

**A Prospective, Randomized, Parallel Group, Double-Blinded Clinical Trial
Comparing Lymphoseek and 99m Tc-Sulfur Colloid with Regard to
Preoperative Imaging and Imaging Drug Kinetics and Intraoperative
Lymphatic Mapping and Sentinel Lymph Node Biopsy Findings in Subjects
With Known Breast Cancer**

Investigator Initiated Clinical Study Protocol No. KHNIC-P14-001

Date of Protocol: April 29, 2015

Principal Investigator: Arash Kardan, MD

Sub-investigators: Roxane Weighall, DO
Carol Sawmiller, MD

Clinical Facility: Kettering Medical Center
3535 Southern Blvd
Kettering, Ohio 45429

Revision History	Release Date
A (Original)	October 20, 2014
B (Amendment 1)	April 29, 2015

-CONFIDENTIAL-

SYNOPSIS

Study Title	A Prospective, Randomized, Parallel Group, Double-Blinded Clinical Trial Comparing Lymphoseek and ^{99m}Tc -Sulfur Colloid with Regard to Preoperative Imaging and Imaging Drug Kinetics and Intraoperative Lymphatic Mapping and Sentinel Lymph Node Biopsy Findings in Subjects With Known Breast Cancer
Clinical Phase	4
Name of Radioactive Drug Substances	Lymphoseek® and ^{99m}Tc -Sulfur Colloid (^{99m}Tc -SC)
Dose(s)	An intradermal dose of 0.5 mCi of one of the ^{99m}Tc radiopharmaceutical agents will be administered to each subject. The total administered volume will be 0.5 mL for each radiopharmaceutical.
Route of Administration	Intradermal injection
Duration of Treatment	A single injection of one of the ^{99m}Tc radiopharmaceutical agents
Indication	Lymphoseek (technetium Tc 99m tilmanocept) Injection is indicated for lymphatic mapping with a hand-held gamma counter to assist in the localization of lymph nodes draining a primary tumor site in patients with breast cancer or melanoma and guiding sentinel lymph node biopsy using a hand-held gamma counter in patients with clinically node negative squamous cell carcinoma of the oral cavity. Technetium Tc 99m Sulfur Colloid Injection is a radioactive diagnostic agent indicated for use as follows: In adults, to assist in the: <ul style="list-style-type: none">localization of lymph nodes draining a primary tumor in patients withbreast cancer or malignant melanoma when used with a hand-held gamma counter.evaluation of peritoneo-venous (LeVeen) shunt patency in adults.
Study Objectives	Primary Objective <ul style="list-style-type: none">To compare the preoperative radiotracer kinetics (rate of injection site clearance and rate of sentinel lymph node [SLN] uptake) for Lymphoseek and ^{99m}Tc-SC. Secondary Objectives <ul style="list-style-type: none">To compare the number and range of intraoperatively detected SLNs identified by Lymphoseek and ^{99m}Tc-SC on an agent cohort basisTo compare differences in the ratio of intraoperative counts for Lymphoseek vs ^{99m}Tc-SC for the hottest harvested axillary SLN relative to the primary intradermal injection site.To compare subject pain tolerance (i.e., subject's perceived level of discomfort) at the injection site for Lymphoseek vs ^{99m}Tc-SC.To compare pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases for Lymphoseek vs ^{99m}Tc-SC.

Inclusion Criteria	<ol style="list-style-type: none">1. The patient must be female and 18 years of age or older.2. The patient must be a preoperative clinical Tis, T1, T2, T3, T4, as well as clinical N0 and clinical M0 breast cancer3. The patient must have a diagnosis of primary breast cancer.4. The patient must be a candidate for surgical intervention, either with lumpectomy and sentinel lymph node biopsy (SLNB) or with mastectomy and SLNB, as the treatment of her breast cancer.5. The patient must have an ECOG performance status of Grade 0 – 26. The patient must provide written informed consent with HIPAA authorization before participating in the study
Exclusion Criteria	<ol style="list-style-type: none">1. The patient has clinical or radiological or pathologic evidence of metastatic cancer, including any abnormal or enlarged clinical palpable lymph nodes or core biopsy/surgical biopsy/fine needle aspiration (FNA) evidence of malignant cell within any lymph nodes.2. The patient has a known hypersensitivity to vital blue dye (VBD) in a case where vital blue dye was planned for use during SLNB.3. The patient has a positive pregnancy test or is lactating.4. The patient has had prior surgery in the upper, outer quadrant, areola or axilla to the indicated breast.5. The patient has had pre-surgical neoadjuvant chemotherapy or previous breast radiation.6. The patient has participated in another investigational drug study during the 30 days prior to signing informed consent.
Concomitant Medications	All medications taken by the subject for seven days prior to surgery will be recorded.
Study Rationale	To compare the kinetics and efficacy of two functionally different diagnostic agents, Lymphoseek (CD206 receptor targeted) and ^{99m}Tc -SC (non-specific mapping agent) in intraoperative lymphatic mapping (ILM) and SLNB.
Study Design	Single center, blinded, randomized, parallel-group, comparative study of Lymphoseek and ^{99m}Tc -SC in the preoperative and intraoperative detection of lymph nodes in subjects with known breast cancer. All subjects will receive a single dose of 50 μg Lymphoseek radiolabeled with 0.5 mCi Tc 99m or 0.5 mCi of ^{99m}Tc -SC. Subjects may also receive up to 1 mL of vital blue dye (VBD) as a companion ILM agent. All radio-labeled agents will be administered in a single intradermal injection.
Methodology	Subjects will participate in 3 study visits (including the Screening Visit [Visit 1]) over the course of the study period. Screening will include an assessment of eligibility and informed consent, a review of concomitant medications, and a random assignment to a treatment group. During Visit 2, all subjects will receive either a single intradermal injection of Lymphoseek or ^{99m}Tc -SC based upon the treatment group assigned at screening, followed by SPECT imaging conducted in two phases: initial sequential planar imaging at 30 to 60 second intervals for the first 60 minutes and repeated at 90 minutes and 120 minutes, as indicated, for identification of the SLN followed by SPECT/CT for higher resolution imaging in transaxial, coronal, and sagittal planes (see Appendix E). Subjects will then proceed to surgery for lymphatic mapping. Lymphoseek and ^{99m}Tc -SC positivity will be based upon

	radioactivity counts derived from the application of the handheld gamma probe ex vivo. Tumor resection or mastectomy will be performed according to standard procedures. Subjects will be contacted by telephone at day 5 ± 3 days post-surgery (Visit 3) to assess adverse events.
Number of Subjects	<p>The sample size for the study is based on the co-primary endpoints. In order to have an 80% chance of demonstrating that Lymphoseek is superior to $^{99m}\text{TcSC}$ based on the injection site clearance rate, assuming that the mean rate of Lymphoseek is 0.250, the mean rate of $^{99m}\text{Tc-SC}$ is 0.05, and the common standard deviation is 0.15, at least 10 subjects will need to be enrolled in each treatment group.</p> <p>In order to have an 80% chance of demonstrating that Lymphoseek is superior to $^{99m}\text{TcSC}$ based on the SLN uptake rate, assuming that the mean rate of Lymphoseek is 0.1075, the mean rate of $^{99m}\text{TcSC}$ is 0.0875, and the common standard deviation is 0.0009, at least 19 subjects will need to be enrolled in each treatment group.</p> <p>In order to meet the sample size requirements for each endpoint, approximately 40 subjects will be enrolled in the study. These sample size calculations were based on a two-sided $\alpha=0.05$ level of significance for each endpoint.</p>
Approximate Duration of Subject Participation	75 days (includes screening period)
Approximate Duration of Study	14 months
Plan for Statistical Analysis	<p>The co-primary endpoints of the study are preoperative rate of injection site clearance and rate of SLN uptake for Lymphoseek and $^{99m}\text{Tc-SC}$.</p> <p>Superiority hypothesis tests comparing the injection site clearance rate and SLN uptake rate of Lymphoseek to the injection site clearance rate and SLN uptake rate of $^{99m}\text{Tc-SC}$ will be conducted at a two-sided $\alpha=0.05$ (one-sided $\alpha=0.025$) level of significance.</p> <p>The secondary efficacy variables are:</p> <ul style="list-style-type: none">• Number of SLNs identified in each subject• Gamma counts at the primary injection site in the breast and the ex vivo gamma counts of the SLNs removed• Pain scores• Pathology status of SLNs <p>Summary statistics (mean, median, standard deviation, minimum, and maximum) will be computed for each of the secondary variables.</p> <p>All analyses of kinetics and efficacy will be performed on the ITT population, which consists of all enrolled subjects who were randomized, received an injection of Lymphoseek or $^{99m}\text{Tc-SC}$, went to surgery, and had at least one lymph node removed that was confirmed to be lymph tissue and had a known disease status (positive or negative for disease) by histopathology.</p>

