Clinical Trial Protocol: NAV3-19

Study Title:	A Prospective, Open-Label Trial of Comparing the Pathology Status of Lymphoseek®-Identified Sentinel Lymph Nodes Relative to the Pathological Pathology Status of Nonsentinel Lymph Nodes in Nodal Staging of Subjects With Known Cancer of the Cervix Who Are Undergoing Lymph Node Dissection
Study Number:	NAV3-19
Study Phase:	2
Product Name:	Lymphoseek®
[redacted]	[redacted]
Investigators:	Multicenter
Sponsor:	Navidea Biopharmaceuticals
[redacted]	[redacted]

	Date
Original Protocol:	27 May 2015
Amendment 1:	03 November 2015

Confidentiality Statement

This protocol and all of the information relating to it are confidential and proprietary property of Navidea Biopharmaceuticals

SYNOPSIS

Study title	A Prospective, Open-Label Trial of Comparing the Pathology Status of Lymphoseek®-Identified Sentinel Lymph Nodes Relative to the Pathological Pathology Status of Nonsentinel Lymph Nodes in Nodal Staging of Subjects With Known Cancer of the Cervix Who Are Undergoing Lymph Node Dissection
Study phase	Phase 2
Study objective(s)	**Primary** Estimation of the per subject false negative rate (FNR) associated with the pathology status of Lymphoseek-identified sentinel lymph nodes (SLNs) relative to the pathology status of non-SLNs in the pelvic lymphadenectomy. **Secondary** • Estimation of sensitivity, negative predictive value (NPV), and overall accuracy of Lymphoseek-detected SLNs relative to the pathology status of non-SLNs. • Estimation of detection rate of SLNs by Lymphoseek and the rate of tumor detection in non-SLNs. • Determination of the proportion of lymph nodes identified intraoperatively by a dye (e.g., vital blue dye [VBD] or indocyanine green [ICG])that are also identified intraoperatively by Lymphoseek (i.e., concordance using the number of lymph nodes stained by the dye as the denominator). • Determination of reverse concordance comparing dye to Lymphoseek (i.e., using the number of lymph nodes identified intraoperatively by Lymphoseek as the denominator). • Comparison of pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases for Lymphoseek and dye on a per-subject basis. • Comparison of imaging and surgical findings **Safety** • Evaluation of the incidence of adverse events
Study drug	Lymphoseek®
Dose(s)	**50 µg Lymphoseek radiolabeled with 2.0 mCi (74 MBq) 99mTc**

Route of administration	Intramucosal peritumoral injection in the subepithelial layer of the cervix
Duration of treatment	Single administration
Control	Pathology status of non-SLNs in the pelvic lymphadenectomy
US Indication	Lymphoseek is a radioactive diagnostic agent indicated with or without scintigraphic imaging for: • Lymphatic mapping using a handheld gamma counter to locate lymph nodes draining a primary tumor site in patients with solid tumors for which this procedure is a component of intraoperative management. • Guiding sentinel lymph node biopsy using a handheld gamma counter in patients with clinically node negative quamous cell carcinoma of the oral cavity, breast cancer, or melanoma.
Main criteria for inclusion	1. Subject has provided written informed consent with HIPAA authorization 2. Has cervical cancer and is a candidate for surgical intervention, with lymph node dissection being a part of the surgical plan. 3. Is at least 18 years of age at the time of consent 4. Has an ECOG performance status of Grade 0 to 2 5. Has the following International Federation of Gynecology and Obstetrics (FIGO) IA2-IIA1 (≤ 4 cm) staging(Appendix 5). Subjects with a single enlarged/suspicious node on PET/CT will still be considered eligible as consistent with FIGO guidelines. 6. If of childbearing potential, the subject has a negative pregnancy test within 48 hours before administration of Lymphoseek, has been surgically sterilized, or has been postmenopausal for at least 1 year
Main criteria for exclusion	1. The subject has had preoperative chemotherapy, immunotherapy, or radiation therapy within the 30 days prior to Lymphoseek administration 2. Has had previous surgery or radiation to node basins that would be involved in the ILM procedure 3. Has a known allergy to dextran 4. Is breast-feeding or pregnant 5. Before the administration of Lymphoseek, has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical 6. Is scheduled for surgery and/or another invasive procedure other than the primary surgical intervention within the 3 days after Lymphoseek administration

	7. Has received an investigational product within the 30 days prior to Lymphoseek administration
Study design	Prospective, open-label, within-patient, multi-center study of Lymphoseek in the detection of lymph nodes in subjects with known cancer of the cervix. All subjects will receive a single dose of 50 µg Lymphoseek radiolabeled with 2 mCi (74 MBq) Tc 99m.
Methodology	The proposed study includes: screening, enrollment, pre- and post-injection assessments, injection (Lymphoseek and dye), imaging and surgery. **Screening Visit (Day -29 to -1):** The screening visit will include review of trial eligibility, informed consent, collection of medical history, vital signs, physical exams, clinical lab results, and review of medications. **Before Enrollment**: Within 48 hours before Lymphoseek injection, a urine or serum pregnancy test will be performed for subjects of childbearing age. **Before Lymphoseek Injection:** Vital signs will be collected. **Lymphoseek Injection:** Subjects will receive a single dose of 50 µg Lymphoseek radiolabeled with 2 mCi (74 MBq) 99mTc at least 15 minutes but no more than 15 hours before the start of the surgical procedure. Lymphoseek will be administered peritumorally by intramucosal injection. **Post-injection:** Vital signs will be collected. Lymphoscintigraphy will be completed prior to surgery and no later than 20 hours after Lymphoseek injection. **Surgery:** Dye (e.g., VBD or ICG) may be administered in the operating room immediately before surgery. Fifteen minutes to 20 hours after Lymphoseek injection, subjects will undergo surgery to remove the primary tumor, and lymphatic mapping will be performed. **36 Hour Post-injection Follow-up:** Vital sign assessment, physical examination, and review of adverse events and medications will be performed.

Planned trial dates	**Start of study** June 2015	**End of Study** July 2016
Planned number of trial centers / countries	Approximately 4 to 6 sites in the United States	
Number of subjects	Approximately 40 subjects will be enrolled. This sample size was chosen in order to provide exploratory results for the primary endpoint of FNR within this subject population. This study is not powered with respect to testing any statistical hypotheses.	
Primary endpoint	Per subject FNR	
Secondary endpoints	• Per-subject sensitivity, NPV, and accuracy • Rate of tumor detection in SLNs and non-SLNs • Proportion of lymph nodes identified intraoperatively by dye (e.g., VBD or ICG) that are also identified intraoperatively by Lymphoseek (i.e., nodal concordance) • Proportion of lymph nodes identified intraoperatively by Lymphoseek that are also identified intraoperatively by dye (i.e., reverse nodal concordance) • Number of identified lymph nodes per subject by tracing agent (Lymphoseek or dye), per-subject concordance, and per-subject reverse concordance • Comparison of pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases for Lymphoseek and dye on a per-subject basis. • Number and anatomical locations of lymph nodes identified by preoperative Lymphoscintigraphy • Incidence of adverse events	
Plan for statistical analysis	The following analysis populations will be defined for the study: • Intent-to-Treat (ITT): enrolled subjects with administration of test medicine for whom at least one lymph node was removed with histopathology available • Per protocol (PP): ITT subjects without major protocol violations • Safety: enrolled subjects with administration of test medicine Primary endpoint primary analysis: The per subject FNR will	

	be computed using pathologically positive lymph nodes from all subjects in the ITT population. Along with reporting the number of false negative subjects and the observed FNR, an exact two-sided 95% confidence interval will be computed. A sensitivity analysis of the primary endpoint will be computed using the PP population. All analyses of safety will be conducted on the safety population.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

