25 September 2014
EMA/CHMP/264502/2014
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Lymphoseek

International non-proprietary name: tilmanocept

Procedure No.: EMEA/H/C/002085/0000

Note

Assessment report as adopted by the CHMP with all information of a commercial confidential nature deleted.

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Product information

Name of the medicinal product:	Lymphoseek
Applicant:	Navidea Biopharmaceuticals Limited 30 Upper High Street Thame OX9 3EZ UNITED KINGDOM
Active substance:	TILMANOCEPT
International Nonproprietary Name/Common Name:	TILMANOCEPT
Pharmaco-therapeutic group (ATC Code):	Radiopharmaceutical Diagnostic Detection Agent V09IA
Therapeutic indication:	This medicinal product is for diagnostic use only. Radiolabelled Lymphoseek is indicated for imaging and intraoperative detection of sentinel lymph nodes draining a primary tumour in adult patients with breast cancer, melanoma, or localised squamous cell carcinoma of the oral cavity. External imaging and intraoperative evaluation may be performed using a gamma detection device.
Pharmaceutical form:	Kit for radiopharmaceutical preparation
Strength:	250 µg
Route of administration:	Intradermal use,Subcutaneous use, Intratumoral use, Peritumoral use
Packaging:	vial (glass)
Package size:	5 vials (multidose)

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List of abbreviations

AE	adverse event
AUC	area under the curve
BMI	body mass index
CI	confidence interval
CHMP	Committee for Medicinal Products for Human Use
CMC	Chemistry, Manufacturing, and Control
CT	computerized tomography
Da	dalton (unit of molecular weight expression)
DTPA	diethylenetriaminepentaacetic acid
EANM	European Association of Nuclear Medicine
EDE	Effective drug equivalent
ECG	electrocardiogram
EMA	European Medicines Agency
EORTC	European Organisation for Research and Treatment of Cancer
FNR	false negative rate
fTcSC	filtered sulphur colloid
GMP	good manufacturing practice
H	hypothesis (H0, null hypothesis; Ha, alternative hypothesis)
HEK	human embryonic kidney (cells)
hMBR	human mannose binding receptor
HNSCC	head and neck squamous cell carcinoma
hr(s)	hour(s)
%IDSN	percent-of-injected dose in the sentinel node
ID	intradermal
ILM	intraoperative lymphatic mapping
ITT	intent-to-treat
kc	the injection site clearance rate constant
kDa	kilodalton(s) (103 Da)
LSN	absolute sentinel node uptake for Lymphoseek
Lymphoseek	reconstituted solution for injection containing technetium Tc 99m tilmanocept
Lymphoseek Kit	Lymphoseek 250 micrograms, kit for radiopharmaceutical preparation
MBq	megabecquerel (106 Bq) (1 MBq = 0.027 mCi)
MBR	mannose binding receptor (CD206)
MDM	monocyte-derived macrophage
µg	microgram(s) (10-6 grams)
mg	milligram(s) (10-3 grams)
mCi	millicurie(s) (10-3 Curies) (1 mCi = 37 MBq)
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter(s) (10-3 liters)
MRI	magnetic resonance imaging
N or n	sample number in a population (N) or in a subpopulation or other subgroup (n)
nm	nanometer(s) (10-9 meters)
nmol	nanomole(s) (10-9 moles)
NPV	negative predictive value
PC	true concordance rate
PD	pharmacodynamics
PIP	paediatric investigation plan
PK	pharmacokinetics
pmol	picomole(s) (10-12 moles)
PP	per protocol
PT	peritumoral
RITT	Reverse ITT
rhMBR	recombinant human mannose binding receptor
RMP	Risk management plan
SAE	serious adverse event

SAP	Statistical Analysis Plan
SC	subcutaneous
SLN (or SN)	sentinel lymph node (sentinel node)
SLNB	sentinel lymph node biopsy
SOC	system organ class
SPECT/CT	single-photon emission computed tomography/computed tomography
SmPC	Summary of Product Characteristics
Tc	injection site clearance half-life
Tc 99m	technetium-99m metastable isotope (γ emitting; half-life = 6.02 hrs)
TcSC	technetium-99m sulphur colloid
technetium Tc 99m Lymphoseek	radiolabelled drug substance of Lymphoseek
tilmanocept	DTPA Mannosyl Dextran; the drug substance of Lymphoseek, unlabeled
TNM staging	tumour, lymph node, metastasis staging; T stage is represented by Tis (in situ) to T4; N0 represents node negative staging; M0 representing metastasis negative staging
UCSD	University of California, San Diego
Vmax	Maximum elimination rate
VBD	vital blue dye (e.g., Lymphazurin®, Patent Blue V®)

1. Background information on the procedure

Submission of the dossier

The applicant Navidea Biopharmaceuticals Limited submitted on 3 December 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Lymphoseek, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 30 June 2009.

The applicant applied for the following indication

This medicinal product is for diagnostic use only.

Lymphoseek is a diagnostic receptor-targeted radiopharmaceutical used in the delineation and localisation of lymph nodes. Lymphoseek is used intraoperatively for evaluation of tumour-draining lymph nodes with a handheld gamma detection probe, and may be used for complementary preoperative external gamma detection-based imaging.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that tilmanocept was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0303/2012 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity
Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request for consideration

New active Substance status
The applicant requested the active substance tilmanocept contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union

Scientific Advice

The applicant received Scientific Advice from the CHMP on 24 July 2008 and 19 January 2012. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

A new application was filed in the following countries: USA.

The product was not licensed in any country at the time of submission of the application.

1.1. Manufacturers

Manufacturer responsible for batch release

Penn Pharmaceutical Services Ltd.
23-24 Tafarnaubach Industrial Estate
Tredegar, Gwent NP22 3AA
South Wales
United Kingdom

Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Greg Markey Co-Rapporteur: Patrick Salmon

- The application was received by the EMA on 3 December 2012.
- The procedure started on 30 January 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 22 April 2013. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 19 April 2013.
- PRAC RMP Advice and assessment overview, adopted by PRAC on 16 May 2013.
- During the meeting on 30 May 2013, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 31 May 2013.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 22 August 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 26 September 2013.
- PRAC RMP Advice and assessment overview, adopted by PRAC on 10 October 2013.
- During the CHMP meeting on 24 October 2013, the CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 15 November 2013.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 5 December 2013.
- During the CHMP meeting on 19 December 2013, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on 17 February 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the 2nd List of Outstanding Issues to all CHMP members on 26 February 2014.
- During the CHMP meeting on 20 March 2014, the CHMP agreed on a 3rd List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP 3rd List of Outstanding Issues on 20 August 2014.
- Joint Rapporteur/Co-Rapporteur Assessment Report on the responses provided by the applicant, dated 18 September 2014
- During the meeting on 25 September 2014, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Lymphoseek.

2. Scientific discussion

2.1. Introduction

In patients with breast cancer, melanoma, HNSCC, and multiple other solid tumor cancers, the ability to identify whether the cancer has spread greatly influences patients' outcomes and future treatment. Intraoperative lymphatic mapping (ILM) and lymph node biopsy have been used in cancer patients to evaluate the association of the tumour with the lymphatic system and determine whether the primary tumour has spread to the regional lymph nodes. ILM is a procedure whereby a surgeon tracks lymphatic drainage (anatomic nexuses of lymphatic channels) from a tumour or tumour bed using a visually tracked colorimetric agent (such as a vital blue dye [VBD]) and/or a gamma emitting radiolabelled agent (used in conjunction with a gamma camera and/or an intraoperative gamma detection probe). Lymph nodes that are found to contain the injected agent and express such anatomical connections from the tumour have the highest likelihood to harbour metastatic disease and are often called 'sentinel' lymph nodes (or SLNs). These lymph nodes may be selectively removed as extended lymphatic dissection is known to result in significantly increased morbidity in many patients. The pathology assessment of the removed SLNs completes the diagnostic process.

Currently there are two types of agents that are widely employed for mapping lymphatic structures:

1. Radiopharmaceuticals, e.g., Tc 99m-labeled Nanocis, Nanocoll, and sulfur colloid.

2. Colorimetric agents, e.g., VBD, which, worldwide, include but are not limited to Lymphazurin (isosulfan blue), Patent Blue V, and methylene blue.

Worldwide, the most commonly used agents for ILM mapping in breast cancer and melanoma are Tc 99m-labelled colloids. According to the 2009 European Association of Nuclear Medicine (EANM) and European Organisation for Research and Treatment of Cancer (EORTC) General Recommendations for

Sentinel Node Diagnostics the agents most used in Europe are Tc 99m human serum albumin colloids (including albumin nanocolloid, Nanocoll, and Senti-Scint).

This Centralised application concerns Lymphoseek containing tilmanocept, a diagnostic receptor-targeted radiopharmaceutical to be used in the delineation and localisation of lymph nodes.

Lymphoseek accumulates in lymphatic tissue by specifically binding to mannose binding receptors (MBRs; CD206) that reside on the surface of lymph-node resident dendritic cells and macrophages. Lymphoseek is a wholly synthetic macromolecule consisting of multiple units of diethylenetriaminepentaacetic acid (DTPA) and mannose, each attached to a 10 kDa dextran backbone. The mannose moieties act as a substrate for the receptor, and the DTPA serves as a chelating moiety for radiolabelling with Tc-99m. It is claimed that Lymphoseek's small diameter permits enhanced diffusion into lymphatic channels and blood capillaries, resulting in rapid injection site clearance. Upon entry into the blood, it is claimed that the agent binds to receptors in the liver or is filtered by the kidney and accumulates in the urinary bladder.

The Lymphoseek cold kit compromises vials containing tilmanocept (drug substance) to be reconstituted and radiolabelled with Tc-99m, and locally injected near a tumour (i.e., intradermal [ID], subcutaneous [SC], Intratumoral, or peritumoral [PT] injection). Lymphoseek is not intended for systemic/intravenous (IV) injection.

The proposed indication for Lymphoseek (the reconstituted solution for injection containing technetium Tc 99m tilmanocept) was:

> Lymphoseek is a diagnostic receptor-targeted radiopharmaceutical used in the delineation and localisation of lymph nodes. Lymphoseek is used intraoperatively for evaluation of tumour-draining lymph nodes with a handheld gamma detection probe, and may be used for complementary preoperative external gamma detection-based imaging.

The final indication for Lymphoseek was:

> This medicinal product is for diagnostic use only.
>
> Radiolabelled Lymphoseek is indicated for imaging and intraoperative detection of sentinel lymph nodes draining a primary tumour in adult patients with breast cancer, melanoma, or localised squamous cell carcinoma of the oral cavity.
>
> External imaging and intraoperative evaluation may be performed using a gamma detection device.

The medicinal product should only be administered by trained healthcare professionals with technical expertise in performing and interpreting sentinel lymph node mapping procedures.

The recommended dose is 50 micrograms tilmanocept radiolabelled with technetium Tc 99m at 18.5 MBq for same day surgery or 74 MBq for next day surgery. The dose of 50 micrograms should not be adjusted for body weight differences. The total injection amount should not exceed 50 micrograms tilmanocept, with a total maximum radioactivity of 74 MBq (2.0 mCi) per dose.

Following reconstitution and labelling, Lymphoseek is intended to be injected in close proximity to the tumour and used in preoperative gamma detection imaging in conjunction with a stationary gamma camera (scintigraphy), single photon emissioncomputed tomography (SPECT), or SPECT/computerized tomography SPECT/CT, and/or intraoperatively in conjunction with a gamma detection probe to localise sentinel lymph nodes in the lymphatic pathway draining the tumour.

This medicinal product must be radiolabelled before administration to the patient. The radiolabelled product is a clear, colourless solution with no visible particles. Following radiolabelling, administration can

be by either intradermal, subcutaneous, intratumoural, or peritumoural injection. For melanoma, administration is intradermal in single or multiple divided injections. For breast cancer, administration is intradermal, subareolar (single or multiple divided injections) or peritumoural (multiple divided injections). For squamous cell carcinoma of the oral cavity, administration is peritumoural (multiple divided injections).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as kit for radiopharmaceutical preparation containing 250 micrograms of tilmanocept as active substance.

Other ingredients are: trehalose, dihydrate, glycine (E640), sodium ascorbate (E301), stannous chloride dihydrate (E512), sodium hydroxide (E524), hydrochloric acid (E507), nitrogen (E941) and water for injections.

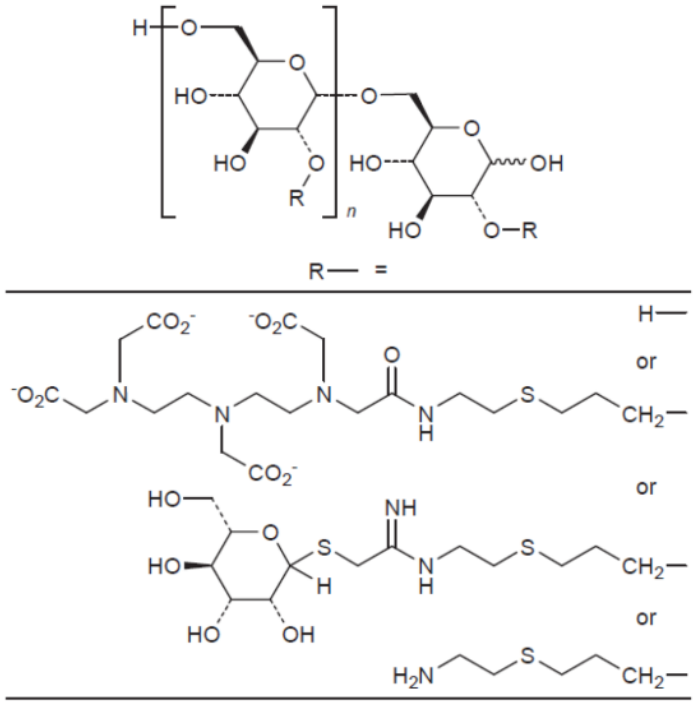
The product is available in type I glass vial with a butyl rubber stopper sealed with a flip-off seal.

2.2.2. Active Substance

General information

Tilmanocept is a new chemical entity and a novel radiodiagnostic imaging agent, targeting the radioisotope, technetium-99m to a mannose-binding protein that resides on the surface of dendritic cells and macrophages. Tilmanocept consists of a macromolecule of multiple units of diethylenetriaminepentaacetic acid (DTPA) and mannose, each attached synthetically to a dextran backbone. The mannose acts as a substrate for the receptor (mannose-binding protein), with the DTPA serving as a chelating agent for labelling with 99mTc.

The chemical name of tilmanocept is dextran, 3 [(2-aminoethyl)thio]propyl 17-carboxy-10, 13, 16-tris(carboxymethyl)-8-oxo-4-thia-7, 10, 13, 16-tetraazaheptadec-1-yl 3-[[2-[[1-imino-2-(D-mannopyranosylthio)ethyl]amino]thio]propyl ether and has the following structure:



The structure of tilmanocept is a mixture of many possible molecular permutations. Techniques that acquire collective or average based signals such as NMR and FT-IR were used to characterise the active substance, which are more appropriate than techniques looking at individual components, such as mass spectrometry.

The active substance, tilmanocept is off-white to buff-coloured powder. Tilmanocept is very soluble in water, insoluble in alcohol and moderately hygroscopic. Since the substance is to be formulated into a true solution prior to lyophilisation during the manufacture of the finished product, its polymorphism was not studied. An average molecule of the substance contains approximately 62 glucopyranosyl units, each with 5 stereo centres, with the side chains having further stereo centres. Therefore, it is not possible to determine the absolute stereochemistry of tilmanocept by typical analytical techniques and this is acceptable.

Manufacture, characterisation and process controls

The active substance is manufactured in one manufacturing site.

Tilmanocept is synthesised in 4 main steps using commercially available well defined starting materials with acceptable specifications.

The manufacture consists in four conjugation steps, allylation, amination of the allyl conjugate, DPTA conjugation and mannose conjugation.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

Specification

The active substance specification includes tests for appearance, assay (HPLC), bacterial endotoxins (Ph Eur), impurities (HPLC), heavy metals, identification (FT-IR, 1H-NMR), residual solvents, total aerobic microbial count (Ph Eur), total combined yeasts and molds count (Ph Eur), amine number (1H-NMR), DTPA Number (UV spectroscopy), mannose number (HPLC), calculated molecular weight and molecular mass distribution (GPC).

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines.

Batch analysis data (6 pilot scale batches) of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on three production scale batches of active substance from the proposed manufacturer stored in HDPE bottles, which differ from the proposed commercial packaging, for 24 months under long term conditions at 25 ºC / 60% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines were provided.

The following parameters were tested: appearance, assay, impurities, water content, total aerobic microbial count, total combined yeasts and moulds, and molecular mass distribution.

The stability results indicate that the drug substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

Lymphoseek is presented as a lyophilised solid to be reconstituted with Sodium Pertechnetate 99mTc Injection to provide a solution of 99mTc-Lymposeek for intradermal, subcutaneous, and epilesional use. The formulation contains excipients (reducing agent, transchelating, bulking, antioxidant and pH-adjusting agents) necessary for the radiolabelling of the product in the radiopharmacy.

The active substance is a powder that is moderately hygroscopic and very water soluble. Evidence of good physico-chemical stability in aqueous solution is provided. The molecule size allows for rapid clearance from the injection site and for easy passage into capillaries.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

An overview was provided of the development of the formulation through clinical development. Phase I studies used a formulation which did not contain trehalose or glycine; upon progression to phase II and III trials, the proposed commercial formulation was chosen.

The starting point for developing the manufacturing process was identifying the critical process parameters that should be monitored or controlled. These were identified as control of oxygen content throughout the manufacturing process as the minimisation of oxidation is important to minimise oxidation

of the excipients stannous chloride dihydrate and sodium ascorbate and control of the pH during compounding. Presence of oxygen was minimised and pH was controlled during development.

Comprehensive details of the development of the lyophilisation process were presented. These include reports of low temperature thermal analysis, lyophilisation cycle refinement and optimisation, a process target confirmation study and boundary studies of the process parameters. Stability batches from these boundary studies were initiated to confirm the acceptability of the resultant dosage form.

The chosen sterilisation method was aseptic filtration of the bulk solution since sterility assurance cannot be applied to a dry blend of powders and terminal sterilisation is not possible due to instability of the excipient stannous chloride dihydrate in aqueous solution.

The differences in the manufacturing process between clinical, stability and proposed commercial batches were presented. These are minor, with increased control for the commercial batches. The differences would not be considered to lead to differences in the quality of the product and are satisfactory.

The development of the container closure system was described adequately and reflected the validated vial type/size at the various sites used in the development of the product. The material (glass and butyl rubber stopper) remained the same throughout the development. Stability results indicated the compatibility of the product with the primary packaging. Leachables studies were described, from which it was concluded that in consideration of the intended low dose and the extremely low levels of leachables, these are not required to be monitored in routine release or stability testing.

Container closure integrity was examined using a microbial ingress test and a dye ingress test. The applicant carried out dye ingress testing on all stability batches in addition to sterility testing, with the aim to eventually use integrity testing in lieu of sterility testing. All microbial ingress and dye ingress results indicated the satisfactory integrity of the proposed container closure system. Residual moisture in the proposed stoppers was determined and considered sufficiently low as to be acceptable.

Manufacture of the product and process controls

The manufacturing process and controls are conventional for an aseptically filled and lyophilized pharmaceutical product. The manufacturing process consists of nine main processes: pre-compounding, compounding, transfer, filtration and filling, lyophilization process, capping and vial washing, and inspection. The process is considered to be a non-standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (container/closure, lyophilized product, and reconstituted solution), reconstitution time, solution pH, residual moisture, osmolality, assay (HPLC-CAD), identification (HPLC-CAD), uniformity of dosage units (Ph Eur), free DTPA (HPLC-DAD), stannous chloride dihydrate content (differential polarography), sodium ascorbate content (HPLC-DAD), sterility (Ph Eur), bacterial endotoxins (Ph Eur) and particulate matter (Ph Eur).

Batch analysis results are provided for 3 commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. The finished product is released on the market based on the above release specifications, through traditional final product release testing

Stability of the product

Stability data is provided in three primary, two process qualification/characterization, and three process validation stability batches stored under long-term conditions of 25°C / 60% RH for up 36 months and under accelerated conditions of 40°C /75% RH for up 6 months. The batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance (container/closure, lyophilized product, reconstituted solution), reconstitution time, pH, residual moisture, osmolality, potency, radiochemical purity, stannous chloride dihydrate content, L-ascorbic acid content, sterility, bacterial endotoxins and particulates.

In-use stability was conducted to determine the shelf-life of reconstituted product as determined by HPLC and ITLC data.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.

Based on available stability data, the shelf-life and storage conditions as stated in the SmPC are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation(s) for future quality development

N/A

2.3. Non-clinical aspects

2.3.1. Introduction

Primary pharmacodynamic studies comprise two *in vitro* studies (NEO3-08, NEO3-10) to evaluate mannose binding receptor binding in human macrophages, and an *in vivo* distribution study of Lymphoseek in rabbits. Safety pharmacology studies comprise two cardiovascular safety studies in beagle dog. Single-dose toxicity studies were conducted in compliance with GLP. Two single dose toxicity

studies were conducted in Sprague Dawley rats and a single subcutaneous dose study was conducted in NZW rabbits. Cardiovascular safety system studies were conducted in the beagle dog.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Three *in vitro* PD studies (NEO3-08, NEO3-08A, and NEO3-10) have been performed.

Table 1: *In Vitro* Studies Using Human Biomaterial

Target Receptor Binding	
Study No.	**Study Title**
NEO3-08	An Integrated Analysis of the *In vitro* Binding Specificity of GMP Lymphoseek® (Kit for the Preparation of Technetium Tc 99m Tilmanocept for Injection) to Human Mannose Binding Receptor-Expressing Macrophages with the Effect of Injection Volume Excursion Modelling and Clinical Results from NEO3-05 Phase 3 of Lymphoseek Concordance with Vital Blue Dye in Breast Cancer and Melanoma Patients
NEO3-08A	*In vitro* Binding Specificity of GMP-grade Lymphoseek® (Tilmanocept) to the Human Mannose Binding Receptor (hMBR) of Viable Human Macrophages and Confirmation of Direct Binding to Recombinant hMBR (rhMBR)
NEO3-10	*In vitro* Binding Study of Tilmanocept with Low and High Mannose Conjugation; Binding to Human Macrophage Mannose Binding Receptor Proteins

The *in vitro* study **(NEO3-08),** with living human macrophages, determined a threshold performance concentration of approximately 1.5 µM for initial injection for clinical applications.

The *in vitro* study **(NEO3-08A)** showed that Lymphoseek bound with human Mannose Binding Receptor (hMBR) on monocyte-derived macrophage (MDM) cells as determined by Western blot and autoradiography; and no non-specific binding was observed. In addition, Lymphoseek binding was evident in recombinant hMBR (rhMBR) transfected human embryonic kidney 293 (HEK 293) but not in the empty vector-transfected HEK 293 cells. Lymphoseek binding was also shown to be competitively inhibited by pre-incubation with unlabelled Lymphoseek. This provided strong evidence that Lymphoseek selectively binds to its intended receptor target, hMBR.

A second *in vitro* study **(NEO3-10)** was conducted to evaluate the effect of varying the number of mannose moieties per molecule of tilmanocept drug substance on *in-vitro* binding to human macrophage hMBRs using a competitive binding paradigm. Binding efficacy was evaluated for tilmanocept batches containing 7.4, 13.6, and 19.1 mannose moieties/dextran and in a reference standard containing 17.2 mannose moieties/dextran. Relative to these batches, binding efficacy was significantly reduced in the tilmanocept batch containing 7.4 mannose moieties/dextran, which is well below the lower boundary of the manufacturing specification range of 12 mannose moieties. From these data, a threshold value for reduced binding performance was estimated at 11.7 mannose moieties per dextran. In *in vitro* studies, technetium Tc 99m tilmanocept exhibited specific and tight binding to human CD206 receptors with a primary binding site affinity of Kd = 2.76 x x 10-11 M.

Figure 1: Inhibition of Cy3-tilmanocept binding to macrophages by increasing concentrations of unlabelled tilmanocept of varying mannose number (corrected for non-specific binding) – Study NEO3-10

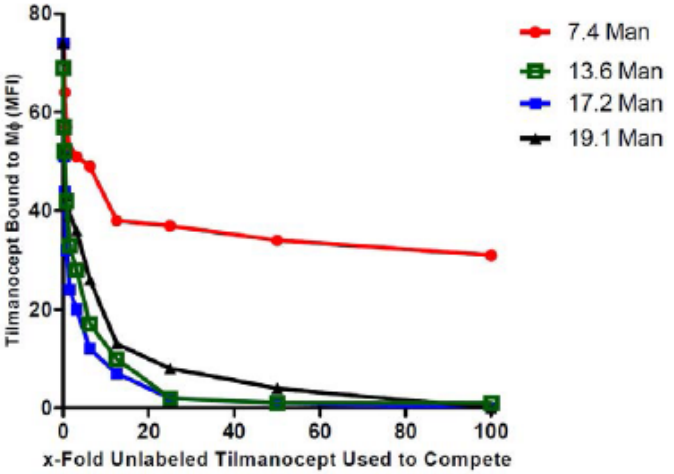


Table 2: Study NEO3-10: AUC summary

Mannose Number	AUC from Figure 1
7.4	3584.0
13.6	435.5
17.2	320.7
19.1	727.9

In Vivo Tissue Distribution of [^{99}Tc]-Lymphoseek in Rabbits (WIL 608002)

Tissue distribution of Tc 99m Lymphoseek was evaluated in New Zealand White rabbits administered a dose of approximately 1.4 µg/kg (5 µg/rabbit) and approximately 140 µCi [5.2 MBq] as a single SC injection into the distal portion of the left thigh. Animals were euthanized, and blood, urine, and tissue samples were collected at 0.25, 1, and 3 hours after dose administration. At 15 minutes and 1 hour after dose administration, approximately 1% of the injected dose of the Tc 99m Lymphoseek equivalents was found in ipsilateral popliteal lymph node, indicating rapid absorption in local lymph node tissue, while none was detected in contralateral popliteal lymph node. At 3 hours postdose, Tc 99m Lymphoseek equivalents had declined from levels found at the 1 hour time point in blood and all tissues with the exception of kidneys, bladder contents, and colon contents, demonstrating ongoing systemic clearance.

Secondary pharmacodynamic studies

No studies evaluating secondary pharmacodynamics were submitted.

Safety pharmacology programme

A summary of the single and repeat dose cardiovascular safety is presented in Table 3.

Table 3: Summary of main findings from the cardiovascular system safety studies

Study ID	Species/Sex/ Number/Group	Dose/Route	GLP	Major findings
TherImmune 1146-102	Beagle dog 2/sex/group	560 µg/kg tilmanocept IV	No	HR decreased in all (4/4) dogs at 1 min postdose, and returned to predose rates by 30 min postdose. Blood pressure showed no treatment-related trends at 1 or 30 min postdose. No treatment-related changes in ECGs, including atrioventricular conduction defects or premature atrial or ventricular complexes, observed at 1 or 30 min postdose. Plasma thromboxane B2 and histamine levels increased at 1 minute postdose, with return towards baseline at 30 minutes. No effects on survival or clinical observations.
Gene Logic 1576-04774	Beagle dog 4/sex/group	Escalating repeated doses (IV) Day 1: Saline Day 2: Saline Day 4: 84 µg/kg tilmanocept Day 6: 420 µg/kg tilmanocept Day 8, 10: 840 µg/kg tilmanocept	Yes	QT interval decreased 24 h postdose (M, 420 µg /kg), increased 2 h postdose (M, 840 µg /kg and F, 420 µg /kg) within normal limits. Systolic and diastolic BP, MAP, and HR elevated at 1 min postdose following all doses including control. Increased HR in controls, 420 µg /kg (M) and 840 mg/kg (F) at 1 min postdose, and 1 min and 24 h postdose in M (420 µg /kg0. Decreased HR in M at 2 h postdose (saline), at 30 min, 2 h, and 4 h postdose (84 µg/kg), at 30 min and 2 h postdose (840 µg/kg), and for F at 30 min postdose (84 µg/kg). Not considered related to the test article. MAP decreased in M at 30 min and 8 h postdose (840 µg/kg). Decreased systolic and diastolic blood pressure, MAP, and heart rate were observed in males at 24 h postdose (420 µg/kg). Not considered test article-related. Slight bradycardia or tachycardia was observed in some ECGs; not considered biologically significant. Body temperature was slightly lower at 4 h postdose (420 µg/kg) for males and was slightly elevated in females (840 µg/kg).

Pharmacodynamic drug interactions

The applicant did not submit drug interaction studies.

2.3.3. Pharmacokinetics

The pharmacokinetic studies comprise a pilot pharmacokinetic study in rats, toxicokinetic analysis of 111-Indium labelled tilmanocept at day 1 and 14 of the pivotal repeat-dose studies in rats and dogs, and a distribution study in rabbits.

The three nonclinical studies that evaluated the PK of labeled tilmanocept formulations and one that used the Tc 99m labeled Lymphoseek drug product are shown in Table 4.

Table 4: Pharmacokinetics Program Overview

Study Type and Duration	Route	Dose	Species
Single dose pilot pharmacokinetics			
Single dose, [In 111]-tilmanocept	SC	52.5 µg/kg	Mongrel dog
Repeated dose toxicity			
14 days, [In 111]-tilmanocept	SC	10.5, 21, and 42 µg/kg	Sprague Dawley rat
14 days, [In 111]-tilmanocept	SC	10.5, 21, and 42 µg/kg	Mongrel dog
Tissue distribution			
Single dose, Tc 99m Lymphoseek	SC	1.41 µg/kg (5 µg/animal)	New Zealand white rabbit

Abbreviation: SC, subcutaneous.

Pilot Pharmacokinetic Study of ^{111}Indium-Lymphoseek in a Mongrel Dog (Study Batelle N106921)

This non-GLP pilot study was performed in a single animal. Administration was by single SC injection of 52.5 µg/kg and approximately 1 mCi [37 MBq] of radioactivity in a volume of 1.0 mL/kg. Cmax was 18.47 ng/mL and was observed at 1 hour (tmax), AUClast was 118.28 hr·ng/mL, and apparent clearance (CL/F) was 326.49 mL/hr/kg.

14-Day Toxicity Study of ^{111}Indium-Lymphoseek in Sprague-Dawley Rats (Study Battelle N106923)

Study Battelle N106923 was conducted to determine the toxicity of tilmanocept (drug substance) when administered via SC injection to Sprague Dawley rats for 14 consecutive days. For the TK groups, 23 animals/sex/group were administered 0, 10.5, 21, or 42 µg /kg. At day 1 and 14, ^{111}Indium-tilmanocept was administered, and at day 2-13 unlabelled drug was used.

An overall mean (± SEM, n=12) absorption half-life value of 0.067 ± 0.01 hours (approximately 4 minutes) was determined. Observed t_{max} values were in close agreement with the fitted values, and there was no apparent dose- or sex-related effect. The group mean fitted t_{max} ranged approximately 7 to 18 minutes. The C_{max} and AUC_{last} results suggest that doses ranging from 10.5 µg/kg (low dose) to 21 µg/kg (mid dose) to 42 µg/kg (high dose) are in a dose-proportional range for the rat, and repeated administration did not appear to have inhibitory or inductive effect. The Vd/F was not dependent on sex or single vs. repeated administration. The overall Vd/F was 1160 ± 50 mL/kg.

Elimination was not dependent on the dose, sex, or single vs. repeated administrations so was not considered saturable. Alpha phase (fast) elimination was well defined for all groups; the overall alpha half-life was 0.540 ± 0.035 hours. The overall beta (slow elimination) half-life (excluding curves with ill-defined terminal linear phases) was 53.8 ± 11.3 hours. The overall central compartment elimination half-life was 3.10 ± 0.70 hours.

Table 5: Summary of Day 1 and Day 14 Toxicokinetic Parameters in Rats After Repeated Administration of Low, Mid, and High Doses of Tilmanocept (SEM) [N106923]

Parameter (units)	Males			Females		
	Low Dose (10.5 µg/kg)	Mid Dose (21 µg/kg)	High Dose (42 µg/kg)	Low Dose (10.5 µg/kg)	Mid Dose (21 µg/kg)	High Dose (42 µg/kg)
Day 1						
Fitted C_{max} (ng/mL)	12.8 (1.3)	16.5 (1.1)	29.1 (3.0)	10.4 (1.0)	12.3 (1.6)	26.2 (2.4)
Obs C_{max} (ng/mL)	13.9 (2.0)	17.8 (2.6)	28.6 (4.9)	10.3 (1.3)	11.1 (0.4)	24.0 (1.3)
Fitted t_{max} (h)	0.121 (0.062)	0.210 (0.035)	0.180 (0.059)	0.301 (0.044)	0.253 (0.070)	0.308 (0.045)
Obs t_{max} (h) [a]	0.167	0.250	0.250	0.500	0.167	0.500
AUC_{last} (ng·h/mL) [a]	20.6	31.7	58.7	17.8	24.2	57.2
V_d/F (mL/kg)	1120 (110)	1040 (120)	1250 (200)	854 (245)	1340 (340)	1110 (240)
CL/F (mL/h/kg)	519 (83)	570 (48)	257 (215)	408 (249)	322 (237)	319 (240)
Day 14						
Fitted C_{max} (ng/mL)	5.62 (0.26)	13.4 (1.1)	25.8 (1.1)	6.10 (0.43)	14.6 (1.1)	19.2 (1.4)
Obs C_{max} (ng/mL)	5.14 (0.46)	12.1 (0.7)	24.9 (1.0)	5.6 (0.55)	13.9 (1.1)	18.7 (2.1)
Fitted t_{max} (h)	0.208 (0.028)	0.205 (0.054)	0.167 (0.030)	0.217 (0.038)	0.212 (0.042)	0.259 (0.040)
Obs t_{max} (h) [a]	0.250	0.250	0.250	0.0833	0.250	0.250
AUC_{last} (ng·h/mL) [a]	12.8	31.6	63.5	16.9	42.5	53.7
V_d/F (mL/kg)	932 (67)	1130 (140)	1310 (80)	1020 (120)	1410 (160)	1360 (180)
CL/F (mL/h/kg)	347 (35)	347 (101)	[6.01 (178.9)] [b]	87.9 (131.3)	[2.56 (244.19)] [b]	158 (162)

- No measure of variability calculated for this observed parameter because of the sparse-sampling study design.
- Unreliable parameter estimates are shown in brackets.

Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; C_{max}, maximum drug concentration; CL/F, apparent total clearance of the drug; Obs, observed; SEM, standard error of the mean; t_{max}, time to reach C_{max}; V_d/F, apparent volume of distribution.

14-Day Toxicity Study of ^{111}Indium-Lymphoseek in Mongrel Dogs (Study Battelle N106922)

Study Battelle N106922 was conducted to determine the toxicity of tilmanocept (drug substance) when administered via SC injection to Sprague Dawley rats for 14 consecutive days. Doses administered were 0, 10.5, 21 and 42 µg /kg/day. Animals were administered 111Indium-tilmanocept on day 1 and 14. Blood samples were collected before dosing, 10, 20 and 30 minutes, and 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 hours after dosing. Urine was collected at 0 to 2, 2 to 6, 6 to 12, 12 to 24, and 24 to 48 hours.

The overall absorption half-life value was approximately 23 minutes. The group mean fitted tmax ranged from approximately 28 to 66 minutes.

Cmax and AUClast were dose and single vs. repeated dose-dependent, but not sex dependent and increased proportionally with increasing dose. These findings indicate that doses ranging from 10.5 to 21 µg/kg are in a dose proportional range for the dog.

The Vd/F was not dependent on sex or single vs. repeated administration. The overall Vd/F was 887 ± 62 mL/kg.

Elimination was not dependent on the dose, sex, or single vs. repeated administrations. Elimination was evaluated using the alpha (fast), beta (slow), and central compartment elimination rate constants and half-lives (Table 6). The alpha phase was well defined for all groups; the overall alpha half-life was 1.32 ± 0.04 hours. The overall beta half-life (excluding curves with plateau-like terminal linear phases) was 87.6 ± 14.9 hours. The overall central compartment elimination half-life was 6.54 ± 0.89 hours.

The overall CL/F was 116 ± 13 mL/hr/kg. Peak equivalent concentrations of [In-111]-tilmanocept in the urine were observed for most male and female groups on Days 1 and 14 during the 2 to 6 hour collection interval. Of the total amount of [In 111]-tilmanocept equivalents eliminated by the kidneys, approximately 90% or more was measured in the urine within 24 hours after dosing on Day 1 and within 12 hours after dosing on Day 14. The group mean percentage of dose excreted in the urine ranged from 26.5% to 43.0%, and the overall mean from all groups was 35.0% ± 1.6%.

Table 6: Summary of Day 1 and Day 14 Toxicokinetic Parameters in Dogs After Repeated Administration of Low, Mid, and High Doses of Tilmanocept (SEM) [N106922]

Parameter (units)	Males			Females		
	Low Dose (10.5 µg/kg)	Mid Dose (21 µg/kg)	High Dose (42 µg/kg)	Low Dose (10.5 µg/kg)	Mid Dose (21 µg/kg)	High Dose (42 µg/kg)
Day 1						
Fitted C_{max} (ng/mL)	8.14 (1.50)	15.6 (1.4)	45.7 (1.8)	10.4 (2.1)	26.5 (1.9)	41.7 (9.7)
Obs C_{max} (ng/mL)	8.17 (1.58)	15.3 (1.6)	42.8 (1.9)	9.56 (1.59)	33.1 (6.6)	40.6 (8.6)
Fitted t_{max} (h)	1.10 (0.08)	0.864 (0.124)	0.518 (0.173)	0.945 (0.303)	0.461 (0.246)	0.960 (0.182)
Obs T_{max} [a] (h)	1.0	1.0	0.1667	1.0	0.5	1.0
AUC_{last} (ng·h/mL)	59.1 (5.1)	95.9 (6.2)	257 (19)	58.1 (2.5)	116 (9)	246 (38)
V_d/F (mL/kg)	988 (180)	871 (82)	596 (131)	617 (88)	666 (190)	764 (275)
CL/F (mL/h/kg) [b]	155	120	46.4	134	83.4	169
Day 14						
Fitted C_{max} (ng/mL)	6.82 (0.83)	10.6 (1.2)	22.1 (4.3)	7.28 (0.87)	12.7 (1.3)	19.5 (1.3)
Obs C_{max} (ng/mL)	6.80 (1.02)	10.5 (1.0)	21.2 (3.6)	7.15 (0.87)	14.6 (3.6)	19.1 (1.7)
Fitted t_{max} (h)	0.963 (0.162)	0.969 (0.192)	0.937 (0.025)	0.889 (0.189)	1.05 (0.14)	1.06 (0.11)
Obs T_{max} (h) [a]	1.0	1.0	1.5	1.0	1.5	1.0
AUC_{last} (ng·h/mL)	51.8 (6.7)	74.8 (8.1)	145 (15)	41.8 (2.2)	88.9 (6.1)	141 (15)
V_d/F (mL/kg)	939 (182)	1110 (210)	1260 (310)	754 (21)	928 (182)	1150 (200)
CL/F (mL/h/kg) [b]	116 (29)	88.1	158	96.0	50.2	170

[a] Median reported for observed T_{max}

[b] No measure of variability is calculated for groups with less than three values used to calculate the mean.

Abbreviations: AUC_{last}, area under the concentration-time curve from time zero to time of last measurable concentration; C_{max}, maximum drug concentration; CL/F, apparent total clearance of the drug; Obs, observed; SEM, standard error of the mean; t_{max}, time to reach C_{max}; V_d/F, apparent volume of distribution.

Tissue Distribution of [^{99m}TC] Lymphoseek in Rabbits (WIL 608002)

TC 99m Lymphoseek in PBS was administered in 11 rabbits (7F/4M) by a single bolus SC injection in the distal portion of the thigh. A single 1.4 µg /kg dose (5 µg/animal) was administered with a resulting radioactive dose of approximately 140 µCi/animal (the actual dose ranged from 131 to 145 µCi [4.8 to 5.4 MBq]). Blood, urine, and tissue samples were collected at 0.25, 1, and 3 hours postdose.

In females, TC 99m Lymphoseek equivalents were widely distributed by 0.25 hours postdose, with highest doses in plasma (7.7%ID), urinary bladder contents (7.7%ID) and injection site skin (6.5%ID). No analysis was performed in males at this time point. At 1 hour postdose TC 99m Lymphoseek equivalents increased substantially at the injection site in females (33.6%ID) and males (24.1%ID) relative to values in females at 0.25 hours postdose. Urinary contents (14.62 and 8.07 %ID in males and females), kidneys (5.86 and 6.67%ID in males and females), and liver (5.28 and 6.66 %ID in male and females) amounts all increased relative to values in females at the 0.25 hour time point, whereas plasma levels appeared to decline. By 3 hours postdose, the highest fraction of dose was present in the urinary bladder contents (34.7%ID in males, 27.4%ID in females). This, in conjunction with moderately elevated levels in the kidneys, indicates urinary excretion is an important route of elimination of Tc 99m Lymphoseek. %ID also increased in gastrointestinal tract, but accounted for <3%ID at any of the time points evaluated. Liver values were highest at approximately 6%ID at the 1 hour time point and decreased to approximately 4%ID at 3 hours after dosing. Approximately 1%ID was found in the draining left popliteal lymph node at each time point evaluated, while little to none was found in the right popliteal lymph node or in either left or right axillary lymph nodes at any time point.

Table 7: Percentages of the Injected Dose (SD) of Tc 99m Lymphoseek in Selected Tissues of Rabbits at 0.25, 1, and 3 Hours After a Single Subcutaneous Injection of 5 µg/animal (approximately 1.4 µg/kg) [WIL 608002]

Tissue	Mean %ID (SD)					
	0.25 hours postdose		1 hour postdose		3 hours postdose	
	M [a]	F [b]	M [a]	F [b]	M [a]	F [b]
Injection site skin	n/a	6.54 (7.03)	24.10 (30.62)	33.64 (6.46)	16.17 (4.64)	23.45 (11.42)
Plasma	n/a	7.71 (0.43)	5.89 (1.88)	5.10 (0.73)	3.64 (0.26)	3.12 (0.30)
Blood cell fraction	n/a	1.92 (0.35)	1.28 (0.47)	1.19 (0.03)	0.79 (0.07)	0.70 (0.18)
Urinary bladder contents	n/a	7.67 (10.51)	14.62 (7.27)	8.07 (8.29)	34.72 (15.70)	27.41 (5.90)
Urinary bladder	n/a	0.05 (0.03)	0.14 (0.08)	0.10 (0.01)	0.29 (0.19)	0.08 (0.00)
Kidneys	n/a	2.81 (0.75)	5.86 (3.41)	6.67 (0.87)	8.34 (0.16)	6.32 (0.97)
Liver	n/a	2.78 (1.06)	5.28 (3.15)	6.66 (0.18)	5.10 (0.68)	3.83 (0.74)
Left popliteal lymph node	n/a	0.68 (0.78)	0.90 (0.44)	0.28 (0.21)	1.18 (1.42)	0.00 (0.00)
Right popliteal lymph node	n/a	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Left axillary lymph node	n/a	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.15)	0.20 (0.08)
Right axillary lymph node	n/a	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Stomach contents	n/a	0.65 (0.63)	0.08 (0.03)	0.34 (0.18)	0.26 (0.26)	0.14 (0.12)
Stomach	n/a	0.33 (0.05)	0.20 (0.12)	0.30 (0.01)	0.21 (0.05)	0.14 (0.09)
Colon	n/a	0.62 (0.09)	0.69 (0.33)	0.93 (0.00)	0.89 (0.26)	0.80 (0.08)
Colon contents	n/a	0.04 (0.01)	0.13 (0.09)	0.21 (0.19)	1.30 (0.51)	1.28 (0.46)
Small intestine	n/a	0.40 (0.04)	0.37 (0.12)	0.57 (0.07)	0.35 (0.11)	0.31 (0.04)
Small intestine contents	n/a	0.09 (0.04)	0.11 (0.07)	0.32 (0.07)	0.14 (0.01)	0.11 (0.01)
Lung	n/a	0.43 (0.08)	0.26 (0.15)	0.22 (0.10)	0.20 (0.00)	0.19 (0.02)
Brain	n/a	0.01 (0.00)	0.01 (0.00)	0.01 (0.01)	0.02 (0.02)	0.01 (0.01)
Flank muscle	n/a	0.07 (0.06)	0.10 (0.10)	0.07 (0.08)	0.02 (0.00)	0.01 (0.00)

[a] Males, n = 0 at 0.25 hours; n = 2 at 1 and 3 hours

[b] Females, n = 3 at 0.25 hours; n = 2 at 1 and 3 hours

Thymus, spleen, thyroid, gall bladder, bone (rib, femur), eyes, testes, and ovaries were examined but not shown here; all were < 0.05%ID at all time points (see source tables for full listing of results).

Abbreviations: F, female %ID, percent of injected dose; M, male; n/a, not applicable; SD, standard deviation.

In a published tissue biodistribution study of [99mTc]DTPA-mannosyl-dextran in New Zealand White rabbits, similar distribution profiles were observed. Following injection to the right rear paw, 1-2% of injected dose was found in the right popliteal lymph node at 15 min, 1 h, and 3h postdose.

Metabolism

No specific metabolism studies were submitted. The applicant provided the following description of the theoretical metabolism of tilmanocept.

Dextran Core: Dextrans are polysaccharides composed of linear glucose residues. They are produced by the enzyme dextran sucrase during growth of various strains of *Leuconostoc* bacteria in media containing sucrose. Dextrans are isotonic and can be stored at room temperature. Dextran is broken down completely to CO_2 and H_2O by dextranase present in spleen, liver, lung, kidney, brain, and muscle at a rate approaching 70 mg/kg every 24 hr.

DTPA: DTPA has been in clinical use for nearly 55 years. Its biodistribution and metabolic fate have been extensively studied. It is currently used as an imaging agent with 99mTc with a Sn2+ reducing agent, exactly like the Lymphoseek radionuclide labelling system.

Mannose: The metabolic fate of mannose is well established. No known untoward metabolites are established as "toxic" from mannose metabolism. Mannose is a well-established molecular species in blood and interstitial fluid (0.55 mM). Mannose is the functional targeting moiety of Lymphoseek (to the mannose binding receptor [MBR; CD206] of the reticuloendothelial cells in the lymph nodes). Its metabolic fate is estimated to be similar to other mannose ligands.

Thioether leashes: The thioether leashes that extend to hold mannose and DTPA as well as free leashes are readily metabolized as other such molecular entities, via the cytochrome P450 pathway. The primary modulators of this degradation are CYP1A2, 3A4, 2B6, 2C9*1, 2C18, 2C19, 2D6*1, and FMO1; disulfoton, CYP1A2, 3A4, 2B6, 2C9*1, 2C9*2, 2C18, 2C19, 2D6*1, and FMO1; sulprofos, CYP1A1, 1A2, 3A4, 2C9*1, 2C9*2, 2C9*3, 2C18, 2C19, 2D6*1, and FMO1; methiocarb, CYP1A1, 1A2, 3A4, 2B6, 2C9*1, 2C19, 2D6*1, and the flavin mono-oxygenases.

Excretion

Specific excretion studies were not conducted in separate studies. Excretion data are available in nonclinical toxicity studies N106923 (Rats) and N106922 (dogs).

In the dog study, the overall central compartment elimination half-life was 6.54 ± 0.89 hours. The apparent volume of distribution (Vd/F) did not appear to be dependent on sex or single vs. repeated administration. Peak [In 111]-tilmanocept concentrations in the urine were observed for most male and female groups on Days 1 and 14 during the 2 to 6 hour collection interval. Of the total amount of [In 111]-tilmanocept equivalents eliminated by the kidneys, approximately 90% or more was measured in the urine within 24 hours after dosing on Day 1 and within 12 hours after dosing on Day 14. The overall percentage of dose excreted in the urine was 35.0% ± 1.6% in this dog study.

In the rat study, the overall central compartment elimination half-life was 3.10 ± 0.70 hours. The analysis of urine samples, obtained from the satellite group animals before dosing on Day 1 and Day 14, and after dosing at the pre-specified time points, indicated that elimination of [In 111]-tilmanocept equivalents in urine was also independent of dose, sex, or single vs. repeated administrations. This result suggests that elimination over repeated doses of [In 111]-tilmanocept remained first order and that no saturation occurred. The overall mean percentage of dose excreted in the urine was 29.1% ± 1.8% in rats.

2.3.4. Toxicology

The proposed human dose is 50 µg Tc 99m Lymphoseek per procedure, equivalent to 0.714 µg/kg if an estimate of 70 kg is used for human body weight. The administration of 14 to 280 µg/kg in single dose animal studies is equivalent to approximately 20 to 390 times that for the anticipated human dose. Administration of 10.5 to 42 µg/kg/day in repeated dose animal studies represent approximately 15 and 60 times the anticipated human dose.

Single dose toxicity

A summary of the single toxicity studies are presented in Table 8.

Table 8: Single dose toxicity studies

Species /Strain	Method of Administration (Vehicle / Formulation)	Dose (µg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (µg/kg)	Noteworthy Findings
Sprague Dawley Rats	SC (SWI/Drug substance, unlabeled)	0 14 140	5M / 5F 5M / 5F 5M / 5F	140 (tilmanocept)	No treatment-related effect on mortality, clinical observations, body weight, clinical pathology, necropsy, or histopathology
Sprague Dawley Rats	SC (sterile saline/Drug product, unlabeled)	0 14 140	5M / 5F 5M / 5F 5M / 5F	140 (unlabeled Lymphoseek)	No treatment-related effect on mortality, clinical observations, body weight, clinical pathology, gross pathology, or histopathology.
New Zealand White Rabbits	SC (SWI/Drug substance, unlabeled)	0 14 140	5M / 5F 5M / 5F 5M / 5F	140 (tilmanocept)	No treatment-related effect on mortality, clinical observations, body weight, clinical pathology, or necropsy findings. Minimal to mild centrolobular hepatocytic hypertrophy noted microscopically in the majority of treated rabbits.
Mongrel Dogs	SC (SWI/Drug substance, unlabeled)	0 42 180 420	4M / 4F 4M / 4F 4M / 4F 4M / 4F	420 (tilmanocept)	No treatment-related effect on mortality, clinical observations, body weight, food consumption, clinical pathology, gross pathology, or organ weight. No systemic toxicity noted. Treatment-related findings were limited to an inflammatory response at the injection site in both sexes at all doses. .

Abbreviations: F, female; GMP, Good Manufacturing Practice; IM, intramuscular; M, male; n/a, not applicable; SC, subcutaneous; SWI, sterile water for injection.

Repeat dose toxicity

The toxicity of tilmanocept drug substance was determined when administered via SC injection to groups of 10 Spague-Dawley rats/sex/group (toxicity) or 23 rats/sex/group (pharmacokinetics) for 14 consecutive days at 0 (vehicle), 10.5, 21 and 42 µg/kg/dose. Blood and urine samples were also collected following the first and last doses to establish a toxicokinetic (TK) profile, using indium-111-radiolabeled tilmanocept ([In 111]- tilmanocept).

There were no treatment related deaths. There were no treatment related overt signs of toxicity or changes in body weight, food consumption, ophthalmologic findings, or physical findings. There were also no treatment related changes in clinical pathology parameters, urinalysis values, organ weights, or macroscopic or microscopic findings.

Based on no treatment-related effects being observed in any dose group, the NOEL for 14 consecutive days of tilmanocept SC administration in rats was considered to be ≥ 42 µg/kg/day.

14-Day Study of ^{111}Indium-Lymphoseek in Mongrel Dogs (Study N106922)

The toxicity of tilmanocept drug substance was determined when administered via SC injection to groups of 12/sex mongrel dogs for 14 consecutive days at dose levels of 0 (vehicle), 0.0105, 0.021, 0.042 mg/kg/dose. Blood and urine samples were also collected on Day 1 and Day 14 to establish a TK profile using [In^{111}]-labeled tilmanocept.

Following at least 15 consecutive doses of tilmanocept, there were no treatment related clinical abnormalities or body weight, food consumption, ophthalmologic, or physical changes. There were no effects on heart rates, electrocardiograms, or interval data. There were also no treatment related changes in clinical pathology parameters, urinalysis values, organ weights, or macroscopic or microscopic changes.

Based on no treatment-related effects being observed in any dose group, the NOAEL for at least 15 consecutive days of tilmanocept SC administration in dogs was considered to be ≥ 42 µg/kg/day.

Genotoxicity

The results of genotoxicity studies are presented in Table 9.

Table 9: Genotoxicity studies

Type of test / study ID / GLP	**Test system**	**Concentrations / Concentration range / Metabolising system**	**Results**
Gene mutations in bacteria AB11LN.503.BTL	Salmonella strains TA98, TA100, TA1535, TA1537 E. Coli WP2uvrA	+/- S9 1.5 - 5000µg/plate- initial assay 15- 5000µg/plate –confirmatory assay	Negative
Gene mutations in mammalian cells AB11LN.704.BTL	L5178Y/TK+/-	+/- S9 500 - 5000µg/ml 4h exposure -S9 10 - 5000µg/ml 24h exposure	4h exposure-negative 24h exposure-equivocal
Chromosomal aberrations *in vivo* AB11LN.123.BTL	Mouse, micronuclei in bone marrow	500, 1000, 2000 mg/kg Single dose	Negative

Carcinogenicity

No carcinogenicity studies were submitted.

Reproduction Toxicity

No studies on reproduction toxicity were submitted.

Local Tolerance

The potential for local irritation from a single intramuscular (thigh) injection was evaluated in two single dose GLP studies in rabbits using either tilmanocept drug substance, or unlabeled Lymphoseek drug product. No treatment-related effects were observed with tilmanocept. Using unlabeled Lymphoseek, mild inflammation and tissue degeneration were seen in one rabbit in the high dose (280 µg/kg) group. No other treatment-related observations were noted.

In the previously reported single subcutaneous dose toxicity study in mongrel dogs with tilmanocept, minimal to mild inflammation was observed at the injection site in some dogs at all doses with mild to moderate inflammation of the subcutis and skeletal muscle vacuolar degeneration.

Other toxicity studies

Antigenicity

An antigenicity study was performed in 50 male guinea pigs to determine the potential of tilmanocept to induce Type 1 systemic hypersensitivity (anaphylactic reactions). Animals were given a single intravenous challenge dose following four weekly subcutaneous sensitization doses. Treatment with tilmanocept at doses of 14.0, 28.0, or 280 µg/kg did not induce any anaphylactic reactions and had no effect on mortality, clinical observations, or changes in body weights. All animals survived until scheduled termination.

Immunotoxicity

No immunotoxicity studies were submitted.

Metabolites

No studies on metabolites were submitted.

Studies on impurities

No studies on impurities were submitted.

2.3.5. Ecotoxicity / environmental risk assessment

The LogKow of Lymphoseek is expected to be << 3.0. Experimental data for Lymphoseek are not available, and the logKow values of the DTPA unit, mannose and the dextran-10 unit were estimated using the EPI SuiteTM KOWWINTM model (US EPA, 2007). An experimental value of the logKow of mannose was available in the model (-3.24). The dextran-10 unit is the backbone that is constructed of several sugar units. The logKow for such backbone consisting of 4 sugar units was calculated to be -7.53, demonstrating that the logKow value of a polymer of mannose-entities will not be higher than that of the individual entities. Finally, the logKow of the DTPA unit was estimated to be -4.9. Therefore, a PBT assessment is not needed for Lymphoseek.

Table 10: Summary of main study results

Substance (tilmanocept):Lymphoseek			
CAS-number (if available): 1185986-76-8			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 or …	-3	Potential PBT (Y/N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	-3	B/not B
	BCF	N/A	B/not B
Persistence	DT50 or ready biodegradability	N/A	P/not P
Toxicity	NOEC or CMR	Not CMR	T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.00025	µg/L	> 0.01 threshold

2.3.6. Discussion on non-clinical aspects

In *in vitro* studies, technetium Tc 99m tilmanocept exhibited specific and tight binding to human CD206 receptors with a primary binding site affinity of Kd = 2.76 x 10^{-11} M. Plasma pharmacokinetic results from the 14 day repeated dose toxicity studies with [In 111]-tilmanocept demonstrated rapid absorption into the circulating blood in both rats and dogs (absorption half-life approximately 4 and 23 minutes, respectively). Observed Cmax values, ranging from 10.3 to 28.6 ng/mL for rats and 6.8 to 42.8 ng/mL for dogs, were in good agreement with the fitted Cmax values, increased proportionately with increasing dose, and were similar for males and females. Fitted tmax values ranged from approximately 7 to 18 minutes for rats and 28 to 66 minutes for dogs, again indicating rapid absorption. The AUC_{1ast} values were also similar for both sexes at a given dose group and increased proportionately with increasing dose in both rats and dogs.

Tilmanocept binds specifically to mannose binding receptors, and therefore off-target interactions are not expected. The absence of secondary pharmacodynamic studies is acceptable.

No specific metabolism studies were conducted to assess metabolite formation following administration of tilmanocept. The metabolism of the constituents of Lymphoseek has been described by the applicant. Lymphoseek localised to the kidneys, bladder and liver, which supports the predicted biliary/faecal route. In the absence of significant findings, particularly renal or hepatic findings, in the repeat dose studies, the absence of comprehensive studies on metabolism and excretion are not considered to pose a safety concern. ICH guidance M3(R2) titled "Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (2010)", states that nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10 percent of total drug-related exposure and at significantly greater level in humans than the maximum exposure seen in the toxicity studies." As lymphoseek is intended to be administered as a single 50 µg dose, it is accepted that given the low dose and frequency of dosing, it is not necessary to quantify all circulating metabolites to the 10 percent level.

Urinary excretion was a major route of elimination in all three studies. In the repeated dose studies in rats and dogs, the overall percentage of dose excreted in the urine (across dose groups) was 29.1% for rats and 35.0% for dogs. Peak excretion generally occurred during the 0 to 2 or 2 to 6 hour post-dose collection periods, and > 90% of the dose excreted by the kidneys was collected by 6 to 24 hours after dosing. Analyses of urine samples after repeated dosing indicated that elimination of radiolabeled tilmanocept in urine was also independent of dose, sex, or single vs. repeated administrations.

All the studies with a pharmacokinetic component were conducted using only one proposed route of administration i.e. the subcutaneous route. There are four proposed clinical routes of injection: intradermal, subcutaneous, intratumoural and peritumoural. However, the subcutaneous route of administration is the most practical route of administration and provides sufficient data for the purpose of non-clinical studies.

In the two single toxicities studies, a single subcutaneous dose of tilmanocept or unlabeled Lymphoseek up to a nominal tilmanocept dose of 140 µg/kg (42 µg/rat) was generally well tolerated. No significant overt signs of toxicity, no treatment related necropsy findings, and no evidence of macroscopic or microscopic histopathological changes were observed with the exception of slight lymphoid hyperplasia of the inguinal lymph node in one study. However, given that no such evidence has been raised in the clinical safety database of over 500 patients, this finding does not lead to any concern and further animal studies are not considered necessary.

In NZW rabbits a single subcutaneous dose of tilmanocept was well tolerated at all dose levels examined up to 140 µg/kg. There was no evidence of any treatment-related effect on mortality, overt signs of toxicity, body weight, gross pathology, or necropsy findings. Minimal to mild hepatocyte centrilobular hypertrophy was noted in treated rabbits and one control rabbit. In a study conducted in guinea pigs. tilmanocept was found not to have antigenic potential.

In a single subcutaneous dose toxicity study in mongrel dogs with tilmanocept, minimal to mild inflammation was observed at the injection site in some dogs at all doses with mild to moderate inflammation of the subcutis and skeletal muscle vacuolar degeneration. The inflammatory reaction was attributed a host response to foreign material, rather than a direct toxic effect of the drug. This reaction was not seen after subcutaneous injection in the other toxicity studies. No significant effects were observed on mortality, overt signs of toxicity, body weight, food consumption, clinical pathology, gross pathology, organ weights, organ-to-body weight ratio or histopathology.

In the *in vitro* mammalian cell gene mutation assay, the results of the 24-hour assay without metabolic activation were ambiguous. However, the total daily dose of the product is low, administered only on one occasion or very infrequently. Therefore, according to the draft ICH M7 guideline, it is expected to be below the limit of toxicological concern.

No CNS and respiratory safety studies were submitted. Based on metabolic body weight the expected accrual in 7.11 ng and ~92.43 ng in the brain and lungs, respectively, which is sufficiently low that no risk is posed to patients. In animals studies at supratherapeutic doses there were no observed respiratory or behavioural changes, or histopathological changes associated with these systems. In the lack of *in vivo* and histopathological findings and no respiratory or CNS signs in over 500 patients, the absence of CNS and respiratory studies is acceptable.

No carcinogenicity studies were submitted. ICH guidance S1A indicates that pharmaceuticals administered infrequently or for short duration of exposure do not require assessment of carcinogenicity unless there is cause for concern. Lymphoseek is intended for single administration. Therefore, the lack of carcinogenicity studies is acceptable.

As the product is indicated for adults aged 18 years and older, the lack of studies in juvenile animals is acceptable.

No reproductive or developmental toxicity studies were submitted. Given the high specificity and rapid clearance, the absence of pathologic changes in reproductive organs in repeated dose toxicity studies, and the minimal duration of patient exposure, the lack of studies on the toxicity to reproduction is acceptable. However, the toxicity of the product when used during pregnancy and lactation remains of concern. This risk has been addressed as part of the RMP.
In summary, non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, acute and repeated dose toxicity, and genotoxicity.

2.3.7. Conclusion on the non-clinical aspects

In conclusion, the non-clinical studies submitted for the marketing authorisation application for tilmanocept were considered adequate and acceptable for the assessment of non-clinical aspects for the product tilmanocept. The lack of carcinogenicity, reproductive and developmental toxicity studies was justified and considered acceptable. The $PEC_{SURFACEWATER}$ (0.00025 µg/L) is below the action limit of 0.01 µg/L, and no other environmental concerns are apparent. Therefore, it is concluded that the product is unlikely to represent a risk for the environment following its prescribed usage in patients.

2.4. Clinical aspects

2.4.1. Introduction

The clinical pharmacology database consisted of three Phase 1 studies (NEO3-A, NEO3-B, and NEO3-C) and one Phase 2 study (NEO3-01). Clinical pharmacology parameters were not assessed in the Phase 3 studies.
The pivotal phase 3 studies were NEO3-05 and NEO3-09 in melanoma and breast cancer patients, and NEO3-06 in patients with head and neck squamous cell carcinoma.

Pharmacotherapeutic group: diagnostic radiopharmaceutical, tumour detection, ATC Code: V09IA09.

GCP

The clinical trials were performed in accordance with GCP as claimed by the applicant. The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 11: Studies in the Lymphoseek Clinical Development Program

Study	Study Design / Cancer Type	Primary Objectives	Dose / Injection method
NEO3-A	Randomized, four-arm, open-label / Primary Breast Cancer	PK and Safety	0.2, 1.0 or 5.0 nmol/PT
NEO3-B	Randomized, four-arm, open-label / Cutaneous Melanoma	PK and Safety	1.0, 5.0 or 10.0 nmol/SC
NEO3-C	Randomized, four-arm, single-blinded / Primary Breast Cancer	PK and Safety	1.0 nmol/ID
NEO3-01	Single-arm, open-label / Breast Cancer and Melanoma	PD and Safety	50 µg/ID or SC
NEO3-05	Single-arm, open-label / Breast Cancer and Melanoma	Efficacy and Safety	50 µg/ID, PT or SA
NEO3-09	Single-arm, open-label / Breast Cancer and Melanoma	Efficacy and Safety	50 µg/ID or SA
NEO3-06	Single-arm, open-label / Head and Neck Squamous Cell Carcinoma	Efficacy and Safety	50 µg/PT
NEO3-14[b]	Meta-analysis / Breast Cancer	Efficacy	n/a
NEO3-15[b]	Meta-analysis / Melanoma	Efficacy	n/a

[b] These retrospective studies compared results for patients in studies NEO3-05 and NEO3-09 to European clinical practice based on published studies.
Abbreviations: PD, pharmacodynamics; PK, pharmacokinetics; PT, peritumoral; SA, subareolar; ID, intradermal

2.4.2. Pharmacokinetics

The PK of Lymphoseek was assessed by examining the injection site clearance rate constant (kc), the injection site clearance half-life (Tc), the percent-of-injected dose in the sentinel node (%IDSN), and the absolute sentinel node uptake for Lymphoseek (LSN).

Table 12: Clinical Pharmacology Studies

Study No.	Study Title	No. of Enrolled Patients
Phase 1		
NEO3-A	A Phase 1 Clinical Trial of a New Receptor-Binding Radiopharmaceutical for Sentinel Node Detection in Breast Cancer	24
NEO3-B	A Phase 1 Study of Tc^{99m} Labelled Lymphoseek Used in Sentinel Lymph Node Mapping in Patients with Cutaneous Melanoma	24
NEO3-C	NCI Phase 1 Study of Lymphoseek® in Patients with Breast Cancer	32

Abbreviations: UCSD, University of California, San Diego

The studies **NEO3-A** and **NEO3-B** evaluated the radiopharmacokinetics of Tc-99m DTPA mannosyl dextran (Lymphoseek) against a known sentinel node imaging agent- filtered sulphur colloid (fTcSC). The methodologies of the studies were similar. The eligible patients were randomised into 2 groups, Groups 1 and 2, evaluated/ imaged with fTcSC and 1.0 nmol Lymphoseek, respectively. Subsequently, further 2 groups were sequentially assigned in both studies to evaluate an additional 2 doses of Lymphoseek.

Radiopharmacokinetic parameters are summarized in the tables below.

Table 13: Summary of Radiopharmacokinetic Parameters in Patients in Study NEO3-A[f]

		Lymphoseek			
Parameter	**Statistic**	**0.2 nmol (N = 6)**	**1.0 nmol (N = 6)**	**5.0 nmol (N = 6)**	**fTcSC (N = 7) [a]**
k_c (hr^{-1})	Mean (SD)	0.278 (0.221)	0.255 (0.147)	0.222 (0.064)	0.014 (0.018)
	p-value		0.012[b,c]		
$\%ID_{SN}$ [d] (%)	Mean (SD)	0.05 (0.10)	0.52 (0.38)	0.21 (0.17)	0.64 (0.62) [e]
	p-value		0.075		
L_{SN} [d] (pmol)	Mean (SD)	0.09 (0.20)	6.53 (2.52)	10.58 (8.43)	Not Measured
	p-value		0.009[b]		

a One patient had bilateral disease and received injections in both breasts.
b One-factor ANOVA
c After Bonferroni correction, all Lymphoseek doses differed from fTcSC ($p < 0.05$).
d Primary sentinel node only.
e For patient with bilateral disease, average of both injections was used.

f Abbreviations: $\%ID_{SN}$, percent-of-injected dose in the sentinel node; fTcSC, filtered Tc 99m sulphur colloid; k_c, injection site clearance rate constant; L_{SN}, absolute sentinel node uptake for Lymphoseek.