TABLE OF CONTENTS

SYNOPSIS.....	2
SIGNATURE PAGE	7
1 INTRODUCTION.....	8
1.1 BACKGROUND.....	8
1.1.1 Intraoperative Lymphatic Mapping and Sentinel Lymph Node Biopsy	8
1.1.2 Lymphoseek.....	9
1.1.3 ^{99m} Tc-Sulfur Colloid (^{99m} Tc-SC).....	9
1.1.4 Study Agents and Prior Use.....	9
1.2 RATIONALE.....	10
1.3 OBJECTIVES/ENDPOINTS	10
2 STUDY POPULATION.....	11
2.1 ELIGIBILITY	11
2.1.1 Inclusion Criteria	11
2.1.2 Exclusion Criteria	11
2.2 NUMBER OF SUBJECTS	12
2.2.1 Screen Failures.....	12
2.2.2 Withdrawal	12
3 RESEARCH PLAN.....	13
3.1 OVERVIEW	13
3.2 VISIT DESCRIPTIONS	13
3.2.1 Informed Consent Visit (Visit 1)	13
3.2.2 Visit 2: Injection and Imaging	14
3.2.3 Telephone Follow Up 5 (+3) days post injection (Visit 3)	17
3.3 COMPLETION/DISCONTINUATION AND WITHDRAWAL OF SUBJECTS.....	17
4 STUDY DRUGS	18
4.1 IDENTIFICATION OF LYMPHOSEEK.....	18
4.2 IDENTIFICATION OF 99MTC-SULFUR COLLOID.....	18
4.3 IDENTIFICATION OF VITAL BLUE DYE.....	18
4.4 DOSAGE AND ADMINISTRATION.....	18
4.5 TREATMENT ASSIGNMENT	18
4.6 BLINDING.....	18
4.7 PACKAGING AND LABELING.....	19
4.8 STORAGE.....	19
4.9 SUPPLY, DISPENSING, AND RETURN.....	19
4.10 DRUG ACCOUNTABILITY	19
5 STATISTICAL AND ANALYTICAL PLANS	20
5.1 INTRODUCTION	20
5.2 RANDOMIZATION METHODS	20
5.3 EFFICACY VARIABLES.....	20
5.3.1 Primary Efficacy Variables.....	20
5.3.2 Secondary Efficacy Variables.....	20
5.4 SAFETY VARIABLES	20
5.5 SAMPLE SIZE JUSTIFICATION.....	21
5.6 STATISTICAL ANALYSES	21
5.6.1 Analysis Populations.....	21
5.6.2 Analysis of Baseline and Demographic Characteristics	21
5.6.3 Analysis of Primary Variables	22
5.6.4 Analysis of Secondary Variables	22
5.6.5 Safety Analyses	23
5.7 HANDLING MISSING VALUES.....	23
5.8 INTERIM ANALYSES	23
6 ADVERSE EVENTS.....	24
6.1 DEFINITION OF ADVERSE EVENT.....	24
7 REFERENCES.....	26

LIST OF APPENDICES

Appendix A	Schedule of Events	28
Appendix B	Performance Status Criteria	29
Appendix C	Links to Product Labels	30
Appendix D	TNM Staging for Breast Cancer Patients.....	31
Appendix E	Imaging Protocol.....	32
Appendix F	Operating Room and Surgical Procedures Flowchart	33

SIGNATURE PAGE

I have reviewed and approve this protocol. My signature assures that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality.

Arash Kardan, MD
Nuclear Medicine
Kettering Medical Center

Date of Signature (DD/MMM/YYYY)

1 INTRODUCTION

1.1 Background

1.1.1 Intraoperative Lymphatic Mapping and Sentinel Lymph Node Biopsy

Since its first description for breast cancer by Krag in 1993 [1] and Giuliano in 1994 [2], intraoperative lymphatic mapping (ILM) and sentinel lymph node biopsy (SLNB) have become standard-of-care technologies widely utilized in the surgical staging and treatment of breast cancer. The utilization of ILM/SLNB for breast cancer has resulted in the reduction in the short-term complications and long-term morbidities, including lymphedema, that are generally associated with axillary lymph node dissection (ALND) [3-6]. Additionally, the utilization of ILM/SLNB has allowed for the avoidance of the need of ALND in cases of early-stage breast cancer with limited sentinel lymph node (SLN) involvement in both cases of breast conserving surgery [7,8] and mastectomy [8].

An ideal lymph node mapping agent for ILM/SLNB should exhibit rapid clearance from the injection site, rapid uptake and high retention within the first draining lymph node(s), and low uptake by subsequent, higher echelon lymph nodes. The ideal lymph node mapping agent should also have biochemical purity, molecular size uniformity, and a high level of biologic and radiation safety. Specifically regarding a radiopharmaceutical lymph node mapping agent, it should possess characteristics for allowing for convenient, rapid, and stable ^{99m}Tc labeling.

For breast cancer, ^{99m}Tc -radiotracer agents and blue dye agents, used separately or in combination, have been described for use during ILM/SLNB [9]. Likewise, multiple injection routes for ILM/SLNB have been described [9]. Definitive evidence for the superiority of the intradermal injection route for the administration of a ^{99m}Tc -radiotracer agent (i.e., ^{99m}Tc -Sulfur Colloid abbreviated here as ^{99m}Tc -SC) during ILM/SLNB is well established from a prospective, randomized clinical trial comparing the intraparenchymal, subareolar, and intradermal injection routes in 400 breast cancer patients [10]. This prospective, randomized clinical trial demonstrated that the intradermal radiotracer injection route: (1) allowed for the greatest success of identification of an axillary SLN; (2) allowed for minimization of the time from injection of radiocolloid to successful identification of an axillary SLN; and (3) resulted in the generation of the ‘hottest’ axillary SLN and the shortest intraoperative time to harvest.

Prior to July 2011, ^{99m}Tc -SC had been used exclusively off-label for all ILM/SLNB procedures performed in the United States over the past 20 years. However, in July 2011, ^{99m}Tc -SC eventually received formal FDA approval in the United States by Pharmalucence, Inc. (Billerica, Massachusetts) for ILM during SLNB for breast cancer [11,12]. Concurrently, within the past 10 years, a novel, soluble, synthetic (i.e., non-biologic), receptor-directed targeting agent, ^{99m}Tc -Tilmanocept (Lymphoseek®, Navidea Biopharmaceuticals, Dublin, Ohio) has been developed for ILM/SLNB [13-16], and has been formally evaluated in two multi-institutional, open-label, nonrandomized, “within-patient,” Phase 3 clinical trials (NEO3-05: May 2008-June 2009; and NEO3-09: July 2010-April 2011) with ^{99m}Tc -Tilmanocept for breast cancer patients (Tis-T4/N0/M0) [17]. Resultantly, as of March 2013, Lymphoseek has now received formal FDA approval in the United States for ILM during SLNB for breast cancer and for melanoma [18] with supplemental approval for guiding sentinel lymph node biopsy for clinically node negative squamous cell carcinoma of the oral cavity in June 2014. It has been well documented in three prospective (but nonrandomized) clinical trials of Lymphoseek [17,19, 20] that ^{99m}Tc -Tilmanocept, for early-stage breast cancer, melanoma, and head and neck squamous cell carcinoma has a high SLN localization/detection rate of > 97% and a low false negative rate of < 5%, demonstrating potentially improved performance over that previously reported within the historical literature for ^{99m}Tc -SC. Both ^{99m}Tc -SC and Lymphoseek are approved radiopharmaceuticals for use in breast cancer ILM/SLNB. However, to date, there is yet to be a prospective, randomized, double-blinded clinical trial that has been conducted at any institution in the United States for directly comparing the preoperative radiotracer

kinetics and the intraoperative imaging findings (i.e., ILM and SLNB findings) for Lymphoseek and ^{99m}Tc -SC.