#	number of (also N or n)
AE	adverse event
ALARA	as low as reasonably achievable
°C	degrees Celsius
cm	centimeters (10^{-2} m)
CRA	clinical research associate
CRO	contract reseach organization
CRF	case report form
DTPA	diethylenetriaminepentaacetic acid
EANM	European Association of Nuclear Medicine
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
°F	degrees Fahrenheit
FIGO	International Federation of Gynecology and Obstetrics
FNR	false negative rate
GCP	Good Clinical Practices
H&E	hematoxylin and eosin
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICG	indocyanine green
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	immunohistochemical
ILM	intraoperative lymphatic mapping
IRB	Institutional Review Board
ITT	intent-to-treat
IV	intravenous
kDa	kilodaltons (molecular weight measure; 10^3 Da)
lbs	pounds
LN	lymph node
Lymphoseek	technetium Tc 99m tilmanocept
max	maximum
MBR	mannose binding receptor (CD206)
mCi	millicurie (10^{-3} Ci; $3.7x10^7$ Becquerel or Bq)
MedDRA	Medical Dictionary for Regulatory Activities
µg	micrograms (10^{-6} g)
min	minimum
mL	milliliters (10^{-3} L)
mm	millimeters (10^{-3} m)
mmHg	millimeters of mercury
NCCN	National Comprehensive Cancer Network

nm	nanometers (10^{-9} m)
nmol	nanomoles (10^{-9} moles)
NPV	negative predictive value
PLND	pelvic lymphadenectomy
PP	per protocol
σ	sigma; standard deviation
SAE	serious adverse event
SAP	Statistical Analysis Plan
SLN	sentinel lymph node
SD	standard deviation
SNMMI	Society of Nuclear Medicine and Molecular Imaging
Sponsor	Navidea Biopharmaceuticals
Tc 99m	technetium-99m metastable isotope; γ-emitting ($t_{½}$ = 6.02 h)
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek)
TNM	tumor, lymph node, metastasis staging
US	United States
USFDA	US Food and Drug Administration
UTI	urinary tract infection
VBD	vital blue dye; Lymphazurin™ as 1% isosulfan blue for injection; USFDA-approved ILM colorimetric agent
WHO	World Health Organization

TRIAL ADMINISTRATIVE STRUCTURE

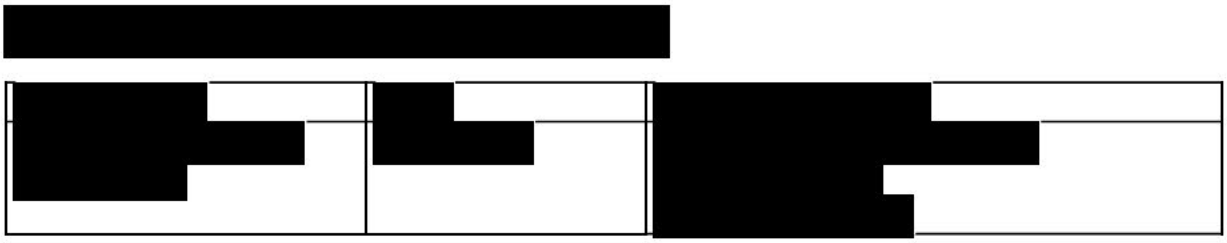
The principal investigator must sign the protocol signature page before trial participant recruitment may start. Likewise, all protocol amendments must be signed and dated by the principal investigator before coming into effect.

The name and address of the participating center, the investigators, and all required signature documents will be maintained in the trial master file (TMF).

In addition to the principal investigator, there are additional on-site roles that may be performed by other sub-investigators:

- Subject referral to the trial
- Review of subject eligibility and medical records
- Safety assessments
- Injection and imaging
- On-site image analysis

Trial personnel not listed in this section are identified in a separate personnel list. This list will be updated as needed. The list of personnel will be available in the center's investigator site file (ISF).



1 INTRODUCTION

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2 STUDY OBJECTIVES

2.1 Primary Objective

Estimation of the per subject FNR associated with the pathology status of Lymphoseek-identified SLNs relative to the pathology status of non-SLNs in the pelvic lymphadenectomy.

2.2 Secondary Objectives

Estimation of sensitivity, negative predictive value, and overall accuracy of Lymphoseek-detected SLNs relative to the pathology status of non-SLNs.

Estimation of the detection rate of SLNs by Lymphoseek and the rate of tumor detection in non-SLNs.

Determination of the proportion of lymph nodes identified intraoperatively by a dye (e.g., VBD or ICG) that are also identified intraoperatively by Lymphoseek (i.e., concordance using the number of lymph nodes stained by the dye as the denominator).

Determination of reverse concordance comparing dye to Lymphoseek (i.e., using the number of lymph nodes identified intraoperatively by Lymphoseek as the denominator).

Comparison of pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases for Lymphoseek and dye on a per-subject basis.

Comparison of imaging and surgical findings.

2.3 Safety Objective

Evaluation of the incidence of adverse events.

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Study Design

This study is a prospective, open-label, within-subject study of Lymphoseek in the detection of lymph nodes in subjects with known cancer of the cervix. The pathology status of non-SLNs in the pelvic lymphadenectomy will serve as the control. All subjects will receive a single dose of 50 µg Lymphoseek (2.0 mCi) prior to ILM. A sample size of 40 was selected in order to provide exploratory results for the primary and secondary objectives. The proposed study includes 3 visits: A screening visit for initial determination of eligibility and evaluation of clinical status (up to 30 days before injection), a baseline visit on the day of surgery, and a 24 ± 12 hour (telephone) safety follow-up.

The Schedule of Events (see Appendix 1) contains a list of all study procedures and time points. Study activities are described in detail in Section 7.

3.2 Justification for Study Design and Population

This study is designed to evaluate the use of Lymphoseek as an ILM and SLNB agent in subjects with with known cancer of the cervix by evaluating the FNR for identifying tumor-positive lymph nodes as determined by histopathological evaluation of sentinel and non-sentinel lymph nodes following pelvic lymphadenectomy. In addition, the the concordance of detection rates of Lymphoseek and of dyes will be determined.

Pelvic lymphadenectomy, with or without ILM using a dye as the mapping agent, is the current standard of care for patients undergoing cervical tumor resection. Therefore, this study will directly evaluate Lymphoseek's performance relative to the standard of care.

3.2.1 Rationale for Subject Selection

No clinical trials have been conducted to evaluate Lymphoseek performance in subjects with cervical cancer. The rationale for evaluating Lymphoseek in this subject population is discussed in Section 1.1.2.

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3.3 Protocol Adherence

Strict adherence to all specifications laid down in this protocol is required for all aspects of the study conduct; the investigator may not modify or alter the procedures described in this protocol. If protocol modifications are necessary, all alterations that are not solely of an administrative nature require a formal protocol amendment (see Section 11.1 for the involvement of International Ethics Committee(s) IEC(s)/ International Review Board(s) IRB(s)).

If an investigator has deviated from the protocol in order to eliminate an immediate hazard to subjects or for other inevitable medical reasons, the investigator shall document all such deviations, including the reasons thereof, and submit the document to the sponsor and the head of the medical institution as applicable.

Participating investigators must have 10 cases of intraoperative lymphatic mapping in gynecologic cancer patients to participate as a surgeon for any subjects enrolled in this trial.

3.4 Study Duration

Subjects will be enrolled for approximately 32 days depending on the duration of the screening window (up to 30 days).

4 STUDY POPULATION

4.1 Eligibility

Subjects who fulfill all respective inclusion and none of the exclusion criteria will be eligible for enrollment into the study. All inclusion/exclusion criteria must be verified before a subject may be considered eligible for injection and imaging (Day 1 procedures). A subject will be considered enrolled in the study on the morning of study day 1 when they arrive at the study site. Written, dated (with time noted) informed consent will be obtained from all subjects. A subject who withdraws consent prior to arrival at the study site on day 1 will be considered a screen failure.

4.1.1 Inclusion Criteria

1. Subject has provided written informed consent with HIPAA authorization
2. Has cervical cancer and is a candidate for surgical intervention, with lymph node dissection being a part of the surgical plan.
3. Is at least 18 years of age at the time of consent
4. Has an ECOG performance status of Grade 0 to 2
5. Has the following International Federation of Gynecology and Obstetrics (FIGO) IA2-IIA1(≤ 4 cm) staging(Appendix 5) Subjects with a single enlarged/suspicious node on PET CT will still be considered eligible as consistent with FIGO guidelines.
6. If of childbearing potential, the subject has a negative pregnancy test within 48 hours before administration of Lymphoseek, has been surgically sterilized, or has been postmenopausal for at least 1 year

4.1.2 Exclusion Criteria

1. The subject has had preoperative chemotherapy, immunotherapy, or radiation therapy within the 30 days prior to Lymphoseek administration
2. Has had previous surgery or radiation to node basins that would be involved in the ILM procedure
3. Has a known allergy to dextran
4. Is breast-feeding or pregnant
5. Before the administration of Lymphoseek, has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical
6. Is scheduled for surgery and/or another invasive procedure other than the primary surgical intervention within the 3 days after Lymphoseek administration
7. Has received an investigational product within the 30 days prior to Lymphoseek

administration

4.2 Recruitment

Subjects will be recruited from oncology practices in accordance with the inclusion and exclusion criteria listed above. Potentially suitable subjects will be asked by their treating physician about their willingness to participate in this study.

4.3 Withdrawal

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time and without providing a reason.

A subject who withdraws consent prior to arrival at the study site on Day 1 will be considered a screen failure.

Should a subject withdraw after administration of the investigational product all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. An explanation should be given of why the subject is withdrawing or being withdrawn from the study.

