Clinical Trial Protocol: NAV3-18

Study Title:	A Prospective, Open-Label, Multicenter Study of Lymphoseek® as a Lymphoid Tissue Targeting Agent in Pediatric Patients With Melanoma, Rhabdomyosarcoma, or Other Solid Tumors Who Are Undergoing Lymph Node Mapping
Study Number:	NAV3-18
Study Phase:	2
Product Name:	Lymphoseek®
IND Number:	061757
Investigators:	Multicenter
Sponsor:	Cardinal Health 414, LLC 7200 Cardinal Pl Dublin, OH 43017
Sponsor Contact:	[redacted]
Medical Monitor:	[redacted]

	Date
Original Protocol:	24 April 2015
Amendment 1:	14 August 2015
Amendment 2:	18 January 2016
Amendment 3:	11 January 2017
Amendment 4:	01 September 2017

SYNOPSIS

Study title	A Prospective, Open-Label, Multicenter Study of Lymphoseek® as a Lymphoid Tissue Targeting Agent in Pediatric Patients With Melanoma, Rhabdomyosarcoma, or Other Solid Tumors Who Are Undergoing Lymph Node Mapping
Study phase	Phase 2
Study objective(s)	**Primary:** To evaluate safety and tolerability of Lymphoseek in pediatric subjects with melanoma, rhabdomyosarcoma, or other solid tumors who are undergoing lymph node mapping. **Secondary:** • To assess the identified lymph node(s) to confirm: the presence/absence of tumor metastases; agent localization per tumor type; degree of localization (nodes per subject both intraoperatively and with preoperative SPECT). • To determine the concordance of in vivo localization rates of Lymphoseek and Lymphazurin™, a vital blue dye (VBD), in tissue excised and histologically confirmed as lymph nodes from those subjects receiving two mapping agents. • To record the change of subject stage based on histopathology and make a descriptive assessment on change in treatment plan
Study drug	Lymphoseek®
Dose(s)	50 µg Lymphoseek radiolabeled with 18.5 MBq (0.5 mCi) ^{99m}Tc
Route of administration	For subjects with melanoma, Lymphoseek will be administered as 1, 2 or 4 intradermal, peritumoral injections or around the excision biopsy site. For subjects with rhabdomyosarcoma or other solid tumor type, Lymphoseek will be administered as intradermal peritumoral injection(s) if anatomically appropriate or, if not possible, injection should be performed as determined clinically appropriate by the surgeon.
Duration of treatment	Single administration
Control (optional)	Lymphazurin (1% isosulfan blue for injection), a VBD, administered by subcutaneous injection immediately before or during the surgical procedure, per the agent labeling. Use of Lymphazurin is optional, consistent with institutional practice.

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 subjects with solid tumors for which this procedure is a component of intraoperative management. • Guiding sentinel lymph node biopsy using a handheld gamma counter in subjects with clinically node negative squamous cell carcinoma of the oral cavity, breast cancer, or melanoma.
Main criteria for inclusion	1. The subject's parent(s)/legal guardian(s) understand(s) and voluntarily signed an informed consent document prior to any study-related assessments/procedures being conducted. Where locally applicable, the subject also understands and voluntarily provides his/her assent prior to any study-related assessments/procedures being conducted. 2. Subject has been diagnosed with melanoma, rhabdomyosarcoma, or other solid tumors where tumor resection or biopsy is planned and lymph node mapping is appropriate. 3. The subject is clinically node negative (cN0) at the time of screening. 4. Age < 18 years 5. Male subjects of childbearing potential must be willing to use a condom during sexual intercourse and shall not father a child during the course of the study or will practice complete abstinence while on study. 6. Female subjects of childbearing potential must agree to the use of two physician-approved contraceptive methods simultaneously or practice complete abstinence while on study.
Main criteria for exclusion	1. The subject has had preoperative radiation therapy. 2. Has had previous surgery or radiation to node basins that would be involved in the intraoperative lymph node mapping (ILM) procedure. 3. Has a known allergy to dextran, or VBD (if intended to be used). 4. Has a history of alcohol abuse or alcohol dependency in the 3 years before study entry, or is an alcoholic or drug addict, as determined by the investigator. 5. Before the administration of Lymphoseek, has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical.

Study design	Prospective, open label, non-randomized, multicenter, blinded pathology assessment study to evaluate the tolerability and the diagnostic utility of Lymphoseek in pediatric subjects with melanoma, rhabdomyosarcoma, or other solid tumors. Subject age will range from neonatal through 17 years. If consistent with institutional practice, subjects may also receive Lymphazurin for a within-subject comparison of lymph node mapping.
Methodology	The proposed study includes 3 visits: A screening visit for initial determination of eligibility and evaluation of clinical status, a baseline visit on the day of surgery, and a 4- to 14-day (in person) safety follow-up to assess subject disease management. **Screening Visit (Day -29 to 0):** The screening visit will include review of trial eligibility, demography, performance status, cancer staging, informed consent, collection of medical history, vital signs, physical exams, electrocardiogram (ECG), review of medications, and clinical laboratory evaluation. A urine or serum pregnancy test will be performed for female subjects of childbearing potential within 48 hours prior to injection. **Baseline Visit (Day 1):** All subjects will receive a single dose of 50 µg Lymphoseek radiolabeled with 18.5 MBq (0.5 mCi) ^{99m}Tc (in a volume of up to 1.0 mL) at least 15 minutes but no more than 8 hours before the start of the surgical procedure. For subjects with melanoma, Lymphoseek will be administered as 1 to 4 intradermal, peritumoral injections or around the excision biopsy site. For subjects with rhabdomyosarcoma or other solid tumor type, Lymphoseek will be administered as intradermal peritumoral injection(s) if anatomically appropriate or, if not possible, injection should be performed as determined clinically appropriate by the surgeon. Radioactive dose, volume, injection site(s), route, and syringe type will be documented. An ECG will be recorded on Day 1 at least 10 minutes after Lymphoseek injection. Vital signs will be recorded at prespecified intervals before and after Lymphoseek administration. After safety measurements have been completed and before surgery, subjects may undergo dynamic and/or planar SPECT or SPECT/CT lymphoscintigraphy according to

	institutional imaging protocols. Imaging should begin at the time of injection when performing dynamic imaging. STATIC planar imaging should begin approximately 15 minutes post injection. At least 15 minutes and no more than 8 hours after Lymphoseek injection, subjects will undergo surgery to remove the primary tumor and all lymph nodes detected during the lymphatic mapping procedure. If in agreement with institutional practice, Lymphazurin will be administered in the operating room immediately before or during surgery. The injection of Lymphazurin will only be performed as deemed appropriate by the surgeon and in accordance with the standard of care at the clinical site. Blood and urine samples will be collected for safety laboratory analyses post-surgery. **Safety follow-up (Day 4 to 14):** Vital sign assessment, physical examination, and review of medications will be performed between 4 to 14 days after Lymphoseek injection. Postsurgical treatment plan; tumor, lymph node, metastasis (TNM) staging; and clinical group will be collected. Adverse events (AE) will be monitored at each visit, starting with the Baseline visit.	
Planned trial dates	**Start of study** August 2015	**End of Study** June 2021
Planned number of trial centers / countries	Up to 10 sites in the United States	
Number of subjects	For the primary analysis of safety, at least 27 subjects administered Lymphoseek will be evaluated: • At least 6 subjects with melanoma • At least 6 subjects with rhabdomyosarcoma Enrollment will be open to up to 15 additional subjects with other tumor types in which ILM and sentinel lymph node biopsy (SLNB) are appropriate. At least 20 of the recruited subjects will be ≤16 years of age.	
Safety endpoints **Primary** **Secondary**	Incidence of AEs Changes over time in vitals signs, laboratory tests, and ECGs	
Diagnostic endpoints	• Proportion of subjects with Lymphoseek-identified lymph nodes (i.e., localization)	

	• Number of Lymphoseek-identified lymph nodes per subject (i.e., degree of localization) • Proportion of subjects who underwent preoperative SPECT lymphoscintigraphy • Comparison of the number of lymph nodes detected with Lymphoseek intraoperatively to the number of nodes detected with preoperative SPECT • Comparison of centralized, blinded pathologic assessment to local pathologic assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases on a per-subject and per-node basis • False negative rates and sensitivity for each detection method in subjects undergoing lymphadenectomy • Upstaging • Change in nodal staging before and after surgery based upon Lymphoseek-identified nodes (and Lymphazurin, if applicable) • Number of changes in treatment plan and relation to lymph nodes identified by Lymphoseek Additionally for those subjects receiving both Lymphoseek and Lymphazurin: • Proportion of subjects with Lymphazurin-identified lymph nodes (i.e., localization) • Number of Lymphazurin-identified lymph nodes per subject (i.e., degree of localization) • Proportion of lymph nodes (and subjects) identified intraoperatively by Lymphazurin that are also identified intraoperatively by Lymphoseek (i.e., nodal and per-subject concordance) • Proportion of lymph nodes identified intraoperatively by Lymphoseek that are also identified intraoperatively by Lymphazurin control (i.e., nodal and per-subject reverse concordance) • Difference between nodal and reverse nodal concordance
Plan for statistical analysis	Analysis populations: • Safety: all enrolled subjects with administration of Lymphoseek • Intent-to-treat (ITT): all enrolled subjects with administration of Lymphoseek (with or without

	Lymphazurin) and complete intraoperative lymphatic mapping • Blue intent-to-treat (BITT): all ITT subjects with administration of Lymphazurin prior to ILM • Per protocol (PP): all ITT subjects without major protocol violations All safety analyses will be conducted on the safety population. The analysis of the diagnostic endpoints will be conducted on ITT, BITT, and PP populations, as applicable. AEs will be summarized using system organ class and preferred term by tumor type and overall for all subjects in the safety population. AEs will also be summarized by severity, relationship to investigational product, 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, quantitative laboratory parameter, and ECG parameter by time point, for each tumor type and overall. The localization rate, degree of localization, difference in preoperative SPECT versus intraoperative localization, agreement proportions of central pathology assessment to local pathology assessment, false negative rates, sensitivity rates, and upstaging, along with 95% confidence intervals, will be computed for each tracer (as applicable) for each tumor type and overall. The proportion of ITT subjects with preoperative SPECT performed, those who had a hot spot on scan, and the time from Lymphoseek injection to hot spot localization, will be summarized by tumor type and overall. Changes in nodal staging will be assessed via shift tables. Changes in treatment plan and relation to lymph nodes will be listed. Concordance endpoint analysis: For the BITT population, the number and proportion of concordant lymph nodes (Lymphoseek to Lymphazurin) will be computed, and a statistical test to determine if the concordance rate is greater than 0.9 will be conducted. In order for the test of the nodal concordance endpoint to successfully reject the null hypothesis that the concordance rate is ≤ 0.9, the lower boundary of the one-sided 95% exact binomial confidence

	interval must exceed 0.9 in the BITT population. In-house and/or literature data indicating that nodes may be considered as an independent sample will supplement the analysis. If the test of the concordance endpoint is met (i.e., the null hypothesis is rejected), then a test for superiority of concordance relative to the secondary diagnostic endpoint of reverse concordance will be conducted using McNemar's test. If the one-sided p-value of the test is <0.05, then the concordance rate of Lymphoseek to Lymphazurin will be shown to be significantly greater than the reverse concordance rate of Lymphazurin to Lymphoseek. The per-subject concordance and per-subject reverse concordance will also be computed along with 95% confidence intervals using the BITT population. No formal interim analyses will be conducted during this study. Data listings and tables will be created on an interim basis for review by a Data and Safety Monitoring Committee (DSMC), which is further described in Section 9.11.