1.1.2 Lymphoseek

Lymphoseek is a radiopharmaceutical tracer that accumulates within lymph nodes by specifically targeting lymphatic reticuloendothelial cells. Lymphoseek is a soluble macromolecule that has a diameter in the 5 to 7 nm range and has a molecular weight of approximately 18 kDa. Lymphoseek consists of multiple units of DTPA (diethylene triamine pentaacetic acid) and mannose, each synthetically attached to a 10-kDa dextran core. Lymphoseek binds to mannose receptors (i.e., CD206) that reside on the surfaces of reticuloendothelial cells (i.e., macrophages and dendritic cells) that specifically reside within lymph nodes. The mannose portion of the macromolecule acts as a ligand binding substrate for the mannose receptor (i.e., CD206) to increase uptake and retention specifically within lymph nodes. The DTPA portion of the macromolecule serves as a chelating agent for the radiolabeling of the macromolecule with ^{99m}Tc . The diameter of Lymphoseek is substantially smaller than that of colloid-based radiolabeled lymph node mapping agents, which generally have a particle diameter size range of < 100 to 200 nm when filtered. The small diameter of Lymphoseek permits enhanced diffusion into lymph nodes and blood capillaries, resulting in a rapid injection site clearance. Upon entry into the blood, Lymphoseek can bind to mannose receptors in the liver, as well as can be filtered and excreted by the kidneys and eliminated via the urinary bladder.

Lymphoseek is a radioactive diagnostic agent indicated for lymphatic mapping with a hand-held gamma counter to assist in the localization of lymph nodes draining a primary tumor site in patients with breast cancer or melanoma.

Lymphoseek is an FDA-approved radiopharmaceutical agent.

1.1.3 ^{99m}Tc -Sulfur Colloid (^{99m}Tc -SC)

While Lymphoseek represents a synthetic, receptor-targeted lymph node mapping agent, ^{99m}Tc -SC represents a non-synthetic/biologic (i.e., containing bovine gelatin), non-specific (i.e., not receptor-targeted) lymph node mapping agent that is passively trapped and accumulates within lymph nodes.

^{99m}Tc -SC is an injectable diagnostic radiopharmaceutical agent that is indicated for:

- (1) In adult patients only, to assist in the: (i) localization of lymph nodes draining a primary tumor in patients with breast cancer when used with a hand-held gamma counter; and (ii) evaluation of peritoneo-venous (LeVeen) shunt patency in adults.
- (2) In adults and pediatric patients, for: (i) imaging areas of functioning reticuloendothelial cells in the liver, spleen and bone marrow; and (ii) studies of esophageal transit and gastroesophageal reflux, and detection of pulmonary aspiration of gastric contents.

^{99m}Tc -SC is an FDA-approved radiopharmaceutical agent.

1.1.4 Study Agents and Prior Use

All additional product information related to each radiopharmaceutical study agent is contained within the Lymphoseek and ^{99m}Tc -SC package inserts, as is shown in [Appendix C](#).

1.2 Rationale

The purpose of this study is to compare the efficacy of two functionally different diagnostic lymph node mapping radiopharmaceutical agents, Lymphoseek and ^{99m}Tc -SC, in a prospective, randomized, double-blinded fashion for ILM and SLNB in breast cancer patients.

1.3 Objectives/Endpoints

Primary Objective

- To compare the preoperative radiotracer kinetics (rate of injection site clearance and rate of SLN uptake) for Lymphoseek and ^{99m}Tc -SC.

Secondary Objectives

- To compare the number and range of intraoperatively detected SLNs identified by Lymphoseek and ^{99m}Tc -SC on an agent cohort basis
- To compare differences in the ratio of intraoperative counts for Lymphoseek vs ^{99m}Tc -SC for the hottest harvested axillary Sentinel Lymph Node (SLN) relative to the primary intradermal injection site.
- To compare subject pain tolerance (i.e., subject's perceived level of discomfort) at the injection site for Lymphoseek vs ^{99m}Tc -SC.
- To compare pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases for Lymphoseek vs ^{99m}Tc -SC.

2 STUDY POPULATION

2.1 Eligibility

Up to 40 evaluable subjects (20 per cohort) will be enrolled in this trial. Evaluable subject is defined as an enrolled subject who was randomized, received an injection of Lymphoseek or ^{99m}Tc -SC, went to surgery, and had at least one lymph node removed. Determination of study eligibility will be established by the investigator on the basis of the inclusion and exclusion criteria.

The inclusion/exclusion criteria are designed to ensure that study subjects have a diagnosis of primary breast cancer and are candidates for surgical intervention as a treatment of breast cancer.

Subjects who fulfill all respective inclusion and none of the exclusion criteria will be eligible for enrollment into the trial. Written, dated informed consent will be obtained from all subjects.

In the event that a subject has bilateral disease, the subject would not be randomly assigned but instead would receive injections of both radiopharmaceuticals, one in each breast. The subject and surgeon will be blinded to which breast is injected with which radiopharmaceutical.

2.1.1 Inclusion Criteria

Patients meeting all of the following inclusion criteria by the end of the screening phase should be considered for admission to the study:

1. The patient must be female and 18 years of age or older.
2. The patient must be a preoperative clinical Tis (see 2.1.4), T1, T2, T3, T4, as well as clinical N0 and clinical M0 breast cancer (see [Appendix D](#)).
3. The patient must have a diagnosis of primary breast cancer.
4. The patient must be a candidate for surgical intervention, either with lumpectomy and SLNB or with mastectomy and SLNB, as the treatment of their breast cancer.
5. The patient must have an ECOG performance status of Grade 0 – 2 (see [Appendix B](#)).
6. The patient must provide written informed consent with HIPAA authorization before participating in the study.

2.1.2 Exclusion Criteria

Patients will not be entered into the study if they meet any of the following exclusion criteria:

1. The patient has clinical or radiological or pathologic evidence of metastatic cancer, including any abnormal or enlarged clinically palpable lymph nodes or core biopsy/surgical biopsy/FNA evidence of malignant cell within any lymph nodes.
2. The patient has a known hypersensitivity to vital blue dye in a case where vital blue dye was planned for use during SLNB.
3. The patient has a positive pregnancy test or is lactating.
4. The patient has had prior surgery in the upper, outer quadrant, areola, or axilla to the indicated breast.

5. The patient has had pre-surgical neoadjuvant radiation or chemotherapy.
6. The patient has participated in another investigational drug study during the 30 days prior to signing informed consent.

2.2 Number of Subjects

The estimated sample size to determine a difference between the two test agents for the co-primary objectives is 19 subjects in each group. It is anticipated that not less than 20 subjects will be randomized and enrolled into each group. (Power = 0.80; $p < 0.05$.)

2.2.1 Screen Failures

Subjects who sign an informed consent form (ICF) but are ultimately not enrolled in the trial will be considered screen failures. Subjects will be assigned a subject identification number at the time the ICF is signed. Once all screening study procedures have been completed and eligibility has been confirmed, the subject will be eligible for enrollment but the subject will not be considered to be enrolled until the morning of drug administration. Demographic data and the reason for the screen failure will be collected for all screen failure subjects.

For subjects who enroll in the trial but withdraw prior to injection of either radiopharmaceutical, enrollment will be extended in order to meet sample size requirements. Enrollment will not be extended for subjects who withdraw for any reason after receiving an injection of Lymphoseek.

2.2.2 Withdrawal

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

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.

The investigator may withdraw a subject from the trial 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 discretion as long as it is in the best interest of the subject
- The investigator terminates the study
- The subject requests to be discontinued from the study

3 RESEARCH PLAN

3.1 Overview

This is a single center, blinded, randomized, parallel-group comparative study of Lymphoseek and ^{99m}Tc -SC colloid in the preoperative and intraoperative detection of excised lymph nodes in subjects with known breast cancer. All subjects will receive a single dose of 50 μg Lymphoseek radiolabeled with 0.5 mCi Tc 99m or 0.5 mCi of sulfur colloid. Subjects may also receive up to 1 mL of VBD as a companion ILM agent followed by massage of the breast adjacent to the area of injection. $\text{Tc}99\text{m}$ -labeled agents will be administered in a total volume of 0.5 mL; all radio-labeled agents will be administered in a single intradermal injection delivered adjacent to the areola in the upper outer quadrant. The approximate duration of the study is 14 months. Subject participation in the study will be defined as the time of patient screening in the clinic in the preoperative period through completion of their ILM/SLNB procedure and Day 5 follow up, which would represent a maximum duration of time of approximately 75 days.