The investigator may withdraw a subject from the study at any time at the discretion of the investigator for any of the following reasons:

- A protocol violation occurs
- A serious or intolerable AE occurs
- A clinically significant change in a laboratory parameter occurs
- At the investigator's/sponsor's discretion as long as it is in the best interest of the subject
- The sponsor or investigator terminates the study
- The subject requests to be discontinued from the study

4.4 Replacement

Subjects will be replaced under the following conditions:

- Subjects who did not receive study medication or did not proceed to surgery or did not undergo lymphatic mapping with lymph node biopsy.

4.5 Subject Identification

After the subject provides written informed consent, the site will assign the subject a 7-digit subject number. Subject numbers are to be assigned in a sequential manner using the following format:

Digits 1 to 2: Trial number "19"
Digits 3 to 4: Site number (e.g., "03")

Digits 5 to 7: Sequential subject number (e.g., “001”, “002”, “003”, etc.)

For example, the first subject consented at Site 03 is subject number “19-03-001”. Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Identification of Investigational Product

Lymphoseek® (also known as technetium Tc 99m tilmanocept) is a radiopharmaceutical that accumulates in lymphatic tissue by binding to mannose binding receptors (CD206) that reside on the surfaces of dendritic cells and macrophages.

5.2 Investigational Product Dosage and Administration

Subjects will receive 50 µg tilmanocept radiolabeled with 2 mCi (74 MBq) Tc 99m administered as four separate injections. The total injection volume for each subject is 0.4 or 1.0 mL administered across all four syringes (0.1 or 0.25 mL each).

The dose will be prepared in 1 mL BD Luer-Lok syringes with a 0.5 inch needle. The needle should be replaced at the discretion of the investigator in accordance with site practices. A 22- to 24-gauge spinal or potocky needle is commonly used. In order to achieve accurate delivery of small volumes, final syringe volume adjustments will be made at the pharmacy to accommodate volume retained in the syringe hub and needle such that air should not be added to the syringe and no syringe flushing should occur.

Subjects will receive Lymphoseek by injection in a manner consistent with the European Association of Nuclear Medicine (EANM) clinical and technical guidelines for lymphoscintigraphy and sentinel node localization in gynaecological cancers as adopted by the Society of Nuclear Medicine and Molecular Imaging (SNMMI) (Giammarile et al., 2014). The agents for lymphatic mapping will be administered peritumorally as subepithelial (submucosal) instillations into the 4 quadrants of the cervix at the 12, 3, 6, and 9 o'clock positions whenever possible. Lymphoseek should not be injected directly into the tumor. For those subjects with little remaining normal cervical tissue, injections should be administered in the tissue surrounding the tumor with adjustment of the injection positions as necessary. Injection placement in relation to the cervix will be documented in the trial source. Subjects will be scheduled for surgery after imaging and no later than 20 hours after Lymphoseek injection and imaging. Just before surgical incision, a dye (e.g., VBD or ICG) may be injected in close proximity to the tumor. Preoperative evaluation of the subject will follow routine institution practice.

5.3 Dye Dosage and Administration

A dye, such as Lymphazurin (isosulfan blue 1%) or indocyanine green, may be administered to subjects as part of their standard of care. The injection will be performed as deemed appropriate by the surgeon and in accordance with the standard of care at the clinical site.

5.4 Treatment Assignment

In this open-label non-randomized study, all subjects will receive the same Lymphoseek treatment.

[redacted]

5.6 Drug Logistics and Accountability

The investigator (or designated personnel) will confirm receipt of the investigational product in writing and will use the investigational product only within the framework of this clinical study and in accordance with this study protocol. For each subject he/she will keep a record of the investigational product dispensed and store all other forms that accompanied the delivery of the radiolabeled investigational product to the clinical site. These documents are to be filed in the investigator site file. Overall drug accountability and reconciliation will be completed by the sponsor or its representative. A list of investigational product vials or syringes and other materials that were returned, or destroyed, must be prepared and signed by the principal investigator or an appropriately qualified designee as documented in the study site responsibility sheet. An overall accountability and reconciliation form of the investigational product will also be prepared and completed. If there are any discrepancies, an explanation for these must be provided.

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapy

All medications taken 30 days prior to Lymphoseek injection through the 24-hour post-injection follow-up will be documented. Any subject that has had preoperative chemotherapy, immunotherapy, or radiation therapy within the 30 days prior to Lymphoseek admnistration will be excluded from the study.

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 SCHEDULE OF EVALUATIONS AND VISIT DESCRIPTION

7.1 Schedule of Evaluations

Evaluations will be performed during a period of 2-3 days, in addition to a screening period of 30 days maximum. A schedule of evaluations is provided in the Schedule of Study Events (see Appendix 1).

7.2 Visit Description

7.2.1 Screening Visit (Day -29 to Day 0)

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the eCRF and enrollment log
- Demography – Date of birth, race and ethnicity
- Medical history – Medical history will be obtained on all study subjects. All relevant prior medical conditions will be recorded in the CRFs. Documented medical conditions will also note the month/year of onset and if the condition is active.
- Cancer staging – Tumor characteristics including FIGO staging information will be collected.
- ECOG performance status will be collected
- Vital signs – Vital signs will include respiratory rate, pulse rate, and systolic and diastolic blood pressures. Prior to obtaining blood pressures and pulse, the subject should be in a resting position for at least 1 minute. Body weight and height without shoes will be recorded only at the screening evaluation.
- Physical exam – Physical examinations will include examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, pelvis, lymph nodes, musculoskeletal, and nervous system. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Clinical laboratory tests - study subjects will have blood obtained for hematology and chemistry (See Section 8.8.2).
- Concomitant medications (within 30 days before injection).
- Final check of inclusion/exclusion criteria

7.2.2 Before Enrollment

Within 48 hours prior to receiving the Lymphoseek injection, a urine or serum pregnancy test will be conducted for female subjects of childbearing age.

All subjects will be questioned to determine if a change in medical history or concomitant medications has occurred since the last visit.

7.2.3 Preparation of Lymphoseek

[redacted]

The following medications are not permitted as co-injected drugs: local anesthetics, e.g., procaine, xylocaine, lidocaine, or carbocaine.

7.2.4 Before Injection

Before receiving the Lymphoseek injection vital signs will be collected. Vital signs will include respiratory rate, pulse rate, and systolic and diastolic blood pressures. A window of up to 10 minutes before injection is allowed. Prior to obtaining blood pressures and pulse, the subject should be in a resting position for at least 1 minute. Body weight and height measurements are not required.

7.2.5 Injection of Lymphoseek

Injection of Lymphoseek will be at study time 0:00. Subjects will receive 50 µg tilmanocept radiolabeled with 2.0 mCi (74 MBq) Tc 99m by injection as described in Section 5.2.

7.2.6 Post Injection

Vital signs will be collected at 10 minutes, 30 minutes, and 1 hour post-injection. Vital signs will include respiratory rate, pulse rate, and systolic and diastolic blood pressures. Before blood pressures and pulse are obtained, the subject should be sitting quietly in a resting position for at least 1 minute. Body weight and height measurements are not required. Allowable windows for vital sign collection are as follow:

0:10, 0:30 = ± 3 minutes
1:00 = ± 5 minutes

7.2.7 Day 1: Imaging and Surgery

SPECT-CT will be performed prior to surgery as described in Section 8.3 and in a manner consistent with the EANM clinical and technical guidelines for lymphoscintigraphy and sentinel node localization in gynaecological cancers as adopted by the SNMMI (Giammarile et al., 2014). All findings will be documented and recorded on the appropriate case report form (CRF). Dye (e.g., VBD or ICG) may be administered in the operating room immediately before surgerical incision. The injection will be performed as deemed appropriate by the surgeon and in accordance with the standard of care at the clinical site. Imaging shall be completed prior to surgery and no greater than 20 hours after Lymphoseek injection, subjects will undergo ILM, SLN(s) will be harvested and surgery to remove the

primary tumor will be completed. Location of both hot and dye-identified nodes will be recorded on the sponsor-provided intraoperative worksheets.

7.2.8 24 ± 12 hours Postsurgical Safety Follow-up (telephone)

7.2.9 Adverse events and medications will be reviewed.End of Study

The 24 ± 12 hours Postsurgical Safety Follow-up call will serve as the end of study visit. FIGO staging will be reassessed post surgery by the PI and included with the final disposition.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Forty female subjects 18 years of age or greater with known cervical cancer will be enrolled. Cancer characteristic including tumor size, cancer type and FIGO staging will be collected.

8.1.2 Medical and Surgical History

Medical and surgical history will be obtained on all trial subjects. All relevant medical conditions (current and prior), month/year of onset, and if the condition is currently active will be recorded in the CRFs. Common accepted medical terminology should be used.