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LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ADR	adverse drug reaction
AJCC	American Joint Committee on Cancer
ALARA	as low as reasonably achievable
BITT	blue intent-to-treat
°C	degrees Celsius
CD206	the mannose binding receptor
cm	centimeters (10^{-2} m)
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
DICOM	Digital Imaging and Communications in Medicine
DSMC	Data and Safety Monitoring Committee
DTPA	diethylenetriaminepentaacetic acid
ECG	electrocardiogram
EDE	effective dose equivalent
°F	degrees Fahrenheit
FNR	false negative rate
GCP	Good Clinical Practices
H&E	hematoxylin and eosin
ICH	International Conference on Harmonization
HNSCC	with head and neck squamous cell cancer
$\%ID_{SN}$	percent of injected dose in the sentinel node(s)
IHC	immunohistochemical
ILM	intraoperative lymphatic mapping
IRB	Institutional Review Board
ISF	investigator site file
ITT	intent-to-treat
keV	kiloelectron-volt
LN	lymph node
Lymphoseek	technetium Tc 99m tilmanocept
mCi	millicurie (10^{-3} Ci; $3.7x10^{7}$ Becquerel or Bq)
MedDRA	Medical Dictionary for Regulatory Activities
MBq	Megabecquerel
µg	micrograms (10^{-6} g)
mGy	Milligray
mL	milliliters (10^{-3} L)
mm	millimeters (10^{-3} m)
mmHg	millimeters of mercury
MRI	magnetic resonance imaging
µSv	Microsievert

µm	micrometers (10^{-6}m)
nm	nanometers (10^{-9} m)
NOAEL	no observed adverse effect level
OLINDA	Organ Level Internal Dose Assessment
PP	per protocol
QC	quality control
RBC	red blood cell
σ	sigma; standard deviation
SAE	serious adverse event
SCC	squamous cell carcinoma
SLN	sentinel lymph node
SLNB	sentinel lymph node biopsy
SPECT/CT	single photon emission computed tomography/computed tomography
Sponsor	Cardinal Health 414, LLC (Cardinal)
SUSAR	Suspected, Unexpected, Serious Adverse Reaction
^{99m}Tc	technetium-99m metastable isotope; γ-emitting (t½ = 6.02 h)
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek)
TMF	trial master file
TNM	tumor, lymph node, metastasis staging
US	United States
FDA	Food and Drug Administration
VBD	vital blue dye; Lymphazurin™ as 1% isosulfan blue for injection; USFDA-approved ILM colorimetric agent

TRIAL ADMINISTRATIVE STRUCTURE

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

The name and address of the participating centers, 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 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 each center's investigator site file (ISF).

LABORATORIES AND OTHER INSTITUTIONS FOR TRIAL CONDUCT

Responsibility	Name	Affiliation / Address
Statistical analysis, data management, and programming	[redacted]	[redacted]
Central laboratory (clinical laboratory analyses)	[redacted]	[redacted]
Central ECG Reader	[redacted]	[redacted] Heights, MO 63043
Pathology laboratory	[redacted]	[redacted]

1 INTRODUCTION

1.1 Background

Intraoperative Lymphatic Mapping (ILM) and Sentinel Lymph Node Biopsy (SLNB) in Adult Cancer Patients

In patients with malignant melanoma and breast cancer, lymph node status is often a strong predictor of outcome and influences the course of treatment a patient may follow after surgery. In an effort to reduce the morbidity and cost of detecting lymph node metastases, surgical oncologists have developed a method by which the sentinel lymph node(s) (SLN); i.e., the first node or nodes in a draining basin), which have the highest probability of harboring cancer, is/are identified intraoperatively and removed. This technique, called ILM with SLNB, has extremely high negative predictive values for both melanoma (Morton et al., 2006) and breast cancer (Giuliano et al., 1994) metastases. The two largest trials for melanoma (Morton et al., 2005 and Rossi et al., 2006), reported false negative rates of 6.3% and 14.7%, respectively. Morton et al. (2006) showed a false negative rate of 3.4%. SLNB has also been evaluated with positive results in adult patients being surgically treated for head and neck squamous cell carcinoma (Thompson et al., 2012; Stoeckli, 2007; Melkane et al., 2012; Broglie et al., 2011; Marcinow et al., 2013), colon cancer (Dionigi et al., 2007; van der Zaag et al., 2012), prostate cancer (Ponholzer et al., 2012), cervical cancer (Gortzak-Uzan et al., 2010; Bats et al., 2012), and lung cancer (Taghizadeh et al., 2013).

During an ILM procedure with SLNB, a radioactive tracing agent such as Lymphoseek, is injected at the site of the primary tumor and follows the drainage path of the tumor to the nearest lymph node(s). After injection of the radioactive agent, external scintigraphic imaging (i.e., lymphoscintigraphy or preoperative single photon emission computed tomography [SPECT]) can be performed to assist in the preoperative localization of nodes. A handheld gamma detection device is then used to track the pathway of the tracing agent. Lymph nodes in the potential drainage pathway are identified using the radioactive tracing agent.

Lymphoscintigraphy and intraoperative mapping with a handheld gamma detection device are relatively noninvasive techniques for mapping the lymphatic drainage patterns to track whether cancer cells have metastasized from the primary tumor to the local lymph nodes. Once a specific area of lymph drainage has been identified by the tracer, the SLN(s) can be removed surgically and tested in the pathology laboratory. Lymphatic mapping with Lymphoseek allows prompt visualization of the lymphatic system, produces high-quality images, and delivers a low radiation dose to the patient. In addition, good regional lymph node retention is seen with Lymposeek, thus improving the success rate of intraoperative gamma probe localization. In combination with surgical localization, lymphoscintigraphy allows preoperative and intraoperative identification of the SLNs in patients with breast cancer and melanoma, obviating the need for radical lymph node dissection in most patients and possibly prolonging their survival.

ILM and SLNB in Pediatric Rhabdomyosarcoma and Melanoma Patients

Rhabdomyosarcoma: Rhabdomyosarcoma is a malignancy derived from muscles that are attached to the bones (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0002402/). It occurs most commonly on the structures of the head and neck, the urogenital tract, and the arms or legs. Rhabdomyosarcoma occurs predominately in children and is the most common soft tissue tumor in children under 18 years of age (Arndt and Crist, 1999). Roughly two-thirds of cases occur in patients between the ages of 1 and 10. According to the American Cancer Society (2014), about 350 cases of rhabdomyosarcoma are newly diagnosed each year in the United States. Because rhabdomyosarcomas are complex cancers and because radiation and chemotherapy treatments can have long-term consequences, patients with rhabdomyosarcomas are usually treated at centers with significant experience in treating this type of cancer (Gosiengfiao et al., 2012; Walterhouse and Watson, 2007). Most rhabdomyosarcomas can be classified into one of two histological or pathophysiological subtypes: embryonal rhabdomyosarcoma (Li et al., 2013) or alveolar rhabdomyosarcoma (Nordberg et al., 2012; Tuna et al., 2012). These subtypes differ in their prognoses, optimal treatments, and various genetic and gene expression alterations. For either type, accurate disease staging including lymph node status relative to the spread of the cancer is a key prognostic variable and an important determinant of post-surgical therapies (Rodeberg et al., 2011; Baker et al., 2000; Bisogno et al., 2012; Blakely et al., 2003; La et al., 2011; Wharam et al., 2004; Leaphart and Rodeberg, 2007).

Despite the well-established importance of accurate disease staging and removal of nodal metastases in rhabdomyosarcoma patients and the established safety and efficacy of ILM and SLNB in adult cancer patients, there have been few studies of ILM and SLNB in pediatric rhabdomyosarcoma patients. In seven studies published since 2000 (Neville et al., 2000; McMulkin et al., 2003; Gow et al., 2008; Kayton et al., 2008, Weiss et al., 2011; De Corti et al., 2009; Parida et al., 2012), ILM and SLNB procedures were performed in 28 pediatric rhabdomyosarcoma patients. Positive nodes were identified in 6 of the 28 patients (21.4%). These studies demonstrated that these procedures were technically feasible and were not associated with complications.

The limited clinical experience of ILM and SLNB in pediatric rhabdomyosarcoma patients can be contrasted with the experience related to regional lymph node dissections in this same population of patients. As far back as 1987, Lawrence et al. reviewed medical records of 592 children who were surgically treated for rhabdomyosarcoma, identifying 81 (14%) with histologically proven metastases to regional lymph nodes. This study showed that lymph node metastases were highly associated ($p = 0.001$) with poorer survival. In 2004, the Intergroup Rhabdomyosarcoma Study reported on the outcomes of 405 children treated for rhabdomyosarcomas (Wharam et al., 2004). This study found that "Lymph node involvement at diagnosis was the single factor most predictive of increased total local failure risk…" More recently (La et al., 2011), an evaluation of 226 children being treated for rhabdomyosarcomas of the extremities was performed with 55 (24%) having been found to have lymph node metastases. It was concluded that evaluation of lymph nodes was necessary for disease staging and optimal treatment. These findings are examples of a much wider literature showing the importance of lymph node evaluations in rhabdomyosarcoma patients;

the published clinical experience with regional lymph node dissections in this patient population extends to well over a thousand patients. In adult cancer patients, ILM and SLNB have been evaluated in many thousands of patients and have been found to be safe and effective alternatives to regional lymph node dissections, allowing for less surgery-related trauma and a lower incidence of post-surgical lymphedema.

Melanoma: Melanomas in children are relatively rare, occurring at about the same rate as rhabdomyosarcomas (Mills and Messina, 2009; Lange et al., 2007; Lewis, 2008), although the reported incidence of pediatric melanoma appears to be rising (Linabery and Ross, 2008; Downard et al., 2007; Strouse et al., 2005). Girls are more likely than boys to be diagnosed with melanomas (Berk et al., 2010).

