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APPLICATION NUMBER:

202207Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202207
Submission date: August 10, 2011
Drug: Lymphoseek®: Kit for the Preparation of Technetium Tc 99m Tilmanocept for Injection
Sponsor: Navidea Biopharmaceuticals (formerly Neoprobe Corporation)
Indication: (b) (4)

Reviewing Division: Division of Medical Imaging Products

Background Comments:
The pharmacology/toxicology reviewer and team leader in the Division of Medical Imaging Products reviewed the nonclinical information for Technetium Tc 99m Tilmanocept and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Carcinogenicity and developmental and reproductive toxicity studies have not been conducted with Technetium Tc 99m Tilmanocept. This is acceptable because the product is a radiopharmaceutical that is used acutely.

Conclusions:
I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. Calling Technetium Tc 99m Tilmanocept a radioactive diagnostic agent for its Established Pharmacologic Class is consistent with other drugs in the class. I concur with the labeling changes suggested in the pharm/tox review.

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/s/

PAUL C BROWN
07/26/2012

`DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202-207
Submission type/Number:	Orig-1/eCTD sequence number 0000
Supporting document/s:	0001
Applicant's letter date:	August 10, 2011
CDER stamp date:	August 10, 2011
Product:	Lymphoseek®: Kit for the Preparation of Technetium Tc 99m Tilmanocept for Injection
Proposed Indication:	Lymphoseek®: (b) (4)
Applicant:	Neoprobe Corporation, 425 Metro Place north, Suite 300, Dublin, OH 43017. Company Name changed to: "Navidea Biopharmaceuticals, Inc." (eCTD 0011, Jan 05, 2012, §1.2, page 1 of 1). Address: unchanged
Review Division:	Division of Medical Imaging Products (HFD-160)
Reviewer:	Olayinka A. Dina, D.V.M., Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Division Director:	Rafel Dwaine Rieves, M.D.
Project Manager:	Alberta Davis-Warren, B.S.

Template Version: September 1, 2010

Disclaimer

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TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

The radioactive diagnostic agent evaluated in NDA 202-207 is Technetium Tc 99m Tilmanocept for Injection. The unlabeled drug substance, Tilmanocept, is Diethylenetriaminepentaacetic acid (DTPA)-Mannosyl-Dextran. DTPA-Mannosyl-Dextran, as used in this review, refers to Tilmanocept. The drug product, Technetium Tc 99m-labeled Tilmanocept is proposed [redacted] (b) (4)

DTPA-Mannosyl-Dextran accumulates in lymphatic tissue bound to mannose binding receptors present on the surface of dendritic cells and macrophages. The recommended adult dose is 50 µg. In the clinical setting, Technetium 99mTilmanocept for Injection is proposed to be administered locally via peritumoral, subcutaneous, subareolar or intradermal routes, as appropriate. The subcutaneous route was commonly used in the nonclinical studies.

1.2 Brief Discussion of Nonclinical Findings

1.2.1 Pharmacology

Primary pharmacodynamics: Primary pharmacodynamic studies, conducted to analyze the binding specificity of drug product, showed that DTPA-Mannosyl-Dextran has a specific binding interaction with mannose binding receptors (MBRs) natively expressed on the surface of human lymphatic system-derived macrophages with a high binding affinity. Receptor specificity and affinity of radiolabeled DTPA-mannosyl-dextran were demonstrated by *in vitro* binding assay. The equilibrium dissociation constant for binding to the MBR was 0.12±0.07 nmol/L.
No secondary pharmacology studies were performed.

Safety pharmacology: Two *in vivo* safety pharmacology studies were conducted in beagle dogs to evaluate the cardiovascular pharmacology effects of intravenously administered DTPA-Mannosyl-Dextran. In the first study (non-GLP) in conscious, non-telemetered dogs, the single administered dose of DTPA-Mannosyl-Dextran (560 µg/kg/dog) had no effect on survival, clinical signs, ECG or blood pressure. In the second study (GLP), DTPA-Mannosyl-Dextran, administered to telemetered dogs at doses up to 840 µg/kg, by dose escalation, was well tolerated. There were no adverse effects on the cardiovascular system, body temperature or body weight. NOAEL was determined as 560 µg/kg (364-fold) and 840 µg/kg (or 546-fold MHD) respectively for the evaluated cardiovascular studies.

CNS and respiratory safety studies were not conducted.

1.2.2 Pharmacokinetics

Four nonclinical pharmacokinetic (PK) studies were conducted to evaluate the PK profile of ^{111}In- or Technetium Tc 99m Tilmanocept. Plasma and urine PK parameters were determined in a single dose non-GLP pilot study in a mongrel dog. Two 14-day repeated dose toxicity studies were conducted in Sprague-Dawley rats and mongrel dogs. Tissue distribution was evaluated in rabbits. This GLP distribution study was conducted as one of three bridging studies with Technetium Tc 99m Tilmanocept.

In the rat and dog repeat-dose toxicity studies, data based on ^{111}In-DTPA-Mannosyl-Dextran administered by the subcutaneous route, showed that PK was similar across both species and sex. Absorption was rapid with a half life of 4 min and 23 min in the rat and dog, respectively. C_{max} ranged between 10.3 and 28.6 ng/mL in the rat and 6.8-42.8 ng/mL in the dog. T_{max} was 7.8 min in the rat and 28-66 min in the dog. AUC increased dose-proportionally in both species. Excretion was by the urinary route and the elimination half life was 3 and 6.5 hours in the rat and dog, respectively.

The findings of the tissue distribution study in rabbits indicated that Technetium Tc 99m Tilmanocept administered by the subcutaneous route, was widely distributed in the body in male and female animals. In females, at 15 minutes postdose, organs with higher percentage amounts of the injected dose (%ID) included the skin injection site (~7%), plasma (~10%), urinary bladder contents (7.7%), both the liver kidney (3%) and gastrointestinal tract (approximately 2%). In general, lower amounts were reported in most organs of distribution at 1h and 3h time points except in the kidneys, urinary bladder and colon contents. The increased amounts of Technetium Tc 99m Tilmanocept the major excretory organs were indicative of continued absorption and excretion**.** Also at 15 min postdose, greater than 1% of the subcutaneously injected dose of Technetium Tc 99m Tilmanocept was found in the popliteal lymph node of the treated leg in female rabbits. Evidence of presence of Technetium Tc 99m Tilmanocept in the popliteal lymph node ipsilateral to the injection site, in contrast to its lack in the contralateral popliteal node, was indicative increased absorption of injected Technetium Tc 99m Tilmanocept from the injection site.

1.2.3 Toxicology

Single-dose and repeat-dose toxicity: Single-dose toxicity studies were performed in rats, rabbits and dogs while repeated dose toxicity studies were conducted in rats and dogs. The single- and repeat-dose studies showed no evidence of potential toxicity of Technetium Tc 99m Tilmanocept and NOAEL was the top dose administered in each study except in one single-dose acute toxicity study in rabbits in which the NOAEL could not be determined. All toxicity studies were GLP-compliant. Toxicity studies were conducted primarily with the unformulated drug substance.

Genetic toxicology: Three studies were performed to assess the genotoxic potential of DTPA-Mannosyl-Dextran. DTPA-Mannosyl-Dextran was negative in the *in vitro* bacterial reverse mutation, *in vitro* L5178Y/TK$^{+/-}$ Mouse Lymphoma and in the *in vivo* bone marrow micronucleus assays.

Carcinogenicity: Carcinogenicity studies were not performed

Reproductive toxicity: Reproductive and Developmental toxicity studies were not conducted. A waiver request was submitted

1.2.4 Special Toxicology

Antigenicity: Antigenicity study, conducted in male guinea pigs showed that DTPA-Mannosyl-Dextran (Tilmanocept), administered up to 280 µg/kg did not induce any anaphylactic reactions in male guinea pigs after four weekly subcutaneous sensitization doses and an intravenous challenge dose. Adverse reactions were elicited in Ovalbumin/CFA-treated positive control animals. NOAEL was 280 µg/kg (or 72.7x MHD).

Local Irritation: Two local irritation studies were designed to determine the irritant potential of a single intramuscular dose of DTPA-Mannosyl-Dextran in NZW rabbits. In both studies, DTPA-Mannosyl-Dextran had no effect on survival, clinical observations or injection site histopathology at doses up to 286 µg/kg or 111-fold MHD ((b) (4) 1146-104) and up to 280 µg/kg or 109-fold MHD ((b) (4) 1576-06069).

Impurities: Impurities identified in the drug product (Technetium Tc 99m Tilmanocept) are (b) (4) the drug product is being developed for single dose administration, no specific toxicity studies with these impurities was conducted.

1.3 Recommendations

1.3.1 Approvability

Approval is recommended based on Pharmacology/Toxicology data evaluated

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following changes in the label would more appropriately reflect findings from preclinical studies

3 pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

Studies on reproductive fertility have not been conducted.

2 Drug Information

2.1 Drug

The radioactive diagnostic agent evaluated in NDA 202-207 is Technetium Tc 99m Tilmanocept Injection.

CAS Registry Number (Optional)

Table 1: Tilmanocept / Technetium Tc 99m Tilmanocept CAS Number

Compound	CAS #
Unlabeled drug substance (Tilmanocept or DTPA-Mannosyl-Dextran)	1185986-76-8
Technetium Tc 99m Tilmanocept Injection	1262984-82-6

Source: Reviewer's Table constructed from sponsor data

Generic Name

Table 2: Tilmanocept / Technetium Tc 99m Tilmanocept Generic Name

Compound	Generic name
Unlabeled drug substance	DTPA-Mannosyl-Dextran
Technetium Tc 99m Tilmanocept Injection	Technetium Tc 99m DTPA-Mannosyl-Dextran[1]

[1]United States Adopted Name [USAN] for the radiolabeled product
Source: Reviewer's Table constructed from sponsor data

Code Name
N/A

Chemical Name

Table 3: Chemical Name of Tilmanocept / Technetium Tc 99m Tilmanocept

Compound	Chemical name
DTPA-Mannosyl-Dextran	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-(Dmannopyranosylthio) ethyl]amino]ethyl]thio]propyl ether
Technetium Tc 99m Tilmanocept	^{99m}Tc-Diethylenetriaminepentaacetic acid-mannosyl-dextran; Technetium-99mTc, 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-(Dmannopyranosylthio) ethyl]amino]ethyl]thio]propyl ether

Source: Reviewer's Table constructed from sponsor data

Proprietary and Non-proprietary Names

Proprietary Name: Lymphoseek® - Kit for the Preparation Technetium Tc 99m Tilmanocept injection or Technetium Tc 99m Lymphoseek® injection

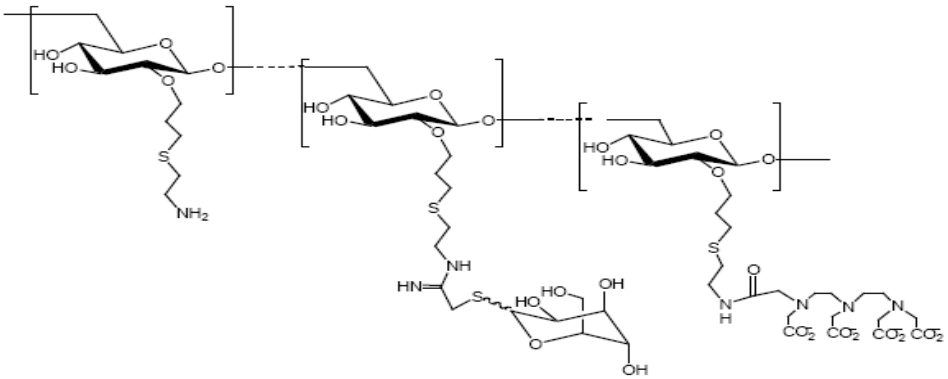
Non-proprietary Name: No INN has been given for Technetium Tc 99m Tilmanocept Injection (§ 3.2.P.2.1.1.3)

Molecular Formula/Molecular Weight

$C_{722}H_{1266}N_{62}O_{439}S_{46}$ (calculated average) / 15,281 - 23,454 g/mol (lower - upper MW range)

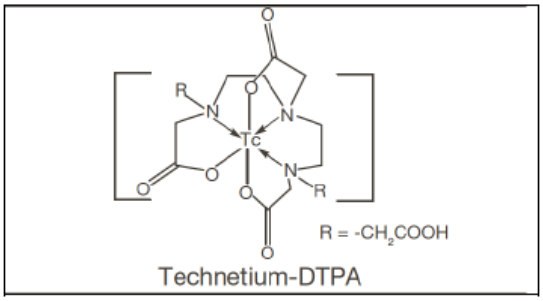
Structure or Biochemical Description

Figure 1: Structure of DTPA-Mannosyl-Dextran (Tilmanocept) [1]



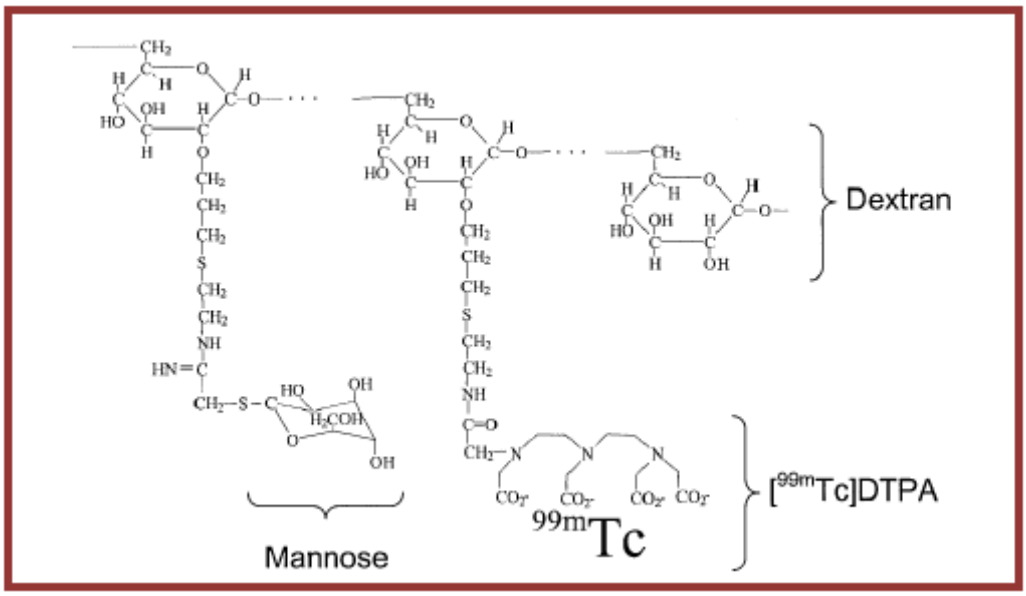
[1]Source: Sponsor's submission (§ 2.3.S.1.2, page 5 of 49)

Figure 2: Possible Structure of Technetium Tc 99m-DTPA Complex[1]



[1]According to the sponsor, the exact structure of Technetium Tc 99m-DTPA is unknown

Figure 3: Chemical Construct of Technetium Tc 99m Tilmanocept Injection [1]



[1]Source: Sponsor data, NEO3-08, Appendix 7.2 – Protocol and Amendments, § 4.2.1.1.1, pages 82 of 262 and 14 of 262)

Pharmacologic Class:

Technetium Tc 99m Tilmanocept is a radioactive diagnostic agent

Manufacturer:

Table 4: Drug substance and product

Name	Type of compound	Manufacturer
Unlabeled Tilmanocept	Drug substance [1]	(b) (4)
Technetium Tc 99m Tilmanocept Injection	Drug product [2]	

Reviewer's Table from sponsor data based on [1]§ 3.2.S.2.1, page 1 of 1; [2]§ 3.2.P.3.1, page 2 of 6 and nonclinical overview § 2.4, page 4 of 22

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 61,757 (Technetium Tc 99m Tilmanocept; (b) (4)

2.3 Drug Formulation

2.3.1 Introduction

The to-be-marketed product, Technetium Tc 99m Tilmanocept Injection is composed of Tilmanocept Mannosyl-Dextran and a chelating agent, diethylenetriaminepentaacetic acid (DTPA). The DTPA moiety chelates the Technetium Tc 99m at the time of drug preparation. Upon subcutaneous injection, the Mannosyl-Dextran directs the radio-chelate to lymphoid tissue for radioimaging. Technetium Tc 99m Tilmanocept injection is proposed for administration within a few minutes to a few hours after preparation. The radiolabeled Tilmanocept has a half-life of 6 h. DTPA-Mannosyl-Dextran formulation comprises DTPA-Mannosyl-Dextran (drug substance), stannous chloride (b) (4) sodium ascorbate (b) (4)

The qualitative and quantitative composition of the lyophilized DTPA-Mannosyl-Dextran formulation (0.25 mg vial) is shown in Tables 5 and 6.

Table 5: Formulation of DTPA-Mannosyl-Dextran [1, 2, 3]

Component	Amount per vial (mg)	Function	Quality Standard
Tilmanocept	0.25	Drug Substance	Company Standard
Glycine	0.5	(b) (4)	USP, Ph. Eur.
Sodium Ascorbate	0.5		USP, Ph. Eur.
Trehalose, Dihydrate	20		NF
Stannous Chloride, Dihydrate	0.075		NF, Ph. Eur.
			(b) (4)
Nitrogen		(b) (4)	NF, Ph. Eur.
Water For Injection			USP, Ph. Eur.

* Removed during processing

[1]Source: Sponsor's Table 1, § 3.2.P.1, Description and Composition of DTPA-Mannosyl-Dextran, page 3 of 5; [2]Formulation also includes Nitrogen (b) (4) Quality Standard = NF, Ph. Eur, source of information: Nonclinical Introduction, page 5 of 6, § 2.6.1). Water for injection (b) (4) Quality Standard = USP, Ph. Eur.) was removed during processing, source: sponsor Table 1, Nonclinical Introduction, page 5 of 6, § 2.6.1); [3]According to the sponsor, the active pharmaceutical ingredient (API) in the final product is Technetium Tc 99m Tilmanocept in phosphate buffered saline (PBS)

Table 6: Composition of Technetium Tc 99m Tilmanocept Injection [1, 2]

Component	Amount per unit	Function
Lymphoseek (Tilmanocept) 0.25 mg	(b) (4)	
Sodium Pertechnetate Tc 99m		
Sterile Saline for Injection		
Sterile Buffered Saline		

[1]Source: Sponsor Table 3, page 5 of 5, § 3.2.P.1, Description and Composition of the Drug Product; [2]In this review, the drug product for consistency is referred t as Technetium Tc 99m Tilmanocept Injection.

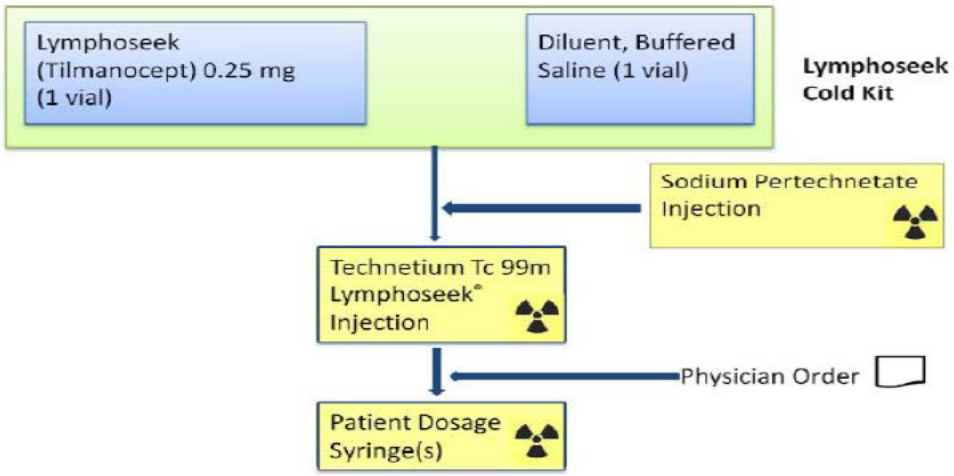
Table 7: Components and Composition of Buffered Saline [1, 2]

Component	Amount per unit (%)	Function
Potassium Phosphate	0.04	(b) (4)
Sodium Phosphate · $7H_2O$	0.11	
Sodium Chloride	0.5	
Phenol	0.4	
Water For Injection	q.s.	

[1]Source: sponsor Table 2, page 4 of 5, 3.2.P.1.4; [2]According to the sponsor, because the reconstitution pH of the DTPA-Mannosyl-Dextran formulation (b) (4) The diluent is sterile buffered saline (4.5 mL), (b) (4) Item SB2507145 (DMF number (b) (4) .This commercially available diluent contains sodium and potassium phosphates with 0.5% NaCl (b) (4) (Source: Sponsor Table 2, page 4 of 5, § 3.2.P.1.4)

2.3.2 Preparation of Technetium Tc 99m Tilmanocept Injection

Figure 4: Schema for Preparation of Technetium Tc 99m Tilmanocept Injection dosage form [1]



[1]Source: § 2.3.1 Introduction, page 4 of 13, Quality overall summary); the radiolabeling is performed in a nuclear pharmacy by persons qualified and approved to use radionuclides (§ 3.2.P.1, page 5 of 5, Description and composition of the drug)

2.3.3 Drug formulations

Different formulations were used for the nonclinical studies, namely, unlabeled and radiolabeled (Cy3) drug substance, [DTPA-Mannosyl-Dextran]; and unlabeled and labeled (Technetium Tc 99m) drug product. Appropriate bridging studies have been completed with the final formulation to demonstrate equivalency.

Table 8: Drug formulations

Formulation		Name	Alternate Name(s)
1	Non-radiolabeled drug substance	Tilmanocept	DTPA-Mannosyl-Dextran
2	Non-radiolabeled formulated drug product	Lymphoseek	Unlabeled DTPA-Mannosyl-Dextran
3	Radiolabeled drug product [1]	Technetium Tc 99m Tilmanocept for injection	Tc 99m Lymphoseek; Lymphoseek®
3	Non-radiolabeled formulated drug product	Lymphoseek	Unlabeled DTPA-Mannosyl-Dextran
4	^{111}In-radiolabeled drug substance	Indium 111-labeled-DTPA-Mannosyl-Dextran	^{111}In-DTPA-Mannosyl-Dextran [2]

Reviewer's Table constructed from sponsor's submission; [1] = Tc 99m radiolabel was used in the proposed market product [2] = ^{111}In-radiolabel. In this review, Technetium Tc 99m Tilmanocept for injection denotes Tc 99m Lymphoseek. DTPA-Mannosyl-Dextran is used in reference to Tilmanocept (drug substance); NOTE: Cyanine-3 (Cy3)-labeled Tilmanocept (Pharmacology Written Summary, § 2.6.2, Table 1, page 6 of 20) was also used for fluorescent-labeling of DTPA-Mannosyl-Dextran in the *in vitro* study NEO3-10 (page 10 of 40).

Table 9: List of Nonclinical studies and Drug formulations used

Study No.	Study category/Type	Test article	Species/Tissue
Vera et al (2001)	Pharmacodynamics	Technetium Tc 99m Tilmanocept	Homogenized liver
NEO3-08		Technetium Tc 99m Tilmanocept [5]; Unlabeled Tilmanocept [4]	Human macrophages
NEO3-08A		Technetium Tc 99m Tilmanocept [5]; Unlabeled Tilmanocept [4]	
NEO3-10		Cy3-labeled-Tilmanocept; UnlabeledTilmanocept [4]	
1146-102	Safety Pharmacology	Unlabeled Tilmanocept [1]	Dog
1576-04774			
N106921	Pilot PK	^{111}Indium-Tilmanocept; Unlabeled Tilmanocept [1]	Dog
608002	Distribution PK	Technetium Tc 99m Tilmanocept [6];	Rabbit
1146-100	Single dose Toxicity	Unlabeled Tilmanocept [3]	Rat
1576-06049		Unlabeled Tilmanocept [2]	Rat
1146-101		Unlabeled Tilmanocept [3]	Rabbit
1146-104	Single dose Toxicity (local tolerance)	Unlabeled Tilmanocept [3]	Rabbit
1576-06069	Single dose Toxicity (local tolerance)	Unlabeled Tilmanocept [2]	Rabbit
1576-04582	Single dose Toxicity	Unlabeled Tilmanocept [1]	Dog
N106923	Repeated Dose Toxicity (including PK/TK)	^{111}Indium-Tilmanocept [1]; Unlabeled Tilmanocept [1]	Rat
N106922			Dog
AB11LN.704.BTL	Genotoxicity; mammalian cell gene mutation assay	Unlabeled Tilmanocept [1]	LY 5178Y/TK$^{+/-}$Mouse lymphoma cells
AB11LN.503.BTL	Genotoxicity; Bacterial reverse mutation assay		*S.typhimurium and E.coli*
AB11LN.123.BTL	Genotoxicity; Mammalian erythrocyte micronucleus test		Mice
1576-04583	Antigenicity	Unlabeled Tilmanocept [1]	Guinea pig

Reviewer's Table constructed from sponsor's submission; Tilmanocept = DTPA-Mannosyl-Dextran; [1,2,3,4] = drug substance batch information (source: sponsor Table 2.6.7.4, § 2.6.7, page 7 of 23); [1] = DTPA-Mannosyl-Dextran batch No. M50840 (development lot); [2] = DTPA-Mannosyl-Dextran Batch No. M51415 (cold kit drug substance for drug product lot NFR-00015-04706-011); [3] = DTPA-Mannosyl-Dextran batch No. DMD02dec99 (from UCSD); [4] = DTPA-Mannosyl-Dextran batch No. M60834; [5]Technetium Tc 99m Tilmanocept (Lymphoseek) Kit Lot No. NMK003; [6] = Technetium Tc 99m Tilmanocept (Lymphoseek) Kit Lot Nos. NMK001-077, NMK001-078, and NMK001-076

Reviewer's Note: Three studies (NEO3-08, NEO3-08A, and [(b) (4)]6008002) were conducted with Technetium Tc 99m Tilmanocept Injection. The single dose toxicity studies, [(b) (4)] 1576-06049 and 1576-06069, were conducted in rats and rabbits, respectively, using GMP-grade drug substance (DTPA-Mannosyl-Dextran).

2.3.4 Routes of Administration: Technetium Tc 99m Tilmanocept Injection will be locally administered by the peritumoral (PT), subcutaneous (SC), subareolar (SA) or intradermal (ID) routes as appropriate, depending on the tumor type and location Technetium Tc 99m Tilmanocept Injection is not intended for systemic administration. The subcutaneous route was used for most nonclinical studies.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None of the impurities are of concern.

Table 10: Specified Impurities in DTPA-Mannosyl-Dextran (drug substance)[1]

Batch No.	Purity (%)	Specified Impurities [(b) (4)] (% w/w)	[(b) (4)] (% w/w)	Study Number	Type of Study
Proposed Specifications:	[(b) (4)]			—	—
M50840 (developmental lot)	95.7	[(b) (4)]	Not detected	[(b) (4)] 1576-04582	Single dose toxicity in dogs
				[(b) (4)] N106922	14-Day toxicity in dog
				N106923	14-Day toxicity in rat
				[(b) (4)] 704.BTL	Genotoxicity (in vitro)
				503.BTL	Genotoxicity (in vitro)
				123.BTL	Genotoxicity (in vivo)
				1576-04583	Antigenicity study in guinea pig
M51415 (drug substance for drug product lot NFR-00015-04706-011)	99.2	Not detected	Not detected	1576-06049	Single dose toxicity in rats
				1576-06069	Local tolerance in rabbits
DMD02dec99 (from UCSD)	Not available	Not available	Not available	[(b) (4)] 1146-100	Single dose toxicity in rats
				1146-101	Single dose toxicity in rabbits
				1146-104 *	Local tolerance in rabbits

* Though batch number documentation is not available for this study, this batch number is assumed based on two other studies conducted with this batch in the same year (2000) at the same testing facility. All other tilmanocept nonclinical studies were conducted during or after 2005.

Abbreviations: DTPA, diethylenetriaminepentaacetic acid; UCSD, University of California at San Diego.

Source: 3.2.S.4.4, Table 2: Batch Analysis

[1]Source: Sponsor's Table 2.6.7.4: Toxicology – Drug substance, page 6 of 23, § 2.6.7 (Toxicology tabulated summaries)

Reviewer's Comments: Based on the description of "drug substance" and "drug product", the term Lymphoseek, with or without the trademark notation (®) is understood to represent the to-be-marketed, labeled and diluted drug product, i.e., Technetium Tc 99m Tilmanocept Injection. The specified impurities in DTPA-Mannosyl-Dextran were

quantified as [redacted] Technetium Tc 99m Tilmanocept Injection is proposed for single dose administration and no specific toxicity studies of these impurities were conducted.

2.6 Proposed Clinical Population and Dosing Regimen

2.6.1 Clinical population

Clinical studies using Technetium Tc 99m Tilmanocept Injection were conducted in patients with melanoma or breast cancer, [redacted]

2.6.2 Dosage regimen

2.6.2.1 Radioactive dose: The lyophilized Tilmanocept (DTPA-Mannosyl-Dextran) formulation will be radiolabeled with Technetium Tc 99m based on the proposed post-surgery schedule [redacted] as shown in Table 11:

Table 11: Dosage regimen for Technetium Tc 99m Tilmanocept Injection [1, 2]

Post-injection Surgery Schedule	Dose[2] of Technetium Tc 99m Tilmanocept (mCi)	Recommended Adult Dose (µg)
Same day (30 min – 15 hrs)	0.5 (18.5 MBq)	50
[redacted]		

[1]Source: Reviewer's Table constructed from sponsor's data (§ 2.5.1.7, Clinical overview: page 17 of 54); [2]The total radiation dose Technetium Tc 99m Tilmanocept in a single injection [redacted]

2.6.2.2 Dose volume: Total injection volume would be no greater than 1 mL and no less than 0.1 mL for each tumor. Individual injection aliquot would not exceed 0.5 mL or be less than 0.1 mL.