8.1.3 ECOG Performance Status

All subjects will be evaluated at screening for Eastern Cooperative Oncology Group (ECOG) performance status (Table 2). The critera below will be used to assign the ECOG grade.

Table 2. ECOG Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

8.1.4 Prior and Concomitant Medication

All prior medication(s) used within the last 30 days before injection and concomitant medications will be documented. Medications administered for general surgical support and anesthesia related to surgery do not need to be captured in the CRFs.

8.2 Lymphoseek Preparation and Injection

[redacted]

Injection of Lymphoseek will be at study time 0:00. Subjects will receive an injection of 50 µg tilmanocept radiolabeled with 2.0 mCi (74 MBq) Tc 99m as described in Section 5.2.

Local anesthetics are not permitted as co-injected drugs:, e.g., procaine, xylocaine, lidocaine or carbocaine.

8.3 Lymphoscintigraphy (SPECT/SPECT-CT Image Acquisition)

The SPECT camera used to obtain the images should be equipped with a low-energy, high-resolution collimator with a 20% window centered over a 140keV peak. The subject should be imaged in the same position used for surgery with arms positioned out of the field of view. Dynamic imaging should start at the time of injection and planar imaging should commence between 15 minutes and 20 hours post injection Subjects should void their bladder just prior to imaging. It is strongly recommended that each site should perform all imaging acquisitions per their camera manufacturer and model parameters and in accordance with their institutional practices. All imaging should be completed prior to surgery no greater than 20 hours after injection.

For Sites using a SPECT camera:

Dynamic Imaging (optional)	Planar Imaging (mandatory)
Start at time of injection and continuously aquire until nodal uptake is seen, but for no longer than 1 hour.	To start after dynamic imaging (if performed)or to begin no sooner than 15 minutes post injection. Total acquisition time should not exceed 1 hour.

For sites using a SPECT/CT hybrid camera:

Dynamic Imaging (optional)	Planar Imaging (mandatory)
CT portion should be done prior to injection and start of SPECT. SPECT portion should start at time of injection and continuouly aquire until nodal uptake is seen, but for no longer than 1 hour.	To start after dynamic imaging (if performed) or to begin no sooner than 15 minutes post injection. Total acquisition time should not exceed 1 hour. A CT scan for attenuation correction should be performed prior to each planar image.

Low-dose CT parameters for each institution's camera make/model should be utilized. SPECT data should be iteratively reconstructed and attenuation correction should be applied.

All images (non-corrected, corrected SPECT , CT and fused SPECT/CT) should be reviewed by a radiologist for quality. [redacted]

[redacted] The number and anatomical location of each probable lymph node(s) identified preoperatively will be documented and captured in the eCRFs.

8.4 Surgical Procedures, Lymphatic Mapping, and Lymph Node Biopsy

8.4.1 Timing of Surgical Procedures

Between 1 to 20 hours after injection and imaging of Tc 99m Lymphoseek, subjectss will proceed to surgery for intraoperative lymphatic mapping.

8.4.2 Lymphoseek-Designated "Hot" Nodes

Room Background Count

Using the handheld gamma detection system a room background shall be determined by recording three 2-second counts or one 10-second count obtained at least 30 cm away from the injected subject and any other radiation source.

Normal Tissue Background Count

Using the handheld gamma detection system, identify an anatomical region greater than 30 cm from the Lymphoseek injection site and not over the abdomen. A set of three 2-second counts or one 10-second count on non-lymphoid tissue (background counts) are obtained to set the threshold criteria.

In Vivo

Lymphoseek positivity is based on radioactivity counts derived from the application of the handheld gamma probe in vivo, where such counts must satisfy the threshold criterion of greater than the quantity of 3 times the square root of the mean normal tissue background count (i.e., standard deviation) added to the mean normal tissue background count (referred to as the "3σ rule"). Any nodal count NOT meeting this threshold criterion will be considered a negative finding (not localized). Use Appendix 2 to assist in determining the 3σ level from the normal tissue background level.

Ex Vivo

Lymphoseek positivity is based on radioactivity counts derived from the application of the handheld gamma probe ex vivo (excised from the subject), where such counts must satisfy the threshold criterion of greater than the quantity of 3 times the square root of the mean normal tissue background count (i.e., standard deviation) added to the mean normal tissue background count (referred to as the "3σ rule"). Any nodal count NOT meeting this threshold criterion will be considered a negative finding (not localized). Use Appendix 2 to assist in determining the 3σ level from the normal room background level.

8.4.3 Surgical Lymph Node Identification

1. If a dye (e.g., VBD or ICG) is to be used, it should be injected according to the standard practice of the clinical site.
2. On entering the subcutaneous tissue, a dye-containing lymphatic vessel or lymph node may be seen. If a dye-containing lymphatic vessel is seen, carefully trace it to the proximal LN. In vivo counts should be recorded and LN removed.

   OR

   If no dye-containing lymphatic vessel or lymph node is seen, then using a combination of visualization and the handheld gamma detector, explore the lymphatic basin for the LNs. As dissection proceeds towards the LN, repeated use of the handheld gamma detector is helpful in maintaining a line of site to the lymph node. The count rate will increase as dissection approaches the Lymphoseek-containing lymph node(s).
3. Once a LN has been identified, in vivo counts should be taken prior to excision. In vivo counts will consist of a set of three 2-second counts or one 10-second count (depending on the device) over the lymph node. Determination of a positive finding (i.e., localization) is based on the **In Vivo** definition above. Any LN count not meeting this threshold criterion will be considered a negative (non-localized) finding.
4. The method used to identify all LN(s) will be recorded (i.e., dyed appearance, Lymphoseek positive finding, or both) prior to excision.
5. To confirm the in vivo procedure, a set of three 2-second counts or one 10-second count will be recorded for the excised lymph nodes. The mean count of the ex vivo LN will be compared to the mean of normal tissue background counts, and the threshold criterion used to determine a positive finding for the in vivo LNs will be applied to the ex vivo specimens. The appearance and time of excision of the lymph node will be documented. Determination of a positive finding (i.e., localization) is based on the **Ex Vivo** definition above. Ex vivo counts must be performed on all excised lymph nodes

Table 3 describes the procedures that should be followed based on the in vivo and ex vivo status of the tissue that has been excised.

Table 3. Handling of Excised Tissue

In Vivo Assessment	Ex Vivo Assessment	Procedure
Radioactive Status		
positive (hot)	positive (hot)	Send to pathology for further evaluation.
positive (hot)	negative (not hot) or no counts obtained	Incorrect node harvested. Send to pathology for further evaluation. Return to resection bed.
negative or no counts (not hot)	positive (hot)	Comment on why lymph node was removed. Send to pathology for further evaluation.
Dye Status		
dye-identified node	N/A	ANY dye-identified node is sent to pathology for further evaluation regardless of radioactive status.

6. A thorough evaluation of the remaining lymphatic basin should be undertaken since more than one LN may be found. This evaluation should include a combination of palpation, visualization, and gamma detector survey for evidence of dye and/or increased gamma detector count rates.
7. Probing of the area will be complete when all selected node counts are negative by use of the threshold criterion. The surgeon will continue with visualization and palpation according to local practice to ensure that no grossly positive LNs remain at the site of resection.

   If a subject has neither a dye-identified node nor any "hot" nodes, continue with visualization and palpation according to your medical expertise.

Pelvic lymphadenectomy will be performed in accordance with institutional standards. Additional surgical internvention such as radical hysterectomy should be performed in accordance with the subject's surgical plan and the discretion of the surgeon.

All removed LNs (SLNs and NSLNs) must be sent to pathology for using the minimum requirements of histopathological evaluation outlined in the trial pathology manual.

For the purposes of analysis, a sentinel lymph node is defined as being hot ex vivo (lymph nodes with counts above the 3σ rule threshold).

8.5 Histopathology of Lymph Nodes

8.5.1 Local Lymph Node Evaluation

Institutions with SLN ultra staging protocols in place as the standard of care will not be required to deviate from their methods when the minimum recommendation of the trial pathology manual have been met. Institutions that do not routinely employ ultra staging in the assessment of SLNs will be required to assess SLNS in accordance with the trial pathology manual as a minimum. The pathological evaluation of SLNs will include at a minimum serial sectioning and staining (H&E and if negative IHC additionally). The pathological evaluation of NSLNs at a minimum will include bifurcation and staining with H&E. Minimum requirements for fixation, sectioning and staining are outlined in the trial pathology manual.

Final pathology results must reflect consistent numbering and labeling from intraoperative worksheets to the final report. SLNs must be individually identified in the final pathology report.