Compared with the experience in rhabdomyosarcoma patients, ILM and SLNB have been extensively evaluated in pediatric patients with melanomas. A review of the literature on the use of ILM and SLNB in pediatric melanoma patients (Neville et al., 2000; Gow et al., 2008; De Corti et al., 2009; Berk et al., 2010; Zuckerman et al., 2001; Toro et al., 2003; Pacella et al., 2003; Butter et al., 2005; Roaten et al., 2005; Shah et al., 2006; Livestro et al., 2007; Topar and Zelgar, 2007; Howman-Giles et al., 2010; Moore-Olufemi et al., 2011) yielded the following conclusions:

- Pediatric melanoma patients are more likely to have positive SLNs than adult melanoma patients and young children more so than adolescents, i.e., age is inversely proportional to SLN positivity.
- ILM and SLNB are safe and effective alternatives to regional lymph node dissections and can be used similarly to ILM and SLNB use in adult melanoma patients.
- SLNB provides information that can guide post-surgical treatment decisions.

Due to the success of ILM and SLNB in pediatric melanoma cases and the acceptance of these procedures as the standard of care in adult melanoma patients, most children undergoing surgeries to remove melanomas have these procedures, even though ILM agents have not been approved by the Food and Drug Administration (FDA) for use in children. (Raval et al. 2010) reviewed pediatric melanoma cases from the National Cancer Data Base in which 461 of 671 (68.7%) children in the database who were treated for melanoma between 2003 and 2007 had SLNBs; 25.6% of the patients undergoing SLNB had pathology-confirmed lymph node metastases.

Vital Blue Dye (VBD)

Lymphazurin (1% isosulfan blue for injection) is a sterile aqueous solution for subcutaneous administration. Lymphazurin is used for visualization of the lymphatic system draining the region of injection. Lymphazurin and other VBDs depend solely on their inherent color in order to provide visualization of the lymphatic structures, effectively requiring line-of-sight as the method of feedback to the surgeon. This requires the surgeon to hunt, via dissection of the tissue between the injection site and any flow-to point, in order to acquire the line-of-sight as these agents have no particular specificity for localization in the tissues of interest. VBD's reliability as a singly applied ILM agent has come under question in the literature

(Neves et al., 2010; Montgomery et al., 2002; Gur et al., 2011; Aydogan et al., 2008; Kang et al., 2010).

Lymphoseek

Lymphoseek is a radiotracer that accumulates in lymphatic tissue by binding to a mannose binding receptor that resides on the surface of dendritic cells and macrophages within the nodes. Lymphoseek is a macromolecule consisting of multiple units of DTPA (diethylenetriaminepentaacetic acid) and mannose, each synthetically attached to a 10 kilodalton dextran backbone. The mannose acts as a substrate for the receptor and the DTPA serves as a chelating agent for labeling with ^{99m}Tc. Standard lymphosintigraphy has been performed using ^{99m}Tc sulfur colloid particles (50 to 800 nm in size) that are nonselectively removed by lymph node phagocytosis. Lymphoseek has a diameter of about 7 nm (Vera et al., 2001), which is substantially smaller than current radiolabeled agents used for targeting lymphoid tissue. Lymphoseek's small diameter permits enhanced diffusion into lymph nodes and blood capillaries, resulting in a rapid injection site clearance. Lymphoseek has demonstrated less injection pain perception by patients (Unkart et al., 2015), faster injection site transit, more successful localization of lymph nodes, and detection of more lymph nodes in the lymphatic drainage pathways in subsets of patients receiving Lymphoseek compared with other subsets having sulfur colloid (Ellner et al., 2003; Wallace et al., 2007a; Wallace et al., 2007b; Wallace et al., 2003; Wallace et al., 2009).

Lymphoseek is approved by the US (United States) Food and Drug Administration for use in lymphatic mapping to assist in the localization of lymph nodes draining a primary tumor site in patients with solid tumors for which this procedure is a component of intraoperative management and for guiding SLNB in patients with clinically node negative squamous cell carcinoma (SCC) of the oral cavity, breast cancer, or melanoma.

Lymphoseek is approved in the European Union (EU) for use in imaging and intraoperative detection of sentinel lymph nodes draining a primary tumor in adult patients with breast cancer, melanoma, or localized squamous cell carcinoma of the oral cavity.

1.2 Previous Nonclinical Research and Clinical Trial Experience with Tilmanocept and Lymphoseek

A detailed evaluation of safety and efficacy data from nonclinical and clinical studies can be found in the accompanying Investigator's Brochure supplied by Cardinal. In addition, the data described in this protocol are reported in peer-reviewed publications, including nonclinical studies (Hoh et al., 2003), the Phase 1 clinical trials (Ellner et al., 2003; Wallace et al., 2007a; Wallace et al., 2007b; Wallace et al., 2003; Wallace et al., 2009), the Phase 2 clinical trial (Leong et al., 2011) and the Phase 3 studies (Tokin et al., 2012; Sondak et al., 2012; Wallace et al., 2013; Marcinow et al., 2013; Agrawal et al., 2015).

1.2.1 Nonclinical Evaluations

Nonclinical studies with Lymphoseek or tilmanocept demonstrated that the drug selectively binds to its intended receptor (the CD206 mannose binding receptor), is appropriately

distributed for radiodetection of lymphatic tissue, and is well tolerated by rats, rabbits, guinea pigs, and dogs.

Pharmacokinetics data obtained from nonclinical studies demonstrated rapid absorption into the plasma. Urinary excretion was a major pathway of elimination. Lymphoseek exhibited rapid clearance from the injection site, rapid uptake by the local lymph node, and low uptake by the remaining lymph nodes. Tilmanocept was well tolerated at all doses tested in nonclinical safety pharmacology studies and in single and repeated dose toxicology studies in rats, rabbits, and dogs. In some studies in rabbits and dogs, tilmanocept acted as a local irritant of the subcutis or skeletal muscle, and induced mild inflammation and tissue degeneration. The no-observed adverse-effect level (NOAEL) was 42 µg/kg/day. Tilmanocept was not mutagenic or genotoxic in vitro or in vivo. No signs or symptoms of hypersensitivity were observed in a study in guinea pigs.

1.2.2 Clinical Pharmacokinetics and Pharmacodynamics

In Phase 1 clinical trials, Lymphoseek showed rapid injection site clearance (approximately 2 to 3 hours). Absolute uptake in the primary sentinel lymph node was dose-related for Lymphoseek, although relative nodal uptake (%ID_{SN}) overall was independent of dose and ranged from 0.05%ID_{SN} to 1.81%ID_{SN}.

In a Phase 2 trial, Lymphoseek was highly effective in identifying tumor-draining lymph nodes (i.e., overall, a “hot spot” from Lymphoseek was identified in 93.0% of patients for whom preoperative lymphoscintigraphy was performed, and the per patient intraoperative localization rate was 96.2%). Diagnostic performance of in vivo Lymphoseek findings relative to pathology assessment of tumor tissue indicated a high per tissue sensitivity estimate (overall, 92.0%). The overall false negative rate (FNR) for pathology was 8.0%, supporting the accuracy of Lymphoseek in identifying lymph nodes with a high potential for containing tumor metastases in the lymphatics draining the tumor bed.

1.2.3 Clinical Efficacy

Clinical efficacy was evaluated in two pivotal Phase 3 clinical trials in adult subjects with breast cancer and melanoma, comparing lymph node detection of Lymphoseek and VBD. Lymphoseek demonstrated a statistically significant concordance rate with VBD (meta-analysis concordance rate = 99.99%).

The detection concordance between Lymphoseek and VBD was similar among subjects with melanoma and subjects with breast cancer (meta-analysis concordance rate = 99.99% for both populations). Lymphoseek also demonstrated a higher sensitivity for detecting tumor-positive (as confirmed by pathology) lymph nodes, corresponding to a decreased FNR when compared with VBD on a per node basis. The corresponding sensitivity rate in detection of lymph nodes most likely to be positive for tumor cells for Lymphoseek was 99.99%, compared with 78.02% for VBD. The FNR for VBD (21.98%) was higher than the FNR for Lymphoseek (0.01%). These findings suggest that, when VBD is used as the imaging agent, there is an increased risk of missing the detection of tumor-involved lymph nodes and incorrectly staging cancer patients. The Lymphoseek-only findings (pathology-positive nodes

that were hot/not blue) suggest that Lymphoseek was markedly more effective in identifying lymph nodes that harbored disease than was VBD.

A third pivotal Phase 3 clinical trial was conducted in adult subjects with oral cavity or cutaneous SCC. Subjects received an injection of Lymphoseek prior to surgery for excision of the primary tumor and Lymphoseek-guided SLN dissection followed by planned elective neck dissection. All excised lymph nodes (Lymphoseek-identified SLNs as well as non-SLNs) underwent histopathologic evaluation for presence of metastatic disease. The primary endpoint in this trial was the Lymphoseek FNR (subjects with pathology-positive lymph node(s) not identified by Lymphoseek). Thirty-nine subjects had at least one pathology-positive lymph node, and Lymphoseek detected nodes with positive pathology in all but one patient. The FNR in this trial was 2.56%.

1.2.4 Clinical Safety

More than 134,000 patients in clinical trials and US commercial use for ILM with SLNB have been exposed to Lymphoseek, and there have been no safety signals, no deaths due to drug, and no SAEs (serious adverse events) due to Lymphoseek. There are no known drug interactions leading to contraindications with the use of Lymphoseek.

1.2.5 Radiation Exposure (Subjects)

Subjects' exposure to Lymphoseek delivered as indicated in this protocol will be similar to that already established for adult breast cancer, melanoma and head/neck squamous cell carcinoma patients. The radiation absorbed dose for adults is included in the FDA-reviewed package insert for the approved product. The OLINDA (for Organ Level INternal Dose Assessment) software (Stabin et al., 2005) was used to calculate radiation exposure. For pediatric subjects, the effective dose equivalents range from 355 µSv (15-year-old male) to 1232 µSv (1-year-old female). Effective dose equivalents for pediatric subjects of various ages as well as effective dose by organ is shown in Table 1; values for adults subjects are also provided for reference.

The effective dose equivalents for other technetium ^{99m}Tc-based evaluations in children are: 1) bone scan = 1,800 to 3,000 µSv; and 2) thyroid scan ≈ 3,000 µSv.

1.2.6 Radiation Exposure (Health Care Workers and Care Givers)

Healthcare worker exposure to Lymphoseek delivered as indicated in this protocol will be similar to that already established for adult breast cancer, melanoma and head/neck squamous cell carcinoma patients. Highest whole body doses (µSv) received by healthcare works can be estimated for nuclear medicine staff as < 0.2, surgeons as 1.9, nurses as 0.2 and pathology staff as 2.5 such that greater than 400 procedures with Lymphoseek would have to be performed prior to reaching an annual exposure of 1000 µSv for non-radiation workers or greater then 20,000 procedures prior to reaching an annual exposure of 50,000 µSv for radiation workers.