2.7 Regulatory Background

The sponsor submitted a New Drug Application (NDA) for Lymphoseek® (Technetium Tc 99m Tilmanocept Injection) in accordance with section 505(b) of the Federal Food, Drug and Cosmetic Act (FDCA) on August 10, 2011. Reference number for the Application is NDA 202-207.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Published article: Vera et al (2001). A synthetic macromolecule for sentinel node detection: ^{99m}Tc-DTPA-Mannosyl-Dextran. The Journal of Nuclear Medicine, 42(6):951-959

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 Modeling and Clinical Results from NEO3-05 Phase 3 Study of Lymphoseek Concordance with Vital Blue Dye in Breast Cancer and Melanoma Patients"
NEO3-08A (Nonclinical study report addendum): "*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)"

Safety Pharmacology
(b) (4)-1146-102: "Safety Pharmacology Study of DTPA Mannosyl Dextran in Dogs"
(b) (4) – 1156-04774: "A Cardiovascular Safety Pharmacology Study in Beagle Dogs"

Pharmacokinetics
Distribution
(b) (4)608002: "A Tissue Distribution Study of [^{99m}Tc]-Lymphoseek in rabbits"

Other Pharmacokinetic Studies
Toxicology components of single dose ((b) (4)N106921) and repeated dose (b) (4) N 106923 and (b) (4) 106922) as described below

Single-Dose Toxicity
Rat (Subcutaneous)
(b) (4) -1146-100: "Acute Toxicity Study of DTPA Mannosyl Dextran in Rats"
(b) (4) 1576-06049: "Lymphoseek DTPA Mannosyl Dextran: An Acute Toxicity Study in Sprague-Dawley Rats"
Rabbit (Subcutaneous)
(b) (4) -1146-101: "Acute Toxicity Study of DTPA Mannosyl Dextran in Rabbits"
Rabbit (Intramuscular)(Note: (b) (4)-1146-104 and (b) (4)1576-06069 are also cited as Local Tolerance studies)
Dog (Subcutaneous)
(b) (4)– 1576-04582: "DTPA Mannosyl Dextran: A Single-dose Subcutaneous Toxicity Study in mongrel Dogs"

Repeat-Dose Toxicity
Rat (Subcutaneous, Short)
(b) (4) N 106923: "14-Day Toxicity of ^{111}Indium-Lymphoseek in Sprague-Dawley Rats"
Dog (Subcutaneous, Short)
(b) (4) N 106922: "14-Day Toxicity of ^{111}Indium-Lymphoseek in Mongrel Dogs"

Genotoxicity
In Vitro Studies
(b) (4) AB11LN.704BTL: '*In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay)"
(b) (4) AB11LN.503BTL: "Bacterial Reverse Mutation Assay"

In Vivo Studies (including supportive toxicokinetics evaluations)
(b) (4) AB11LN.123BTL: "Mammalian Erythrocyte Micronucleus Test"

Local Tolerance

(b) (4) -1146-104: "Study of the Irritant Potential of DTPA-Mannosyl Dextran in New Zealand White Rabbits"
(b) (4) 1576-06069: "Lymphoseek™ DTPA-Mannosyl-Dextran: A Study of the Irritant Potential in New Zealand White Rabbits"

Other Toxicity Studies
Antigenicity
(b) (4) 1576-04583: "DTPA-Mannosyl-Dextran: A Systemic Hypersensitivity (Anaphylaxis) Study in Guinea Pigs"

3.2 Studies Not Reviewed

Primary Pharmacology
NEO3-10: "*In Vitro* Binding Study of Tilmanocept with Low and High Mannose Conjugation; Binding to Human Macrophage Mannose Binding Receptor Proteins"

Pharmacokinetics
(b) (4) N 106921: "Pilot Pharmacokinetic Study of ^{111}Indium-Lymphoseek in a Mongrel Dog"

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

4.1.1.1 Mechanism of action: Technetium Tc 99m Tilmanocept accumulates in lymphatic tissue and binds onto mannose binding receptors ((MBRs) or mannose-binding protein residing on the surface of dendritic cells and macrophages. Receptor-bound ligand (i.e., MBR-Lymphoseek receptor-ligand complex) can be detected by gamma planar imaging, gamma detection probe or by single-photon emission computed tomography (SPECT)

4.1.1.2 Mannose Binding Receptor: Mannose binding receptors are located on the surface of resident dendritic cells and macrophages. The intended receptor target for Lymphoseek is the human MBR (hMBR). The mannose receptor is a C-type lectin (CLEC) carbohydrate binding protein domain. The C-type designation is based on their requirement of calcium for binding. Two types of mannose receptors are expressed in humans, each encoded by a specific gene (macrophage mannose receptor 1 and 2 or MRC1 and MRC2, respectively). MRC1 is variously known as C-type mannose receptor 1, C-type lectin domain family 13 member (CLEC 13D) or CD206.The mannose component of the Lymphoseek macromolecule acts as a substrate for the MBR receptor while the DTPA serves as a chelating agent for radiolabeling with Technetium Tc 99m.. The small mean diameter (~7nm) of DTPA-Mannosyl-Dextran enhances its diffusion into lymphatic channels and blood capillaries. As shown in Figure 5, MBR consists of

(b) (4)

Source: Sponsor data, Appendix 7.2 – Protocol and Amendments, § 4.2.1.1.1, page 9 of 262)

4.1.2 Primary Pharmacology

Reports of the following published article; in *vitro* (NEO3-08 and NEO3-08A) and *in vivo* ((b) (4) 608002) studies were evaluated:

1. Publication -Vera et al (2001): A synthetic macromolecule for sentinel node detection: 99mTc-DTPA-Mannosyl-Dextran.
2. NEO3-08: "An Integrated Analysis of the *In Vitro* Binding Specificity of GMP Lymphoseek® (Kit for the preparation of Technetium Tc 99mTilmanocept for Injection) to Human Mannose Binding Receptor-Expressing Macrophages with the Effect of Injection Volume Excursion Modeling and Clinical Results from NEO3-05 Phase 3 Study of Lymphoseek Concordance with Vital Blue Dye in Breast Cancer and Melanoma Patients"
3. NEO3-08A: "*In Vitro* Binding Specificity of GMP-grade Lymphoseek® Lymphoseek® (Tilmanocept) to the Human Mannose Binding Receptor (hMBR) of Viable Human Macrophages and Confirmation of Direct Binding to Recombinant hMBR (rhMBR)". NEO3-08A was submitted as an amendment to the NEO3-08 final report.
4. (b) (4) 608002: 'A Tissue Distribution Study of [99mTc]-Lymphoseek in Rabbits", (Reviewed under Pharmacokinetic studies).

4.1.2.1: In vitro binding Study: Vera et al (2001) – "A synthetic macromolecule for sentinel node detection: ^{99m}Tc-DTPA-Mannosyl-Dextran".

Introduction: The authors describe the synthesis and preliminary biologic testing of DTPA-Mannosyl-Dextran. Although the paper notes synthesis, radiolabeling, and *in vitro* binding and biodistribution of DTPA-Mannosyl-Dextran, the focus of this review is the *in vitro* binding of the drug substance to the Mannose Binding Receptor (MBR).

Key study findings: Receptor specificity and affinity of radiolabeled DTPA-mannosyl-dextran were demonstrated by *in vitro* binding assay. The equilibrium dissociation constant for binding to the MBR using homogenized liver was 0.12 ± 0.07 nmol/L.

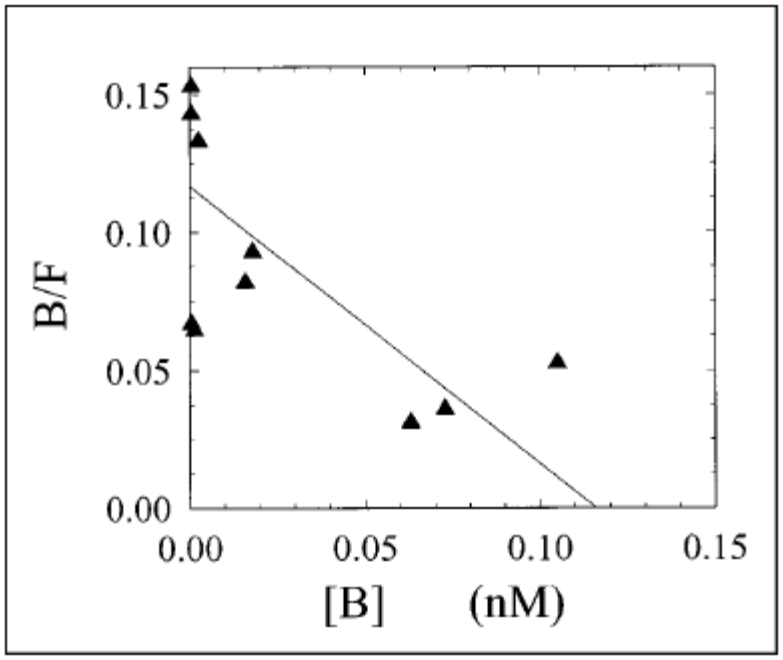
Objective: This study was designed to demonstrate, *inter alia*, the receptor binding of Lymphoseek to the mannose-terminated glycoprotein receptor (or Mannose Binding Receptor, MBR) and specifically to demonstrate that ^{99m}Tc-DTPA-mannosyl-dextran had an ultrahigh receptor affinity for the MBR.

Binding Assay: Receptor affinity was measured by Scatchard assay of the rabbit liver. According to the authors, previous studies of the mannose binding protein receptor (Kawasaki et al, 1980) had shown equivalent affinities for liver and lymph node binding. The equivalent dissociation constant for binding to homogenized rabbit liver was measured as previously described (Vera et al., 1995). Briefly, DTPA-Mannosyl-Dextran was labeled with 2.2 Gbq Tc 99m and diluted to 7 concentrations ranging from 2.1×10^{-6} to 2.1×10^{-11} mol/L and incubated with homogenized liver in 200 µL assay buffer comprising 1.0 mol/L NaCl, 0.05 mol/L$CaCl_2$, $MgCl_2$ 0.05 mol/L, Tris-HCl, and 0.1% human serum albumin at 37^0C for 30 min. Bound and free activity were separated by ultracentrifugation. Following 2 washes with 200 µL buffer, the ultracentrifuge tube and the washings were placed in scintillation vials and assayed for radioactivity at 100-200 keV. The equilibrium dissociation constant, K_D; the receptor concentration, [R]; and N1

(ratio representing the amount of nonspecifically-bound ligand), were calculated using the computer program LIGAND (Munson and Rodbard, 1980).

Results: ^{99m}Tc-DTPA-mannosyl-dextran demonstrated both receptor specificity and affinity. The equilibrium dissociation constant for binding to the mannose binding receptor was 0.12±0.07 nmol/L. Specific binding data are shown in the Scatchard plot (Figure 6):

Figure 6: In vitro assay of Technetium Tc 99m Tilmanocept binding to rabbit homogenized liver



Source: Figure 7 (Vera et al, 2001). K_D = 0.12 ± 0.07 nmol/L; [B] = bound concentration; B/F = bound-to-free ratio; each symbol represents a data point corrected for nonspecific binding by N1 (0.273 ± 0.011). The Scatchard line was calculated by LIGAND and yielded an x-axis intercept of 0.116 ± 0.074 nmol/L which is the receptor concentration (Bmax)

Discussion: Technetium Tc 99m Tilmanocept accumulates in lymphatic tissue by binding to a receptor that resides on the surface of macrophages in the lymph node (Hoh et al., 2003; Kawasaki et al., 1978, 1980). According to the sponsor, Technetium Tc 99m Tilmanocept, with an equivalent dissociation constant (K_D) of 0.12 nmol/L ranked among the most avid labeled neoglycoconjugates. These include ^{99m}Tc-DTPA-galactosyl-polylysine (K_D = 0.33 nmol/L Vera et al., 1995), iodine-labeled galatosyl-neoglycoalbumin (K_D = 0.14 nmol/L, Vera et al., 1984), and gallium-labeled deferoxamine-neoglycoalbumin, K_D = 0.19 nmol/L; Vera et al., 1992).

Conclusion: The authors concluded that Technetium Tc 99m Tilmanocept is a member of a new class of receptor-targeting diagnostic agents with a mannosyl-

neoglycoconjugate base with a highly substituted dextran backbone density of sites for the attachment of receptor substrates and imaging reporters.

Reviewer's comments: The Scatchard plot of bound [B] ligand / unbound ligand [U] versus [B]) was used to assess reversible ligand- receptor binding interactions. In this study, the plot is linear and ligand binding achieved equilibrium. The study determined that Technetium Tc 99m-radiolabeled DTPA-mannosyl-dextran () showed both receptor specificity and affinity for the mannose-binding receptor.

Overall, I agree with the results and conclusion of the *in vitro* binding assay.

4.1.2.2: Study No. NEO3-08

Study Title [a]: An integrated analysis of the *In vitro* binding specificity of GMP Lymphoseek® (Kit for the preparation of Technetium Tc 99m Lymphoseek for injection) to human mannose binding receptor-expressing macrophages with the effect of injection volume excursion modeling and clinical results from NEO3-05 phase 3 study of Lymphoseek concordance with Vital Blue Dye in breast cancer and melanoma patients	
Volume # and Page #:	Module 4, § 4.2.1.1; 64 pages (Revision C)
Conducting laboratory and location:	(b) (4)
Study #:	NEO3-08 (In vitro study)
Sponsor:	Neoprobe Corporation
Date of Study:	10/07/2009 – 11/26/2009 (Duration: 7 weeks)
GLP Compliance:	Yes (), No (x); Pharmacology tabulated summary, Source: § 2.6.3, Table 2.6.3.1, page 2 of 6
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Technetium Tc 99m Tilmanocept injection or Tc 99m Lymphoseek (GMP-compliant), Lot #: NMK003, % purity >90% of total radioactivity; Specific activity: 5 mCi/250 µg (page 10 of 10, Appendix 7-1, Appendix to Final Report) containing The DTPA-Mannosyl-Dextran (Lot #: M60834; §4.2.1.1.1, Appendix 7.5 [Protocol & amendments], pages 3-4 of 6)
Manufacturer(s):	DTPA-Mannosyl-Dextran and Tc 99m Lymphoseek (Technetium Tc 99m Tilmanocept injection): (b) (4) (§3.2.S.2.1, page 1 of 1; (§3.2.P.3.1, page 2 of 6, respectively). Radiolabeling was conducted by (b) (4)
Manufacturing standard:	GMP
Tissue/Cells:	Cells: Macrophages derived from 3 human donors of screened blood. Cells were validated for the expression of mannose receptors
Doses/Vehicle:	Technetium Tc 99m Tilmanocept injection: 0.654-30.0 µM Vehicle = Phosphate buffered saline (PBS)
Duration/route:	N/A; *in vitro* study
Treatment groups :	None

Reviewer's Table constructed from sponsor's data; [a] Report NEO3-08 (Revision C, Final date April 20, 2011)

Objective: This *in vitro* study was designed to determine the specificity and binding affinity (K_D) of Technetium Tc 99m Tilmanocept injection (drug product) to the mannose

binding receptor (MBR) natively expressed on the surface of human lymphatic-system derived macrophages and dendritic cells.

Key study findings: It was determined from this *in vitro* study that macrophage binding receptors (MBRs) were expressed of on the surface of human macrophages and that Lymphoseek had a specific binding interaction with native MBRs with high binding affinity. The results of this study demonstrated that Technetium Tc 99m Tilmanocept selectively binds to its intended receptor (hMBR).

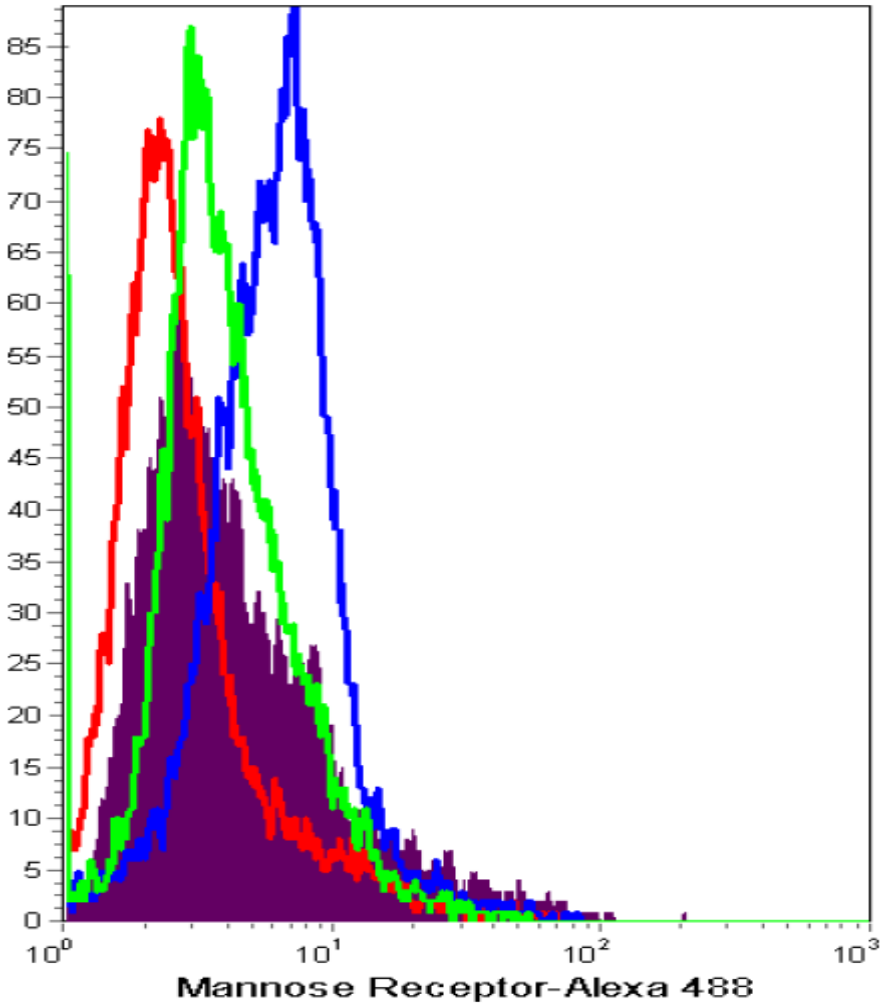
Study design: Macrophages were obtained from blood collected from three human donors used as sources of monocytes destined to differentiate into macrophages. Blood samples were de-identified from the three corresponding human donors and re-labeled by color as patients Blue, Black and Red. The blood was validated for the expression of mannose receptors by methods previously described in published literature. Macrophages were grown as fixed cells to six-well plates (9.5 cm^2) and the binding rate of Lymphoseek determined. Each determination was performed in triplicate at four concentrations (0.654, 1.47, 7.35, and 30 µM) reflecting the different concentrations of Technetium Tc 99m Tilmanocept employed in clinical study NEO3-05. Evaluations were conducted at 0-4^{0}C to prevent receptor internalization. Studies were run from at 5, 20, 35, 65 and 90 min. Unlabeled (cold) compound added, for nonspecific binding, were >95 times that of labeled Lymphoseek (Technetium Tc 99m Tilmanocept) for any given assay concentration. All samples were counted in an automatic gamma well counter. The main assay endpoints were binding kinetics ((maximum reaction/binding velocity or rate (V_{max}), one-half V_{max} ($0.5V_{max}$), and equilibrium dissociation constant (K_D)), and Hill coefficient. Data analysis was performed using GraphPad Prism (ver. 5.02) and Statmate (ver. 2.0) programs. The binding model assumes that all receptors were equally accessible to ligands; that receptors were either free or bound to ligand; that binding did not alter the ligand or receptor, and that binding was reversible.

Results:

1. Confirmation of Mannose receptor surface expression on human donor macrophages after two weeks culture *in vitro* (Figure 7):

The color scans in the figure indicate relative fold-changes in the expression of the number (cell number is constant for each scan). Blue indicates a relative fold-change of approximately 6.5-fold. There is heterogeneity in population (receptor/cell). Nevertheless, the scans confirm the differentiation dependent expression of the receptor and its surface expression validation.

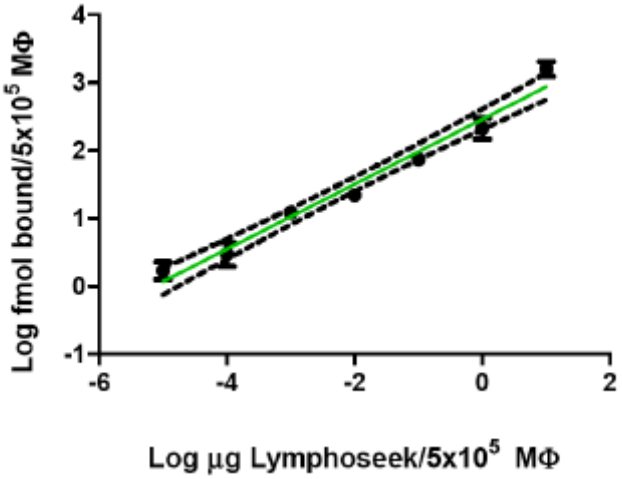
Figure 7: Mannose receptor expression on human donor macrophages[1]



[1]Sponsor's Figure 4 (page 29 of 64, study report NEO3-08); Sponsor's legend: Figure 4 is a flow evaluation of the surface expression of the human mannose receptor on donor macrophages after two weeks culture *in vitro*. Purple = Isotype control; Red = Undifferentiated Cells (Day 1); Green = Partial Differentiation (Day 5); Blue = Differentiated Cells (Day 14).

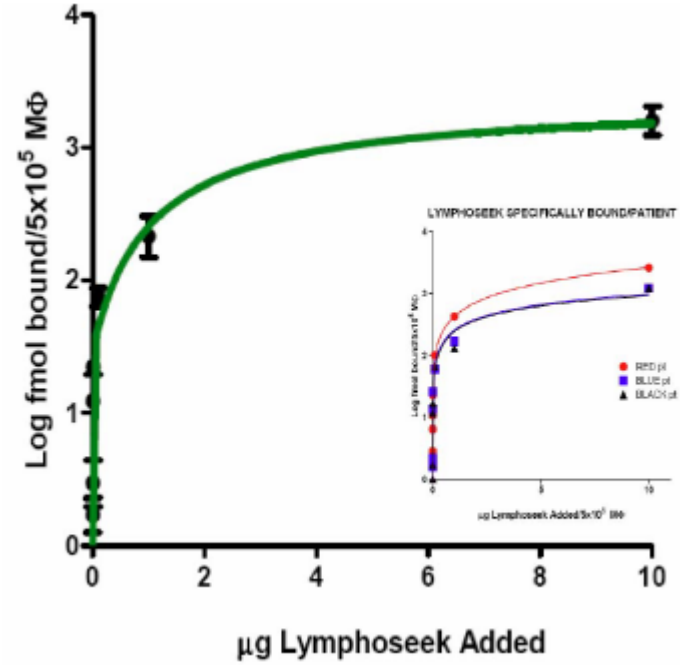
2. Concentration Effect of Lymphoseek – K_D and Receptor Number:
The specific binding of Lymphoseek to MBR at 0 - 4^0C for 3 patients combined are expressed as log fmol Lymphoseek bound per $5x10^5$ macrophages are described in Figure 8-10.

Figure 8: Technetium Tc 99m Tilmanocept (Lymphoseek) specifically bound / 5 x 10^5 Macrophages (Φ) [1]



[1] = Sponsor Figure 10 (page 34 of 64, Study Report No. NEO3-08)

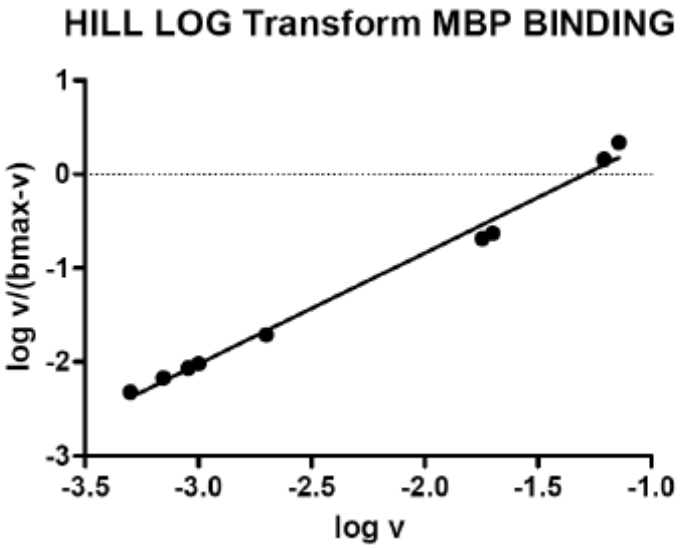
Figure 9: Technetium Tc 99m Tilmanocept Specifically Bound / 5x10^5 MΦ (All Patient Replicates Combined) [1]



Best Available Copy

[1] = Sponsor Figure 11 (page 35 of 64, Study Report No. NEO3-08)

Figure 10: Hill Plot (Three Patient Combined Data) [1]



[1] = Sponsor Figure 13 (page 34 of 64, Study Report No. NEO3-08)

The overall response parameters of the binding of Technetium Tc 99m Tilmanocept to the MBR are outlined in Table 12:

Table 12: Summary Data from Concentration-Dependent Binding of Technetium Tc 99m Tilmanocept to Multisite MBR *in Vitro* at 0-4^{0}C [1]

Plot Parameter	3 Patient Mean	30.0 µM	7.35 µM	1.47 µM	0.654 µM
Hill number [a]	1.98±0.50	←	-----	-----	-----
K_d Sites 1°	$2.758x10^{-11}$ M	-----	-----	-----	-----
K_d Sites 2°	$3.166x10^{-11}$ M	-----	-----	-----	-----
Mean Sites / MΦ [b]	$3.53x10^5 \pm 0.67x10^5$ [c]				
Relative $0.5V_{max}$ per Injection Volume NEO3-05 (V_{max}; Figure 14)	----- (V_{max} approached at ~2.0 mL)	355 fmol at 30 min	43 fmol at 32 min	6 fmol at 30 min	1.2 fmol at 35 min

[a] Cooperativity at 30.0 µM; Hill number is > 1.0, indicating some cooperativity.

[b] Does not indicate mean receptor number per MΦ since there are multiple binding sites per receptor (8 known sites/receptor); can be expressed as B_{max}.

[c] Estimated by calculation

[1] = Sponsor's Table 5 (page 38 of 64, Study Report NEO3-08)

Summary of Binding results: The results of the binding studies indicated that DTPA-Mannosyl-Dextran expectedly performed as a ligand molecule in its specific binding interaction with the native macrophage MBR.

Binding analysis revealed multiple binding sites with K_D values of $2.76x10^{-11}$ M and $3.17x\ 10^{-11}$ M.

A Hill coefficient of >1.0 was determined indicating a positive cooperativity of ligand binding. In a positive cooperating ligand binding reaction, binding of a ligand to the receptor increases its affinity for other molecules.

The Hill coefficient describes the fraction of a macromolecule saturated by ligand as a function of the ligand concentration. The Hill coefficient was often used in biochemical characterization, in which the binding of a ligand to a macromolecule was often enhanced if there are other ligands present on the same molecule,

Conclusions: Based on the results, the sponsor concluded that mannose binding receptors (MBRs) were expressed on the surface of human macrophages and that Technetium Tc 99m Tilmanocept had a specific binding interaction with these native MBRs. The sponsor also concluded there were multiple binding sites for Technetium Tc 99m Tilmanocept and that these sites were positively affected by multiple ligand interactions. The sponsor also concluded that the Technetium Tc 99m Tilmanocept performed as expected as a ligand molecule for the human mannose binding receptor (hMBR).

Reviewer's comments: The drug substance DTPA-Mannosyl-Dextran binds to CD206. Many tumor antigens are heavily glycosylated, such as tumoral mucins, and/or attached to tumor cells by mannose residue-containing glycolipids (Dangaj et al., 2011). Evidence from literature showed that tumor-infiltrating macrophages respond to microenvironmental signals by developing a tumor-associated phenotype via a high expression of the mannose receptor (MR, CD206). Although macrophages highly express the mannose binding receptor, macrophages expressing CD206 (MRC1) accumulate in other tissues. Aron-Wisnewski et al (2009) showed that macrophages accumulate in adipose tissue and stain along with CD40 and CD163 surface markers by immunochemistry. Further, complex carbohydrate structures are essential molecules of infectious microbes and host cells and are involved in cell signaling associated with inflammation and immune responses. The uptake of mannosyl-tailed glycans is usually carried out by macrophages, dendritic cells and other professional phagocytes to trigger MHC-class I and MHC-class II restricted antigen presentation and to promote T-cell effector responses (Reginier-Vigoroux, 2003; Laskarin et al., 2005; Baetas-da-Cruz et al., 2009).

The reviewer agrees with the sponsor's conclusion that Technetium Tc 99m Tilmanocept binds directly to macrophage binding receptors (MBRs) expressed on human macrophage. More importantly, the results of preliminary *in vitro* binding studies described in Vera et al (2001) provide a more direct description of the binding characteristics of Technetium Tc 99m Tilmanocept onto mannose binding receptors using liver macrophages. Although Vera et al (2001) used liver macrophages in the study as a source of MBRs (CD206), these receptors are known to be present at other locations in the body and the results are applicable to lymph nodes.

4.1.2.3: Report No. NEO3-08A (Amendment to NEO3-08)

Study Title: *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)	
Volume # and Page #:	Module 4, § 4.2.1.1; 18 pages
Conducting laboratory and location:	(b) (4)
Study #:	NEO3-08A (*In vitro* study); Amendment to NEO3-08
Sponsor:	Neoprobe Corporation
Date of Study:	01/02/2011 – 04/10/2011
GLP Compliance:	Yes (), No (x)
QA report:	Yes (x), No (x)
Drug, lot #, and % purity:	Drug Product: Technetium Tc 99m Tilmanocept Lot #: NMK003, % purity >90% of total radioactivity Specific activity: 5 mCi/250 µg (page 10 of 10, Appendix 7-1, Appendix to Final Report) (Drug substance in Technetium Tc 99m Tilmanocept: DTPA-Mannosyl-Dextran Lot #: M60834) *(§4.2.1.1.1, Appendix 7-5 [Protocol & amendments], pages 3-4 of 6)* No Reference substances were used in the study
Manufacturer(s):	DTPA-Mannosyl-Dextran and Technetium Tc 99m Tilmanocept: (b) (4) (*§3.2.S.2.1, page 1 of 1; (§3.2.P.3.1, page 2 of 6, respectively)* Radiolabeling was conducted by (b) (4)
Manufacturing standard:	GMP
Tissue/Cells:	Cells: Monocyte-derived macrophage (MDM) monolayers; Blood obtained from healthy human donors.
Doses/Vehicle:	Technetium Tc 99m Tilmanocept: 20 µg/0.8mCi DTPA-Mannosyl-Dextran: 100, 250, or 500 µg/mL Vehicle: Phosphate buffered saline (PBS) containing Ca^{2+} and Mg^{2+}
Duration/route:	N/A, *in vitro* study
Treatment groups :	None

Reviewer's Table constructed from sponsor's data

Objective : The purpose of this *in vitro* study was to evaluate the direct binding of Technetium Tc 99m Tilmanocept to its intended native human mannose binding receptor (hMBR).

Key study findings: The results indicated that Technetium Tc 99m Tilmanocept was neither recognized by, nor was bound to the empty vector components for carrying the gene for hMBR. The specificity of Tc 99m Tilmanocept binding to hMBR was further confirmed by Western blotting analysis. Based on ARG analysis, there was no evidence that the binding of Tc 99m Tilmanocept to hMBR was recognized by binding at the 181 kDa band

Study design: Technetium Tc 99m Tilmanocept was tested in the presence of rhMBR recovered from rhMBR-expressing host HEK 293 cells containing the plasmid carrying the MBR gene (CD 206). Technetium Tc 99m Tilmanocept binding to hMBR was also assessed by ARG evaluation.