Recruitment will take place at study investigator's offices. The investigator will describe the study to potential subjects and answer any questions about the patient's study eligibility. The surgical plan and additional study related procedures will be discussed with the patient. A study consent form will be offered to the subject for them to take home and discuss with family and/or friends.

3.2 Visit Descriptions

3.2.1 Informed Consent Visit (Visit 1)

3.2.1.1 Informed Consent

All potential study subjects will be informed of the investigational nature of this research study and will be required to read, agree to, and sign a statement of informed consent prior to participation. Consent will be obtained using a current IRB/IEC approved informed consent form.

3.2.1.2 Screening and Enrollment Activities

Screening procedures must occur within 45 days prior to Lymphoseek or ^{99m}Tc -SC injection.

Screening procedures include:

- A review of recent medical history obtained when the surgical plan was discussed with the patient.
- Demography – Date of birth, gender, and race will be collected.
- Physical examination at the screening visit or in the preceding 45 days with documented findings available.
- Collection of vital signs - heart rate, blood pressure, respiratory rate, and temperature.
- A record of medications taken seven days prior to the screening visit will be obtained.
- Relevant medical history – Medical history will be obtained on all study patients. All relevant prior medical conditions will be recorded. Medical conditions will be documented by body system, noting the year of onset and if the condition is active. Additionally, significant illnesses experienced within one week prior to screening will be recorded. Common accepted medical terminology should be used.

- Previously determined pathology findings from breast biopsy
- Eligibility assessment – Conducted by the Investigator and/or his/her designated representative.
- Explanation of the details of the pain assessment rating scale.

3.2.2 Visit 2: Injection and Imaging

Randomization assignment (see Section 4.5) will be determined just prior to the injection visit when the order for the test article is placed by the nuclear medicine technician. Either Lymphoseek or ^{99m}Tc -SC will be injected at Visit 2. The respective dosage, administration, and SPECT imaging procedures for both agents are described below.

3.2.2.1 Pre-Injection

Prior to receiving the test agent injection, adverse event monitoring will begin.

Pregnancy testing for women of child bearing potential. A urine or serum pregnancy test will be collected before receiving the Lymphoseek or ^{99m}Tc -SC injection. If the urine pregnancy test is positive, the subject will be withdrawn from the study.

3.2.2.2 Injection of Lymphoseek and ^{99m}Tc -SC

The appropriate dose of ^{99m}Tc used to label tilmanocept or sulfur colloid should be ordered from the Nuclear Medicine Department or radiolabeling facility once the subject has been scheduled for surgery.

Injection of Lymphoseek or ^{99m}Tc -SC will be at study time 0:00. The only method of injection that will be used is a single intradermal administration.

Intradermal Administration: The injection dose of ^{99m}Tc radiopharmaceutical agent will be administered at a dose of 0.5 mCi and a volume of 0.5 mL, using a tuberculin-type syringe with a 25-gauge or 27-gauge needle. ^{99m}Tc -SC should be filtered with a 0.22 micron filter. The injection will be performed using a single, intradermal injection near the areola in the upper, outer quadrant. This technique will not differ based on study group. The subject and physician administering the agent will be blinded to the drug administered. No breast massage will be allowed immediately after the intradermal injection of the radiopharmaceutical is performed. No local anesthetics agents will be allowed to be administered prior to or at the time of or in combination with the intradermal injection of the ^{99m}Tc radiopharmaceutical agents.

Immediately after injection, subjects will be asked to rate their level of discomfort using the Wong-Baker FACES pain rating scale [21], which ranges from a minimum score of zero (no pain) to a maximum score of ten (worst pain possible). The scale includes a visual analog scale with the following categories, each accompanied by a cartoon face indicating the level of pain: 0 No Hurt, 1-2 Hurts Little Bit, 3-4 Hurts Little More, 5-6 Hurts Even More, 7-8 Hurts Whole Lot, 9-10 Hurts Worst. This scale will be shown and explained to the subjects at the screening visit, and a copy will be provided for the subject to take home. Immediately after injection of Lymphoseek or ^{99m}Tc -SC, the subject will be asked to verbally rate the level of pain experienced by indicating a number between 0 and 10, based on this scale.

3.2.2.3 SPECT Imaging

The lymphoscintigraphy will be performed in two phases: (1) evaluation of the initial rapid absorption and transit phase through lymphatics to the sentinel lymph node(s), (2) additional efforts to facilitate the exact location of the SLN for surgical excision.

Subjects will be injected with the assigned radiopharmaceutical agent (0.5 mCi) per the randomization schema.

Subjects will undergo standard sequential planar SPECT imaging at 30 to 60 seconds intervals per the procedure detailed in [Appendix E](#). A SPECT/CT will also be performed for higher resolution imaging in transaxial, coronal, and sagittal planes. Vital signs (temperature, respiratory rate, pulse rate, blood pressure) will be collected on each subject just prior to injection, and post-injection at 15, 30, 60 and 120 minutes, respectively, \pm 5 minutes, and recorded on the appropriate study form. Following injection of the test article and image acquisition the subject will undergo surgery according to her surgical plan on the day of imaging.

3.2.2.4 Operating Room and Surgery (Overview)

Operating room and surgical procedures are described in [Appendix F](#).

The surgeon will be provided with the preoperative imaging results. The surgeon will perform an initial external survey of the subject with the hand-held gamma detection probe and gamma detection unit in the operating room area and will determine the site(s) of 99m Tc radiopharmaceutical agent localization.

Once the above image review and initial external survey are completed, the subject's surgical procedure will begin. If the planned surgery is lumpectomy the SLN survey and biopsy will occur prior to the lumpectomy. Conversely, per the operating surgeon's standard approach with mastectomy, SLN survey and biopsy will be completed immediately following the mastectomy with a flap of mammary tissue remaining attached during the SLN survey and biopsy. All intraoperative data will be recorded on a specifically designed SLNB data collection sheet by a designated clinical research coordinator for the study. All data from the specifically designed SLNB data collection sheet form will then be transcribed into and maintained in a prospective SLNB computerized database by a designated clinical research associate for the study.

All excised lymph nodes will be examined by a pathologist during the surgical case to determine any positive findings of malignancy per frozen section. See Section 3.2.2.4.1 below for details of the specific surgical procedures for SLN detection and biopsy and pathology evaluation.

3.2.2.4.1 Lymphoseek and 99m Tc-SC Designated “Hot” Nodes

Ex Vivo

Lymphoseek and 99m Tc-SC 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 10% or more of the ex vivo count of the hottest node (referred to as the “10% rule”) [22]. Any nodal count NOT meeting this threshold criterion will be considered a negative finding (not localized).

3.2.2.4.2 Surgical Procedures

After preoperative imaging procedures have been completed, subjects will proceed to surgery for lymphatic mapping. Excision of the primary tumor will be performed at the same time and in the same setting.

1. Intraoperatively, subjects will undergo injection of VBD, unless contraindicated, (1% isosulfan blue) via the method and injection route of choice of the operating surgeon. Breast massage may be performed immediately after VBD injection.
2. Using the handheld gamma detection system for three 2-second counts or one 10-second count obtain and record the reading for the room “background” count. Then, identify an anatomical

region on non-lymphoid tissue, greater than 20 cm from the injection site, and not over the abdomen to collect and record a second reading of three 2-second counts or one 10-second count to determine the “normal tissue” count to set the threshold criteria. Three 2-second counts or one 10-second count will be used for all subsequent readings.

