this synthetic radiotracer was specifically designed for sentinel lymph node mapping. The molecule has a molecular weight of 35.8 kDa and a diameter of 7.1 nm and consists of a dextran backbone and 8 DTPA groups. The DTPA groups are used for the chelation of Tc 99m Technetium.

55 mannose residues are covalently bound to the dextran backbone. These 55 mannose residues provide high receptor affinity to the mannose receptor CD 206 which is highly enriched on the surface of macrophages and dendritic cells. Lymphoseek has a dissociation constant of 0.12 nmol/L. This low dissociation constant ensures the effective uptake of the agent by the lymph node and improved retention and thus a lower distal lymph node accumulation than seen for the nonreceptor-targeted filtered Tc 99m-sulfur colloid [27]. Accordingly, Lymphoseek accumulates in fewer lymph nodes with higher specificity [28].

Furthermore, the dextran backbone guarantees rapid injection site clearance which entails the rapid diffusion from the injection site through lymph and blood vessels. In fact, neutral dextrans show an interstitial diffusion coefficient 10-fold greater than for proteins of the same hydrodynamic radius, a property which has been attributed to hydrophilicity and flexibility of the dextrans and the absence of charge. A radiotracer that clears more rapidly from the injection site, even if the tracer delivers equal activity to the sentinel node, will provide higher signal-to-noise ratio by reducing the unspecific background and thus better a detection rate than an agent with slower clearance [27].

While Lymphoseek has been FDA-approved in breast cancer, melanoma, and head and neck cancer [29], no published studies exist for the use of Lymphoseek in endometrial cancer.

2.3 Rationale

Lymphoseek is the first FDA-approved receptor-targeted lymphatic mapping agent. Lymphoseek is a small synthetic molecule that carries multiple mannose residues and is specifically recognized by the mannose receptor which is highly enriched on the cell surface of macrophages and dendritic cells. High-affinity receptor binding, guarantees 100% extraction of the agent by the lymph node and abrogates tracer leakage and distal lymph node accumulation. In addition to its SLN superior accumulation as a consequence of specific lymph node uptake, through rapid interstitial passive diffusion and lymph drainage, Lymphoseek clears the injection site within 10 minutes and accumulates in sentinel lymph nodes where it remains bound for up to 30 hours.

We hypothesize that through these different mechanisms of action, the implementation of Lymphoseek into our sentinel lymph node algorithm for endometrial cancer will significantly improve bilateral and overall detection rates. The preoperative imaging using SPECT/CT and not only planar lymphoscintigraphy will furthermore provide critical preoperative information about the location of sentinel lymph nodes, which is especially important in scenarios where the sentinel lymph node maps to atypical locations such as the pre-sacral and para-aortic regions [30]. The true incidence of these less frequent sentinel lymph node locations is unknown, since they cannot be reliably assessed using thus-far available tracers including nonspecific radioactive

tracers, or fluorescent or non-fluorescent dyes. With the a-priori knowledge of the preoperative SPECT/CT imaging, the intraoperative use of Indocyanine Green will continue to visually guide the surgeon in situ. A laparoscopic gamma counter will help confirm thus identified sentinel lymph nodes and the removal in their entirety. We believe that the proposed combined approach of Lymphoseek and Indocyanine Green will improve sentinel lymph node detection rates and thereby decrease the surgical morbidity associated with a complete lymphadenectomy.

2.4 Study Design

This is an open-label, single-center single-group prospective diagnostic trial.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Refer to the Participant Eligibility Checklist in Appendix A.

3.1 Inclusion Criteria

- The patient has histological diagnosis of cancer of the endometrium of any histology or grade.
- The patient should have received no prior treatment for her endometrial cancer.
- The patient has clinically uterine confined disease.
- The patient is a candidate for minimal invasive surgery, with sentinel lymph node assessment with IC-GREEN planned as part of standard of care.
- The patient has an Eastern Cooperative Group (ECOG) performance status of 0-2.
- If age less than or equal to 55 years, the patient has a negative pregnancy test within 72 hours before administration of Lymphoseek, has been surgically sterilized, or has been postmenopausal for at least 1 year.
- The patient has provided written informed consent.
- The patient is at least 18 years of age at the time of consent.