The pathology evaluation of each node shall be reported as follows:

A - Macrometastases (> 2 mm)

B - Micrometastases (≤ 2.0 mm and ≥ 0.2 mm)

C - Isolated tumor cells or < 0.2 mm

D - No observable tumor presence

8.6 Pharmacokinetics

No pharmacokinetic investigation will be performed in this study.

8.7 Safety

8.7.1 Adverse Events

8.7.1.1 Definition of Adverse Event

The definitions below follow International Conference on Harmonization (ICH) – Good Clinical Practice (GCP) (see also ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

By definition for this study, all untoward medical occurrences beginning on the day of injection through the 24 hour post injection assessment are to be reported as AEs. Untoward medical events occurring prior to the day of injection will be captured in medical history. SAEs will be reported from the time of consent through the end of participation.

8.7.1.2 Categories for Adverse Event Assessment

All AEs will be assessed and documented by the investigator according to the categories detailed below.

Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 8.7.1.5.

Severity

The intensity of an AE is classified according to the following categories, taking into account the possible range of the intensity of the event:

- Mild The adverse event is transient and easily tolerated by the subject.
- Moderate The adverse event causes the subject discomfort and interrupts the subject's usual activities

- Severe The adverse even causes considerable interference with the subject's usual activities and may be incapacitating or life-threatening.

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationships to investigational product and dye

The investigator will use the following definitions to assess the relationship of the adverse event to the use of investigational product:

Definitely related: Event can be fully explained by administration of the investigational product.

Probably related: Event is most likely to be explained by administration of the investigational product rather than the subject's clinical state or other agents/therapies.

Possibly related: Event may be explained by administration of the investigational product or by the subject's clinical state or other agents/therapies.

Probably not related: Event is most likely to be explained by the subject's clinical state or other agents/therapies, rather than the investigational product.

Definitely not related: Event can be fully explained by the subject's clinical state or other agents/therapies.

For causality assessments, events meeting the categories of definitely, probably, or possibly related will be considered to be related to investigational product.

Causal relationship to study procedure

The investigator will use the following definitions to assess the relationship of the adverse event to the study procedure:

Definitely related: Event can be fully explained by the study procedure.

Probably related: Event is most likely to be explained by the study rather than the subject's clinical state or other agents/therapies.

Possibly related: Event may be explained by the study procedure or by the subject's clinical state or other agents/therapies.

Probably not related: Event is most likely to be explained by the subject's clinical state or other agents/therapies, rather than the study procedure.

Definitely not related: Event can be fully explained by the subject's clinical state or other agents/therapies.

For causality assessments, events meeting the categories of definitely, probably, or possibly related will be considered to be related to the study procedure.

8.7.1.3 Assessments and Documentation of Adverse Events

Any AE (observed, volunteered, or elicited) should be recorded in detail in the source documenation.

The following information is required:

- The date and time of onset of any AE
- The date and time the AE ends
- The seriousness of the AE will be assessed by the investigator. If the investigator deems that an AE qualifies as an SAE, a special form provided by the sponsor should be completed and the event must be immediately reported to the sponsor. A definition of serious adverse events is provided below.
- The maximum intensity (mild, moderate, or severe)
- Whether drug treatment was administered for the event
- The relationship of the AE to the investigational product, dye and to study conduct (for definitions, see above)
- Did the AE cause study discontinuation
- The outcome of the AE (Resolved, Resolved with sequelae, Ongoing, Unknown, Lost to Follow-up, Death)

AEs will be coded according to an internationally recognized dictionary (Medical Dictionary for Regulatory Activities or MedDRA).

8.7.1.4 Expected Adverse Events

Expected Conduct-Related AEs

AEs are determined by the investigator. If in the investigator's opinion/experience an event is expected and not uncommon for that surgery (i.e., nausea in the recovery room), and it is not deemed different in severity from other episodes observed in the same situation, then it is up to the investigator's discretion to determine the event to be adverse.

An example of an expected conduct-related event is postoperative pain at the site of the incision.

[redacted]

Precautionary Measures

Special precautionary measures are not considered to be necessary for this study. In case of emergency, standard emergency procedures will be employed.

Unexpected Adverse Events

An unexpected adverse event is defined as an adverse reaction that in nature and severity is not consistent with the applicable product information (e.g., Investigator's Brochure).

Any adverse experience that is not listed in the current Investigator's Brochure or which is, with regard to the specificity or severity, not consistent with the risk information shall be regarded as unexpected.

Examples would be (a) acute renal failure listed in the Investigator's Brochure with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis. "Unexpected" as used in this definition refers to an adverse drug experience that has not been previously observed and included in the product information, rather than from the perspective of such experience not being anticipated from the pharmacological properties of the investigational product.

8.7.1.5 Serious Adverse Events

Definition of Serious Adverse Events

Definition

The following SAE definition is based on ICH guidelines and the final rule issued by the Food and Drug Administration (FDA) and effective 06 Apr 1998. It is to be applied to AEs (defined in Section 8.7.1.1).

An SAE is classified as any untoward medical occurrence that at any dose

- results in death, or
- is life threatening, or
- requires inpatient hospitalization or prolongation of existing hospitalization, or

- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect, or
- is an important medical event (see paragraphs below).

The term 'life threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether it is appropriate to report an AE as serious also in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm or blood dyscrasias or convulsions that do not result in subject hospitalization.

Hospitalizations for planned elective surgeries will not be considered serious adverse events.

Actions and reporting obligations in case of serious adverse events

The investigator should take appropriate diagnostic and therapeutic measures to minimize the risk to the subject. If any SAE occurs over the course of the study, investigators or other site personnel will inform Navidea Biopharmaceutical representatives within one day (i.e., within 24 hours) of becoming aware of the SAE. [redacted]. For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately.

Pregnancy will have the same time reporting obligations to the sponsor as SAEs. Upon notification, Navidea will provide a form for collection of pregnancy information.

All SAEs must also be recorded on the Adverse Event eCRFs.

Notification of the IECs/IRBs

The sponsor and/or the investigator will notify the IECs/IRBs about all relevant events (e.g., Serious Adverse Events [SAEs] and Suspected, Unexpected, Serious Adverse Reactions [SUSARs]) according to all applicable regulations.

Notification of the authorities

The sponsor will process and report all relevant events (e.g., SAEs, SUSARs) to the authorities according to all applicable regulations.

Sponsor's notification of the investigators

The sponsor will inform all investigators about reported relevant events (e.g., SAEs, SUSARs) according to all applicable regulations.

8.7.2 Further Safety Assessments

8.7.2.1 Physical Examination

Complete physical examinations will be conducted according to the Schedule of Study Events (see Appendix 1).

Physical examination will be performed for the following body systems:

- General appearance
- Skin/dermatological
- Eyes, ears, nose, throat
- Head and neck (including thyroid)
- Lungs
- Heart
- Abdomen (liver, kidney, spleen, gastrointestinal)
- Pelvis
- Lymph nodes
- Musculoskeletal
- Nervous system

8.7.2.2 Vital Signs

Vital signs comprise the measurement of systolic and diastolic blood pressure, respiration, and heart rate. All vital signs will be measured after the subject has been in a resting position for at least 1 minute. Heart rate should be measured immediately before or immediately after blood pressure measurement.

Vital signs will be measured at screening, baseline (0-10 minutes before investigational product injection), 10±3, 30±3, 60±5 minutes post injection, and after scanning.

Any clinically significant change from baseline that results in a change in subject management will be considered an AE.

8.8 Other Procedures and Variables

8.8.1 Blood Sampling

8.8.2 Laboratory Data for Subject Characterization

Clinical laboratory tests to be evaluated in this study include hematology (complete blood count) and serum chemistry (basic metabolic panel). Blood samples will be obtained only at screening in accordance with the Schedule of Events. Table 4 shows the minimum parameters to be assessed:

Table 4. Minimum Clinical Laboratory Parameters

Hematology	hemoglobin (Hb), platelets, white blood cell (WBC)
Serum chemistry	creatinine, chloride, potassium, sodium, carbon dioxide (CO2)/bicarbonate, blood urea nitrogen (BUN)

All laboratory reports must be promptly reviewed for clinical significance by the investigator, and upon review, initialed and dated by the investigator.

Good clinical practice would suggest that a copy of the safety laboratory results be available to the subject and to the subject's referring physician.

The expected amount of blood to be withdrawn is shown in Table 5.

Table 5. Approximate Amount of Blood Withdrawn

	mLs
Chemistry	5
Hematology	4
Total	9

9 STATISTICAL METHODS

9.1 Introduction

This study is a prospective, open-label study conducted at approximately 4 to 6 study centers in the US. The objectives of the statistical analyses are to establish the safety and efficacy of Lymphoseek in targeting lymphoid tissue in subjects with cervical cancer who are undergoing lymph node mapping.