Table 14: Summary of Radiopharmacokinetic Parameters in Study NEO3-B[c]

		Lymphoseek				
Parameter (Units)	**Statistic**	**1.0 nmol (N = 6)**	**5.0 nmol (N = 6)**	**10.0 nmol (N = 6)**	**All Doses (N = 18)**	**fTcSC (N = 6)**
k_c (hr^{-1})	Mean (SD)	0.338 (0.146)	0.396 (0.143)	0.227 (0.092)	0.320 (0.141)	0.047 (0.020)
	p-value [a]	< 0.001	< 0.001	0.036	< 0.001	
T_c (hr)	Mean (SD)	2.05 (0.89)	1.75 (0.62)	3.05 (1.25)	2.17 (0.96)	14.7 (6.3)
	p-value [a]	< 0.001	< 0.001	< 0.001	< 0.001	
$\%ID_{SN}$ [b]	Mean (SD)	0.50 (0.80)	0.35 (0.27)	0.58 (0.41)	0.48 (0.52)	1.22 (1.52)
	p-value	No significant differences (p-value not available)				
L_{SN} [b] (pmol)	Mean (SD)	5.01 (8.02)	17.5 (13.7)	58.2 (41.2)	Not Calculated	Not Measured

[a] Lymphoseek dose vs. fTcSC.
[b] Primary sentinel node only.
[c]

Abbreviations: $\%ID_{SN}$, percent-of-injected dose in the sentinel node; fTcSC, filtered Tc 99m sulphur colloid; k_c, injection site clearance rate; L_{SN}, absolute sentinel node uptake for Lymphoseek; SD, standard deviation, T_c, injection site clearance half-life.

In study NEO3-A, Lymphoseek was studied at three doses (0.2, 1.0, and 5.0 nmol; 500 µCi each), and all three doses were cleared from the injection site faster than fTcSC (500 µCi). Similar results were also observed with the three doses studied (1.0, 5.0, and 10.0 nmol; 500 µCi each) in study NEO3-B.

While the absolute uptake of Lymphoseek into the primary sentinel lymph node was dose-dependent, the percentage of dose reaching the primary sentinel node appeared to be independent of dose.

The 1.0 nmol Lymphoseek dose, in study NEO3-A, resulted in comparable distribution to the primary sentinel node with fTcSC treatment.

The study **NEO3-C** was conducted to optimize the imaging protocol and obtain preliminary efficacy information, including injection site retention and sentinel node localization at 3 and 16 hours post-administration; to provide an assessment of the clearance kinetics of the radiotracer, Lymphoseek; and to quantify its imaging and detection properties relative to Tc-99 sulphur colloid.

Lymphoseek was eliminated from the injection site significantly faster than Tc-SC: the mean elimination half-life was 2.6 hours for Lymphoseek compared with 27 hours for Tc-SC.

Table 15: Summary of Injection Site Clearance Parameters in Patients with Breast Cancer

Parameter (Units)	Statistic	Lymphoseek (N = 8)	Tc 99m Sulphur Colloid [b] (N = 3)
k_c (hr^{-1})	Mean (SD)	0.299 (0.130)	0.027 (0.008)
	Min, Max	0.177, 0.599	0.019, 0.036
	Median	0.270	0.027
	p-value	0.007	
T_c (hr)	Mean (SD)	2.60 (0.808)	27.1 (8.70)
	Min, Max	1.16, 3.92	19.5, 36.6
	Median	2.58	25.3
	p-value	< 0.0001	

Computations presented in these tables are from unedited data provided to Navidea by UCSD. Analyses were conducted in accordance with the UCSD protocol planned analyses (one-way ANOVA).

Patients who received filtered and unfiltered Tc 99m sulphur colloid are combined in this group.
Note: Data shown to three significant figures.
Abbreviations: k_c, injection site clearance rate; SD, standard deviation; T_c, injection site clearance half-life.

Summary statistics on the %IDSN were analysed for each radiopharmaceutical within the respective post-surgical time groups (i.e., 3 and 16 hours after injection). Although the values were numerically lower for Lymphoseek, there were no significant differences between the imaging agents in either time group.

Table 16: Summary of Sentinel Node Uptake in Patients with Breast Cancer

		3 Hours Between Injection and Surgery		16 Hours Between Injection and Surgery	
Parameter	Statistic	Lymphoseek (N = 5)	fTcSC (N = 5)	Lymphoseek (N = 13)	uTcSC (N = 5)
$\%ID_{SN}$	Mean (SD)	1.68 (1.22)	2.78 (5.46)	1.81 (2.19)	3.66 (3.20)
	Min, Max	0.42, 3.70	0.00, 12.49	0.00, 8.09	0.16, 7.49
	Median	1.47	0.00	0.94	3.55
	p-value	0.670		0.175	

[a] Computations presented in these tables are from unedited data provided to Navidea by UCSD. Analyses were conducted in accordance with the UCSD protocol planned analyses (one-way ANOVA).
Note: Data shown to three significant figures.
Abbreviations: $\%ID_{SN}$, percent-of-injected dose in the sentinel node; SD, standard deviation.

Table 17: Summary of Radiopharmacokinetic Parameters Across Phase 1 Studies

Parameter	Statistic	Lymphoseek	TcSC [g]	p-value

(Units) and Study		0.2 nmol	1.0 nmol	5.0 nmol	10.0 nmol		
k_c (hr^{-1})							
NEO3-A	Mean (SD)	0.278 (0.221)	0.255 (0.147)	0.222 (0.064)	—	0.014 (0.018)	0.012 [a]
NEO3-B	Mean (SD)	—	0.338 (0.146)	0.396 (0.143)	0.227 (0.092)	0.047 (0.020)	≤ 0.036 [b]
NEO3-C	Mean (SD)	—	0.299 (0.130)	—	—	0.027 (0.008)	0.007 [e]
T_c (hr)							
NEO3-A	Mean (SD)	NC	NC	NC	—	NC	
NEO3-B	Mean (SD)	—	2.05 (0.89)	1.75 (0.62)	3.05 (1.25)	14.7 (6.3)	< 0.001 [c]
NEO3-C	Mean (SD)	—	2.60 (0.808)	—	—	27.1 (8.70)	<0.0001 [e]
$\%ID_{SN}$ [d] (%)							
NEO3-A	Mean (SD)	0.05 (0.10)	0.52 (0.38)	0.21 (0.17)	—	0.64 (0.62)[f]	0.075 [e]
NEO3-B	Mean (SD)	—	0.50 (0.80)	0.35 (0.27)	0.58 (0.41)	1.22 (1.52)	Not significant
NEO3-C (3 hr)	Mean (SD)	—	1.68 (1.22)	—	—	2.78 (5.46)	0.670 [e]
NEO3-C (16 hr)	Mean (SD)	—	1.81 (2.19)	—	—	3.66 (3.20)	0.175 [e]
L_{SN} [d] (pmol)							
NEO3-A	Mean (SD)	0.09 (0.20)	6.53 (2.52)	10.58 (8.43)	—	Not Measured	0.009 [e]
NEO3-B	Mean (SD)	—	5.01 (8.02)	17.5 (13.7)	58.2 (41.2)	Not Measured	
NEO3-C	Mean (SD)	—	NC	—	—	NC	

a All Lymphoseek doses vs. fTcSC (ANOVA); after Bonferroni correction, $p < 0.05$ for each Lymphoseek dose vs. fTcSC.

b $p = 0.036$ for Lymphoseek 10.0 nmol vs. fTcSC; for all other comparisons of Lymphoseek (individual doses and all doses combined) vs. fTcSC, $p < 0.001$.

c $p < 0.001$ for each dose and all doses combined of Lymphoseek vs. fTcSC.

d Primary sentinel node only.

e p-value from ANOVA.

f Includes an average of both injections for the patient with bilateral disease.

g fTcSC for NEO3-A and NEO3-B; combined fTcSC and uTcSC for NEO3-C calculations of k_c and T_c, or fTcSC for 3 hours and uTcSC for 16 hours for $\%ID_{SN}$.

Abbreviations: —, dose not studied; $\%ID_{SN}$, percent-of-injected dose in the sentinel node; k_c, injection site clearance rate; L_{SN}, absolute sentinel node uptake for Lymphoseek; NC, not calculated; SD, standard deviation; T_c, injection site clearance half-life; TcSC, Tc 99m sulphur colloid (filtered or unfiltered).

Absorption

The applicant did not submit studies on absorption of tilmanocept.

Distribution

Injection-Site Clearance

Injection site clearance was examined in three Phase 1 studies (NEO3-A, NEO3-B, and NEO3-C).

The injection site clearance rates were similar for all doses of Lymphoseek, and all doses of Lymphoseek were cleared significantly faster from the injection site than the comparator, technetium 99m sulphur colloid.

The mean Lymphoseek injection site clearance half-life was approximately 2 to 3 hours vs. approximately 15 to 27 hours for TcSC. Although the patient populations in the studies were different (two studies in women with breast cancer and one study in men and women with melanoma), injection site clearance rates were similar for all doses of Lymphoseek.

Lymph Node Uptake

Lymph node uptake was examined in three Phase 1 studies. After a single PT, ID, or SC dose, Lymphoseek readily dispersed and localised. L_{SN} was dose-related for Lymphoseek (ranging from 0.09 pmol at the 0.2 nmol dose to 58.2 pmol at the 10.0 nmol dose). Lymphoseek relative uptake (%IDSN) was generally independent of dose and ranged from 0.05%IDSN to 1.81%IDSN, while the TcSC values ranged from 0.64%IDSN to 3.66%.

Exposure relevant for safety evaluation / Estimated radiation exposure

The radiation doses estimated or measured in patients on the pivotal clinical studies are listed in the tables below.

The radiation absorbed dose values for breast cancer patients in NEO3-A are shown in the Table 18. The effective dose equivalent (EDE) is 1.60x10-2 mSv/MBq for males and 1.79x10-2 mSv/MBq for females. A 18.5 MBq (0.5 mCi) dose of Lymphoseek would yield a radiation exposure of 296 to 330 µSv. For patients with breast cancer, tissues with the highest estimated radiation absorbed dose at the 18.5 MBq activity are injection site (1.659 milligray [mGy]), ovary (0.187 mGy), and kidney (0.186 mGy).

Table 18: Radiation Absorbed Dose for a 50 µg Dose of Lymphoseek in Breast Cancer Patients, mGy (rad)

	Radiation Labelling Index MBq (mCi) / 50 µg Lymphoseek		
Target Organ	**18.5 MBq (0.5 mCi)**	**37 MBq (1 mCi)**	**74 MBq (2 mCi)**
brain	0.003 (0.0003)	0.006 (0.0006)	0.012 (0.0012)
breast (injection site)	1.659 (0.1659)	3.3181 (0.3318)	6.6362 (0.6636)
gallbladder wall	0.0349 (0.0035)	0.0698 (0.007)	0.1397 (0.014)
LLI wall	0.0123 (0.0012)	0.0246 (0.0025)	0.0493 (0.0049)
small intestine	0.0101 (0.001)	0.0203 (0.002)	0.0405 (0.0041)
stomach	0.0184 (0.0018)	0.0369 (0.0037)	0.0738 (0.0074)
ULI wall	0.0125 (0.0012)	0.0249 (0.0025)	0.0499 (0.005)
kidney	0.1863 (0.0186)	0.3727 (0.0373)	0.7453 (0.0745)
liver	0.0324 (0.0032)	0.0648 (0.0065)	0.1295 (0.013)
lungs	0.0374 (0.0037)	0.0747 (0.0075)	0.1494 (0.0149)
muscle	0.0092 (0.0009)	0.0184 (0.0018)	0.0368 (0.0037)
ovaries	0.187 (0.0187)	0.374 (0.0374)	0.7479 (0.0748)
red marrow	0.0127 (0.0013)	0.0254 (0.0025)	0.0509 (0.0051)
bone	0.0177 (0.0018)	0.0354 (0.0035)	0.0707 (0.0071)
spleen	0.0285 (0.0029)	0.057 (0.0057)	0.1141 (0.0114)

testes	0.0501 (0.005)	0.1003 (0.01)	0.2006 (0.0201)
thymus	0.1168 (0.0117)	0.2336 (0.0234)	0.4673 (0.0467)
thyroid	0.088 (0.0088)	0.176 (0.0176)	0.352 (0.0352)
urinary bladder	0.0586 (0.0059)	0.1171 (0.0117)	0.2342 (0.0234)
total body	0.0195 (0.0019)	0.039 (0.0039)	0.078 (0.0078)
EDE (males, µSv)	**296.0**	**592.1**	**1184.2**
EDE (females, µSv)	**330.2**	**660.5**	**1321.0**

The radiation absorbed dose values for melanoma patients (NEO3-B) are shown in the Table 19. The EDE is 1.09x10-2 mSv/MBq for males and 1.36x10-2 mSv/MBq for females. A 18.5 MBq (0.5 mCi) dose of Lymphoseek would yield a radiation exposure of 202 to 251 µSv. For patients with melanoma, tissues with the highest estimated radiation absorbed dose at the 18.5 MBq activity are injection site (0.790 mGy), ovary (0.299 mGy), and kidney (0.278 mGy).

Table 19: Radiation Absorbed Dose for a 50 µg Dose of Lymphoseek in Melanoma Patients, mGy (rad)

Target Organ	**Radiation Labelling Index MBq (mCi) / 50 µg Lymphoseek**		
	18.5 MBq (0.5 mCi)	**37 MBq (1 mCi)**	**74 MBq (2 mCi)**
brain	0.0927 (0.0093)	0.1854 (0.0185)	0.3708 (0.0371)
breast (injection site) [a]	0.7903 (0.079)	1.5806 (0.1581)	3.1613 (0.3161)
gallbladder wall	0.0712 (0.0071)	0.1424 (0.0142)	0.2849 (0.0285)
LLI wall	0.057 (0.0057)	0.1141 (0.0114)	0.2281 (0.0228)
small intestine	0.0594 (0.0059)	0.1188 (0.0119)	0.2377 (0.0238)
stomach	0.0562 (0.0056)	0.1123 (0.0112)	0.2246 (0.0225)
ULI wall	0.0582 (0.0058)	0.1163 (0.0116)	0.2327 (0.0233)
kidney	0.278 (0.0278)	0.5561 (0.0556)	1.1121 (0.1112)
liver	0.0929 (0.0093)	0.1859 (0.0186)	0.3717 (0.0372)
lungs	0.0599 (0.006)	0.1198 (0.012)	0.2395 (0.024)
muscle	0.0451 (0.0045)	0.0902 (0.009)	0.1804 (0.018)
ovaries	0.2991 (0.0299)	0.5982 (0.0598)	1.1963 (0.1196)
red marrow	0.0507 (0.0051)	0.1014 (0.0101)	0.2027 (0.0203)
bone	0.0878 (0.0088)	0.1756 (0.0176)	0.3512 (0.0351)
spleen	0.0598 (0.006)	0.1197 (0.012)	0.2394 (0.0239)
testes	0.1043 (0.0104)	0.2086 (0.0209)	0.4172 (0.0417)
thymus	0.0577 (0.0058)	0.1153 (0.0115)	0.2306 (0.0231)
thyroid	0.0464 (0.0046)	0.0927 (0.0093)	0.1855 (0.0185)
urinary bladder	0.1401 (0.014)	0.2802 (0.028)	0.5605 (0.056)
total body	0.0547 (0.0055)	0.1094 (0.0109)	0.2187 (0.0219)
EDE (males, µSv)	**202.4**	**404.8**	**809.7**
EDE (females, µSv)	**251.1**	**502.2**	**1004.4**

[a] Due to the differences in injection sites among melanoma patients, the injection site was assumed to be the breast for the purposes of this calculation, as it represents the nearest anatomical construct for the skin from the anatomical sites appropriately included in the estimates.

Table 20: Estimated Dose Absorbed from Lymphoseek in Patients with Breast Cancer[a]

Estimated Radiation Absorbed Dose for Breast Cancer, mGy/MBq	
Target Organ	**Adults**
brain	0.0002
breast (injection site)	0.0897
gall bladder wall	0.0019
lower large intestine wall	0.0007
small intestine	0.0005
stomach	0.0010
upper large intestine wall	0.0007
kidney	0.0101
liver	0.0018
lungs	0.0020
muscle	0.0005
ovaries	0.0101
red marrow	0.0007
bone	0.0010
spleen	0.0015
testes	0.0027
thymus	0.0063
thyroid	0.0048
urinary bladder	0.0032
total body (blood)[b]	0.0011
Effective Dose (E) (males, mSv/MBq)	**0.01600**
Effective Dose (E) (females, mSv/MBq)	**0.01785**

[a] Calculated from data of 18 breast cancer patients who received four peritumoural injections of 4, 20, and 100 microgram doses of Lymphoseek.

[b] Blood represents total body exposure segregated from independent measurements of other organs and tissues.

Table 21: Estimated Dose Absorbed from Lymphoseek in Patients with Melanoma[a]

Estimated dose absorbed per activity administered, mGy/MBq	
Target Organ	**Adults with Melanoma**
brain	0.0050
breast (injection site)	0.0427
gall bladder wall	0.0038
lower large intestine wall	0.0031
small intestine	0.0032
stomach	0.0030
upper large intestine wall	0.0031
kidney	0.0150
liver	0.0050
lungs	0.0032
muscle	0.0024

ovaries	0.0162
red marrow	0.0027
bone	0.0047
spleen	0.0032
testes	0.0056
thymus	0.0031
thyroid	0.0025
urinary bladder	0.0076
total body (blood)[b]	0.0030
Effective Dose (E) (males, mSv/MBq)	**0.01094**
Effective Dose (E) (females, mSv/MBq)	**0.01357**

[a] Calculated from data of 18 melanoma patients who received four intradermal injections of 20, 100, and 200 microgram doses of Lymphoseek.

[b] Blood represents total body exposure segregated from independent measurements of other organs and tissues.

Elimination

Upon entry into the blood, the agent binds to receptors in the liver or is filtered by the kidney and accumulates in the urinary bladder. The amount of the accumulated radioactive dose in the liver, kidney, and bladder reached a maximum 1 hour post administration of Lymphoseek and was approximately 1% to 2% of the injected dose in each tissue.

Dose proportionality and time dependencies

The applicant provided data on the numbers of counts available in the sentinel node compartment for the isotopic doses for surgery day-of injection versus day-after injection. The data is presented in Table 22.

Table 22: mCi and MBq, Disposition in Sentinel Nodes After Time Period (Hours After Injection)[a]

Hours Post Injection	0	6	12	15	16	20	24	30
	Surgery Day of Injection				Surgery Day After Injection			
mCi, MBq Part of Whole Injected dose Remaining	0.5 18.5	0.25 9.3	0.13 4.6	0.09 1.6	---	---	---	---
Bq/Node disposition/ probe count time[b]	$1.85x10^6$	$9.25x10^5$	$4.63x10^5$	$3.26x10^5$	---	---	---	---
mCi, MBq Part of Whole Injected dose Remaining	2.0 74.0	---	---	---	0.315 11.7	0.20 7.4	0.13 4.6	0.063 2.3
Bq/Node disposition/ probe count time[b]	---	---	---	---	$1.17x10^6$	$7.4x10^5$	$4.62x10^5$	$2.31x10^5$

[c] Based on 1% of injected dose localized in sentinel node compartment and standard 10-second probe count times; from clinical trial data and dosimetry evaluations in breast cancer and melanoma patients.

[d] Bq/Node disposition/Probe count time = Disintegrations/Node disposition/10-second probe count time.

Special populations

The applicant did not submit studies in special populations.

Pharmacokinetic interaction studies

The applicant did not submit pharmacokinetic interaction studies.

Pharmacokinetics using human biomaterials

The applicant did not submit pharmacokinetic studies using biomaterials.

2.4.3. Pharmacodynamics

Mechanism of action

The applicant did not submit clinical studies on the mechanism of action of tilmanocept.

Primary and Secondary pharmacology

Phase 2 clinical study, NEO3-01

In this study, the PD properties of Lymphoseek were assessed in patients with melanoma or breast cancer. The primary objective of this study was to determine preoperative and intra-operative lymphoscintigraphic localisation of lymph node(s) in the lymphatic pathways draining the primary site of melanoma or breast cancer using Lymphoseek as a radiotracer.

Each patient received 50 µg Lymphoseek, with a recommended activity of 11 to 185 MBq (0.3 to 5.0 mCi). Injections were made ID or SC in close proximity to the primary tumour.

Preoperative evaluations followed normal practice and could include lymphoscintigraphy. ILM could occur between 15 minutes and 30 hours post-injection, depending on surgical schedule. VBD injection at surgery was allowed as an adjunct lymphatic mapping agent, but not required by the protocol.

Intraoperatively, based on the investigator's intraoperative assessment, Lymphoseek localised at least one tissue sample in 75 of the 78 per protocol patients (96.2%), and the localisation rate was similar between the two tumour types (97.9% of patients with melanoma and 93.5% of patients with breast cancer). On a per tissue basis, of the 180 specimens identified *in vivo*, 171 (95.0%) were identified by Lymphoseek and all 171 tissues were subsequently determined to be lymphoid tissue in pathology.

The effect of time between Lymphoseek injection and surgery on the localisation rate was also evaluated. In patients with melanoma, the time interval made no difference in the localisation rate (97.5% for same-day vs. 100% for next-day surgery). However, in patients with breast cancer, the same day surgery group had a 95.5% localisation rate compared with 88.9% in the next-day surgery group. However this difference is not statistically significant (p = 0.5032, Fisher's exact test).

Injection Volume Effects (NEO3-05)

The main source of the non-concordance in study NEO3-05 was the deviation from the volume of injection by 2 study sites (05 and 06). The results for this study have been for these sites alone, for all sites together, and with the exclusion of these 2 sites. Following the observation that injection volume deviations affected the concordance rates, and non-clinical study NEO3-08 was initiated to study this effect.

Table 23: Condordance rates for blue dye and Lymphoseek by study sites

Part A – Non-Discordant Sites (NA)				
Site #	Total Surgeries	Total Blue and Hot Nodes (B+/H+)	Total Blue and Not Hot Nodes (B+/H-)	Concordance Rate by Site
01	3	5	0	100%
02	51	67	1	98.5%
03	5	3	1	75%
04	2	3	0	100%
07	---	---	---	---
08	---	---	---	---
09	7	8	1	88.88%
10	---	---	---	---
11	---	---	---	---
12	20	41	0	100%
13	---	---	---	---
Combined	**88**	**127**	**3**	**97.69%**
			Between Sites (Not Weighted)	**93.73% ± 10.15%**
Part B – Discordant Sites (AS)				
Site #	Total Surgeries	Total Blue and Hot Nodes (B+/H+)	Total Blue and Not Hot Nodes (B+/H-)	Concordance Rate by Site
05	19	19	7	73%
06	7	7	5	58.3%
Combined	**26**	**26**	**12**	**68.4%**
			Between Sites (Not Weighted)	**65.65% ± 10.39%**

The *in vitro* study NE03-08 with human MDMs was conducted to evaluate injection volume effects on the behaviour and specificity of the binding of Lymphoseek to the hMBR, and was precipitated by the observed discordance in the Phase 3 study NEO3-05 between results from Lymphoseek and the clinical standard, VBD in breast cancer and melanoma patients. A break-point performance concentration of approximately 1.5 µM for initial injection was determined for clinical applications. Volume excursion (excessive injection volumes of greater than 4 mL of Lymphoseek, per se) may significantly alter 0.5 Vmax, thus potentially affecting the expected binding performance in patients.

The results of this study confirmed Lymphoseek's specific binding interaction with the hMBR and supported injection volume excursion as the root cause of discordance in the performance of Lymphoseek at two clinical sites in the NEO3-05 study. On the basis of this study, preferred clinical single injection volumes for Lymphoseek were set to 0.1 to 0.5 mL, with total injection volume limits set at 0.1 to 1.0 mL.

2.4.4. Discussion on clinical pharmacology

The radiopharmacokinetic parameters for Lymphoseek were evaluated in three Phase 1 studies. The patient populations in the studies were different two studies in women with breast cancer, men and women with melanoma in the third. The injection site clearance rates were similar for all doses of Lymphoseek, and all doses of Lymphoseek were cleared significantly faster from the injection site than the comparator, TcSC (mean clearance half-life, 1.75 to 3.05 hours for Lymphoseek vs. 14.7 to 27.1 hours for TcSC).

In one Phase 1 study in breast cancer patients, Lymphoseek at all three doses tested (4, 20, and 100 micrograms) exhibited fast injection site clearance (elimination rate constants in the range of 0.222/h to 0.278/h). Uptake of technetium Tc 99m tilmanocept into the primary sentinel node increased dose dependently (p=0.009): Lymphoseek injection at 4, 20, and 100 micrograms produced primary sentinel node levels (LSN) of 0.09 ± 0.20 pmol, 6.53 ± 2.52 pmol, and 10.58 ± 8.43 pmol of technetium Tc 99m tilmanocept, respectively. The percent-of-injected dose reaching the primary sentinel node (%IDSN) was 0.05% ± 0.10%, 0.52% ± 0.38%, 0.21% ± 0.17% in the 4, 20, and 100 microgram Lymphoseek dose groups, respectively. The plasma %ID per gram for two dose levels peaked at 4 hours; the mean values for the 4 and 100 microgram doses were 0.0090%/g ± 0.0048%/g and 0.0089%/g ± 0.0046%/g, respectively. The 20 microgram dose peaked at 2.5 hours with a mean %ID/g of 0.0023%/g ± 0.0005%/g.

In the second Phase 1 study in breast cancer patients in which patients were injected with 20 micrograms Lymphoseek, the mean elimination rate constant of technetium Tc 99m tilmanocept was 0.299/h and the drug half-life at the injection site was 2.6 h. The %IDSN was 1.68% ± 1.22% in the 3 hour injection to surgery group and 1.81% ± 2.19% in the Lymphoseek 16 hour injection to surgery group.

In the Phase 1 study in melanoma patients, Lymphoseek at all three doses tested (20, 100, and 200 micrograms) cleared the injection site with elimination rate constants in the range of 0.227/h to 0.396/h, resulting in drug half-life at the injection site of 1.75 to 3.05 h). Uptake of technetium Tc 99m tilmanocept into the primary sentinel node increased dose-dependently: Lymphoseek injection at 20, 100, and 200 micrograms produced LSN values of 5.01 ± 8.02 pmol, 17.5 ± 13.7 pmol, and 58.2 ± 41.2 pmol of technetium Tc 99m tilmanocept, respectively. The %IDSN taken up into the primary lymph node was 0.50% for the 20 microgram dose, 0.35% for the 100 microgram dose, 0.58% for the 200 microgram dose of Lymphoseek. The plasma %ID per gram for two dose levels peaked at 15 minutes; the mean values for the 20 and 200 microgram doses were 0.0104%/g ± 0.0135%/g and 0.0065%/g ± 0.0082%/g, respectively. The 100 microgram dose peaked at 1 and 2 hours with a mean %ID/g of 0.0018%/g ± 0.001%/g at each timepoint.

Two of the Phase 1 studies included a range of Lymphoseek drug doses (doses of 0.2, 1.0, and 5.0 nmol in NEO3-A and 1.0, 5.0, and 10.0 nmol in NEO3-B). In these studies, the lowest dose tested (0.2 nmol; NEO3-A) failed to localise lymphatic structures in greater than 60% of patients, indicating that this dose would likely be suboptimal. The next higher doses (1.0 and 5.0 nmol) were not significantly different in intraoperative imaging performance. L_{SN} (amount of Lymphoseek at a sentinel node) of Lymphoseek was dose-dependent in the NEO3-A and NEO3-B studies, with the amount increasing with increasing dose. In all three studies, %IDSN of Lymphoseek was generally independent of dose, with mean uptake of 0.5% to 1.81% across the four drug doses. This uptake was numerically lower than that for the TcSC (0.64% to 3.66%), though the uptake among treatment groups was not significantly different. Each 250 microgram vial contains an excess of product. However, it is recommended that the vial be prepared as instructed and a 50 microgram aliquot be used for a single patient dose. Individual injection volumes should not exceed 0.5 mL or be less than 0.1 mL. Total injection volume should be no greater than 1.0

mL and no less than 0.1 mL. Dilution of the product in volumes greater than 1.0 mL could affect the *in vivo* disposition of Lymphoseek. For instructions for preparation and control of the radiochemical purity of the radiopharmaceutical, see section 12. For patient preparation and dosimetry information, see section 4.4 and 11 of the SmPC.

An overall analysis of dose performance predicted that a dose of 50 µg Lymphoseek (~2.7 nmol) would provide clinically relevant localisation as well as minimising the overall exposure to Lymphoseek. Additionally, the Phase 1 NEO3-C study, using a single Lymphoseek dose of 1.0 nmol, evaluated two labelling doses (18.5 MBq [0.5 mCi] and 37 MBq [1.0 mCi]) between same day and next day surgery procedures. No significant differences were indicated between Lymphoseek radiolabelling amounts in terms of uptake into the sentinel nodes at the 3 hour (18.5 MBq) or 16 hour (37 MBq) post-surgery injection times. The recommended minimum time for imaging is 15 minutes post injection. Intraoperative lymphatic mapping may begin as early as 15 minutes post injection. Patients scheduled for surgery on the day of injection will receive 18.5 MBq technetium Tc 99m labelled product. Administration should occur within 15 hours of the scheduled time of the surgery and intraoperative detection. Patients scheduled for surgery on the day after injection will receive 74 MBq technetium Tc 99m labelled product. Administration should occur within 30 hours of the scheduled time of the surgery and intraoperative detection.

Data for the blood and plasma pharmacokinetics are also limited (up to 6 hours). There is no elimination data from plasma post 6 hours, however it is accepted that the circulating concentrations are very low and the radioactive half-life is relatively short. Technetium Tc 99m tilmanocept is eliminated primarily through the kidneys. The metabolism of technetium Tc 99m tilmanocept has not been investigated experimentally. Tilmanocept may be metabolised in the liver to its component molecules, namely dextran (which is renally excreted and/or further metabolised to glucose), mannose (an endogenous sugar) and diethylenetriaminepentaacetic acid (which is renally excreted). As with all general metabolites, especially those in which the liver plays a measurable roll of elimination, some biliary elimination of technetium Tc 99m tilmanocept is also likely to occur.

The %ID for liver, kidneys, and bladder as calculated from the whole body scans of breast cancer patients at 1, 2.5, and 12 hours after administration was below 2.6% at all times (all dose levels combined). The %ID for liver, kidneys, and bladder as calculated from the whole body scans of melanoma patients at 1 and 12 hours after administration ranged from 1.1% to 3.1% at 1 hour, and all decreased to less than 1% by 12 hours.

No data on excretion has been provided. However, as the dose is low and given infrequently and the radioactive half-life is short, this is accepted.

The applicant has discussed the metabolic fate based on the three main tilmanocept constituents – dextran, mannose and diethylenetriaminepentaacetic acid (DTPA), all of which are constituents of medical products or are themselves medical products approved for other uses or intents. These constituents are, within Lymphoseek, bound together and each constituent is known to be metabolised/ eliminated in routes as previously established in the assessment of the constituents themselves. Any of the main constituents of tilmanocept formed as a result of hepatic metabolism, would be eliminated by well recognised routes. Information on elimination has been provided in section 5.2 of the SmPC regarding the possible metabolism of Lymphoseek and the clearance of main expected metabolites.

Extensive dose-range and adjustment studies with the medicinal product in normal and special populations have not been performed. The pharmacokinetics of technetium Tc 99m tilmanocept in patients with renal or hepatic impairment have not been characterised (see section 5.2).There were no data collected in patients with hepatic or renal impairment. Therefore, caution is advised when dosing in these subjects and statements have been introduced in the SmPC in section 4.4 with the following

wording: Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. The estimated radiation dose to the patient would not exceed 0.69 mSv even if none of a 74 MBq dose (2.0 mCi) were eliminated (see section 4.2).

No dedicated drug-drug interaction studies were submitted. However, it is recommended that Lymphoseek is not co-injected (mixing) with any other product (e.g., VBD) because of the potential influence of what is termed the fluid dynamic effect due to the added volume of the co-injected product. It has been determined that adding additional tracing agents or other injectants temporally or anatomically proximal to Lymphoseek could alter the performance of Lymphoseek. Therefore, the following warning has been included in section 4.5 of the SmPC: Adding very large volumes of tracing agents or other injectants temporally or anatomically proximal to Lymphoseek could affect the *in vivo* disposition of Lymphoseek. Additional tracing agents should not be injected within 30 minutes of Lymphoseek administration.

2.4.5. Conclusions on clinical pharmacology

The CHMP was of the opinion that the clinical pharmacology studies submitted by the applicant were adequate. There was some missing information on the metabolism of Lymphoseek. Since Lymphoseek is to be administered on very rare occasions at very low doses, the magnitude of the risks are low and pose no further concerns. There is also missing information on the use of the medicinal product in hepatic and renal impaired patients and information on the importance of hepatic and renal clearance was missing. The risks for these patient populations have been addressed with a warning and precaution of use in the SmPC. The CHMP considered that the benefit risk balance was not affected by this missing information and all concerns have been adequately addressed in the RMP and SmPC.

2.5. Clinical efficacy

2.5.1. Dose response studies

The localization of Lymphoseek in the primary sentinel node was compared between dosing groups within disease (breast cancer in NEO3-A and melanoma in NEO3-B) and for combined data from both diseases. Forty-eight patients were included in the combined data analysis: 36 who received Lymphoseek and 12 who received fTcSC. Within the group receiving Lymphoseek; 6, 12, 12, and 6 patients, received 0.2, 1.0, 5.0, and 10.0 nmol doses, respectively.