3.2 Exclusion Criteria

- The patient has clinical or radiological evidence of metastatic disease.
- The patient has a known hypersensitivity to Indocyanine Green and/or Lymphoseek.
- The patient has a history of a prior loop electrosurgical excision procedure (LEEP) or cone procedure performed on her cervix.
- The patient has participated in another investigational drug study within 30 days of scheduled surgery.
- The patient has an iodine allergy.
- The patient is pregnant or lactating.

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient

information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Study Timeline

Primary Completion:

The study will reach primary completion 12 months from the time the study opens to accrual.

Study Completion:

The study will reach study completion 24 months from the time the study opens to accrual.

4. TREATMENT PLAN

In this study, the patient will receive cervical injections of Lymphoseek 1 – 24 hours prior to surgery and subsequent SPECT/CT imaging preoperatively. Intraoperatively, following anesthesia induction, Indocyanine Green will be injected into the uterine cervix. Using near-infrared imaging, efferent lymphatic vessels and lymph nodes will be visualized and confirmed by detected radioactivity using a laparoscopic gamma counter. The preoperatively obtained SPECT/CT images will help guide the surgery. The laparoscopic gamma counter will confirm that sentinel lymph node tissue was removed by recording gamma counts before and after lymph node removal.

Subject participation in this study comprises the following:

- **Screening**

    - Screening begins after informed consent is obtained.
    - The screening window is Day 1 to Day 42 after signing of informed consent.
    - Screening assesses the subject's eligibility to participate as determined by inclusion/ exclusion criteria.

- **Sentinel lymph node assessment**

    - Preoperatively, 1 - 24 hrs prior to surgery, the patient will receive in the nuclear medicine suite of Stanford Hospital injections of 0.125 mCi or 0.5 mCi mL each Lymphoseek into the uterine cervix at 3, 6, 9, and 12 o'clock. Then, the patient will undergo SPECT/ CT imaging. Immediate and delayed static planar images will be obtained with and without a transmission source. SPECT-CT of the abdomen and pelvis will be obtained for further characterization. The imaging process will take approximately 1 hour.

- The patient will undergo minimally invasive endometrial cancer surgery. The SPECT/ CT images will be available for review to the surgeon prior to the start of the surgery.
- In the operating room, following anesthesia induction, the uterine cervix of the patient will be injected with about 2.0 mL total of Indocyanine Green. The surgery will start with the identification and dissection of sentinel lymph nodes.

- **Follow up (duration of the study)**
  - The patient will be observed preoperatively after Lymphoseek injection in the nuclear medicine suite and postoperatively in the recovery room for possible allergic reactions or any other adverse events.
  - There will be a routine postoperative visit to be performed that will be conducted in person or remotely via telemedicine between 14-28 days after surgery. This will be a telemedicine visit where the patient will collect the weight, vitals and the physical examination and AE evaluation will be conducted by assistance of the physician remotely. There are no risks to participants for missing any follow up procedures that can't be conducted remotely.

4.1 Criteria for Removal from Study

Subjects MUST discontinue study treatment by cancelling the tilmanocept injection and SPECT/CT scan for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason).
- Any clinical AE, laboratory abnormality, or intercurrent illness that, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject.
- Discovery of metastatic disease in the operating room.
- Pregnancy.
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

All subjects who discontinue study treatment should comply with protocol-specified follow-up procedures as outlined in Section 7.2. The only exception to this requirement is when a subject withdraws consent for all study procedures or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness). If a subject was withdrawn before completing the study, the reason for withdrawal must be entered on the appropriate case report form (CRF) page.

4.2 Alternatives

Injection with Indocyanine Green alone for sentinel lymph node mapping and biopsy in clinical uterine confined endometrial cancer is currently considered standard of care.

5. INVESTIGATIONAL AGENTS

5.1 Lymphoseek

Kit for the preparation of Lymphoseek contains five Tilmanocept Powder vials, each contain 250 mcg of tilmanocept from which 50 mcg is intended for administration to a patient.

5.1.1 Handling and dispensing of Lymphoseek

Lymphoseek must be stored in a secure area according to local regulations. It must be stored in the original packaging at controlled room temperature 20-25° C, excursions permitted to 15-30° C. Radiolabeled Lymphoseek must be stored in radiation shielding at room temperature and used within 6 hours of preparation.