Primary caregiver exposure has not been quantified but is assumed to be less than the exposure of providing nursing care. Excreta from patients contains very low level radiation and will effectively decay to nil in 72 hours. Patients discharged for home pose essentially no risk to their co-habitants through regulator social contact.

Table 1. Estimated Radiation Absorbed Dose for a 0.5 mCi, 50 µg Dose of Lymphoseek in Intradermal Injection of Cancer Patients
(Reference Patient is Intradermal Injection of Lymphoseek; Units *in mGy)*

Target Organ	Adult 70 kg	15 Year Old Phantom 55 kg	10 Year Old Phantom 33.5 kg	5 Year Old Phantom 20.3 kg	1 Year Old Phantom 12.1 kg
brain	0.003	0.0036	0.00519	0.00759	0.01119
gallbladder wall	0.0349	0.04188	0.060377	0.088297	0.130177
lower large intestinewall	0.0123	0.01476	0.021279	0.031119	0.045879
small intestine	0.0101	0.01212	0.017473	0.025553	0.037673
stomach	0.0184	0.02208	0.031832	0.046552	0.068632
upper large intestine wall	0.0125	0.015	0.021625	0.031625	0.046625
kidney	0.1863	0.22356	0.322299	0.471339	0.694899
liver	0.0324	0.03888	0.056052	0.081972	0.120852
lungs	0.0374	0.04488	0.064702	0.094622	0.139502
muscle	0.0092	0.01104	0.015916	0.023276	0.034316
ovaries	0.187	0.2244	0.32351	0.47311	0.69751
red marrow	0.0127	0.01524	0.021971	0.032131	0.047371
bone	0.0177	0.02124	0.030621	0.044781	0.066021
spleen	0.0285	0.0342	0.049305	0.072105	0.106305
thymus	0.1168	0.14016	0.202064	0.295504	0.435664
thyroid	0.088	0.1056	0.15224	0.22264	0.32824
urinary bladder	0.0586	0.07032	0.101378	0.148258	0.218578
total body	0.0195	0.0234	0.033735	0.049335	0.072735
EDE (males, mGy)	**0.2960**	**0.3552**	**0.5121**	**0.7488**	**1.1040**
EDE (females, mGy)	**0.3302**	**0.3962**	**0.5712**	**0.8354**	**1.2316**

2 STUDY OBJECTIVES

2.1 Primary Objective

To evaluate safety and tolerability of Lymphoseek in pediatric subjects with melanoma, rhabdomyosarcoma, or other solid tumors who are undergoing lymph node mapping.

2.2 Secondary Objectives

- To assess the excised lymph node(s) to confirm: the presence/absence of tumor metastases; agent localization per tumor type; degree of localization (nodes per subject both intraoperatively and with preoperative SPECT.
- To determine the concordance of in vivo localization rates of Lymphoseek and Lymphazurin, a vital blue dye (VBD), in tissue excised and histologically confirmed as lymph nodes from those subjects receiving two mapping agents.
- To record the change of subject stage based on histopathology and make a descriptive assessment on change in treatment plan.

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Study Design

This is a prospective, open label, non-randomized, multicenter, blinded pathology assessment study to evaluate the tolerability and the diagnostic utility of Lymphoseek in pediatric subjects from neonatal to less than 18 years of age with melanoma, rhabdomyosarcoma, or other solid tumors where ILM and SLNB is appropriate and tumor resection or biopsy is planned. In accordance with local site practice, subjects may also receive Lymphazurin for a within-subject comparison of lymph node mapping.

The proposed study includes 3 visits: A screening visit for initial determination of eligibility and evaluation of clinical status, a baseline visit on the day of surgery, and a 4-to 14-day safety (in person) follow-up to assess subject disease management.

The Schedule of Study 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 pediatric subjects with melanoma, rhabdomyosarcoma, or other solid tumors for which ILM and SLNB is useful, and by determining the concordance of detection rates of Lymphoseek and of Lymphazurin in the subjects who have utilized both agents. In addition, for each agent used, the presence or absence of tumor in the nodes, the number of nodes detected per tumor type, and the change in patient staging will be determined.

As the primary objective of this study is the safety of Lymphoseek in this population, Lymphazurin is being used to the extent possible as an optional control in this study for ILM endpoints because it would not be feasible to evaluate Lymphoseek's performance relative to another gamma-emitting radiopharmaceutical (i.e., technetium sulfur colloid) in a within-subject study design. A meaningful comparison of radiopharmaceuticals in a between-subject design would necessitate the enrollment of large numbers of subjects, which again would not be feasible, given the relatively small incidence of pediatric solid tumors for which ILM and SLNB is indicated. Additionally, the use of Lymphazurin is a concomitant procedure that provides real-time intraoperative assessment between Lymphoseek and Lymphazurin. Lastly, Lymphazurin is an appropriate comparator because lymphatic mapping with a VBD is recognized and authorized in the US and multiple EU countries as an experiential clinical practice agent, it has been a forming agent in the development of the theory/practice of SLNB, and, importantly, its use is supported by ASCO-SSO (Wong et al, 2012) and EANM-EORTC sentinel node guidelines in melanoma (Chakera et al, 2009). However, due to the potential for allergic reactions within this vulnerable population, the use of Lymphazurin is optional and should be used consistent with instutitional practice.

3.2.1 Parent/Guardian

The subject's parent(s) or guardian(s) will assist in responding to questions and answer questions during phone calls, etc., for the duration of the study. The parent(s) or guardian(s) will accompany the study subject to the clinical appointments throughout the study and supply information as needed.

3.2.2 Rationale for Subject Selection

No clinical trials have been conducted to evaluate Lymphoseek performance in pediatric cancer patients. This study will gather feasibility and safety data in pediatric subjects diagnosed with rhabdomyosarcoma, melanoma, or other solid tumors for whom tumor resection or biopsy is planned and lymph node mapping is indicated. Melanoma and rhabdomyosarcoma represent the most frequently occurring pediatric solid tumor types for which ILM is an appropriate procedure.

3.2.3 Justification for Radiation Exposure With Proposed Lymphoseek Radioactive Dose

In nuclear medicine imaging, the diagnostic quality of an image is dependent upon the number of radioactive events (or counts) detected by the camera in the regions of interest. The number of recorded events (counts) increases with the duration of the acquisition period and, for a given acquisition period, with the radioactivity administered. Although the injected radioactivity should then be high, the higher the applied radioactivity dose, the higher the radiation exposure to the subject. Thus, in nuclear medicine, the optimal radioactive dose for a given radiopharmaceutical is usually defined as the lowest radioactive dose that renders an image of sufficient quality to provide high diagnostic confidence, commonly referred to as ALARA (as low as reasonably achievable) practices for radiation exposure.

Calculated pediatric effective dose equivalents range from 0.355 mGy (15 year old male) to 1.232 mGy (1 year old female) as as shown in Table 1 for pediatric subjects of various age.

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 Institutional Review Board(s) (IRB).

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.

3.4 Study Duration

Subjects will be enrolled for approximately 1.5 months 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 enrollment into the study. 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. The subject's parent(s)/legal guardian(s) understand(s) and voluntarily signed an informed consent document prior to any study-related assessments/procedures being conducted. Where locally applicable, the subject also understands and voluntarily provides his/her assent prior to any study-related assessments/procedures being conducted.
2. Subject has been diagnosed with melanoma, rhabdomyosarcoma, or other tumor where tumor resection or biopsy is planned and lymph node mapping is appropriate.
3. The subject is clinically node negative (cN0) at the time of screening.
4. Age < 18 years
5. Male subjects of childbearing potential must be willing to use a condom during sexual intercourse and shall not father a child during the course of the study or will practice complete abstinence while on study.
6. Female subjects of childbearing potential must agree to the use of two physician-approved contraceptive methods simultaneously or practice complete abstinence while on study.

4.1.2 Exclusion Criteria

1. The subject has had preoperative radiation therapy.
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 or VBD (if intended to be used).
4. Has a history of alcohol abuse or alcohol dependency in the 3 years before study entry, or is an alcoholic or drug addict, as determined by the investigator.
5. Before the administration of Lymphoseek has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical.

4.1.3 Justification of Selection Criteria

Rhabdomyosarcoma and melanoma represent the most frequently occurring pediatric solid tumor types for which ILM is an appropriate procedure. While most cases are expected to occur in children greater than 2 years of age, younger children who present with these tumor types and meet the other eligibility criteria will not be excluded.

4.2 Recruitment

Subjects will be recruited in accordance with the inclusion and exclusion criteria listed above from oncology or pediatric practices. Potentially suitable subjects will be asked by their treating specialist about their willingness to participate in this study. At least 20 of the recruited subjects will be ≤16 years of age.

The minimum number of subjects who will be recuited for each age range are indicated in Table 2.

Table 2. Minimum enrollment per age group

Age Range	Minimum number of enrolled and injected subjects
1 month to < 2 years	1
2 to <6 years	3
6 to <12 years	3
12 to <18 years	3

The first three subjects between the ages of 2 to 17 will be recruited and injected. Once these three subjects have completed the Day-8 safety evaluation, enrollment will be put on hold until the Data Safety Monitoring Committee (DSMC) reviews all available safety information and agrees to proceed with additional enrollment. If the DSMC concurs, study enrollment will then be open to all age cohorts (1 month to <18 years). The DSMC will then determine the frequency to meet based on review of the available trial information.

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 adverse event (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 or parent/guardian 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 and/or parent or guardian provides written informed consent/assent, 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 "18"
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 "18-03-001".

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 an injection of 50 µg tilmanocept radiolabeled with 0.5 mCi ^{99m}Tc. Lymphoseek may be administered to a subject as a single injection or as multiple injections. The recommended total injection volume for each subject is 0.1 or 0.5 mL administered in a single syringe; 0.5 or 1.0 mL in two syringes (0.25 mL or 0.5 mL each); or 0.4 or 1.0 mL administered in four syringes (0.1 mL or 0.25 mL each). For subjects with melanoma, Lymphoseek will be administered as 1, 2 or 4 intradermal, peritumoral injections or around the excision biopsy site. For subjects with rhabdomyosarcoma or other solid tumors, Lymphoseek will be administered as intradermal peritumoral injection(s) if anatomically appropriate or, if not possible, injection should be performed as determined clinically appropriate by the surgeon.

All doses will be provided in 1 mL BD Luer-Lok™ syringes. Each syringe will be delivered with a ½ inch 25-30 gauge needle that can remain or can be replaced with an alternate needle as selected by the surgeon or nuclear medician physician as most appropriate for the individual subject's tumor type. The final administered dose will be ± 20 % of the intended dose (0.4 to 0.6 mCi).

5.3 Vital Blue Dye

Any investigational site with current experience and standard practice with VBD will use Lymphazurin (1% isosulfan blue injection). Other dyes should not be used. Lymphazurin is commercially available and will be ordered and administered to subjects at the start of or during the surgical procedure as part of their standard of care, per institutional practice. 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. The use of Lymphazurin is optional, consistent with institutional practice.