There was a significant variation in the absolute amount localized for the Lymphoseek 0.2 nmol dose compared with the 5.0 and 10.0 nmol doses. This was due in large part to the lack of localization in four of the six patients in the 0.2 nmol dose group. This non-localization appeared to be related the small dose (0.2 nmol) since none of the patients receiving higher doses of Lymphoseek lacked localization. These data indicate that 0.2 nmol is a suboptimal dose for effective localization and use in anatomic delineation of lymphatic structures. The differences between other groups in the relative amount of Lymphoseek localised were less striking. The 1.0 and 5.0 nmol doses were not significantly different. The dose of 1.0 nmol was considered to be unacceptably proximal to the dose of 0.2 nmol, and it was determined that the 1.0 nmol dose could create a potentially less robust clinical practice due to increased risk of non-localization. The higher dose of 5.0 nmol provided no significant gains over the 1.0 nmol dose in imaging performance. The dose of 50 µg Lymphoseek (~2.7 nmol) was selected to provide successful localization (i.e., reduce the chance of non-localization) while exposing patients to less drug.

Additionally, the NEO3-C study, using a single Lymphoseek dose of 1.0 nmol, evaluated two Tc-99m labelling doses (18.5 MBq [0.5 mCi] and 37 MBq [1.0 mCi]) between same day and next day surgery procedures. No significant differences were indicated between Lymphoseek radio-labelling amounts in terms of the %IDSN at the 3 hour (18.5 MBq) or 16 hour (37 MBq) post-surgery injection times.

2.5.2. Main studies

NEO3-05: A Phase 3, Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek and Vital Blue Dye as Lymphoid Tissue Targeting Agents in Patients With Known Melanoma or Breast Cancer Who Are Undergoing Lymph Node Mapping

Methods

Study Participants

The main inclusion criteria were as follows:
1. The patient had provided written informed consent with HIPAA authorization before participating in the study, as had his/her responsible caregiver, if applicable.

2. The patient was a candidate for surgical intervention with lymphatic mapping being a part of the surgical plan.

3. The patient was at least 18 years of age at the time of consent.

4. The patient had an ECOG performance status of Grade 0 – 2.

5. The patient had a clinical negative node (N0) status at the time of study entry.

6. If of childbearing potential, the patient had a negative pregnancy test within 72 hours prior to administration of Tc 99m Lymphoseek, had been surgically sterilized, or had been postmenopausal for at least 1 year.

For melanoma patients, additional criteria were:

7. For melanoma patients, patients had to have been diagnosed with primary melanoma.

For breast cancer patients, additional criteria were:

8. The patient had a diagnosis of primary breast cancer.

9. The patient had a diagnosis of pure ductal carcinoma in situ (DCIS) or non-invasive carcinoma if lymph node biopsy was part of the surgical plan.

The main exclusion criteria were as follows:

1. The patient was pregnant or lactating.

2. The patient had clinical or radiological evidence of metastatic cancer including palpably abnormal or enlarged lymph nodes (i.e., all patients were to be any T, but N0 and M0).

3. The patient had a known hypersensitivity to Lymphazurin or Patent Blue V.

4. The patient was currently participating in another investigational drug study.

For melanoma patients, additional exclusion criteria were as follows:

6. The patient had received preoperative chemotherapy, immunotherapy, or radiation therapy.

7. The patient was diagnosed with a prior invasive melanoma that would occur on the same body region or potentially draining to the same nodal basin, or the patient had truncal or extremity primary melanoma and previously had breast cancer potentially draining to the same axillary nodal basin.

8. The patient had undergone node basin surgery of any type or radiation to the nodal basin(s) potentially draining the primary melanoma.

9. The patient had undergone a wide excision for their primary melanoma (>1 cm in dimension) or complex reconstruction (rotation, free flap, or skin graft of any type).

10. The patient had bilateral primary breast cancers or multiple tumours within the breast.

For breast cancer patients, additional exclusion criteria were as follows:

11. The patient had had prior surgical procedures such as breast implants, reduction mammoplasty, or axillary surgery.

12. The patient was scheduled for bilateral mastectomy for any reason.

13. The patient had received preoperative radiation therapy to the affected breast or axilla.

Treatments

In this open-label, single arm, within-patient comparative study, all patients were to undergo ILM by experienced physicians. Prior to surgery, patients received 50 µg Tc 99m Lymphoseek injected in close proximity to the primary tumour. The interval between injection and ILM could range from 15 minutes to 30 hours, depending on surgical scheduling.

Patients scheduled for surgery on the day of injection received 0.5 mCi Tc 99m Lymphoseek (50 µg) and patients scheduled for surgery the next day received 1.0 mCi Tc 99m Lymphoseek (also 50 µg). At or near the time of ILM (prior to incision), VBD was injected in close proximity to the tumour. Preoperative evaluation of the patient followed standard clinical practice and could include lymphoscintigraphy.

Prior and Concomitant Therapy

All medications taken or administered by the patient for seven days prior to surgery were recorded on the source documents. All concomitant medications post-surgery through Day 30 were recorded on the source documents. Documentation included the name of the drug, dose level, frequency, route, reason for use, and the start/stop dates. Medications administered for anaesthesia and analgesics related to surgery did not need to be captured in the CRFs.

Any chemotherapy regimens were prohibited prior to surgery for patients enrolled in this study.

Objectives

The primary objective was the concordance between Tc 99m Lymphoseek and VBD in the in vivo detection of the excised lymph node(s) as confirmed by pathology.

The secondary objective was the assessment of the resected lymph node(s) to confirm the presence/absence of tumour metastases.

The safety objective was the evaluation of patient safety through observation of adverse events, clinical laboratory tests, vital signs, ECGs, and physical examinations.

Outcomes/endpoints

The ***primary efficacy endpoint*** was the proportion of lymph nodes identified intraoperatively by localization of VBD (by blue hue) that were also identified intraoperatively by localization of Tc-99m Lymphoseek (by 3σ rule). This was a per node concordance measure that used the number of lymph nodes stained by VBD as the denominator. Concordance was achieved when a VBD-stained lymph node was also detected by Tc-99m Lymphoseek based on the handheld gamma probe count(s) satisfying the threshold criterion. Any lymph node count not meeting this threshold criterion was considered a negative (i.e., Tc-99m Lymphoseek non-localized) finding.

The ***primary safety endpoints*** were based on the evaluation of adverse events, clinical laboratory tests, vital signs, ECGs, and physical examinations.

The ***secondary efficacy endpoints*** were:

- The primary concordance rate calculated on a per patient basis. This per patient estimate of concordance was the number of patients for whom all VBD-stained lymph nodes were also Tc-99m Lymphoseek hot divided by the number of patients in the ITT population.
- The reverse concordance rates (based on the reverse intent-to-treat [RITT] population; both per node and per patient), where VBD localization was compared against Tc-99m Lymphoseek localization, treating Tc-99m Lymphoseek as comparator or "truth" standard. These proportions used similar numerators as the concordance variables, but used the number of lymph nodes or patients that were identified intraoperatively by Tc-99m Lymphoseek as the denominator.

Other efficacy endpoints included measures of Tc-99m Lymphoseek detection and VBD detection relative to pathological finding of metastases in the excised lymph nodes, and calculations of sensitivity and false negative rates (FNRs) for each detection method in all patients undergoing lymphadenectomy.

Exploratory endpoints were based on subgroup analyses of the primary and secondary efficacy measures by study site and by tumour type (melanoma vs. breast cancer). In addition, data regarding medical history and co-medication were summarized and exploratory analyses were conducted as needed.

Sample size

Exploratory results from the Phase 2 NEO3-01 study showed an intraoperative concordance rate between Tc-99m Lymphoseek and VBD of 0.944 for melanoma and breast cancer patients. An observed concordance rate of at least 0.95 was assumed.

The hypothesis of H0: $P \leq 0.90$ versus Ha: $P > 0.90$ was evaluated by a one-sided exact test of a binomial proportion with a nominally stated α-level = 0.05. Given an assumed observed concordance rate of 0.95 and a target Type II error rate of 0.20 (80% power), the minimum sample size estimate was 203 VBD-stained lymph nodes that fulfilled the definition of the ITT population. The exact α-level and power of the test were 0.0495 and 85.89%, respectively.

The number of patients needed to satisfy the sample size requirement of 203 VBD stained nodes was a random variable, and depended on two observed quantities: the proportion of patients having at least one VBD-stained lymph node and the number of such nodes per patient. Data from the Phase 2 NEO3-01 study suggested that 0.90 of the patients would have at least one VBD-stained node. The number of VBD nodes per patient was expected to range from 1.0 (by definition) to 1.5 for those patients with these nodes. As a crude estimate, 226 ITT patients (203 blue dye nodes/0.90 blue dye nodes/patient) were needed to yield 203 VBD-stained nodes. This estimate assumed that each patient having a VBD stained node would contribute at most one such node for analysis. From the NEO3-01 study, approximately 0.95

of the enrolled patients met criteria that were similar to the ITT definition used for this trial. Thus, to obtain the desired number of ITT-based patients and VBD stained lymph nodes, the patient estimate of 226 patients was upwardly adjusted to 238 patients (226 patients/0.95).

Patient accrual was restricted such that no more than 60% of the 203 VBD-stained lymph nodes came from a certain cancer type. That is, either cancer type (breast cancer, melanoma) could not contribute more than 122 VBD-stained nodes to the primary analysis of concordance.

Randomisation

This was an open-label, single arm, within-patient comparative study. Concordance between techniques in identifying lymph nodes before excision was evaluated on data collected within each patient. After meeting protocol-specified eligibility criteria, each patient was assigned a unique patient number identifying him/her within the trial. Once assigned, all patients received a single 50 µg dose of Tc 99m Lymphoseek.

Blinding (masking)

This was an open-label, single arm (nonrandomized) study.

Statistical methods

Four prospectively planned population definitions were used for analysis and reporting, in the pivotal studies.

The ITT population consisted of those eligible patients (and nodes excised from such patients) who signed informed consent, were injected with Tc-99m Lymphoseek and VBD, underwent surgery, and had one or more lymph nodes stained blue intraoperatively by VBD for which the pathologist confirmed the type (i.e., lymph node versus non-lymph node) and contents (e.g., tumour cells).

A PP population was limited to those ITT patients (and nodes excised from such patients) for whom there were no associated major protocol violations that impacted the assessment of efficacy.

The safety population consisted of all patients (and nodes excised from such patients) who had signed informed consent and received any injection of Tc-99m Lymphoseek.

The safety PP population was limited to those safety patients (and nodes excised from such patients) for whom there were no associated major protocol violations that impacted the analyses.

The primary analysis population of the primary and secondary efficacy endpoints was the ITT population including all study sites; the secondary endpoints related to pathology were based on the safety population; and supportive analyses used the PP and safety PP populations.

One additional population was defined in the study the NEO3-05 study- the RITT (reverse ITT) population which included all enrolled patients (and their lymph nodes) who were injected both Tc-99m Lymphoseek and VBD, who underwent surgery and had at least one lymph node detected intraoperatively by Tc-99m Lymphoseek, and for whom the tissue type (lymphatic/non-lymphatic) and pathology status (presence/absence of tumour cells) had been confirmed for the excised tissue.

The secondary efficacy endpoints of reverse concordance (per node and per patient) used the RITT population.

For both NEO3-05 and NEO3-09, the primary measure of efficacy was the concordance rate between Lymphoseek and vital blue in the in vivo localization of lymph node(s) prior to their excision.

The primary measure of concordance, based on a "per node" calculation, was of the form:

$$P_{C1} = \frac{\text{\# of nodes that were VBD-stained and were also Lymphoseek hot}}{\text{\# of VBD-stained nodes}}$$

The following hypotheses were tested on the primary endpoint of the study using an exact binomial test of the null hypothesis (i.e., that the true concordance rate PC1 was ≤ 0.90) with a one-sided significance level of α=0.05 for NEO3-05, or a two-sided significance level of α=0.05 (one-sided α=0.025) for NEO3-09.

Again, for both Phase 3 studies, a secondary measure of concordance was based on a "per patient" estimate (PC2). This measure was calculated as:

$$P_{C2} = \frac{\text{\# of patients for whom all VBD-stained nodes were also Lymphoseek hot}}{\text{\# of patients with at least one VBD-stained node}}$$

Analysis of concordance using the per patient derivation was based on calculating a point estimate and a 95% exact binomial confidence interval. A formal statistical test of the per patient concordance endpoint was not performed.

Additionally, the following secondary efficacy variables were analyzed for both studies:

- The proportions of excised lymph nodes that were positive by pathology for four groupings of VBD and Lymphoseek findings were examined (i.e., blue/hot, blue/not hot, not blue/hot, and not blue/not hot). The denominator for each calculation was the total number of nodes excised from the safety population.
- Sensitivities and false negative rates (FNRs) were calculated separately for Lymphoseek and VBD in patients undergoing lymphadenectomy. Sensitivity was based on the number of excised nodes that were identified by a mapping agent and that were pathology positive (i.e., contained tumour) divided by the number of pathology-positive nodes. The FNR was calculated as the number of excised nodes that were missed by a mapping agent (i.e., not hot or not blue) and pathology-positive divided by the number of pathology-positive nodes.

Statistical tests of hypotheses were not performed for these secondary efficacy endpoints analyses. Instead, point estimates and 95% exact binomial confidence intervals were calculated.

The reverse concordance rate (PC3), based on a per node calculation, was as follows:

$$P_{C3} = \frac{\text{\# of Lymphoseek hot nodes that were VBD-stained}}{\text{\# of Lymphoseek hot nodes}}$$

The number and proportion of reverse concordant nodes for each tumour type were computed. A 95% confidence interval was calculated for the overall reverse concordance rate. To note, reverse concordance was calculated, and a statistical test of PC3 vs. 0.90 using a two-sided significance level of α=0.05, was conducted for NEO3-05 as defined in a supplemental statistical analysis plan (SAP). However, both reverse concordance and superiority testing were incorporated prospectively into the NEO3-09 study.

For superiority testing, the following hypotheses, where PC3 was the reverse concordance rate (of VBD relative to Lymphoseek) and PC1 was the concordance rate (of Lymphoseek relative to VBD), were conducted using McNemar's test with a two-sided significance level of α=0.05 (one-sided α=0.025). This test was conducted in NEO3-09 only after passing the primary endpoint of concordance of Lymphoseek relative to VBD. The statistical hypotheses were as follows:

$$H_0: P_{C1} \leq P_{C3}$$
vs.
a $$H_a: P_{C1} > P_{C3}$$

A secondary measure of reverse concordance was also based on a per patient estimate. This measure was calculated as:

$$P_{C4} = \frac{\text{\# of patients for whom all Lymphoseek hot nodes were VBD-stained}}{\text{\# of patients with at least one in vivo hot node}}$$

The number and proportion of reverse concordant patients for each tumour type were computed. A 95% exact binomial confidence interval was computed for the overall reverse concordance rate per patient.

Results

Participant flow

Overall, 195 patients were screened and enrolled into the study. A total of 26 (13.3%) patients were withdrawn from the study. Of those enrolled patients, 179 (91.8%) received study drug administration, nine (4.6%) were considered to be screen failures, six (3.1%) withdrew consent prior to receiving an injection of Tc 99m Lymphoseek, and one (0.5%) did not receive Tc 99m Lymphoseek due to unavailability of the study drug at the time of injection ("Other").

Of those 179 patients receiving Tc 99m Lymphoseek, 169 (86.7% of the enrolled population) completed the study. Ten patients were withdrawn from the study after study drug administration: seven patients were considered to have had a protocol violation, two patients were lost to follow-up, and one patient did not map any radioactivity with Tc 99m Lymphoseek following injection ("Other").

No patients discontinued due to AEs and there were no deaths. A summary of patient disposition is shown in Table 24.

Table 24: Patient disposition – Study NEO3-05 (all enrolled patients)

		Cancer Type			
		Melanoma (N=94)	Breast Cancer (N=99)	Primary Diagnosis Not Reported (N=2)	Overall (N=195)
Completed		78 (83.0%)	91 (91.9%)	0 (0.0%)	169 (86.7%)
Withdrawn		16 (17.0%)	8 (8.1%)	2 (100.0%)	26 (13.3%)
	Screen Failure	5 (5.3%)	2 (2.0%)	2 (100.0%)	9 (4.6%)
	Adverse Event	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Protocol Violation	4 (4.3%)	3 (3.0%)	0 (0.0%)	7 (3.6%)
	Lost To Follow-Up	2 (2.1%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
	Withdrawal Of Consent	4 (4.3%)	2 (2.0%)	0 (0.0%)	6 (3.1%)
	Death	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Other	1 (1.1%)	1 (1.0%)	0 (0.0%)	2 (1.0%)

Recruitment

The study started on 12 June 2008 (first patient enrolled) to 18 June 2009 (last patient completed). This was a single dose study, with safety follow-up at 30 days post-injection.

Conduct of the study

There were three revisions to the original protocol (07 February 2008). Noteworthy changes are summarized here. However, all protocol revisions occurred prior to first patient enrollment and thus did not have an effect on the conduct of the study.

Version II (13 March 2008): This amendment revised the original protocol to allow for the screening visit to occur up to 30 days prior to enrollment. Lymphoscintigraphy was now to be performed at surgery for all patients. Neoprobe now provided Lymphoseek as well as Patent Blue V to the study sites.

Version IIB (09 April 2008): This amendment contained the following updates:

The two stage design was removed.

The maximum sample size was increased from 177 vital blue stained nodes and 193 expected patients to 203 VBD stained nodes and 238 expected patients.

A supportive measure of the primary efficacy endpoint was added using the number of patients with VBD stained nodes as the denominator.

The calculation of sensitivity/false negative rate for each detection method was added as a secondary efficacy endpoint.

The modified ITT population was revised to be the ITT population; this study population must have pathology determination of the type and contents (i.e., tumor cells) of the excised tissue. The PP population was to include all ITT patients with no major protocol violations.

The alternative hypothesis was changed from $P \geq 0.95$ to $P \geq 0.90$.

A list of acceptable gamma detection devices was added, and detectors were allowed if they were only able collect counts in 10-second intervals.

Methods of injection and appropriate injection volumes were included: breast cancer patients could have intradermal, periareolar, subareolar, or peritumor injections and melanoma patients were to have intradermal injection.

A procedure for pathological evaluation the excised tissue was specified. Evaluation was to include serial sectioning with H&E staining as well as IHC staining. IHC staining for melanoma patients was to use the MART-1/melan-A melanocyte differentiation antigen and/or HMB-45 antibody; IHC staining for breast cancer patients was to use the Anti- Cytokeratin CAM 5.2 reagent.

Version III (20 May 2008): This protocol revision requested sites to use Lymphazurin (isosulfan blue 1%) for the VBD. However, if it was unavailable, Patent Blue V could be used and would be supplied by the Sponsor. The radiolabeling procedure was updated to include an option for a final preparation volume of 0.5 mL for a total injection volume of 0.1 mL.

Baseline data

The baseline demographic characteristics of the patients in study NEO3-05 are listed in the 2 tables below.

Table 25: Summary of patient demographics – Study NE03-05 (safety population)

Demographics Variable	Category	Safety Population (N=179)
Gender	Male	51 (28.5%)
	Female	128 (71.5%)
Age (years)	Mean (SD)	58.5 (14.12)
	Min, Max	20, 90
Race	White	169 (94.4%)
	Black	4 (2.2%)
	Asian	5 (2.8%)
	American Indian Or Alaskan Native	0 (0%)
	Native Hawaiian Or Other Pacific Islander	1 (0.6%)
Ethnicity	Hispanic	7 (3.9%)
	Non Hispanic	171 (95.5%)
	Missing/Did not report	1 (0.6%)
Cancer Type	Melanoma	85 (47.5%)
	Breast Cancer	94 (52.5%)
Weight (lbs)	Mean (SD)	175.9 (44.67)
	Min, Max	70, 345
Height (inches)	Mean (SD)	65.6 (4.30)
	Min, Max	43, 78

Abbreviations: SD=standard deviation; min=minimum, max=maximum; lbs=pounds

Numbers analysed

Four analysis populations were prospectively planned and analysed. The ITT population (N=158) was the primary population for efficacy evaluation. The per protocol (PP) population (N=156) was used for supportive efficacy evaluation, consisting of the ITT population lacking major protocol violations. The safety population (N=179) consisted of all eligible patients injected with Tc 99m Lymphoseek. The safety PP population (N=176) was used for supportive safety evaluation, consisting of the safety population lacking major protocol violations.

A revised Statistical Analysis Plan defined two additional data analyses for the analysis of efficacy: one additional population, a reverse ITT population (N=167), and a new subgroup, defined as all study sites except Study Sites 05 and 06. This subgroup analysis included an ITT subgroup that excluded Study Sites

05 and 06 (N=136), a PP subgroup that excluded Study Sites 05 and 06 (N=135), a safety subgroup that excluded Study Sites 05 and 06 (N=152), and a safety PP subgroup that excluded Study Sites 05 and 06 (N=150).

Outcomes and estimation

Concordance Rate between Tc-99m Lymphoseek and Vital Blue Dye (All Sites)

Nodal level: Of 256 lymph nodes in the ITT node population, 239 nodes were detected by both VBD and Tc-99m Lymphoseek. The corresponding nodal concordance rate (P_{C1}) was 93.36%.

The exact binomial test of this result, against the null hypothesis H0: PC1 ≤ 0.90, was statistically significant at the 0.05 one-sided α level (p=0.0401).

The corresponding nodal concordance rates for melanoma and breast cancer patients were 97.52% and 89.63%, respectively.

Table 26: Count and proportion of concordant nodes (ITT population) – Study NEO3-05

	Number (Proportion) of Concordant Nodes in ITT Population (Total ITT Nodes=256)	95% Exact Binomial Confidence Interval for Proportion	1-Sided p-Value for Exact One-Sample Test of H_0: $P_{C1} \leq 0.90$ [b]
Concordant Nodes [a]	239 (0.9336)	(0.8958, 0.9608)	0.0401
Concordant Nodes from Melanoma Patients (Nodes from Melanoma Patients=121)	118 (0.9752)		
Concordant Nodes from Breast Cancer Patients (Nodes from Breast Cancer Patients=135)	121 (0.8963)		

[a] Concordant Nodes = Nodes that were determined in vivo to be both "blue" (due to presence of vital blue dye) and "hot" (due to presence of Tc 99m Lymphoseek).

[b] Evaluated against one-sided α = 0.05 (per the study protocol).

The concordance was slightly higher in the PP population:

Table 27: Count and proportion of concordant nodes – Study NEO3-05

	Number (Proportion) of Concordant Nodes in PP Population (Total Nodes = 251)	95% Exact Binomial Confidence Interval for Proportion	1-Sided P-Value for Exact One-Sample Binomial Test of H_0 : $P_{C1} \leq 0.90$
Concordant Nodes - Nodes that were determined in-vivo to be both "blue" (due to presence of vital blue dye) and "hot" (due to presence of Lymphoseek)	237 (0.9442)	(0.9082, 0.9692)	0.0088
Concordant Nodes from Melanoma Subjects (Nodes from Melanoma Subjets = 121)	118 (0.9752)		
Concordant Nodes from Breast Cancer Subjects (Nodes from Breast Cancer Subjets = 130)	119 (0.9154)		

At a patient level (ITT; N=158), the overall concordance rate (PC2) was 92.41%, with concordance remaining higher among melanoma patients (96.00%) than among breast cancer patients (89.16%).

Table 28: Count and proportion of concordant patients – Study NEO3-05 (ITT population)

	Number (Proportion) of Concordant Patients in ITT Population (N=158)	95% Exact Binomial Confidence Interval for Proportion
Concordant Patients [a]	146 (0.9241)	(0.8711, 0.9601)
Concordant Melanoma Patients (Total Melanoma Patients=75)	72 (0.9600)	
Concordant Breast Cancer Patients (Total Breast Cancer Patients=83)	74 (0.8916)	

[a] Concordant Patients = Patients for whom all nodes that were determined in vivo to be "blue" (due to the presence of vital blue dye) were also determined to be "hot" (due to presence of Tc 99m Lymphoseek).

When using the supportive PP population (N=156), the overall concordance rate (P_{C2}) was 92.95%, with concordance remaining higher among melanoma patients (96.00%) than among breast cancer patients (90.12%).

ITT concordance rates by site is shown below, where concordance is shown by site and increasing average Tc-99m Lymphoseek injection volume.

Table 29: Concordance by Lymphoseek injection volume – Study NEO3-05 (ITT population)

Average Volume of Tc 99m Lymphoseek Injection (mL)	Overall Concordance Rate	Number of ITT Nodes	Study Site
0.12	1.00	7	07
0.15	0.99	75	02
0.40	1.00	22	10
0.40	1.00	1	11
0.40	1.00	3	13
0.42	0.95	20	08
0.53	1.00	13	04
0.55	0.75	4	03
1.99	1.00	42	12
2.00	0.91	22	09
3.00	1.00	5	01
3.63	**0.73**	**26**	**05**
8.00	**0.67**	**15**	**06**
8.00	1.00	1	15

Pathology Results from Excised Lymph Nodes (All Sites)

A total of 380 lymph nodes were excised, and evaluated for histology and pathology, from the safety population. All but one was confirmed to be lymphoid tissue. It was later noted that this was incorrectly entered into the database as "not lymphoid tissue." However, the database was not changed because of this post-study finding.

Of the 380 excised lymph nodes, 41 (10.79%) were pathology-positive for presence of tumour cells.

A total of 192 lymph nodes from breast cancer patients were excised; of these, 23 (11.98%) were pathology-positive for presence of tumour cells. A total of 188 lymph nodes from melanoma patients were excised; of these, 18 (9.57%) were pathology-positive for presence of tumour cells.

Of these 41 pathology-positive lymph nodes, 38 nodes were identified by Tc-99m Lymphoseek, 33 nodes were identified by VBD, and 32 nodes were identified by both Tc-99m Lymphoseek and VBD. The corresponding sensitivity rate in detection of lymph nodes positive for tumour cells was higher for Tc-99m Lymphoseek (0.9268) compared with VBD (0.8049). The false negative rate for VBD (0.1951) was more than 2.5 times the false negative rate for Tc-99m Lymphoseek (0.0732).

Table 30: Sensitivities and false negative rates for Lymphoseek and VBD – Study NEO3-05 (safety population)

	Number of Nodes	Proportion/Rate (N=41)	95% Exact Binomial Confidence Interval for Proportion
Vital Blue Dye + / Pathology +	33	--	--
Vital Blue Dye - / Pathology +	8	--	--
Tc 99m Lymphoseek + / Pathology +	38	--	--
Tc 99m Lymphoseek - / Pathology +	3	--	--
Vital Blue Dye Sensitivity	--	0.8049	(0.6513, 0.9118)
Tc 99m Lymphoseek Sensitivity	--	0.9268	(0.8008, 0.9846)
Vital Blue Dye False Negative Rate	--	0.1951	(0.0882, 0.3487)
Tc 99m Lymphoseek False Negative Rate	--	0.0732	(0.0154, 0.1992)
The number of safety nodes in this table includes all pathology-positive nodes with a valid in vivo assessment.			

Table 31: Summary of nodes in safety population by Lymphoseek, VBD and pathology status in all study sites – Study NEO3-05 (safety population)

	Breast Cancer	Melanoma	Cumulative by Row
Total Excised Nodes	192	188	380
Total Pathologically +	23 (11.98%)	18 (9.57%)	41 (10.79%)
Total Pathologically -	169 (88.02%)	170 (90.43%)	339 (89.21%)
Tc 99m Lymphoseek + / Vital Blue Dye + / Pathology +	16 (8.33%)	16 (8.51%)	32 (8.42%)
Tc 99m Lymphoseek - / Vital Blue Dye + / Pathology +	1 (0.52%)	0 (0.00%)	1 (0.26%)
Tc 99m Lymphoseek + / Vital Blue Dye - / Pathology +	4 (2.08%)	2 (1.06%)	6 (1.58%)
Not Detected / Pathologically+	2 (1.04%)	0 (0.00%)	2 (0.53%)
Tc 99m Lymphoseek + / Vital Blue Dye + / Pathology -	105 (54.69%)	102 (54.26%)	207 (54.47%)
Tc 99m Lymphoseek - /Vital Blue Dye + / Pathology -	13 (6.77%)	3 (1.60%)	16 (4.21%)
Tc 99m Lymphoseek + /Vital Blue Dye - / Pathology -	45 (23.44%)	53 (28.19%)	98 (25.79%)
Not Detected / Pathologically-	6 (3.13%)	12 (6.38%)	18 (4.74%)

The denominator for percentages in this table is the number of total excised nodes with a valid in vivo assessment per cancer type and overall.

Concordance Rate Using the Reverse ITT Analysis Population (i.e., Reverse Concordance)

Based on the reverse ITT concordance population (N=343 nodes from 167 patients), nodal concordance of VBD against Tc-99m Lymphoseek was 69.68% (239 nodes). That is, less than 70% of nodes found positive by Tc-99m Lymphoseek were detected by VBD.

Reverse concordant nodes are those nodes that were determined *in vivo* to be both "blue" (due to presence of VBD) and "hot" (due to presence of Tc-99m Lymphoseek). Reverse concordant patients are those patients for whom all nodes that were determined *in vivo* to be "hot" were also determined to be "blue". There was 68.21% nodal concordance in melanoma patients (118 out of 173 nodes) and 71.18% nodal concordance in breast cancer patients (121 out of 170 nodes). The exact binomial test of this result against the same null hypothesis used for primary concordance, H0: PC3 ≤ 0.90, was not statistically significant (p=1.0000).

On a patient level, the overall reverse concordance was 56.89% (95 out of 167 patients), with reverse concordance higher among breast cancer patients (62.07%) than among melanoma patients (51.25%).

Concordance between Tc-99m Lymphoseek and Vital Blue Dye (Excluding Sites 05 and 06)

These analyses were supplemental analyses conducted excluding Sites 05 and 06 owing to the complete excursion from protocol injection volumes.

Of 215 total ITT nodes evaluated in this subgroup, 210 were identified by both VBD and Tc-99m Lymphoseek, giving a concordance rate of 97.67% and the exact binomial test of this result against the null hypothesis, H0: PC1 ≤ 0.90, was statistically significant (p<0.0001). In this population, nodal concordance rates from melanoma and breast cancer patients were similar (97.48% and 97.92%, respectively).

At a patient level (ITT subgroup; N=136), the overall concordance rate (PC2) was 96.32% (131 patients), with concordance being similar between melanoma patients (95.89%) and breast cancer patients (96.83%).

Table 32: Count and proportion of concordance nodes without sites 05 and 06 – Study NEO3-05 (ITT population)

	Number (Proportion) of Concordant Nodes in ITT Population (Total ITT Nodes=215)	95% Exact Binomial Confidence Interval for Proportion	1-Sided p-Value for Exact One-Sample Test of H_0: $P_{C1} \leq 0.90$ [b]
Concordant Nodes [a]	210 (0.9767)	(0.9466, 0.9924)	<0.0001
Concordant Nodes from Melanoma Patients (Nodes from Melanoma Patients=119)	116 (0.9748)		
Concordant Nodes from Breast Cancer Patients (Nodes from Breast Cancer Patients=96)	94 (0.9792)		

[a] Concordant Nodes = Nodes that were determined in vivo to be both "blue" (due to presence of vital blue dye) and "hot" (due to presence of Tc 99m Lymphoseek).
[b] Evaluated against one-sided α = 0.05 (per the study protocol).

Table 33: Count and proportion of concordance patients without sites 05 and 06 – Study NEO3-05 (ITT population)

	Number (Proportion) of Concordant Patients in ITT Population (N=136)	95% Exact Binomial Confidence Interval for Proportion
Concordant Patients [a]	131 (0.9632)	(0.9163, 0.9880)
Concordant Melanoma Patients (Total Melanoma Patients=73)	70 (0.9589)	
Concordant Breast Cancer Patients (Total Breast Cancer Patients=63)	61 (0.9683)	

[a] Concordant Patients = Patients for whom all nodes that were determined in vivo to be "blue" (due to the presence of vital blue dye) were also determined to be "hot" (due to presence of Tc 99m Lymphoseek).

Pathology Results from Excised Lymph Nodes (Excluding Study Sites 05 and 06)

A total of 327 lymph nodes were excised, and evaluated for histology and pathology, for this subgroup of the safety population (excluding the Sites 05 and 06). All were confirmed to be lymphoid tissue, and 38 (11.62%) were pathologically positive for presence of tumour cells.

A total of 145 lymph nodes from breast cancer patients were excised; of these, 21 (14.48%) were pathology-positive for presence of tumour cells.

A total of 182 lymph nodes from melanoma patients were excised; of these, 17 (9.34%) were pathology-positive for presence of tumour cells.

Of the 38 positive lymph nodes (excluding Sites 05 and 06), 36 nodes were identified by Tc-99m Lymphoseek, 31 nodes were identified by VBD, and 31 nodes were identified by both Tc-99m Lymphoseek and VBD. In this population, the corresponding sensitivity rate in detection of lymph nodes positive for tumour cells also was higher for Tc-99m Lymphoseek (0.9474) compared with VBD (0.8158). The false negative rate for VBD (0.1842) was more than 3.5 times the false negative rate for Tc-99m Lymphoseek (0.0526).