Waterproof gloves, effective radiation shielding, and appropriate safety measures will be used when preparing and handling Lymphoseek. As a radiopharmaceutical Lymphoseek will be used under the control of physicians who are qualified by specific training and experience in the safe use and handling of radionuclides, and whose experience and training have been approved by the appropriate governmental agency authorized to license the use of radionuclides.

5.1.2 Preparation and administration of Lymphoseek

Each patient will receive 4 injections of 0.125 mCi into the cervix at the 12, 3, 6, and 9 o'clock positions for the same day of surgery (0.125 mCi x 4 or 0.5 mCi +/- 20%) for the same day of surgery.

Each patient will receive 4 injections of 0.5 mCi into the cervix at 12, 3, 6, and 9 o' clock positions for the next day surgery (0.5 mCi x 4 or 2.0 mCi +/- 20%) for the next day of surgery.

As per manufacturer's instructions, the reconstitution volume will be 2.5 mL.

Radiolabeling

Technetium Tc 99m pertechnetate, the sodium injection solution from a technetium Tc 99m generator within 8 hours of its elution will be used. Using a sterile syringe, approximately 92.5 MBq (2.5 mCi) of Technetium Tc 99m pertechnetate sodium injection solution will be drawn in 0.7 mL volume. The syringe will be assayed for technetium Tc 99m activity in a dose calibrator. The radioactivity amount, the vial volume, date and time, expiration time and lot number will be written on the radioactive product vial label and affixed to the Tilmanocept Powder vial. The vial will be placed in a radiation shield, the septum sanitized with an alcohol wipe.

Under aseptic conditions the Technetium Tc 99m pertechnetate sodium injection solution will be added to the Tilmanocept Powder vial. Without withdrawing the needle, an equal volume of headspace gas will be removed. After removing the needle, the vial will be gently shaken to mix contents and then incubated at room temperature for at least 15 minutes.

Reconstitution

Then, the supplied DILUENT for Lymphoseek will be added aseptically to the radiolabeled product in the Tilmanocept Powder vial to bring the volume to the reconstituted vial volume of 2.5 mL prior to filling the patient dose in syringe(s). To normalize pressure, withdraw an equal volume of headspace gas.

For quality control, the reconstituted vial will be assayed for total radioactivity using a dose calibrator. The activity of the syringes will be assayed in the dose calibrator before application to the patient.

Physical Characteristics

Technetium Tc 99m decays by isomeric transition with a half-life of approximately 6 hours. The principal photon that is useful for detection and imaging studies is Gamma-2 with a mean energy of 140.5 keV and a mean disintegration of 89.1%.

External Radiation

The linear mass energy absorption attenuation coefficient for Tc 99m is 18.9 cm^{-1}. The first half-value layer is 0.037 cm of lead (Pb). The use of a 0.25 cm thick standard radiation lead shield will attenuate the radiation emitted by millicurie amounts of technetium Tc 99m by a factor of about 100. The half-life of technetium Tc 99m is approximately six hours.

5.2 Indocyanine Green

Indocyanine Green (IC-GREEN®) is a sterile, lyophilized green powder containing 25 mg of Indocyanine Green with no more than 5% sodium iodide. Indocyanine Green (IC-GREEN®) is supplied in a kit containing six 25-mg Indocyanine Green (IC-GREEN®) vials and six 10-mL sterile water for injection, USP ampules.

5.2.1 Handling and dispensing of Indocyanine Green

As with all injectable drugs, care should be taken when handling and preparing Indocyanine Green. Whenever possible, it should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of injectable agents, applying aseptic technique.

The Indocyanine Green (IC-GREEN®) powder should be dissolved with the sterile water for injection, USP provided and the solution should be used within 6 hours after it is prepared. The Indocyanine Green (IC-GREEN®) kit will be stored at 20-25°C.

5.2.2 Preparation and administration of Indocyanine Green

Cervical injection by the investigator of 0.5 mL each of the 0.5 mg/mL Indocyanine Green solution at 12, 3, 6, and 9 o'clock follows anesthesia induction.