Preparation of human monocyte-derived macrophages (MDMs): Human monocyte-derived macrophages (MDM) monolayers were prepared from healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood and cultured in Teflon wells for 5 days in the presence of 20% autologous serum.

Comparison of the binding characteristics of Technetium Tc 99m Tilmanocept to MDM hMBR and HEK 293 rhMBR: Binding characteristics of Technetium Tc 99m Tilmanocept to native human donor-derived hMBR and HEK 293-expressed recombinant form of hMBR (rhMBR) were compared. The comparisons are intended to provide integrative support for the specificity of MBR (native hMBR and rhMBR) for Technetium Tc 99m Tilmanocept

Results: The findings of this study describe (1) the Western blotting analysis of Technetium Tc 99m Tilmanocept binding to rhMBR (HEK 293) using autoradiographic imaging and a determination rhMBR specificity (2) Technetium Tc 99m Tilmanocept binding to hMBR (MDMs) using the Western blotting technique:

Technetium Tc 99m Tilmanocept binding to rhMBR – The lysate from cells expressing the complete hMBR gene insert showed a substantial band in the autoradiograph where Technetium Tc 99m Tilmanocept (20 μg/0.8 mCi) was bound at the 181 kDa band. The results (Figure below) showed that Technetium Tc 99m Tilmanocept was neither recognized by nor was bound to the empty vector components for carrying the gene for hMBR. The binding of Technetium Tc 99m Tilmanocept was specific as preincubation of the HEK 293 host cells with 10x unlabeled Technetium Tc 99m Tilmanocept abolished any banding at the 181 kDa level. The specificity of Technetium Tc 99m Tilmanocept binding to hMBR was further confirmed by Western blotting.

Figure 11: Autoradiograph of HEK 293 cell lysates with rhMBR and Tc 99m Technetium Tc 99m Tilmanocept without exposure to 10x 'cold' Lymphoseek and pre-incubation with 10x Lymphoseek (B)

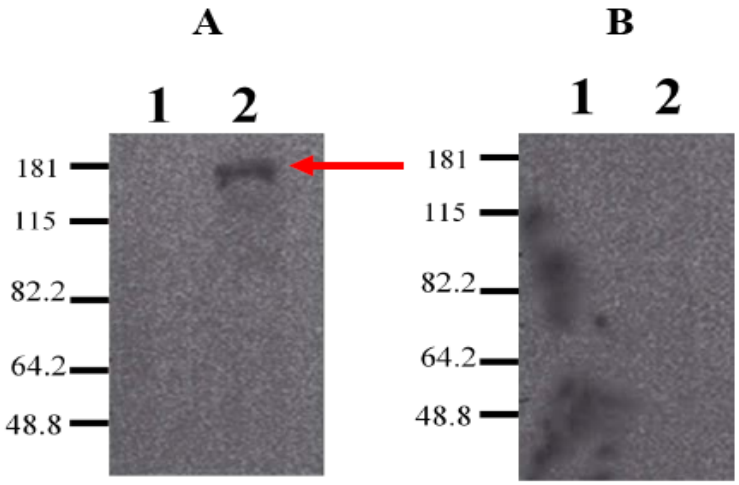


Figure legend (adapted by Reviewer from sponsor's Figure 3 in submission, § 4.2.1.1.1 (sub-section 3.1.1), Appendix 7.1, page 12 of 18) highlights the following findings:

- Figure describes ARG of Western blotting (probed with anti-rhMBR) of HEK 293 cell lysates
- Lanes A-1 and B-1: Lysates from HEK 293 transfected with an empty plasmid vector alone.
- Lanes A-2 and B-2: Lysates from host cells with parent plasmid expressing full length rhMBR
- The cell lysates in A: Incubated with Technetium Tc 99mTilmanocept (20 µg/mL/0.8 mCi)
- Cell lysates in B were also incubated with Tc 99m Tilmanocept but pre-incubated with exposure to 10x unlabeled Lymphoseek (200 µg/mL) for 1 hour
- The absence of bands in Lane 1 (A and B) indicated that neither the cell lysates nor the empty plasmid vectors contain any protein recognized by anti-rhMBR to which Lymphoseek binds (dots in Lane 1B were water artifacts due to film development)
- hMBR native band confirmation: 181 kDa (red arrow; Lane A-2, rhMBR) corresponds to native hMBR. The corresponding band in B-2 was totally mitigated in the presence of 10X cold Lymphoseek

Technetium Tc 99mTilmanocept binding to hMBR (MDMs) – Based on ARG analysis, there was no evidence that binding of Tc 99m Lymphoseek to hMBR was recognized by binding to any other protein outside the 181 kDa band and all lanes A-F (Figures below) indicated binding (ARG banding) of Lymphoseek at this kDa level. The results further confirm the specificity of Lymphoseek to 181 kDa hMBR band

Figure 12: Autoradiograph of human macrophage cell lysates incubated with 20ug/0.8mCi Tc 99m Tilmanocept without pre-incubation exposure to unlabeled Lymphoseek

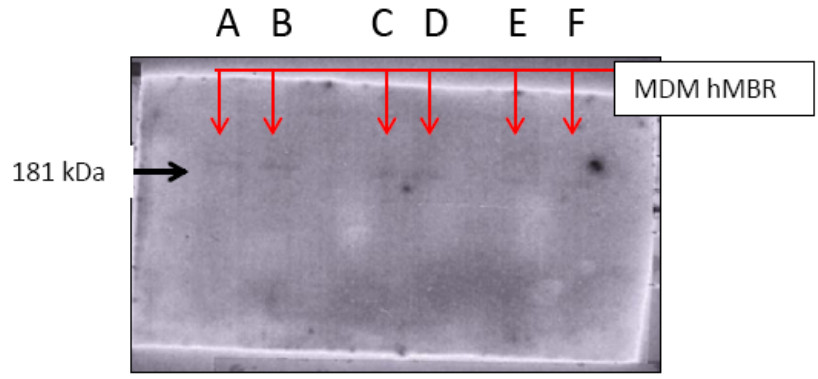
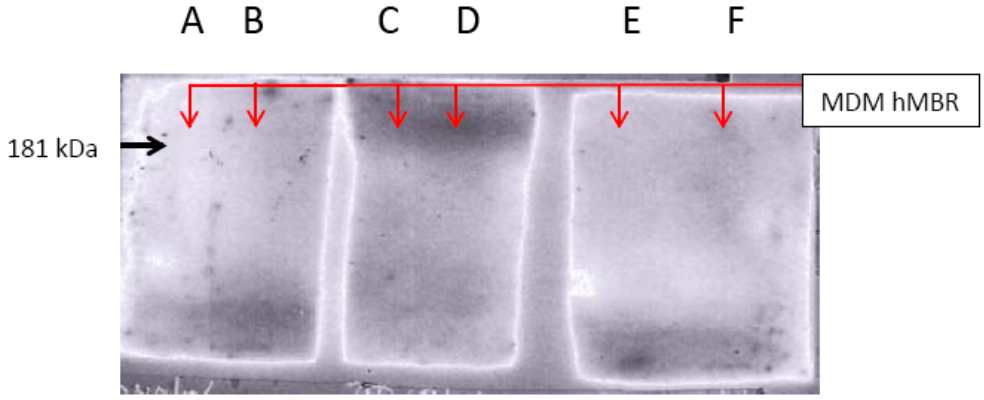


Figure 13: Autoradiograph of human macrograph cell detergent lysates pre-incubated with either 5x (A, B), 12.5x (C, D), or 25x (E, F) unlabeled Tilmanocept, subsequently incubated with Tc 99m Tilmanocept



Legend (adapted by Reviewer from sponsor's Figures 4 and 5 in submission, § 4.2.1.1.1 (sub-section 3.2.1), Appendix 7.1, page 13-14 of 18):

- Lanes A-F represents equal amounts of the lysate and correspond to parallel lanes in both figures
- Figures represent an autoradiograph of the nitrocellulose blot of MDM detergent lysates with Tc 99m Lymphoseek (20 ug/0.8 mCi).
- All lysates in the lower figure were pre-incubated with increasing levels of unlabeled Lymphoseek for 2 hours with either 5x (A, B), 12.5x (C, D), or 25x (E, F) unlabeled Tilmanocept. Banding at all concentrations of pre-incubation with unlabeled Tilmanocept was abolished

Conclusions: The affinity of a compound for its receptor is important in the evaluation and interpretation of drug performance. Lymphoseek binds to native macrophage binding receptor (hMBR). The study confirmed the binding of Lymphoseek, either as the

Tc 99m-labeled drug or as the DTPA-Mannosyl-Dextran – unlabeled drug substance. Based on Autoradiographic and Western blot analysis, the results indicated that there was binding of labeled or unlabeled DTPA-Mannosyl-Dextran to human macrophage binding receptor (hMBR).

Reviewer's comments: I agree with the sponsor that Lymphoseek showed affinity to the native human macrophage binding receptor.

4.2 Secondary Pharmacology

No secondary pharmacology studies were performed

4.3 Safety Pharmacology

Two *in vivo* safety pharmacology studies were conducted in beagle dogs. The objective was to evaluate cardiovascular safety

4.3.1 Study No. 1146-102:

Study Title: Safety Pharmacology Study of DTPA-mannosyl-dextran in Dogs	
Study #:	(b) (4) No. 1146-102
Volume # and Page #:	eCTD Module 4, §4.2.1.3; 1-26 pages
Conducting laboratory and location:	(b) (4)
Sponsor and Address:	Neoprobe Corporation, 425 Metro Place North, Suite 300, Dublin, Ohio 43017-1367
Date of Study initiation:	June 21, 2000
GLP Compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran; no control(s) tested
Animals/species/strain/sex per dose:	Dog/Beagle (b) (4) 4 dogs: 2/sex
Age:	4-6 months
Weight:	5-10 kg
Doses/Vehicle:	560 μg/kg/dog
Duration/route:	Single dose/intravenous
Dose volume:	0.125 mL/kg

Reviewer's Table constructed from sponsor's data

Objective: The purpose of this study was to evaluate the acute pharmacological effects of a single intravenous dose of DTPA-Mannosyl-Dextran in dogs

Key study findings: 560 μg/kg/dog of DTPA-Mannosyl-Dextran, administered to beagle dogs via a single intravenous dose, had no effect on survival, clinical signs, ECG and BP. Although HR decreased 1 min post-administration, the HR at 30-min post-treatment was comparable to the pre-dose measurement. At both 1 and 30 min, plasma $TXB_{2\ and}$ histamine levels were increased compared to their pre-dose levels.

Study design: The study design and methods are summarized in Tables 13 and 14.

Table 13: DTPA-Mannosyl-Dextran Dose multiples (Study No. 1146-102)

	Single dose (μg)
Dose (μg/kg) in Dogs (1 dose group; 2/sex)	560
Dose multiples (based on BSA)	364x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Table 14: Summary of Methods/Observations (Study No. 1146-102)

Protocol	**Method, frequency and/or objectives**
Clinical observations	Twice/day Evidence of mortality, morbidity and toxicity
Body weight	Individual BW /Prior to dosing
Blood samples	4 mL in 5 mL EDTA tubes; all dogs/Prior to dosing and 1 min and 30 min pose-dose immediately after ECG recordings
Plasma	Frozen at -80^{0}C; Analyzed at (b) (4) for Thromboxane B2 and Histamine
ECG, BP and HR	12-lead ECG (I, II, III, aVR, V1, V2, V3, V4, V5, and V6); Parameters recorded prior to dosing, and at 1 min and 30 min post-dose. Results section described ECG findings from a 9-lead ECG (I, II, III, aVR, aVL, aVF, CV_5RL (rV_2), CV_6LL (V_2) and V_{10}) recorded at a paper speed of 25 mm/sec or 50 mm/sec and sensitivity of 1 cm/mv
Termination	Dogs were returned to colony at end of study
Dose Formulation Analysis	Yes ( ), No (x)

Reviewer's Table constructed from sponsor's data; ECG = electrocardiography; BP = Blood pressure; HR = Heart rate

Results

Mortality: No deaths were reported

Clinical observations: No adverse effects of DTPA-mannosyl-dextran reported

Clinical pathology
Thromboxane B2 (TXB_2): At 1 min post-dose, the plasma TXB_2 predose levels (237.22 - 309.31 pg/mL) increased 2.5-6.8 folds. Levels declined 30 min post-dose but remained at 1.7-2.8x pre-dose levels
Histamine: Changes in plasma histamine (pre-dose: 0.13 - 0.20 ng/mL) increased to 1.2 - 8.8 folds at 1 min post-dose but declining to 0.9-2.2x the pre-dose level at 30 min

Table 15: Thromboxane B2 and histamine data (Study No. 1146-102)

Animal Number	Sex	Day 1					
		Thromboxane B2 (pg/mL)			Histamine (ng/mL)		
		Predose	1- Minute Postdose	30-Minutes Postdose	Predose	1-Minute Postdose	30-Minutes Postdose
10217	M	280.58	1917.37	794.69	0.15	1.32	0.33
10218	M	309.31	785.96	546.37	0.13	0.15	0.12
10219	F	277.73	814.65	467.41	0.16	0.30	0.20
10219	F	237.22	837.23	487.59	0.20	0.37	0.17
M = Male; F = Female							

Source: Sponsor's Table 2, page 15, Safety Pharmacology Report 1146-102

ECG, BP, and HR

ECG: The results of a 9-lead ECG following a single-dose (560 μg/kg/dog) intravenous administration of DTPA-mannosyl-dextran in beagle dogs did not cause any electrocardiographic abnormalities, atrioventricular conduction defects or premature atrial or ventricular premature complexes in any dog. No ECG raw data was included in the results or Appendix 1 (page 11 of 26 of report).

Blood pressure (BP) and Heart rate (HR): Pretreatment BP ranged from 123/76 to 146/88 mmHg (systolic/diastolic). There was no treatment-related effect at the 1- and 30-min post-dose periods. The test article did not appear to have any effect on the HR. Pre-dose HR ranged from 93-123 beats/min but decreased in all dogs at 10min post-dose to 69-116 beats/min. HR returned to pre-dose levels 30-min post dose.

Dose Formulation Analysis: Yes ( ); No (x)

Conclusions: A dose of 560 μg/kg/dog of DTPA-Mannosyl-Dextran, administered to beagle dogs via a single intravenous dose, had no effect on survival, clinical signs, ECG and BP. Although the heart rate (HR) decreased 1 min post-administration, the HR at 30-min post-treatment was comparable to the pre-dose measurement. At both 1 and 30 min, plasma Thromboxane B_2 and histamine levels were increased compared to their pre-dose levels.

Reviewer's comments: In evaluating ECG in this non-GLP study, a discrepancy was observed in the methods and results section in which a 12-lead ECG protocol was described in the methods and the results of a 9-lead ECG was reported in the results. No raw data of the ECG was included in the results or Appendix as indicated on page 11 of 26 of the study report. Control animals were not evaluated and it was not possible to determine if the changes observed in plasma Thromboxane B_2 or histamine can be attributed to DTPA-Mannosyl-Dextran or if these changes were due to the stress of the ECG procedure on the dogs. Although TXB_2, an inactive metabolite of Thromboxane A2 (TXA_2), is almost completely cleared in urine and is not involved in platelet activation and aggregation, its precursor, TXA_2 is involved in platelet activation and aggregation. Monitoring of anti-platelet therapy is applied in instances of high risk for re-thrombosis or bleeding and TXB_2 is one of the parameters being measured to assess pharmacological inhibition of platelet function. TXA_2 synthesis is the target of aspirin, which inhibits cyclooxygenase-1 (COX-1, also known as prostaglandin G/H synthase 1, prostaglandin-endoperoxide synthase 1 or prostaglandin H2 synthase 1). COX-1 is an

enzyme that plays a central role in the biosynthesis of prostaglandins from arachidonic acid. The clinical significance of COX-1 is that it can be inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin. Histamine triggers the inflammatory response and inflammatory stimuli such as histamine, thrombin, VEGF and other factors can result in a dissociation of cell-cell junctions between endothelial cells and cytoskeleton contraction leading to a widened intercellular space and trans-endothelial flux. Based on a consideration of these reasons, it is advised that potential increases in TXB_2 and histamine levels be closely monitored following the administration of Lymphoseek drug product. Both TXB_2 and histamine were further examined in the GLP study 1576-04774 that examined the cardiovascular safety pharmacology study in beagle dogs. It can be concluded from the results of this non-GLP study are preliminary. The study does not include controls and the findings were generally inconclusive.

4.3.2 Study No. (b) (4) 1576-04774:

Study Title: "DTPA-Mannosyl-Dextran: A Cardiovascular Safety Pharmacology Study in Beagle Dogs"	
Study #:	(b) (4) 1576-04774
Volume # and Page #:	eCTD Module 4, § 4.2.1.3.1; pages 1-136
Conducting laboratory and location:	(b) (4)
Sponsor and Address:	Neoprobe Corporation, 425 Metro Place North, #300, Dublin, OH 43017-1367
Date of Study initiation:	June 14, 2005
GLP Compliance:	Yes (x), No (), § 2.6.3 (page 6 of 6) Signed: 12/14/2005 (page 3 of 136)
QA report:	Yes (x), No () Signed: 12/14/2005 (page 4-5 of 136)
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran, Lot #: M50840, % purity – assumed 100%; (b) (4)
Doses/Vehicle:	0, 0.084, 0.42, 0.84 mg/kg / 0.9% saline (Lot #: 14-052-JT, % purity – 98.1%; CoA; page 26-27 of 136; (b) (4)
Dose volume :	0.125 mL/kg
Frequency of Dosing /Duration/route:	Dose escalation on Study Day 1, 2, 4, 6, 8 and 10; intravenous bolus injection (cephalic vein)
Animals/species/strain/sex per dose:	Dog (non-naive)/Beagle (b) (4) 4/sex
Age:	14 -15 months (at first dosing)
Weight:	7-11 kg; Males (10.1-11.0kg), Females (7.1-8.2 kg)
Treatment groups:	Described under study design

Reviewer's Table constructed from sponsor's data; Protocol Deviations: None that affected study integrity or conclusions

Objectives: The aim of this study was to determine cardiovascular safety following intravenous administration of DTPA-Mannosyl-Dextran to male and female beagle dogs.

Key study findings: DTPA-Mannosyl-Dextran at doses up to 0.84 mg/kg (840 µg/kg) was well tolerated in beagle dogs. There were no effects on cardiovascular parameters and other parameters evaluated. Based on the findings, the NOAEL was greater than the high dose of 0.84 mg/kg (or 546x MHD).

Study design: The design for this study is described in Table 16:

Table 16: Study design - [redacted] (b) (4) 1576-04774 [1, 2]

Neoprobe Corporation [redacted] (b) (4) Study No. 1576-04774

Text Table 4: Group Designation and Dosage Levels

Dose	Study Day	Treatment	Dose Level		Dose Concentration (mg/mL)	Animal Numbers	
			nmol/kg	mg/kg		M	F
1	1	Saline	0	0	0		
2	2	Saline	0	0	0		
3	4	DTPA-mannosyl-dextran	2.8	0.084	0.672	12157-12160	12161-12164
4	6	DTPA-mannosyl-dextran	14	0.42	3.36		
5	8	DTPA-mannosyl-dextran	28	0.84	6.72		
6	10	DTPA-mannosyl-dextran	28	0.84	6.72		

M = male; F = female

[1] Source: Sponsor Table ([redacted] (b) (4) Study No. 1576-04774; page 13 of 136)
[2] Dose levels in nmol/kg: The sponsor submitted [redacted] (b) (4) Errata 1576-04774 indicating that the nmol/kg calculation values (Sponsor's Text Table 4, page 13, Table 16 above). According to the Sponsor, the error did not change the conclusions of the study

Table 17: DTPA-Mannosyl-Dextran Dose multiples (Study No. 1156-04774)

Administration	Intravenous		
	Low dose	Mid dose	High dose
Dose (µg/kg) in Dogs	84	420	840
Dose multiples (based on BSA)	55x	273x	546x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 µg or 30.8 µg/m^2 based on BSA)

Table 18: Summary of Methods/Observations (Study No. 1576-04774)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside: ≥ Twice/day; Mortality and moribundity, general health and signs of toxicity Clinical: Prior to each dose; Evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavioral

	patterns
Body weight	Prior to dosing
ECG, BP and HR	ECG waveform, systolic and diastolic pressure and mean arterial blood pressure (MAP) and heart rate were recorded by Telemetry (b) (4)) Telemetry System (DataQuest A.R.T. 3.1) for approximately 10 min prior to dosing and continuously from approximately 0 to 130 min postdose, and for approximately 10 min, at 4, 8, and 24 h postdose on study days 2, 4, 6, and 8. HR and QTc were based on QT and HR from representative ECG waveforms (Fridericia correction)
Clinical Pathology	Collection day: Blood samples were collected on study days 1 and 10 (Dose 1 and 6) Target collection time: 1 and 30 min, and 2, 4, 8 and 24 h postdose Collection site: Jugular vein Volume collected: Approximately 4mL per time point Tubes used: 2.0 mL containing dipotassium EDTA Plasma: Tubes were inverted several times, stored on wet ice, and centrifuged at ~3000 rpm 10 min at 4^0C. Plasma was transferred to microcentrifuge tubes and stored at -75 ± 10^0C. All plasma samples were transferred to (b) (4) (on dry ice) and analyzed for histamine and Thromboxane B2 (TXB_2) levels. *All postdose samples were collected within 1 min of the target time
Termination	All animals were returned to the stock colony following the last blood collection on SD 11
Dose formulation Analysis	Yes (x), No ( ); Data in Appendix 2

Reviewer's Table constructed from sponsor's data; ECG = Electrocardiography; BP = Blood pressure; HR = Heart rate

Results

Clinical signs: There were no mortalities and all dogs survived to study completion. A finding of soft feces, observed in both sexes in control and treatment groups, was not treatment-related

Body weight: Doses of DTPA-Mannosyl-Dextran up to the high dose (840 μg/kg or 546x MHD) had no effect on body weight or body weight changes

Cardiovascular: DTPA-Mannosyl-Dextran, administered up to 0.84 mg/kg had no effect on QT interval (QT), corrected QT (QTc), systolic or diastolic blood pressures mean arterial pressure, body temperature or ECG. When compared to the predose level, there were slight but significant changes in QT in males and females, occurring at the mid dose (0.42 mg/kg) and high dose (0.84 mg/kg) on study days (SD) 6 and 8. The changes were within normal limits (0.15 - 0.26 s) and were not considered as

biologically significant. No atrioventricular conduction defects or atrial / ventricular premature beats were observed. The QTc interval was not altered at any dose.

Table 19 provides a summary of ECG changes observed:

Table 19: Summary of changes in QT interval in dogs (study 1156-04774)

Sex	Study day (SD)	Dose (mg/kg)			
		0	0.084	0.42	0.84
M	6	-	-	shorter at 24h postdose	-
	8	-	-	-	longer at 2h postdose
F	6	-	-	longer at 2h postdose	

Reviewer's Table based on sponsor's data; M, F = males, females

Four hours post dose on study day 6 (SD6), a slightly lowered body temperature was observed in males administered 0.42 mg/kg. As this finding did not occur in any other group it was considered incidental. Blood pressure and heart rate changes, summarized in Table 20, were inconsistent, incidental and not test article-related.

Table 20: Summary of cardiovascular changes in dogs (Study 1156-04774)

Sex	Parameter	Study Day	Postdose time point	Dose (mg/kg)			
				0	0.084	0.42	0.84
M/F	SP/DP/MAP/HR (↑)	2	1 min	✓	✓	✓	✓
M	↓ HR	2	2h	✓			
M	↓ HR	4	30min, 2h, 4h				✓
M	↓ HR	8	30min, 2h				✓
F	↓ HR	4	30min				✓
M	MAP	8	30min, 8h				✓
M	SP/DP/MAP/HR (↓)	6	24h			✓	

Reviewer's Table based on sponsor's data; M, F = males, females; SP/DP/MAP/HR = systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate; ✓ = occurrence of parameter

Histamine and Thromboxane analysis

DTPA-mannosyl-dextran at doses up to 0.84 mg/kg had no effect on Histamine or Thromboxane B_2 levels. Significantly lower levels of Thromboxane B_2 were observed in control males at 2h and 8h postdose on SD1.

Dose Formulation Analysis: Test article formulations were prepared on the day of dosing by dissolving DTPA-Mannosyl-Dextran in sterile saline. Saline was used as supplied. Thirty-two (32) formulation samples were analyzed using a spectrophotometric method. The stability of the dose formulations was determined in [redacted] (b) (4) 1576-04582. The standard curves for determination of concentrations of DTPA-mannosyl-

dextran (Lymphoseek) dosing formulations were within acceptable limits for accuracy based on relative error (RE) (RE ± 10%) and precision (coefficient of variation ± 15%) of standard replicate concentrations, as well as, strength of linearity within the curve (correlation coefficient, R ≥ 0.98). All DTPA-Mannosyl-Dextran dosing formulations were within acceptance limits for precision

Conclusions: The sponsor concluded that intravenous injection of DTPA-mannosyl-dextran at doses up to 0.84 mg/kg (840 μg/kg) was well tolerated in beagle dogs. There were no effects on cardiovascular parameters and other parameters evaluated.

Table 21: Determination of NOAEL in Study 1156-04774

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality and/or clinical signs	> 840	546x
Body weight changes		
Cardiovascular:		
(i) ECG	>840	
(ii) BP/HR		
Clinical Pathology:	>840	
Histamine and Thromboxane B2		

Reviewer's Table

Reviewer's comments: The NOAEL was established as the high dose of 0.84 mg/kg (or 546x MHD). Overall, I agree with the sponsor's conclusions.

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5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Introduction: Four studies were conducted to evaluate the pharmacokinetic (PK) profile of Lymphoseek as shown in Table 22 below. The specific test article in each of these studies was noted as shown in the Table. Plasma and urine PK parameters were determined using a single dose pilot PK non-GLP study in a mongrel dog and two 14-day repeated dose toxicity studies in Sprague-Dawley rats and mongrel dogs. The single-dose pilot PK study (N106921) was not reviewed. Tissue distribution (b) (4) 608002) was evaluated using NZW rabbits. The tissue distribution study, performed as one of three bridging studies was conducted using Technetium Tc 99m Tilmanocept. An overview of the PK studies is summarized in Table 22:

Table 22: Overview of Pharmacokinetic Studies[1, 2]

Study #	Study Type & Test Article(s)	Animals (Species/Sex & No.)	Dose (μg/kg), [mCi/kg]	x-MHD (BSA)	Route
N106921 [D]	2-day single-dose pilot PK; ^{111}In-labeled and unlabeled DTPA-Mannosyl-Dextran (drug substance)	Mongrel dog 1F	52.5 [~1]	341x	SC
N106923 [D]	14-day repeat-dose toxicity study; ^{111}In-labeled and unlabeled DTPA-Mannosyl-Dextran	SD Rat 23M,23F	(L) 10.5 [0.125] (M) 21.0 [0.125] (H) 42.0 [0.125]	20.5x (21x) 40.9x (41x) 81.8x (82x)	SC
N106922 [D]	14-day repeat-dose toxicity study; ^{111}In-labeled and unlabeled DTPA-Mannosyl-Dextran	Mongrel dog 3M,3F	(L) 10.5 [0.125] (M) 21.0 [0.125] (H) 42.0 [0.125]	68.2x (68x) 136.4 (136x) 272.7x (273x)	SC
(b) (4) 608002 [F]	1-day single-dose tissue distribution; Technetium Tc 99m Tilmanocept	NZW Rabbit 4M,7F	1.41 (5 μg/animal); [~140 μCi/animal]	5.49x (6x)	SC

[1]Source: Reviewer's Table adapted from sponsor data (§ 2.6.4, Pharmacokinetic Written Summary, Tables 2 and 3, page 7-8 of 27; (§ 2.6.4, Pharmacokinetic Tabulated Summary, pages 2-9 of 9)
[2]Abbreviations: SD, Sprague-Dawley; PK, Pharmacokinetic; x-MHD, Dose multiples of maximum human dose (based on body surface area [BSA]; human dose = 50 μg total dose; calculations assume a 60kg body weight in humans) SC, subcutaneous; NZW, New Zealand White; Technetium Tc 99m Tilmanocept (drug product); L, M, H = Low, Mid and High dose levels, respectively; [D] = (b) (4) study; [F] = (b) (4) study

5.1.1.2 Study No. (b) (4) 608002:

Study title: A Tissue Distribution Study of [99m Tc]-Lymphoseek in rabbits	
Study no.:	(b) (4) 608002
Report date:	7/24/2007
Study report location:	eCTD Module 4, § 4.2.2.3.1; pages 1-97
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/3/2007 (page 6 of 97)
GLP compliance:	Yes (x), No ( ), page 2 of 97 Signed: 7/24/2007
QA statement:	Yes (x), No ( ), page 21 of 97 Signed: 7/24/2007
Drug, lot #, and % purity:	Technetium Tc 99m Tilmanocept Lot #s, NMK001-077 (#1); NMK001-078 (#2) and NMK001-076 (#3), page 7 of 97

Objective: The objective of this study was to determine the pharmacokinetics (PK) and tissue distribution of Technetium Tc 99m Tilmanocept in male and female rabbits following subcutaneous (SC) administration.

Key Study Findings: At 15 min postdose, Technetium Tc 99m Tilmanocept was widely distributed in the body in female rabbits with about 10% of the injected dose in the blood and 3% in both liver and kidneys. Evidence of absorption and excretion was apparent at 15 minutes post-dosing with 7.7% of the administered dose contained in the urinary bladder contents and approximately 2% distributed throughout the GIT and its contents. At 1 and 3 h postdose, Technetium Tc 99m Tilmanocept decreased in the blood and most tissues of both male and female rabbits. However, increased amount of Technetium Tc 99m Tilmanocept injection was detected in kidneys and urinary bladder contents and colon contents which were indicative of continued absorption and excretion Technetium Tc 99m Tilmanocept. Less than 0.05 %ID was obtained in the thymus, spleen, thyroid, bone (rib, femur), eyes, testes and ovaries. Lastly, at 15 min postdose, greater than 1% of the subcutaneously injected dose Technetium Tc 99m Tilmanocept was found in the popliteal lymph node of the treated leg in female rabbits in contrast to the contralateral popliteal lymph node indicating a rapid absorption into the draining lymph node ipsilateral to the injection site.