A study center is defined as a treatment administration site or group of treatment administration sites under the control and supervision the same Primary Investigator.

9.2 Randomization Methods

There is no randomization planned for this study. All enrolled subjects will receive Lymphoseek along with an optional administration of either VBD or ICG.

9.3 Pooling of Study Centers

There will be no selective pooling of study centers. Subjects from all study centers will be pooled for the analysis.

9.4 Efficacy Variables

9.4.1 Primary Efficacy Variables

The primary efficacy variable is the per subject false negative rate (FNR), which is the number of subjects with pathologically negative Lymphoseek-identified SLN(s) (or no SLNs were detected) and at least one pathologically positive non-SLN divided by the number of subjects with at least one pathologically positive node (SLN or non-SLN)

9.4.2 Secondary Efficacy Variables

The secondary efficacy variables are the following:

- Per-subject sensitivity, defined as the number of subjects with at least one pathologically positive SLN divided by the number of subjects with at least one pathologically positive node (SLN or non-SLN)

- Per-subject negative predictive value (NPV), defined as the number of subjects with no pathogically positive lymph nodes (SLNs or non-SLNs) divided by the number of subjects with pathologically negative Lymphoseek-identified SLN(s) (or no SLNs were detected)

- Per-subject accuracy, defined as the proportion of subjects whose lymph nodes were either all pathologically negative (i.e, true negative subject) or at least one SLN was

pathologically positive (i.e., true positive subject) divided by the number of subjects in the analysis population

- Rate of tumor detection in SLNs, defined as the proportion of subjects with at least one pathologically positive SLN divided by the number of subjects in the analysis population
- Rate of tumor detection in non-SLNs, defined as the proportion of subjects with at least one pathologically positive non-SLN divided by the number of subjects in the analysis population
- Nodal concordance, defined as the proportion of lymph nodes identified intraoperatively by dye (e.g., VBD or ICG) that are also identified intraoperatively by Lymphoseek
- Subject concordance, defined as the number of subjects whose lymph nodes that were identified intraoperatively by dye were also all identified intraoperatively by Lymphoseek
- Reverse nodal concordance, defined as the proportion of lymph nodes identified intraoperatively by Lymphoseek that are also identified intraoperatively by dye
- Subject reverse concordance, defined as the number of subjects whose lymph nodes that were identified intraoperatively by Lymphoseek were also all identified intraoperatively by dye
- Number of SLNs per subject
- Number of lymph nodes identified intraoperatively by dye
- Number and anatomical locations of lymph nodes identified by preoperative Lymphoscintigraphy

9.5 Pharmacokinetic Variables

Not applicable.

9.6 Safety Variables

9.6.1 Primary Safety Variables

The primary safety variables for this study are:

- Vital signs (screening, at pre- and 10, 30 and 60 minutes post-injection timepoints)
- Adverse events

9.7 Sample Size Justification

Approximately 40 subjects diagnosed with cervical cancer will be enrolled in the study. This sample size was chosen in order to provide exploratory results for the primary endpoint of per subject FNR within this subject population. This study is not powered with respect to testing any statistical hypotheses.

It is anticipated that approximately 20% of the enrolled subjects will have at least one pathologically positive node, resulting in a total of 8 subjects available for the primary endpoint analysis. With an expected FNR of 12.5%, i.e., 1 false negative subject, a sample size of 8 subjects will allow for an exact 95% confidence interval half-width of 26% on the true subject FNR. Due to the asymmetric nature of the exact binomial confidence interval, the half-width described in this protocol is simply half the entire width of the confidence interval and not the distance between the estimate and the confidence limits.

9.8 Handling of Missing Data

There will be no missing value imputation used in this study. All endpoints will be analyzed with subjects who have non-missing data (i.e., a complete case analysis).

9.9 Statistical Analysis

9.9.1 Analysis Populations

The following analysis populations will be defined:

Intent-to-treat (ITT): The ITT population will include all enrolled subjects with administration of test medicine for whom at least one lymph node was removed with histopathology available.

Per protocol (PP): The PP population will include all ITT subjects without major protocol violations.

Safety: The safety population will include all enrolled subjects with administration of test medicine.

The analysis of the efficacy endpoints will be conducted on ITT and PP populations. The primary analysis set will be the ITT analysis set. All safety analyses will be conducted on the safety population.

9.9.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics will be summarized for all subjects in the safety population. Continuous variables will be displayed via summary statistics (mean, median, sample size, standard deviation, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

9.9.3 Analysis of Primary Efficacy Variables

The per-subject FNR will be computed as follows using all ITT subjects with at least one pathologically positive lymph node:

$$FNR_{subj} = \frac{\text{Subjects with at least one pathologically positive non-SLN and no pathologically positive SLNs (i.e., false negative subject)}}{\text{Subjects with at least one pathologically positive node (SLN or non-SLN)}}$$

Along with reporting the number of false negative subjects and the observed per-subject FNR, an exact two-sided 95% confidence interval will be computed.

The above analyses will be repeated using the PP analysis population.

9.9.4 Analysis of Secondary Efficacy Variables

The per-subject sensitivity will be computed as follows using all ITT subjects with at least one pathologically positive lymph node:

$$SENS_{subj} = \frac{\text{Subjects with at least one pathologically positive SLN (i.e., true positive subject)}}{\text{Subjects with at least one pathologically positive node (SLN or non-SLN)}}$$

Along with reporting the number of true positive subjects and the observed per-subject sensitivity, an exact two-sided 95% confidence interval will be computed.

The per-subject NPV will be computed as follows using all ITT subjects with pathologically negative Lymphoseek-identified SLN(s) (or no SLNs were detected):

$$NPV_{subj} = \frac{\text{Subjects with no pathologically positive nodes (SLNs and non-SLNs) (i.e., correctly predicted negative subjects)}}{\text{Subjects with no pathologically positive SLNs or subjects with no SLNs}}$$

Along with reporting the number of correctly predicted negative subjects and the observed per-subject NPV, an exact two-sided 95% confidence interval will be computed.

The per-subject accuracy will be computed as follows using all ITT subjects:

$$\frac{\text{Subjects whose lymph nodes were either all pathologically negative (i.e, true negative subjects) or subjects with at least one pathologically positive SLN}}{}$$

$$ACC_{subj} = \frac{\text{(i.e., true positive subjects)}}{\text{All ITT subjects}}$$

Along with reporting the total number of true negative and true positive subjects and the observed per-subject accuracy, an exact two-sided 95% confidence interval will be computed.

The rate of tumor detection in SLNs will be computed as follows using all ITT subjects:

$$\text{Rate of SLN Tumor Detection} = \frac{\text{Subjects with at least one pathologically positive SLN}}{\text{All ITT subjects}}$$

Along with reporting the total number of subjects with at least one pathologically positive SLN and the observed rate of SLN tumor detection, an exact two-sided 95% confidence interval will be computed.

The rate of tumor detection in non-SLNs will be computed as follows using all ITT subjects:

$$\text{Rate of SLN Tumor Detection} = \frac{\text{Subjects with at least one pathologically positive non-SLN}}{\text{All ITT subjects}}$$

Along with reporting the total number of subjects with at least one pathologically positive non-SLN and the observed rate of non-SLN tumor detection, an exact two-sided 95% confidence interval will be computed

The nodal concordance, P_1, will be computed as follows using lymph nodes from all subjects in the ITT population:

$$P_1 = \frac{\text{Number of SLNs identified intraoperatively by VBD or ICG}}{\text{Number of lymph nodes identified intraoperatively by VBD or ICG}}$$

9.9.4.1 Along with reporting the number of SLNs indentified intraoperatively by VBD or ICG and the observed nodal concordance, an exact two-sided 95% confidence interval will be computed.

The subject concordance, P_2, will be computed as follows using all subjects in the ITT population:

$$P_2 = \frac{\text{Subjects whose lymph nodes that were intraoperatively identified by VBD or ICG were also all identified by Lymphoseek}}{\text{Subjects with at least one lymph node intraoperatively identified by VBD or ICG}}$$

Along with reporting the number of subjects in the numerator and the observed subject concordance, an exact two-sided 95% confidence interval will be computed.

The reverse nodal concordance, P_3, will be computed as follows using lymph nodes from all subjects in the ITT population:

$$P_3 = \frac{\text{Number of SLNs identified intraoperatively by VBD or ICG}}{\text{Number of SLNs}}$$

Along with reporting the number of SLNs indentified intraoperatively by VBD or ICG and the observed reverse nodal concordance, an exact two-sided 95% confidence interval will be computed.

The subject reverse concordance, P_4, will be computed as follows using all subjects in the ITT population:

$$P_4 = \frac{\text{Subjects whose SLNs were also all identified by VBD or ICG}}{\text{Subjects with at least one SLN}}$$

Along with reporting the number of subjects in the numerator and the observed reverse subject concordance, an exact two-sided 95% confidence interval will be computed.