5.3 Availability

Lymphoseek will be provided already reconstituted and radiolabeled by outside pharmacy. Indocyanine Green (IC-GREEN ®) will be provided by the Pharmacy of Stanford Hospital and

prepared in the operating room by the registered nurse in the usual fashion.

6. DOSE MODIFICATIONS

There will not be any dose modifications for either Lymphoseek or Indocyanine Green. If anaphylactic or urticarial reactions occur, the patient will be treated with the appropriate agents, e.g. epinephrine, antihistamines, and corticosteroids.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

7.1.1 Potential Adverse Events associated with Lymphoseek

The most common adverse reactions (incidence <1%) are injection site irritation and/or pain. In open label, single-arm clinical trials, 553 patients with either breast cancer, melanoma, or squamous cell carcinoma of the oral cavity, skin, lip received Lymphoseek. No patient experienced serious adverse reaction. Injection site irritation (0.7%) and pain (0.2%) were reported [29].

Lymphoseek may pose a risk of hypersensitivity reactions due to its chemical similarity to dextran. No hypersensitivity reactions have been reported in the literature.

7.1.1.1 Radiation Risks with Lymphoseek

Any radiation-emitting product may increase the risk for cancer. Adherence to dose recommendations and safe handling will minimize the risk of excessive radiation exposure to either patients or health care workers which includes the radiation exposure in the operating room (OR). No additional protection for the OR staff is required.

7.1.2 Potential Adverse Events associated with Indocyanine Green

Most common adverse reactions are anaphylactic or urticarial reactions. They have been reported in patients with and without history of allergy to iodine. If such reactions occur, the patient will be treated with the appropriate agents, e.g. epinephrine, antihistamines, and corticosteroids. In breast cancer and melanoma, extensive information on Lymphoseek's safety exist but we will continue to monitor and report its safety in this population. Monitoring for adverse events to Lymphoseek will start after its injection into the uterine cervix.

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v4.03. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent,

Protocol Document, or Investigator's Brochure, and related to the investigation. All Serious Adverse Events (SAEs) will be tracked until resolution, or until 30 after the last dose of the study treatment.

SAEs CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

8. STUDY CALENDAR

	Pre-Study 0 – 42 days before surgery	Pre-OP 1 – 24 hours before surgery	Intra- OP	Post-OP/ Off Study[d] 14 – 28 days after surgery
Lymphoseek injection		X[a]		
IC-GREEN injection			X[b]	
Informed consent	X			
Demographics	X			
Medical history	X			
Physical exam	X			X
Vital signs	X			X
Height	X			
Weight	X			X
Performance status	X			
Adverse event evaluation		X	X	X
SPECT/CT imaging		X		
Laparoscopic gamma detection			X	
Laparoscopic near-infrared fluorescence detection			X	
B-HCG		X[c]		

a: Lymphoseek: 0.125 mCi x 4 each at 3, 6, 9, 12 o'clock (or 0.5 mCi +/- 20%) in the uterine cervix for same day surgery;
0.5 mCi x 4 each at 3, 6, 9, 12 o'clock (or 2.0 mCi +/- 20%) in the uterine cervix for next day surgery
b: IC-GREEN: injection of 0.5 mL each at 3, 6, 9, 12 o'clock in the uterine cervix after induction of anesthesia.
c: Serum or urine pregnancy test (women of childbearing potential).
d: Off-study evaluation at postop visit, i.e., 14 – 28 days after surgery, in person or remote telemedicine visit.

9. MEASUREMENTS

9.1 Primary Outcome

Bilateral SLN Detection (yes/no): For each subject, an assessment will be made of whether there was bilateral sentinel lymph node detection. A sentinel lymph node will be defined as being detected if it is detected on the pre-operative SPECT/CT imaging scan or intraoperatively using near-infrared imaging or the handheld gamma detection device. If at least one SLN is detected on each side, then this outcome will be considered a 'Yes'.

9.2 Secondary Outcome

(1) Overall detection (yes/no): For each subject, an assessment will be made of whether there was overall sentinel node detection. A sentinel lymph node will be defined as being detected if it is detected on the pre-operative SPECT/CT imaging scan or intraoperatively using near-infrared imaging or the handheld gamma detection device. If at least one SLN is detected (on any side), then this outcome will be considered a 'Yes'.