Study design

Methods	
Dose:	1.41 (5 µg/animal); Radioactive dose ~140 µCi/animal
Frequency of dosing:	Once
Route of administration:	Subcutaneous; bolus injection
Dose volume:	40 µL
Formulation/Vehicle:	Technetium Tc 99m Tilmanocept radionuclide / PBS solution; Radiolabeling was done at (b) (4) while radioactive Technetium was obtained from (b) (4)
Analyte:	Total Technetium Tc 99m by gamma counting (cpm), (b) (4)
Species/Strain:	Rabbit; New Zealand White Rabbit (HRA:[NZW] SPF), (b) (4)
Number/Sex/Group:	4M / 7F
Age:	Approximately 20 weeks old (at receipt)
Weight:	On day of dosing: Males (3183 - 3700 g); Females (3247 - 3951 g)
Unique study design:	None
Deviation from study protocol:	No significant deviations reported

Methods: The animals were divided into three groups based on the time of post-administration euthanasia as shown in Table 23:

Table 23: Study Design (Study No. (b) (4) 608002)

Designation of Groups

Group	Dose Level Per Animal (µCi)	Dose Volume	Number of Animals	Time of Euthanasia (Hours Post-Dose)
1	145	40 µL	3F	0.25
2	131	40 µL	2M/2F	1
3	145	40 µL	2M/2F	3

M = Male, F = Female

The test article Technetium Tc 99m Tilmanocept in phosphate-buffered saline solution, was administered at a dose of 5 µg/animal to 7 female and 4 male New Zealand White rabbits as a bolus subcutaneous injection to the distal portion of the thigh in a volume of 40 µL/animal using a 1mL syringe and 25-gauge needle. Radioactive dose was approximately 140 µCi/animal; Range: 131-145 µCi). Following dosing, group 1 rabbits

(3 females) remained restrained until they were euthanized 15 minutes post-dosing. However, all animals in groups 2 and 3, comprising of 2 males and 2 females each, were placed back in wire-mesh cages until euthanized at 1- and 3-hours post dosing, respectively. Blood, urine and tissue samples were collected from all animals at 0.25h (15 min), 1h, and 3h postdose immediately after euthanasia. Only female animals were evaluated at the 15 min (0.25h) time point. All animals were euthanized by an intravenous injection of sodium pentobarbital via a marginal ear vein. Following euthanasia, each animal was weighed and an intravenous blood sample obtained via the vena cava was collected into anticoagulant tubes containing sodium heparin. Blood samples were centrifuged for plasma and blood cellular fractions. Samples were analyzed, were possible in duplicate, for total Technetium Tc 99m radioactivity using gamma counting techniques. Where appropriate quantitative analytical results were expressed in terms of Technetium Tc 99m Tilmanocept equivalents based on the theoretical specific activity of the test article in the dosing formulation or as percent of the administered dose. Mean (±SD) % injected dose (ID) of Technetium Tc 99m Tilmanocept in tissues was based on the amount of Technetium Tc 99m Tilmanocept in 40 μL of dose solution. Tissue samples collected are listed in Table 24:

Table 24: Tissues and Organs collected after euthanasia

Sample List	
Brain	Stomach
Thymus	Stomach contents
Kidneys	Bone (femur)
Liver	Muscle (flank)
Lung	Small intestine
Spleen	Small intestine contents
Thyroid	Left popliteal lymph node
Gall bladder	Right popliteal lymph node
Bone (rib)	Left axillary lymph node
Eyes	Right axillary lymph node
Colon	Urinary Bladder
Colon contents	Urinary Bladder Contents
Testes (males)	Injection site
Ovaries (females)	

Results: The percentage of injected dose (%ID±SD) following a subcutaneous administration of Technetium Tc 99m Tilmanocept found in selected tissues and organs is summarized in Table 25

At 15 min postdose, Technetium Tc 99m Tilmanocept was widely distributed in the body in female rabbits with about 10% (7.71%) of the injected dose in the blood plasma and 3% in both liver and kidneys (2.78% and 2.81%), respectively.

Evidence of absorption and excretion was apparent at 15 minutes post-dosing with 7.7% of the administered dose contained in the urinary bladder contents and approximately 2% distributed throughout the GIT and its contents. The %ID injected compound in the urinary bladder contents increased progressively up to 27.41% (females) and 34.72% (males) at 3h post dose.

At 1 and 3 h postdose, Technetium Tc 99m Tilmanocept decreased in the blood and most tissues of both male and female rabbits. However, increased amount of Technetium Tc 99m Tilmanocept was detected in kidneys and urinary bladder contents and colon contents which were indicative of continued absorption and excretion Technetium Tc 99m Tilmanocept.

Although at 15 min postdose, greater than 1% of the subcutaneously injected dose of Technetium Tc 99m Tilmanocept was found in the ipsilateral popliteal lymph node of the treated (left) leg in female rabbits while in contrast, none was detected in the contralateral popliteal lymph node. Technetium Tc 99m Tilmanocept in amounts ranging from zero to negligible were obtained in right and left axillary lymph nodes; brain, and flank muscle at 15 min in females and at 1 and 3h in both sexes.

Less than 0.05%ID (not shown in Table 25) was obtained in the thymus, spleen, thyroid, bone (rib, femur), eyes, testes and ovaries.

Table 25: [1]Percentages injected dose (%ID±SD) of Technetium Tc 99m Tilmanocept in tissues of rabbit at 0.25, 1 and 3 h after a single SC injection of 5 μg/animal

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)

[1]Sponsor's Table (Table 8, Pharmacokinetics Written Summaries, § 2.6.4; page 23 of 27);
[a] = Males, n = 0 at 0.25h, n = 2 at 1 and 3h; [b] =females, n = 3 at 0.25h and n = 2 at 1 and 3h

Conclusions: At 15 min postdose, Technetium Tc 99m Tilmanocept was widely distributed in the body in female rabbits with about 10% of the injected dose in the blood and 3% in both liver and kidneys. Evidence of absorption and excretion was apparent at 15 minutes post-dosing with 7.7% of the administered dose contained in the urinary bladder contents and approximately 2% distributed throughout the GIT and its contents. At 1 and 3 h postdose, Technetium Tc 99m Tilmanocept decreased in the blood and most tissues of both male and female rabbits. However, increased amount Technetium Tc 99m Tilmanocept injection was detected in kidneys and urinary bladder contents and colon contents which were indicative of continued absorption and excretion Technetium Tc 99m Tilmanocept. Less than 0.05% ID was obtained in the thymus, spleen, thyroid, bone (rib, femur), eyes, testes and ovaries. Lastly, at 15 min postdose, greater than 1% of the subcutaneously injected dose of Technetium Tc 99m Tilmanocept was found in

the popliteal lymph node of the treated leg in female rabbits in contrast to the contralateral popliteal lymph node indicating a rapid absorption into the draining lymph node ipsilateral to the injection site.

Reviewer's comments: I agree with the sponsor's conclusion that Technetium Tc 99m Tilmanocept was well distributed in the body in male and female rabbits following subcutaneous administration. Results of the study indicated evidence of absorption and excretion as early as 0.25h post administration. I also agree with the sponsor that the increased amounts of Technetium Tc 99m Tilmanocept in the kidneys, urinary bladder and colon contents was indicative of continued absorption and excretion of Technetium Tc 99m Tilmanocept. Since the cut off time point for measuring distribution was 3h, it could not be ascertained, based on this data provided what the distribution pattern in the body would be post-3h especially as there was evidence of increasing excretion in the kidneys, bladder and colon contents. I also agree that the finding of a greater amount of Technetium Tc 99m Tilmanocept injection in the ipsilateral popliteal lymph node at 15 minutes post-injection in contrast to corresponding right popliteal node indicated an increased and rapid absorption into the draining (ipsilateral) lymph node of the injection site.

5.2 Toxicokinetics

(TK findings are included and reviewed in the repeated dose toxicity studies)

6 General Toxicology

Introduction: Four single-dose toxicity studies were conducted in the rat (2 studies), rabbit (I study) and the dog (1 study) . Two repeat-dose studies were evaluated one each in the rat and dog. Other toxicity studies included 3 genotoxicity studies, 1 antigenicity and 2 local tolerance studies. All the toxicity studies were GLP-compliant. According to the sponsor, Studies conducted to determine the toxicity of DTPA-Mannosyl-Dextran toxicity, were conducted with the unformulated drug substance. In order to evaluate the safety of the final formulation, representative of the excipient profile of the final drug product, three bridging studies: Tissue Distribution ((b) (4) 608002), Irritant toxicity ((b) (4) 1576-06069), and a rat acute toxicity study ((b) (4) 1576-06049). Report (b) (4) 608002 was reviewed under PK studies. An overview of the toxicology study reports is shown in Table 26:

Table 26: Overview of Toxicology Studies [1, 2]

Study #	Study Type, Test Article & Lot No.	Species / Sex & No.	Dose (μg/kg) [G] (except mg/kg in Study AB11LN.123.BTL)	X MHD (BSA)	Route
Single Dose Toxicity					
1146-100 [A] (GLP)	14-day single dose Tox, Unlabeled Drug Substance, Batch No. DMD02dec99	SD Rat 5/sex/group	0 (veh)		SC
			(L) 14	2.7x	
			(H) 140	27x	
1146-101 [A] (GLP)	14-day single dose Tox, Unlabeled Drug Substance, Batch No. DMD02dec99	NZW Rabbit 5/sex/group	(veh) 0		SC
			(L) 14	5.5x	
			(H) 140	55x	
1576-06049 [B] (GLP)	14-day Single dose Tox, Unlabeled Drug Substance, Batch No. M51415 for product Lot #: NFR-C0015-040706	SD Rat 5/sex/group	(veh) 0		SC
			(L) 14	2.7x	
			(H) 140	27x	
1576-04582 [C] (GLP)	14-day Single dose Tox Unlabeled Drug Substance, Batch No. M50840	Mongrel dog 4/sex/group	(veh) 0		SC
			(L) 42	27x	
			(M) 180	117x	
			(H) 420	273x	
Repeated Dose Toxicity					
N106923 [D] (GLP)	14-day Repeat dose Tox; ^{111}In-labeled and unlabeled Drug Substance, Batch No. M50840	SD Rat; 10/sex/group	(veh) 0		SC
			(L) 10.5	2x	
			(M) 21	4x	
			(H) 42	8x	
N106922 [D] (GLP)	15 or 16-day Repeat dose Tox; ^{111}In-labeled and unlabeled Drug Substance, Batch No.	Mongrel dog; 10/sex/group	(veh) 0		SC
			(L) 10.5	7x	
			(M) 21	14x	

	M50840		(H) 42	27x	
Genotoxicity					
AB11LN.704. BTL [E] (GLP)	Mutagenesis assay, 4h and 24h exposures, Unlabeled Drug Substance, Batch No. M50840	LY5178/TK $^{+/-}$ Mouse Lymphoma cells	4h exposure: 2000, 2500, 3000, 4000, 5000 μg/mL 24h exposure: 10, 50, 250, 1000, 5000 μg/mL		*In vitro*
AB11LN.503. BTL [E] (GLP)	Reverse mutation assay, 48-72h incubation, Unlabeled Drug Substance, Batch No. M50840	*S. typhimurium* & *E. coli*	1.5, 5.0, 15, 50, 150, 500, 1500, 5000 μg/plate		*In vitro*
AB11LN.123. BTL [E] (GLP)	Mouse Micronucleus Assay (24-48h), Unlabeled Drug Substance, Batch No. M50840	ICR mice	0, 500, 1000, 2000 **mg/kg**		IP
Antigenicity Tox Study					
1576-04583 [B] (GLP)	Sensitization/Challenge; Unlabeled Drug Substance, Batch No. M50840	Hartley Guinea Pig 5 groups of 10M/group	Sensitization dose: (veh) 0 (L) 14 (M) 28 (H) 280 Ovalbumin (+ve control): 0.5 mg/kg Challenge dose: 280 14 28 280 Ovalbumin (+ve control): 1.0 mg/kg	L: 3.6x M: 7.3x H: 72.7x	SC & IV
Local Tolerance Study (Final study reports are located under single dose toxicity studies)					
1146-104 [A] (GLP)	Unlabeled Drug Substance, Batch No.	NZW Rabbit; 3/sex/group	0 (veh) (L) 143	55.7x	IM

	DMD02dec99		(H) 286	111.4x	
1576-06069 [B] (GLP)	Unlabeled Drug Substance, Batch No. M51415 for Product Lot #: NFR-C0015-040706	NZW Rabbit; 10/sex/group	0 (veh)		IM
			(L) 140	54.5x	
			(H) 280	109.1x	

[1]*Source:* Reviewer's Table adapted from sponsor data (§ 2.6.4, Pharmacokinetic Written Summary, Tables 2 and 3, page 7-8 of 27; (§ 2.6.4, Pharmacokinetic Tabulated Summary, pages 2-9 of 9)
[2]*Abbreviations:* Tox, Toxicity; SD, Sprague-Dawley; PK, Pharmacokinetic; x MHD, Dose multiples of maximum human dose (based on body surface area [BSA]; human dose = 50 µg total dose; calculations assume a 60kg body weight in humans); SC, subcutaneous; IV, intravenous; IM, intramuscular; NZW, New Zealand White; Drug Substance (DTPA-Mannosyl-Dextran); Drug Product (Technetium Tc 99m Tilmanocept); L, M, H = Low, Mid and High dose levels, respectively; [A] = (b) (4) study; [B] = (b) (4) study; C = (b) (4) study; [D] = (b) (4) study; [E] = (b) (4) veh = vehicle control; [G] = Test article doses are given as µg/kg unless otherwise stated

6.1 Single-Dose Toxicity

6.1.1 Study No. (b) (4) 1146-100:

Study title: Acute Toxicity Study of DTPA-Mannosyl-Dextran in Rats	
Study no.:	1146-100
Study report location:	eCTD Module 4, § 4.2.3.1.1; pages 1-146
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 31, 2000
GLP compliance:	Yes (x), No (), page 4 of 146 Signed: 06/02/2005
QA statement:	Yes (x), No (), page 5 of 146 Signed: 06/02/2005
Drug, lot #, and % purity:	Unlabeled drug product (DTPA-Mannosyl-Dextran), Lot #, DMD02dec99, % Purity-assumed 100% (page 9 of 146)

Objective: The purpose of this study was to determine the toxicity of a single subcutaneous dose of DTPA-Mannosyl-Dextran in male and female Sprague-Dawley rats

Key Study Findings: There was no treatment-related effect on survival, clinical observations, body weight, clinical chemistry, necropsy and histopathology findings and DTPA-Mannosyl-Dextran was well-tolerated up to the high (140.0 mg/kg or 27x MHD) when administered to Sprague-Dawley rats. A finding of increased BUN in males on day 8 was not accompanied by a concomitant increase in creatinine or histopathological changes that would signal renal disease. Similarly, an elevated ALT in high dose females on day 1 was not accompanied by histopathological changes in the liver. The changes in mean BUN and ALT were substantially outside range of normal, not dose-dependent and were considered as probably biologically insignificant. Based on the results, NOAEL was determined as 140 mg/kg.

Methods	
Doses:	0 (vehicle), 14 and 140 μg/kg (diluted in sterile water for injection)
Frequency of dosing:	Single dose; Sterile water for injection, USP; Lot # 8101111 (b) (4)
Route of administration:	Subcutaneous injection (in right hind footpad)
Dose volume:	0.08 mL/kg (based on body weight on day 1)
Formulation/Vehicle:	Unlabeled drug substance (DTPA-Mannosyl-Dextran) / 0.9% Sodium Chloride (control article), Lot #: G951624, (b) (4)
Species/Strain:	Rats / Sprague-Dawley, (b) (4)
Number/Sex/Group:	30 rats randomized to 5/sex/group (3 groups/sex)
Age:	7-9 weeks (at study initiation)
Weight:	Males (186.4 -220.5 g), Females (154.7-195.9g) At treatment: ~150-200 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Listed in Appendix 7 (page 146 of 146

Study design: Following randomization, animals were grouped as follows:

Table 27: Study groups and dose levels (Study No. 1146-100)

Group	Subcutaneous Dose (μg/kg)	Number of Rats	
		Males	Females
1 (veh)	0	5	5
2 (LD)	14	5	5
3 (HD)	140	5	5

Source: Reviewer's Table adapted from Sponsor's Study Design, (b) (4) Study 1146-100, page 12 of 146; veh = vehicle; LD, HD = Low and High Dose, respectively

Table 28: DTPA-Mannosyl-Dextran Dose multiples ((b) (4) 1146-100)

Administration	Subcutaneous		
		Low Dose	High Dose
Dose (μg/kg) in Rats	0 (vehicle)	14	140
Dose multiples (based on BSA)	N/A	2.7x	27x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (proposed human dose = 50 μg or 30.8 μg/m^2 based on BSA)

Observations and Results

Observations:

Table 29: Summary of Methods/Observations (Study No. 1146-100)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside; Twice/day; Mortality and moribundity, general health and signs of toxicity Physical: At randomization, prior to treatment, weekly thereafter until termination
Body weight	At randomization, prior to treatment, weekly thereafter until termination
Clinical Pathology	Blood samples for hematology and serum chemistry was obtained from all animals prior to termination via the orbital sinus in all fasted, anesthetized surviving animals Hematology: WBC count and differential, RBC count and cell morphology, Hb, MCV, PCV, MCH, Platelet count, MCHC, Reticulocytes (ABRETI, Absolute reticulocyte count) Clinical Chemistry: Na^+, K^+, Cl^-, Total protein, albumin, Ca^{2+}, Phosphate, Total bilirubin, urea nitrogen, Lactate dehydrogenase, Creatinine, AST, ALT, Globulin, ALP, Cholesterol, Triglycerides, A/G ratio, Glucose, Creatinine kinase
Termination/ Gross pathology	14 days postdose (SD15); by CO_2, asphyxiation, and exsanguination
Histopathology	Tissue preservation: 10% neutral-buffered formalin Tissues: Liver, lymph nodes (left and right popliteal, injection site (right foot pad), ovary or testes, bone marrow smear
Urinalysis	No data
Dose formulation Analysis	Yes ( ), No (x)

Reviewer's Table constructed from sponsor's data; all abbreviations are standard clinical pathology terms. Abbreviations were listed on page 23 of 146, Appendix 5

Results

Mortality: No deaths were reported and all animals survived to their scheduled sacrifice

Clinical Signs: No test-related adverse finding was reported

Body Weight: No significant changes in body weight were observed in all treatment groups

Feed Consumption: Not evaluated

Ophthalmoscopy: Not evaluated

ECG: Not evaluated

Hematology: No test-related adverse finding was reported

Clinical Chemistry: Slight but significant increases were observed in mean BUN and mean ALT high dose females on day 15. There was no increase in either parameter in male rats. The changes were seen in one sex, were not associated with histopathological changes and were considered not biologically significant. No other statistically significant findings were observed in the clinical chemistry panel

Urinalysis: Not evaluated

Gross Pathology: No gross pathology tissue lesions were reported

Organ Weights: Not evaluated

Histopathology
Adequate Battery: Yes (x), No ( )
Peer Review: Yes ( ), No (x)

Histological Findings: Microscopic findings were minimal and included a slight liver inflammation in animals spanning all study groups. Hypoplasia was observed in one popliteal lymph node in a control group male

Special Evaluation: None

Toxicokinetics: Not evaluated

Dosing Solution Analysis: No information provided

Conclusions: Based on the results, there was no treatment-related effect on survival, clinical observations, body weight, clinical chemistry, necropsy and histopathology findings and DTPA-mannosyl-dextran was well-tolerated up to the high (140.0 mg/kg or 27x MHD) when administered to Sprague-Dawley rats. The NOAEL in rats was therefore 140 mg/kg

Table 30: Determination of NOAEL in Study No. 1146-100

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality and/or clinical signs	>140	27x
Body weight changes		
Hematology		

Reviewer's Table

Reviewer's comments: The sponsor considered the increased BUN in males as equivocal. It was not accompanied by a concomitant increase in creatinine or histopathological changes that would signal renal disease. The elevated ALT in high dose females was not accompanied by histopathological changes in the liver. Although the changes in mean BUN and ALT were outside the range of normal, they were not

dose-dependent and were considered as probably biologically insignificant. Overall, I agree with the sponsor's conclusions.

6.1.2 No. (b) (4) Study No. 1576-06049:

Study title: Lymphoseek™ DTPA-Mannosyl-Dextran: An Acute Toxicity Study in Sprague Dawley Rats	
Study no.:	1576-06049
Study report location:	eCTD Module 4, § 4.2.3.1.1; pages 1-102
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 21, 2006
GLP compliance:	Yes (x), No ( ), page 3 of 102 Signed: 01/19/2007
QA statement:	Yes (x), No (), page 4 of 102 Signed: 01/18/2007
Drug, lot #, and % purity:	Lymphoseek™ DTPA-Mannosyl-Dextran i.e., (Unlabeled Tilmanocept) for drug product Lot #, NFR-C0015-040706 (Neoprobe; Dublin, OH), % purity 95%, page 9 of 102

Objective : The purpose of this study was to determine the potential toxicity of a single subcutaneous dose of the unlabeled drug substance (DTPA-Mannosyl-Dextran) in Sprague Dawley rats.

Key Study Findings: The results showed no evidence of potential toxicity following a single dose of subcutaneously administered unlabeled Lymphoseek-DTPA-Dextran otherwise known as DTPA-Mannosyl-Dextran at 14 and 140 μg/kg in Sprague-Dawley rats. At the doses tested, DTPA-Mannosyl-Dextran did not cause any mortality or adverse clinical signs. Although there was a dose-related decrease in lactate dehydrogenase activity in females at both administered doses of DTPA-Mannosyl-Dextran on study day 15, no treatment-related effects on other clinical chemistry parameters, hematology, or gross pathology were observed. Incidental gross and concomitant microscopic lesions indicating lymphoid hyperplasia was observed in the right inguinal lymph node. DTPA-Mannosyl-Dextran administered at the dose of 140 μg/kg (or 27x MHD) appeared to be generally well-tolerated based on the findings of this single-dose toxicity study in Sprague-Dawley rats. NOAEL for this single dose toxicity study was therefore 140 μg/kg or 27x MHD.

Methods	
Doses:	0 (vehicle), 14 and 140 µg/kg
Frequency of dosing:	Single dose on study day 1
Route of administration:	Subcutaneous [a] (proposed route of administration in humans)
Dose volume:	0.1-mL fixed dose
Formulation/Vehicle:	Unlabeled drug substance / Sterile saline (b) (4), Lot #: 39-093-JT; % Purity – Assumed 100%
Species/Strain:	Rats / Sprague-Dawley (b) (4)
Number/Sex/Group:	30 rats; 5/sex/group (3 groups/sex); Animals were randomized to the group using computer-generated random numbers
Age:	7-9 weeks (at study initiation)
Weight:	Males (305.0-329.8 g), Females (205.5-233.4 g)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Protocol amendments and deviations are reported in Appendix 8, Report (pages 95-102)

[a] In the Study errata ((b) (4) 1576-06049), the sponsor made the corrections listed in Table 31 below: (1) That the route of administration was subcutaneous and **not** intravenous (2) That the test article was not radiolabeled.

Table 31: Study Errata – Study No. 1576-06049

Error Location	Error Content	Statement of Effect
Summary, Page 6 Discussion / Conclusion, Page 18	The term intravenous is used in place subcutaneous. Neoprobe review findings: The report states the route of administration is subcutaneous throughout the report, except in the two error locations. The study protocol in Appendix 8 specifically states a subcutaneous delivery method (see section XII on page 89 of the protocol). This is a typographical error.	Error does not change the conclusions of the study.
Appendix 1, Page 25	The test article was not radiolabeled for this study, yet the Certificate of Analysis shows a radiochemical purity value. Neoprobe review findings: The wrong Certificate of Analysis was used. It is a Certificate of Analysis for stability at a 12 week timepoint instead of a 0 week timepoint. A radiochemical purity test is completed for stability data on 12 week samples. Attached to this errata is the correct Certificate of Analysis for a 0 week timepoint.	Error does not change the conclusions of the study.

Source: Sponsor data: Table 1 (Errata list), § 4.2.3.1.1, page 1 of 3

Study design: Following randomization, animals were divided into groups as follows:

Table 32: Study Design and Dose groups (Study No. 1576-06049)

Group	Treatment	Subcutaneous Dose (μg/kg)	Number of Dogs	
			Males	Females
1 (veh)	Saline	0	5	5
2 (LD)	Test Article	14	5	5
3 (HD)	Test Article	140	5	5

Source: Reviewer's Table adapted from Sponsor's Study Sponsor Table (Text Table 4), page 12 of 102; veh = vehicle; LD, HD = Low, and High Dose, respectively

Table 33: DTPA-Mannosyl-Dextran Dose multiples (Study No. 1576-06049)

Administration	Subcutaneous		
		Low Dose	High Dose
Dose (μg/kg) in rats	0 (vehicle)	14	140
Dose multiples (based on BSA)	N/A	2.7x	27x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area ((calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Observations and Results

Observations:

Table 34: Summary of Methods/Observations (Study No. 1576-06049)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside: ≥ Twice/day; Mortality and moribundity, general health and signs of toxicity Clinical: SD1 (predose), SD8, SD15; SD1 (postdose, 1 ± 0.25 h) Physical: Skin and fur characteristics, injection site, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior patterns, and time of onset, location, dimensions, appearance, and progression of grossly visible or palpable masses: At randomization, prior to treatment, weekly thereafter until termination
Body weight	At randomization, prior to treatment (predose), SD8 and SD15 (fasted)
Clinical Pathology	Blood samples for hematology (≥ 0.5mL) and serum chemistry (≥ 1 mL) obtained from all animals prior to termination; jugular puncture; overnight fasting before collection Hematology: Standard hematology tests (Text Table 3,

	Appendix 5, page 39 of 102) Clinical Chemistry: Standard serum clinical chemistry panel (Text Table 2, Appendix 5, page 38 of 102)
Termination/ Gross pathology	14 days postdose (SD15); by CO_2, asphyxiation, and exsanguination
Histopathology	Tissue preservation: Appendix 7 Tissues: According to SOP (injection site, liver, mandibular and inguinal lymph nodes, ovary, testes, and uterus
Urinalysis	No data
Dose formulation Analysis	Yes (x), No ( )

Reviewer's Table; SD = study day; According to the sponsor's protocol amendment (Page 95 of 102), data collection for body weights changes, food consumption data and organ weights was not performed

Results

Mortality: Treatment with DTPA-Mannosyl-Dextran did not cause mortality and all animals survived to their scheduled termination.

Clinical Signs: No test article-related effects observed

Body Weights: No test article-related effects observed

Feed Consumption: Not evaluated

Ophthalmoscopy: Not evaluated

ECG: Not evaluated

Hematology: There were no test article-related effects in selected hematology parameters

Clinical Chemistry: No apparent effects were observed on clinical pathology parameters. Compared to controls, there were slight changes in chloride (CL), lactate dehydrogenase (LDH) activity and aspartate aminotransferase (AST) activities on SD15. A minimal increase in CL was observed in group 2 males, a decrease in AST in group 3 females, and a decrease occurred in LDH in females in groups 2 and 3. The findings are summarized in Table 35:

Table 35: [1]Summary of clinical chemistry findings on SD15 (Study No. 1576-06049)

Group/Dose (n=5/sex/group)	**1** **Controls** (0 μg/kg)	**2** **Low dose** (14 μg/kg)	**3** **High dose** (140 μg/kg)
Dose multiple (x MHD)		2.7x	27x

	M	F	M	F	M	F
CL (mmol/L)	105.6±1.5		*↑ 107±0.5	-	-	-
LDH (IU/L)		6712.4±3234.3	-	*↓ 3235.6±1657.01	-	*↓ 2487.6±785.02
AST (IU/L)		1438.8±19.7	-	-	-	*↓ 114.8±11.8

[1] Reviewer's Table constructed from sponsor data; M, F = male, female; *↑, *↓ = significant increase or decrease; CL, Chloride; LDH, Lactate dehydrogenase; AST, Aspartate dehydrogenase

Urinalysis: Not evaluated

Gross Pathology: Treatment with DTPA-Mannosyl-Dextran generally had no effect on gross pathology. However, on SD15 uterine distension and a bilateral reddish discoloration of inguinal and mandibular lymph nodes were observed in 1/5 females administered the low dose.

Organ Weights: Data on organ weights was not collected (see deviations from study protocol)

Histopathology
Adequate Battery: Yes (x), No ( )
Peer Review: Yes ( ), No (x)

Histological Findings: There appeared to be an increase in the number of animals with mild incidence of lymphoid hyperplasia of the right inguinal lymph node, a finding that appeared test article-related. All other microscopic findings were not consistent with acute toxicity due to test article.

Special Evaluation: None

Toxicokinetics: Not evaluated

Dosing Solution Analysis:
No information was provided. Homogeneity testing was not performed

Conclusions: The results showed no evidence of potential toxicity following a single dose of subcutaneously administered unlabeled DTPA-Mannosyl-Dextran at 14 and 140 μg/kg in Sprague-Dawley rats. At the doses tested, DTPA-Mannosyl-Dextran did not cause any mortality or adverse clinical signs. Although there was a dose-related decrease in lactate dehydrogenase activity in females at both administered doses of DTPA-Mannosyl-Dextran on study day 15, no treatment-related effects on other clinical chemistry parameters, hematology, or gross pathology were observed. Incidental gross and concomitant microscopic lesions indicating lymphoid hyperplasia was observed in the right inguinal lymph node. DTPA-Mannosyl-Dextran administered at the dose of 140 μg/kg (or 27x MHD) appeared to be generally well-tolerated based on the findings of

this single-dose toxicity study in Sprague-Dawley rats. NOAEL for this single dose toxicity study was therefore 140 μg/kg or 27x MHD.

Table 36: Determination of NOAEL in Study No. 1576-06049

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality and/or clinical signs	>140	27x
Body weight changes		
Hematology, clinical chemistry		
Gross pathology and histopathology		

Reviewer's Table

Reviewer's comments: The changes in clinical chemistry observed on SD15 namely, a minimal increase in chloride level in group 2 (14 μg/kg) males and decreased aspartate dehydrogenase in group 3 (140 μg/kg) were not dose-related. However, the decrease in lactate dehydrogenase observed in females in both groups 2 and 3 was dose- but not sex-related. I agree with the conclusion of the sponsor that the changes observed in the clinical chemistry parameters resulted from individual animal variation. Similar changes were observed in control animals and the effects were neither dose- nor sex-dependent. Secondly, the occurrence on study day 15 of uterine distension and bilateral discoloration of inguinal and mandibular lymph nodes observed in 1 of 5 females receiving the low dose was probably incidental and spontaneous. Since these findings were observed in only in one animal (No. 29063; group 2F), was not dose-dependent and did not occur in other females or animals of the opposite sex. Overall, I agree with the study results and conclusions.