3. Collect and record counts over the injection site, and then conduct an initial transcutaneous survey to identify the area of increased radioactivity that is separate from the injection site. The survey should include all of the potential areas, such as the axilla, medial chest, ipsilateral supraclavicular area, and ipsilateral neck.
4. Once an area of increased radioactivity is identified in the axilla, an incision should be placed to make as direct an approach as possible to the lymph node. Subjects will then undergo SLNB using a hand-held gamma detection system/probe to detect radiopharmaceutical (“hot”) uptake and using visual inspection to detect blue dye (“blue”) uptake.
5. An in vivo count will be collected prior to excision of each respective lymph node and an ex vivo account will be collected immediately following excision of each lymph node.
 - a. A sentinel lymph node (SLN) is defined as any lymph node that was either “hot” and “blue”, “hot” only, or “blue” only, or a palpable, enlarged, hardened node.
 - b. A “hot” SLN is defined as the node with the greatest number of counts based on radioactivity or counts derived from the application of the handheld gamma detector in vivo, or any node that satisfies the 10% rule of 10% or more of the ex vivo count of the hottest node. Any nodal count not meeting this 10% rule will be considered a negative finding (not localized).
 - c. A “blue” SLN is defined as any lymph node that is visibly stained blue, has a visible contiguous blue-stained afferent lymphatic, or both. Therefore, a very thorough evaluation of the lymphatic basin should be undertaken during the SLNB procedure. This should include gamma detection probe survey (for the detection of ^{99m}Tc radiopharmaceutical agent localization to lymph nodes), palpation, direct visualization (for the detection of vital blue dye localization to lymph nodes).
 - d. Digital palpation (for the detection of intraoperatively clinically grossly suspicious (i.e., enlarged or hard) lymph nodes) will be conducted as well. It is important to note that one or more SLN may be found at the time of SLNB [22].
6. All removed lymph nodes must be sent to pathology for further evaluation. Prior to any pathological evaluation, confirmation of ex vivo counts per tissue should be conducted. The pathological evaluation of lymph node(s) will include serial sectioning with H&E staining as well as IHC stain.
 - a. In those cases in which intraoperative frozen section analysis is performed and all SLNs are determined to be negative for malignancy on intraoperative frozen section evaluation, no further removal of axillary lymph nodes will be undertaken, unless otherwise determined necessary at the discretion of the operating surgeon.
 - b. In those cases in which intraoperative frozen section analysis is performed and the SLNs are determined to be positive for malignancy on intraoperative frozen section evaluation, a formal axillary lymph node dissection (levels I and II) may be performed at the discretion of the operating surgeon, or may be excluded at the discretion of the operating

surgeon if the subject fits the criteria for exclusion of formal axillary lymph node dissection (as based upon the ACOSOG Z0011 criteria).

- c. Any axillary lymph node that is neither “hot” or “blue”, but is determined to be intraoperatively clinically grossly suspicious (i.e., enlarged or hard), will also be removed. In those cases in which any clinically grossly suspicious lymph nodes removed intraoperatively are determined to be positive for malignancy on intraoperative frozen section evaluation, a formal axillary lymph node dissection may be performed at the discretion of the operating surgeon.
- d. In those cases in which no SLN can be intraoperatively identified at the time of ILM and SLNB procedure, a formal axillary lymph node dissection may be performed at the discretion of the operating surgeon.

3.2.2.5 Post-Operative Procedures

3.2.2.5.1 Histological Evaluation of Resected Lymph Nodes

All excised lymph nodes should undergo histopathological evaluation for the presence of tumor according to the current institutional standards. Final pathology results need to reflect consistent numbering and labeling from intraoperative worksheets to the final report. SLNs need to be individually identified in the final pathology report.

3.2.3 Telephone Follow Up 5 (+3) days post injection (Visit 3)

- Adverse event assessment

3.3 Completion/Discontinuation and Withdrawal of Subjects

A subject will be considered to have completed the study when she completes the follow up assessment 5 ± 3 days after injection. If a subject is discontinued at any time after enrollment in the study, the Investigator will make every effort to complete the follow up/premature discontinuation assessments.

4 STUDY DRUGS

4.1 Identification of Lymphoseek

Lymphoseek is a commercially available radiopharmaceutical imaging agent. Information on the product and its use are located in the US Structured Product Label ([Appendix C](#)).

4.2 Identification of ^{99m}Tc -Sulfur Colloid

^{99m}Tc -Sulfur Colloid is a commercially available radiopharmaceutical imaging agent. Information on the product and its use are located in the US Structured Product Label ([Appendix C](#)).

4.3 Identification of Vital Blue Dye

Subjects shall also undergo injection of VBD (1% isosulfan blue see [Appendix C](#)) via the method and injection route of choice of the operating surgeon. Isosulfan blue is commercially available for use as part of standard of care. The localization of VBD is detected by visual inspection only at the time of the SLNB procedure. VBDs flow through the lymphatic channels/vessels, and then passively drain into the lymph nodes in a non-targeted fashion, and are recognized by the visual presence of a blue color within the lymphatic channels/vessels and the draining lymph node, making these structures visually discernible from surrounding tissues. Allergic reaction to the VBDs are rare, but have been previously reported to vary from 0.7% and 2.7% incidences [24].

4.4 Dosage and Administration

Each trial participant will receive either an injection of Lymphoseek (50 μg) or ^{99m}Tc -SC with a total activity dose amounting to 0.5 mCi. These injections will contain a total volume of 0.5 mL.

4.5 Treatment Assignment

In this blinded, randomized, parallel-group study, all subjects will receive an injection of either Lymphoseek or ^{99m}Tc -SC. A computer-generated list of random numbers will be used for subject allocation. Randomization sequence will be created and stratified with a 1:1 allocation using a fixed block size of 8. Randomization envelopes, numbered with sequential subject ID, will be prepared by unblinded research office personnel. Each envelope will contain a card identifying the test article assigned to that subject ID. The randomization envelopes will be maintained in a secure fashion in the Nuclear Medicine department, with access available only to the technician who prepares the test article. Study personnel will inform the nuclear medicine technician when a subject is scheduled for pre-surgical injection and provide the subject ID number. The nuclear medicine technician will select the corresponding envelope to the subject ID number and prepare the appropriate test article assigned to that subject ID. The nuclear medicine technician preparing the test article will initial the card, return the card to the envelope, and place the used envelope (containing the initialed randomization card) at the end of the envelope stack (used envelopes are to be maintained as a record of test article identification). On a corresponding hard copy log the nuclear medicine technician will record the subject name, ID, and group assignment.

4.6 Blinding

Participants, investigators, radiologists, pathologists and staff will be kept blinded to the allocation throughout the trial. It is not necessary to un-blind information on any participant during the trial unless a drug-related safety signal arises during the study. The nuclear medicine technician preparing the radiolabeled product will not be blinded to treatment.

4.7 Packaging and Labeling

The ^{99m}Tc radiopharmaceutical agents are prepared in injection form and are identical in appearance and are to be dispensed in syringes that are identified with the subject name, ID number, and date (Section 4.5). The person conducting the injection is blinded and will, thus, receive only the syringes for injection labelled in the manner described above. Only one injection per subject will be performed with the exception of subjects who have bilateral breast cancer as addressed in Section 2.1.

4.8 Storage

Lymphoseek, Sulfur Colloid, and Isosulfan Blue, will be obtained from commercial sources and should be stored according to the package insert instructions.

4.9 Supply, Dispensing, and Return

Lymphoseek and ^{99m}Tc -SC will be radiolabeled at cGMP qualified facilities. The radiopharmaceutical doses can be tracked from their arrival through their disposition. A history of all the exams and doses a subject receives in Nuclear Medicine will be maintained by the PI using a system that ensures regulatory compliance.

Lymphoseek and ^{99m}Tc -SC will be prepared and injected intradermally according to the instructions and indications on the manufacturer US Structured Package Label (See [Appendix C](#)).

No local anesthetic agents (such as procaine, xylocaine, lidocaine, or carbocaine) will be permitted to be injected with either radiopharmaceutical, separately, or as a part of the combined solution for injection.

4.10 Drug Accountability

The investigator is responsible for accounting for all trial drugs discussed in the previous section.

Each time Lymphoseek, Sulfur Colloid, and Isosulfan Blue are dispensed to subjects enrolled into this study, the following information at a minimum will be recorded on the Drug Accountability Log: the patient's subject number, manufacturer, lot or batch number, radiolabeling facility, radiolabeled date and date delivered to Nuclear Medicine. At the completion or termination of the trial, a final drug accountability review and reconciliation must be completed, and any discrepancies must be investigated and their resolution documented.

5 STATISTICAL AND ANALYTICAL PLANS

5.1 Introduction

This is a prospective, double-blind, two-treatment parallel study conducted at a single study center. Once screened, subjects are enrolled in the trial for a period of up to 75 days, which includes a 5 ± 3 day follow up period. The objectives of the statistical analyses are to establish the effectiveness of Lymphoseek relative to ^{99m}Tc -SC.