5.5 Packaging and Labeling

Tilmanocept Powder kits ready for radiolabeling will be shipped and stored at the region-specific Cardinal Health radiopharmacy or clinical site radiopharmacy performing the radiolabeling. Tilmanocept Powder is provided in a 250-microgram vial. A carton contains five vials of tilmanocept. One kit, which is one tilmanocept vial, should be used for each

subject. The carton also contains five shield labels and 25 syringe labels. This package has been designed specifically for tilmanocept and protects the vials during shipment, handling, and storage. Cardinal will provide a radiolabeling protocol and Quality Control worksheets. If the clinical site uses a Cardinal Health radiopharmacy, the Cardinal Health radiopharmacy will radiolabel tilmanocept with 0.5 mCi of ^{99m}Tc and deliver single or multiple syringes that are ready for injection to the clinical site radiopharmacy.

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 Day 4 to 14 follow-up will be documented.

6.2 Post-Study Therapy

Not applicable.

7 SCHEDULE OF EVALUATIONS AND VISIT DESCRIPTION

7.1 Schedule of Evaluations

Evaluations will be performed during a period of up to 14 days post Lymphoseek administration, following a screening period of maximum 30 days. A schedule of evaluations is provided in the Schedule of 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 (case report form) and enrollment log
- Demography – Date of birth, gender, and race
- Medical history – Medical history will be obtained on all study subjects. All relevant prior medical conditions will be recorded in the eCRFs. Documented medical conditions will also note the month/year of onset and if the condition is active.
- Cancer staging – tumor, lymph node, metastasis staging (TNM) (Edge, 2010) information will be collected.
- Performance status will be collected using the Lansky (Lansky et al, 1987) or Karnofsky (Karnofsky et al, 1948) scale as determined by age.
- Vital signs – Vital signs will include body temperature (°F or °C), 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 (in pounds) and height (in inches) without shoes will be recorded only at the screening evaluation.
- ECG – The ECG (electrocardiogram) reading will be assessed for clinical significance by the investigator, and any clinically significant abnormal findings will be noted on the subject's medical history and should be transmitted to the Central ECG Reader within 1 business day.
- Physical exam – Physical examinations will include examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, 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 and urine samples obtained for hematology, chemistry, and urinalysis (See Section 8.8.2). [Alternatively, blood and urine may be collected on Day 1 if performed before Lymphoseek injection.]
- Concomitant medications (within 30 days before surgery).
- 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 potential as defined as having reached menarche.

Subject care provider/guardian 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

The indicated dose of Lymphoseek should be ordered from the Cardinal Health radiopharmacy or clinical site radiopharmacy by the Nuclear Medicine Department once the subject has been scheduled for surgery.

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

7.2.4 Day 1: Before Injection

Before receiving the Lymphoseek injection, vital signs will be collected. Vital signs will include body temperature (°F or °C), respiratory rate, radial pulse rate, and systolic and diastolic blood pressures. Prior to obtaining blood pressures and pulse, the subject should be resting quietly for 1 minute. Body weight and height measurements are not required.

Each syringe will be assayed individually and recorded in the source documentation prior to injection.

7.2.5 Day 1: Injection of Lymphoseek

Injection of Lymphoseek will be at study time 0:00. Subjects will receive 50 µg tilmanocept radiolabeled with 0.5 mCi ^{99m}Tc by injection as described in Section 5.2.

7.2.6 Day 1: Post Injection

Vital signs will be collected at 10 minutes, 30 minutes, and 1 hour post-injection and after surgery. Vital signs will include body temperature (°F or °C), respiratory rate, pulse rate, and systolic and diastolic blood pressures. Before blood pressures and pulse are obtained, the subject should be resting quietly for 1 minute. Body weight and height measurements are not required. Allowable windows for vital sign collection are as follows:

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

An ECG will be recorded on Day 1 at least 10 minutes after Lymphoseek injection. The ECG reading will be reviewed by the investigator, and any clinically significant abnormal findings will be noted as an AE.

Each syringe will be assayed individually and recorded in the source documentation after injection.

7.2.7 Day 1: Imaging and Surgery

Dynamic and/or planar SPECT or SPECT/CT imaging may be performed prior to surgery as described in Section 8.3, and any finding will be documented and recorded on the appropriate CRF.

Vital signs and ECGs will be recorded as described in Section 7.2.6.

If Lymphazurin is to be used, it should be administered in the operating room at the start of or during surgery prior to transcutaneous surveying. The injection will be performed as deemed appropriate by the surgeon and in accordance with the standard of care at the clinical site. Fifteen minutes to 8 hours after Lymphoseek injection, subjects will undergo surgery to remove the primary tumor, ILM will be performed, and SLN(s) will be harvested. Locations of both hot and blue (when applicable) nodes will be recorded.

7.2.8 Day 1: After Surgery

Clinical laboratory evaluations (chemistry, hematology and urinalysis), review of medications, and AE monitoring will be performed. Medications administered for anesthesia related to surgery do not need to be captured in the eCRFs.

7.2.9 Day 4 to 14: Postsurgical Safety Follow-up

Vital sign assessment, physical examination, review of medications, and AE monitoring will be performed. The post surgical treatment plan and TNM staging will be collected for all subjects. Clinical group will be collected for subjects with rhabdomyosarcoma.

7.2.10 End of Study

The Day 4 to 14 Follow-up visit will serve as the end of study visit.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Male or female pediatric subjects aged <18 years (at least 20 of the subjects will be ≤16 years of age) will be enrolled. For the primary analysis, at least 27 subjects will be injected with Lymphoseek. This population of subjects will include at least 6 subjects with melanoma, at least 6 subjects with rhabdomyosarcoma, with enrollment open to up to an additional 15 subjects with other solid tumors for which ILM and SLNB are appropriate. At least 25 subjects will complete the 4-to 14-day follow up.

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 eCRFs. Common accepted medical terminology should be used.

8.1.3 Staging

All subjects will be assigned TNM classification and clinical stage prior to the primary surgical intervention. After the primary surgical intervention, TNM classification will be updated along with the pathological stage. The TNM Staging System is based on the extent of the tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M). Table 3, Table 4, Table 6, and Table 7 include applicable definitions for rhabdomyosarcoma (National Cancer Institute, 2015, http://www.cancer.gov) and melanoma (American Joint Cancer Staging Manual, 7th edition). For staging of other tumor types, the TNM staging will be applied as made available by the AJCC. Subjects with rhabdomyosarcoma will be additionally classified by clinical group (National Cancer Institute, 2015) which is based on the extent of the disease and how completely it is removed during the primary surgical intervention at the end of their trial participation as indicated in Table 5.

Table 3. Rhabdomyosarcoma TNM Classification

Term	Definition
Favorable	Orbit; nonparameningeal head and neck; genitourinary tract other than kidney, bladder, and prostate; biliary tract.
Unfavorable	Any site other than favorable.
T1	Tumor confined to anatomic site of origin (noninvasive).
T2	Tumor extension and/or fixation to surrounding tissue (invasive).
A	Tumor ≤5 cm in maximum diameter.
B	Tumor >5 cm in maximum diameter.
N0	No clinical regional lymph node involvement.
N1	Clinical regional lymph node involvement.
NX	Regional lymph nodes not examined; no information.
M0	No metastatic disease.
M1	Metastatic disease.

Table 4. Rhabdomyosarcoma Staging

Stage	Definition
1	Favorable sites. T1 or T2 any size. N0 or N1 or NX. M0.
2	Unfavorable sites. T1 or T2, less than or equal to 5 cm. N0 or NX. M0.
3	a: Unfavorable sites. T1 or T2, less than or equal to 5 cm. N1 M0.
	b: Unfavorable sites. T1 or T2, greater than 5 cm. N0 or N1 or NX. M0.
4	Any site. T1 or T2, any size. N0 or N1 or NX. M1.

Table 5. Clinical Group Rhabdomyosarcoma

Group	Definition
I	Localized tumor, completely removed with microscopically clear margins and no regional lymph node involvement.
II	Localized tumor, completely removed with: (a) microscopic disease at the margin; (b) regional disease with involved, grossly removed regional lymph nodes without microresidual disease; **or** (c) regional disease with involved nodes, grossly removed but with microscopic residual and/or histologic involvement of the most distal node from the primary tumor.
III	Localized tumor, incompletely removed with gross, residual disease after: (a) biopsy only **or** (b) gross major resection of the primary tumor (>50%).
IV	Distant metastases are present at diagnosis. This category includes: (a) radiographically identified evidence of tumor spread **and** (b) positive tumor cells in cerebral spinal fluid, pleural, or peritoneal fluids, or implants in these regions.

Table 6. Melanoma TNM Classification

Term	Definition
Tis	Melanoma in situ
T1a	≤1.0 mm in thickness without ulceration; mitosis <1/mm^2
T1b	≤1.0 mm in thickness with ulceration or mitoses ≥1/mm^2
T2a	1.01–2.0 mm in thickness without ulceration
T2b	1.01–2.0 mm in thickness with ulceration
T3a	2.01–4.0 mm in thickness without ulceration
T3b	2.01–4.0 mm in thickness with ulceration
T4a	>4.0 mm in thickness without ulceration
T4b	>4.0 mm in thickness with ulceration
N0	No regional metastases
N1	1 regional lymph node metastasis
N1a	1 regional node metastasis with micrometastasis
N1b	1 regional node metastasis with macrometastasis
N2	2–3 regional lymph node metastases
N2a	2–3 regional lymph node metastases with micrometastasis
N2b	2–3 regional lymph node metastases macrometastasis
N2c	In transit met(s)/satellite(s) without metastatic lymph nodes
N3	≥4 regional lymph node metastases; or matted nodes; or in transit met(s)/satellite(s) with metastatic lymph node(s)
NX	Regional lymph nodes not examined; no information.
M0	No detectable evidence of distant metastases
M1a	Metastases to skin, subcutaneous, or distant lymph nodes and normal serum LDH
M1b	Metastases to lung and normal serum LDH
M1c	Metastases to all other visceral sites and normal serum LDH; or distant metastases to any site and elevated serum LDH

Table 7. Melanoma Staging

Term	Definition
Stage 0	Tis, N0, M0
Stage IA	T1a, N0, M0
Stage IB	T2a, N0, M0 or T1b, N0, M0
Stage IIA	T2b, N0, M0 or T3a, N0, M0
Stage IIB	T3b, N0, M0 or T4a, N0, M0
Stage IIC	T4b, N0, M0
Stage III	Any T, N1-N3, M0
Stage IIIA	T1a, N1a, M0 or T2a, N1a, M0 or T3a, N1a, M0 or T4a, N1a, M0 or T1a, N2a, M0 or T2a, N2a, M0 or T3a, N2a, M0 or T4a, N2a, M0
Stage IIIB	T1b, N1a, M0 or T2b, N1a, M0 or T3b, N1a, M0 or T4b, N1a, M0 or T1b, N2a, M0 or T2b, N2a, M0 or T3b, N2a, M0 or T4b, N2a, M0 or T1a, N1b, M0 or T2a, N1b, M0 or T3a, N1b, M0 or T4a, N1b, M0 or T1a, N2b, M0 or T2a, N2b, M0 or T3a, N2b, M0 or T4a, N2b, M0 or T1a, N2c, M0 or

Term	Definition
	T2a, N2c, M0 or T3a, N2c, M0 or T4a, N2c, M0
Stage IIIC	T1b, N1b, M0 or T2b, N1b, M0 or T3b, N1b, M0 or T4b, N1b, M0 or T1b, N2b, M0 or T2b, N2b, M0 or T3b, N2b, M0 or T4b, N2b, M0 or T1b, N2c, M0 or T2b, N2c, M0 or T3b, N2c, M0 or T4b, N2c, M0 or Any T, N3, M0
Stage IV	Any T, any N, M1(a, b, or c)

8.1.4 Performance Status

All subjects will be evaluated at screening for performance status (Table 8). Karnofsky Scale will be used for children 16 years of age and older. The Lansky scale will be used for subjects less than 16 years of age. The table below will be used to assign the performance score.