6.1.3 Study No. 1146-101:

Study title: Acute Toxicity Study of DTPA-Mannosyl-Dextran in Rabbits	
Study no.:	1146-101
Study report location:	eCTD Module 4, § 4.2.3.1.1; pages 1-153
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3 1,2000
GLP compliance:	Yes (x), No ( ), page 4 and 9 of 153 Signed: 06/02/2000
QA statement:	Yes (x), No ( ), page 5 of 153 Signed: 06/02/2000
Drug, lot #, and % purity:	DTPA-mannosyl-dextran (unlabeled drug product; DTPA-Mannosyl-Dextran), Lot #, DMD02dec99, (Neoprobe; Dublin, OH), % purity assumed to be 100%, page 9 of 153

Objective: The purpose of this study was to determine the toxicity of a single subcutaneous dose of DTPA-mannosyl-dextran in New Zealand White rabbits.

Key Study Findings: DTPA-mannosyl-dextran, administered as a single subcutaneous injection at dose levels of 0, 14 and 140 µg/kg, did not cause any treatment-related effect on survival, clinical observations, body weight, hematology and serum chemistry parameters, necropsy and histopathological findings in NZW rabbits. Microscopically, there was a finding of a minimal to mild hepatocytic hypertrophy in all treated female rabbits, 8/10 males and 1/5 control male rabbit. Based on the results, the test article, subcutaneously administered DTPA-mannosyl-dextran appeared well-tolerated at all dose levels evaluated. Although NOAEL was determined by the sponsor as the high dose (140 µg/kg) or 55x MHD, the reviewer is of the opinion that since the centrilobular hepatocytic hypertrophy observed in virtually all treated rabbits of both sexes was treatment-related, the NOAEL could not be determined based on the findings of this study.

Methods	
Doses:	0 (vehicle), 14 and 140 µg/kg (diluted in sterile water for injection; USP; Lot #: 8101111, (b) (4) % purity:100%
Frequency of dosing:	Single dose, on study day 1 (page 147 of 153)
Route of administration:	Subcutaneous (SC) in right hind footpad
Dose volume:	0.033 mL/kg (page 147 of 153)
Formulation/Vehicle:	Unlabeled drug substance (DTPA-Mannosyl-Dextran) / 0.9% Sodium Chloride (control article), Lot #: G951624, (b) (4)
Species/Strain:	Rabbits/HsdOkd:NZW; (b) (4)
Number/Sex/Group:	30; 5/sex/group. Animals were randomized to the group using computer-generated random numbers
Age:	2-3 months (at study initiation)
Weight:	Males (1.93-2.22 kg), Females (1.98-2.32 kg)
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	Deviations listed in Appendix 7, page 153 of 153

Study Design

Table 37: Study Design and Dose groups (Study No. 1146-101)

Group	Subcutaneous Dose (µg/kg)	Number of Rats	
		Males	Females
1 (veh)	0	5	5
2 (LD)	14	5	5
3 (HD)	140	5	5

Source: Reviewer's Table adapted from Sponsor's Study Design, (b) (4) Study 1146-101, and Page 12 of 153; veh = vehicle; LD, HD = Low and High Dose, respectively

Table 38: DTPA-Mannosyl-Dextran Dose multiples (Study No.1146-101)

Administration	**Subcutaneous**		
		Low Dose	High Dose
Dose (μg/kg) in Rats	0 (vehicle)	14	140
Dose multiples (based on BSA)	N/A	5.5x	55x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Observations and Results

Observations

Table 39: Summary of Methods/Observations (Study No. 1146-101)

Protocol	**Method, frequency and/or objectives**
Clinical observations	≥ Twice/day; Mortality and moribundity, general health and signs of toxicity Physical: Detailed physical examination at each weighing interval
Body weight	At randomization, prior to treatment (predose), and weekly thereafter
Clinical Pathology	Blood samples were obtained from non-anesthetized prior to termination by puncture of the medial ear artery following an overnight fast Hematology: Blood samples for hematology collected in EDTA tubes. Standard hematology parameters measured were listed in Appendix 4 (page 38 of 153) Clinical Chemistry: Serum samples were collected in serum chemistry tubes. Standard serum clinical chemistry panel (Appendix 4, page 38 of 153)
Termination/Gross pathology	14 days postdose (SD15); by sodium pentobarbital injection into marginal ear vein followed by exsanguination (Appendix 3)
Histopathology	Tissue preservation: 10% neutral-buffered formalin (Appendix 4) Tissues: According to SOP (liver, lymph nodes (left and right popliteal), injection site (footpad, right only), ovary or testis, bone marrow smear)
Urinalysis	No data (not required)
Dose formulation Analysis	Yes (x), No ( )

Reviewer's Table

Results

Mortality: All animals survived to scheduled sacrifice

Clinical Signs: No adverse clinical observations were reported

Body Weights: There were no test article-related changes in body weight

Feed Consumption: Not evaluated

Ophthalmoscopy: Not evaluated

ECG: Not evaluated

Clinical Pathology: There were no effects on hematology parameters. However, when compared to controls there was a significant increase in the mean absolute neutrophil count in group 2 (low dose) females, and significant decreases in the mean absolute lymphocyte count in high dose (group 3) females and mean creatinine kinase activity in high dose (group 3) males. Overall, no test article-related effects were observed in serum chemistry parameters. The findings are summarized in Table 40:

Table 40: [1]Summary of hematology and serum chemistry findings on SD15

Group/Dose (n=5/group) Dose multiple (x MHD)	**1 Controls** (0 μg/kg)		**2 Low dose** (14 μg/kg) 5.5x		**3 High dose** (140 μg/kg) 55x	
	M	F	M	F	M	F
Mean ABNEUT	-	1.75±0.4	-	*↑ 3.85±1.3	-	-
Mean ABLYM	-	3.48±0.15	-	-	-	*↓ 2.46±0.45
Mean CK	1073.8±292.2	-	-	-	*↓ 668.8±87.5	-

[1] Reviewer's Table constructed from sponsor data; M, F = male, female; *↑, *↓ = statistically significant ($p \leq 0.05$) increase or decrease; ABNEUT, absolute neutrophil count; ABLYM, absolute lymphocyte count; CK, creatine kinase activity

Urinalysis: Not performed

Gross Pathology: There were no adverse macroscopic findings

Organ Weights: No data submitted

Histopathology

Adequate Battery**:** Yes (x), No ( )
Peer Review**:** Yes ( ), No (x)

Histological Findings: A minimal to mild finding of centrilobular hepatocytic hypertrophy was reported treated females (10/10) and males (8/10) rabbits. A similar finding was observed in 1/5 control male animal.

Special Evaluation: None

Toxicokinetics: Not evaluated

Dose Formulation Analysis: Not provided. Homogeneity testing was not performed.

Conclusions: The sponsor concluded there was no evidence that DTPA-mannosyl-dextran, when administered once as a single subcutaneous injection at dose levels of 0, 14 and 140 μg/kg, caused any treatment-related effect on survival, clinical observations, body weight, hematology and serum chemistry parameters, necropsy and histopathological findings in NZW rabbits. Microscopically, there was a finding of a minimal to mild hepatocytic hypertrophy in all treated female rabbits, 8/10 males and 1/5 control male rabbit which may be due to an increased liver metabolism. Based on the results, the test article, subcutaneously administered DTPA-mannosyl-dextran appeared well-tolerated at all dose levels evaluated. NOAEL was determined as the high dose (140 μg/kg) or 55x MHD.

Table 41: Determination of NOAEL in Study No. 1146-101

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality and/or clinical signs	Not determined from the study[1]	
Body weight changes		
Hematology, clinical chemistry		
Gross pathology and histopathology		

Reviewer's Table; [1] NOAEL could not be determined by the reviewer based on the finding of centrilobular hepatocytic hypertrophy in virtually all treated animals of both sexes.

Reviewer's comments: There were no test article-related effects in serum chemistry parameters and the differences observed in hematological parameters (increase in absolute neutrophil count; decreased absolute lymphocyte count and decrease in mean creatine kinase activity) were not dose- or sex-dependent, lacked histopathological correlation, and were not biologically significant. The reviewer however does not agree with the sponsor conclusion that the minimal to mild centrilobular hepatocytic hypertrophy observed microscopically in 1 control male, 8/10 males and 10/10 female rabbits was not treatment-related, and the disproportionate increase in incidence from control to treated animals would argue otherwise. The implication of this histopathological finding is not completely clear. Since this finding was reported in almost all treated rabbits (low and high dose groups combined), the NOAEL cannot be determined, contrary to the conclusion of the sponsor.

6.1.4 Study No. (b) (4) 1576-04582:

Study title: DTPA-Mannosyl-Dextran: A Single-dose Subcutaneous Toxicity Study in Mongrel Dogs	
Study no.:	1576-04582
Study report location:	eCTD Module 4, § 4.2.3.1.1; pages 1-215
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 19, 2005
GLP compliance:	Yes (x), No ( ), page 4 of 215 Signed: 12/15/2005
QA statement:	Yes (x), No ( ), page 5 of 215 Signed: 12/15/2005
Drug, lot #, and % purity:	DTPA-mannosyl-dextran (unlabeled drug substance), (b) (4) Lot # M50840, % purity: Assumed 100%

Objective: The purpose of this study was to determine the toxicity of a single subcutaneous dose of unlabeled substance DTPA-mannosyl-dextran in mongrel dogs.

Key Study Findings: Based on the findings, subcutaneously-administered DTPA-mannosyl-dextran at doses up to and including 420 µg/kg was well tolerated in mongrel dogs. Only injection site inflammatory response occurring in both sexes was noted as a treatment-related effect. The NOAEL was established as 420 µg/kg.

Methods	
Doses:	0 (saline vehicle), 42, 180, 420 µg/kg.
Frequency of dosing:	Single dose; on study day 1 (SD1)
Route of administration:	Subcutaneous (mid-scapular region)
Dose volume:	0.2 mL/kg based on body weight on SD-1
Formulation/Vehicle:	Unlabeled drug substance (DTPA-mannosyl-dextran)/ saline (0.9% USP; Lot #: 14-052-JT, (b) (4) and Purity assumed 100%)
Species/Strain:	Dog / Mongrel, (b) (4)
Number/Sex/Group:	32 dogs; 4/sex/group
Age:	4-6 months (at 1st dose)
Weight:	Males (7.0-11.3 kg); Females (7.0-9.6 kg)
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	protocol deviations were listed in appendix 14, page 215 of 215

Study Design

Table 42: Study Design and Dose groups (Study No.1576-04582)

Group	Treatment	Subcutaneous Dose (μg/kg)	Number of Dogs	
			Males	Females
1 (veh)	Saline	0	4	4
2 (LD)	DTPA-mannosyl-dextran	42	4	4
3 (MD)	DTPA-mannosyl-dextran	180	4	4
4 (HD)	DTPA-mannosyl-dextran	420	4	4

Source: Reviewer's Table adapted from Sponsor's Study Design (page 13 of 215); veh = vehicle; LD, MD, HD = Low, Mid and High Dose, respectively

Table 43: DTPA-Mannosyl-Dextran Dose multiples (Study No. 1576-04582)

Administration	Subcutaneous			
		Low dose	Mid dose	High dose
Dose (μg/kg) in Rats	0 (vehicle)	42	180	420
Dose multiples (based on BSA)	N/A	27x	117x	273x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Observations and Results

Observations

Table 44: Summary of Methods/Observations (Study No. 1576-04582)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside: ≥ Twice/day Clinical: Prior to dosing on SD 1, weekly thereafter and at termination for mortality and moribundity, general health and signs of toxicity; evaluation of skin and fur characteristics, eye and mucous membranes, injection site, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns
Body weight	Prior to dosing on SD 1, weekly thereafter, prior to termination (non-fasted), and at termination (fasted)
Feed consumption	Daily
Clinical Pathology	Blood and urine were collected for clinical pathology prior to initiation of dosing and prior to termination (SD 15) via jugular puncture for serum chemistry, hematology and coagulation (page 14 of 215) Serum chemistry: ~2.5 mL in serum separator tubes Hematology: ~2.0 mL in 2.0 mL tubes containing dipotassium EDTA) Coagulation: ~1.8 mL in tubes containing sodium citrate
Termination/Gross pathology	Termination: Sodium pentobarbital overdose and exsanguination on SD15

	Necropsy: 14 days postdose (SD15). Protocol-specified samples were preserved in 10% neutral buffered formalin
Histopathology	Tissues: According to SOP
Urinalysis	Overnight fasted animals with water available; Clear pan collection of total urine available at point of collection using conical tube; Parameters evaluated based on SOP (Appendix 8)
Dose formulation Analysis	Yes (x), No ( )

Reviewer's Table based on sponsor data

Results

Mortality: Treatment with DTPA-mannosyl-dextran at doses up to the high dose of 420 μg/kg (or 0.42 mg/kg) had no effect on mortality and all animals survived until study completion.

Clinical Signs: There were no defined treatment-related effects on clinical signs. Observations noted in the report as incidental include fecal abnormalities (diarrhea, and mucoid, soft and discolored feces), frothy and food emesis, erythema and abscess formation

Body Weights: No treatment-related effects on the absolute body weight or body weight changes were observed up to 420 μg/kg of the test article

Feed Consumption: Treatment with DTPA-mannosyl-dextran at doses up to 420μg /kg had no effect on food consumption.

Ophthalmoscopy: Not performed

ECG: Not performed

Hematology: There were no statistically significant differences in hematological parameters between control and treated animals on Study days 1 and 15

Clinical Chemistry: On study day 1, there was a slight significant increase in alkaline phosphatase (ALKP) in low dose (42 μg/kg) group males compared to controls. A similar significant increase in ALKP was observed on SD-15 in males administered the low and high (420 μg/kg) doses. Female dogs treated with the mid (180 μg/kg) dose had a significant increase in chloride and a decreased CO_2 at the high dose. No differences from controls were observed with other measured clinical chemistry parameters. The findings are summarized (data not shown) in Table 45:

Table 45: [1]Summary of serum chemistry findings (Study 1576-04582)

Group/Dose	1 Control 0 μg/kg		2 Low dose 42 μg/kg		3 Mid dose 180 μg/kg		4 High dose 420 μg/kg	
	M	F	M	F	M	F	M	F
SD1								
ALKP			*↑					
SD15								
ALKP			*↑				*↑	
CL					*↑			
CO_2								*↓

[1]Reviewer's Table constructed from sponsor data; M, F = male, female; *↑, *↓ = statistically significant ($p \leq 0.05$) increase or decrease; SD1, SD15, study days 1 and 15; ALKP, alkaline phosphatase; CL, chloride; CO_2, carbon dioxide

Urinalysis: There were no statistically significant differences in the assessed urinalysis parameters between control and treated animals on study days 1 and 15

Gross Pathology: Treatment with DTPA-Mannosyl-Dextran had no effect on gross pathology when administered at doses up to 420 μg/kg. Incidental and spontaneous findings observed at the high dose namely, a discolored liver in a control male and bronchial lymph node enlargement in 1 female were not dose-related effects.

Organ Weights: There were no treatment-related effects on absolute or relative organ weights up to the high dose (420 μg/kg). No organ-to-body weight or organ-to-brain weight ratios were noted at any administered dose. Incidental findings included a significantly higher absolute adrenal gland weight with no corresponding change in relative organ weight ratios, was noted in high dose females. This finding was not noted in males and had no pathologic correlation

Histopathology
Adequate Battery: Yes (x), No ( )
Peer Review: Yes ( ), No (x)

Histological Findings: Histopathologic examination did not reveal any systemic toxicity attributable to the test substance up to the high dose. Microscopic findings, consisting of minimal to mild chronic active inflammation of the hypodermis and perimysial connective tissue, were limited to the injection site in both sexes.

Special Evaluation: None

Toxicokinetics: Not performed

Dosing Solution Analysis: All DTPA-mannosyl-dextran dosing formulations were within acceptance limits for precision (coefficient of variation ± 15%) and accuracy (relative error ±10%).

Conclusions: Based on the findings, subcutaneously-administered DTPA-mannosyl-dextran at doses up to and including 420 μg/kg was well tolerated in mongrel dogs. Injection site inflammatory response, occurring in both sexes, was noted as a treatment-related effect. The NOAEL for this study was established as 420 μg/kg. On the clinical observations that these were made, the sponsor concluded, often in single cases, in either control or treated animals. There was no correlation with dose or sex. The sponsor's conclusion was confirmed by an examination of the data. I agree.

Table 46: Determination of NOAEL in Study No. 1576-04582

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality and/or clinical signs	>420	273x
Body weight changes		
Feed consumption		
Hematology, clinical chemistry		
Gross pathology histopathology		
Organ weights		
Histopathology		

Reviewer's Table

Reviewer's comments: The slight but significant increase in alkaline phosphatase (ALKP) in males in the low dose group on SD1 and on SD15 in males in the low and high dose groups; the significant increase in chloride in mid dose females and a decreased CO_2 in high dose group females were considered due to individual animal variation and not to the test article. The differences were minimal, lacked a corresponding change in animals in the opposite sex or were not dose-dependent. I agree with the sponsor's conclusions.

6.2 Repeat-Dose Toxicity

6.2.1 Study No. (b) (4) N106923:

Study title: 14-Day Toxicity Study of ^{111}Indium-Lymphoseek In Sprague-Dawley Rats	
Study no.:	N106923
Study report location:	eCTD Module 4, § 4.2.3.2.1; pages 1-407
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 11, 2005 (1st dose)
GLP compliance:	Yes (x), No ( ), page 4 of 407 Signed: 12/16/2005
QA statement:	Yes (x), No ( ), page 5 of 407 Signed: 12/16/2005
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran, Lot #: M50840, % purity ^{111}Indium-DTPA-Mannosyl-Dextran, Lot #: % purity 93.1% (average), page 25 of 407

Key Study Findings: There were no treatment related effects observed in all the dose groups tested and the no observed effect level (NOAEL) following a 14-day consecutive administration of subcutaneous administration of DTPA-Mannosyl-Dextran was considered to be at least 420 μg/kg/day (or 8x MHD)

Methods	
Doses:	0 (vehicle control), 10.5, 21, and 42 μg/kg (or 0, 0.210, 0.0420, and 0.084 mg/mL)
Frequency of dosing:	DTPA-Mannosyl-Dextran: Repeated dose consecutively for 14 days; ^{111}Indium-Lymphoseek: Single dose on Days 1 and 14 Note: The test article is ^{111}Indium-DTPA-Mannosyl-Dextran and not Lymphoseek as might be suggested by the name
Route of administration:	Subcutaneous (SC) injection
Dose volume:	0.5 mL/kg body weight on SD-1
Formulation/Vehicle:	DTPA-Mannosyl-Dextran in PBS, unlabeled and [^{111}In]-labeled; glycine HCl buffer was used to adjust the radiolabeled formulation pH / Phosphate buffered saline (PBS)
Species/Strain:	Rat / Sprague-Dawley [Crl:CD(SD) IGS BR], (b) (4)
Number/Sex/Group:	218 rats (109/sex) were used; Core group (toxicity):10/sex/group (4 groups including controls) Satellite (Pharmacokinetic) group: 23/sex/group (no controls)
Age:	6 weeks (at receipt); 7 weeks (at 1st administration)
Weight:	Males (185.8-305.6 g); Females (147.7-228.9 g) Deviation: "At initiation of dosing (SD-1, 8/1/2005) and on SD-28, 8/19/2005), 28 of 40 core group females and all of the pharmacokinetic group females weighed less than the 200-400 g stipulated by the protocol" (Appendix A, page 25; study report page 69 of 407)
Satellite groups:	Yes (x), ( ) Satellite groups for pharmacokinetics: 23/sex/group (3 groups; no control group)
Unique study design:	Repeated dose toxicity study with toxicokinetics
Deviation from study protocol:	Deviations are described in Appendix A, pages 68-99 of study report

In the Study Errata to Report, the sponsor made the corrections listed in Table 47:

Table 47: Study Errata – Study No. N106923

Error Location	Error Content	Statement of Effect
Appendix G Page G-2 and Page G-6	The number of animals in the PK group. The study report states 23/sex/group (e.g., Summary section), while the TK report in Appendix G states 13/sex/group. Neoprobe review findings: The study used 23 rats per sex per group (low, medium, high dose) for pharmacokinetic data, further divided into 20/sex/group for blood/plasma collection and 3/sex/group for urine collection. The core subset consisted of 10 rats per sex per group (vehicle, low, medium, high). The "13" in Appendix G is erroneous since the collected data confirm 23 rats/sex/group were used.	Error does not change the conclusions of the study.

Study Design; A total of 218 Sprague-Dawley rats (109/sex) divided into 4 dose groups were used for the study. 10 rats/sex/group were assigned to the core toxicity group and were administered the test article or vehicle (phosphate buffered saline) subcutaneously for 14 consecutive days. Twenty three (23) rats/sex/group were assigned to the PK study comprising 3 DTPA-Mannosyl-Dextran doses (low, mid and high) with no control group (i.e., groups 2-4 and no control group 1). Prior to Day 1 and on Day 14, PK animals received the ^{111}Indium-labeled drug formulation at a target dose of 0.125 mCi/kg. On days 1 through 13, PK rats (groups 2-4) received a daily dose of the unlabeled compound. 20 rats/sex/group rats were sampled for blood and plasma while three (3) rats/sex/group were sampled for urine. The study design is summarized in Tables 48 and 49.

Table 48: Study Design and Dose group (Study No: N106923)

Groups [A]	Core Group (CG) [2]		Pharmacokinetic Group (PK)		
	Unlabeled DTPA-Mannosyl-Dextran[1]		Unlabeled DTPA-Mannosyl-Dextran[1]	^{111}Indium-DTPA-Mannosyl-Dextran[2]	
	Dose (µg/kg)	Dose Days	Dose Days	Radioactive dose (mCi/kg)	Dose Schedule / TK days
1 (veh)	0	1-14	NA	NA	NA
2 (LD)	10.5		1-13	0.125	Prior to Day 1, 14
3 (MD)	21				
4 (HD)	42				

Source: Reviewer's Table adapted from Sponsor's Study Design (page 13 of 407); veh = vehicle; LD, MD, HD = Low, Mid and High Dose, respectively; NA = not applicable; [1] = unlabeled DTPA-Mannosyl-Dextran; [2] = ^{111}Indium-labeled DTPA-Mannosyl-Dextran; [A] = animals in all groups were administered the respective test article via the subcutaneous route.

Table 49: Dose groups and numbers of animals (Study No.106923)

Groups [A]		Core Toxicology (CG)[1]		Pharmacokinetic (PK) Group[2]		Total
#	Dose(µg/kg)	M	F	M	F	
1 (veh)	0	10	10	0	0	20
2 (LD)	10.5	10	10	23	23	66
3 (MD)	21.0	10	10	23	23	66
4 (HD)	42.0	10	10	23	23	66
						218

Source: Reviewer's Table adapted from Sponsor's Study Design (page 13 of 407); veh = vehicle; LD, MD, HD = Low, Mid and High Dose, respectively; M, F = Male, Female; [1] = unlabeled DTPA-Mannosyl-Dextran; [2] = ^{111}Indium-labeled DTPA-Mannosyl-Dextran; [A] = animals in all groups were administered the respective test article via the subcutaneous route.

Table 50: Unlabeled DTPA-Mannosyl-Dextran - Dose Multiples (Study No: N106923)

Administration	**Subcutaneous**			
		Low dose	Mid dose	High dose
Dose (µg/kg) in Rats	0 (vehicle)	10.5	21	42
Dose multiples (based on BSA)	N/A	2x	4x	8x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 µg or 30.8 µg/m^2 based on BSA

Observations and Results

Observations

Table 51: Summary of Methods/Observations (N 106923)

Protocol	Method, frequency and/or objectives
Clinical observations	Twice/day (a.m and p.m) for moribundity and mortality per SOP. Cage side observation for evidence of toxicity and injection site reaction 2x/day
Body weight	Individual body weights were recorded pre-study for group assignment, on Days 1 and 8, prior to dosing for both the core and pharmacokinetic groups and prior to dosing on Day 14 for the pharmacokinetic groups. Core group animal body weights were also recorded on Day 14 (post-dose) and on the day of necropsy (fasted)
Feed/water consumption	*ad libitum* access to their daily ration of pelleted rodent diet (Harlan Teklad Certified 2018C) except during periods of scheduled fasts. Feed and water were monitored under (b) (4) SOPs. Food consumption was quantitatively determined for all core group animals beginning on Day 1,

	then weekly thereafter
Ophthalmic examination	Performed based on SOPs
PK analysis	Based on SOPs
Clinical Pathology	Core group animals were fasted (at least 12 hours) prior to blood collection for hematology, coagulation and serum chemistry parameters prior to necropsy on Day 15. Blood samples for hematology and serum chemistry from the retro-orbital sinus according to facility SOP using EDTA anticoagulant tubes hematology samples. No anticoagulant was used for serum samples. Serum was separated by centrifugation. Rats were bled via the abdominal aorta or vena cava for coagulation parameters. Animals were bled under CO2/O2 anesthesia according to facility SOP. Coagulation tubes contained sodium citrate as an anticoagulant. Hematology, serum chemistry parameters evaluated were listed on page 21 of 407
Urinalysis	All core group animals were housed overnight and fasted for at least 12 hours in cages prior to scheduled necropsy on Day 15.The parameters evaluated were listed on page 22 of 407
Termination/Gross pathology	Core group rats (10 rats/sex/group) were necropsied on Day 15; the day after the final dose was administered. Pharmacokinetic animals had blood or urine collected, and were euthanized without necropsy following their final collection.
Histopathology	Adequate Battery: Yes (x), No ( ); Histopathology tissues and organs weighed are listed in Table 53. Peer Review: Yes ( ), No (x)
Dose formulation Analysis	Yes (x), No ( ), page 15 of 407 and Appendix F (page 222 of 407)

Reviewer's Table based on sponsor data

Results

Mortality: There were no deaths reported and all animals survived to their scheduled necropsy.

Clinical Signs: No clinical observations were attributed to the test article. There were reports of alopecia in males and female animals in both core (treatment and controls) and PK groups and some instances of minor abrasions in PK dosage groups. Alopecia or abrasion was reported in 1-2 animals/group. One low dose core group female had a thin appearance for 2 days, a finding that was claimed as being due to partial malocclusion of frontal incisors and was resolved by veterinary care. Normal feeding was thereafter restored in this individual animal. NOAEL based on clinical signs was 42.0 μg/kg or 8-fold HD)

Body Weights: No statistically-significant differences were noted in body weight

Feed Consumption: No statistically-significant differences were noted in quantitative food consumption

Ophthalmoscopy: No abnormalities of the eye or its surrounding tissues were observed

ECG: ECG was not evaluated

Hematology: No treatment-related effects were observed

Clinical Chemistry: No treatment-related effects were observed

Urinalysis: On Day 15, the urine specific gravity, calcium, inorganic phosphorus, and potassium all increased in males administered the test article when compared to the corresponding vehicle-treated males. None of the increases in urine inorganic phosphorus were statistically different from values in male vehicle rats. The increases in group mean urine specific gravity, calcium, and potassium in mid-dose males differed statistically from values in male vehicle rats. Urine sodium and chloride results were increased in treated males, but only slightly and without statistical significance. Urine volume was slightly decreased in the three male treatment groups, albeit non-significantly. These findings are summarized in Table 52:

Table 52: Effect of Unlabeled DTPA-Mannosyl-Dextran on urinary chemistry[1]

Parameter	Males				Females
	Dose (μg/kg)				
	Veh (0)	LD (10.5)	MD (21.0)	HD (42.0)	
Specific Gravity	1.012 ± 0.005	↑ 1.014 ± 0.006	*↑ **1.022 ± 0.012**	↑ 1,019 ± 0.010	No similar trends as observed in males
Calcium (mg/dL)	1.1 ± 0.5	↑ 1.5 ± 0.6	*↑ **2.1 ± 0.9**	↑ 1.7 ± 0.8	
Inorg. Phosphorus (mg/dL)	74.6 ± 31.0	↑ 96.1 ± 37.9	↑ 135.6 ± 86.6	↑ 133.3 ± 90.0	
Potassium (mEq/L)	64.2 ± 29.1	↑ 83.5 ± 40.7	*↑ **128.8 ± 83.4**	↑ 113.7 ± 64.5	
Urine sodium (mEq/L)	18 ± 6	↑ 20 ± 6	↑ 26 ± 14	↑ 26 ± 15	
Urine chloride mEq/L)	19 ± 6	↑ 20 ± 10	↑ 34 ± 21	↑ 35 ± 19	
Urine volume (mL)	13.5 ± 1.9	↓ 11.7 ± 3.5	↓ 9.8 ± 4.9	↓ 10.7 ±3.9	

[1] Reviewer's Table based on sponsor data; values are mean ± SD (standard deviation); n values not shown in Table; Veh = vehicle; LD, MD, HD = Low, Mid and High dose; ↑, ↓ =

increased or decreased; * = p ≤ 0.05; M = male; No significant effects were observed in the following urinary indices: Glucose, Protein, pH, Ketones, Urobilinogen, Bilirubin, Occult blood, Color, Appearance, White blood cells, Red blood cells, Casts, Epithelial cells, Mucus, Sperm, Bacteria, Yeast, Amorphous sodium, and Crystals

Gross Pathology: Two gross lesions were noted in core group males namely, dilated right renal pelvis (1/10 vehicle group) and an enlarged mandibular lymph node (1/10 mid dose [21.0 μg/kg]) males. No corresponding findings were observed in females. Histopathology confirmed the renal pelvic dilation and also revealed the lymphoid hyperplasia in the lymph node

Terminal body weight: The mean terminal body weight of rats in the treatment groups were similar to those of the respective controls

Organ Weights: There was a statistically significant (p ≤ 0.05) decrease in the mean absolute, percent organ-to-terminal body weight and percent organ-to-brain weight results for the adrenal glands of the 10.5 μg/kg males when compared with male vehicle controls. The NOAEL, based on organ weight data was determined as the high dose (42 μg/kg or 8x MHD).