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

5.2 Randomization Methods

Subjects who meet all inclusion and exclusion criteria will be enrolled in the study on Day 0 prior to surgery. Enrolled subjects will be randomized to one of two treatment groups: Lymphoseek or ^{99m}Tc Sulfur Colloid. See section 4.5 above for specifics on the randomization procedure.

5.3 Efficacy Variables

5.3.1 Primary Efficacy Variables

The co-primary efficacy endpoints are the injection site clearance rate and the SLN uptake rate for each radiopharmaceutical. Injection clearance rates and SLN uptake rates will be determined by planar SPECT imaging and by SPECT/CT. Subjects will undergo standard sequential planar imaging at 30 to 60 seconds intervals until the sentinel lymph node is seen. Once a sentinel node is located, a SPECT/CT will be performed for higher resolution imaging in transaxial, coronal, and sagittal planes.

5.3.2 Secondary Efficacy Variables

The secondary efficacy variables are:

- Number of SLNs identified in each subject
- Gamma counts at the primary injection site in the breast and the ex vivo gamma counts of the SLNs removed
- Pain scores
- Pathology status of SLNs

For the purposes of the study, an SLN is defined as a lymph node that meets the 10% rule as detailed in Section 3.2.2.4. The number of SLNs will be recorded for each subject, as well as the ex vivo gamma counts for each SLN removed. The gamma counts at the injection site will also be recorded.

Subjects will report their pain scores once during the injection procedure. Scores will be reported on a scale of zero (no pain) to a maximum score of ten (worst pain possible).

All SLNs removed will be sent for histological evaluation according to the methods described in Section 3.2.2.5.1. Each node will be characterized as positive or negative for disease.

5.4 Safety Variables

The safety analysis variables, collected on all subjects at the time points indicated, are as follows:

- Adverse Events
- Vital Signs (screening, pre injection, and 15, 30, 60, 90, 120 minutes post injection)

5.5 Sample Size Justification

The sample size for the study is based on the co-primary endpoints. In order to have an 80% chance of demonstrating that Lymphoseek is superior to $^{99m}\text{Tc-SC}$ based on the injection site clearance rate, assuming that the mean rate of Lymphoseek is 0.250, the mean rate of $^{99m}\text{Tc-SC}$ is 0.05, and the common standard deviation is 0.15, at least 10 subjects will need to be enrolled in each treatment group.

In order to have an 80% chance of demonstrating that Lymphoseek is superior to $^{99m}\text{Tc-SC}$ based on the SLN uptake rate, assuming that the mean rate of Lymphoseek is 0.1075, the mean rate of $^{99m}\text{Tc-SC}$ is 0.0875, and the common standard deviation is 0.0009, at least 19 subjects will need to be enrolled in each treatment group. These estimates are based on the sample size methods described in Dupont 1998.

In order to meet the sample size requirements for each endpoint, up to 40 evaluable subjects will be enrolled in the study (see 5.7.1 intent-to-treat). These sample size calculations were based on a two-sided $\alpha=0.05$ level of significance for each endpoint.

5.6 Statistical Analyses

The statistical analyses for the efficacy and demographic variables are described below. Additional analyses of the secondary efficacy variables may be described in the Statistical Analysis Plan (SAP) for the study. Unless otherwise stated, all hypothesis tests will be conducted using a two-sided $\alpha=0.05$ (one-sided $\alpha=0.025$) level of significance.

5.6.1 Analysis Populations

The following analysis populations will be defined.

Intent-to-treat (ITT) Population – The ITT population includes all enrolled subjects who were randomized, received an injection of Lymphoseek or $^{99m}\text{Tc-SC}$, went to surgery, and had at least one lymph node removed that was confirmed to be lymph tissue and had a known disease status (positive or negative for disease) by histopathology.

Safety Population – The safety population includes all enrolled subjects who were randomized and received an injection of Lymphoseek or $^{99m}\text{Tc-SC}$.

The analysis of the primary and secondary efficacy variables will be conducted using the ITT population. The analysis of all demographic data will be conducted using the safety population. All subjects will be analyzed according to the treatment they received.

5.6.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics of the safety population will be summarized by treatment group and overall. Continuous variables will be summarized via mean, median, standard deviation, minimum, maximum, and number of non-missing observations. Categorical variables will be summarized via counts and percentages.

5.6.3 Analysis of Primary Variables

Injection Site Clearance Rate

The injection site clearance rate for each ITT subject will be computed using the SPECT/CT imaging data. Gamma counts will be obtained at the injection site by standard sequential planar imaging at 30 to 60 seconds intervals until the sentinel lymph node is seen. Once a sentinel node is located, a SPECT/CT will be performed for higher resolution imaging in transaxial, coronal, and sagittal planes. The average clearance rate for each radiopharmaceutical will be computed and the following test will be conducted using a two-sample t-test at a two-sided $\alpha=0.05$ (one-sided $\alpha=0.025$) level of significance:

$$H_0: \mu_{LS} \leq \mu_{SC} \text{ vs. } H_A: \mu_{LS} > \mu_{SC},$$

where μ_{LS} is the average injection site clearance rate of Lymphoseek and μ_{SC} is the average injection site clearance rate of ^{99m}Tc -SC.

SLN Uptake Rate

The SLN uptake rate for each ITT subject will be computed using the SPECT/CT imaging data. Gamma counts will be obtained at the injection site by standard sequential planar imaging at 30 to 60 seconds intervals until the sentinel lymph node is seen. Once a sentinel node is located, a SPECT/CT will be performed for higher resolution imaging in transaxial, coronal, and sagittal planes. Figures showing the percent of peak activity in the node versus time will be constructed for each radiopharmaceutical. The average uptake rate for each radiopharmaceutical will be computed and the following test will be conducted using a two-sample t-test at a two-sided $\alpha=0.05$ (one-sided $\alpha=0.025$) level of significance:

$$H_0: \mu_{LS} \leq \mu_{SC} \text{ vs. } H_A: \mu_{LS} > \mu_{SC},$$

where μ_{LS} is the average SLN uptake rate of Lymphoseek and μ_{SC} is the average SLN uptake rate of ^{99m}Tc Sulfur Colloid.

In order for the study to be successful relative to the co-primary endpoints, both of the null hypotheses for injection site clearance rate and SLN uptake rate must be rejected in favor of their respective alternatives.

5.6.4 Analysis of Secondary Variables

Number of SLNs

The number of SLNs identified for each ITT subject will be recorded, and summary statistics (mean, median, standard deviation, minimum, and maximum) on the number of SLNs will be displayed for each treatment group.

Ex Vivo and Injection Site Gamma Counts

The ratio of the ex vivo gamma counts of the hottest SLN removed to the counts at the primary injection site in the breast will be computed for all subjects in the ITT population as follows:

$$R = \frac{\text{Ex vivo gamma counts of hottest SLN removed}}{\text{Gamma counts at primary injection site}}$$

If three 2-second counts were obtained for either the ex vivo measurements or the injection site measurement, then the average of the counts will be used in the ratio calculation. If one 10-second count was obtained, then that single count will be used in the ratio calculation.

It is assumed that the ratio data of each treatment group is lognormal, therefore a log transformation (base e) of the data will be also performed for each subject as follows:

$$\text{LnR} = \log_e(R)$$

Summary statistics of the ratio (R) and the log ratio (LnR) will be displayed for each treatment group and will include the mean, median, and standard deviation. In addition, a 95% confidence interval for the mean ratio and the mean log ratio will be computed.

Pain Scores

Each subject will rate their level of discomfort using the Wong-Baker FACES pain rating scale immediately after injection. The following summaries will be computed for each treatment group:

- Proportion of subjects with a 0 score (No Hurt)
- Proportion of subjects with a score of 1-2 (Hurts a Little Bit)
- Porportion of subjects with a score of 3-4 (Hurts Little More)
- Porportion of subjects with a score of 5-6 (Hurts Even More)
- Porportion of subjects with a score of 7-8 (Hurts Whole Lot)
- Porportion of subjects with a score of 9-10 (Hurts Worst)
- Mean, median, standard deviation, minimum, and maximum of pain scores

Pathology Status of SLNs

The pathology status of SLNs will be assessed on a per subject basis. The number and percent of subjects who have at least one pathology-positive SLN will be calculated for each treatment group.

5.6.5 Safety Analyses

Incidence of adverse events as described in section 6 below will be tabulated by treatment group.