Table 8. Performance Status Scale

Karnofsky Scale (age ≥16 years)		Lansky Scale (age <16 years)	
100	Normal, no complaints, no evidence of disease	100	Fully active
90	Able to carry on normal activity	90	Minor restriction in physically strenuous play
80	Normal activity with effort	80	Restricted in strenuous play, tires more easily, otherwise active
70	Cares for self, unable to carry on normal activity or to do work	70	Both greater restrictions of, and less time spent in active play

Karnofsky Scale (age ≥16 years)		Lansky Scale (age <16 years)	
60	Requires occasional assistance but is able to care for most needs	60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	50	Considerable assistance required for any active play, fully able to engage in quiet play
40	Disabled, requires special care and assistance	40	Able to initiate quiet activities
30	Severely disabled, hospitalization indicated, although death not imminent	30	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	20	Limited to very passive activity initiated by other (e.g., TV)
10	Moribound, fatal process progressing rapidly	10	Complete disabled, not even passive play

8.1.5 Prior and Concomitant Medication

All prior medication used within the last 30 days before the first screening examination and concomitant medications will be documented. Medications administered for general anesthesia related to surgery do not need to be captured in the eCRFs.

8.2 Lymphoseek Preparation and Injection

The 0.5 mCi dose of Lymphoseek should be ordered from the Cardinal Health or clinical site radiopharmacy once the subject has been scheduled for surgery.

Injection of Lymphoseek will be at study time 0:00. Subjects will receive an injection of 50 µg tilmanocept radiolabeled with 0.5 mCi ^{99m}Tc +/- 20% as described in Section 5.2.

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

8.3 SPECT/SPECT-CT or Other Gamma Image Acquisition and Image Transfer

After safety measurements have been completed and before surgery, subjects may undergo lymphoscintigraphy according to institutional imaging protocols.The camera used to obtain images should be equipped with a low-energy, high-resolution collimator and be peaked at 140 KeV with a 20% window centered over the peak. The subject should be imaged in the same position used for surgery so that there is no shift if the sentinel node is marked on the skin whenever possible. Imaging for melanoma and rhabdomyosarcoma/other solid tumors subjects may commence at the time of injection (if performing dynamic imaging). If

performed, imaging should not conclude less than15 minutes post injection and should be completed no later than 60 minutes after injection.

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.

Once a sentinel node is identified, a radioactive marker should be placed over skin while the subject is under the camera detector, keeping the SLN in the field of view. The marker should be moved until the marker and SLN overlap, this area should be marked with waterproof ink.

All images should be reviewed by a radiologist for quality prior to the subject being sent to surgery.

If collected, a deidentified Digital Imaging and Communications in Medicine (DICOM) copy of all images should be transmitted to Cardinal using the provided source worksheet and file pathway provided by your Project Manager.

8.4 Surgical Procedures, Lymphatic Mapping, and Lymph Node Biopsy

8.4.1 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 20 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

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 the Gamma Detector Counts Calculation Sheet, Appendix 2 to assist in determining the 3σ level from the normal tissue background level.

Ex Vivo

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.

8.4.2 Surgical Lymph Node Identification

1. If Lymphazurin is used, inject it into the dermis around the primary tumor site (see Lymphazurin insert for instructions) in accordance with institutional standards. No more than 3 mL will be injected.
2. Conduct an initial transcutaneous survey to identify the area of increased radioactivity that is separate from the injection site. The survey should include the entire lymph node (LN) basin(s) at risk.
3. Once an area of increased radioactivity is identified, an incision should be placed to make as direct an approach as possible to the LNs.
4. On entering the subcutaneous tissue, a blue lymphatic vessel or blue lymph node may be seen. If a blue lymphatic vessel is seen, carefully trace it proximally to a blue LN. In vivo counts should be recorded and LN removed.

   OR

   If no blue lymphatic vessel or blue lymph node is seen, or if Lymphazurin is not used, 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).
5. Once an 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.
6. The method used to identify all LN(s) will be recorded (i.e., blue appearance, positive finding, or both) prior to excision.
7. 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 9 describes the procedures that should be followed based on the in vivo and ex vivo status of the tissue that has been excised.

Table 9. 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.
Vital Blue Dye Status		
Blue node	N/A	ANY blue node is sent to pathology for further evaluation regardless of radioactive status.

8. 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 blue and/or increased gamma detector count rates.
9. 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 blue node nor any "hot" nodes, continue with visualization and palpation according to your medical expertise.

All removed LNs must be sent to pathology for further evaluation. The pathological evaluation of lymph node(s) will include serial sectioning and staining as described below (see also the Pathology Manual for additional details). For the purposes of analysis, a sentinel lymph node is defined as either being hot in vivo (lymph nodes with counts above the 3σ rule threshold) or blue (when applicable).

8.5 Histopathology of Lymph Nodes

8.5.1 Local Lymph Node Evaluation

Final pathology results need to reflect consistent individual numbering and labeling from intraoperative worksheets to the final pathology report. All LNs should be fixed in 10% neutral phosphate buffered formalin for 6 to 24 hours, processed, and embedded in paraffin. All lymph nodes greater than 0.5 cm should be bisected transversely to the long axis or along the long axis prior to fixation. The entire lymph node should be submitted for histological

evaluation. Local pathology assessment of the lymph nodes should be conducted according to the institution's current practices.

8.5.2 Preparation and Shipping of Lymph Node Samples to Central Laboratory

Paraffin blocks can be submitted directly to the central laboratory for slide and stain processing. Alternatively, sites can send slides. If slides are to be sent, the local pathology laboratory will section the tissue into levels from the superficial surface to produce three levels. Each level will be cut such that it is 750 microns (0.75 mm) deeper than the previous level. Eight unstained slides will be generated from each level of tissue and should be cut at 3 to 5 μm thickness and placed on plus glass slides. In the event that the tissue thickness in a given block is less than 0.25 inches thick, only two levels will be cut, with 8 slides submitted from each level.

The blocks or unstained slides will be shipped to the central laboratory using kits supplied to the trial site along with instructions and prepaid shipping labels. All slides will be maintained by the central laboratory until trial completion. Contact information and additional preparation and shipping instructions are maintained in the Study Operations Manual and Pathology Manual.

Shipments as well as questions regarding logistics, quality assurance, or documentation can be directed to the central pathology laboratory.

8.5.3 Central Laboratory Processing of Samples

The central pathology laboratory will process samples with hematoxylin and eosin (H&E) and immunohistochemistry (IHC) stains. Processing will be in accordance with the detailed guidelines specified in the Pathology Manual.

Central pathology laboratory results will be entered into the eCRFs by designated central pathology laboratory staff.

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 4- to 14-day follow-up assessment are to be reported as AEs. Additionally, untoward medical events occurring prior to the day of injection will only be captured as AEs if they are related to a study procedure. SAEs will be reported from the time of consent through the end of participation. All adverse events should be followed until resolution or stabilization as determined by the investigator.

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 relationship to investigational product and/or Lymphazurin (VBD)

The investigator will use the following definitions to assess the relationship of the AE to the use of investigational product and/or Lymphazurin:

Definitely related: Event can be fully explained by administration of the investigational product and/or Lymphazurin.

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

Possibly related: Event may be explained by administration of the investigational product and/or Lymphazurin 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 and/or Lymphazurin.

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

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

Causal relationship to study procedure

The investigator will use the following definitions to assess the relationship of the AE 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 documentation.

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 a serious adverse event (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 and to study conduct (for definitions, see above)
- 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: The use of an intravenous blood collection and indwelling venous cannula for the purpose of blood sampling and administration of investigational product may be accompanied by mild bruising and also, in rare cases, by transient inflammation of the vessel wall. After initial irritation, the presence of an indwelling cannula is usually painless and hardly noticeable. The same applies to single vein punctures for blood sampling.

Expected Adverse Drug Reactions

The definition below follows ICH-GCP (see also ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting):

Adverse Drug Reaction (ADR): In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered as ADR. The phrase 'responses to a medicinal product' means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility (definitely, probably, possibly), i.e., the relationship cannot be ruled out.

Investigational Product-Related Risks

In all completed clinical trials of Lymphoseek, involving 553 subjects, only 3 events (breast pain and injection site pain reported by subjects with breast cancer and injection site irritation reported by a subject with head and neck squamous cell cancer) were deemed definitely related to the administration of Lymphoseek by the investigator. The most common adverse reactions (incidence <1%) are injection site irritation and/or pain.

Adverse events from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

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 Drug Reactions

An unexpected adverse drug reaction 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 or other acute events that fall within the definition of SAE for planned elective surgeries, surgeries anticipated as part of the subject's primary surgical intervention such as reconstruction or plastic surgeries or resulting from extended surgical requirements that are secondary to intraoperative findings unknown at the initiation of surgery 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 the Cardinal Health Drug Safety Group via phone immediately at (800) 618-2768. Written notification of the SAE will be faxed within one day (i.e., within 24 hours) of

becoming aware of the SAE to (855) 793-7501 or emailed to npssafety@cardinalhealth.com. 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, Cardinal will provide a form for collection of pregnancy information.

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

Notification of the IRBs

The sponsor and/or the investigator will notify the 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 Events (see Appendix 1). Any clinically significant change from baseline that results in a change in subject management will be considered an AE.

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)
- Lymph nodes

- Musculoskeletal
- Nervous System

8.7.2.2 Vital Signs

Vital signs comprise the measurement of systolic and diastolic blood pressure, body temperature, respiration, and heart rate. All vital signs will be measured after the subject should be resting quietly for 1 minute. Heart rate should be measured immediately before or immediately after blood pressure measurement.