Histopathology
Adequate Battery: Yes (x), No ( )
Peer Review: Yes ( ), No (x)

Histological Findings: Some microscopic findings were identified in treated and vehicle rats but none was determined as treatment-related. The tissues and organs examined findings are summarized in Table 53. Tissues and organs not examined are listed in the footnotes to Table 53:

Table 53: Histopathological findings[1]

Tissues	Finding(s)	Sex/Group/Dose (μg/kg)							
		Males (n=10)				**Females** (n=10)			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
		0	10.5	21.0	42.0	Veh	10.5	21.0	42.0
Brain	epidermal inclusion cyst								
	minimal	0	0	0	0	0	0	0	1
Epididymis	interstitial mononuclear cells infiltration	7	0	0	9	0	0	0	0
Heart	myocardial mononuclear cell infiltration								
	minimal	2	0	0	3	0	0	0	0
Kidney	nephropathy								
	minimal	1	0	0	1	1	0	0	1
	mild	1	0	0	2	0	0	0	0
	pelvic dilation								
	mild	1	0	0	0	0	0	0	0
	mineralization								

	minimal	0	0	0	0	3	0	0	3
	mild	0	0	0	0	0	0	0	1
Liver	mononuclear cell infiltration								
	minimal	0	0	0	0	0	0	0	9
	mild	0	0	0	0	0	0	0	1
	bile duct hyperplasia								
	minimal	10	0	0	9	10	0	0	10
	midzonal fatty change								
	minimal	1	0	0	1	0	0	0	0
Mandibular LN	lymphoid hyperplasia								
	marked	0	0	1	0	0	0	0	0
Lung	lymphoid hyperplasia								
	minimal	3	0	0	4	3	0	0	1
Prostate	mononuclear cell infiltration								
	minimal	3	0	0	3	-	-	-	-
Stomach	mononuclear cell infiltration of tunica submucosa	3	0	0	2	0	0	0	2
	minimal								
Thyroid gland	thyroglossal duct cyst	1	0	0	0	0	0	0	0
	present								
Trachea	mononuclear cell infiltration of tunica propria								
	minimal	2	0	0	3	1	0	0	2
	mild	1	0	0	0	0	0	0	0

[1] Reviewer's Table based on sponsor data (Table 27, pages 52-55 of 407); LN = lymph node; No microscopic lesions were observed in the following organs/tissues: adrenal glands, aorta, bone, bone marrow, cecum, cervix, colon, duodenum, esophagus, eye, femur, ileum, jejunum, mesenteric lymph nodes, mammary gland, optic nerve, ovary, pancreas, parathyroid, rectum, rib, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, spleen, sternum, testes, thymus, urinary bladder, uterus and vagina

Table 54: Reviewer's comment(s) on the Histopathology findings[1]

Tissue/Organ	Dose-dependent effect	Sex-related	Effect present in controls	Comment(s)
Brain	N	N	N	Isolated incident; occurred in 1 HD female
Epididymis	N	NA	✓M	some incidences; not dose- or sex-dependent; seen only in HD males
Heart	N	N	✓M	
Kidney				
nephropathy	N	✓M, ✓F	✓M, ✓F	sex- but not dose-dependent; also seen in M,F controls
pelvic dilation	N	N	✓M	not dose- or sex-dependent; seen only in 1 M control
mineralization	N	N	✓F	Isolated incident; not dose- or sex-dependent; occurred in 1 HD female

Liver				
cell infiltration	N	N	N	some incidents; not dose- or sex-dependent; occurred in HD females
bile duct hyperplasia	N	✓M, ✓F	✓M, ✓F	sex- but not dose-dependent; also seen in M,F controls
fatty change	N	N	✓M	some incidences; not dose- or sex-dependent; seen only in 1 HD male and 1 M control
Mandibular LN	N	N	N	Isolated incident; occurred in 1 MD female
Lung	N	✓M, ✓F	✓M, ✓F	some incidences; not dose- or sex-dependent; seen in HD M,F and M,F controls
Prostate	N	NA	✓M	not dose-dependent; seen 3 HD rats
Stomach	N	✓M, ✓F	✓M	not dose- but sex-related; seen also only in M controls
Thyroid gland	N	N	✓M	isolated incident occurring only in 1 M control
Trachea	N	✓M, ✓F	✓M, ✓F	Few not-dose dependent incidences occurring in HD M,F and respective controls

[1] Reviewer's Table based on summary of histopathology findings; ✓= Yes; N = No; M, F = males, females; NA = not applicable; MD, HD = mid dose or high dose

Special Evaluation
None

Toxicokinetics

- The PK profile of a 14-day toxicity study of subcutaneously-administered ^{111}Indium-DTPA-Mannosyl-Dextran in male and female Sprague-Dawley rats followed a 2-compartment model with first order absorption and elimination kinetics as the best fit for the plasma concentration curves
- The absorption was relatively fast with well-defined peak concentration, time-to-peak concentration and a biphasic elimination phase
- No dosage, sex or single versus repeated administration effects were observed on the absorption TK parameters. Absorption $t_{1/2}$ was 0.067 ± 0.01 h (about 4 min).
- Cmax increased dose-proportionately and values were similar for males and females. Mean Cmax values on Day 1 and Day 14 for the administered doses are shown in the summary Table 55.
- Tmax was not dependent on dosage, sex or single versus repeated administration
- Systemic exposure (AUC0-last) values were similar for both sexes of a given dose group, increased dose-proportionately and were not affected by repeated dosing
- Volume of distribution (Vd F) was similarly not dependent on sex and was not affected by single versus repeated administration. The overall, V_d was 1160 ± 50 mL/kg

- Elimination, evaluated by alpha, beta or central compartment elimination constants was not dependent on dose, sex or single versus repeated administration. The elimination profile is given in the PK summary (Table 55)
- The mean overall clearance (CL) value of 333 ± 46 mL/hr/kg also was not dependent on sex or single versus repeated administration
- Peak urinary ^{111}Indium-Lymphoseek concentrations were observed for both sexes in the different dose groups on Days 1 and 14 at between 0-6 h post-dose (i.e., the 0 to 2 and 2 to 6 h collection intervals). Approximately 90% of ^{111}Indium-Lymphoseek eliminated by the kidney was measured at the 2-6 h interval on Day 1 and at 6-12 h interval on Day 14. The percentage of the radiolabeled Lymphoseek eliminated via the kidney was similar across dosage groups, sex and single versus repeated administration. A mean of 29.1±1.8 (SEM) percent of the administered dose was eliminated via urine

Table 55: PK Satellite Group (Toxicokinetic mean values)[1]

Parameter	Day	Dose groups					
		1 (10.5 μg/kg)		2 (21.0 μg/kg)		3 (42.0 μg/kg)	
		M	F	M	F	M	F
Cmax (ng/mL)	1	12.8±1.3	10.4±1.0	16.5±1.1	12.3±1.6	29.1±3.0	26.2±2.4
	14	5.62±0.26	6.10±0.43	13.4±1.1	14.6±1.1	25.8±1.1	19.2±1.4
Tmax (h) Range	1	Males (0.12 – 0.21); Females (0.25 – 0.31)					
	14	Males (0.17 – 0.21); Females (0.21 – 0.26)					
Vd (mL/kg)		1160 ± 50					
AUC(0-last) (ng/mL* h)	1	20.6	17.8	31.7	24.2	58.7	57.2
	14	12.8	16.9	31.6	42.5	63.5	53.7
Apparent CL (mL/h/kg)		333 ± 46					
Dose recovered in urine (%)	1	30.3	34.2	31.0	32.4	29.4	20.7
	14	32.9	40.2	33.4	19.2	23.3	22.3

[1]Reviewer's Table based on sponsor's data

Dosing Solution Analysis: The formulations prepared on Day 1 were analyzed (except the control dose). The concentrations of the Lymphoseek formulations were within ±10% of target and within the acceptance limit for precision (±15% of target). The dose formulation analysis is acceptable.

Conclusions: There were no treatment related effects observed in all the dose groups tested and the no observed effect level (NOAEL) following a 14-day consecutive administration of subcutaneous administration of Lymphoseek was considered to be at least 420 μg/kg/day (or 8x MHD).

Table 56: Determination of NOAEL in Study No. N106923

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality		
Clinical signs		

Body weight changes	>42.0	8x
Feed consumption		
Ophthalmoscopy		
Hematology, clinical chemistry		
Gross pathology histopathology		
Organ weights		
Histopathology		

Reviewer's Table

Reviewer's comments: Based on the sporadic nature of observed incidences of dilated renal pelvis macroscopic incidences in mid-dose males only, the fact that the findings were neither dose- nor sex-related, the sponsor concluded a lack of relationship of the incidences to treatment. According to the sponsor, renal pelvic dilation is known to occur spontaneously in Sprague-Dawley rats without evidence of inflammation changes and was generally associated with congenital urinary tract development. I agree that the cause of the lymph node enlargement is uncertain but could not be associated with Lymphoseek. The sponsor did not consider the decrease in adrenal gland weights in males treated with the low dose to be toxicologically significant nor due to the test article since the decrease was not continued in the males administered higher doses of DTPA-Mannosyl-Dextran. I agree that there was no similar trend in treated females. Also, other mean organ weight results in the treated groups of rats were similar to those of the appropriate vehicle group. Lastly, the reported multiple histopathology findings were often observed also in control animals, were generally not dose-dependent, were observed at only one test-article dose (commonly the high dose), and were often isolated, single animal incidences (or occasionally involved several animals). Based on these findings, the reviewer concluded that these findings were occasional occurrences and were not toxicologically significant. Overall, the reviewer agrees with the findings and conclusions and based on histopathological findings NOAEL was the high dose (42 μg/kg or 8x MHD)

6.2.2 Study No. (b) (4) N 106922

Study title: 14-Day Toxicity Study of ^{111}Indium-Lymphoseek In Dogs	
Study no.:	N106922
Study report location:	eCTD Module 4, § 4.2.3.2.2; pages 1-464
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 23, 2005; 1st dose: July 13, 2005
GLP compliance:	Yes (x), No ( ), page 4 of 464 Signed: 11/22/2005
QA statement:	Yes (x), No ( ), page 5 of 464 Signed: 1/22/2005
Drug, lot #, and % purity:	Unlabeled drug substance (DTPA-Mannosyl-Dextran), Lot #: M50840 (Neoprobe Corp.) % purity ^{111}Indium-DTPA-Mannosyl-Dextran, Lot #: % purity: 89.3% (Day 1), 87.1% (Day 14), 88.2% (average), Appendix E (Chemistry report) page 170 of 464

Objective: The purpose of this study was to determine the acute toxicity of Lymphoseek when administered via subcutaneous injection to mongrel dogs for at least 14 consecutive days

Key Study Findings: There were no treatment-related effects in dogs at all the doses tested for all the endpoints assessed in this 14-day repeated dose toxicity study. Plasma and urine samples collected from male and female dogs and analyzed for ^{111}Indium-Lymphoseek equivalents and to determine TK parameters and urinary excretion patterns indicated a relatively fast absorption that was unaffected by dosage, sex, or repeated administrations. The no observed adverse effect level (NOAEL) for repeated subcutaneous administration of Lymphoseek was considered to be at least 42.0 μg/kg/day.

Methods	
Doses:	0 (vehicle control), 10.5, 21, and 42 µg/kg (or 0, 0.105, 0.210, and 0.420 mg/mL)
Frequency of dosing:	Unlabeled Lymphoseek: Repeated dosing (consecutively for 15 days or 16, if required) ^{111}Indium-Lymphoseek: Single dose on Days 1 and 14
Route of administration:	Subcutaneous injection
Dose volume:	0.1 mL/kg
Formulation/Vehicle:	DTPA-Mannosyl-Dextran in PBS, unlabeled and [^{111}In]-labeled; glycine HCl buffer was used to adjust the radiolabeled formulation pH / Phosphate buffered saline (PBS)
Species/Strain:	Dog / Mongrel; (b) (4)
Number/Sex/Group:	Core group: 12/sex/group, (4 groups including controls)
Satellite groups:	Yes. Satellite groups for pharmacokinetics:3/sex/group (3 groups; no control group)
Age:	4-6 months (at arrival); 6-8 months (at initiation of dosing)
Weight:	Males (6.6-12.2 kg); 6.3-10.1 kg) on Day 1 of dose administration
Satellite groups:	Yes. Satellite groups for pharmacokinetics:3/sex/group (3 groups; no control group)
Unique study design:	Repeated dose toxicity study with toxicokinetics
Deviation from study protocol:	Deviations are described in Appendix A, pages 66-94 of study report

Study Design: A total of 24 dogs (12/sex), divided into 4 dose groups (3 dogs/sex/group) and administered the test article or vehicle (phosphate buffered saline) subcutaneously for 14 consecutive days, were used. On Days I and 14, animals in Groups 2 - 4 received DTPA-Mannosyl-Dextran radiolabeled with ^{111}Indium at a target dose of 0.125 mCi/kg and were bled at specified time points for toxicokinetic analysis (see 7of procedures and observations). On Days 2 -13 and Days 15 through the day prior to necropsy, animals in groups 2-4 received a single, daily dose of non-radiolabeled DTPA-Mannosyl-Dextran. Vehicle-treated animals (Group I) were administered a single daily dose of phosphate buffered saline on Days 1-14. Toxicity was evaluated based on body weights, clinical observations, food consumption (qualitative), physical and ophthalmological examinations, ECGs, clinical pathology and gross and microscopic pathology findings. Tables 57 and 58 summarize the study design:

Table 57: Group Assignment and Dose levels (Study No: N106922)

Groups [A]	Core Group		Pharmacokinetic Group		
	Unlabeled DTPA-Mannosyl-Dextran		Unlabeled DTPA-Mannosyl-Dextran	^{111}Indium-DTPA-Mannosyl-Dextran	
	Dose (µg/kg)	Dose Days	Dose Days	Radioactive dose (mCi/kg)	Dose Schedule / TK days
1 (veh)	0	1-14	NA	NA	NA
2 (LD)	10.5		2-15; and 16 (for 2 males and 1 female/group)	0.0125	On Days 1, 14
3 (MD)	21				
4 (HD)	42				

Source: Reviewer's Table adapted from Sponsor's Study Design (page 14 of 464); veh = vehicle; LD, MD, HD = Low, Mid and High Dose, respectively; NA = not applicable; Group 1 (veh) dogs received a single daily dose of phosphate buffered saline on Days 1 through 15 (and Day 16 for 2 males and 1 female); [1] = unlabeled DTPA-Mannosyl-Dextran; [2] = ^{111}Indium-labeled DTPA-Mannosyl-Dextran; [A] = animals in all groups were administered the respective test article via the subcutaneous route.

Table 58: Dose Groups and numbers of animals (Study No: N106922)

Groups [A]		Unlabeled DTPA-Mannosyl-Dextran		^{111}Indium-DTPA-Mannosyl-Dextran	Total
#	Dose(µg/kg)	M	F		
1 (veh)	0	3	3		6
2 (LD)	10.5	3	3	Yes	6
3 (MD)	21.0	3	3		6
4 (HD)	42.0	3	3		6
					24

Source: Reviewer's Table adapted from Sponsor's Study Design (page 13 of 407); veh = vehicle; LD, MD, HD = Low, Mid and High Dose, respectively; M, F = Male, Female; [A] = animals in all groups were administered the respective test article via the subcutaneous route.

Table 59: Unlabeled DTPA-Mannosyl-Dextran Dose multiples (Study No: (b) (4) 1576-04583)

Administration	Subcutaneous			
		Low dose	Mid dose	High dose
Dose (µg/kg) in Rats	0 (vehicle)	10.5	21	42
Dose multiples (based on BSA)	N/A	7x	14x	27x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 µg or 30.8 µg/m^2 based on BSA)

Observations and Results

Observations

Table 60: Summary of Methods/Observations (N 106922)

Protocol	Method, frequency and/or objectives
Clinical observations	Twice/day (a.m and p.m) for morbidity and mortality per SOP. Cage side observation for evidence of toxicity and injection site reaction 2x/day
Body weight	Individual body weights were recorded on Days 1, 8 and 14, prior to dosing and on the day of necropsy (fasted)
Food consumption	Food consumption (qualitative by visual examination) will be recorded for all animals beginning Day I and daily during the study except when fasting is required for scheduled necropsy or clinical pathology bleeds
Ophthalmic examination	Performed based on SOPs prior study and on Days 12 and 13
ECG	ECGs were performed once pretest and again on all animals on Days 2 and 15 within 1h ± 15 min post dosing. ECG tracings of leads I, II, III, aVR, aVL, aVF, and V10 were collected at a paper speed of 50 mm/second. Analysis included heart rate and duration of PR, QRS, and QT intervals. Tracings were evaluated for rhythm and morphology
Clinical Pathology (Hematology, Coagulation and Serum Chemistry parameters based on SOPs; page 10 of 464)	Blood samples for hematology, coagulation and serum chemistry parameters were collected (via jugular vein) after an overnight fast from all animals prior to randomization, and prior to dosing on Day 14. EDTA was used as an anticoagulant for blood samples collected for hematology. Tubes used for serum chemistry analysis did not contain any anticoagulant. Sodium citrate anticoagulant was used in tubes to collect blood for coagulation parameters. Day 14 samples for serum chemistry and coagulation analysis from animals in the low, mid, and high dose groups were frozen until indirect monitoring indicated they were no longer radioactive (34 days). Slides were prepared for possible morphology evaluation from Day 14 samples collected for hematology parameters from low, mid, and high dose animals
Urinalysis (Parameters were evaluated based on SOPs; page 11 of 464)	Urine collection: Urine was collected from each animal in Groups 2, 3, and 4 on Days 1 and 14 prior to dosing (collected on the morning of Day 13) and after each of the following target intervals following dose administration: 0-2, 2-6, 6-12, 12-24, and 24-48 h. On Days -1 and 12, urine collection was initiated (overnight, at least 16 h) from each low, mid and high dosage group dog. The urine and cage rinse samples were weighed to the nearest 0.1 g

	Microscopic analysis of urine content was not performed
Termination/Gross pathology (Tissues collected based on SOPs; page 24 of 464)	On Day 16 (1M and 2F/group) or Day 17 (2M and 1F/group), were fasted overnight, euthanized via barbiturate overdose, exsanguinated and necropsied. Terminal body weights were recorded. Necropsy was performed based on SOPs
Histopathology	Adequate Battery: Yes (x), No ( ); Histopathology tissues and organs weighed are listed in the Tables below Peer Review: Yes ( ), No ( )
Toxicokinetics (Plasma)	Blood (~5 mL/sample) was collected from Groups 2, 3, and 4 animals on Days 1 and 14: prior to dosing (collected on Day 13 for the Day 14 pre-dose collection), 10, 20, and 30 minutes and 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 h post dosing via the jugular or cephalic vein. Blood sample tubes (with sodium heparin anticoagulant), were maintained at room temperature until centrifuged within 1 h after collection. Plasma was transferred into labeled polypropylene cryogenic vials and maintained on dry ice until analyzed and results presented as ng-equivalents/mL
Urine and Plasma Analysis	Plasma, urine, and cage rinse samples were analyzed for radioactivity (counts per minute; cpm) using a gamma counter. The cpm values were used to calculate the ng/mL concentration of ^{111}In-Lymphoseek equivalents of each plasma sample and, for the urine/cage rinse samples, the ng/mL concentration of ^{111}In -Lymphoseek equivalents, which were used to calculate the percent of dosage excreted in the urine
Dose formulation Analysis	Yes (x), No ( )

Reviewer's Table based on sponsor data

Results

Mortality
No mortalities were reported

Clinical Signs
Diarrhea and emesis were reported in vehicle and test article-treated dogs. Abnormal digestive signs (diarrhea, soft, mucoid or reddened feces and emesis) were observed at the low, mid and high doses of the test compound. The findings are summarized in Table 61:

Table 61: Summary of abnormal clinical observations

Dose (mg/kg)	Category	Subcategory	Number of Animals Affected	First Day Observed	Last Day Observed	Total Number of Observations
Males						
0	DIGESTIVE SYSTEM	DIARRHEA	1	6	6	1
0.0210	DIGESTIVE SYSTEM	SOFT FECES	1	3	14	4
Females						
0	DIGESTIVE SYSTEM	DIARRHEA	2	7	11	2
		EMESIS	1	4	4	1
0.0105	DIGESTIVE SYSTEM	EMESIS	1	3	5	2
		FECES/REDDENED	1	6	6	1
		MUCOID FECES	1	2	2	1
		SOFT FECES	1	4	11	3
0.0210	DIGESTIVE SYSTEM	MUCOID FECES	1	14	14	1
0.0420	DIGESTIVE SYSTEM	DIARRHEA	1	8	8	1
		EMESIS	2	11	16	2
		MUCOID FECES	1	11	11	1
		SOFT FECES	1	11	12	2

Source: Sponsor's Table (page37 of 464)

Body Weights: There was no statistically-significant change in group mean body weight

Feed Consumption: Although there appeared to be lowered food consumption (by qualitative assessment) in the first 2 days after dosing, overall, there were no differences in food consumption

Ophthalmoscopy: No ophthalmic abnormalities were observed

ECG: ECGs were normal and there was no evidence of ectopic activity

Clinical Chemistry: Due to the presence of radioactivity, the Day 14 hematology slides were maintained for 34 days prior to analysis and according to the sponsor, it was not possible to accurately determine cell counts or RBC parameters. Samples for coagulation and serum chemistry determinations were frozen and held for 34 days prior to analysis:

Hematology: No effects were observed on hematology parameters due to the administration of DTPA-Mannosyl-Dextran

Coagulation: No effects were observed on coagulation parameters due to the administration of DTPA-Mannosyl-Dextran

Serum chemistry: There were no findings related to the administration of Lymphoseek

Urinalysis: Mean values of urine volume were decreased, and the group mean specific gravity as well as the concentrations of inorganic phosphorus, sodium, potassium and chloride were increased in the males administered the mid (21 μg/kg) and high (42 μg/kg) doses. These findings were not observed in treated females. No other urinalysis results indicated any drug-treatment effects

Gross Pathology

Terminal body weight: In males, the mean terminal body weight of dogs in the treatment groups was decreased, albeit non-significantly, compared to control group males. A similar finding was not observed in females

Gross lesions: A small-sized prostate was observed in one control group male but not in any DTPA-Mannosyl-Dextran treatment groups. Lung adhesions confirmed microscopically as due to pleural adhesions were observed in one high dose group (42 μg/kg) male and one low dose group (10.5 μg/kg) female. Pulmonary nodule was observed in one vehicle group female. No other gross findings were reported

Organ Weights:
Spleen: In males treated with the mid dose (21 μg/kg), the mean absolute splenic weight was significantly ($p \leq 0.05$) larger (88.03 ± 24.17 g) compared to vehicle-treated controls (44.83 ± 12.53 g). The organ-to-body weight (1.003 ± 0.330%), but not the organ-to-brain weight ratio, was also statistically significant ($p \leq 0.05$) when compared to controls (0.447 ± 0.054%).

Thymus: The mean thymic organ-to-body weight ratio (0.106 ± 0.002%) for males in the high dose group (42 μg/kg) and the mean epididymis organ-to-body weight ratio (0.039 ± 0.003 %) in the low dose (10.5 μg/kg) were statistically ($p \leq 0.05$) higher compared to respective control values (Thymus: [0.083 ± 0.005%]; epididymis [0.024 ± 0.005]). The absolute weights of both thymus and epididymides were not statistically different from vehicle controls

Histopathology
Adequate Battery: Yes (x), No ( )
Peer Review: Yes (x), No ( ); Internal peer review was performed

Histological Findings: None of the observed histopathological findings were treatment-related

Special Evaluation: None

Toxicokinetics

- The PK profile of a 14-day toxicity study of ^{111}In-DTPA-Mannosyl-Dextran) in male and female mongrel dogs followed a 2-compartment model with first order absorption and elimination kinetics as the best fit for the plasma concentration curves
- Absorption was fast (absorption half-life was 0.38±0.03 h i.e., 22.8 min)
- There was no dosage, sex, or single vs. repeated administration effects on the absorption TK parameters
- C_{max} increased dose-proportionately with increase in dose and values for a given dose were similar for both sexes but decreased from Day 1 to Day 14. Mean C_{max} values on Day 1 and Day 14 for both sexes are summarized in Table 62
- T_{max} was not dependent on dose, sex or single versus repeated administration

- Volume of distribution (Vd_F) was also not dependent on dose, sex or single versus repeated administration. The overall apparent volume of distribution was 887±62 mL/kg
- Systemic exposure (AUC_{last}) values were similar for both males and females for a given dose group, increased dose-proportionally with increase in dose, and for a given dose level, decreased from Day 1 to Day 14
- Elimination was similarly not dependent on dose, sex or single vs. repeated administrations. Elimination was evaluated using the alpha, beta, and central compartment elimination rate constants and the overall alpha, beta, and central compartment half-life values were 1.32±0.04, 87.6±14.9, and 6.54±0.89 h, respectively
- The overall apparent clearance (CL_F), 116±13 mL/hr/kg, was not dependent on sex or single vs. repeated administration
- Peak urinary ^{111}Indium-Lymphoseek equivalent concentrations were observed for male and female groups on Days 1 and 14 in the 2-6 h collection interval. Of the total amount of ^{111}Indium-Lymphoseek equivalents eliminated by the kidneys, approximately 90% (or greater), was measured in the urine within 24 h postdose on Day 1 or within 12 h on Day 14. The % total dosage excreted in the urine was similar across dosage groups, and was not dependent on sex, and single vs. repeated administration. The overall percent of dosage excreted in the urine was 35.0±1.6 percent

Table 62: PK Satellite Group (Toxicokinetic mean values)[1]

Parameter	Day	Dose groups					
		1 (10.5 µg/kg)		2 (21.0 µg/kg)		3 (42.0 µg/kg)	
		M	F	M	F	M	F
C_{max} (ng/mL)	1	8.1±1.5	10.4±2.1	15.6±1.4	26.5±1.9	45.7±1.8	41.7±9.7
	14	6.8±0.8	7.3±0.9	10.6±1.2	12.7±1.3	22.3±4.3	19.5±1.3
T_{max} (h) Range	1	Males (0.52 - 1.10); Females (0.46 - 0.96)					
	14	Males (0.94 – 0.97); Females (0.89 – 1.1)					
Vd (mL/kg)		887 ± 62					
AUC(0-last) (ng/mL* h)	1	20.6	17.8	31.7	24.2	58.7	57.2
	14	12.8	16.9	31.6	42.5	63.5	53.7
Apparent CL (mL/h/kg)							
Dose recovered in urine (%)	1	30.3	34.2	31.0	32.4	29.4	20.7
	14	32.9	40.2	33.4	19.2	23.3	22.3

[1]Reviewer's Table based on sponsor's data

Dosing Solution Analysis: Formulations of non-radiolabeled Lymphoseek in phosphate-buffered saline (PBS) at target concentrations of 0, 10.5, 21.0, and 42.0 µg/mL were prepared fresh daily and used for dosing on Days 2 through 13, 15, and 16. The formulations prepared on Day 2 were analyzed. The formulations met acceptance criteria for accuracy (within 10% of target) and precision (within 15% of target).

Conclusions: There were no treatment-related effects in dogs at all the doses tested for all the endpoints assessed in this 14-day repeated dose toxicity study. Plasma and urine samples collected from male and female dogs and analyzed for ^{111}Indium-Lymphoseek equivalents and to determine TK parameters and urinary excretion patterns indicated a relatively fast absorption that was unaffected by dosage, sex, or repeated administrations. The no observed adverse effect level (NOAEL) for repeated subcutaneous administration of Lymphoseek was considered to be at least 42.0 μg/kg/day.

Table 63: Determination of NOAEL (Study No. N106922)

Parameter evaluated	NOAEL (μg/kg)	Dose multiples
Mortality	>42.0	27x
Clinical signs		
Body weight changes		
ECG		
Feed consumption		
Ophthalmoscopy		
Hematology, coagulation, serum chemistry		
Urinalysis		
Gross pathology histopathology		
Organ weights		
Histopathology		

Reviewer's Table

Reviewer's comments: Overall, I agree with the findings and conclusions. Specific comments are noted as follows:

I agree with the sponsor that due to the small decreases in the mean terminal body weight observed in treated males, the lack of statistical significance and the absence of similar decreases in terminal body weight in females, the decrease in weight was not test article-related. Although the sponsor argued that the trend for a higher mean splenic weight in treated dogs of either sex was not considered a treatment-related effect due to a lack of clear statistical significance and a high normal variability in splenic weights that has been associated with barbiturate anesthesia. If this argument were true, a similar tendency would have occurred also in females. No similar finding was reported in DTPA-Mannosyl-Dextran-treated female dogs. However, since this effect was not dose-dependent, this reviewer accepts that the higher splenic weight was not treatment-related. Lastly, the sponsor considered the ratio organ-to-body weight findings in the thymus and epididymides were not treatment-related due to the sporadic nature of their occurrence and because they were consistent with variation in the weights of these organs frequently seen in untreated dogs. If this argument were true, a similar tendency would have occurred also in female thymic organ-to-body weight ratio. However, since the thymic organ-to-body weight effect was not observed in females at the corresponding dose in females nor was the effect dose-dependent (same lack of

dose-dependence argument for the epididymis effect in males), this reviewer accepts that the observed effects were not treatment-related. NOAEL was therefore determined as the top dose (42 μg/kg (27x MHD).

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7 Genetic Toxicology

Introduction: Genotoxicity assessment of Lymphoseek was performed using *in vitro* non-mammalian (bacterial reverse mutation) assay, *in vitro* mammalian cell gene mutation test (L5178Y/TK$^{+/-}$ mouse Lymphoma) assay and the *in vivo* mammalian erythrocyte micronucleus test. DTPA-Mannosyl-Dextran (drug substance) was used in all the three studies as test article.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay	
Study no.:	(b) (4) AB11LN.503.BTL
Study report location:	eCTD Module 4, § 4.2.3.3.1.2, pages 1-58
Conducting laboratory and location:	(b) (4) Sponsor: Neoprobe Corporation, 425 Metro Place North, Suite 300, Dublin, OH 43017-1367
Date of study initiation:	5/25/2005 (page 6 of 97)
GLP compliance:	Yes (x), No ( ), page 2 of 58 Signed: 11/10/2005
QA statement:	Yes (x), No ( ), page 3 of 58 Signed: 11/10/2005
Drug, lot #, and % purity:	Lymphoseek (DTPA-Mannosyl-Dextran), lot #: 50840 – CoA page 57 of 58; purity: N/A

Objective: The purpose of this study is to evaluate the mutagenic potential of DTPA-Mannosyl-Dextran by measuring its ability to induce reverse mutation at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvr*A in the presence and in the absence of S9-activation

Key Study Findings: In the *in vitro* bacterial reverse mutation assay (Ames test) with the direct plate incorporation method, DTPA-Mannosyl-Dextran was not mutagenic in the absence or presence of extrinsic metabolic (S9) activation. None of the five tester strains showed an increase in revertant mutant colonies. Growth inhibition of the background lawn was not observed and there were no precipitates in the agar. Under the conditions of this study, Lymphoseek, tested at a concentration up to 5000 μg/plate, was negative in the bacterial reverse mutation assay.