(2) Location and number of SLNs detected by Lymphoseek: The location and number of sentinel lymph nodes detected by Lymphoseek will be assessed. A sentinel lymph node will be defined as being detected by Lymphoseek if it is detected using the pre-operative SPECT/CT imaging scan and/or intraoperatively using the gamma detection device.

(3) Location and number of SLNs detected by Indocyanine Green: The location and number of sentinel lymph nodes detected by Indocyanine Green will be assessed. A sentinel lymph node will be defined as being detected by Indocyanine Green if it is detected intraoperatively using near-infrared imaging only and not by the gamma detection device.

(4) Adverse events and severe adverse events will be monitored and reported.

10. REGULATORY CONSIDERATIONS

10.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

10.2 Data and Safety Monitoring Plan

The investigators will review AEs and SAEs in real time and report these events according to the Stanford Cancer Center Cancer Clinical Trials Office Standard Operating procedures.

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the

monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

10.3 Data Management Plan

The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document outcomes for data analysis. Case report forms will be developed and maintained both on paper and electronically using the REDCap database system by the clinical research team. CRFs will be kept in a locked office, only accessible to the research team.

11. STATISTICAL CONSIDERATIONS

11.1 Statistical Design

This is an open-label, single cohort, feasibility study to assess the detection rate of bilateral sentinel lymph nodes with concordant use of Lymphoseek and Indocyanine Green in patients with endometrial cancer.

11.2 Interim analyses

No interim analysis will be conducted.

11.3 Descriptive Statistics and Exploratory Data Analysis

We will provide descriptive statistics for the study population. The primary and secondary outcomes will be summarized using frequencies and percentages.

11.4 Primary Analysis

11.4.1. Analysis Population

The analysis population will include all patients who complete the study procedures specified above. We do not expect missing data or loss-to-follow up since the outcome is ascertained at the time of surgery. Patients who do not adhere to the study treatment plan of both injections, SPECT/CT and surgery will not be included in the analysis.

11.4.2. Analysis Plan

The analysis for this study is primarily descriptive as the primary objective is to determine the detection rate of bilateral SLNs. The bilateral SLN detection rate will be calculated as the number of patients with bilateral SLN detection divided by the total number of patients. If the bilateral detection rate is at least 80%, we will deem this approach promising in detecting bilateral SLNs.

11.5. Secondary Analysis

11.5.1 Analysis Population

The population for secondary analysis will include all patients in the safety population, i.e. the analysis population plus those women who underwent lymphoseek injection but were removed from the study for any reason.

11.5.2 Analysis Plan

Descriptive statistics will be used to analyze the secondary outcomes. The overall detection rate of sentinel lymph nodes will be calculated as the number of patients with overall SLN detection divided by the total number of patients. To assess the safety of the concurrent use Lymphoseek and Indocyanine Green, we will calculate the number and rate of adverse events. We will describe the location and number of Lymphoseek-positive SLNs by method of detection (pre-operative or intraoperative). We will calculate the number of patients with SLNs found by Lymphoseek and by Indocyanine Green and conduct a McNemar's test (or mid-*p* McNemar's test) to determine the concordance of Lymphoseek-positive and Indocyanine Green-positive sentinel lymph nodes.

11.6 Sample Size

11.6.1 Accrual estimates

100 patients are seen annually at our gynecologic oncology clinics and would be considered eligible to participate in this study. We expect 33% to be willing to participate in the study. We anticipate accruing 30 patients over one year/s.

11.6.2 Sample size justification

To address our primary objective, we plan to enroll 30 patients who have concurrent use of lymphoseek and indocyanine green. The sample size is based on a convenience sample from patients routinely followed in the Stanford gynecologic oncology clinic. With 30 participants and assuming a true bilateral detection rate of 80%, the probability of correctly concluding that our approach has an acceptable bilateral detection rate (i.e. 24 patients being detected with bilateral SLNs) is 61%. The probability of incorrectly concluding this (assuming that the true detection rate is 69% - the rate observed with current practice at Stanford) is low at 13%. We will deem the

approach used in this study promising if we observe a bilateral detection rate of at least 80%. The exact 95% confidence interval for the assumed bilateral detection rate of 80% is 61% to 92%.