Vital signs will be measured at screening, baseline (before investigational product injection), 10, 30, 60 minutes post injection (Day 1), and at the 4- to 14-day follow-up visit.

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

8.7.2.3 Electrocardiogram

A standard 12-lead ECG will be obtained at screening and on Day 1 at least 10 minutes after Lymphoseek injection. The ECG will be measured with the subject in a resting position for at least 1 minute. No continuous ECG monitoring will be required.

On-site investigator's responsibilities

The immediate cardiac safety of the subject will be ensured by the on-site qualified physician. Any 12-lead ECG intervals, waveform abnormalities, and rhythm changes that are clinically significant in that they result in a change in subject management will be considered an AE. In the case of an SAE, once SAE notification is decided upon, investigators are required to follow the procedure described for SAE notification and document abnormal ECG findings (intervals and waveforms). Any interval data or abnormal waveform finding that resulted in an AE (i.e., change of patient management) must be followed for the duration of the subject's participation in the study until normalization or return to baseline takes place.

Each 12-lead ECG tracing must be signed and dated and stored in the subject's source documentation.

8.8 Other Procedures and Variables

8.8.1 Blood Sampling

8.8.2 Laboratory Data Related to Screening and Safety

Clinical laboratory tests to be evaluated in this study include hematology, serum chemistry, and urinalysis. Blood and urine samples for safety will be obtained according to the Schedule of Events (Appendix 1) and in accordance with NIH blood volume limits. Table 10 shows the parameters to be assessed:

Table 10. Minimum Clinical Laboratory Parameters

Hematology	Hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes, red blood cells (RBC), RBC Morphology, white blood cells (WBC)
Serum chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (gamma-GT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, globulin, Bicarbonate, blood urea nitrogen (BUN)
Urinalysis	Color, clarity, specific gravity, pH, bilirubin, glucose, ketones, leukocyte esterase, nitrites, protein, urobilinogen

A central laboratory will be used for all blood and urine samples to be determined in this trial.

The central laboratory will provide the necessary kits to collect the blood and urine samples and will also provide appropriate information regarding shipping of the samples.

Care is to be taken not to potentially produce an erroneous value, e.g., by inappropriate use of the tourniquet or forceful withdrawal of blood. Each original laboratory report will be retained in the subject's files.

All laboratory reports must be promptly reviewed by the investigator, and upon review, initialed and dated by the investigator. Change(s) in post-dose test values considered clinically significant, which would require either additional control or therapy and, in case of disturbing or influencing factor(s) on values/samples, details of the appropriate value(s) and the source of disturbance or influence (e.g., quality of sample, co-medication, etc.) are to be recorded. 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 following are general guidelines and are provided in order to assist the investigator in evaluating changes in laboratory values after the administration of investigational product. Since from previous clinical experience, these changes are unexpected, in all instances laboratory error must be considered and ruled out. One common cause of error is dilution of the blood sample, which occurs when blood is drawn through a fluid-filled catheter.

Changes in most individual laboratory values cannot be interpreted without concomitant/concordant changes in related parameters. For example, a decrease in hematocrit of approximately 5 percentage points (49% to 44%) at 24 hours post injection can be considered clinically significant, but without a similar change in hemoglobin and red blood cells (RBC), laboratory error must be ruled out. Shifts in hematocrit may also occur with changes in subject's hydration status. With comparable decreases in hematocrit, hemoglobin and RBC, the investigator must evaluate the subject for blood loss/hemorrhage, especially at

the site of venipuncture. If coagulopathy or hemolysis is suspected, appropriate follow-up, including diagnostic tests and treatment, should be undertaken. Decreases in platelet count by greater than 30% of baseline value or an absolute value of less than 50,000/μL after a normal baseline value should also be followed up to rule out laboratory error or an underlying problem, such as a coagulopathy.

Increases in 2 or more liver function and related parameters (alkaline phosphatase, AST, ALT, and bilirubin) that result in values twice that of the upper limit of the normal range must be followed up to rule out any acute liver and/or cardiac illness. The investigator must obtain the appropriate tests as indicated by the subject's clinical status. Changes in sodium, potassium and chloride that are greater than 20% of baseline value must be followed up.

In all instances of changes in multiple parameters, regardless of the magnitude, the investigator must evaluate the subject in light of the clinical signs and symptoms and institute appropriate treatment. There may be other abnormal laboratory changes not specifically noted above that the investigator may want to follow up as appropriate for a particular subject.

Any change in laboratory value which results in a change in subject management (additional controls or treatment required) will be reported as a clinically significant change. Clinically significant changes in laboratory parameters which are not the result of laboratory error are to be recorded as AEs.

Any clinically significant changes in laboratory values are to be followed up with repeated tests at appropriate intervals (as determined by the investigator and the medical monitor) until the values return to baseline level or until the abnormality is explained by the investigator. Such additional tests should also be done at the central laboratory and will be entered into the database.

The amount of blood to be withdrawn is shown in Table 11.

Table 11. Amount of Blood Withdrawn

	Screening	After Surgery
Hematology	2 to 3 mL	2 to 3 mL
Chemistry	1 to 3 mL	1 to 3 mL
Total	3 to 6 mL	3 to 6 mL

For subjects that weigh less than 2 kg, no more than 3 mL/kg of blood can be drawn at either time point. Blood and urine specimens will be sent to a central vendor for analysis. Specimen collection, packaging, and shipping instructions are provided in a separate laboratory manual.

9 STATISTICAL METHODS

9.1 Introduction

This study is a prospective, open-label study conducted at up to 10 study centers. The objectives of the statistical analyses are to establish the safety and diagnostic utility of Lymphoseek in targeting lymphoid tissue in pediatric patients with melanoma, rhabdomyosarcoma, or other solid tumors 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 principal investigator.

9.2 Randomization Methods

There is no randomization planned for this study. All enrolled subjects will receive Lymphoseek, and Lymphazurin as an optional control agent if consistent with institutional practice.

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 Safety Variables

9.4.1 Primary Safety Variable

The primary safety variable is the incidence of adverse events.

9.4.2 Secondary Safety Variables

The secondary safety variables are the following:

- Vital signs (screening, at pre- and post-injection time points, and at the 4-to 14-day follow-up visit)
- Laboratory parameters (screening and after surgery)
- ECG parameters (screening and at the post-injection time point)

9.5 Pharmacokinetic Variables

Not applicable.

9.6 Diagnostic Variables

9.6.1 Primary Diagnostic Variables

The primary diagnostic variables for this study are:

- Subject localization rate (defined as the proportion of subjects with Lymphoseek-identified lymph nodes)
- Number of lymph nodes identified intraoperatively by Lymphoseek per subject
- Proportion of subjects who underwent preoperative SPECT or SPECT/CT
- Proportion of subjects with a lymph nodes identified preoperatively using SPECT or SPECT/CT
- Number of lymph nodes identified preoperatively using SPECT or SPECT/CT
- Agreement of the number of nodes identified by preoperative SPECT or SPECT/CT to intraoperative localization
- Subject and nodal agreement of central pathology assessment with local pathology assessment of the excised lymph node(s) to confirm the presence/absence of tumor metastases
- Nodal false negative rate for Lymphoseek-identified nodes (using blinded central pathology assessment and local pathology assessment)
- Nodal sensitivity for Lymphoseek-identified nodes (using blinded central pathology assessment and local pathology assessment)
- Upstaging (defined as the proportion of patients with pathology-positive lymph nodes who had at least one pathology-positive lymph node that was identified by Lymphoseek and had no other pathology-positive lymph nodes [i.e., identified by any other method])
- Change in subject nodal staging before and after surgery based upon Lymphoseek-identified nodes
- Changes in treatment plan and relation to lymph nodes identified by Lymphoseek

9.6.2 Secondary Diagnostic Variables

The secondary diagnostic variables for this study are for those subjects receiving both Lymphoseek and Lymphazurin:

- Subject localization rate for Lymphazurin
- Number of lymph nodes identified intraoperatively by Lymphazurin per subject
- Nodal concordance (defined as the proportion of lymph nodes identified intraoperatively by Lymphazurin that are also identified intraoperatively by Lymphoseek)
- Reverse nodal concordance (defined as the proportion of lymph nodes identified intraoperatively by Lymphoseek that are also identified intraoperatively by Lymphazurin)
- Subject concordance
- Subject reverse concordance
- Nodal false negative rate for Lymphazurin-identified nodes (using blinded central pathology assessment and local pathology assessment)
- Nodal sensitivity for Lymphazurin-identified nodes (using blinded central pathology assessment and local pathology assessment)

- Upstaging (defined as the proportion of patients with pathology-positive lymph nodes who had at least one pathology-positive lymph node that was identified by Lymphazurin and had no other pathology-positive lymph nodes (i.e., identified by any other method)
- Change in subject nodal staging before and after surgery based upon Lymphazurin-identified nodes

9.7 Sample Size Justification

Approximately 27 subjects will be enrolled into the study, at least 6 of whom have been diagnosed with melanoma and at least 6 of whom have been diagnosed with rhabdomyosarcoma. Enrollment will be open to up to 15 additional subjects with other tumor types in which ILM and sentinel lymph node biopsy (SLNB) are appropriate. At a minimum, 20 of the enrolled subjects will be ≤16 years of age and at least 25 subjects will complete the 4- to 14-day follow up. The sample size was chosen in order to provide a reasonable amount of data to assess the safety and diagnostic performance of Lymphoseek within this limited pediatric subject population.

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:

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

Intent-to-treat (ITT): The ITT population will include all protocol-eligible subjects with administration of Lymphoseek (with or without Lymphazurin) and complete intraoperative lymphatic mapping.

Blue intent-to-treat (BITT): The BITT population will include all ITT subjects who were also administered Lymphazurin prior to ILM.

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

All safety analyses will be conducted on the safety population. The analysis of the diagnostic endpoints will be conducted on ITT, BITT, and PP populations, as applicable.

9.9.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics will be summarized by tumor type and overall 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 Safety Variables

All AEs will be observed for each subject from enrollment 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 by tumor type and overall for all subjects in the safety population. AEs will also be summarized by severity, relationship to investigational product, and relationship to study procedure. All AEs will be listed.

9.9.4 Analysis of Secondary Safety Variables

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 time point, for each tumor type and overall. The pre-injection time point will serve as baseline. If there are multiple vital signs taken at any time point, then the latest set of vital signs will be used for the analysis. All vital sign data 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 quantitative laboratory parameter by time point, for each tumor type and overall. The screening time point will serve as baseline. If there are multiple labs taken at any time point, then the latest set of labs will be used for the analysis. All clinical laboratory data, including any qualitative urinalysis results, 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 ECG parameter by time point, for each tumor type and overall. The screening time point will serve as baseline. If there are multiple ECGs taken at any time point, then the latest set of ECGs will be used for the analysis. All ECG data will be listed.