The average number of SLNs per subject will be computed, along with a 95% confidence interval.

The average number of lymph nodes identified intraoperatively by dye per subject will be computed, along with a 95% confidence interval.

The average number of lymph nodes identified by preoperative Lymphoscintigraphy per subject will be computed, along with a 95% confidence interval.

The number and percent of lymph nodes identified by preoperative Lymphoscintigraphy will be reported per anatomical location.

The above analyses of secondary variables will be repeated using the PP analysis population.

9.9.5 Safety Analyses

All AEs will be observed for each subject from injection until termination from the study. Additionally, all SAEs will be observed for each subject from time of consent until termination from the study. Prior to analysis, all AEs will be coded using MedDRA. Based on these coded terms, AEs will be summarized using system organ class and preferred term for all subjects in the safety population. AEs will also be summarized by severity, relationship to investigational product, relationship to dye, and relationship to study procedure. All AEs will be listed.

Summary statistics (mean, median, sample size, standard deviation, minimum, and maximum) will be computed on the raw and change from baseline values for each vital sign parameter by timepoint. The pre-injection timepoint will serve as baseline. If there are multiple vital signs taken at any timepoint, then the latest set of vital signs will be used for the analysis. All vital sign data will be listed.

Additional analyses of safety variables for this study may be conducted as described in the Statistical Analysis Plan document.

9.10 Interim Analyses

No formal interim analyses will be conducted during this study.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

Data required according to this protocol is captured in the subject's source documentation and are to be entered into the eCRFs (provided by the sponsor) as soon as possible.

10.1.1 Electronic CRF design

Electronic data capture with the sponsor's eCRF will be used for collecting all data generated during the study. The eCRF application has a built-in plausibility check system, forcing the investigators to answer the questions in the appropriate manner, and auto-checks for missing data. The system type and eCRF details will be outlined in a separate document that will be provided by the sponsor and maintained in the TMF.

10.2 Monitoring

This study will be monitored regularly by a clinical research associate (CRA) from the sponsor or a contract research organization (CRO). Monitoring procedures include one or more visits designed to clarify all prerequisites before the study starts. Interim monitoring visits will take place on a regular basis according to a schedule fixed by mutual agreement. During these visits, the CRA will check for completion of the entries on the eCRFs, their compliance with the protocol and with GCP, and will compare the eCRF entries with the source data.

All data recorded in the eCRF will be captured in the source documentation.

The CRA will verify the correct use of the investigational product. The investigational product will not be supplied to the investigator site prior to a favorable opinion from the IRB/IEC and the regulatory authority and, if appropriate, from the radiation protection authorities.

In addition, the CRA will determine whether all AEs and SAEs have been appropriately reported (including adherence to the time periods required for SAEs).

10.3 Data Processing

Study data documentation will be maintained specifying all relevant aspects of data processing for the study (including data validation, cleaning, correcting, releasing). This documentation will be stored in the TMF.

For data coding (e.g., AEs, medication, medical/surgical history), internationally recognized and accepted dictionaries will be used. These and the processes used for coding will be specified in the data management plan.

10.4 Auditing

A member of the sponsor's (or a designated CRO) quality assurance unit may arrange to visit the investigator in order to audit the performance of the study at the study site and the study documents originating there. The auditor(s) will usually be accompanied by a CRA or the study team lead. The investigator will be informed about the outcome of the audit.

In addition, inspections by health authority representatives and IEC(s)/IRB(s) are possible at any time. The investigator is to notify the sponsor of any such inspection immediately.

10.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution, or private practice. Where the archiving procedures do not meet the minimum timelines required by the sponsor, alternative arrangements must be made to ensure the availability of the source documents for the required period.

The investigator/institution notifies the sponsor if the archival arrangements change (e.g., relocation or transfer of ownership).

The investigator site file is not to be destroyed without the sponsor's approval.

The investigator's contract will contain all regulations relevant for the study center.

10.6 Premature Termination of the Study

Termination by the Sponsor

The sponsor may terminate the study at a site any time for any of the following reasons:

1. Failure to enroll subjects
2. Protocol violations
3. Inaccurate or incomplete data
4. Unsafe or unethical practices
5. Questionable safety of the investigational product
6. Suspected lack of efficacy of the investigational product
7. Administrative decision

Termination by the Investigator

If the Investigator terminates the study prematurely, the Investigator must do the following:

- Return all unused investigational products and related study materials to the sponsor.
- Provide the IRB/IEC and the sponsor with a written statement describing why the study was terminated prematurely. Prompt compliance with this requirement is essential so that the sponsor may comply with its regulatory obligations.

10.6.1 Study as a Whole

The sponsor retains the right to prematurely terminate the study as a whole at any time.

At the discretion of the sponsor, the entire study may be canceled for medical reasons (e.g. unanticipated adverse events). In addition, the sponsor retains the right to end the study at any time if the study cannot be conducted as specified in the protocol.

In case of premature termination or suspension of the study, the principal investigator/sponsor will promptly inform the investigator/institutions, regulatory authorities, and IRB/IEC of the termination or suspension and the reason for that.

10.6.2 Study Participant

Individual subjects may be withdrawn from the study according to the criteria specified in Section 4.3.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

The planning and conduct of this clinical study are subject to national laws. Only when all of the requirements of the appropriate regulatory authority have been fulfilled will the study begin. The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and the ICH-GCP Guidelines of 17 Jan 1997. At the discretion of the investigator, the entire study may be canceled for medical reasons. In addition, the sponsor retains the right to end the study for medical-scientific or GCP-relevant reasons. In case of premature termination the investigators, IRB/IECs and Regulatory Authorities will be informed by the Study Manager. As required by local law, current safety-relevant information will be provided to the IEC / IRB and the regulatory authorities by the sponsor. The sponsor will also inform all investigators about relevant safety events according to the applicable regulations.

11.2 Subject Information and Consent

All relevant information on the study will be summarized in the subject consent form and additionally as required by the investigator's institution in an integrated subject information and consent sheet. A sample informed consent form (ICF) is provided as a document separate to this protocol.

Based on this subject ICF, the investigator will explain all relevant aspects of the study to each subject, before her entry into the study (i.e., before examinations and procedures associated with selection for the study are performed).

The investigator will also mention that written approval of the IRB/IEC has been obtained.

Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Following this informative discussion, the subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the subject voluntarily agrees to sign the ICF and has done so, may he/she enter the study. Additionally, the investigator or his/her designee will personally sign and date the form. The subject will receive a duplicate of the signed and dated form.

The investigator will record in the source documentation the consent process including the time and date of obtaining informed consent.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

The ICF and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol which necessitates a change to the content of the subject information and/or the written ICF. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised ICF. Any revised written ICF and written information must receive the IRB/IEC's approval/favorable opinion in advance of use.

11.3 Financing/Financial Disclosure

Each investigator (including principal and/or any subinvestigators; as well as their spouses and dependent children) who is directly involved in the treatment or evaluation of research subjects has to provide a financial disclosure according to all applicable legal requirements. All relevant documentation will be filed in the sponsor trial master file and the investigator site file, as appropriate.

11.4 Publication Policy

The sponsor will be responsible for determining when any trial results should be published. The sponsor will work jointly with the investigator(s) to publish information in a timely manner. The investigator(s) shall not submit any information gleaned under the direct support or sponsorship of the sponsor to journals or professional societies without the prior written approval of the sponsor. A "publication" is meant to include any abstract, letter, manuscript or public announcement in any form or length that contains information gleaned under the direct support or sponsorship of the sponsor.

11.5 Subject Injury

In general, if a subject is injured as a direct result of the investigational product but not due to medical negligence on the part of the principal investigator or study staff, the sponsor will pay for reasonable and necessary medical treatment for the injury, to the extent the expenses are not covered by the subject's medical insurance, a government program, or other responsible third party. If laws or regulations of the locality in which the trial is taking place require additional payment of expenses, the sponsor shall comply with such law or regulation. Where applicable, the sponsor has taken specific national insurance.