5.7 Handling Missing Values

In the statistical analysis of the efficacy and safety endpoints of the study, all subjects who have missing values for a given endpoint will not be used in the respective analysis.

5.8 Interim Analyses

There are no formal interim analyses planned for this study.

6 ADVERSE EVENTS

The occurrence of any drug-related adverse events is not expected.

Normal risks of nuclear medicine scans due to the injection of a radiopharmaceuticals include allergic reactions, swelling, infections, intravenous injuries, bruising, pain and discomfort. These risks, if encountered, would occur as the result of the scheduled clinical lymphoscintigraphy or ILM.

6.1 Definition of Adverse Event

The term "adverse event," is synonymous with the term "adverse experience," which is used by the FDA.

An **adverse event** is any untoward, undesired, and/or unplanned clinical event in the form of signs, symptoms, disease, or laboratory testing, or physiological observations occurring in a human participating in a clinical study with a study drug, regardless of causal relationship. This includes the following:

- Any clinically significant worsening of a preexisting condition.
- Any recurrence of a preexisting condition.
- An AE occurring from overdose of a study drug whether accidental or intentional (i.e., a dose higher than that prescribed by a health care professional for clinical reasons).
- An AE occurring from abuse of a study drug (i.e., use for nonclinical reasons)
- An AE that has been associated with the discontinuation of the use of a study drug.

Note: A procedure is not an AE, but the reason for a procedure may be an AE.

Note: Adverse events are determined by the Investigator. If in the Investigator's opinion/experience an event (i.e. nausea in the recovery room) is expected and not uncommon for that surgery, 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.

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

- Death.
- Life-threatening situation (see below).
- Inpatient hospitalization or prolongation of an existing hospitalization (see below).
- Persistent or significant disability or incapacity (see below).
- Congenital anomaly or birth defect.

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include: allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not require hospitalization; or development of drug dependency or drug abuse.

A **life-threatening adverse event** is any AE that places the subject at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that might have caused death had it occurred in a more severe form but that did not create an immediate risk of death as it actually occurred. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though drug-induced hepatitis of a more severe nature can be fatal.

Hospitalization is to be considered only as an overnight admission. Hospitalization or prolongation of a hospitalization is a criterion for considering an AE to be serious. In the absence of an AE, the participating Investigator should not report hospitalization or prolongation of hospitalization on a form. This is the case in the following situations:

Hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol. Day or night survey visits for biopsy or surgery required by the protocol are not considered serious.

Hospitalization or prolongation of hospitalization is part of a routine procedure followed by the study center (e.g., stent removal after surgery). This should be recorded in the study file.

Hospitalization for survey visits or annual physicals fall in the same category.

In addition, a hospitalization planned before the start of the study for a preexisting condition that has not worsened does not constitute an SAE (e.g., elective hospitalization for a total knee replacement due to a preexisting condition of osteoarthritis of the knee that has not worsened during the study).

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

If there is any doubt whether the information constitutes an AE or SAE, the information is treated as an AE or SAE.

7 REFERENCES

1. Krag DN, Weaver DL, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surg Oncol* 1993;2(6):335-339; discussion 340.
2. Giuliano AE, Kirgan DM, Guenther JM, Morton. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220(3):391-401.
3. Petrek JA, Senie RT, Peters M, Rosen PP. Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. *Cancer* 2001;92(6):1368-1377.
4. Lucci A, McCall LM, Beitsch PD, Whitworth PW, Reintgen DS, Blumencranz PW, Leitch AM, Saha S, Hunt KK, Giuliano AE; American College of Surgeons Oncology Group. Surgical complications associated with sentinel lymph node dissection (SLND) plus axillary lymph node dissection compared with SLND alone in the American College of Surgeons Oncology Group Trial Z0011. *J Clin Oncol* 2007; 20;25(24):3657-3663.
5. McLaughlin SA, Wright MJ, Morris KT, Giron GL, Sampson MR, Brockway JP, Hurley KE, Riedel ER, Van Zee KJ. Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: objective measurements. *J Clin Oncol* 2008;26(32):5213-5219.
6. McLaughlin SA, Wright MJ, Morris KT, Sampson MR, Brockway JP, Hurley KE, Riedel ER, Van Zee KJ. Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: patient perceptions and precautionary behaviors. *J Clin Oncol* 2008;26(32):5220-5226.
7. Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, Leitch AM, Saha S, McCall LM, Morrow M.. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA* 2011;305(6):569-575.
8. Galimberti V, Cole BF, Zurrida S, Viale G, Luini A, Veronesi P, Baratella P, Chifu C, Sargentini M, Intra M, Gentilini O, Mastropasqua MG, Mazzarol G, Massarut S, Garbay JR, Zgajnar J, Galatius H, Recalcati A, Littlejohn D, Bamert M, Colleoni M, Price KN, Regan MM, Goldhirsch A, Coates AS, Gelber RD, Veronesi U; International Breast Cancer Study Group Trial 23-01 investigators. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. *Lancet Oncol* 2013;14(4):297-305.
9. Burak WE Jr, Agnese DM, Povoski SP. Advances in the surgical management of early stage invasive breast cancer. *Curr Probl Surg* 2004;41(11):882-935.
10. Povoski SP, Olsen JO, Young DC, Clarke J, Burak WE, Walker MJ, Carson WE, Yee LD, Agnese DM, Pozderac RV, Hall NC, Farrar WB. Prospective randomized clinical trial comparing intradermal, intraparenchymal, and subareolar injection routes for sentinel lymph node mapping and biopsy in breast cancer. *Ann Surg Oncol* 2006;13(11):1412-1421.
11. Pharmalucence Announces FDA Approval for Use of Sulfur Colloid Injection to Locate Lymph Nodes in Breast Cancer Patients. Business Wire (A Berkshire Hathaway Company). Available at: <http://www.businesswire.com/news/home/20110722005806/en/Pharmalucence-Announces-FDA-Approval-Sulfur-Colloid-Injection>.

12. Technetium Tc 99m sulfur colloid Prescribing Information. Pharmalucence. Billerica, MA 01821. Available at:
<http://www.pharmalucence.com/images/Sulfur%20Colloid%20Package%20Insert%20August%202012.pdf>.
13. Vera DR, Wallace AM, Hoh CK, Mattrey RF. A synthetic macromolecule for sentinel node detection: (99m)Tc-DTPA-mannosyl-dextran. *J Nucl Med* 2001;42(6):951-959.
14. Wallace AM, Hoh CK, Vera DR, Darrah DD, Schulteis G. Lymphoseek: a molecular radiopharmaceutical for sentinel node detection. *Ann Surg Oncol* 2003;10(5):531-538.
15. Wallace AM, Hoh CK, Darrah DD, Schulteis G, Vera DR. Sentinel lymph node mapping of breast cancer via intradermal administration of Lymphoseek. *Nucl Med Biol* 2007;34(7):849-853.
16. Wallace AM, Hoh CK, Limmer KK, Darrah DD, Schulteis G, Vera DR. Sentinel lymph node accumulation of Lymphoseek and Tc-99m-sulfur colloid using a "2-day" protocol. *Nucl Med Biol* 2009;36(6):687-692.
17. Wallace AM, Han LK, Povoski SP, Deck K, Schneebaum S, Hall NC, Hoh CK, Limmer KK, Krontiras H, Frazier TG, Cox C, Avisar E, Faries M, King DW, Christman L, Vera DR. Comparative evaluation of [(99m)Tc] Tilmanocept for sentinel lymph node mapping in breast cancer patients: results of two phase 3 trials. *Ann Surg Oncol* 2013;20(8):2590-2599.
18. FDA Approves Navidea's Lymphoseek® (technetium Tc 99m tilmanocept) Injection for Use in Lymphatic Mapping. The first receptor-targeted radiopharmaceutical approved for lymphatic mapping in breast cancer and melanoma patients. Navidea Biopharmaceuticals. Dublin, OH. Available at: <http://ir.navidea.com/phoenix.zhtml?c=68527&p=irol-newsArticle&id=1795818>.
19. Sondak VK, King DW, Zager JS, Schneebaum S, Kim J, Leong SP, Faries MB, Averbook BJ, Martinez SR, Puleo CA, Messina JL, Christman L, Wallace AM. Combined analysis of phase III trials evaluating [⁹⁹mTc]tilmanocept and vital blue dye for identification of sentinel lymph nodes in clinically node-negative cutaneous melanoma. *Ann Surg Oncol* 2013;20(2):680-688.
20. Marcinow AM, Hall N, Byrum E, Teknos TN, Old MO, Agrawal A. 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 Sep;139(9):895-902.
21. Wong DI, Baker CM. Pain in children: comparison of assessment scales. *Pediatr Nurs* 1988; 14:9-17.
22. Povoski SP, Young DC, Walker MJ, Carson WE, Yee LD, Agnese DM, Farrar WB. Re-emphasizing the concept of adequacy of intraoperative assessment of the axillary sentinel lymph nodes for identifying nodal positivity during breast cancer surgery. *World J Surg Oncol* 2007;5:18.
23. Martin RC, Edwards MJ, Wong SL, Tuttle TM, Carlson DJ, Brown M, Noyes D, Glaser RL, Vennekotter DJ, Turk PS, Tate PS, Sardi A, Cerrito PB, McMasters KM. Practical guidelines for optimal gamma probe detection of sentinel lymph nodes in breast cancer: Results of a multi-institutional study. *Surgery* 2000; 128:139-44
24. Bézu C, Coutant C, Salengro A, Daraï E, Rouzier R, Uzan S. Anaphylactic response to blue dye during sentinel lymph node biopsy. *Surg Oncol* 2011;20(1):e55-59.