Table 34: Sensitivities and false negative rates for Lymphoseek and VBD – Study NEO3-05 (safety population without sites 05 and 06)

Table 12 Sensitivities and False Negative Rates for Tc 99m Lymphoseek and Vital Blue Dye (Safety Population Excluding Sites 05 and 06)			
	Number of Nodes	Proportion/Rate (N=38)	95% Exact Binomial Confidence Interval for Proportion
Vital Blue Dye + / Pathology +	31	--	--
Vital Blue Dye - / Pathology +	7	--	--
Tc 99m Lymphoseek + / Pathology +	36	--	--
Tc 99m Lymphoseek - / Pathology +	2	--	--
Vital Blue Dye Sensitivity	--	0.8158	(0.6567, 0.9226)
Tc 99m Lymphoseek Sensitivity	--	0.9474	(0.8225, 0.9936)
Vital Blue Dye False Negative Rate	--	0.1842	(0.0774, 0.3433)
Tc 99m Lymphoseek False Negative Rate	--	0.0526	(0.0064, 0.1775)
The number of safety nodes in this table includes all pathology-positive nodes with a valid in vivo assessment.			

Sensitivities and false negative rates are also displayed by disease type.

Of the 21 pathology positive lymph nodes from breast cancer patients, 19 (0.9048) nodes were identified by Tc-99m Lymphoseek, 15 (0.7143) nodes were identified by VBD, and 15 (0.7143) nodes were identified by both Tc-99m Lymphoseek and VBD. Of the 17 pathology-positive lymph nodes from melanoma patients, 17 (1.0000) were identified by Tc-99m Lymphoseek and 16 (0.9412) were identified by VBD.

The corresponding false negative rate for VBD in breast cancer patients (0.2857; 6/21) three times as high as the false negative rate for Tc-99m Lymphoseek (0.0952; 2/21). The false negative rate for Tc-99m Lymphoseek in melanoma was zero, whereas that of VBD was 0.0588 (1/17).

Table 34: Summary of nodes in safety population by Lymphoseek, VBD and pathology status – Study NEO3-05 (safety population without sites 05 and 06)

Table 13 Summary of Nodes in Safety Population by Tc 99m Lymphoseek, Vital Blue Dye, and Pathology Status (Safety Population Excluding Sites 05 and 06)			
	Breast Cancer	Melanoma	Cumulative by Row
Total Excised Nodes	145	182	327
Total Pathologically +	21 (14.48%)	17 (9.34%)	38 (11.62%)
Total Pathologically -	124 (85.52%)	165 (90.66%)	289 (88.38%)
Tc 99m Lymphoseek + / Vital Blue Dye + / Pathology +	15 (10.34%)	16 (8.79%)	31 (9.48%)
Tc 99m Lymphoseek - / Vital Blue Dye + / Pathology +	0 (0.00%)	0 (0.00%)	0 (0.00%)
Tc 99m Lymphoseek + / Vital Blue Dye - / Pathology +	4 (2.76%)	1 (0.55%)	5 (1.53%)
Not Detected / Pathologically +	2 (1.38%)	0 (0.00%)	2 (0.61%)
Tc 99m Lymphoseek + / Vital Blue Dye + / Pathology -	79 (54.48%)	100 (54.95%)	179 (54.74%)
Tc 99m Lymphoseek - / Vital Blue Dye + / Pathology -	2 (1.38%)	3 (1.65%)	5 (1.53%)
Tc 99m Lymphoseek + / Vital Blue Dye - / Pathology -	41 (28.28%)	56 (30.77%)	97 (29.66%)
Not Detected / Pathologically -	2 (1.38%)	6 (3.30%)	8 (2.45%)
The denominator for percentages in this table is the number of total excised nodes with a valid in vivo assessment per cancer type and overall.			

Summary of Study NEO3-05

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Title: A Phase 3, Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek and VBD as Lymphoid Tissue Targeting Agents in Patients With Known Melanoma or Breast Cancer Who Are Undergoing Lymph Node Mapping			
Study identifier	**NEO3-05**		
Design	Phase 3, prospective, open label, multicentre, single arm, within patient, comparative study of Lymphoseek and VBD		
	Duration of main phase:		9 months
Treatments groups (within patient comparison) Melanoma: 94 patients enrolled/ 78 completed Breast cancer: 99 patients enrolled/ 91 completed.	Test		Technetium Tc-99m Lymphoseek Injection was given in 50 µg doses by intradermal, periareolar, subareolar, or peritumoral injection in close proximity to the primary tumour.
	Comparator		The above injection was followed by injection of **vital blue due**, and followed by intraoperative lymph nodal mapping.
Endpoints and definitions	Primary endpoint	Nodal concordance	Per node concordance rate of Tc99m lymphoseek relative to blue dye

	Secondary endpoint	Patient concordance	Per patient concordance rate of Tc 99m lymphoseek relative to blue dye
	Secondary endpoint	Detection categories	Proportions
	Secondary endpoint	Vital Blue Dye	Sensitivity and false negative rate
	Secondary endpoint	Tc99m Lymphoseek	Sensitivity and false negative rate
	Supplemental outcome	Nodal reverse concordance	Per node "reverse" concordance rate of vital blue dye relative to Tc99m lymphoseek
	Supplemental outcome	Per patient "reverse" concordance	Per patient "reverse" concordance rate of vital blue dye relative to Tc99m lymphoseek
Database lock	12 June 2008 (first patient enrolled) to 18 June 2009 (last patient completed)		

Results and Analysis

Analysis description	**Primary Analysis**
Analysis population and time point description	Intent to treat

Descriptive statistics and estimate variability for nodal concordance	Treatment group	ITT	ITT without AS1 and AS2
	Total number of excised nodes	256	215
	Concordant nodes (L/VBD) *Proportion of concordant nodes*	239 0.9336	210 0.9767
	95% C.I. p-Value	0.8958-0.9608 p=0.0401	0.9466-0.9924 p<0.0001
	Total excised melanoma nodes	121	119
	Concordant melanoma nodes *Proportion of concordant nodes*	118 0.9752	116 0.9748
	Total excised breast cancer nodes	135	96

	Concordant breast cancer nodes *Proportion of concordant nodes*	121 0.8963	94 0.9792
Descriptive statistics and estimate variability for patient concordance	Number Patients concordance	158	136
	Concordant patients (L/VBD) *Proportion of concordant patients*	146 0.9336	131 0.9767
	95% C.I.	0.8711-0.9601	0.9163-0.9880
	Total melanoma patients	75	73
	Concordant melanoma patients *Proportion of concordant patients*	72 0.9600	70 0.9589
	Total breast cancer patients	83	63
	Concordant breast cancer patients *Proportion of concordant patients*	74 0.8916	61 0.9683

Total excised nodes	Safety N=380	Safety without AS1 and AS2 N=327
Pathology-positive nodes	41	38
VBD+/L+/Path+	32	31
VBD+/L-/Path+	1	0
VBD-/L+/Path+	6	5
VBD-/L-/Path+	2	2
Total VBD+/Path+	33	31
Total VBD-/Path+	8	7
Total L+/Path+	38	36
Total L-/Path+	3	2

False Negative Rates	ITT		ITT without AS1 and AS2
True FNR lymphoseek (%)/total nodes	1.17%		0.93%
True FNR VBD (%)/Total nodes	3.13%		3.25%
Sensitivities	Safety N=380		Safety without AS1 and AS2 N=327
True FNR (%) lymphoseek /Path+ nodes	7.32%		5.25%
Tue FNR VBD (%)/Path+ nodes	19.51%		18.42%
Reverse concordance	Treatment group	ITT	ITT without AS1 and AS2
	Total excised nodes	343	306
	Reverse concordance nodes (VBD/L) *Proportion of reverse concordant nodes* 95% C.I. p-value	239 0.6968 0.6451-0.7450 P=1.000	210 0.6863 0.6310-0.7379 P=1.000
	Total patients	167	144
	Reverse concordant patients (VBD/LS) *Proportion of concordant patients* 95% C.I.	95 0.5689 0.4901-0.6451	78 0.5417 0.4567-0.6249
Notes	AS1 refers to study site 5 data; and AS2 refers to study site 6 data.		

Title: A Phase 3, Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek and VBD as Lymphoid Tissue Targeting Agents in Patients With Known Melanoma or Breast Cancer Who Are Undergoing Lymph Node Mapping

Study identifier	**NEO3-05**	
Design	Phase 3, prospective, open label, multicentre, single arm, within patient, comparative study of Lymphoseek and VBD	
	Duration of main phase:	9 months

Treatments groups (within patient comparison) Melanoma: 94 patients enrolled/ 78 completed Breast cancer: 99 patients enrolled/ 91 completed.	Test		Technetium Tc-99m Lymphoseek Injection was given in 50 µg doses by intradermal, periareolar, subareolar, or peritumoral injection in close proximity to the primary tumour.
	Comparator		The above injection was followed by injection of **vital blue due**, and followed by intraoperative lymph nodal mapping.
Endpoints and definitions	Primary endpoint	Nodal concordance	Per node concordance rate of Tc99m lymphoseek relative to blue dye
	Secondary endpoint	Patient concordance	Per patient concordance rate of Tc 99m lymphoseek relative to blue dye
	Secondary endpoint	Detection categories	Proportions
	Secondary endpoint	Vital Blue Dye	Sensitivity and false negative rate
	Secondary endpoint	Tc99m Lymphoseek	Sensitivity and false negative rate
	Supplemental outcome	Nodal reverse concordance	Per node "reverse" concordance rate of vital blue dye relative to Tc99m lymphoseek
	Supplemental outcome	Per patient "reverse" concordance	Per patient "reverse" concordance rate of vital blue dye relative to Tc99m lymphoseek
Database lock	12 June 2008 (first patient enrolled) to 18 June 2009 (last patient completed)		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat		

Descriptive statistics and estimate variability for nodal concordance	Treatment group	ITT	ITT without AS1 and AS2
	Total number of excised nodes	256	215
	Concordant nodes (L/VBD) *Proportion of concordant nodes*	239 0.9336	210 0.9767

	95% C.I. p-Value	0.8958-0.9608 p=0.0401	0.9466-0.9924 p<0.0001
	Total excised melanoma nodes	121	119
	Concordant melanoma nodes *Proportion of concordant nodes*	118 0.9752	116 0.9748
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	Concordant breast cancer nodes *Proportion of concordant nodes*	121 0.8963	94 0.9792
Descriptive statistics and estimate variability for patient concordance	Number Patients concordance	158	136
	Concordant patients (L/VBD) *Proportion of concordant patients*	146 0.9336	131 0.9767
	95% C.I.	0.8711-0.9601	0.9163-0.9880
	Total melanoma patients	75	73
	Concordant melanoma patients *Proportion of concordant patients*	72 0.9600	70 0.9589
	Total breast cancer patients	83	63
	Concordant breast cancer patients *Proportion of concordant patients*	74 0.8916	61 0.9683

Total excised nodes	Safety N=380		Safety without AS1 and AS2 N=327
Pathology-positive nodes	41		38
VBD+/L+/Path+	32		31
VBD+/L-/Path+	1		0
VBD-/L+/Path+	6		5
VBD-/L-/Path+	2		2
Total VBD+/Path+	33		31
Total VBD-/Path+	8		7
Total L+/Path+	38		36
Total L-/Path+	3		2
False Negative Rates	ITT		ITT without AS1 and AS2
True FNR lymphoseek (%)/total nodes	1.17%		0.93%
True FNR VBD (%)/Total nodes	3.13%		3.25%
Sensitivities	Safety N=380		Safety without AS1 and AS2 N=327
True FNR (%) lymphoseek /Path+ nodes	7.32%		5.25%
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Reverse concordance	Treatment group	ITT	ITT without AS1 and AS2
	Total excised nodes	343	306
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	Total patients	167	144
	Reverse concordant patients (VBD/LS) *Proportion of concordant patients* 95% C.I.	95 0.5689 0.4901-0.6451	78 0.5417 0.4567-0.6249
Notes	AS1 refers to study site 5 data; and AS2 refers to study site 6 data.		

Study NEO3-09: A Phase 3, Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek and Vital Blue Dye as Lymphoid Tissue Targeting Agents in Patients With Known Melanoma or Breast Cancer Who Are Undergoing Lymph Node Mapping

Methods

Study Participants

The main inclusion and exclusion criteria were the same as for study NEO3-05.

Treatments

The treatment regimen was similar to study NE03-05. Technetium Tc 99m Lymphoseek Injection was given in a 50 µg dose by intradermal or subareolar injection in close proximity to the primary tumor, followed by injection of VBD and ILM. Formulated, unlabelled Lymphoseek (tilmanocept) 0.25 mg drug product was radiolabeled with Tc 99m at 0.5 or 2.0 mCi, depending on time of surgery.

Each investigational site used Lymphazurin (1% isosulfan blue for injection) as the VBD agent. Instructions for preparing and administering Lymphazurin were provided in the package insert. This agent was administered near the tumor site (according to the tumor type) via injection at or near the time of ILM (prior to incision).

Objectives

The objectives were the same as for the studies NE03-05 and NE03-06.

The ***primary objective*** of efficacy was the concordance between Tc-99m Lymphoseek and VBD in the *in vivo* detection of the excised lymph node(s) as confirmed by pathology.

The ***secondary objective*** was the assessment of the resected lymph node(s) to confirm the presence/absence of tumour metastases.

The ***safety objective*** was the evaluation of patient safety through observation of adverse events, clinical laboratory tests, vital signs, ECGs, and physical examinations.

Outcomes/endpoints

The endpoints were similar to studies NE03-05 and NE03-06.

The ***primary efficacy endpoint*** was the proportion of lymph nodes identified intraoperatively by localization of VBD (by blue hue) that were also identified intraoperatively by localization of Tc-99m Lymphoseek (by 3σ rule). This was a per node concordance measure that used the number of lymph nodes stained by VBD as the denominator. Concordance was achieved when a VBD-stained lymph node was also detected by Tc-99m Lymphoseek based on the handheld gamma probe count(s) satisfying the threshold criterion. Any lymph node count not meeting this threshold criterion was considered a negative (i.e., Tc-99m Lymphoseek non-localized) finding.

The ***first secondary efficacy variable*** was a supportive measure of the primary concordance rate calculated on a per patient basis. This per patient estimate of concordance was the number of patients for whom all VBD-stained lymph nodes were also Tc-99m Lymphoseek hot divided by the number of patients in the ITT population.

The next ***secondary efficacy variables*** were the reverse concordance rates (based on the reverse intent-to-treat [RITT] population; both per node and per patient), where VBD localization was compared against Tc-99m Lymphoseek localization, treating Tc-99m Lymphoseek as comparator or "truth" standard. These proportions used similar numerators as the concordance variables, but used the number of lymph nodes or patients that were identified intraoperatively by Tc-99m Lymphoseek as the denominator.

Other secondary measures of efficacy included measures of Tc-99m Lymphoseek detection and VBD detection relative to pathological finding of metastases in the excised lymph nodes, and calculations of sensitivity and false negative rates (FNRs) for each detection method in all patients undergoing lymphadenectomy.

Exploratory endpoints were based on subgroup analyses of the primary and secondary efficacy measures by study site and by tumour type (melanoma vs. breast cancer). In addition, data regarding medical history and co-medication were summarized and exploratory analyses were conducted as needed.

The ***primary safety endpoints*** were based on the evaluation of adverse events, clinical laboratory tests, vital signs, ECGs, and physical examinations.

Sample size

The hypothesis to be tested was the same as in NEO3-05, however, this time a one-sided α-level of 0.025 was used. Given the proportions specified for testing (assumed concordance rate of 0.96) and a target Type II error rate of 0.10 (90% power), the minimum sample size required was 196 VBD-stained lymph nodes that fulfilled the definition of the ITT population. The exact one-sided α-level and power of the test were 0.0207 and 0.904, respectively.

The number of patients needed to satisfy the sample size requirement of 196 VBD-stained nodes was a random variable, and depended on two observed quantities: the proportion of patients having at least one VBD-stained lymph node and the number of such nodes per patient. Based on the average number of ITT nodes per patient in the Phase 3 NEO3-05 study (1.5806 ITT nodes/patient), approximately 124 ITT patients were estimated to be needed to be enrolled to produce 196 VBD-stained nodes. From NEO3-05, approximately 13% of the patients injected with Tc 99m Lymphoseek did not meet ITT criteria (did not express a blue node *in vivo* or did not have VBD injected). Thus, to obtain the desired number of ITT patients and VBD-stained lymph nodes, the estimate of 124 ITT patients needed required that approximately 143 patients needed to be injected with Tc 99m Lymphoseek (124 ITT patients/0.87 ITT patients per injected). Additionally, there were approximately 8% of the patients enrolled in NEO3-05 who were early withdrawals. This brought the enrolment estimate for this study up to 155 (143 injected patients/0.92 injected patients per enrolled).

Randomisation

The study was designed as a single arm study.

Blinding (masking)

The study was designed as an open label study.

Statistical methods

The statistical methods used to analyse the study population and the primary and secondary measures were the same as for study NEO3-05.

Results

Participant flow

A total of 165 patients were screened, and 163 patients were enrolled into the study. Two patients were evaluated to be screen failures.

Of those patients enrolled, 93.3% (152/163) completed the study.

A total of 11 (6.7%) patients were withdrawn from the study; no patients discontinued due to adverse events, and there were no deaths in this study.

Ten patients were withdrawn from the study prior to injection of Tc-99m Lymphoseek: four withdrew consent, and six were unable to be treated before completion of the study (Other: withdrawn due to Sponsor request/before deadline).

One patient withdrew after study drug injection; and one patient was lost-to-follow-up.

Summaries of patient disposition are provided in the table below.

Table 35: Patient disposition – Study NEO3-09

		Tumor Type		
		Melanoma (N=86)	Breast Cancer (N=77)	Overall (N=163)
Completed		76 (88.4%)	76 (98.7%)	152 (93.3%)
Withdrawn		10 (11.6%)	1 (1.3%)	11 (6.7%)
	Adverse Event	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Protocol Violation	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Lost to Follow-up	0 (0.0%)	1 (1.3%)	1 (0.6%)
	Withdrawal of Consent	4 (4.7%)	0 (0.0%)	4 (2.5%)
	Other	6 (7.0%)	0 (0.0%)	6 (3.7%)

Recruitment

The study started on the 07 July 2010 (first patient enrolled) to 29 April 2011 (last patient completed). This was a single dose study, with safety follow-up at 30 days post-injection.

Conduct of the study

There were no major protocol violations in this study. There were no violations of inclusion criteria and one violation of exclusion criteria: one patient was indicated to have a tumour with a Breslow depth less than 0.75 mm. The patient was enrolled, and a waiver was granted, because the Breslow depth was unable to be determined at the time of screening due to primary tumour location underneath the right index fingernail. Final pathology confirmation of disease revealed tumour with Clark Level 4 (4.1 mm Breslow depth). This patient, therefore, met all inclusion and exclusion criteria and was included in the study analyses.

The largest contribution of protocol deviations involved vital sign and laboratory measurements; these deviations were independently reviewed by medical consultants and were considered not clinically significant. Thus, the deviations were considered not to have significantly influenced study results.

There no were episodes of noncompliance within the safety population of patients receiving the study agent, Tc-99m Lymphoseek, at the indication total dose (50 µg).

Baseline data

The baseline demographic and disease characteristics of the patients in study NEO3-09 are listed in the 2 tables below.

Table 36: Summary of patient demographics – Study NEO3-09 (safety population)

Age (years)	
N	153
Mean ± SD	59.3 ± 12.40
Median	61.0
Min – Max	26 – 88
Gender	
Male	49 (32.0%)
Female	104 (68.0%)
Race	
White	142 (92.8%)
Black	4 (2.6%)
Asian	6 (3.9%)
American Indian or Alaskan Native	0 (0.0%)
Native Hawaiian or Other Pacific Islander	1 (0.7%)
Ethnicity	
Hispanic	5 (3.3%)
Non-Hispanic	137 (89.5%)
Not Reported	11 (7.2%)
Tumor Type	
Melanoma	76 (49.7%)
Breast Cancer	77 (50.3%)
Height (in)	
N	153
Mean ± SD	66.52 ± 4.079
Median	66.00
Min – Max	59.0 – 81.2
Weight (lbs)	
N	153
Mean ± SD	181.36 ± 48.739
Median	175.00
Min - Max	94.0 – 372.6

Table 37: Baseline disease characteristics and ECOG performance status – Study NEO3-09 (safety population)

Disease Characteristic	Category	Tumor Type: Melanoma (N=76)	Tumor Type: Breast Cancer (N=77)	Overall (N=153)
Current Clinical T Staging	TX	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Tis	0 (0.0%)	6 (7.8%)	6 (3.9%)
	T1	10 (13.2%)	63 (81.8%)	73 (47.7%)
	T2	33 (43.4%)	7 (9.1%)	40 (26.1%)
	T3	20 (26.3%)	0 (0.0%)	20 (13.1%)
	T4	13 (17.1%)	1 (1.3%)	14 (9.2%)
Current Clinical N Staging	N0	76 (100.0%)	77 (100.0%)	153 (100.0%)
	N1	0 (0.0%)	0 (0.0%)	0 (0.0%)
	N2	0 (0.0%)	0 (0.0%)	0 (0.0%)
	N3	0 (0.0%)	0 (0.0%)	0 (0.0%)
	N4	0 (0.0%)	0 (0.0%)	0 (0.0%)
Current Clinical M Staging	M0	76 (100.0%)	77 (100.0%)	153 (100.0%)
ECOG Performance Status	0	72 (94.7%)	69 (89.6%)	141 (92.2%)
	1	3 (3.9%)	8 (10.4%)	11 (7.2%)
	2	1 (1.3%)	0 (0.0%)	1 (0.7%)
	3	0 (0.0%)	0 (0.0%)	0 (0.0%)
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)

[a] The denominator for all percentages calculated in this table is the number of patients in the safety population.

Numbers analysed

The total number of accrued patients was prospectively estimated to be between 124 and 155 in order to provide the estimated number of VBD-stained lymph nodes to power the primary outcome; 165 patients were enrolled; 153 patients were injected with Tc 99m Lymphoseek.

A total of five populations were planned and analysed: the intent-to-treat (ITT) population (N=133), the reverse ITT (RITT) population (N=152), the per protocol (PP) population (N=133), the safety population (N=153), and the safety PP population (N=153).

Outcomes and estimation

Concordance Rate between Tc-99m Lymphoseek and Vital Blue Dye

All 229 lymph nodes in the ITT node population that were detected by VBD (blue) were also detected by Tc-99m Lymphoseek (hot nodes; ≥3σ counts). The primary endpoint, the corresponding nodal concordance rate (PC1), was thus 100%, and the exact binomial test of this result, against the null hypothesis H0: PC1 ≤ 0.90, was highly significant at $p<0.0001$

Additionally, the concordance rates for nodes from melanoma and breast cancer patients were also 100%.

Since the PP and ITT populations were identical, results were the same for the PP population.

Table 38: Count and proportion of concordant nodes – Study NEO3-09

	No. (Proportion) of Concordant Nodes (Total ITT Nodes = 229)	95% Exact Binomial Confidence Interval for Proportion	1-Sided p-Value for Exact 1-Sample Binomial Test of H_0: $P_{C1} \leq 0.90$
Concordant Nodes [a]	229 (1.0000)	(0.9840, 1.0000)	<0.0001
From Melanoma Patients (Nodes from Melanoma Patients = 116)	116 (1.0000)		
From Breast Cancer Patients (Nodes from Breast Cancer Patients = 113)	113 (1.0000)		

[a] Concordant nodes are nodes that were determined in vivo to be blue (due to presence of VBD) that were also hot (due to presence of Tc 99m Lymphoseek).

The concordance rate for every study site was 100%. The average volume of Tc-99m Lymphoseek injected at each study site ranged from 0.14 to 2.60 ml.

At a patient level, the overall concordance rate of detection by both VBD and Tc-99m Lymphoseek in the ITT population (N=133) was 100%. Similarly, the concordance rates for melanoma and breast cancer patients were both 100%. Since the PP and ITT populations were identical, results were the same for the PP population.

Table 39: Count and proportion of concordant patients – Study NEO3-09

	No. (Proportion) of Concordant Patients (ITT = 133)	95% Exact Binomial Confidence Interval for Proportion
Concordant Patients [a]	133 (1.0000)	(0.9726, 1.0000)
Concordant Melanoma Patients (Total Melanoma Patients = 65)	65 (1.0000)	
Concordant Breast Cancer Patients (Total Breast Cancer Patients = 68)	68 (1.0000)	

[a] Concordant patients are patients for whom all nodes that were determined in vivo to be blue (due to presence of VBD) were also hot (due to presence of Tc 99m Lymphoseek).

Reverse Concordance Rate and Superiority

Based on the RITT analysis population (N=378 nodes from 152 patients), nodal reverse concordance of VBD against Tc-99m Lymphoseek was 60.58% (229 nodes). There was 58.88% reverse concordance in melanoma patients (116 out of 197 nodes), and 62.43% reverse concordance in breast cancer patients (113 out of 181 nodes). The one-sided p-value for the test of reverse concordance against the null hypothesis H0: PC1 ≤ PC3 was significant (p<0.0001).

Table 40: Count and proportion of reverse concordant nodes – Study NEO3-09 (RITT population)

	No. (Proportion) of Reverse Concordant Nodes (Total RITT Nodes = 378)	95% Exact Binomial Confidence Interval for Proportion	1-Sided p-Value for Test of H_0: $P_{C1} \leq P_{C3}$
Reverse Concordant Nodes [a]	229 (0.6058)	(0.5546, 0.6554)	<0.0001
From Melanoma Patients (Nodes from Melanoma Patients = 197)	116 (0.5888)		
From Breast Cancer Patients (Nodes from Breast Cancer Patients = 181)	113 (0.6243)		

[a] Reverse concordant nodes are nodes that were determined in vivo to be hot (due to presence of Tc 99m Lymphoseek) that were also blue (due to presence of VBD).

Additionally, a one-sided test of reverse concordance against the anticipated rate of 90% (i.e., null hypothesis H0: PC3 ≤ 0.90) was not significant (p=1.0000).

On a patient level, the overall reverse concordance rate of VBD against Tc-99m Lymphoseek in the RITT population was 50% (76 out of 152 patients). There was 49.33% reverse concordance in melanoma patients (37 out of 75 patients), and 50.65% reverse concordance in breast cancer patients (39 out of 77 patients).

Table 41: Count and proportion of reverse concordant patients – Study NEO3-09 (RITT population)

	No. (Proportion) of Reverse Concordant Patients (RITT = 152)	95% Exact Binomial Confidence Interval for Proportion
Reverse Concordant Patients [a]	76 (0.5000)	(0.4179, 0.5821)
Reverse Concordant Melanoma Patients (Total Melanoma Patients = 75)	37 (0.4933)	
Reverse Concordant Breast Cancer Patients (Total Breast Cancer Patients = 77)	39 (0.5065)	

[a] Reverse concordant patients are patients for whom all nodes that were determined in vivo to be hot (due to presence of Tc 99m Lymphoseek) were also blue (due to presence of VBD).

Pathology Diagnostic Performance

In the safety population (N=379 nodes in 153 patients), 40 nodes (10.55%) were determined to be pathologically positive. Of the 40 pathology-positive nodes, 30 were detected by both Tc-99m Lymphoseek and VBD, 10 were detected only by Lymphoseek, and none were detected by only VBD or by neither detection method. Since the safety PP and safety populations were identical, results were the same for the safety PP population.

Table 42: Number and Proportion of Pathology-Positive Nodes by Lymphoseek and VBD Detection Categories – Study NEO3-09 (Safety Population)

Total Number of Safety Population Nodes = 379			
Detection Category for Pathology-Positive Nodes	**No. of Nodes**	**Proportion of Safety Nodes**	**95% Exact Binomial Confidence Interval for Proportion**
Vital Blue Dye + / Tc 99m Lymphoseek +	30	0.0792	(0.0540, 0.1111)
Vital Blue Dye + / Tc 99m Lymphoseek –	0	0.0000	(0.0000, 0.0097)
Vital Blue Dye – / Tc 99m Lymphoseek +	10	0.0264	(0.0127, 0.0480)
Vital Blue Dye – / Tc 99m Lymphoseek –	0	0.0000	(0.0000, 0.0097)
Total Pathology-Positive Nodes	40	0.1055	

In the safety population, 40 of the 40 pathology-positive nodes were detected by Tc-99m Lymphoseek, and 30 of the 40 were detected by VBD. The corresponding sensitivity rate for detection of lymph nodes for tumour cells was 100% for Tc-99m Lymphoseek, compared to 75% for VBD. The false negative rate was 25% for VBD and 0% for Tc-99m Lymphoseek. Since the safety PP and safety populations were identical, results were the same for the safety PP population.

Table 43: Sensitivities and False Negative Rates for Tc-99m Lymphoseek and VBD – Study NEO3-09 (Safety Population)

Total Number of Pathology-Positive Nodes = 40			
Category	**No. of Nodes**	**Proportion / Rate**	**95% Exact Binomial Confidence Interval for Proportion**
Vital Blue Dye + / Pathology +	30		
Vital Blue Dye – / Pathology +	10		
Tc 99m Lymphoseek + / Pathology +	40		
Tc 99m Lymphoseek – / Pathology +	0		
Vital Blue Dye Sensitivity		0.7500	(0.5880, 0.8731)
Tc 99m Lymphoseek Sensitivity		1.0000	(0.9119, 1.0000)
Vital Blue Dye False Negative Rate		0.2500	(0.1269, 0.4120)
Tc 99m Lymphoseek False Negative Rate		0.0000	(0.0000, 0.0881)

A summary of excised nodes for each detection category is shown in the table below. All 379 safety nodes, pathologically positive or negative, were detected by Tc-99m Lymphoseek.

In patients with melanoma, for the 28 pathology-positive nodes, 20 were detected by both Tc-99m Lymphoseek and VBD, and eight by Tc-99m Lymphoseek only.

In patients with breast cancer, for the 12 pathology-positive nodes, 10 were detected by both Tc-99m Lymphoseek and VBD, and two by Tc-99m Lymphoseek only.

Overall, for the 339 pathology-negative nodes, 199 were detected by both Tc-99 m Lymphoseek and VBD and 139 by Tc-99m Lymphoseek only.

Table 44: Summary of Excised Nodes for Each Detection Category – Study NEO3-09 (Safety Population)

Category	Melanoma (N=76)	Breast Cancer (N=77)	Overall (N=153)
Total Excised Nodes	198	181	379
Total Pathology+	28 (14.1%)	12 (6.6%)	40 (10.6%)
Total Pathology–	170 (85.9%)	169 (93.4%)	339 (89.4%)
Tc 99m Lymphoseek+/Vital Blue Dye+/Pathology+	20 (10.1%)	10 (5.5%)	30 (7.9%)
Tc 99m Lymphoseek–/Vital Blue Dye+/Pathology+	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tc 99m Lymphoseek+/Vital Blue Dye–/Pathology+	8 (4.0%)	2 (1.1%)	10 (2.6%)
Tc 99m Lymphoseek+/Vital Blue Dye+/Pathology–	96 (48.5%)	103 (56.9%)	199 (52.5%)
Tc 99m Lymphoseek–/Vital Blue Dye+/Pathology–	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tc 99m Lymphoseek+/Vital Blue Dye–/Pathology–	73 (36.9%)	66 (36.5%)	139 (36.7%)

There were 33 patients with at least one pathology-positive lymph node (21 for melanoma, 12 for breast cancer). Of these, there were 10 patients (8 melanoma and 2 breast cancer) for whom all of their pathology-positive lymph nodes were hot, but at least one was not blue. Of these 10 patients, six patients had all of their pathology-positive lymph nodes hot and not blue. No patients had any of their pathology-positive lymph nodes blue and not hot. Therefore, 3.92% (6/153) of the safety population were upstaged by Tc-99m Lymphoseek findings alone, and none was upstaged by VBD.

Table 45: Summary by Patient of Mapping Agent-Missed Pathology-Positive Nodes – Study NEO3-09 (Safety Population)

Category[a,b,c]	Melanoma (N=76)	Breast Cancer (N=77)	Overall (N=153)
No. of Patients with ≥ 1 Pathology+ Lymph Node	21	12	33
No. of Patients Such That All Pathology+ Lymph Nodes are Both Hot and Blue	13 (61.90%)	10 (83.33%)	23 (69.70%)
No. of Patients Such That All Pathology+ Lymph Nodes are Hot but at Least One is Not Blue	8 (38.10%)	2 (16.67%)	10 (30.30%)
No. of Patients Such that All Pathology+ Lymph Nodes are Hot and Not Blue	4 (19.05%)	2 (16.67%)	6 (18.18%)
No. of Patients Such That All Pathology+ Lymph Nodes are Blue but at Least One is Not Hot	0 (0.00%)	0 (0.00%)	0 (0.00%)
No. of Patients Such that All Pathology+ Lymph Nodes are Blue and Not Hot	0 (0.00%)	0 (0.00%)	0 (0.00%)
No. of Patients Such That at Least One Pathology+ Lymph Node is Not Hot and at Least One is Not Blue	0 (0.00%)	0 (0.00%)	0 (0.00%)
No. of Patients Such that All Pathology+ Lymph Nodes are Not Hot and Not Blue	0 (0.00%)	0 (0.00%)	0 (0.00%)

[a] Palpable masses are not included in this table.

[b] Patients are only counted once per category for which they qualify.

[c] The denominator for all percentages is the total number of patients with at least one pathology-positive lymph node with the respective tumor type.