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Appendix 1 Schedule of Events

Assessment	Screen (Day -29 to Day 0)	Pre- and Post-Injection Day 1 (hour: minute relative to Lymphoseek injection)								Safety Follow-up Call (24 ± 12 hours post-injection)
		-00:10	0:00	00:10 ± 3	0:15	0:30 ± 3	1:00 ± 5	20:00	After Scanning	
Informed Consent	X									
Entry Criteria	X									
Medical History and Demography	X									
ECOG Status & Cancer Staging	X									
Vital Sign Assessment[a]	X	X		X		X	X			
Physical Examination	X									
Review of Medications	X	X								X
Clinical Laboratory Evaluation	X									
Urine or Serum Pregnancy Test[b]	X									
Lymphoseek Administration			X							
Lymphoscintigraphy[e]			X							
VBD or ICG Administration[c]									X	
Surgery, Probing and Harvest Lymph Nodes[d]									X	
Adverse Event Monitoring		X	X	X	X	X	X		X	X

[a] Body weight and height will only be collected at screening
[b] Pregnancy testing should be performed within 48 hours before Lymphoseek injection
[c] Dye (e.g., VBD or ICG) may be administered immediately before the start of surgery
[d] Surgery, node probing and harvesting should occur no later than 20 hours after Lymphoseek injection
[e] Lymphoscintigraphy (SPECT or SPECT-CT) should occurbetween 15 minutes and 20 hours after Lymphoseek injection

Appendix 2 Gamma Detector Counts Calculation Sheet

Gamma Detector Counts Calculation Sheet

Locate the number in the left column that corresponds to the average of three 2 second counts or one 10 second count.

	3Σ		3Σ		3Σ		3Σ
1	4.00	53	74.84	105	135.74	157	194.59
2	6.24	54	76.05	106	136.89	158	195.71
3	8.20	55	77.25	107	138.03	159	196.83
4	10.00	56	78.45	108	139.18	160	197.95
5	11.71	57	79.65	109	140.32	161	199.07
6	13.35	58	80.85	110	141.46	162	200.18
7	14.94	59	82.04	111	142.61	163	201.30
8	16.49	60	83.24	112	143.75	164	202.42
9	18.00	61	84.43	113	144.89	165	203.54
10	19.49	62	85.62	114	146.03	166	204.65
11	20.95	63	86.81	115	147.17	167	205.77
12	22.39	64	88.00	116	148.31	168	206.88
13	23.82	65	89.19	117	149.45	169	208.00
14	25.22	66	90.37	118	150.59	170	209.12
15	26.62	67	91.56	119	151.73	171	210.23
16	28.00	68	92.74	120	152.86	172	211.34
17	29.37	69	93.92	121	154.00	173	212.46
18	30.73	70	95.10	122	155.14	174	213.57
19	32.08	71	96.28	123	156.27	175	214.69
20	33.42	72	97.46	124	157.41	176	215.80
21	34.75	73	98.63	125	158.54	177	216.91
22	36.07	74	99.81	126	159.67	178	218.02
23	37.39	75	100.98	127	160.81	179	219.14
24	38.70	76	102.15	128	161.94	180	220.25
25	40.00	77	103.32	129	163.07	181	221.36
26	41.30	78	104.50	130	164.21	182	222.47
27	42.59	79	105.66	131	165.34	183	223.58
28	43.87	80	106.83	132	166.47	184	224.69
29	45.16	81	108.00	133	167.60	185	225.80
30	46.43	82	109.17	134	168.73	186	226.91
31	47.70	83	110.33	135	169.86	187	228.02
32	48.97	84	111.50	136	170.99	188	229.13
33	50.23	85	112.66	137	172.11	189	230.24
34	51.49	86	113.82	138	173.24	190	231.35
35	52.75	87	114.98	139	174.37	191	232.46
36	54.00	88	116.14	140	175.50	192	233.57
37	55.25	89	117.30	141	176.62	193	234.68
38	56.49	90	118.46	142	177.75	194	235.79
39	57.73	91	119.62	143	178.87	195	236.89
40	58.97	92	120.77	144	180.00	196	238.00
41	60.21	93	121.93	145	181.12	197	239.11
42	61.44	94	123.09	146	182.25	198	240.21
43	62.67	95	124.24	147	183.37	199	241.32
44	63.90	96	125.39	148	184.50	200	242.43
45	65.12	97	126.55	149	185.62	201	243.53
46	66.35	98	127.70	150	186.74	202	244.64
47	67.57	99	128.85	151	187.86	203	245.74
48	68.78	100	130.00	152	188.99	204	246.85
49	70.00	101	131.15	153	190.11	205	247.95
50	71.21	102	132.30	154	191.23	206	249.06
51	72.42	103	133.45	155	192.35	207	250.16
52	73.63	104	134.59	156	193.47	208	251.27

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page 1

Appendix 3 TNM and FIGO Cancer Staging Definitions

American Joint Committee on Cancer

Cervix Uteri Cancer Staging

7th EDITION

Definitions

Primary Tumor (T)

TNM CATEGORIES	FIGO STAGES	
TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
Tis*		Carcinoma in situ (preinvasive carcinoma)
T1	I	Cervical carcinoma confined to uterus (extension to corpus should be disregarded)
T1a**	IA	Invasive carcinoma diagnosed only by microscopy. Stromal invasion with a maximum depth of 5.0 mm measured from the base of the epithelium and a horizontal spread of 7.0 mm or less. Vascular space involvement, venous or lymphatic, does not affect classification
T1a1	IA1	Measured stromal invasion 3.0 mm or less in depth and 7.0 mm or less in horizontal spread
T1a2	IA2	Measured stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread 7.0 mm or less
T1b	IB	Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a/IA2
T1b1	IB1	Clinically visible lesion 4.0 cm or less in greatest dimension
T1b2	IB2	Clinically visible lesion more than 4.0 cm in greatest dimension
T2	II	Cervical carcinoma invades beyond uterus but not to pelvic wall or to lower third of vagina
T2a	IIA	Tumor without parametrial invasion
T2a1	IIA1	Clinically visible lesion 4.0 cm or less in greatest dimension
T2a2	IIA2	Clinically visible lesion more than 4.0 cm in greatest dimension
T2b	IIB	Tumor with parametrial invasion
T3	III	Tumor extends to pelvic wall and/or involves lower third of vagina, and/or causes hydronephrosis or nonfunctioning kidney
T3a	IIIA	Tumor involves lower third of vagina, no extension to pelvic wall
T3b	IIIB	Tumor extends to pelvic wall and/or causes hydronephrosis or nonfunctioning kidney
T4	IVA	Tumor invades mucosa of bladder or rectum, and/or extends beyond true pelvis (bullous edema is not sufficient to classify a tumor as T4)

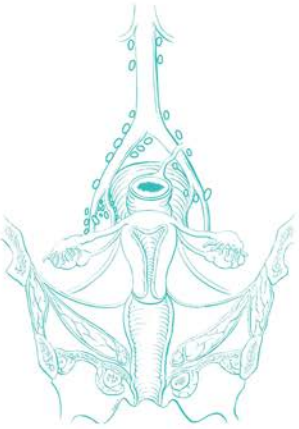
Regional Lymph Nodes (N)

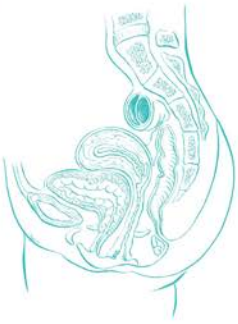
TNM CATEGORIES	FIGO STAGES	
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1	IIIB	Regional lymph node metastasis

Distant Metastasis (M)

TNM CATEGORIES	FIGO STAGES	
M0		No distant metastasis
M1	IVB	Distant metastasis (including peritoneal spread, involvement of supraclavicular, mediastinal, or paraaortic lymph nodes, lung, liver, or bone)

ANATOMIC STAGE/PROGNOSTIC GROUPS (FIGO 2008)			
Stage 0*	Tis	N0	M0
Stage I	T1	N0	M0
Stage IA	T1a	N0	M0
Stage IA1	T1a1	N0	M0
Stage IA2	T1a2	N0	M0
Stage IB	T1b	N0	M0
Stage IB1	T1b1	N0	M0
Stage IB2	T1b2	N0	M0
Stage II	T2	N0	M0
Stage IIA	T2a	N0	M0
Stage IIA1	T2a1	N0	M0
Stage IIA2	T2a2	N0	M0
Stage IIB	T2b	N0	M0
Stage III	T3	N0	M0
Stage IIIA	T3a	N0	M0
Stage IIIB	T3b	Any N	M0
	T1–3	N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1





Notes

*FIGO no longer includes Stage 0 (Tis).

**All macroscopically visible lesions—even with superficial invasion—are T1b/IB.

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Appendix 5 Investigator's Signature

Study Title:	A Prospective, Open-Label Trial of Lymphoseek®-Identified Sentinel Lymph Nodes Relative to the Pathological Status of Nonsentinel Lymph Nodes in Subjects With Known Cancer of the Cervix Who Are Undergoing Lymph Node Dissection
Study Number:	NAV3-19
Original Protocol Date:	27 May 2015
Amendment 1 Protocol Date:	03 November 2015

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____

<enter name and credentials>
<enter title>
<enter affiliation>
<enter address>
<enter phone number>