11.6.3 Effect size justification

The bilateral detection rate using current techniques at Stanford is approximately 69%. Based on this, we would consider a bilateral detection rate of 80% to be promising.

12. REFERENCES

1. Mikuta, J.J., *International Federation of Gynecology and Obstetrics staging of endometrial cancer 1988.* Cancer, 1993. **71**(4 Suppl): p. 1460-3.
2. Benedetti Panici, P., et al., *Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial.* J Natl Cancer Inst, 2008. **100**(23): p. 1707-16.
3. group, A.s., et al., *Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study.* Lancet, 2009. **373**(9658): p. 125-36.
4. Abu-Rustum, N.R., et al., *Sentinel lymph node mapping for grade 1 endometrial cancer: is it the answer to the surgical staging dilemma?* Gynecol Oncol, 2009. **113**(2): p. 163-9.
5. Holloway, R.W., et al., *Sentinel lymph node mapping and staging in endometrial cancer: A Society of Gynecologic Oncology literature review with consensus recommendations.* Gynecol Oncol, 2017. **146**(2): p. 405-415.
6. Rossi, E.C., et al., *A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study.* Lancet Oncol, 2017. **18**(3): p. 384-392.
7. Renz, M., et al., *Immediate intraoperative sentinel lymph node analysis by frozen section is predictive of lymph node metastasis in endometrial cancer.* J Robot Surg, 2019.
8. Cabanas, R.M., *An approach for the treatment of penile carcinoma.* Cancer, 1977. **39**(2): p. 456-66.
9. Krag, D.N., et al., *Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial.* Lancet Oncol, 2010. **11**(10): p. 927-33.
10. Giuliano, A.E., et al., *Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial.* JAMA, 2011. **305**(6): p. 569-75.
11. Levenback, C.F., et al., *Lymphatic mapping and sentinel lymph node biopsy in women with squamous cell carcinoma of the vulva: a gynecologic oncology group study.* J Clin Oncol, 2012. **30**(31): p. 3786-91.
12. Oonk, M.H., et al., *Size of sentinel-node metastasis and chances of non-sentinel-node involvement and survival in early stage vulvar cancer: results from GROINSS-V, a multicentre observational study.* Lancet Oncol, 2010. **11**(7): p. 646-52.
13. Network, N.C.C., *NCCN clinical practice guidelines in oncology (NCCN Guidelines) Uterine Neoplasms.* NCCN.org, 2017. **version 1.2017**.
14. Niikura, H., et al., *Tracer injection sites and combinations for sentinel lymph node detection in patients with endometrial cancer.* Gynecol Oncol, 2013. **131**(2): p. 299-303.
15. Sawicki, S., P. Lass, and D. Wydra, *Sentinel Lymph Node Biopsy in Endometrial Cancer--Comparison of 2 Detection Methods.* Int J Gynecol Cancer, 2015. **25**(6): p. 1044-50.
16. Tanner, E.J., et al., *Factors associated with successful bilateral sentinel lymph node mapping in endometrial cancer.* Gynecol Oncol, 2015. **138**(3): p. 542-7.
17. Holloway, R.W., et al., *A Prospective Cohort Study Comparing Colorimetric and Fluorescent Imaging for Sentinel Lymph Node Mapping in Endometrial Cancer.* Ann Surg Oncol, 2017. **24**(7): p. 1972-1979.
18. Sinno, A.K., et al., *A comparison of colorimetric versus fluorometric sentinel lymph node*