Appendix A: Schedule of Study Events

Assessment	Screen (Day -45 to Day -1)	Pre- injection Day 0 30 to 60 minutes pre- injection	Post-Injection Lymphoseek/ ^{99m} Tc-SC Day 0 (hour:minute)						Surgery ^a 5:00- 10:00	Follow- up (Day) 5 ± 3)
			0:00	0:15	0:30	1:00	1:30	2:00		
Informed consent	X									
Entry criteria and randomization	X									
Medical history	X									
Physical examination	X ^c									
Vital signs	X		X ^d	X	X	X	X	X		
Urine or serum pregnancy test		X ^c								
Lymphoseek or ^{99m} Tc-SC administration			X							
Pain assessment		X	X ^d							
Vital blue dye administration									X	
Imaging – SPECT and SPECT/CT				X	X	X	X	X		
Surgery, ILM, and harvest lymph nodes									X ^a	
Adverse event monitoring	X	X	X	X	X	X	X	X	X	X ^b

- a. The interval between administration of ^{99m}Tc agent and surgery will vary according to triage and the scheduling of surgery.
- b. Adverse event assessments may be collected telephonically.
- c. Urine or serum pregnancy test must be completed and determined to be negative preceding administration of Lymphoseek or ^{99m}Tc-SC in women of childbearing potential.
- d. Time point 0:00 is before Lymphoseek or ^{99m}Tc-SC injection (a 5 minute pre-injection window is permitted).
- e. Physical examinations done within 30 days of injection may be used even if conducted prior to obtaining informed consent if they are ordered and conducted at the direction of the Investigator as part of patient standard of care.

Appendix B: Performance Status Criteria

ECOG Performance Status ^a	
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

^a Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

Appendix C: Links to Product Labels

Link to ^{99m}TC-Tilmanocept Structured Product Label:

<http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=6681aa14-5ae3-4143-9d58-c2d92a143c03>

Link to ^{99m}TC-Sulfur Colloid Structured Product Label:

<http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=eeaf70fd-46bd-4fec-ba9d-2366fdf07888>

Link to Isosulfan Blue Structured Product Label:

<http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=cbee4532-238b-4f91-86ae-f8c0c1b6d7da>

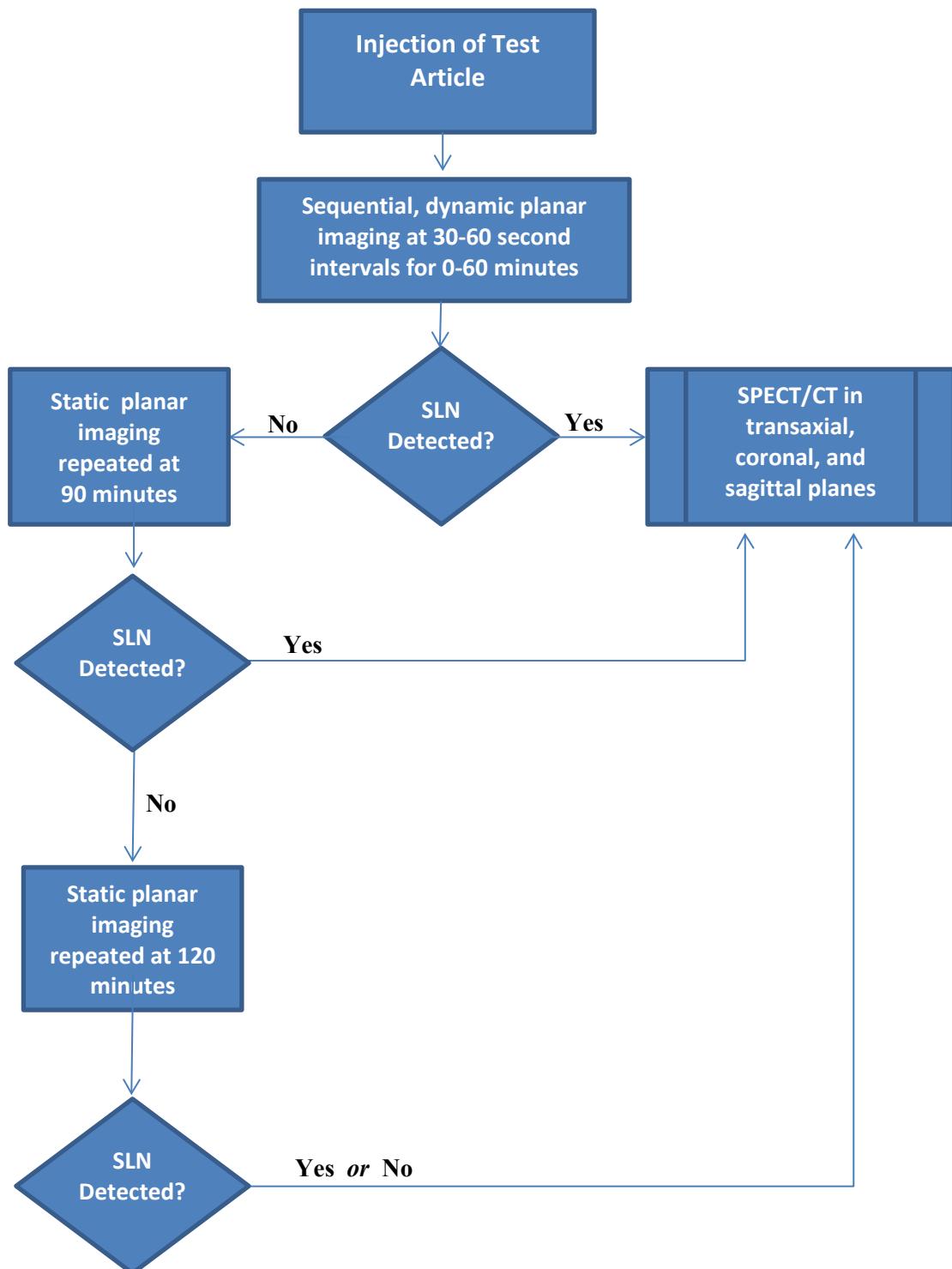
Appendix D: TNM Staging for Breast Cancer Patients

Grade	Description
Primary Tumor (T)	
TX	Primary tumor cannot be evaluated
T0	No evidence of primary tumor
Tis	Carcinoma in situ: Intraductal carcinoma, lobular carcinoma in situ, or Paget's disease of the nipple with no tumor
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 5 cm in greatest dimension
T3	Tumor more than 5 cm in greatest dimension
T4	Tumor of any size with direct extension to chest wall or skin
Lymph Node (N)	
NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral axillary lymph node(s)
N2	Metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures
N3	Metastasis to ipsilateral internal mammary lymph node(s)
Distance Metastasis (M)	
MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis (includes metastasis to ipsilateral supraclavicular lymph node(s))

Source: American Joint Committee on Cancer, Manual for Staging of Cancer 6th Edition

Staging of Cancer 6th Edition , Melanoma

Appendix E: IMAGING PROTOCOL



Appendix F: Operating Room and Surgical Procedures Flowchart