There were 19 patients in whom VBD failed to detect any nodes. Of those 19 patients, four had pathology-positive nodes. The pathology rate (percent of patients with pathology positive nodes) in these patients was 21.1% (4/19). In the remaining portion of patients, i.e., 133 patients for whom VBD detected at least one lymph node, 29 patients had at least one pathology-positive node. The pathology rate in these 133 patients was 21.8%. However, of the 33 patients with pathology-positive nodes, Tc-99m Lymphoseek detected 100% of those nodes. Therefore, Tc-99m Lymphoseek pathology elucidation rate was clinically important in the overall assessment of 21.1% of the VBD patient assessment failures (4/19) for disease status, and potentially influential in post-surgical therapy for these patients.

Summary of Study NEO3-09

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Title: A Phase 3, Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek and Vital Blue Dye as Lymphoid Tissue Targeting Agents in Patients With Known Melanoma or Breast Cancer Who Are Undergoing Lymph Node Mapping			
Study identifier	**NEO3-09**		
Design	Phase 3, prospective, open label, multicentre, single arm, within patient, comparative study of Lymphoseek and VBD (Lymphazurin as 1% isosulfan blue of injection)		
Treatments (within patient comparison) *ITT: 133 patients* *Melanoma: 65 patients* *Breast cancer: 68 patients*	Test		**Technetium Tc-99m Lymphoseek®** Injection was given in 50 µg doses by intradermal, periareolar, subareolar, or peritumoral injection in close proximity to the primary tumour.
	Comparator		The above injection was followed by injection of **vital blue due**, and followed by intraoperative lymph nodal mapping.
Endpoints and definitions	Primary endpoint	Concordance rate between LS and a VBD in the *in vivo* detection of lymph node(s), per nodal level; P_{C1}.	The proportion of lymph nodes identified *in vivo* by VBD that were also identified *in vivo* by LS. $P_{C1} = \frac{\text{\# of nodes that were VBD-stained and were also Lymphoseek hot}}{\text{\# of VBD-stained nodes}}$
	Secondary endpoint	"Per patient" concordance rate between LS and a VBD in the *in vivo* detection of lymph node(s); P_{C2}.	$P_{C2} = \frac{\text{\# of patients for whom all VBD-stained nodes were also Lymphoseek hot}}{\text{\# of patients with at least one VBD-stained node}}$
	Secondary endpoint	Sensitivity and False Negative Rate	VBD sensitivity: $\frac{\text{Path+/ VBD+}}{\text{All Path+ Nodes}}$ VBD FNR: $\frac{\text{Path+/VBD–}}{\text{All Path+ Nodes}}$
			LS sensitivity: $\frac{\text{Path+/LS+}}{\text{All Path+ Nodes}}$ LS FNR: $\frac{\text{Path+/LS–}}{\text{All Path+ Nodes}}$
	Secondary endpoint	Reverse concordance rate, per nodal level; P_{C3}.	$P_{C3} = \frac{\text{\# of Lymphoseek hot nodes that were VBD-stained}}{\text{\# of Lymphoseek hot nodes}}$ $H_0: P_{C1} \leq P_{C3}$ vs. $H_a: P_{C1} > P_{C3}$

	Secondary endpoint	Reverse concordance rate, per patient level; P_{C4}.	$P_{C4} = \frac{\#\text{ of patients for whom all Lymphoseek hot nodes were VBD-stained}}{\#\text{ of patients with at least one in vivo hot node}}$

Results and Analysis

Analysis description	**Primary Analysis**
Analysis population and time point description	Intent to treat

Descriptive statistics and estimate variability	Treatment group	Concordant nodes (total ITT nodes=229)	Concordant patients (total ITT patients=133)	Reverse concordant nodes (total RITT nodes=378)	Reverse concordant patients (total RITT patients=152)
	Number (proportion) of concordant nodes/patient	229 (1.000)	133 (1.000)	229 (0.6058)	76 (0.5000)
	95% Exact binomial CI for proportion	0.9840-1.000	0.9726-1.000	0.5546-0.6554	0.4179-0.5821
	p-value	<0.0001[a]		<0.0001[b]	

Sensitivities and FNR	category	No of Path+ nodes (total=40)	Proportion/rate	95% exact binomial CI for proportion
	Vital blue dye+/pathology+	30	-	-
	Vital blue dye-/pathology+	10	-	-
	Lymphoseek +/Pathology +	40	-	-
	Lymphoseek -/Pathology +	0	-	-
	Vital blue dye sensitivity	-	0.7500	0.5880-0.8731
	Lymphoseek sensitivity	-	1.000	0.9119-1.000
	Vital blue dye NPV	-	0.2500	0.1269-0.4120
	Lymphoseek NPV	-	0.000	0.000-0.0881

a= p-value of P_{c1} vs 0.90; b=p-value of P_{C1} vs P_{C3}

NEO3-06: A Phase 3, Prospective, Open-Label, Multicenter Study of Lymphoseek-Identified Sentinel Lymph Nodes (SLNs) Relative to the Pathological Status of Non-Sentinel Lymph Nodes in an Elective Neck Dissection (END) in Cutaneous Head and Neck and Intraoral Squamous Cell Carcinoma

Methods

Study Participants

Inclusion criteria

1. The patient provided written informed consent with Health Information Portability and Accountability Act (HIPAA) authorization before participating in the trial.

2. The patient had a diagnosis of primary SCC of the head and neck either cutaneous or intraoral that was anatomically located in the mucosal lip, buccal mucosa, lower alveolar ridge, upper alveolar ridge, retromolar gingival (retromolar trigone), floor-of-the-mouth, hard palate or oral (mobile) tongue and was stage T1-T4a, N0, M0.

3. Clinical nodal staging (N0) was confirmed by negative results from contrast CT scan or gadolinium-enhanced MRI or lateral and central neck ultrasound within 30 days of the planned lymphadenectomy. PET scan could not have been used for this evaluation.

4. Imaging of the regional nodal basin was performed within 30 days of the planned lymphadenectomy.

5. The patient was a candidate for surgical intervention, with ILM and END included in the surgical plan.

6. Patients with prior malignancy were allowed provided the patient met both of the following criteria:

• Underwent potentially curative therapy for all prior malignancies and was deemed low risk for recurrence.

• No malignancy for the past 5 years (except effectively treated basal cell or squamous cell skin cancer, carcinoma in situ of the cervix effectively treated with surgery alone, lobular carcinoma in situ of the ipsilateral or contralateral breast treated with surgery alone, or carcinoma of the mouth that was in situ or minimally invasive), and no evidence of recurrence.

7. The patient was at least 18 years of age at the time of consent.

8. The patient had an Eastern Cooperative Oncology Group (ECOG) status of Grade 0 to 2 (see Appendix 5: Performance Status Criteria of the protocol [Appendix 16.1.1]).

Exclusion criteria

1. The patient had a diagnosis of SCC of the head and neck in the following anatomical areas: non-mobile base of the tongue, oropharynx, nasopharynx, hypopharynx, and larynx.

2. The patient was pregnant or lactating.

3. The patient had clinical or radiological evidence of metastatic cancer to the regional lymph nodes.

4. The patient had a history of neck dissection, or gross injury to the neck that precluded reasonable surgical dissection for this trial, or radiotherapy to the neck.

5. The patient had had other nuclear imaging studies, including technetium, conducted within 2.5 days (60 hours) of injection.

6. The patient was actively receiving systemic cytotoxic chemotherapy.

7. The patient was currently participating in another investigational drug trial or participated within 30 days before consenting.

8. Patient was on immunosuppressive, anti-monocyte, or immunomodulatory therapy.

Treatments

All patients received Lymphoseek, which was administered in the Nuclear Medicine Department or clinic room by a certified nuclear medicine physician or surgeon.

Patients scheduled for surgery on the same day of injection received 0.5 mCi (18.5 MBq) Lymphoseek (50 µg) and patients scheduled for surgery the next day received 2.0 mCi (74 MBq) Lymphoseek (50 µg).

Injection of Lymphoseek was at time point 00:00. Patients received 50 µg of Lymphoseek in the total injection volume of 0.1 to 1.0 mL. Individual injections (aliquots) did not exceed 0.5 mL or were less than 0.1 mL. All injections were peritumoral. A total volume of 0.1 to 0.5 mL was injected from a single syringe in a single injection overlying the primary tumour or a total volume of 0.5 to 1.0 mL was injected from multiple syringes into 4 aliquots at 12:00, 3:00, 6:00 and 9:00 positions of the clock peritumorally, or 5 aliquots at 12:00, 3:00, 6:00 and 9:00 positions of the clock peritumorally and to the deepest side of the tumour.

Patients with intraoral SCC and cutaneous SCC had a same-day surgery injection radiolabeled with 0.5 mCi (18.5 MBq) Tc 99m (recommended time from injection to surgery 1 to less than 15 hours) or 2.0 mCi (74 MBq) Tc 99m for injections on day before surgery (recommended time from injection to surgery 15 to 30 hours).

Objectives

Primary Objective:

To determine the false negative rate (FNR) associated with Lymphoseek-identified SLNs relative to the pathology status of non-SLNs. [The FNR was the ratio of false negatives/(true positives + false negatives). The estimate was made on a per-patient basis and relative to those patients with pathology-positive nodes.]

Secondary Objectives:

To determine the sensitivity, negative predictive value (NPV), and overall accuracy of Lymphoseek-detected SLNs relative to the pathology status of non-SLNs. Additional secondary objectives included the detection rate of SLNs by Lymphoseek and the rate of tumor detection in non-SLNs.

Safety Objective:

To evaluate patient safety through observation of adverse events (AEs), clinical laboratory tests, vital signs, electrocardiograms (ECGs), and physical examinations.

Outcomes/endpoints

Efficacy Analysis:

The primary efficacy endpoint was the FNR associated with Lymphoseek-identified SLNs relative to non-SLNs. Lymphoseek-identified lymph nodes constituted the *in vivo*-identified lymph nodes such that, when counted with a handheld gamma detector, counts met or exceeded the defined 3 sigma gamma counting rule (3σ rule).

The estimate was made on a per-patient basis and relative to those patients with pathology-positive nodes as assessed by local or central pathology. Where both pathology assessments were conducted but differed (i.e., local laboratory results were negative but central laboratory results were positive), the central pathology results determined the final pathology status of the node.

Lymphoseek positivity was based on radioactivity or counts derived from the application of the handheld gamma detector *in vivo*, where such counts satisfied the 3σ rule of greater than the quantity of 3 square roots of the mean background count (i.e., 3 standard deviations [SD]) added to the mean background count. Any nodal count not meeting this 3σ rule was considered a negative finding (not localized and/or not detected as an SLN).

Once *in vivo* SLN(s) were identified by the presence of Lymphoseek, they were removed and their ex vivo radioactivity status was also determined. All tissue excised to the extent that such tissue represented anatomical locations that harbored SLN(s) were also counted ex vivo with a handheld gamma detector. Once probing was complete, the remaining non-SLNs were also removed and assessed by pathology. All tissues were labeled and documented with regard to patient, anatomical location, and Lymphoseek status (+/- with regard to *in vivo* comparison to the 3σ rule)

An initial local pathology status of the SLNs was determined via hematoxylin and eosin (H&E) staining. This primary trial site evaluation after bifurcation through the long axis of the lymph nodes consisted of sectioning the node every 2 to 3 mm producing at least 3 levels through the node for evaluation. Had this initial evaluation of Lymphoseek-positive nodes been positive for any tumor presence, this was recorded and no further local pathology evaluation was required. If this initial pathology evaluation was negative, additional sectioning and staining at the site were permitted based on institutional standards. Pathology status for tumor in all non-SLNs was categorized as positive or negative based on the slide derived from the single bifurcation.

Secondary measures of efficacy included selected measures of diagnostic performance (sensitivity, NPV, and overall accuracy) of Lymphoseek-identified SLN(s) in relationship to the non-SLN(s).

The secondary efficacy variables were the following proportions and rates:

• sensitivity of Lymphoseek to detect patients with at least 1 pathology-positive SLN (sensitivity = 1-FNR)

• negative predictive value (NPV) of Lymphoseek for detection of patients with at least 1 pathology-positive SLN

• overall accuracy of Lymphoseek in classifying patients with at least 1 pathology-positive lymph node versus those with all negative nodes

• by-patient rate (proportion) of lymph node detection by Lymphoseek

• by-patient rate (proportion) of tumor detection in SLNs

• by-patient rate (proportion) of tumor detection in all lymph nodes (both SLNs and non- SLNs)

A true negative was defined as a patient for whom all SLNs identified by Lymphoseek were pathology negative or no SLNs were identified and all non-SLNs were pathology negative. A true positive was defined as a patient for whom at least one Lymphoseek-identified SLN was pathology positive. Patients lacking an SLN detected by Lymphoseek but having an otherwise positive non-SLN were counted as a false negative.

The by-patient rate (proportion) of the SLN detection by Lymphoseek, the by-patient rate (proportion) of tumor detection in SLNs, and the by-patient rate (proportion) of tumor detection in all lymph nodes were determined as additional secondary measures of efficacy.

Exploratory analyses used the primary and secondary efficacy variables, categorized by trial site, anatomical tumor location, and time of surgery relative to Lymphoseek administration. There were no new exploratory efficacy variables.

Safety:

Safety was evaluated by examining the incidence of AEs, changes over time in laboratory tests, vital signs, ECGs, and physical examination findings.

Sample size

A total of 392 patients were expected to be accrued in order to yield 114 patients with 1 or more pathology-positive lymph nodes from the END (SLNs and/or non-SLNs). At the prospectively planned interim analysis, a total of 85 patients were injected (thus, analyzed for safety), 83 patients were in the intent-to-treat (ITT) population (i.e., must have been injected, gone to surgery, and had at least 1 SLN or non-SLN removed with a known pathology status), and 39 patients in the ITT were pathology positive.

Randomisation

The study was non-randomised.

Blinding (masking)

The study was a single arm study.

Statistical methods

For the primary endpoint, the FNR was calculated as the ratio of false negatives/(true positives + false negatives). More specifically, the FNR was defined as follows:

FNR = (# of patients with ≥ 1 pathology-positive lymph node for whom Lymphoseek did not detect any pathology-positive SLNs) ÷ (total # of patients with ≥ 1 pathology-positive lymph node)

	≥ 1 Lymph Nodes (SLN or non-SLN) Are Pathology Positive	All Lymph Nodes (SLN and non-SLN) Are Pathology Negative
≥ 1 Lymphoseek Detected Lymph Node(s) (SLNs) Are Pathology Positive	a	N/A (b = 0)
Lymphoseek Detected Lymph Nodes (SLNs) Are All Pathology Negative (or no SLNs exist)	c	d

Source: NEO3-06 SAP version 2.0 (Appendix 16.1.9)
Abbreviations: N/A = not applicable; SLN = sentinel lymph nodes.

The primary endpoint was summarized by use of a point estimator and a 2-sided exact 95% confidence interval (CI) computed on a binomial proportion. The point estimate for FNR was simply the observed rate. The statistical hypotheses.

H0: FNR ⩾ 0.14 vs. Ha: FNR < 0.14
were tested using a 1-sided significance level of α=0.025 (2-sided significance level of 0.05), such that if the upper limit of the 95% CI for the FNR was less than 0.14, the null hypothesis was rejected in favor of the alternate hypothesis.

In order to maintain the overall level of significance at the 1-sided α = 0.025 level after a prospectively planned interim analysis using α = 0.02486, this test would have been performed using an adjusted 1-sided α = 0.00032 at the completion of the trial if the trial was not stopped for efficacy at the interim analysis.

For the secondary measures, sensitivity was calculated as one minus the FNR (1 – FNR). The remaining measures of diagnostic performance were defined as follows:

• Sensitivity of Lymphoseek to detect patients with at least 1 pathology-positive lymph node:

Sensitivity = 1 – FNR = a/(a+c)

• Negative predictive value (NPV) of Lymphoseek for detection of patients with at least 1 pathology-positive lymph node:

NPV = # patients with a true negative/# patients with true negative or false negative

NPV = d/(c+d)

• Overall accuracy of Lymphoseek in classifying patients with at least 1 pathology-positive lymph node versus those with all negative nodes:

Overall accuracy = # patients with a true positive or a true negative/# ITT patients

Accuracy = (a+d)/(a+c+d)

• A by-patient rate (proportion) of tumor detection in all lymph nodes taken from the END (both SLNs and non-SLNs):

Overall Proportion of Patients with Tumors = (a+c)/(a+c+d)

The final 2 secondary endpoints were calculated as shown below:

• A by-patient rate (proportion) of SLN detection (i.e., lymph node detection by Lymphoseek):

Rate of SLN Detection = # of patients with SLNs total # of patients in ITT

• A by-patient rate (proportion) of tumor detection in SLNs:

Proportion of Patients = # of patients with ≥ 1 pathology-positive SLN with Tumors in SLN(s) total # of patients in ITT

Point estimates and CIs were used to summarize the secondary endpoints and subgroup analyses. These estimators were based on patient counts. The CIs were calculated using exact methods with a 95% coverage probability.

Summarization of safety data included AEs, laboratory tests, ECGs, physical examinations, and vital signs.

Prospectively Planned Interim Analysis

A group sequential design was used as a guideline to monitor the ongoing FNR results. The stopping rule was based on a 1-sided test, assumed 2 unequally spaced analysis points (i.e., after 38 and 114 patients with a pathology-positive SLN and/or non-SLN), and had α (1-sided) and 1-β probabilities equal to 0.025 and 0.90, respectively. The interim and final alpha levels were computed using the Wang-Tsiatis stopping boundaries:

$$l = -ck\ \rho\ -1/\ 2\ k$$

$$u = ck\ \rho\ -1/\ 2\ k$$

The shape parameter (ρ) was set to 1.0, while k represented the analysis number (1 or 2) and c was set so that the type I error was equal to α. Early stopping occurred for positive (low FNR) results if the interim significance level for a 1-sided exact test of binomial proportion, after ≥ 38 patients with a pathology-positive lymph node, was ≤ 0.02486.

An independent DSMC reviewed the interim results and made recommendations regarding early stopping and/or changes in the trial design.

Results

Participant flow

A total of 117 patients were screened: 101 patients were enrolled into the trial and 16 patients were evaluated to be screen failures. Of the 101 enrolled patients, 85 patients were injected with Lymphoseek and all 85 (84.2%) patients completed the trial. Of the 101 patients who were enrolled into the trial, a total of 16 (15.8%) patients were withdrawn.

Table 46: Patient disposition – Study NEO6-09 (all screened patients)

	Tumor Type		
	Cutaneous (N=8)	Intraoral (N=109)	Overall (N=117)
Screen Failure	2	14	16
Enrolled	6	95	101
Injected	6	79	85
Completed	6 (100.0%)	79 (83.2%)	85 (84.2%)
Withdrawn	0	16 (16.8%)	16 (15.8%)
Adverse Event	0	0	0
Protocol Violation	0	0	0
Lost to Follow-up	0	0	0
Withdrawal of Consent	0	12 (12.6%)	12 (11.9%)
Death	0	0	0
Other	0	4 (4.2%)	4 (4.0%)

Recruitment

A total of 117 patients were screened: 101 patients were enrolled into the trial and 16 patients were evaluated to be screen failures.

Conduct of the study

The original trial protocol was revised 7 times with main changes being related to update of sample size, clinical trial information, naming conventions and editorial changes, clarification on the pathology process and procedures, collection of slides sections and ICH staining for central pathology, clarification on the administration of the medicinal product and the detection of radioactivity. Further amendments were made on the SAP.

Major protocol violations occurred for 9 intraoral-tumor patients in this trial. Four of the 9 patients had violations of Lymphoseek dosing (either too low or too high dose). Two patients had violations of END (END was performed before SLN mapping for one patient and not performed for another patient. Other major violations occurred for 1 patient each: imaging of the regional nodal basin was performed outside of the 30-day window and no waiver was requested; no *in vivo* count was found for a third lymph node for a patient; and an informed-consent violation occurred for a patient (an ultrasound to confirm nodal status was performed the day before the patient was consented for the trial).

Baseline data

Table 47: Demographics data – Study NEO3-09 (safety population)

Demographics Variable	Category	Safety Subjects (N=85)
Gender	Male	64 (75.3%)
	Female	21 (24.7%)
Race	White	83 (97.6%)
	Black	1 (1.2%)
	Asian	0
	American Indian or Alaskan Native	1 (1.2%)
	Native Hawaiian or Other Pacific Islander	0
Ethnicity	Hispanic	10 (11.8%)
	Non-Hispanic	75 (88.2%)
Tumor Type	Cutaneous	6 (7.1%)
	Intraoral	79 (92.9%)

Demographics Variable	Mean	SD	N	Min	Max	Median
Age of Patient (years)	60.79	12.832	85	23.00	87.00	59.00
Height of Patient (inches)	67.93	3.393	84	61.20	75.00	68.00
Weight of Patient (pounds)	189.09	49.534	85	85.00	355.00	186.30

Table 48: Baseline disease characteristics and ECOG PS – Study NEO3-09 (safety population)

		Tumor Type		
Disease Characteristic	Category	Cutaneous (N=6)	Intraoral (N=79)	Overall (N=85)
Current Clinical T Staging	TX	0	0	0
	TIS	0	0	0
	T1	0	26 (32.9%)	26 (30.6%)
	T2	6 (100.0%)	40 (50.6%)	46 (54.1%)
	T3	0	7 (8.9%)	7 (8.2%)
	T4	0	2 (2.5%)	2 (2.4%)
	T4a	0	4 (5.1%)	4 (4.7%)
Current Clinical N Staging	N0	6 (100.0%)	79 (100.0%)	85 (100.0%)
	N1	0	0	0
	N2	0	0	0
	N3	0	0	0
	N4	0	0	0
Current Clinical M Staging	M0	6 (100.0%)	78 (98.7%)	84 (98.8%)
	MX	0	1 (1.3%)	1 (1.2%)
ECOG Performance Status	0	5 (83.3%)	53 (67.1%)	58 (68.2%)
	1	1 (16.7%)	21 (26.6%)	22 (25.9%)
	2	0	5 (6.3%)	5 (5.9%)
	3	0	0	0
	4	0	0	0

Numbers analysed

Three patient populations were used to analyze and report the data: A total of 3 populations were analyzed in this trial: the ITT population (N=83), the PP population (N=75), and the safety population (N=85). The ITT population included all patients injected with Lymphoseek, who underwent surgery, and who had 1 or more SLNs or non-SLNs with a known pathology status. The PP population included all ITT patients who lacked major protocol violations where such violations compromised the assessment of the efficacy endpoints. The safety population included patients with signed informed consent and who received any injection of Lymphoseek.

A total of 18 patients were excluded from the number of all enrolled patients (N=101) to define the ITT population (N=83): 16 of these patients were excluded because they were not injected with Lymphoseek

and 2 patients were excluded because they did not have intraoperative SLNB or END, and therefore had no SLN or non-SLN with known pathology.

The analysis of the primary and secondary endpoints and subgroup analyses were made using the ITT population as defined, whereas the PP patients were used in a supportive manner.

Exploratory analyses, which used the primary and secondary efficacy variables, were conducted using the ITT population. All analyses of safety were based on the safety population, as were summaries of baseline data.

Outcomes and estimation

Primary Analysis (False Negative Rate of Lymphoseek)

The primary efficacy endpoint was the FNR for patients in the ITT population with at least 1 pathology-positive lymph node that was not an SLN. Lymphoseek detection of pathology-positive lymph nodes is presented in Table 49.

Table 49: Lymphoseek detection of pathology-positive lymph nodes – Study NEO3-06 (ITT population)

		Pathology	
		+	-
	Lymphoseek Detection:	**≥ 1 Lymph Nodes (SLN or non-SLN) Are Pathology Positive**	**All Lymph Nodes (SLN and non-SLN) Are Pathology Negative**
+	≥ 1 Lymphoseek Detected Lymph Node(s) (SLNs) Are Pathology Positive	38 True Positive	N/A (0)
-	Lymphoseek Detected Lymph Nodes (SLNs) Are ALL Pathology Negative (or no SLNs exist)	1 False Negative	44 True Negative

Source: Appendix Figure 1
Abbreviations: N/A=not applicable; non-SLN(s)=non-sentinel lymph node(s); SLN(s)=sentinel lymph node(s).
False positives (upper left quadrant) were not applicable to the analysis as lymph nodes did not fit into this category (Nodes cannot be both pathology positive and pathology negative.).

The FNR of lymphoseek in the ITT population is provided in Table 50.

Table 50: False negative rate of Lymphoseek – Study NEO3-06 (ITT population)

	Number of Patients	False Negative Rate and 95.03% Exact Binomial Confidence Interval	One-Sided p-Value for Exact Binomial Test of H_0: FNR ≥ 0.14
False Negative Patients - Patients that had ≥ 1 pathology-positive lymph node, none of which were detected by Lymphoseek	1		
All Pathology-Positive Patients - Patients that had ≥ 1 pathology positive lymph node	39		
		0.0256 (0.0006, 0.1349)	0.0205

Source: Appendix Table 16
Abbreviations: FNR=false negative rate; H_0=null hypothesis; ITT=intent-to-treat.

Secondary endpoints

Sensitivity of Lymphoseek

The sensitivity of lymphoseek in the ITT population is provided in Table 51.

Table 51: Sensitivity of Lymphoseek – Study NEO3-06 (ITT population)

	Number of Patients	Sensitivity and 95% Exact Binomial Confidence Interval
True Positive Patients - Patients that had ≥ 1 pathology-positive lymph node that was detected by Lymphoseek	38	
All Pathology-Positive Patients - Patients that had ≥ 1 pathology-positive lymph node	39	
		0.9744 (0.8652, 0.9994)

Source: Appendix Table 18
Abbreviations: ITT=intent-to-treat.

The sensitivity of Lymphoseek in the PP population, where Lymphoseek detected pathology-positive nodes in 35 of 36 patients, was 0.9722 (95% CI, 0.8547 to 0.9993).

Negative Predictive Value

The negative predictive value of lymphoseek in the ITT population is provided in Table 52.

Table 52: Negative predictive value of Lymphoseek – Study NEO3-06 (ITT population)

	Number of Patients	Negative Predictive Value and 95% Exact Binomial Confidence Interval
True Negative Patients - Patients for whom all lymph nodes (SLNs and non-SLNs) were pathology negative	44	
Patients Predicted to be Pathology Negative - Patients for whom all Lymphoseek detected lymph nodes (SLNs) were pathology negative or no SLNs exist	45	
		0.9778 (0.8823, 0.9994)

Source: Appendix Table 20
Abbreviations: ITT=intent-to-treat; non-SLN(s)=non-sentinel lymph node(s); SLN(s)=sentinel lymph node(s).

The sensitivity of Lymphoseek in the PP population, where Lymphoseek detected pathology-positive nodes in 35 of 36 patients, was 0.9722 (95% CI, 0.8547 to 0.9993).

Overall Accuracy of Lymphoseek

The overall accuracy of Lymphoseek in the ITT population is provided in Table 53.

Table 53: Overall accuracy of Lymphoseek – Study NEO3-06 (ITT population)

	Number of Patients	Overall Accuracy and 95% Exact Binomial Confidence Interval
True Positive and True Negative Patients - Patients for whom all pathology-positive lymph nodes were detected by Lymphoseek (all were SLNs) or for whom all lymph nodes (SLNs and non-SLNs) were pathology negative	82	
All Patients in ITT Population - Patients who were injected with Lymphoseek, had surgery, and had at least one lymph node removed for which a pathology status was determined	83	
		0.9880 (0.9347, 0.9997)

Source: Appendix Table 22
Abbreviations: ITT=intent-to-treat; non-SLN(s)=non-sentinel lymph node(s); SLN(s)=sentinel lymph node(s).

In the PP population, 74 of the 75 patients were true positives and true negatives, which corresponded to an overall accuracy of 0.9867 (95% CI, 0.9279 to 0.9997).

Rate of Sentinel Lymph Node Detection By Lymphoseek

The rate of *in vivo* sentinel lymph node detection by Lymphoseek (i.e., SLNs as defined by the trial protocol) in the ITT population is provided in Table 54.

Table 54: Rate of *in vivo* sentinel lymph node detection in patients by lymphoseek - Study NEO3-06 (ITT population)

	Number of Patients	Rate of SLN Detection and 95% Exact Binomial Confidence Interval
Patients with SLNs Detected - Patients for whom Lymphoseek identified at least one lymph node, i.e., patients with at least one sentinel lymph node	81	
All Patients in ITT Population - Patients who were injected with Lymphoseek, had surgery, and had at least one lymph node removed for which a pathology status was determined	83	
		0.9759 (0.9157, 0.9971)

Source: Appendix Table 24
Abbreviations: ITT=intent-to-treat; SLN(s)=sentinel lymph node(s).

In the PP population, Lymphoseek identified at least 1 SLN in 74 of 75 patients, corresponding to a lymph node detection rate of 0.9867 (95% CI, 0.9279 to 0.9997).

Other secondary outcomes:

Lymphoscintigraphy was performed in 100% of patients overall: 6 and 79 patients with cutaneous and intraoral tumor types, respectively. For 92.9% of patients undergoing lymphoscintigraphy, hot spots representing hot tissues other than the injection site were noted on a scan.

Table 55: Summary of lymphoscintigraphy and *in vivo* findings per patient[a]

	LS Result			
	Patient Has Hot Spot (N=77)	Patient Does Not Have Hot Spot (N=6)	LS and Lymphoseek In Vivo Summary Results	
Lymphoseek In Vivo Status[b]				
Patient is Hot in Vivo	77 (100.0%)	4 (66.7%)		
Patient is Not Hot in Vivo	0	2 (33.3%)	Total Percent of LS and In Vivo Agreement[c]	95.2%

Source: Supplemental Table 3

Abbreviations: LS=lymphoscintigraphy; SLN=sentinel lymph node.

[a] Summary table contains results on those safety patients who had nonmissing LS result data and at least one node removed in vivo with a nonmissing pathology status. Patients who had LS performed but did not go to surgery or had no lymph node in vivo data collected were not included in this table.

[b] Lymphoseek "hot" status determined by a patient having ≥ 1 SLN.

[c] Calculated using the total counts in the upper left and lower right cells of the table divided by the total patient count in all 4.

The average number of SLNs detected per ITT patient was 3.9 hot nodes per patient.

The rate of tumor detection in SLNs in the ITT population is provided in Table 56.

Table 56: By-patient proportion of tumours detected in sentinel lymph nodes – Study NEO3-06 (ITT population)

	Number of Patients	Proportion of Patients with Tumor(s) in SLN(s) and 95% Exact Binomial Confidence Interval
True Positives - Patients that had ≥ 1 pathology-positive lymph node that was detected by Lymphoseek	38	
All Patients with ≥ 1 SLN - Patients who had at least one lymph node detected by Lymphoseek	81	
		0.4691 (0.3573, 0.5833)

Source: Appendix Table 26
Abbreviations: ITT=intent-to-treat; SLN(s)=sentinel lymph node(s).

Of the 74 patients in the PP population with at least 1 SLN, 35 patients had at least one pathology-positive lymph node that was detected by Lymphoseek. This result corresponded to a tumour-detection rate in SLNs of 0.4730 (95% CI, 0.3557 to 0.5925).

The rate of tumour detection in all lymph nodes in the ITT population is provided in Table 57.

Table 57: By-patient proportion of tumours detected in all lympho nodes – Study NEO3-06 (ITT population)

	Number of Patients	Proportion of Patients with Tumor(s) in All Lymph Nodes and 95% Exact Binomial Confidence Interval
Pathology-positive Patients - Patients that had ≥ 1 pathology-positive lymph node	39	
All Patients in ITT Population - Patients who were injected with Lymphoseek, had surgery, and had at least one lymph node removed for which a pathology status was determined	83	
		0.4699 (0.3593, 0.5826)

Source: Appendix Table 28
Abbreviations: ITT=intent-to-treat.

Of the 75 patients in the PP population, 36 patients had at least 1 pathology-positive lymph node. This result corresponded to a tumor-detection rate in all lymph nodes of 0.4800 (95% CI, 0.3631 to 0.5985).