Methods	
Strains:	*Salmonella typhimurium* tester strains (TA98, TAI00,TA1535 and TA1537) and E. coli tester strain WP2 *uvr*A
Concentrations in definitive study:	Direct plate incorporation method with and without metabolic activation: 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate of Lymphoseek
Basis of concentration selection:	Selection of dose levels for the confirmatory mutagenicity assay was based on the toxicity and precipitation profile of the test article assessed in an initial toxicity-mutation assay. The highest dose for the confirmatory mutagenicity assay was selected to give some indication of toxicity without exceeding 5 mg/plate.
Negative control:	Water (for test article); DMSO (for positive controls except Water for sodium azide)
[1]Positive controls:	[2]With S9-activation: 2-aminoanthracene (1.0 μg for *Salmonella* strains; 10 μg for *E. coli* strain WP2 uvrA) Without S9-activation: 2-nitrofluorene (1.0 μg), sodium azide (1.0 μg), 9-aminoacridine (75 μg) and methyl methanesulfonate (1000 μg) Vehicle for positive controls: Water for sodium azide; otherwise, DMSO
Formulation/Vehicle:	DTPA-Mannosyl-Dextran / sterile distilled water (CAS #: 7732-18-5)
Incubation & sampling time:	- Direct plate incorporation test / 48 - 72 hrs - 3 replicate plates ± S9-activation - 3 independent assays - Colonies were counted after incubation with test, negative and positive control articles

[1] Concentration of positive controls provided as μg/plate in parenthesis; [2] Metabolizing System: Aroclor-induced rat liver S9

Initial toxicity-mutagenicity assay: The initial and repeat toxicity-mutation assays were used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test article were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.

Confirmatory mutagenicity assay: The confirmatory mutagenicity assay was used to evaluate and confirm the mutagenic potential of the test article. Five or six dose levels of test article along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective

minimal agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test article, vehicle control and positive controls were plated in triplicate.

Table 64: Positive controls used in the initial and confirmatory tests

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (Aldrich Chemical Co., Inc.) Lot No. 09106PS Exp. Date 14-Sep-2005 CAS No. 613-13-8 Purity 97.4%	1.0
WP2 *uvr*A			10
TA98	None	2-nitrofluorene (Aldrich Chemical Co., Inc.) Lot No. 08708HS Exp. Date 08-Mar-2006 CAS No. 607-57-8 Purity 99.9%	1.0
TA100, TA1535		sodium azide (Sigma Chemical Co.) Lot No. 073K0119 Exp. Date 31-Jul-2006 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine (Sigma Chemical Co.) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (Aldrich Chemical Co., Inc.) Lot No. 03715TB Exp. Date 27-Jan-2007 CAS No. 66-27-3 Purity 99.9%	1,000

Source: Sponsor Table (b) (4) study AB11LN.503.BTL page 7 of 58)

Study Validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996, and OECD, 1998). Positive controls produced expected responses and induced marked increases in revertant colony numbers with all strains. Dose selection for the plate incorporation method was adequate. The S9 concentration was within acceptable limits.

Results

- DTPA-Mannosyl-Dextran was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9

- In the initial toxicity-mutation assay used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation, no positive mutagenic response was observed and no precipitate was observed
- The maximum dose tested in this phase, 5000 μg per plate, was achieved using a concentration of 50 mg/mL and a 100 μL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 μg per plate.
- Although no background lawn toxicity was observed, reductions in revertant counts were observed beginning at 500, 1500 or at 5000 μg per plate with some conditions. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 μg per plate
- In the confirmatory mutagenicity assay to evaluate and confirm the mutagenic potential of the test article, no precipitate was observed. Although no background lawn toxicity was observed, a reduction in revertant counts was observed with tester strain TA1535 in the absence of S9 activation beginning at 1500 μg per plate
- The results are shown in the following Tables 65-68

Table 65: Ames test (Initial Toxicity-Mutation Assay without S9-activation)

			Initial Toxicity-Mutation Assay – Revertent Colony Counts (mean [SD])				
Metabolic Activation	**Test Article**	**Dose Level or Concentration (μg / plate)**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
Without Activation	Vehicle	100 μL/plate	17 (8)	180 (2)	23 (5)	7 (0)	28 (1)
	Tilmanocept	1.5	17 (1)	189 (1)	22 (4)	5 (6)	26 (0)
		5.0	17 (8)	185 (4)	21 (8)	7 (1)	30 (3)
		15	14 (6)	173 (3)	27 (3)	5 (2)	35 (10)
		50	17 (5)	161 (0)	28 (3)	6 (4)	29 (1)
		150	20 (2)	168 (11)	18 (4)	9 (0)	32 (6)
		500	22 (2)	186 (25)	15 (8)	1 (0)	23 (2)
		1500	22 (7)	207 (8)	20 (4)	3 (2)	19 (8)
		5000	15 (4)	160 (4)	3 (4)	1 (1)	11 (5)
	2-Nitrofluorene	1.0	171 (12)	—	—	—	—
	Sodium azide	1.0	—	549 (18)	603 (109)	—	—
	9-Aminoacridine	75	—	—	—	473 (129)	—
	Methyl methanesulfonate	1000	—	—	—	—	156 (4)

Source: Sponsor Table (Toxicology Tabulated Summaries, § 2.6.7.8 A, page 14 of 23)

Table 66: Ames test (Initial Toxicity-Mutation Assay with S9-activation)

			Initial Toxicity-Mutation Assay – Revertent Colony Counts (mean [SD])				
Metabolic Activation	**Test Article**	**Dose Level or Concentration (µg / plate)**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
With Activation	Vehicle	100 µL/plate	27 (8)	203 (1)	12 (2)	9 (3)	31 (11)
	Tilmanocept	1.5	26 (8)	193 (8)	13 (9)	8 (1)	23 (4)
		5.0	27 (2)	201 (8)	15 (1)	7 (3)	23 (1)
		15	32 (1)	214 (33)	11 (2)	12 (1)	30 (4)
		50	27 (1)	182 (13)	13 (5)	8 (1)	35 (11)
		150	28 (11)	199 (2)	13 (4)	13 (1)	36 (11)
		500	29 (1)	195 (1)	17 (5)	10 (3)	36 (7)
		1500	33 (6)	208 (18)	18 (6)	7 (3)	14 (5)
		5000	28 (8)	148 (34)	10 (1)	2 (0)	11 (4)
	2-Aminoanthracene	1.0	450 (41)	612 (12)	114 (15)	99 (1)	—
		10	—	—	—	—	293 (125)

Source: Sponsor Table (Toxicology Tabulated Summaries, § 2.6.7.8 A, page 15 of 23)

Table 67: Ames test (Confirmatory Mutagenicity Assay without S9-activation)

			Confirmatory Mutagenicity Assay – Revertent Colony Counts (mean [SD])				
Metabolic Activation	**Test Article**	**Dose Level or Concentration (µg / plate)**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
Without Activation	Vehicle	100 µL/plate	37 (4)	138 (4)	37 (9)	9 (3)	32 (6)
	Tilmanocept	15	—	—	—	7 (3)	—
		50	32 (3)	129 (18)	34 (4)	4 (2)	23 (7)
		150	32 (5)	130 (22)	21 (7)	5 (1)	27 (2)
		500	32 (12)	144 (12)	19 (6)	5 (3)	18 (6)
		1500	36 (6)	140 (4)	17 (2)	5 (2)	13 (3)
		5000	20 (3)	92 (16)	14 (5)	8 (3)	18 (3)
	2-Nitrofluorene	1.0	134 (22)	—	—	—	—
	Sodium azide	1.0	—	445 (37)	341 (21)	—	—
	9-Aminoacridine	75	—	—	—	496 (81)	—
	Methyl methanesulfonate	1000	—	—	—	—	134 (19)

Source: Sponsor Table (Toxicology Tabulated Summaries, § 2.6.7.8 A, page 15 of 23)

Table 68: Ames test (Confirmatory Mutagenicity Assay with S9-activation)

			Initial Toxicity-Mutation Assay – Revertent Colony Counts (mean [SD])				
Metabolic Activation	**Test Article**	**Dose Level or Concentration (µg / plate)**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
With Activation	Vehicle	100 µL/plate	33 (5)	137 (11)	12 (1)	7 (2)	29 (3)
	Lymphoseek	50	39 (3)	137 (10)	11 (3)	9 (1)	33 (2)
		150	32 (2)	141 (14)	13 (4)	7 (2)	32 (6)
		500	33 (4)	140 (17)	10 (4)	6 (1)	24 (4)
		1500	34 (6)	136 (2)	11 (1)	10 (1)	22 (3)
		5000	25 (7)	128 (10)	10 (7)	4 (2)	15 (3)
	2-Aminoanthracene	1.0	755 (199)	658 (54)	82 (15)	70 (20)	—
		10	—	—	—	—	190 (25)

Source: Sponsor Table (Toxicology Tabulated Summaries, § 2.6.7.8 A, page 16 of 23)

Conclusion: Under the conditions of this study, Lymphoseek, tested at a concentration up to 5000 μg/plate, was negative in the bacterial reverse mutation assay.

Reviewer's comments: I agree with the sponsor's conclusions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)	
Study no.:	(b) (4) AB11LN.704.BTL
Study report location:	eCTD Module 4, § 4.2.3.3.1.1, pages 1-41
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 23, 2005
GLP compliance:	Yes (x), No ( ), page 2 of 41 Signed: 11/11/2005
QA statement:	Yes (x), No ( ), page 3 of 41 Signed: 11/11/2005
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran, lot #: 50840 - CoA, page 41 of 41; purity: N/A

Objective: The purpose of this study was to evaluate the genotoxic potential of DTPA-Mannosyl-Dextran using the *in vitro* mammalian system, LY5178Y/TK+/- mouse lymphoma mutagenesis assay performed in the presence and absence of Arochlor-induced rat liver S9metabolic activation. Assessment of the genotoxic potential was based on quantitation of forward mutations at the thymidine kinase locus of L5178 mouse lymphoma cells and the sizing of the resulting colonies.

Key Study Findings: The sponsor concluded that DTPA-Mannosyl-Dextran was negative with and without S9 activation with a 4 h exposure. Since a result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level, a determination of equivocal finding was made at the 24h exposure in the absence of activation because two cloned cultures treated with 500 μg/mL DTPA-Mannosyl-Dextran exhibited mutant frequencies in the above range when compared to solvent controls. However, all other concentrations were < 55 mutants per 10^6 clonable cells compared to the solvent control.

Methods	
Cell line:	L5178Y/TK$^{+/-}$ Mouse lymphoma cells clone 3.7.2 (b) (4)
Concentrations in definitive study:	**4h exposure (with or without S9-activation):** 2000, 2500, 3000, 4000 and 5000 µg/mL **24h exposure (without activation):** 10, 50, 250, 1000 and 5000 µg/mL
Basis of concentration selection:	A preliminary dose range finding assay was performed to select optimal doses for the definitive mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.5 - 5000 µg/mL in both the absence and presence of S9-activation with a 4-hour exposure and without activation with a 24-hour exposure. Based on the preliminary assay, concentrations chosen for cloning were 2000, 2500, 3000, 4000, and 5000 µg/mL, with and without S9 activation.
Negative control:	Deionized water (CAS 7732-18-5)
Positive control:	**With S9:** 7,12-dimethyl-benz(a)anthracene (7,12-DMBA; CAS 57-97-6, lot # 31K1185) **Without S9:** Methyl methanesulfonate (MMS; CAS 66-27-3, lot #03715TB) **Vehicles for positive control:** Deionized water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5) and ethanol (CAS 64-17-5).
Formulation/Vehicle:	DTPA-Mannosyl-Dextran / sterile distilled water (CAS #: 7732-18-5; Lot #: 1252969, (b) (4))
Incubation & sampling time:	4h exposure (with or without S9-activation) 24h exposure (without activation)

Metabolizing System: Aroclor 1254-induced rat liver S9-fraction using male Sprague-Dawley rats (Aroclor-1254 (500 mg/kg; single i/p) was injected 5 days prior to sacrifice

Dose selection criteria: Preliminary dose range finding assay was performed to select doses for the definitive assay. Concentrations tested were: 0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/mL.
No visible precipitate was present at any concentration in the treatment medium
Suspension growth relative to the solvent controls (relative suspension growth, RSG) was 85% without activation with a 4-h exposure, 113% with S9-activation with a 4-h exposure, and 13% without activation with a 24-h exposure at 5000 µg/mL.
Based on the results of the toxicity test, the concentrations tested in the initial mutagenesis assay ranged from 500-5000 µg/mL for both the non-activated and S9-activated cultures with a 4-h exposure. The concentrations tested in the extended treatment assay ranged from 10-5000 µg/mL for non-activated cultures with a 24-h exposure. Based on these findings, the concentrations chosen for the definitive assays

were 2000, 2500, 3000, 4000 and 5000 μg/mL at the 4h exposure (with or without S9-activation) and 10, 50, 250, 1000 and 5000 μg/mL at the 24h exposure (without activation)

Lymphoseek solubility: Soluble in water

Cytotoxic endpoints: Preliminary dose range-finding assay (Relative Suspension Growth, RSG) and Relative Total Growth (RTG) for the definitive assay

Sponsor's criteria for positive results:
A result was considered positive if a concentration-related increase in mutant frequency was observed and one or more concentrations with 10% or greater total growth exhibited mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level. If the spontaneous mutant frequency was >100 mutants per 10^6 clonable cells, a doubling of mutant frequency over the background was also required
A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.
Lastly, a result was considered negative if the mutant frequency in treated cultures was fewer than 55 mutants per 10^6 clonable cells over the background level

Study Validity
According to the sponsor, the following criteria must be met for the mutagenesis assay to be considered valid:
Negative controls: The average spontaneous mutant frequency of the solvent control cultures must be within 20-120 TFT (trifluorothymidine-resistant)-resistant mutants of L5178Y/$TK^{+/-}$ of mouse lymphoma cells per 10^6 surviving cells. Low spontaneous mutant frequencies, i.e., 20-40 mutants per 10^6 surviving cells, are considered acceptable if small colony recovery is demonstrated. The cloning efficiency of the solvent control group must be greater than 50%
Positive controls: At least one concentration of each positive control must exhibit mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS (methy lmethanesulfonate) positive control must show an increase in both small and large colonies.
All criteria for a valid study were met as described in the protocol.

Results
- The maximum DTPA-Mannosyl-Dextran concentration in treatment medium was 5000 μg/mL in the preliminary assay
- No visible precipitate was present at any concentration in treatment medium
- Concentrations for the mutation assay was based on reduction of suspension growth relative to the solvent control and a substantial toxicity (i.e., suspension growth of ≤ 50% of the solvent control) was observed only at ≥ 50 μg/mL without activation with a 24h exposure
- Based on the results of the preliminary toxicity assay, the initial mutagenesis assay tested DTPA-Mannosyl-Dextran concentrations of 500 - 5000 μg/mL in both non-activated and S9-activated cultures, after a 4h exposure. Under these

conditions, no visible precipitate was observed in treatment medium at any concentration

- The concentrations chosen for cloning were 2000, 2500, 3000, 4000 and 5000 μg/mL in the with and without of S9-activation
- Based on a result being considered negative if the mutant frequency in treated cultures was less than 55 mutants/10^6 clonable cells over the background level, no cloned cultures exceeded this criterion. There was also no concentration-related increase in mutant frequency
- Concentrations of 10-5000 μg/mL, based on the preliminary toxicity assay, were used for non-activated cultures with a 24h exposure in the extended treatment assay results
- No visible precipitate was present at any concentration in treatment medium.
- The concentrations selected for cloning were 10, 50, 250, 1000, and 5000 μg/mL.
- Two cloned cultures treated with 500 μg/mL DTPA-Mannosyl-Dextran exhibited mutant frequencies between 55-99 mutants per 10^6 clonable cells over that of the solvent control but did not meet the criteria to be considered positive. All other concentrations were < 55 mutants per 10^6 clonable cells compared to the solvent control
- The trifluorothymidine (TFT)-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2-1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in both small colonies (thus verifying the adequacy of the methods used to detect small colony mutants) and large colonies
- The results are described in the following Tables 69 and 70:

Table 69: L5178Y/TK+/- Mouse Lymphoma Assay in the absence or presence of S9-activation after a 4h exposure[1]

		Without Exogenous Metabolic Activation (4 hour exposure)			With Exogenous Metabolic Activation (4 hour exposure)		
Test Article	Dose Level or Concentration (μg/mL)	Mutant Frequency [a,b]	Induced Mutant Frequency [b,c]	% Total Growth	Mutant Frequency [a,b]	Induced Mutant Frequency [b,c]	% Total Growth
Solvent (water)	0	73 / 69	—	—	54 / 65	—	—
Tilmanocept	2000	81 / 59	10 / -11	83 / 89	62 / 80	3 / 21	94 / 88
	2500	59 / 61	-11 / -10	85 / 85	52 / 53	-7 / -6	102 / 105
	3000	64 / 54	-7 / -17	78 / 96	55 / 72	-4 / 13	103 / 106
	4000	50 / 61	-21 / -10	86 / 83	45 / 62	-15 / 3	94 / 93
	5000	58 / 68	-12 / -3	93 / 78	74 / 69	15 / 10	97 / 111
Methyl methanesulfonate	15	441	370	21	—	—	—
	20	790	719	9	—	—	—
7,12-Dimethylbenz(a)anthracene	2.5	—	—	—	202	143	63
	4	—	—	—	259	200	50

[1] Source: Sponsor Table; [a] Mutant frequency per 10^6 surviving cells; [b] Results shown as x / y represent results from two duplicate cultures; [c] Induced mutant frequency per 10^6 surviving cells = mutant frequency minus the average mutant frequency of solvent controls

Table 70: L5178Y/TK+/- Mouse Lymphoma Assay in the absence of S9-activation after a 24h exposure1

Test Article	Dose Level or Concentration (µg/mL)	Without Exogenous Metabolic Activation (24 hour exposure)		
		Mutant Frequency [a,b]	Induced Mutant Frequency [b,c]	% Total Growth
Solvent (water)	0	42 / 61	—	—
Tilmanocept	10	58 / 44	7 / -8	107 / 83
	50	59 / 62	7 / 10	65 / 69
	250	68 / 70	16 / 18	41 / 33
	1000	106 / 104	54 / 53	28 / 25
	5000	123 / 114	71 / 62	17 / 21
Methyl methanesulfonate	5	372	320	33
	7.5	684	633	16

[1] Source: Sponsor Table; [a] Mutant frequency per 10^6 surviving cells; [b] Results shown as x / y represent results from two duplicate cultures; [c] Induced mutant frequency per 10^6 surviving cells = mutant frequency minus the average mutant frequency of solvent controls

Conclusions: The sponsor concluded that DTPA-Mannosyl-Dextran was negative with and without S9 activation with a 4 h exposure. The result was equivocal at the 24h exposure in the absence of activation because two cloned cultures treated with 500 µg/mL DTPA-Mannosyl-Dextran exhibited mutant frequencies between 55-99 mutants per 10^6 clonable cells over that of the solvent control but did not meet the criteria to be considered positive. All other concentrations were < 55 mutants per 10^6 clonable cells compared to the solvent control. A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.

Reviewer's comments: I agree with the sponsor's conclusions.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mammalian Micronucleus Test	
Study no:	AB11LN.123.BTL
Study report location:	eCTD Module 4; 4.2.3.3.2.1, pages 1-37
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/24/2005 (page 4 of 37)
GLP compliance:	Yes (x), No ( ), page 2 of 37 Signed: 11/11/2005 [ICH S2A (1996), ICH S2B (1997) and OECD (1998); page 8 of 37]
QA statement:	Yes (x), No ( ), page 3 of 37 Signed: 11/11/2005
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran[1], lot #: 50840 - CoA, page 37 of 37; purity: N/A

[1] DTPA-Mannosyl-Dextran, NOT Lymphoseek, was used in all nonclinical studies except where stated otherwise. This notation is fully clarified in Table 9 above.

Objective: The purpose of this study was to evaluate the clastogenic potential of the test article as measured by its ability to induce micronucleated polychromatic erythrocytes in mouse bone marrow.

Key Study Findings: A single IP administration of DTPA-Mannosyl-Dextran at doses up to 2000 mg/kg did not induce a significant increase in the incidence of micronucleated PCEs in male or female ICR mice and DTPA-Mannosyl-Dextran was considered negative in the mouse micronucleus assay.

Methods	
Doses in definitive study:	500, 1000 and 2000 mg/kg body of DTPA-Mannosyl-Dextran[1]
Frequency of dosing:	Single dose
Route of administration:	Intraperitoneal injection
Dose volume:	20 mL/kg BW (Test and control articles)
Formulation/Vehicle:	DTPA-Mannosyl-Dextran / Sterile Water for Injection (SWI; CAS #: 7732-18-5 (b) (4)
Species/Strain:	Mouse / ICR; (b) (4)
Age:	6-8 weeks
Weight (g):	Pilot study: Males (30.1-33.8); Females (21.1-23.6) Definitive study: Males (26.5-31.9); Females (20.9-23.9)
Number/Sex/Group:	Pilot study: 4 group of 2 male mice and 1 group of 5 mice/sex Definitive study: 7 groups 5mice/sex/group
Satellite groups:	none
Basis of dose selection:	Based on result of a dose range finding pilot toxicity study
Negative control:	Sterile Water for Injection, USP
Positive control:	Cyclophosphamide monohydrate (CP; 50 mg/kg; CAS number 6055-19-2; (b) (4))
Sampling time:	24 and 48 h

[1] DTPA-Mannosyl-Dextran, NOT Lymphoseek, was used in all nonclinical studies except where stated otherwise. This notation is fully clarified in Table 9 above.

Methods

Study Design:

1. Pilot toxicity study: Mice were randomly assigned to one group of 5 mice per sex and to 4 groups of 2M mice. 5 mice per sex were exposed to DTPA-Mannosyl-Dextran at a dose of 2000 mg/kg and 2M mice each received 1, 10, 100 or 1000 mg/kg. The test article was administered in a volume of 20 mL/kg via a single intraperitoneal injection. Mice were observed after dosing and thereafter daily for 3 days for clinical signs of toxicity. Body weights were recorded before dose administration and 1 and 3 days postdose.

2. Definitive study: Mice were assigned to 7 study groups comprising five mice per sex. An additional group of 5 mice per sex was designated as a replacement group to be used in the event of mortality at the high dose. As indicated in the above Table, animals in five of the groups were treated either with the controls (vehicle or positive) or with DTPA-Mannosyl-Dextran at a dose of 500, 1000 or 2000 mg/kg and were euthanized 24 h after treatment. Animals in the other two groups were treated either with the vehicle control article or DTPA-Mannosyl-Dextran at a dose of 2000 mg/kg and were euthanized 48 hours after treatment. The study design is shown in Table 71:

Table 71: Study Design (Definitive study) [1]

Treatment (20 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
Vehicle Control: Water	10	5	5
Test Article: Lymphoseek			
Low dose (500 mg/kg)	5	5	0
Mid dose (1000 mg/kg)	5	5	0
High dose (2000 mg/kg)	15*	5	5
Positive Control: CP (50 mg/kg)	5	5	0

*Including 5 replacement animals per sex to ensure the availability of five animals for micronucleus analysis

[1] DTPA-Mannosyl-Dextran, NOT Lymphoseek, was used in all nonclinical studies except where stated otherwise. This notation is fully clarified in Table 9 above.

Study Validity

Criteria for a valid test: The mean incidence of micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control. The incidence of micronucleated polychromatic erythrocytes in the positive control group must be significantly increased relative to the vehicle control group ($p \leq 0.05$).

Results:

Pilot toxicity study:

- No mortality was observed during the course of the pilot study.
- Piloerection was observed in M/F mice at 2000 mg/kg and lethargy was seen in males at 2000 mg/kg. All other animals treated with DTPA-Mannosyl-Dextran were normal in the period study
- The results of the pilot study are summarized in the Table 72:

Table 72: Pilot Toxicity study of IP DTPA-Mannosyl-Dextran in ICR mice [1]

Table 8.0-1: Pilot Toxicity Study - Clinical Signs Following a Single Intraperitoneal Dose of Lymphoseek in ICR Mice

		Number of Animals With Observed Signs/Total Number of Animals Dosed		Number of Animals Died/Total Number of Animals Dosed	
Treatment (20 mL/kg)	Observation	Males	Females	Males	Females
Lymphoseek 1 mg/kg	Normal	0/2	N/A	0/2	N/A
10 mg/kg	Normal	0/2	N/A	0/2	N/A
100 mg/kg	Normal	0/2	N/A	0/2	N/A
1000 mg/kg	Normal	0/2	N/A	0/2	N/A
2000 mg/kg	Lethargy Piloerection	5/5 5/5	0/5 5/5	0/5	0/5

N/A = female mice were not dosed as per study design

[1] DTPA-Mannosyl-Dextran, NOT Lymphoseek, was used in all nonclinical studies except where stated otherwise. This notation is fully clarified in Table 9 above.

Definitive study:

- No mortality was reported during the definitive study. However, lethargy and piloerection were observed in M/F mice at the high dose 2000 mg/kg.
- Animals treated with DTPA-Mannosyl-Dextran or vehicle, were normal after dose administration and throughout the duration of the study.
- After bone marrow was collected 24 and 48 h after dose administration, polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) of the bone marrow were examined microscopically for the presence of micronuclei There was no reduction in the ratio of PCEs to total erythrocytes in the DTPA-Mannosyl-Dextran -treated groups relative to the vehicle control groups for males at 24 hours after dose administration and for males and females at 48 hours after dose administration.
- Reductions of up to 17% in the ratio of PCEs to total erythrocytes were observed in the 24h female, DTPA-Mannosyl-Dextran-treated groups relative to the concurrent vehicle control
- There was no significant increase in micronucleated polychromatic erythrocytes in test article-treated groups relative to the respective vehicle control groups was observed in male or female mice at 24 or 48 hours after dose administration ($p > 0.05$)
- The results of the definitive study are summarized in Tables 73 and 74:

Table 73: Findings of the Definitive study in mice sacrificed 24h post-dose

Definitive Micronucleus Study							
						Micronucleated Polychromatic Erythrocytes (PCEs)	
Treatment	Sex	Hour	# Mice	PCEs/Total Erythrocytes (Mean [SD])	Change from Control (%)	Number per 1000 PCEs (Mean [SD])	Number per PCEs Scored
Vehicle	M	24	5	0.380 (0.04)	—	0.1 (0.22)	1 / 10 000
	F	24	5	0.564 (0.07)	—	0.2 (0.27)	2 / 10 000
Tilmanocept 500 mg/kg	M	24	5	0.452 (0.06)	19	0.2 (0.27)	2 / 10 000
	F	24	5	0.504 (0.06)	-11	0.2 (0.27)	2 / 10 000
1000 mg/kg	M	24	5	0.395 (0.04)	4	0.1 (0.22)	1 / 10 000
	F	24	5	0.55 (0.06)	-2	0.3 (0.45)	3 / 10 000
2000 mg/kg	M	24	5	0.441 (0.02)	16	0.2 (0.45)	2 / 10 000
	F	24	5	0.468 (0.07)	-17	0.6 (0.65)	6 / 10 000
Cyclophosphamide monohydrate 50 mg/kg	M	24	5	0.379 (0.05)	0	18.4 (5.47)	184 [a] / 10 000
	F	24	5	0.385 (0.05)	-32	16.9 (3.73)	169 [a] / 10 000

Table 74: Findings of the Definitive study in mice sacrificed 48h post-dose

Definitive Micronucleus Study							
						Micronucleated Polychromatic Erythrocytes (PCEs)	
Treatment	Sex	Hour	# Mice	PCEs/Total Erythrocytes (Mean [SD])	Change from Control (%)	Number per 1000 PCEs (Mean [SD])	Number per PCEs Scored
Vehicle (sterile water for injection)	M	48	5	0.406 (0.01)	—	0	0 / 10 000
	F	48	5	0.403 (0.07)	—	0.2 (0.27)	2 / 10 000
Tilmanocept 2000 mg/kg	M	48	5	0.415 (0.05)	2	0	0 / 10 000
	F	48	5	0.436 (0.04)	8	0.1 (0.22)	1 / 10 000

[a] $p \leq 0.05$ compared to control (Kastenbaum-Bowman tables).

Abbreviations: —, none; F, female; IP, intraperitoneal; M, male; SD, standard deviation; USP, United States Pharmacopeia.

Conclusion: Under the conditions of this study, a single IP administration of DTPA-Mannosyl-Dextran at doses up to 2000 mg/kg did not induce a significant increase in the incidence of micronucleated PCEs in male or female ICR mice and DTPA-Mannosyl-Dextran was considered negative in the mouse micronucleus assay

Reviewer's comments: I agree with the sponsor's conclusions.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not conducted

9 Reproductive and Developmental Toxicology

Not conducted

10 Special Toxicology Studies

10.1 Local Tolerance Studies

Introduction: The potential for DTPA-Mannosyl-Dextran to cause local irritation was evaluated in two single dose toxicity studies in rabbits namely: (b) (4) 1146-104, in which DTPA-Mannosyl-Dextran drug substance was used; and (b) (4) 1576-06069. Both compounds were administered by a single intramuscular injection.

10.1.1 Study No. (b) (4) 1146-104:

Study title: Study of the Irritant potential of DTPA-mannosyl-dextran in New Zealand White rabbits	
Study no.:	(b) (4) 1146-104
Study report location:	eCTD Module 4; 4.2.3.1.1, pages 1-46
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/8/2000
GLP compliance:	Yes (x), No ( ), page 3 of 46 Signed: 12/28/2000
QA statement:	Yes (x), No ( ), page 4 of 46 Signed: 12/28/2000
Drug, lot #, and % purity:	DTPA-mannosyl-dextran, Batch No. DMD02dec99, % purity: N/A

Objective: This study was designed to determine the irritant potential of a single intramuscular dose of DTPA-mannosyl-dextran in New Zealand White (NZW) rabbits

Key Study Findings: Intramuscular injection of DTPA-mannosyl-dextran (DTPA-Mannosyl-Dextran) at doses up to 286 µg/kg /kg had no effect on survival, clinical observations, injection site, or injection site histopathology in New Zealand White rabbits

Methods	
Doses:	0 (saline/vehicle control), 143 and 286 μg/kg BW
Frequency of dosing:	Single dose
Route of administration:	Intramuscular
Dose volume:	0.2 mL/kg (fixed volume)
Formulation/Vehicle:	DTPA-mannosyl-dextran / saline vehicle. Test article was reconstituted with injectable normal saline
Species/Strain:	Rabbit / New Zealand White (b) (4)
Number/Sex/Group:	9 rabbits; 3/sex/group; 3 groups
Age:	11-13 weeks
Weight range:	Males (2625-2922 g); Females (2747 to 3174 g); 1F (No. 11671; 3174 g) was outside range required by protocol
Deviation from study protocol:	No significant deviations

Study Design:

Table 75: Group Design and Dose groups (Study No. (b) (4) 1146-104)

Group	Intramuscular Dose (μg/kg)	Number of Rabbits	
		Males	Females
1 (veh)	0	3	3
2 (LD)	143	3	3
3 (HD)	268	3	3

Source: Reviewer's Table adapted from Sponsor's Study Design, (b) (4) Study 1146-104, page 10 of 46; veh = vehicle; LD, HD = Low and High Dose, respectively

Each rabbit in Groups 2 and 3 (3/sex/group) received a single intramuscular injection into the right thigh of 143 and 286 μg/kg, respectively of DTPA-Mannosyl-Dextran. The control rabbits (Group 1, 3/sex) received a single dose of normal saline. The injection site was shaved at least 24 h prior to the injection.