mapping during robotic surgery for endometrial cancer. Gynecol Oncol, 2014. **134**(2): p. 281-6.
19. Blessing, W.D., et al., *A comparison of methylene blue and lymphazurin in breast cancer sentinel node mapping.* Am J Surg, 2002. **184**(4): p. 341-5.
20. Desai, P.H., et al., *Accuracy of robotic sentinel lymph node detection (RSLND) for patients with endometrial cancer (EC).* Gynecol Oncol, 2014. **135**(2): p. 196-200.
21. Ballester, M., et al., *Detection rate and diagnostic accuracy of sentinel-node biopsy in early stage endometrial cancer: a prospective multicentre study (SENTI-ENDO).* Lancet Oncol, 2011. **12**(5): p. 469-76.
22. Bats, A.S., et al., *The sentinel node technique detects unexpected drainage pathways and allows nodal ultrastaging in early cervical cancer: insights from the multicenter prospective SENTICOL study.* Ann Surg Oncol, 2013. **20**(2): p. 413-22.
23. Touhami, O., et al., *Predictors of non-sentinel lymph node (non-SLN) metastasis in patients with sentinel lymph node (SLN) metastasis in endometrial cancer.* Gynecol Oncol, 2015. **138**(1): p. 41-5.
24. Jewell, E.L., et al., *Detection of sentinel lymph nodes in minimally invasive surgery using indocyanine green and near-infrared fluorescence imaging for uterine and cervical malignancies.* Gynecol Oncol, 2014. **133**(2): p. 274-7.
25. Plante, M., et al., *Sentinel node mapping with indocyanine green and endoscopic near-infrared fluorescence imaging in endometrial cancer. A pilot study and review of the literature.* Gynecol Oncol, 2015. **137**(3): p. 443-7.
26. Vera, D.R., et al., *A synthetic macromolecule for sentinel node detection: (99m)Tc-DTPA-mannosyl-dextran.* J Nucl Med, 2001. **42**(6): p. 951-9.
27. Baker, J.L., et al., *Comparison of [(99m)Tc]tilmanocept and filtered [(99m)Tc]sulfur colloid for identification of SLNs in breast cancer patients.* Ann Surg Oncol, 2015. **22**(1): p. 40-5.
28. Wallace, A.M., et al., *Lymphoseek: a molecular radiopharmaceutical for sentinel node detection.* Ann Surg Oncol, 2003. **10**(5): p. 531-8.
29. FDA, *Prescribing information on Lymphoseek*. 2016.
30. Marcinow, A.M., et al., *Use of a novel receptor-targeted (CD206) radiotracer, 99mTc-tilmanocept, and SPECT/CT for sentinel lymph node detection in oral cavity squamous cell carcinoma: initial institutional report in an ongoing phase 3 study.* JAMA Otolaryngol Head Neck Surg, 2013. **139**(9): p. 895-902.

APPENDICES

APPENDIX A**: Participant Eligibility Checklist**

A Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist must be retained in the patient's study file and the study's Regulatory Binder.

The study coordinator, treating physician and an independent reviewer must verify that the participant's eligibility is accurate, complete, and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

Protocol Title:	**Concurrent use of Lymphoseek and Indocyanine Green for sentinel lymph node detection in endometrial cancer – a prospective trial**
Protocol Number:	
Principal Investigator:	**Amer Karam, MD**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. Age 18 or older	☐	☐	
2. Patient has histological diagnosis of cancer of the endometrium.	☐	☐	
3. Patient should have received no prior treatment.	☐	☐	
4. Patient has a clinical negative node status at study entry.	☐	☐	
5. Patient is a candidate for minimal invasive surgery with lymph node assessment.	☐	☐	
6. Eastern Cooperative Group (ECOG)	☐	☐	

performance status of 0-2			
7. If age less than or equal to 55 years, the patient has a negative pregnancy test within 72 hours before administration of Lymphoseek, has been surgically sterilized, or has been postmenopausal for at least 1 year.	☐	☐	
8. Willing and able to provide a written informed consent document for the trial.	☐	☐	
Exclusion Criteria (From IRB approved protocol)			
1. Patient has participated in another investigational drug study within 30 days of scheduled surgery.	☐	☐	
2. Patient has clinical or radiological evidence of metastatic disease.	☐	☐	
3. Patient has a history of a prior loop electrosurgical excision procedure (LEEP) or cone procedure performed on her cervix.	☐	☐	
4. Patient has a history of previous abdominal retroperitoneal surgery or abdominal/pelvic lymphadenectomy.	☐	☐	
5. Patient has a known hypersensitivity to Indocyanine Green and/or Lymphoseek.	☐	☐	
6. The patient has an iodine allergy	☐	☐	
7. The patient is pregnant or lactating.	☐	☐	

*****All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	