Ancillary analyses

Table 58: Sensitivities and false negative rates for Lymphoseek and VBD – Summary of efficacy (safety population)

Table 16. Sensitivities and False Negative Rates for Lymphoseek and Vital Blue Dye
Neoprobe Corporation - Integrated Summary of Efficacy
Safety Population (N=332)

Total Number of Pathology-Positive Nodes = 81

	NEO3-05			NEO3-05			Meta-Analysis		
	Number	Prop./ Rate	CI*	Number	Prop./ Rate	CI*	Number	Prop./ Rate	CI*
Vital Blue Dye + / Pathology +	33			30			63		
Vital Blue Dye - / Pathology +	8			10			18		
Tc 99m Lymphoseek + / Pathology +	38			40			78		
Tc 99m Lymphoseek - / Pathology +	3			0			3		
Vital Blue Dye Sensitivity		0.8049	(0.6513, 0.9118)		0.7500	(0.5880, 0.8731)		[illegible]	(0.6502, 0.8702)
Lymphoseek Sensitivity		0.9268	(0.8008, 0.9866)		1.0000	(0.9119, 1.0000)		0.9559	(0.9068, 1.0000)
Vital Blue Dye False Negative Rate		0.1951	(0.0882, 0.3487)		0.2500	(0.1269, 0.4120)		0.2198	(0.1298, 0.3098)
Lymphoseek False Negative Rate		0.0732	(0.0154, 0.1992)		0.0000	(0.0000, [illegible])		0.0001	(0.0000, 0.0032)

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Summary of efficacy for trial NEO3-06

Title: A Phase 3, Prospective, Open-Label, Multicenter Study of Lymphoseek-Identified Sentinel Lymph Nodes (SLNs) Relative to the Pathological Status of Non-Sentinel Lymph Nodes in an Elective Neck Dissection (END) in Cutaneous Head and Neck and Intraoral Squamous Cell Carcinoma			
Study identifier	**NEO3-06**		
Study Design	Prospective, open-label, multicentre trial of Lymphoseek (technetium Tc 99m tilmanocept) Injection in the detection of tumour-draining SLNs in patients with known cutaneous or intraoral squamous cell carcinoma of the head and neck.		
Treatments *Planned: 114 pathology positive patients.* *At interim analysis* *ITT: 83 patients*	Test		**Technetium Tc-99m Lymphoseek** Injection was given in 50 µg doses by peritumoral injection in close proximity to the primary tumour.
Endpoints and definitions	Primary endpoint	False negative rate (FNR)-associated with Lymphoseek-identified SLNs relative to non-SLNs. All pathology-positive patients	FNR = (# of patients with ⩾ 1 pathology- positive lymph node for whom Lymphoseek did not detect any pathology-positive SLNs) ÷ (total # of patients with ⩾ 1 pathology-positive lymph node). H0: FNR ≥ 0.14 vs. Ha: FNR < 0.14 Patients that had ≥ 1 pathology positive lymph node
	Secondary endpoint	Sensitivity of Lymphoseek to detect patients with at least 1 pathology-positive lymph node	True Positive Patients (TPP) = Patients that had ≥ 1 pathology –positive lymph node that was detected by Lymphoseek Sensitivity = 1 – FNR
	Secondary endpoint	Negative Predictive value) of Lymphoseek for detection of patients with at least 1 pathology-positive lymph node	True negative Patients (TNP)= patients for whom all lymph nodes (SLNs and non-SLNs) were pathology negative $NPV = \frac{\#\text{ patients with a true negative}}{\#\text{ patients with true negative or false negative}}$ Patients predicted to be pathology negative = patients for whom all Lymphoseek detected lymph nodes (SLNs) were pathology negative or no SLNs exist

	Secondary endpoint	Overall accuracy in classifying patients with at least 1 pathology-positive lymph node versus those with all negative nodes	True positive and True negative patients= patients for whom all pathology –positive lymph nodes were detected by Lymphoseek (all were SLNs) or for whom all lymph nodes (SLNs and non-SLNs) were pathology negative All patients in ITT population = patients who were injected with Lymphoseek, had surgery, and had at least one lymph node removed for which a pathology status was determined Overall accuracy = $\frac{\text{\# patients with a true positive or a true negative}}{\text{\# ITT patients}}$

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	False negative rate	N of patients	FNR (95.03% CI)	p-value
	False negative patients	1	0.0256 (0.0006-0.1349)	0.0205
	All pathology-positive patients	39		
	Sensitivity	N of patients	Sensitivity and 95% C.I.	
	true positive patients	38	0.9744 0.8652-0.9994	
	All pathology-positive patients	39		
	Overall Accuracy	N of patients	Overall accuracy and 95% C.I.	
	True positive and true negative patients	82	0.9880 0.9347-0.9997	
	All patients in ITT population	83		
Notes	FNR=false negative rate; ITT=intent to treat; nonSLNs=non-sentinel lymph node(s); SLNs=sentinel lymph node(s)			

Analysis performed across trials (pooled analyses and meta-analysis)

Two retrospective meta-analyses (NEO3-14 and NEO3-15) were also conducted, contrasting Lymphoseek against radiocolloidal agents (e.g., Nanocoll or Nanocis), the colloidal agents being utilized on the basis of European clinical practice of ILM and wherein these agents may have been used in conjunction with a vital VBD. The results of this meta-analysis are presented in the tables below.

Table 59: Summary of preoperative lymphoscintigraphy utilisation – patient level

	Tumour Type		
	Melanoma	**Breast Cancer**	**Overall**
NEO3-05 Safety Population [n]	85	94	179
Lymphoscintigraphy was performed [m (%)]	85 (100.0%)	82 (87.2%)	167 (93.3%)
NEO3-09 Safety Population [n]	76	77	153
Lymphoscintigraphy was performed [m (%)]	76 (100.0%)	58 (75.3%)	134 (87.6%)
Combined Safety Population [n]	161	171	332
Lymphoscintigraphy was performed [m (%)]	161 (100.0%)	140 (81.9%)	301 (90.7%)
NEO3-05 Safety Population – without NEO3-05 Sites 05 and 06 [n]	80	72	152
Lymphoscintigraphy was performed [m (%)]	80 (100.0%)	68 (94.4%)	148 (97.4%)
Combined Safety Population – without NEO3-05 Sites 05 and 06 [n]	156	149	305
Lymphoscintigraphy was performed [m (%)]	156 (100.0%)	126 (84.6%)	282 (92.5%)

Table 60: Summary of hot spot localisation rates – patient level

	Tumour Type		
	Melanoma	**Breast Cancer**	**Overall**
NEO3-05 Lymphoscintigraphy Population [n]	85	82	167
Hot spot was identified [m (%)]	83 (97.6%)	67 (81.7%)	150 (89.8%)
NEO3-09 Lymphoscintigraphy Population [n]	76	58	134
Hot spot was identified [m (%)]	76 (100.0%)	58 (100.0%)	134 (100.0%)
Combined Lymphoscintigraphy Population [n]	161	140	301
Hot spot was identified [m (%)]	159 (98.8%)	125 (89.3%)	284 (94.4%)
NEO3-05 Lymphoscintigraphy Population – without NEO3-05 Sites 05 and 06 [n]	80	68	148
Hot spot was identified [m (%)]	78 (97.5%)	65 (95.6%)	143 (96.6%)
Combined Lymphoscintigraphy Population – without NEO3-05 Sites 05 and 06 [n]	156	126	282
Hot spot was identified [m (%)]	154 (98.7%)	123 (97.6%)	277 (98.2%)

Table 61: Summary of lymphoscintigraphy and *in vivo* agreement per patient

	Tumour Type		
	Melanoma	**Breast Cancer**	**Overall**
Agreement Between LS and *In vivo* Results – NEO3-05			
Evaluable Patients [n]	83	81	164
Agreement [m (%)]	81 (97.6%)	70 (86.4%)	151 (92.1%)
Agreement Between LS and *In vivo* Results – NEO3-09			
Evaluable Patients [n]	75	58	133
Agreement [m (%)]	75 (100.0%)	58 (100.0%)	133 (100.0%)
Agreement Between LS and *In vivo* Results – Combined			
Evaluable Patients [n]	158	139	297
Agreement [m (%)]	156 (98.7%)	128 (92.1%)	284 (95.6%)
Agreement Between LS and *In vivo* Results – NEO3-05 without Sites 05 and 06			
Evaluable Patients [n]	78	67	145
Agreement [m (%)]	76 (97.4%)	65 (97.0%)	141 (97.2%)
Agreement Between LS and *In vivo* Results – Combined without NEO3-05 Sites 05 and 06			
Evaluable Patients [n]	153	125	278
Agreement [m (%)]	151 (98.7%)	123 (98.4%)	274 (98.6%)

Table 62: Count and proportion of concordant nodes for NEO3-05 and NEO3-09

	ITT Population (N=291)		
	NEO3-05 (Total ITT Nodes[a]=256)	**NEO3-09 (Total ITT Nodes[a]=229)**	**Meta-Analysis (Total ITT Nodes[a]=485)**
Number (Proportion) of Concordant Nodes[a]	239 (0.9336)	229 (1.0000)	468 (0.9999)
95% Confidence Interval for Proportion	(0.8958, 0.9608)	(0.9840, 1.0000)	(0.9986, 1.0000)
1-Sided p-Value[b] for One-Sample Test of H_0: $P_{C1} \leq 0.90$	0.0401	<0.0001	<0.0001
Melanoma[c] (Total ITT Nodes=237)	118 (0.9752)	116 (1.0000)	234 (0.9999)
Breast Cancer[d] (Total ITT Nodes=248)	121 (0.8963)	113 (1.0000)	234 (0.9999)

[a] Concordant Nodes – Nodes that were determined *in vivo* to be "blue" (due to presence of vital blue dye) were also "hot" (due to presence of Lymphoseek).

[b] α=0.05 for NEO3-05 (per protocol); α =0.025 for NEO3-09 (per protocol); α = 0.025 for meta-analysis

[c] Concordant Nodes from Melanoma Patients.

[d] Concordant Nodes from Breast Cancer Patients.

Table 63: Count and proportion of concordant nodes for NEO3-05 and NEO3-09 – without site 05 and 06

	ITT Population (N=269)		
	NEO3-05 (Total ITT Nodes=215)	**NEO3-09 (Total ITT Nodes=229)**	**Meta-Analysis (Total ITT Nodes=444)**
Number (Proportion) of Concordant Nodes[a]	210 (0.9767)	229 (1.0000)	439 (0.9999)
95% Confidence Interval for Proportion	(0.9466, 0.9924)	(0.9840, 1.0000)	(0.9986, 1.0000)
1-Sided p-Value[b] for One-Sample Test of $H_0 : P_{C1} \leq 0.90$	<0.0001	<0.0001	<0.0001
Melanoma[c] (Total ITT Nodes=235)	116 (0.9748)	116 (1.0000)	232 (0.9999)
Breast Cancer[d] (Total ITT Nodes=209)	94 (0.9792)	113 (1.0000)	207 (0.9999)

[a] Concordant Nodes – Nodes that were determined *in vivo* to be "blue" (due to presence of vital blue dye) were also "hot" (due to presence of Lymphoseek).

[b] α=0.05 for NEO3-05 (per protocol); α =0.025 for NEO3-09 (per protocol); α=0.025 for meta-analysis

[c] Concordant Nodes from Melanoma Patients

[d] Concordant Nodes from Breast Cancer Patients

Retrospective meta-analyses

In addition, **NEO3-14** and **NEO3-15** are meta-analysis studies on intraoperative lymph node mapping (ILM) agents in breast cancer patients (NEO3-14) or melanoma patients (NEO3-15) based solely on the European clinical practice of ILM and sentinel lymph node biopsy (SLNB). The primary objectives were to evaluate the following key clinical efficacy endpoints:

- the localization rate of the *in vivo* detection of the excised lymph node(s)
- the degree of localization as measured by the number of localized nodes per patient

NEO3-14: The efficacy results for patients with **breast cancer** from the Phase 3 studies NEO3-05 and NEO3-09 were analysed against seven European studies identified from the literature *(Ref: 1-7)*. The majority of the published studies used colloids in conjunction with VBD, and results for the combined use of these agents was compared to the use of Lymphoseek alone. The Localization Rate Benchmark Population was 6,313 patients, yielding an estimated localization rate of 0.9683 and a 95% Confidence Interval of 0.9529 to 0.9837.

Table 64: Breast cancer benchmark meta-analysis for localisation rate – Study NEO3-014

Localization Rate Benchmark Population: N=6313

Meta-Analysis Method	Number of Studies	Total Number of Patients	Estimated Localization Rate	95% Confidence Interval for Rate
Random Effects Model	7	6313	0.9683	(0.9529, 0.9837)

Patients from four studies were included in the Degree of Localization Benchmark Population (N=1528), yielding an estimated degree of localization of 1.8396 and an exact 95% Confidence Interval for the degree of localization of 1.5873 to 2.0919.

Table 65: Breast cancer benchmark meta-analysis for degree of localisation – Study NEO3-014

Degree of Localization Benchmark Population: N=1528

Meta-Analysis Method	Number of Studies	Total Number of Patients	Estimated Degree of Localization	Exact 95% Confidence Interval for Degree of Localization
Random Effects Model	4	1528	1.8396	(1.5873, 2.0919)

The Lymphoseek population for NEO3-14 consisted of 148 patients. The number or proportion of Lymphoseek-localized patients using fixed effects meta-analysis was 146 (0.9991). The 95% Confidence Interval for Proportion was 0.9921 to 1.0000. The 1-sided p-value for One-Sample Test of H0 (p ≤0.9529) was < 0.0001.

Table 66: Count and proportion of Lymphoseek-localised breast cancer patients – Study NEO3-014

Lymphoseek Population: N=148

	Fixed Effects Meta-Analysis (N=148)
Number (Proportion) of Lymphoseek-Localized Patients	146 (0.9991)
95% Confidence Interval for Proportion	(0.9921, 1.0000)
1-Sided p-Value for One-Sample Test of H_0: P ≤ 0.9529	<0.0001

Table 67: degree of Lymphoseek-localised breast cancer patients – Study NEO3-014

Lymphoseek Population: N=148

	Fixed Effects Meta-Analysis (N=148)
Mean of Lymphoseek-Localized Nodes per Patient	2.08
95% Confidence Interval for Mean	(1.9052, 2.2626)
1-Sided p-Value for One-Sample Test of H_0: μ ≤ 1.5873	<0.0001

The mean (standard deviation) of Lymphoseek-localized nodes per patient was 2.08 for the fixed effects meta-analysis. The 95% Confidence Interval for Mean was 1.9052 to 2.2626. The 1-sided p-value for One-Sample Test of H0 (μ ≤ 1.5873) was < 0.0001.

NEO3-15: The efficacy results for patients with **melanoma** from the Phase 3 studies NEO3-05 and NEO3-09 were analysed against six European studies identified from the literature *(Ref: 8-13)*. The majority of the published studies used colloids in conjunction with VBD, and results for the combined use of these agents was compared to use of Lymphoseek alone.

The Localization Rate Benchmark Population was 2,909 patients, yielding an estimated localization rate of 0.9798 and a 95% Confidence Interval of 0.9685 to 0.9910.

Table 68: Melanoma benchmark meta-analysis for localisation rate – Study NEO3-015

Localization Rate Benchmark Population: N=2909

Meta-Analysis Method	Number of Studies	Total Number of Patients	Estimated Localization Rate	95% Confidence Interval for Rate
Random Effects Model	6	2909	0.9798	(0.9685, 0.9910)

Patients from four of the six studies were included in the Degree of Localization Benchmark Population (N=2226), yielding an estimated degree of localization of 1.9629 and an exact 95% Confidence Interval for the degree of localization of 1.7005 to 2.2252.

Table 68: Melanoma benchmark meta-analysis for degree of localisation – Study NEO3-015

Degree of Localization Benchmark Population: N=2226

Meta-Analysis Method	Number of Studies	Total Number of Patients	Estimated Degree of Localization	Exact 95% Confidence Interval for Degree of Localization
Random Effects Model	4	2226	1.9629	(1.7005, 2.2252)

The Lymphoseek population for NEO3-15 consisted of 153 patients. The number or proportion of Lymphoseek-localized patients using fixed effects meta-analysis was 150 (0.9989). The 95% Confidence Interval for Proportion was 0.9919 to 1.0060. The 1-sided p-value for One-Sample Test of H0 ($p \leq 0.9685$) was < 0.0001.

Table 69: Count and proportion of Lymphoseek-localised melanoma patients – Study NEO3-015

Lymphoseek Population: N=153

	Fixed Effects Meta-Analysis (N=153)
Number (Proportion) of Lymphoseek-Localized Patients	150 (0.9989)
95% Confidence Interval for Proportion	(0.9919, 1.0000)
1-Sided p-Value for One-Sample Test of H_0: $P \leq 0.9685$	<0.0001

The mean (standard deviation) of Lymphoseek-localized nodes per patient was 2.30 for the fixed effects meta-analysis. The 95% Confidence Interval for Mean was 2.0827 to 2.5077. The 1-sided p-value for One-Sample Test of H0 ($\mu \leq 1.7005$) was < 0.0001.

Table 70: Degree of Lymphoseek localisation in melanoma patients – Study NEO3-015

Lymphoseek Population: N=153

	Fixed Effects Meta-Analysis (N=153)
Mean of Lymphoseek-Localized Nodes per Patient	2.30
95% Confidence Interval for Mean	(2.0827, 2.5077)
1-Sided p-Value for One-Sample Test of H_0: $\mu \leq 1.7005$	<0.0001

Supportive studies

The study NEO3-01 served as a pilot study for the two, phase 3, pivotal studies, and the results are discussed as supportive evidence of the efficacy of Lymphoseek.

Table 71: Summary of study design for study NEO3-01

Study	# of Study Centers	Study Period and Enrollment	Design Control Type	Study and Control Drugs Dose, Route, Regimen	Study Objectives	# Patients Entered/ Completed	Duration	Gender M/F Median age (Range)	Diagnosis Inclusion Criteria	Primary Endpoints
NEO3-01	5 (U.S.)	**Date of first enrollment:** 24 August 2006 **Date of last completed:** 08 August 2007 **Study Period:** 1 year **Enrollment:** 80 patients planned/ 84 patients enrolled	**Design:** Phase 2, multicenter, open label, single arm study to evaluate the safety and efficacy of LS as a lymphoid tissue targeting agent in patients with known or suspected melanoma or breast cancer **Control:** Historical	**Drug:** LS **Dose:** 50 µg **Route:** SC or ID injection **Regimen:** Single dose 50 µg LS by injection, in close proximity to the primary tumor	**Primary:** To determine the preoperative and intra-operative lymphoscinti-graphic localization of lymph node(s) in the lymphatic pathways draining the primary site of melanoma or breast cancer using LS as a radiotracer. **Secondary:** To assess the resected lymph node(s) to confirm tissue type and presence or absence of tumor metastases **Safety:** To evaluate patient safety	**Melanoma:** 52 / 45 **Breast:** 32 / 28	Up to 31 days (from study drug adminis-tration to 30 days post-surgery)	**M:** 31 **F:** 53 **Age:** 55 (22, 89)	Males and females, at least 18 years of age, with known or suspected melanoma or breast cancer who were candidates for surgical interventi on and clinically node negative, for whom lymph node mapping was part of the surgical plan, and for whom nodal status was a signif-	The in vivo localiza-tion rate (i.e., detection rate) of LS in tumor-draining lymph nodes compared to historical controls (the historical localiza-tion rates of other mapping agents (colloid and/or dye) from the literature).

Overall, 84 patients were enrolled, with mean age of 55.5 years (range 22 to 89 years). Most of the patients were female (63.1%) and Caucasian (94.0%).

The results of preoperative patient evaluation using Lymphoseek are summarized in the table below. Lymphoscintigraphy was performed in 57 of the 80 injected patients, and a “hot spot” was located in 53 of these patients (93.0%).

Table 71: Preoperative Lymphoscintigraphy with Lymphoseek by Tumour Type (Safety Population; NEO3-01 Study)

		Melanoma (N = 49)	Breast Cancer (N = 31)	Overall (N = 80)
Lymphoscintigraphy performed [a]				
Yes	n (%)	48 (98.0%)	9 (29.0%)	57 (71.3%)
No	n (%)	1 (2.0%)	22 (71.0%)	23 (28.8%)
Hot spot located on scan [b]				
Yes	n (%)	46 (95.8%)	7 (77.8%)	53 (93.0%)
No	n (%)	2 (4.2%)	2 (22.2%)	4 (7.0%)

Percentage was calculated using the number of patients injected with Lymphoseek as the denominator.

Percentage was calculated using the number of patients injected with Lymphoseek with a scan as the denominator.

Overall, based on the investigator's intraoperative assessment, Lymphoseek localized in at least one tissue sample in 75 of the 78 PP patients (96.2%). Lymphoseek was localized in lymphoid tissue for 97.9% of patients with melanoma and for 93.5% of patients with breast cancer. Overall, and for each type of tumour, the localization rate was significantly greater than 80%, the predesigned statistical endpoint.

Most patients (79.5%) had their surgery the same day as being injected with Lymphoseek. Only 20.5% were injected and went to surgery the next day.

The "per tissue" *in vivo* results for Lymphoseek localization were similar between the two tumour types for the same day of injection: 97.7% (melanoma) vs. 92.6% (breast cancer).

For the next day surgery, 100% of the tissue specimens from patients with melanoma were localized compared with 83.3% tissue specimens from patients with breast cancer.

A total of 46 out of 47 patients (97.9%) with melanoma localized Lymphoseek to at least one tissue, with similar rates between same day and next day surgery groups. For patients with breast cancer, the "per patient" localization rate was 93.5% overall, with 95.5% (21/22) for same day injection and surgery and 88.9% (8/9) for next day surgery. These disease specific rates were not significantly different as per Fisher's exact test (p = 0.5032).

Table 72: Pathology Findings – Study NEO3-01 Study (Per Protocol Population[a])

		Melanoma (N = 47)	Breast Cancer (N = 31)	Overall (N = 78)
No. of tissue specimens received and assessed by pathology	n	108	72	180
No. of specimens confirmed to be lymphoid tissue	n (%)	108 (100.0%)	72 (100.0%)	180 (100.0%)
Patients with lymphoid tissue	n	47	31	78
Patients with localized lymphoid tissue according to the investigator's assessment	n (%)	46 (97.9%)	29 (93.5%)	75 (96.2%)
Lymphoid tissue with tumour present	n (%)	16 (14.8%)	9 (12.5%)	25 (13.9%)
Patients with pathology(+) lymphoid tissue	n (%)	11 (23.4%)	9 (29.0%)	20 (25.6%)

All patients who received an injection of Lymphoseek, had a completed intraoperative survey of the tumour site, and had at least one resected tissue specimen confirmed to be lymphoid tissue by the site pathologist

Note: Palpable mass data are not included. All probe results are based on the calculated 3σ rule with the investigator's assessment. Table reflects pathology specimens that had an intraoperative survey.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The application for sentinel node detection in breast cancer and melanoma was supported by 2 pivotal studies and NEO3-06 and NEO3-09 and by 2 retrospective meta- analyses. Supportive evidence was also provided by the phase 2 study NEO3-01.

The 2 pivotal studies (NEO3-05 and NEO3-09) in breast cancer and melanoma patients, were conducted comparing the lymph nodal detection rates, using Lymphoseek, with the detection rates using VBD, with the same patients. The primary endpoint in both studies was the concordance rate between Lymphoseek and VBD, i.e., the rate of Lymphoseek positivity in patients/ nodes that were positive with VBD. The CHMP concern, regarding the pivotal studies, was previously highlighted in the scientific advice provided to the Applicant, in that the current EU standard for sentinel lymph nodal detection used radiocolloids labelled with 99m-technetium. At the same time the difficulty in comparing 2 radiopharmaceuticals within the same patient was also acknowledged. For the study NEO3-05, in the ITT analysis, the applicant claims success based on a one-sided interval of 5%. This is generally not acceptable, and the confidence interval does not exclude 90%. Therefore non-inferiority has not been rigorously demonstrated, though it is accepted that it is very close. However, the results of this study are corroborated by the subsequently conducted study NEO3-09, in which there are no concerns regarding the statistical methodology. The applicant has further conducted a meta-analysis of the results of both the pivotal studies.

The CHMP had concerns that the comparison of Lymphoseek was to vital blue due not to radiocolloids (described as the standard in the CHMP scientific advice). In order to address this concern and provide a comparison with current EU standard, radiocolloids, the applicant conducted 2 meta-analyses of published literature of radiocolloids used in sentinel lymph nodal detection. One was conducted for studies in patients with breast cancer, and one in patients with melanoma. The methodology and dose of radiation in most of the studies chosen for the meta-analyses was similar to the Lymphoseek pivotal studies. Therefore, the CHMP considered the meta-analyses as supportive evidence for the application.

The indication for sentinel node detection in patients with head and neck squamous cell carcinomas is supported by the pivotal study NEO3-06. The Study NEO3-06 evaluated, as the primary endpoint, the false negative rate on using Lymphoseek for sentinel node detection in head and neck squamous cell cancers. For this study the DSMC recommended stopping the trial for efficacy (which ended in approximately 40 months) because of the positive (low) false negative rate (FNR), which reached the statistical significance specified for the stopping rule.

Efficacy data and additional analyses

Breast cancer and malignant melanoma:

In the analyses of the two pivotal Phase 3 studies in breast cancer and melanoma, Lymphoseek demonstrated a statistically significant concordance rate with VBD. Additionally, Lymphoseek demonstrated a statistically superior concordance rate for lymph node detection when compared with the reverse concordance rate of VBD. Therefore, a high proportion of nodes that were VBD positive were detected by Lymphoseek, whereas a lower proportion of nodes that were Lymphoseek positive were detected by VBD.

The detection concordance between Lymphoseek and VBD was similar among patients with melanoma and patients with breast cancer.

Lymphoseek also demonstrated a higher sensitivity for detecting pathology-positive lymph nodes, corresponding to a decreased false negative rate when compared with VBD, on a per node basis.

Retrospective meta-analyses from breast cancer and malignant melanoma:

The retrospective meta-analyses compared Lymphoseek results, obtained in the two pivotal Phase 3 studies, to data from recent SLN peer-evaluated mapping studies of European practice (i.e., using colloids in conjunction with VBD) in patients with known breast cancer or melanoma. The applicant claimed superiority by comparing the results of Lymphoseek with the lower boundary of the confidence interval for the estimate of the European studies. This was considered not an acceptable proof of superiority.

However, for the localisation rate the lower end of the confidence interval for Lymphoseek did lie above the upper end of that of the European studies for both breast and melanoma, and for the degree of localisation, even though the confidence intervals did not exclude one another, those of Lymphoseek did lie above the point estimate for the European studies. From this data, it was concluded that Lymphoseek was at least not inferior to the European standards.

Head and neck squamous cell carcinomas:

The primary end-point of the pivotal study in head and neck squamous cell cancer patients was the evaluation of the false negative rate.

There were 39 patients that were found to have cancer in at least one loco-regional node. Only in one patient was the sentinel node that was identified by Lymphoseek found to be negative and any other node positive. This gives a false negative rate of 2.56% (95% CI, 0.06% to 13.49%) for sentinel node localisation. This was significantly better than the predicted performance ($p<0.0205$). Lymphoseek sensitivity was therefore 0.9744 (95% CI, 0.8652 to 0.9994); NPV was 0.9778 (95% CI, 0.8823 to 0.9994); and overall accuracy was 0.9880 (95% CI, 0.9347 to 0.9997). No differences in analyses by clinical trial site or by anatomical location of the primary tumour yielded any observable effect.

The applicant's description of a false negative rate (i.e., when the "hot" node(s) removed are examined histologically and found to have no evidence of cancer but other "cold" nodes in the block dissection are found to contain cancer) was considered acceptable by the CHMP. However, the less rigorous examination of the "non-sentinel nodes", i.e., nodes not taking up Lymphoseek; as compared to the rigorous examination of the Lymphoseek designated "sentinel nodes" was a concern. It was considered that the less rigorous examination of the non-sentinel nodes would have underestimated the false negative rate.

In their response, the applicant referred to clinical practice guidelines and expert opinions regarding sentinel node detection, to justify the asymmetrical examination of the sentinel and non-sentinel nodes. The applicant also provided evidence from the literature to demonstrate that a more thorough pathology review of non-SLN rarely leads to false negatives being found. Review of literature showed 2 studies conducted with radiocolloids which did perform rigorous examination of non-sentinel nodes as is expected of sentinel nodes (Christensen et al 2011; Stoeckli et al 2007). These studies demonstrated that sentinel nodes were never missed by the diagnostic and that therefore, there is no need in future to perform rigorous examination of the non-sentinel nodes, when this specific method of detecting sentinel nodes is employed. Reasons have been provided to show that the performance of Lymphoseek is at least similar to radiocolloids, as seen in patients with breast cancer and malignant melanoma. In addition further statistical evaluation of the results based on the radiocolloid data from the literature, demonstrates that the chances of having any additional pathology-positive patients in the 44 pathology-negative (pN0) patients of Study NEO3-06 are extremely low at less than 0.36% (0.0036).

This issue was discussed at the SAG. The SAG acknowledged that the histological examination of lymph nodes as performed in the study reflects current practice and did not raise particular concerns regarding a potential under-estimation of the false negative rate with respect to a more intensive sampling (the false-negative rate would not be expected to change significantly). Therefore, based on the applicant's responses and the opinion of the SAG (see below) concerning the methodology of the histopathological examination, the CHMP considered that the indication in head and neck cancer was not approvable. Therefore, based on the opinion of the SAG and the inclusion criteria of patients in the pivotal trial NEO3-06, the CHMP restricted the indication to localised squamous cell carcinoma of the oral cavity.

The CHMP also noted that the results of this study would have been useful in the assessment of the impact of Lymphoseek on "diagnostic thinking" and "patient management" of Lymphoseek in head and neck cancer. It was discussed that SLN dissection is not standard clinical practice for head and neck cancers. However, it is acknowledged that impact of Lymphoseek on "diagnostic thinking" and "patient management" has been demonstrated in breast and melanoma cancers.

The European Medicines Agency has deferred the obligation to submit the results of studies with Lymphoseek in one or more subsets of the paediatric population for visualisation of lymphatic drainage of solid malignant tumours for diagnostic purposes (see section 4.2 for information on paediatric use).

Supportive study

The data from the supportive study NE0-01 also confirmed that Lymphoseek has a high specificity for lymph nodes relative to other tissues as all tissues identified by Lymphoseek (171) were histologically confirmed to be lymphoid tissue. Overall, Lymphoseek showed a high per tissue sensitivity rate (92%) and a low false negative rate (8%) in the tumour-positive lymph nodes, which suggests that Lymphoseek is accurately identifying lymph nodes that have a high potential for containing tumour metastases.

Additional expert consultation

Following a CHMP request, a Scientific Advisory Group meeting was convened on 11 July 2014 to provide advice on the list of questions adopted by the CHMP at its April 2014 meeting. The SAG final answers to the questions from the CHMP are as follows:

1. **Is sentinel LN identification of value in the staging and/or pre-operative evaluation of patients with head and neck cancer?**

   Based on the available data, the value of sentinel-node identification in head and neck cancer (more specifically, oral cavity cancer) in terms of clinical outcome is currently unknown. Although conceptually one could envisage a place for this approach (in view of the expected reduction of morbidity associated with the ability to avoid neck dissection), there is a concern that recurrence may be adversely affected. The data presented in the application did not include clinical outcomes and do not allow to address this risk. Further studies on clinical outcomes compared to standard approaches are warranted.

2. **If so, would this be applicable to head and neck cancer in general or is there a need to restrict by tumour type and/or location?**

   In view of the general concerns expressed in the answer to question No. 1 (lack of clinical outcome data to establish the benefits and risks of this approach), there is no convincing evidence-based support for the approach as a whole and it is therefore not possible to propose evidence-based restrictions.

In oral cavity cancer, sentinel node detection is performed in some experienced institutions but is not an established approach based on generally acceptable scientific standard. In published studies and local guidelines, sentinel-node detection is often restricted to T1-T2, N0 squamous cell oral cavity cancer in patients without prior treatment for head and neck cancer. This restriction is mainly driven by the risk of recurrence associated with more advanced stages. Also, T3-T4 tumours generally require neck dissection anyway, so that sentinel lymph node detection is generally not relevant. Furthermore, in T3-T4 tumours, extensive tumour invasion may stop the tracer from entering the node, leading to false-negative results.

3. **To what extent might it be reasonable to extrapolate the data in breast cancer and melanoma to other tumour types and locations, including head and neck cancer, with respect to identification of the sentinel node(s).**

It is not possible to extrapolate the benefits of this approach from melanoma and breast cancer to oral cavity cancer. This is due to important differences between these diseases, for instance in terms of drainage patterns by tumour type (e.g., rapid lymphatic drainage in melanoma; sentinel node activity outside the expected basin in breast cancer and melanoma), practical aspects of the nuclear medicine procedure (e.g., interpretation criteria in case of multiple "hot spots" in head and neck cancer), as well as biology (e.g., prevalence of loco regional disease versus systemic spread) and additional treatments that may affect outcome (systemic versus local therapy).

4. **The claim for an indication for Lymphoseek in the evaluation of patients with head and neck cancer is supported by a study intended to evaluate the false negative rate for identification of tumour positive nodes. Is the study methodology satisfactory and do the presented results reliably establish the false negative rate? Specifically, is the SAG reassured that the histological examination of nodes not identified as sentinel by Lymphoseek is sufficient and does not raise concerns regarding under-estimation of the false negative rate?**

The submitted study is not considered satisfactory from a methodological point of view since it does not provide information about the long term clinical outcome (locoregional relapse) and the reliability to evaluate false negative rate associated with Lymphoseek when compared to EU accepted standard methods (radiocolloids).

When evaluated per lymph node (as it was presented), the false negative (FN) rate and the negative predictive values (NPV) have the disadvantage to depend on the number of lymph nodes which have been resected: a large number of resected lymph nodes will lead to a large number of negative lymph nodes (true negative TN) and thus to a "dilution" of the small number of false negative nodes [NPV = TN/(TN+FN)]. This approach could be of interest in a comparative study (which is not the case of the study presented) with the adequate statistical corrections for clustered data. A patient-based approach is needed, with a clear definition of FN and TN patients.

However, it is acknowledged that the histological examination of lymph nodes as performed in the study reflects current practice and did not raise particular concerns regarding a potential under-estimation of the false negative rate with respect to a more intensive sampling (the false-negative rate would not be expected to change significantly).

The lack of an appropriate control (radiocolloids) and long term results (locoregional relapse rate) remain major issues.

5. **Are such data sufficient in principle to establish the place of Lymphoseek in the staging and/or pre-operative evaluation of patients with head and neck cancer? Does the SAG consider that the benefits outweigh any risks relating to false negative evaluation?**

The data submitted are not sufficient to establish the benefits and risks of lymphoseek (see answers to questions No. 1-4). Although the benefits (in view of the expected reduction of morbidity associated with the ability to avoid neck dissection and its rare complications) can be assumed, the risk of an increase in the rate of recurrence is not known and therefore is not considered acceptable.