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

9.9.5 Analysis of Primary Diagnostic Variables

The localization rate, defined as the proportion of ITT subjects that had at least one lymph node identified intraoperatively, along with a 95% confidence interval will be computed for Lymphoseek for each tumor type and overall.

The average number of lymph nodes identified intraoperatively by Lymphoseek per subject, along with a 95% confidence interval, will be computed for each tumor type and overall.

The proportion of ITT subjects who had preoperative SPECT performed will be summarized by tumor type and overall. The proportion of patients who had a hot spot on the scan will also be summarized by tumor type and overall. For those patients with a hot spot, the time from Lymphoseek injection to hot spot localization will be summarized.

The average number of lymph nodes identified with preoperative SPECT per subject, along with a 95% confidence interval, will be computed for Lymphoseek by tumor type and overall.

The average difference in the number of lymph nodes identified with preoperative SPECT per subject and the number of lymph nodes identified intraoperatively per subject, along with a 95% confidence interval, will also be computed.

The nodal and subject agreement of central pathology assessment to local pathology assessment will be computed as follows:

$$\text{Nodal Agreement} = \frac{\text{Lymph nodes whose local and central pathology assessments were either both positive or both negative}}{\text{Lymph nodes with non-missing local and central pathology results}}$$

$$\text{Subject Agreement} = \frac{\text{Subjects whose local and central pathology assessments agree}}{\text{Subjects with at least one lymph node with non-missing local and central pathology results}}$$

Where agreement on the subject level means that a subject either has at least one pathology-positive node with local and central pathology or has all pathology-negative nodes with local and central pathology. For the analysis of nodal agreement and subject agreement, only nodes that have non-missing local and central pathology results will be considered. Along with the agreement proportions, a 95% confidence interval will be computed for each endpoint. Subgroup analyses by tumor type will also be performed within the ITT and PP analysis populations.

The nodal false negative rate will be computed for Lymphoseek as the number of pathology-positive lymph nodes that were missed intraoperatively by the tracer divided by the total number of pathology-positive lymph nodes. A 95% confidence interval will also be computed. False negative rates also will be calculated separately for the blinded central pathology assessment and the local pathology assessment.

The nodal sensitivity will be computed for Lymphoseek as the number of pathology-positive lymph nodes identified intraoperatively by the tracer divided by the total number of pathology-positive lymph nodes. A 95% confidence interval will also be computed. Sensitivities also will be calculated separately for the blinded central pathology assessment and the local pathology assessment.

The number and proportion of subjects upstaged by Lymphoseek will be computed along with a 95% confidence interval, where upstaging by an individual tracer is defined as a subject who had at least one pathology-positive node identified by one tracer and no pathology-positive nodes identified by the other tracer.

The change in subject nodal staging will be assessed via shift tables. A shift table for Lymphoseek will be produced to show the number of subjects with each combination of pre-surgery and post-surgery nodal staging. Each subject's post-surgery staging will be based on the pathology results of those nodes identified by Lymphoseek. For the purposes of this analysis, a subject may have two different post-surgery nodal staging results, one based on Lymphoseek-identified nodes and the other based on Lymphazurin-identified nodes (see Section 9.9.6).

Changes in treatment plan and relation to lymph nodes identified by Lymphoseek will be listed.

9.9.6 Analysis of Secondary Diagnostic Variables

The localization rate, defined as the proportion of BITT subjects that had at least one lymph node identified intraoperatively, along with a 95% confidence interval will be computed separately for Lymphazurin for each tumor type and overall.

The average number of lymph nodes identified intraoperatively by Lymphazurin per subject, along with a 95% confidence interval, will be computed for each tumor type and overall.

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

$$P_1 = \frac{\text{Number of lymph nodes identified intraoperatively by Lymphoseek and Lymphazurin}}{\text{Number of lymph nodes identified intraoperatively by Lymphazurin}}$$

An exact one-sided 95% confidence interval will be computed in order to test the following hypotheses at a one-sided α=0.05 level of significance:

$$H_0: P_1 \leq 0.9 \text{ vs. } H_1: P_1 > 0.9$$

If the lower boundary of the confidence interval is greater than 0.9, then the study will have successfully demonstrated the performance of Lymphoseek relative to the nodal concordance endpoint.

Assessment as to whether lymph nodes can be considered as an independent sample will be evaluated using in-house and/or literature data. The details of this analysis will be described in the statistical analysis plan.

Subgroup analyses by tumor type will also be performed for the BITT population, but no hypotheses will be tested by tumor type.

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

$$P_3 = \frac{\text{Number of lymph nodes identified intraoperatively by Lymphoseek and Lymphazurin}}{\text{Number of lymph nodes identified intraoperatively by Lymphoseek}}$$

In addition to the above proportion, an exact one-sided 95% confidence interval will be computed.

If the null hypothesis for the nodal concordance endpoint is rejected, then the following test of superiority of nodal concordance relative to reverse nodal concordance will be conducted at a one-sided α=0.05 level of significance using McNemar's test:

$$H_0: P_1 \leq P_3 \text{ vs. } H_1: P_1 > P_3$$

If the one-sided p-value from McNemar's test is less than 0.05, then the nodal concordance of Lymphoseek to Lymphazurin (P_1) will be shown to be significantly greater than the reverse nodal concordance of Lymphazurin to Lymphoseek (P_3).

The per-subject concordance, P_2, and per-subject reverse concordance, P_4, will be computed as follows using all subjects in the BITT population:

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

and

$$P_4 = \frac{\text{Subjects whose lymph nodes that were intraoperatively identified by Lymphoseek were also all identified by Lymphazurin}}{\text{Subjects with at least one lymph node intraoperatively identified by Lymphoseek}}$$

In addition to the above proportions, exact one-sided 95% confidence intervals will be computed.

The nodal false negative rate will be computed for Lymphazurin as the number of pathology-positive lymph nodes that were missed intraoperatively by the tracer divided by the total number of pathology-positive lymph nodes. A 95% confidence interval will also be computed. False negative rates also will be calculated separately for the blinded central pathology assessment and the local pathology assessment.

The nodal sensitivity will be computed for Lymphazurin as the number of pathology-positive lymph nodes identified intraoperatively by the tracer divided by the total number of pathology-positive lymph nodes. A 95% confidence interval will also be computed. Sensitivities also will be calculated separately for the blinded central pathology assessment and the local pathology assessment.

The number and proportion of subjects upstaged by Lymphazurin will be computed along with a 95% confidence interval, where upstaging by an individual tracer is defined as a subject who had at least one pathology-positive node identified by one tracer and no pathology-positive nodes identified by the other tracer.

The change in subject nodal staging will be assessed via shift tables. A shift table for Lymphazurin will be produced to show the number of subjects with each combination of pre-surgery and post-surgery nodal staging. Each subject's post-surgery staging will be based on the pathology results of those nodes identified by Lymphazurin. For the purposes of this analysis, a subject may have two different post-surgery nodal staging results, one based on Lymphoseek-identified nodes and the other based on Lymphazurin-identified nodes.

9.10 Interim Analyses

No formal interim analyses will be conducted during this study.

Data listings and tables of safety and diagnostic data will be created on an interim basis for review by a DSMC. Refer to Section 9.11 and the DSMC Charter for further details on the data handling and analysis procedures.

9.11 Data and Safety Monitoring Committee

An independent DSMC will review the interim results and make recommendations regarding early stopping and/or changes in the study design. Specifics of the DSMC role are detailed in a separate DSMC Charter.

The first three subjects between the ages of 2 to 17 will be recruited and injected. Once these three subjects have completed the Day 8 safety evaluation, enrollment will be put on hold until the DSMC reviews all available safety information and agrees to proceed with additional enrollment. If the DSMC concurs, study enrollment will then be open to all age cohorts (1 month to <18 years). The DSMC will then determine the frequency to meet based on review of the available trial information.

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, 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 documented 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 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 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 ISF 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 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, IRBs and Regulatory Authorities will be informed by the Project Manager. As required by local law, current safety-relevant information will be provided to the 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 or minor assent document. A sample informed consent form is provided as a document separate to this protocol.

Based on this subject informed consent form, the investigator will explain all relevant aspects of the study to each subject and subject's parent(s)/legal guardian(s), before his/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 has been obtained.

Each subject and the subject's parent(s)/legal guardian(s) 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's parent(s)/legal guardian(s) will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the subject's parent(s)/legal guardian(s) voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator or his/her designee will personally sign and date the form, too. The subject's parent(s)/legal guardian(s) will receive a duplicate of the signed and dated form.

The investigator will record in the source documentation 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 informed consent form and any other written information provided to subjects and subject's parent(s)/legal guardian(s) 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 informed consent form. 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 informed consent form. Any revised written informed consent form and written information must receive the IRB'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 TMF and the ISF, 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 will provide electronic evidence of 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 (4 to 14 days; in person)
		Before Injection	0:00	00:10	0:30	1:00	Surgery	After Surgery	
Informed Consent	X								
Entry Criteria	X								
Medical History and Demography	X								
Performance Status	X								
Cancer Staging	X								X
Vital Signs[a]	X	X		X	X	X			X
ECG	X			X					
Physical Examination	X								X
Review of Medications	X							X	X
Collect Blood and Urine for Central Lab[b]	X							X	
Urine or Serum Pregnancy Test[c]	X								
Lymphoseek Administration			X						
Lymphoscintigraphy SPECT/CT[d]			X						
Lymphazurin Administration[e]							X		
Surgery and SLNB[f]							X		
Treatment Plan									X
Adverse Event Monitoring		X	X	X	X	X	X	X	X

[a] Body weight and height will only be collected at screening
[b] Blood and urine may be collected on Day 1 if performed before Lymphoseek injection
[c] Pregnancy testing should be performed within 48 hours before Lymphoseek injection
[d] Imaging may begin at the time of injection when performing dynamic imaging STATIC planar imaging should begin approximately 15 minutes post injection
[e] When used, vital blue dye will be administered at the start of or during surgery in accordance with the standard of care at the clinical site
[f] Surgery, node probing, and harvesting should occur between 15 minutes and 8 hours after Lymphoseek injection

Appendix 2 Gamma Detector Counts Calculation Sheet

Appendix 4 Investigator's Signature

Study Title:	A Prospective, Open-Label, Multicenter Study of Lymphoseek® as a Lymphoid Tissue Targeting Agent in Pediatric Patients With Melanoma, Rhabdomyosarcoma, or Other Solid Tumors Who Are Undergoing Lymph Node Mapping
Study Number:	NAV3-18
Original Protocol Date:	24 April 2015
Amendment 1 Date:	14 August 2015
Amendment 2 Date:	18 January 2016
Amendment 3 Date:	11 January 2017
Amendment 4 Date:	01 September 2017

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>