Table 76: DTPA-Mannosyl-Dextran Dose multiples (Study No. (b) (4) 1146-104)

Administration	Intramuscular		
		Low Dose	High Dose
Dose (μg/kg) in Rats	0 (vehicle)	143	286
Dose multiples (based on BSA)	N/A	56x	111x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Observations and Results

Observations:

Table 77: Summary of Methods/Observations (Study No. (b) (4) 1146-104)

Protocol	Method, frequency and/or objectives
Clinical observations	Twice/day (a.m and p.m with at least 6 h between observations) Cage-side observation was performed for mortality, moribundity, and toxicity Special clinical observations: The area of the injection was examined for signs of erythema immediately after injection and at approximately 2, 4, and 6 h post-injection and daily thereafter. The irritation was scored and recorded for both edema and erythema according to grades specified in SOPs
Body weights	Individual body weights were recorded at randomization, prior to dosing on day 1 of study and at acute and final sacrifices on days 4 and 15, respectively
Termination	Scheduled: Acute and final scheduled sacrifices on day 4 and 15, respectively Unscheduled: Animals sacrificed due to moribundity or found dead will undergo a gross necropsy and have tissues collected
Histopathological examination of injection site	Tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically
Dose formulation Analysis	Yes), No (x)

Reviewer's Table based on sponsor data

Results

Mortality: No deaths were reported during the study

Clinical observations: There were no adverse clinical observations noted following administration of DTP A-mannosyl-dextran

Body weight: There were no changes in body weight

Histopathology: On day 4 (acute sacrifice), 2 control males exhibited inflammation of the dermis and focal fibrosis, respectively. No similar findings were observed in test article-treated animals. On day 15 (final sacrifice), mild focal hemorrhage was observed in the subcutis of one female control animal. A second female had chronic inflammation of the panniculus muscle.

Conclusion: Intramuscular injection of DTPA-mannosyl-dextran (DTPA-Mannosyl-Dextran) at doses up to 286 μg/kg /kg had no effect on survival, clinical observations, injection site, or injection site histopathology in New Zealand White rabbits

Table 78: Determination of NOAEL in Study No. (b) (4) 1146-104

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality	>286	111x
Clinical signs		
Body weight changes		
Histopathology		

Reviewer's Table

Reviewer's Comments: I agree with the results and conclusion of the study

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10.1.2 Report No. (b) (4) 1576-06069

Study title: Lymphoseek™ DTPA-mannosyl-dextran: A study of the irritant potential in New Zealand White rabbits	
Study no.:	(b) (4) 1576-06069
Study report location:	eCTD Module 4; 4.2.3.1.1, pages 1-77
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/16/2006
GLP compliance:	Yes (x), No ( ), page 3 of 77 Signed: 1/19/2007
QA statement:	Yes (x), No ( ), page 4 of 77 Signed: 1/18/2000
Drug, lot #, and % purity:	DTPA-mannosyl-dextran (cold kit drug product), for drug product Lot #: NFR-C0015-040706, % purity, 95%

Objective: The purpose of this study was to determine the irritant potential of a single intramuscular dose of DTPA-Mannosyl-Dextran in New Zealand White rabbits

Key Study Findings: There was no evidence of treatment-related effect on mortality, clinical observations, body weight, injection site observations, or injection site histopathology. However, histopathology of the injection site skeletal muscle revealed mild inflammation with minimal degeneration/necrosis and mineralization in one female administered the high dose (280 μg/kg or 109x MHD). There were no other findings in the histological examination of the injection site in control rabbits or rabbits treated with140 μg/kg. Overall, it appeared that a single intramuscular dose of DTPA-Mannosyl-Dextran up to 280 μg/kg was generally well tolerated. Based on the finding of some inflammatory change, degeneration/necrosis and mineralization of the injection site skeletal muscle, albeit occurring in one single female at the high dose, NOAEL for local tolerance was determined by the reviewer to be140 μg/kg (or 55x MHD).

Methods	
Doses:	0 (saline/vehicle control), 140 and 280 µg/kg BW
Frequency of dosing:	Single dose
Route of administration:	Intramuscular
Dose volume:	0.1 mL/kg (fixed volume)
Formulation/Vehicle:	[a] DTPA-mannosyl-dextran / saline vehicle. Test article will be reconstituted with injectable normal saline
Species/Strain:	Rabbit / New Zealand White (b) (4)
Number/Sex/Group:	9 rabbits; 3/sex/group; 3 groups
Age:	2-3 months/sex (age at first dose)
Weight range:	Males (2.0-2.3 kg); Females (2.0-2.4 kg)
Deviation from study protocol:	No significant deviations

[a] Lymphoseek ™ DTPA-mannosyl-dextran (Lymphoseek product); The sponsor added a study errata (Table 79) to the submission to indicate the test article was not radiolabeled as was shown in the CoA. DTPA-Mannosyl-Dextran was used as the test article.

Table 79: Study Errata (Study No: (b) (4) 1576-06069)

Table 1. Errata List

Error Location	Error Content	Statement of Effect
Appendix 1, Page 25	The test article was not radiolabeled for this study, yet the Certificate of Analysis shows a radiochemical purity value. Neoprobe review findings: The wrong Certificate of Analysis was used. It is a Certificate of Analysis for stability at a 12 week timepoint instead of a 0 week timepoint. A radiochemical purity test is completed for stability data on 12 week samples. Attached to this errata is the correct Certificate of Analysis for a 0 week timepoint.	Error does not change the conclusions of the study.

Study Design

Table 80: Study Design and Dose groups (Study No. (b) (4) 1576-06069)

Group	Intramuscular Dose (µg/kg)	Number of Rabbits	
		Males	Females
1 (veh)	0	3	3
2 (LD)	140	3	3
3 (HD)	280	3	3

Source: Reviewer's Table adapted from Sponsor's Study Design, (b) (4) Study 1146-104, page 10 of 46; veh = vehicle; LD, HD = Low and High Dose, respectively

Table 81: DTPA-Mannosyl-Dextran dose multiples (Study No. (b) (4) 1576-06069)

Administration	Intramuscular		
		Low Dose	High Dose
Dose (μg/kg) in Rats	0 (vehicle)	140	280
Dose multiples (based on BSA)	N/A	55x	109x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 μg or 30.8 $\mu g/m^2$ based on BSA)

Observations and Results

Observations:

Table 82: Summary of Methods/Observations (Study No. (b) (4) 1576-06069)

Protocol	Method, frequency and/or objectives
Clinical observations	Cage-side observation performed 2ce/day Special clinical observations: On SD1 (predose), and prior to necropsy on SD4 or SD15 Cageside and post-dose observations included observation for mortality, moribundity, general health, and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, injection site, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior patterns, and time of onset, location, dimensions, appearance, and progression of grossly visible or palpable masses
Dermal Draize test	Predose, immediately after injection, and at 2, 4, 6 hours (± 0.25 h) on SD 1, and daily thereafter. Dermal Draize observations (shown in sponsor's Table below) included examination of the injection site for signs of edema and erythema
Body weights	SD 1 (predose), and prior to necropsy on SD 4 or SD 15
Termination	On SD 4, the first two males and the first female per group were euthanized by sodium pentobarbital injection and exsanguinated. On SD 15, all remaining animals were euthanized by sodium pentobarbital injection and exsanguinated.
Histopathological examination of injection site	All preserved tissues were embedded, sectioned, stained and examined microscopically
Dose formulation Analysis	Yes), No (x)

Reviewer's Table based on sponsor data

Table 83: Classification of Dermal Draize observations

Text Table 7: Grades for Edema and Erythema

Score	Grade	Edema	Erythema
0	None	No Swelling	Normal color
1	Minimal	Slight swelling; indistinct border	Light pink, indistinct
2	Mild	Defined swelling; distinct border	Bright pink/pale red, distinct
3	Moderate	Defined swelling; raised border (<1 mm)	Bright red; distinct
4	Severe	Pronounced swelling; raised border (≥ 1mm)	Dark red; pronounced

Results

Mortality: DTPA-Mannosyl-Dextran had no effect on mortality or clinical signs. All animals survived to scheduled termination.

Clinical observations: There were no adverse clinical observations noted following administration of Lymphoseek product

Dermal Draize Test: Treatment with Lymphoseek™ DTPA-Mannosyl-Dextran did not affect dermal Draize observations. The results are summarized in Table 84.

Table 84: Summary of findings in Dermal Draize Test

Group/Dose (n=5/group)	**1** Controls (0 µg/kg)		**2** Low dose (140 µg/kg)		**3** High dose (280 µg/kg)	
Dose multiple (x MHD)	0		55x		109x	
Degree of effect seen	M	F	M	F	M	F
minimal					E, Ery (1)	
minimal		E (2)				

Reviewer's Table based on sponsor's data; E, edema, Ery, Erythema; Number in parenthesis = number of animals affected

Body weight: There were no changes in body weight

Gross pathology: Treatment with DTPA-Mannosyl-Dextran had no effect on gross pathology

Histopathology: On SD 15, a test article–related finding consisting of mild, locally extensive, intramuscular subacute inflammation with minimal, multifocal, coagulative degeneration / necrosis and mineralization was observed in a single female rabbit treated with the high dose 280 µg/kg. There were no other test article-related observations

Conclusions: Based on the findings of this study, the sponsor concluded that there was little evidence of DTPA-mannosyl-dextran causing irritation when administered in a single intramuscular dose to NZW rabbits. However a histopathological examination of

the injection site skeletal muscle revealed mild inflammation with minimal degeneration/necrosis and mineralization in one female administered the high dose (280 μg/kg (109x MHD). There were no other findings in the histological examination of the injection site in control rabbits or rabbits treated with140 μg/kg. Overall, the sponsor concluded that a single intramuscular dose of DTPA-Mannosyl-Dextran up to 280 μg/kg was generally well tolerated.

Table 85: Determination of NOAEL in Study No. (b) (4) 1576-06069

Parameter evaluated	NOAEL (μg/kg)	Dose multiple
Mortality	>280	109x
Clinical signs		
Body weight changes		
Gross pathology		
Histopathology	140	55x

Reviewer's Table

Reviewer's Comments: Overall, I agree with the conclusions of this bridging study that DTPA-Mannosyl-Dextran was generally well tolerated following a single intramuscular injection in the rabbit. However, based on the finding of some inflammatory change, degeneration/necrosis and mineralization of the injection site skeletal muscle, albeit occurring in one single female at the high dose, NOAEL for local tolerance was determined by the reviewer to be140 μg/kg (or 55x MHD)

10.2 Antigenicity

10.2.1 Report No. (b) (4) 1576-04583

Table 86: Study No. (b) (4) 1576-04583

Study title: DTPA-Mannosyl-Dextran: A Systemic Hypersensitivity (Anaphylaxis) Study in Guinea Pigs	
Study no.:	(b) (4) 1576-04583
Study report location:	eCTD Module 4; § 4.2.3.7.1.1, pages 1-104
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/20/2005
GLP compliance:	Yes (x), No ( ), page 3 of 104 Signed: 3/22/2007
QA statement:	Yes (x), No ( ), page 4 of 104 Signed: 3/22/2007
Drug, lot #, and % purity:	DTPA-Mannosyl-Dextran (Lymphoseek Ligand), Lot #: M50840, % purity: assumed 100%

Objective: The purpose of this study was to determine the potential of DTPA-Mannosyl-Dextran to induce Type I systemic hypersensitivity (anaphylactic reactions) following an intravenous (IV) challenge after four weekly SC sensitization doses in male guinea pigs

Key Study Findings: DTPA-Mannosyl-Dextran administered up to 280 μg/kg did not induce any anaphylactic reactions in male guinea pigs after four weekly subcutaneous sensitization doses and an intravenous challenge dose. Adverse reactions were expectedly limited to Ovalbumin/CFA-treated animals only

Table 87: Summary of Methods/Observations (Study No. (b) (4) 1576-04583)

Methods	
Doses:	DTPA-Mannosyl-Dextran: 0 (saline vehicle control), 14, 28 and 280 µg/kg Ovalbumin: sensitization (0.5 mg/kg), challenge (1.0/2.0 mg/kg)
Frequency of dosing:	DTPA-mannosyl-dextran: sensitization (4 times), Challenge (once) Ovalbumin: sensitization (once), challenge (once)
Route of administration:	DTPA-mannosyl-dextran (SC); Ovalbumin (IV)
Dose site:	Dorsal scapular; saphenous vein
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	DTPA-mannosyl-dextran / saline [1]Ovalbumin (Lot #: A0200978) prepared in sterile saline at 2mg/mL and mixed 1:1 with Complete Freund's Adjuvant (Appendix 8, page 89 of 104). For challenge on SD36, Ovalbumin formulation was prepared in saline was prepared in saline at 2 mg/mL with no mixing with CFA
Negative control:	0.9% saline
Positive control:	Ovalbumin (Albumin, Egg): 0.5 mg/kg / Complete Freund's Adjuvant
Species/Strain:	Guinea pigs / Hartley; (b) (4)
Number/Sex/Group:	50 males; 10/group; 5 groups
Age:	5-6 weeks (at first dose)
Weight:	369.1 – 484.5 g (at first dose)
Deviation from study protocol:	No significant deviations

[1]Ovalbumin preparation: Ovalbumin was prepared in sterile saline at 2mg/mL and mixed 1:1 with Complete Freund's Adjuvant (Appendix 8, page 89 of 104). For challenge on SD36, Ovalbumin formulation was prepared in saline was prepared in saline at 2 mg/mL with no mixing with CFA

Table 88: Test and Control article Information

Text Table 1: Neat Test and Control Articles

Name	Lot No.	Supplier	Purity	Description
DTPA-Mannosyl-Dextran (Lymphoseek Ligand)	M50840	(b) (4)	Assumed 100%	Tan powder
0.9% Sodium Chloride Injection, USP (Sterile Saline)	29-231-JT		Assumed 100%	Clear liquid
Ovalbumin (Albumin, Egg)	A0200978001		Assumed 100%	White powder
Complete Freund's Adjuvant	5069691		Assumed 100%	Yellow liquid

Source: Sponsor's Table (Study Report, page 9 of 104)

Study Design

Fifty male guinea pigs (10/group) were randomly assigned to one of five groups and received a SC injection of either negative control (sterile saline), positive control (0.5 mg/kg Ovalbumin with Complete Freund's Adjuvant (CFA), or 14, 28 or 280 µg/kg of DTPA-Mannosyl-Dextran on Days 1, 8, 15, and 22 as sensitization doses. On Day 36, animals received an IV challenge dose of positive control (1.0 mg/kg of Ovalbumin, Group 2) or 280, 14, 28, or 280 µg/kg of DTPA-Mannosyl-Dextran for Groups 1, 3, 4, and 5, respectively. The study was terminated on Day 36 following the postdose observation interval. Parameters evaluated included mortality, clinical and cage side observations, postdose observations (anaphylactic reactions), body weights, and body weight changes. The group and dose designations are shown in Table 89:

Table 89: Hypersensitivity Study Group Designations and Dose Levels multiples (Study No. (b) (4) 1576-04583)

Group	Treatment	Dose Level (µg/kg)	
		Sensitization [a]	Challenge [b]
1	Negative control (Saline)	Saline, 0	Tilmanocept, 280
2	Positive control (Ovalbumin)	Ovalbumin, 500	Ovalbumin, 1.0
3	Tilmanocept	Tilmanocept, 14	Tilmanocept, 14
4	Tilmanocept	Tilmanocept, 28	Tilmanocept, 28
5	Tilmanocept	Tilmanocept, 280	Tilmanocept, 280

[a] Sensitization doses were administered SC once on Days 1, 8, 15, and 22.

[b] Challenge doses were administered IV once on Day 36.

Source: Sponsor's Table (Toxicology written summary, § 2.6.6, page 18 of 22)

Table 90: DTPA-Mannosyl-Dextran dose multiples (Study No. (b) (4) 1576-04583)

Administration	Subcutaneous			
	Vehicle	Low Dose	Mid Dose	High Dose
Dose (µg/kg) in Rats	0	14	28	280
Dose multiples (based on BSA)	N/A	3.6x	7.3x	72.7x

Source: Reviewer's Table constructed from sponsor's data; BSA = Body surface area (calculations based on a 60 kg human body weight; proposed human dose = 50 µg or 30.8 µg/m^2 based on BSA)

Observations and Results

Observations

Table 91: Summary of Methods/Observations (Study No. (b) (4) 1576-04583)

Protocol	Method, frequency and/or objectives
Clinical observations	Cage-side observation performed ≥ 2ce/day (mortality, morbidity, general health, and signs of toxicity) Clinical observations: On SD1 (predose), weekly and at termination on SD36 to evaluate skin and fur characteristics, eye and mucous membranes, respiratory, circulatory; autonomic and central nervous systems, and somatomotor and behavior patterns and time of onset, location, dimensions appearance, and progression of grossly visible or palpable masses) Anaphylaxis: Anaphylactic reactions during post-dose observations were scored as shown in Text Table 7 shown below
Body weights	Prior to treatment on SD1, weekly thereafter, and at termination (SD 36)
Blood collection	Blood (~3 mL) was collected prior to termination via puncture of the vena cava into serum separator tubes, allowed to clot at room temperature and then centrifuged. Serum samples were be stored at -75±10°C (page 94 of 104) for possible future antibody analysis. Serum samples were discarded on 3/15/07
Termination	On SD36 after postdose observations without necropsy
Postmortem procedures	Gross necropsy according to SOP on moribund animals. There was no bone marrow collection. No organ weight or tissue preservation and histopathology was performed
Dose Formulation Analysis	Yes (x), No ( ); Acceptable (study report, Appendix 2, page 33-41 of 104)

Reviewer's Table based on sponsor data

Table 92: Scoring of anaphylactic reactions (Sponsor's Text Table 7)

Score	Grade	Signs of Anaphylactic Reactions
0	None	Normal – no identifiable reaction
1	Minimal	Tremors, nose scratching, piloerection, and sneezing
2	Moderate	Dyspnea, wheezing, labored respiration, cyanosis, urination, defecation, and staggered gate
3	Severe[a]	Convulsions, prostration, and death

[a]If any animal exhibited any signs of severe anaphylactic reactions, the animal was euthanized.

Source: Sponsor's Table (page 14 of 104 of study report)

Results

Mortality: DTPA-Mannosyl-Dextran-DTPA-dextran had no effect on mortality or clinical signs. All animals survived to scheduled termination.

Clinical observations: There were no adverse clinical observations noted following administration of DTPA-Mannosyl-Dextran-DTPA-dextran. Adverse effects (anaphylactic reactions) were observed, expectedly, in positive control animals (group 2) that received Ovalbumin. The reactions included lesions on the dorsal thoracic area, bilateral ocular discharge and a languid disposition. There were abrasions and abscesses in the injection area claimed to result from multiple injections of Ovalbumin with CFA.

Body weight: There were no treatment-related changes in body weight.

Conclusions: DTPA-Mannosyl-Dextran (DTPA-Mannosyl-Dextran), administered up to 280 µg/kg did not induce any anaphylactic reactions in male guinea pigs after four weekly subcutaneous sensitization doses and an intravenous challenge dose. Adverse reactions were expectedly limited to Ovalbumin/CFA-treated animals only.

Table 93: Determination of NOAEL in Study No. (b) (4) 1576-04583

Parameter evaluated	NOAEL (µg/kg)	Dose multiple
Mortality	280	109x
Clinical signs		
Body weight changes		
Gross pathology/Histopathology		

Reviewer's Table

Reviewer's Comments: I agree with the findings of the study in which no hypersensitivity reactions were due to the drug substance, DTPA-Mannosyl-Dextran. The study was adequately controlled with both positive (Ovalbumin) and negative (saline) controls. NOAEL for this study was 280 µg/kg. While no hypersensitivity reactions were reported with this dextran-based product, a close monitoring of signs of anaphylaxis is advised since dextrans are known for their negative effects on coagulation and potential for anaphylactic reactions.

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11 Integrated Summary and Safety Evaluation

Introduction: The radioactive diagnostic product evaluated in NDA 202-207 is Technetium Tc 99m-labeled DTPA-Mannosyl-Dextran, or Lymphoseek® (Tc 99m Lymphoseek). The unlabeled drug substance, DTPA-Mannosyl-Dextran, is a dextran-based molecule coupled with DTPA and mannose moieties. Lymphoseek®: Kit for the Preparation of Technetium Tc 99m DTPA-Mannosyl-Dextran for Injection is supplied as a cold kit (non-radiolabeled) containing a sterile, lyophilized preparation of DTPA-Mannosyl-Dextran 0.25 mg in single-se vials and sterile buffered saline diluent. The active ingredient after radiolabeling is Technetium Tc 99m DTPA-Mannosyl-Dextran. Lymphoseek is proposed (b) (4)

(b) (4) Lymphoseek is intended to accumulate in lymphatic tissue bound to mannose binding receptors present on the surface of dendritic cells and macrophages. Lymphoseek is proposed to be administered locally via peritumoral, subcutaneous, subareolar or intradermal routes. The subcutaneous route was commonly used in the nonclinical studies.

Pharmacodynamic studies: Primary pharmacodynamic studies, conducted to analyze the binding specificity of drug product, showed that Lymphoseek has a specific binding interaction with mannose binding receptors natively expressed on the surface of human lymphatic system-derived macrophages with a high binding affinity. The equilibrium dissociation constant for binding to the MBR was 0.12±0.07 nmol/L. No secondary pharmacology studies were performed.

Safety Pharmacology:
Safety pharmacology: Two *in vivo* safety pharmacology studies were conducted in beagle dogs to evaluate the cardiovascular pharmacology effects of intravenously administered DTPA-Mannosyl-Dextran. In the first study (non-GLP) in conscious, non-telemetered dogs, the single administered dose of DTPA-Mannosyl-Dextran (560 μg/kg/dog) had no effect on survival, clinical signs, ECG or blood pressure. In the second study (GLP), DTPA-Mannosyl-Dextran, when administered to telemetered dogs at doses up to 840 μg/kg, by dose escalation, was well tolerated. There were no adverse effects on the cardiovascular system, body temperature or body weight. NOAEL was determined as 560 μg/kg (364-fold) and 840 μg/kg (or 546-fold MHD) respectively for the evaluated cardiovascular studies.

CNS and respiratory safety studies were not conducted.

Pharmacokinetics:
Four nonclinical pharmacokinetic (PK) studies were conducted to evaluate the PK profile of DTPA-Mannosyl-Dextran. Plasma and urine PK parameters were determined using a single dose pilot PK non-GLP study in a mongrel dog. Two 14-day repeated dose toxicity studies in SD rats and mongrel dogs. Tissue distribution was evaluated in NZW rabbits as one of three bridging studies with the Lymphoseek drug product (Technetium Tc 99mTilmanocept Injection).

In the rat and dog repeat-dose toxicity studies, data based on ^{111}In- DTPA-Mannosyl-Dextran administered by the subcutaneous route, showed that PK was similar across both species and sex. Absorption was rapid with a half life of 4 min and 23 min in the rat and dog, respectively. C_{max} ranged between 10.3 and 28.6 ng/mL in the rat and 6.8-42.8 ng/mL in the dog. T_{max} was 7.8 min in the rat and 28-66 min in the dog. AUC increased dose-proportionally in both species. Excretion was by the urinary route and the elimination half life was 3 and 6.5 hours in the rat and dog, respectively.

The findings of the tissue distribution study in rabbits indicated that Technetium Tc 99m Tilmanocept administered by the subcutaneous route, was widely distributed in the body

in male and female animals. In females, at 15 minutes postdose, organs with higher percentage amounts of the injected dose (%ID) included the skin injection site (~7%), plasma (~10%), urinary bladder contents (7.7%), both the liver kidney (3%) and gastrointestinal tract (approximately 2%). In general, lower amounts were reported in most organs of distribution at 1h and 3h time points except in the kidneys, urinary bladder and colon contents. The increased amounts of Technetium Tc 99m Tilmanocept the major excretory organs were indicative of continued absorption and excretion**.** Also at 15 min postdose, greater than 1% of the subcutaneously injected dose of Technetium Tc 99m Tilmanocept was found in the popliteal lymph node of the treated leg in female rabbits. Evidence of presence of Technetium Tc 99m Tilmanocept in the popliteal lymph node ipsilateral to the injection site, in contrast to its lack in the contralateral popliteal node, was indicative increased absorption of injected Technetium Tc 99m Tilmanocept from the injection site.

Toxicology: Single-dose toxicity studies were performed in rats, rabbits and dogs while repeated dose toxicity studies were conducted in rats and dogs. Three studies were performed to assess the genotoxic potential of DTPA-mannosyl-dextran. In addition, one local antigenicity study and two local tolerance studies were performed. All toxicity studies were GLP-compliant. Toxicity studies were conducted primarily with the unformulated drug substance. The single- and repeat-dose showed no evidence of potential toxicity of Lymphoseek and NOAEL was the top dose administered in each study except in one single-dose acute toxicity study in rabbits in which the NOAEL could not be determined. DTPA-mannosyl-dextran (drug substance) was negative in the bacterial reverse mutation, L5178Y/TK$^{+/-}$ mouse Lymphoma, and in the *in vivo* bone marrow micronucleus assays.

Antigenicity study conducted in male guinea pigs showed that DTPA-Mannosyl-Dextran (DTPA-mannosyl-dextran), administered up to 280 μg/kg did not induce any anaphylactic reactions in male guinea pigs after four weekly subcutaneous sensitization doses and an intravenous challenge dose. The two local irritation studies were designed to determine the irritant potential of a single intramuscular dose of DTPA-mannosyl-dextran in NZW rabbits. In both studies, DTPA-Mannosyl-Dextran had no effect on survival, clinical observations or injection site histopathology at doses up to 286 μg/kg or 111-fold MHD ((b) (4) 1146-104) and up to 280 μg/kg or 109-fold MHD ((b) (4) 1576-06069).

Table 94: NOAEL, Safety Margin and prominent finding(s) in Toxicity Studies

Toxicity	Species	NOAEL* (µg/kg) in M/F	Safety margin (x-fold MHD)	Prominent findings (if any)
Single Dose	Rat	140	27x	No adverse effects observed up to and including the high dose
	Rabbit	Not established in this study	ND	Generally, there were no adverse effects observed up to and including the high dose. However, there was a treatment-related finding of centrilobular hepatocytic hypertrophy in animals of both sexes
	Dog	420	273x	No adverse effects observed up to and including the high dose
Repeat-Dose	Rat	42	8x	No adverse effects observed up to and including the high dose
	Dog	42	21x	No adverse effects observed up to and including the high dose
Local Tolerance	Rabbit (1)	286	111x	No adverse effects observed up to and including the high dose
	Rabbit (2)	140[md]	55x	Inflammatory change, degeneration/necrosis and mineralization of the injection site skeletal muscle
Antigenicity	Guinea Pig	280	72.7x	No adverse effects observed up to and including the high dose

Reviewer's Table. * = Unless stated otherwise, the NOAEL is the top dose administered; [md] = mid dose; Rabbit (1) = Local tolerance study conducted in NZW rabbits using the drug substance (DTPA-mannosyl-dextran); Rabbit (2) = Local tolerance (Bridging) study conducted in NZW rabbits using the unlabeled drug product (Lymphoseek); ND = could not be determined.

12 Appendix/Attachments

12. 1 References

Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, Aissat A, Guerre-Millo M, and Clément K (2009) Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J. Clin Endocrinol Metab. 94(11):4619-23

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J Nucl Med 33:1160 –1166

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/s/

OLAYINKA A DINA
06/26/2012

ADEBAYO A LANIYONU
06/27/2012
I concur with Dr. Dina recommendations

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202207. **Applicant:** Neoprobe Corp. **Letter Date:** August 10, 2011.

Drug Name: Lymphoseek. **NDA Type:** 505(b)(i). **Stamp Date:** August 10, 2011.

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		Module 2.4 [nonclinical overview] and 2.6 [nonclinical written and tabulated summaries]; Module 4 [4.2.1(Pharmacology), 4.2.2 (Pharmacokinetics), 4.2.3 (Toxicology), 4.3 (Literature References)]
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Acceptable
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		Acceptable
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	✓		Carcinogenicity, Reproductive/developmental toxicity, and metabolite toxicity studies were not conducted. Respective waivers were submitted in §1.12.13
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	✓		Different formulations were used for the nonclinical studies, namely, unlabeled and radiolabeled drug substance [Tilmanocept] and unlabeled and labeled drug product [Lymphoseek]. Appropriate bridging studies have been completed with the final formulation to demonstrate equivalency. See also comment (ii) below in response to content parameter #10, page 2.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	✓		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	✓		(i). Compliance with GLP regulations for pivotal pharm/tox studies was affirmed in § 2.4 (nonclinical overview, page 9, paragraph) (ii). Appropriate annotation of GLP/non-GLP status was provided for all nonclinical studies submitted § 2.4 (nonclinical overview, Table 1: Nonclinical study list, pages 12-14)

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special Studies/data requested by the Division during pre-submission discussions?	✓		(i). § 2.4.1.2 (Nonclinical testing strategy, page 8, paragraph 2). (ii). Nonclinical General Reviews of Dr. David Bailey (signed off on May 12, 2006, April 25, 2007 and Sept 10, 2010) provide support that required nonclinical studies have been provided in pre-submission discussions
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	✓		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	✓		(i). Impurities were addressed in § 2.4.4.7.2 (nonclinical overview p.20 of 24). (ii). According to P/T review of Dr. David Bailey (signed off April 25, 2007); *"After many nonclinical studies were completed the sponsor modified the drug formulation. To verify that this change did not alter the toxicity of the drug, the Sponsor was requested to conduct bridging studies with the new formulation. These studies have been conducted and from the perspective of nonclinical pharmacology and toxicology, no difference could be discerned between the two formulations"*. (iii). Sponsor confirmed that bridging studies were performed as requested by the Agency (nonclinical overview, p. 8 of 24).
11	Has the applicant addressed any abuse potential issues in the submission?		✓	
12	If this NDA is to support an Rx to OTC switch, have all relevant studies been submitted?		✓	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Olayinka A. Dina, D.V.M., Ph.D.

Reviewing Pharmacologist Date

Adebayo Laniyonu, Ph.D.

Team Leader/Supervisor Date

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/s/

OLAYINKA A DINA
09/19/2011

ADEBAYO A LANIYONU
09/19/2011