There is also a risk of increased recurrence if the procedure is not adequately performed. In trials with multiple institutions, a high number of false-negative results has been observed, which raises concern as to the general reproducibility of the method[14].

6. **If the SAG considers that these data are not sufficient to support the requested indication, what further information would the SAG consider necessary?**

Whether sentinel node biopsy might replace neck dissection in patients with clinically negative neck lymph nodes who suffer from oral squamous cell carcinoma is unknown. A comparative study on clinical outcome against standard therapy is considered necessary in order to establish the benefits and risks of this procedure.

Furthermore, the SAG considered that in the context of the current application (melanoma, breast indications):

- The use of blue dye alone was not the best comparator to use in the breast and melanoma studies since the combination of radio-colloids and blue dye has been established as a superior approach compared to blue dye alone [15]. Nanocolloid is the EU standard for these indication and therefore this compound should have been used as comparator. Furthermore, the blue dye detection being visual only takes into account the axillary basin, whereas the detection of radioactive sentinel node in the internal mammary chains may impact the patient management (resection in some cases, more frequently prophylactic irradiation) [16]. Moreover, lymphatic mapping with blue dye only in melanoma is cumbersome and clearly inferior to radio colloids, especially in aberrant drainages.
- In the meta-analyses presented, the precise number of nodes which have been detected as sentinel lymph nodes should be reported for the different tracers since a high rate of positive detection may artificially reduce the false-negative rate. If all resected lymph nodes are considered sentinel nodes the false negative rate will be 0 and the NPV will be 100%. However, this decreases the interest in the method. The procedure is meant to be a minimally invasive, but accurate staging mechanism. Accordingly, the objective is to resect a very limited number of lymph nodes to reduce the morbidity of lymph node dissection and to be able to analyse those few nodes in details (e.g., thin cuts, immunohistochemistry). The definition sentinel node (in every study) must be addressed against general definitions.

2.5.4. Conclusions on the clinical efficacy

The results from the pivotal studies NEO3-05 and NEO3-09 supported by the meta-analyses provided satisfactory evidence of the efficacy of Lymphoseek in the detection of sentinel lymph nodes in patients with breast cancer or melanoma. The sensitivity and specificity of the diagnostic agent was demonstrated and the impact on diagnostic thinking and patient management is considered self-evident given that lymph node mapping is widely used in breast cancer and melanoma.

The results of the phase 3 study conducted in cutaneous and oral head and neck squamous cell cancer patients are considered supportive of the indication for sentinel node detection in this patient population.

2.6. Clinical safety

Safety data for Lymphoseek is available from the Phase 1, Phase 2, and Phase 3 clinical studies. Patients enrolled into these studies included patients with breast cancer, melanoma, or head and neck squamous cell carcinoma (HNSCC). Data from a total of 542 patients contributed to an integrated safety database.

Primary safety data were available from three Phase 1 studies: NEO3-A and NEO3-C (patients with breast cancer), and NEO3-B (patients with cutaneous melanoma). In addition to the Phase 1 studies, safety data are available from one Phase 2 study (NEO3-01) and two completed Phase 3 studies (NEO3-05 and NEO3-09); all three of which were conducted in patients with melanoma or breast cancer diagnoses. The applicant expanded the safety database to include the data a Phase 3 study in patients with HNSCC undergoing lymphatic mapping (NEO3-06). Data from NEO3-06 patients who received a Lymphoseek injection and underwent surgery as of 10 May 2012 and completed the 30 day follow-up are included in the safety database.

Patient exposure

The patients' disposition and exposure are summarised in the table below.

Table 73: Patient disposition in the safety population

Patient Disposition

		Cancer Type				
		Melanoma	**Breast Cancer**	**HNSCC Cutaneous**	**HNSCC Intraoral**	**Overall**
Enrolled		245	244	6	82	577
Completed[a]		217 (88.6%)	232 (95.1%)	6 (100%)	68 (82.9%)	523 (90.6%)
Withdrawn[a]		28 (11.4%)	12 (4.9%)	0 (0.0%)	14 (17.1%)	54 (9.4%)
Reason for Withdrawal	Adverse Event[a]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Protocol Violation[a]	6 (2.4%)	5 (2.0%)	0 (0.0%)	0 (0.0%)	11 (1.9%)
	Lost to Follow-up[a]	2 (0.8%)	3 (1.2%)	0 (0.0%)	0 (0.0%)	5 (0.9%)
	Withdrawal by subject[a]	11 (4.5%)	2 (0.8%)	0 (0.0%)	11 (13.4%)	24 (4.2%)
	Death[a]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Other[a]	9 (3.7%)	2 (0.8%)	0 (0.0%)	3 (3.7%)	14 (2.4%)

a) The denominator for all percentages in this row is the number of enrolled subjects in their respective column.

Abbreviations: HNSCC, head and neck squamous cell carcinoma

Adverse events

Overall, 39.7% (215/542) of patients reported at least one AE:

- 39.9% (91/228) of patients with melanoma
- 37.9% (91/240) of patients with breast cancer
- 44.6% (33/74) patients with HNSCC

A list of all observed adverse events are in the table below. The highest investigator-rated relationship to Lymphoseek for most adverse events were "not related" or "unlikely related".

Table 74: Number and Percentage of Subjects with Adverse Events (Safety Population, N=542)

	Cancer Type				
Adverse Event Category[a]	**Melanoma (N=228)**	**Breast Cancer (N=240)**	**HNSCC Cutaneous (N=6)**	**HNSCC Intraoral (N=68)**	**Overall (N=542)**
Total Number of Adverse Events	351	419	17	80	867
Subjects with at Least One Adverse Event	91 (39.9%)	91 (37.9%)	5 (83.3%)	28 (41.2%)	215 (39.7%)
Blood and Lymphatic System Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	2 (2.9%)	3 (0.6%)
Cardiac Disorders	4 (1.8%)	2 (0.8%)	1 (16.7%)	3 (4.4%)	10 (1.8%)
Ear and Labyrinth Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Eye Disorders	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	2 (0.4%)
Gastrointestinal Disorders	12 (5.3%)	11 (4.6%)	2 (33.3%)	11 (16.2%)	36 (6.6%)
General Disorders and Administration Site Conditions	12 (5.3%)	11 (4.6%)	0 (0.0%)	6 (8.8%)	29 (5.4%)
Immune System Disorders	0 (0.0%)	4 (1.7%)	0 (0.0%)	1 (1.5%)	5 (0.9%)
Infections and Infestations	17 (7.5%)	20 (8.3%)	0 (0.0%)	8 (11.8%)	45 (8.3%)
Injury, Poisoning and Procedural Complications	22 (9.6%)	18 (7.5%)	0 (0.0%)	2 (2.9%)	42 (7.7%)
Investigations	24 (10.5%)	19 (7.9%)	0 (0.0%)	1 (1.5%)	44 (8.1%)
Metabolism and Nutrition Disorders	4 (1.8%)	3 (1.3%)	2 (33.3%)	5 (7.4%)	14 (2.6%)
Musculoskeletal and Connective Tissue Disorders	3 (1.3%)	8 (3.3%)	0 (0.0%)	7 (10.3%)	18 (3.3%)
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Nervous System Disorders	8 (3.5%)	9 (3.8%)	1 (16.7%)	4 (5.9%)	22 (4.1%)
Psychiatric Disorders	4 (1.8%)	2 (0.8%)	2 (33.3%)	3 (4.4%)	11 (2.0%)
Renal and Urinary Disorders	6 (2.6%)	4 (1.7%)	0 (0.0%)	1 (1.5%)	11 (2.0%)
Reproductive System and Breast Disorders	0 (0.0%)	4 (1.7%)	0 (0.0%)	0 (0.0%)	4 (0.7%)
Respiratory, Thoracic and Mediastinal Disorders	2 (0.9%)	4 (1.7%)	0 (0.0%)	6 (8.8%)	12 (2.2%)
Skin and Subcutaneous Tissue Disorders	8 (3.5%)	7 (2.9%)	1 (16.7%)	1 (1.5%)	17 (3.1%)
Surgical and Medical Procedures	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	2 (0.4%)
Vascular Disorders	4 (1.8%)	7 (2.9%)	3 (50.0%)	4 (5.9%)	18 (3.3%)

[a] Adverse events coded with MedDRA Coding Dictionary Version 12.0.

The denominator for all percentages calculated in this table is the number of subjects with the respective type of cancer in the safety population.

HNSCC = head and neck squamous cell carcinoma

The table below presents the number and percent of patients experiencing ADRs, in this case these are AEs that are rated by the site investigators as possibly, probably, or definitely related to study drug, distributed among 12 system organ classes (SOCs).

Table 75: Number and Percentage of Subjects with Adverse Events, Relation to Lymphoseek=Possibly, Probably or Definitely (Safety Population, N=542)

	Cancer Type				
Adverse Event Category[a]	**Melanoma (N=228)**	**Breast Cancer (N=240)**	**HNSCC Cutaneous (N=6)**	**HNSCC Intraoral (N=68)**	**Overall (N=542)**
Number of Adverse Events	5	8	2	14	29
Subjects with at Least One Adverse Event	3 (1.3%)	8 (3.3%)	1 (16.7%)	4 (5.9%)	16 (3.0%)
Cardiac Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Sinus Tachycardia	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Eye Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Vision Blurred	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Gastrointestinal Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Nausea	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
General Disorders and Administration Site Conditions	0 (0.0%)	4 (1.7%)	0 (0.0%)	1 (1.5%)	5 (0.9%)
Feeling Hot	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Injection Site Irritation	0 (0.0%)	3 (1.3%)	0 (0.0%)	1 (1.5%)	4 (0.7%)
Injection Site Pain	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Injury, Poisoning and Procedural Complications	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	2 (0.4%)
Incision Site Pain	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Seroma	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Wound Dehiscence	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Metabolism and Nutrition Disorders	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Hypercalcaemia	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Musculoskeletal and Connective Tissue Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	3 (4.4%)	4 (0.7%)
Musculoskeletal Pain	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Neck Pain	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.9%)	2 (0.4%)
Pain in Extremity	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Pain in Jaw	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Nervous System Disorders	1 (0.4%)	0 (0.0%)	1 (16.7%)	3 (4.4%)	5 (0.9%)
Aphasia	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (0.2%)
Dizziness	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (0.2%)
Headache	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.9%)	2 (0.4%)

Adverse Event Category[a]	Cancer Type				
	Melanoma (N=228)	Breast Cancer (N=240)	HNSCC Cutaneous (N=6)	HNSCC Intraoral (N=68)	Overall (N=542)
Paraesthesia	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Renal and Urinary Disorders	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Micturition Urgency	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Pollakiuria	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Reproductive System and Breast Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Breast Pain	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Skin and Subcutaneous Tissue Disorders	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Skin Irritation	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Vascular Disorders	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (0.2%)
Flushing	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (0.2%)

[a] Adverse events coded with MedDRA Coding Dictionary Version 12.0.

The denominator for all percentages calculated in this table is the number of subjects with the respective type of cancer in the safety population.

HNSCC = head and neck squamous cell carcinoma

Serious adverse event / deaths / other significant events

Overall, 27 patients (5.0%) reported experiencing at least one serious adverse event. No patients withdrew from the study because of an SAE or AE, and no deaths were reported.

Within the integrated safety database, a total of 29 SAEs were reported for 27 patients (5.0%) in the overall safety population. SAEs were distributed among nine SOCs, with the infections and infestations SOC having the highest incidence (n=7; 1.3%). The only SAE that occurred in more than one patient was cellulitis (4 patients).

Laboratory findings

Haematology

Overall, changes from baseline to post-injection (postoperative) time points were small for haematology parameters, except basophils (overall mean decreased from baseline approximately 40%), eosinophils (overall mean decreased from baseline approximately 50%), lymphocytes (overall mean decreased from baseline approximately 33%), and monocytes (overall mean increased from baseline approximately 28%).

Overall, variability was high (S.D. greater than one-third of the mean) for basophils, eosinophils, leukocytes, lymphocytes, and monocytes.

Shifts from Baseline:

Haematology parameters at baseline and at 6 to 30 hours post-injection were categorized as being below normal range, within normal range, or above normal range. Shift tables for each parameter show the number of patients who moved within these three categories from baseline to the post-injection (postoperative) assessment.

Clinical Chemistry

Overall, changes from baseline to post-injection time points were small for all blood chemistry parameters. Variability was high (S.D.s greater than one-third of the mean) for alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and total bilirubin.

An AE of hyperglycaemia was reported for one patient with high glucose, and one patient with high glucose had a history of non-insulin-dependent diabetes. An AE of hypoglycaemia was reported for one patient with low glucose.

Urinalysis

Mean changes over time

Overall, means and S.D.s, at baseline and during the 6 to 30 hour safety assessment period, for specific gravity, urobilinogen, and pH did not vary from baseline to post-injection time points. Overall, variability was high (SDs greater than one-third of the mean) for urobilinogen.

Of six urinalysis values judged by the investigator as clinically significant, two clinically significant values occurred in two patients at the 6 to 30 hour assessment but baseline values were missing, and four clinically significant values occurred in two patients during both baseline and the 6 to 30 hour safety assessment period in the melanoma and breast cancer disease groups.

None of these abnormal clinical laboratory parameters reported as AEs were judged to be related to study drug.

Shifts from Baseline

Urinalysis parameters (specific gravity, urobilinogen, and pH) at baseline and at 6 to 30 hours post-injection were categorized as being below normal range, within normal range, or above normal range. Shift tables for each parameter show the number of patients who moved within these three categories from baseline to the post-injection assessment. No shifts occurred in greater than 3% of patients comprising the safety population.

Only one urinalysis parameter, specific gravity, displayed a two-category shift in $\geqslant$ 1% of patients. This occurred in 1.1% of patients from above normal range before injection to below normal range post-injection.

Vital Signs, Physical Findings, and Other Observations Related to Safety

Vital Signs

Vital signs at baseline and at 6 to 30 hours post-injection are summarized across cancer type for the pooled studies.

Vital signs monitored at baseline, 15 and 30 minutes, and 1, 2, and 6 to 30 hours post injection included systolic blood pressure, diastolic blood pressure, radial pulse rate, respiratory rate, and body temperature (in degrees Fahrenheit). No clinically significant findings were observed at baseline or during the 6 to 30 hour post-injection assessment period.

Any patients who had post-injection vital signs recorded perioperatively and/or intraoperatively were under the effects of anaesthesia. Due to the influence of anaesthesia and the operating room environment, these vital signs are expected to be decreased and do not represent any effects of Lymphoseek.

Abnormal vital sign results that were reported as AEs include hypertension (in one patient in NEO3-05) and hypotension (in four patients, two in NEO3-09 and two in NEO3-06). The investigator judged the hypertension AE to be mild in severity, not serious, and not related to study drug. The investigator judged one of the hypotension AEs as mild in severity, two of the AEs as moderate in severity, and one as severe. The severe event, occurring in one patient in the HNSCC group, was also judged as serious . This serious AE, like the other AEs of hypotension, was not related to study drug and resolved. The other three events of hypotension were judged as not serious. Only one of these vital sign results reported as an AE (hypertension) did not resolve.

Physical Examination Findings

Findings from the physical examinations remained normal from baseline to post-injection timepoints for most patients as shown in the shift table for physical examination findings. Greater percentages of patients had shifts from abnormal to normal than from normal to abnormal for findings related to skin; neck; head, eye, ear, nose and throat (HEENT) examination; heart; lungs; abdomen; and extremities. For findings related to lymph nodes, more shifts were recorded from normal to abnormal than abnormal to normal and for general appearance, shifts were similar in both directions, from normal to abnormal and from abnormal to normal.

Electrocardiograms

Mean Changes Over Time

Changes in mean ECG parameters, overall, from baseline to the 6 to 30 hour safety assessment period were minimal (increases in heart rate of 5.3 bpm, PR of 5.2 msec, and QT of 4.1 msec; decreases in QRS duration of 0.9 msec and QRS axis of 1.4 degrees) and not clinically significant

Changes in Individual Patients

Of 10 patients with ECG changes that the investigator judged as abnormal and clinically significant, for five of these patients with missing values or values within the normal range at baseline, the abnormal changes occurred during the 6 to 30 hour safety assessment period in the melanoma, breast cancer, and HNSCC disease groups.

Abnormal ECG results that were reported as AEs in the SOC investigations were ECG abnormal and ECG ST-T segment abnormal (in one patient), and ECG T-wave inversion (in two patients). In the SOC cardiac disorders AEs were reported in ten (1.8%) of 542 patients overall and included atrial fibrillation (in three patients); myocardial infarction, sinus bradycardia, and tachycardia (two patients each); and bradycardia, sinus tachycardia, and ventricular extrasystoles (one patient each).

Safety in special populations

The applicant did not submit studies in special populations.

Safety related to drug-drug interactions and other interactions

The applicant did not submit drug-drug interaction and other interaction studies.

Discontinuation due to adverse events

No patients withdrew from the study as a result of adverse events.

Post marketing experience

No report on post-marketing experience was submitted in the application.

2.6.1. Discussion on clinical safety

In the overall safety population, close to 40% of the patient population experienced an adverse event. It should be noted that all patients underwent surgery soon after the administration of Lymphoseek, and a large number of the adverse events are attributed to surgery or other related treatments. Only 3% of the population had an adverse event that was attributed by the investigators to be related to Lymphoseek. Most of these were localised to the site of administration of Lymphoseek. In the 553 patients evaluated in the clinical studies, the most common adverse reactions were injection site irritation (0.7%; 4 of 553 patients) and injection site pain (0.2%; 1 of 553 patients). It is unlikely that there are drug interactions with Lymphoseek, as it is administered to the site and travels to the local lymph glands. Therefore, the lack of drug interaction studies is acceptable. The applicant did not submit studies in special populations such as hepatic impairment, renal impairment and paediatric popualtion. The number of patients is insufficient to make any meaningful conclusions on differences or similarity of adverse events by demographic subpopulations, however racial and gender differences are unlikely to have any major differences in adverse events. Differences in BMI or disease types may influence differences in surgical outcomes but are also considered unlikely to have differences in response to Lymphoseek injection. There is no expectation to see any major differences between the groups. The missing data has been addressed in the RMP.

The incidences of serious adverse events were also low and none were attributed to Lymphoseek, and there were no deaths.

The dose of radioactivity was increased, from 1 mCi to 2 mCi, in anticipation of the extension of time from injection to SLN biopsy. The dose of radiation, however, is well below the 99mTc index for other imaging procedures.

Elderly patients aged 65 or older (32%) were evaluated in clinical studies; no safety issues were identified. No dose adjustment is recommended based on age.

The safety and efficacy of Lymphoseek in children and adolescents below the age of 18 years has not yet been established. No data are available.

The product is contraindicated for patients whish have hypersensitivity to the active substance, to any of the excipients listed in section 6.1 or to any of the components of the radiolabelled pharmaceutical.

Renal and hepatic impairment

Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. The estimated radiation dose to the patient would not exceed 0.69 mSv even if none of a 74 MBq dose (2.0 mCi) were eliminated (see section 4.2).

The patient should be well hydrated before the start of the examination and frequent voiding of urine during the initial hours after examination would reduce radiation exposure to the patient.

This medicinal product contains less than 1 mmol sodium (23 mg) per dose, i.e., essentially ‘sodium-free’.

Precautions with respect to environmental hazard see section 6.6.

When an administration of radiopharmaceuticals to a woman of childbearing potential is intended, it is important to determine whether or not she is pregnant. Any woman who has missed a period should be assumed to be pregnant until proven otherwise. If in doubt about her potential pregnancy (if the woman has missed a period, if the period is very irregular, etc.), alternative techniques not using ionising radiation (if there are any) should be offered to the patient.

There are no data from the use of Lymphoseek in pregnant women. No reproductive toxicity studies in animals were performed, and it is not known if Lymphoseek can cause foetal harm when administered to a pregnant woman.

Radionuclide procedures carried out on pregnant women also involve radiation dose to the foetus. Only essential investigations should therefore be carried out during pregnancy, when the likely benefit far exceeds the risk incurred by the mother and foetus.

It is not known whether technetium Tc 99m tilmanocept is excreted into human milk.

Before administering radiopharmaceuticals to a mother who is breast-feeding consideration should be given to the possibility of delaying the administration of radionuclide until the mother has ceased breast-feeding, and to what is the most appropriate choice of radiopharmaceuticals, bearing in mind the secretion of activity in breast milk. If administration is considered necessary, breast-feeding should be interrupted for 24 hours post injection and the expressed feeds discarded.

Animal fertility studies have not been conducted with Lymphoseek.

Lymphoseek has no or negligible influence on the ability to drive or use machines.

Exposure to ionizing radiation is linked with cancer induction and a potential for the development of hereditary defects. As the effective dose to an adult (70 kg) is 0.69 mSv when the maximal recommended activity of 74 MBq is administered adverse events are expected to occur with a low probability.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the national reporting system listed in Appendix V.

The total injection amount should not exceed 50 micrograms tilmanocept, with a total maximum radioactivity of 74 MBq per dose. Chronic or acute overdose is unlikely to occur given the total injection amount. No clinical consequences were observed at dose levels of 3.7 times the recommended dose of Lymphoseek in humans, or at 390 times the anticipated human exposure of tilmanocept in animals.

In the event of administration of a radiation overdose with tilmanocept the absorbed dose to the patient should be reduced where possible by increasing the elimination of the radionuclide from the body by frequent micturition or by forced diuresis and frequent bladder voiding

In general, radiopharmaceuticals should be received, used, and administered only by authorised persons in designated clinical settings. Their receipt, storage, use, transfer, and disposal are subject to the regulations and/or appropriate licenses of the competent official organisation.

Radiopharmaceuticals should be prepared in a manner which satisfies both radiation safety and pharmaceutical quality requirements. Appropriate aseptic precautions should be taken.

Contents of the vial are intended only for use in the preparation and radiolabelling of Lymphoseek and are not to be administered directly to the patient without first undergoing the preparative procedure. Each 250 microgram vial contains an excess of product. However, it is recommended that the vial be prepared as instructed and a 50 microgram aliquot be used for a single patient dose; any remaining material should be discarded after reconstitution and use.

For instructions on reconstitution and radiolabelling of the medicinal product before administration, see section 12. The radiolabelled product is a clear, colourless solution with no visible particles.

If at any time in the preparation of this product the integrity of this vial is compromised it should not be used.

Administration procedures should be carried out in a way to minimise risk of contamination of the medicinal product and irradiation of the operators. Adequate shielding is mandatory.

The content of the kit before extemporary preparation is not radioactive. However, after sodium pertechnetate (^{99}mTc), Ph.Eur is added, adequate shielding of the final preparation must be maintained.

The administration of radiopharmaceuticals creates risks for other persons from external radiation or contamination from spill of urine, vomiting, etc. Radiation protection precautions in accordance with national regulations must therefore be taken.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

For instructions on the safe preparation of Lymphoseek see the SmPC section 12.

2.6.2. Conclusions on the clinical safety

There are no serious concerns with regards to the clinical safety of Lymphoseek. The incidence of adverse events related to Lymphoseek was low, and the radiation exposure/ absorbed doses are within the acceptable limits.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 2.0, the PRAC considers by consensus that the risk management system for tilmanocept (Lymphoseek) for imaging and intraoperative detection of sentinel lymph nodes draining a primary tumour in adult patients with breast cancer, melanoma, or head and neck cancer is acceptable.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

The applicant identified the following safety concerns in the RMP:

Table 1: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Medication errors Hypersensitivity reactions, including anaphylaxis
Missing information	Use in Patients receiving more than one dose Use in Paediatric Population Use during Lactation Use in Pregnancy Use in Patients with Renal Impairment Use in Patients with Hepatic Impairment

The PRAC agreed.

- **Pharmacovigilance plans**

Table 2: Ongoing and planned studies in the PhV development plan

Activity / Study title (category 1-3)	**Objectives**	**Safety concerns addressed**	**Status**	**Date for submission of interim or final reports**
NAV3-18: A Prospective, Open-Label, Multicenter Comparison Study of Lymphoseek® and Vital Blue Dye as Lymphoid Tissue Targeting Agents in Paediatric Patients with Solid Tumours (interventional, Phase 3)	To determine the concordance of *in vivo* detection rates of Lymphoseek and vital blue dye in excised tissue histologically confirmed as lymph nodes.	Use in children under the age of 18 years.	PIP approved. Study still in planning stage.	Deferred status has been granted for this study. Milestones will be agreed with the Paediatric Committee following approval of the Lymphoseek marketing authorisation application.

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

- **Risk minimisation measures**

Table 3: Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Medication errors	Sections 4.2 'posology and method of administration, 4.4 'special warnings and precautions', and 12 'instructions for preparation of radiopharmaceuticals' provide clear and detailed instructions for the preparation and use of Lymphoseek.	None proposed
Hypersensitivity reactions, including anaphylaxis	Section 4.3 'Contraindications' contraindicates the use of Lymphoseek in patients with a known hypersensitivity to tilmanocept or any of the components of the radiolabelled pharmaceutical.	None proposed
Use in patients receiving more than one dose	Section 4.2 "Posology and method of administration" of the proposed SmPC describes the processes for single dosing of each patient.	None proposed
Use in pregnancy	Section 4.6 "Fertility, pregnancy and lactation" of the proposed SmPC includes the following language to minimise the risk of a patient being exposed to Lymphoseek during pregnancy: *"<u>Women of childbearing potential</u> When an administration of radiopharmaceuticals to a woman of childbearing potential is intended, it is important to determine whether or not she is pregnant. Any woman who has missed a period should be assumed to be pregnant until proven otherwise. If in doubt about her potential pregnancy (if the woman has missed a period, if the period is very irregular, etc.), alternative techniques not using ionising radiation (if there are any) should be offered to the patient.* *<u>Pregnancy</u> There are no data from the use of Lymphoseek in pregnant women. No reproductive toxicity studies in animals were performed, and it is not known if Lymphoseek can cause foetal harm when administered to a pregnant woman. As a precautionary measure, it is preferable to avoid the use of Lymphoseek during pregnancy unless clinically necessary."*	None proposed

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Paediatric use	Section 4.2 "Posology and method of administration" of the proposed SmPC includes the following language to ensure that healthcare professionals know there is no available information on the use of Lymphoseek in the paediatric population: *"The safety and efficacy of Lymphoseek in children below the age of 18 years has not yet been established. No data are available."*	None proposed
Use during lactation	Section 4.6 "Fertility, pregnancy and lactation" of the proposed SmPC includes the following language to minimize the risk of a patient receiving Lymphoseek during breast feeding: *"It is not known whether technetium Tc 99m tilmanocept is excreted into human milk. Because many drugs are excreted into human milk and because of the potential for serious adverse reactions in nursing infants, a decision should be made whether to interrupt nursing after administration of Lymphoseek or not to administer Lymphoseek, taking into account the importance of the drug to the mother. Wherever possible, infant formula feedings should be substituted for breast milk until the technetium Tc 99m has been eliminated from the body. Breast-feeding should be interrupted for 24 hours post injection and the expressed milk discarded."*	None proposed
Use during renal impairment	Section 4.2 'Posology and method of administration' of the proposed SmPC states "*Careful consideration of the activity to be administered in these patients is required since an increased radiation exposure is possible. Extensive dose-range and adjustment studies with the medicinal product in normal and special populations have not been performed. The pharmacokinetics of technetium Tc 99m tilmanocept in patients with renal or hepatic impairment have not been characterised.*"	None proposed

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	Section 4.4 'Special warnings and precautions for use' states "*Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. Tilmanocept is eliminated primarily through the kidneys and patients with renal impairment have the potential of increased radiation exposure. See section 4.2*."	
Use during hepatic impairment	Section 4.2 'Posology and method of administration' of the proposed SmPC states "*Careful consideration of the activity to be administered in these patients is required since an increased radiation exposure is possible. Extensive dose-range and adjustment studies with the medicinal product in normal and special populations have not been performed. The pharmacokinetics of technetium Tc 99m tilmanocept in patients with renal or hepatic impairment have not been characterised.*" Section 4.4 'Special warnings and precautions for use' states "*Careful consideration of the benefit risk ratio in these patients is required since an increased radiation exposure is possible. Tilmanocept is eliminated primarily through the kidneys and patients with renal impairment have the potential of increased radiation exposure. See section 4.2*."	None proposed

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication

The CHMP endorsed this advice without changes.

2.9. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

All tissues detected by Lymphoseek in studies NEO3-05 and NEO3-09, were confirmed to be lymph nodal tissue. The results of these pivotal studies show a greater detection rate with Lymphoseek for all nodes; as well as lower false negative rate with Lymphoseek for pathologically positive lymph nodes, in comparison to vital blue. This is demonstrated by the high concordance rate and the comparatively low reverse concordance rate, between sentinel node detection by Lymphoseek and VBD.

Therefore the efficacy of Lymphoseek in the detection of sentinel nodes in patients with breast cancers or melanoma is considered demonstrated. The beneficial effects (diagnostic performance and patient management) of sentinel node detection in these patient populations are already recognised in clinical practice.

The proposed indication currently includes head and neck cancers. Although there were concerns regarding the methodology of the study, it has been justified that there is very minimal chance for additional positive pathology in the study patients.

Uncertainty in the knowledge about the beneficial effects

The CHMP in previous scientific advice stated that VBD was not considered standard practice in the European setting. This issue was addressed by the applicant by providing retrospective meta-analyses of EU published literature using the CHMP recommended comparator, radiolabelled sulphur colloid. Comparison of the results from the meta-analyses with the results of pivotal studies with Lymphoseek showed that from these indirect comparisons it is possible to conclude that Lymphoseek inferiority to the European standards is unlikely. There are no further uncertainties in the beneficial effects in relation to the breast cancer and melanoma indication. However, for the indication in localised squamous cell carcinoma of the oral cavity, it is acknowledged that although there is increasing interest and recognition of a possible benefit of sentinel node detection in head and neck cancer, it is not recognised as standard practice that is widely accepted and used in the EU. There is a lack of long-term data on the outcome of patients that have been staged and resected based on the detection of sentinel node for localised squamous cell carcinoma of the oral cavity.

Risks

Unfavourable effects

There are no major concerns with regards to the clinical safety of Lymphoseek. The incidence of adverse events related to Lymphoseek appears low, and the radiation exposure/ absorbed doses are within acceptable limits.

Uncertainty in the knowledge about the unfavourable effects

There is uncertainty in the knowledge about the unfavourable effects on patients with impaired renal and hepatic function. There were no data submitted on elimination, excretion and metabolism of Lymphoseek in humans. It is thought that metabolism of Lymphoseek occurs in the liver and that elimination primarily occurs via renal excretion. Therefore, warnings have been included in the SmPC and the risks have been addressed in the risk management plan.

As the overall total patient exposure to Lymphoseek is small, there may be rare events that have not been reported so far. These risks will be managed through the RMP.

Benefit-risk balance

Importance of favourable and unfavourable effects

The efficacy of Lymphoseek in the detection of sentinel lymph nodes in patients with breast cancer, melanoma and localised squamous cell carcinoma of the oral cavity has been demonstrated. In addition, the diagnostic performance and impact on patient management has been established for sentinel lymph node detection with the use of radiocolloids in clinical practice.

The risks associated with the use of Lymphoseek in intraoperative lymph nodal mapping in breast cancer and melanoma appear to be low and manageable.

Discussion on the benefit-risk assessment

Based on the results of the pivotal trials NEO3-05, NEO3-09 and NEO-06 and the supportive data from the meta-analyses, the benefits of Lymphoseek in the imaging of SLN in breast cancer, melanoma and localised squamous cell carcinoma of the oral cavity outweighed the adverse events (injection site irritation and injection site pain). Therefore, the CHMP considers that the benefit-risk balance for Lymphoseek in the indication for imaging and intraoperative detection of sentinel lymph nodes draining a primary tumour in adult patients with breast cancer, melanoma, or localised squamous cell carcinoma of the oral cavity is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Lymphoseek in the diagnosis of “This medicinal product is for diagnostic use only. Radiolabelled Lymphoseek is indicated for imaging and intraoperative detection of sentinel lymph nodes draining a primary tumour in adult patients with breast cancer, melanoma, or localised squamous cell carcinoma of the oral cavity. External imaging and intraoperative evaluation may be performed using a gamma detection device.” is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

CHMP divergent position(s)

Divergent position(s) to the majority recommendation are appended to this report.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that Lymphoseek (tilmanocept) is qualified as a new active substance.

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Appendix

Divergent positions expressed by CHMP members

Some members of the CHMP expressed a divergent position as follows:

Divergent opinion

For the localised squamous cell carcinoma of the oral cavity indication:

There is no convincing evidence-based support for the indication in localised squamous cell carcinoma of the oral cavity. There is only data from a small study in patients with oral squamous cell carcinoma, intended to evaluate the false negative rate for identification of tumour positive nodes. In this study there was a false negative rate of 2.5% (0.6%; 13.5%), i.e. compatible with possible lymph node involvement not detected by Lymphoseek in up to around 10+% of the patients, this is considered too high to be acceptable.

For the breast cancer and melanoma indication:

Regarding the indication for sentinel-node identification in melanoma and breast cancer, it has not been clearly demonstrated that Lymphoseek is at least similar to radiolabelled nanocolloids (which is standard European practice (EMA/150127/2010)) in terms of technical and diagnostic performances. Further cross-over studies on concordance between Lymphoseek and nanocolloids are warranted.

The CHMP members that have a divergent position for all indications are:

_____
Alar Irs

_____
Andrea Laslop

_____
Ján Mazag

_____
Ivana Mikačić

_____
Concepcion Prieto Yerro

_____
Sol Ruiz

The CHMP members that have a divergent position for only the localised squamous cell carcinoma of the oral cavity indication are:

_____
Kristina Dunder