Clinical Trial Protocol: NAV3-24

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and a Comparison to Subcutaneous (SC) Injection in Human Immunodeficiency Virus (HIV) Subjects Diagnosed with Kaposi Sarcoma (KS)
Study Number:	NAV3-24
Study Phase:	1
Product Name:	technetium Tc 99m tilmanocept
IND Number:	132943

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Amendment #3	30 January 2019

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SYNOPSIS

Study Title	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and a Comparison to Subcutaneous (SC) Injection in Human Immunodeficiency Virus (HIV) Subjects Diagnosed with Kaposi Sarcoma (KS)
Study Phase	Phase 1
Study Drug	Technetium Tc 99m tilmanocept injection
Dose(s) and Route of Administration	**Route of Administration:** Administrations of Tc 99m tilmanocept will occur through an intravenous (IV) and subcutaneous (SC) route of injection. For the IV doses, a single syringe will be used and injected as a slow push into the peripheral venous catheter. The preferred site of catheter placement will be the left or right antecubital vein. At the completion of the injection, a 10 cc sterile normal saline flush will be administered. For SC doses, two syringes will be used and injected bilaterally as a slow push into the ankle(s) or the dorsal surface of the foot (feet). **Doses:** Cohort 1 subjects will receive an IV administration of 100 µg/ 5 mCi Tc 99m tilmanocept. Cohort 2 subjects will receive an IV administration of 100 µg/ 10 mCi Tc 99m tilmanocept. Cohort 3 subjects will receive a SC and an IV administration of 200 µg/ 5 mCi Tc 99m tilmanocept approximately one week apart.
Study Objectives	*Primary Objective(s):* To determine the safety of escalating doses of Tc 99m tilmanocept in HIV subjects with biopsy-confirmed KS. *Secondary Objective(s):* • Concordance between clinical assessment/diagnosis and the localization of Tc 99m tilmanocept by planar and/or SPECT (single photon emission computed tomography)/CT (X-ray computed tomography) imaging in cutaneous and non-cutaneous sites of KS. • Qualify and quantify Tc 99m tilmanocept localization intensity on imaging with CD206 locale and quantity by histology and immunohistochemistry (IHC) in biopsied KS lesions to determine optimal IV dose. • Concordance of localization of Tc 99m tilmanocept via IV and SC routes of administration by planar and/or SPECT/CT imaging in cutaneous and non-cutaneous sites of KS. *Exploratory Objective(s):*

	• Quantify HHV8 in biopsied KS lesions by real-time polymerase chain reaction (qPCR) *Safety:* General safety will be evaluated by examining the incidence of adverse events (AEs) and changes over time in laboratory tests, vital signs, ECGs (electrocardiogram) and physical examination findings.
Inclusion Criteria	1. The subject has provided written informed consent with HIPAA (health insurance portability and accountability act) authorization before the initiation of any study-related procedures. 2. The subject is at least 18 years of age at the time of consent. 3. The subject is HIV positive. 4. The subject has a biopsy-confirmed diagnosis of KS and is classified into one of the categories below: a. Confirmed cutaneous KS/oral lesions without edema b. Confirmed cutaneous KS/oral lesions with edema c. Confirmed cutaneous KS/oral lesions with or without edema and suspected non-cutaneous KS due to clinical symptomology or confirmed non-cutaneous KS lesion(s).
Exclusion Criteria	1. The subject is pregnant or lactating. 2. The subject has received chemotherapy or radiation therapy to KS sites within six weeks of enrollment. 3. The subject has known sensitivity to dextran. 4. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration on Day 1. 5. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m tilmanocept on Day 1. 6. Any condition that, in the clinical judgment of the treating physician, is likely to prevent the subject from complying with any aspect of the protocol or that may put the subject at unacceptable risk.
Investigational Product	technetium Tc 99m tilmanocept
Study design	Prospective, single center, open-label, non-randomized, dose escalation, comparative, safety study of Tc 99m tilmanocept in the localization and detection of cutaneous and non-cutaneous lesions in HIV subjects with KS by planar and optional SPECT/CT imaging. Cohort 1 subjects will receive an IV administration of 100 µg/ 5 mCi Tc 99m tilmanocept. Cohort 2 subjects will receive an IV administration of 100 µg/ 10 mCi Tc 99m tilmanocept. Cohort 3 subjects will receive a SC and an IV

<table>
<tr><td></td><td>administration of 200 µg/ 5 mCi Tc 99m tilmanocept approximately one week apart. At least one subject enrolled into each cohort must meet inclusion criteria 4c, as defined above (suspected non-cutaneous or confirmed non-cutaneous KS). For determination of the primary endpoint, a review meeting for safety will be held after the completion of each cohort prior to opening the next dose cohort. Cohorts will be dosed per the following table:
<table>
<tr><th></th><th>Tc 99m tilmanocept Dose</th><th>Route of Administration</th><th>Number of Subjects</th></tr>
<tr><td>Cohort 1</td><td>100 µg/ 5 mCi</td><td>IV</td><td>n = 4</td></tr>
<tr><td>Cohort 2</td><td>100 µg/ 10 mCi</td><td>IV</td><td>n = 4</td></tr>
<tr><td>Cohort 3</td><td>200 µg/ 5 mCi</td><td>IV and SC</td><td>n = up to 6</td></tr>
</table>
Following IV administration, all subjects will have a whole body planar scan with optional SPECT/CT region of interest scanning beginning at 60-75 minutes post-injection. Subjects in Cohort 3 will additionally have a whole body planar scan with optional SPECT/CT region of interest scanning beginning at 60-75 minutes and 4-6 hours following SC injection.</td></tr>
<tr><td>Methodology</td><td>The study includes: screening, enrollment, pre- and post-injection assesments, injection, imaging, and follow-up.

Cohorts 1 and 2:

<u>Visit 1, Screening (Day -29 to Day 0):</u>
The screening visit will include an assessment of eligibility, informed consent, collection of medical history including medications, vital signs, ECG, physical exam including height and weight, assessment of KS lesions, photographs of all visible cutaneous KS lesions, assessment of edema/lymphedema, clinical labs, urinalysis, and serum pregnancy test for subjects of child bearing potential.

<u>Visit 2 (Day 1):</u>
<u>Pre-Tc 99m Tilmanocept Injection</u>
• Before Injection
o Urine pregnancy test will be performed for subjects of child bearing potential within 48 hours of injection
o Concomitant medication review
o Vital signs—within 30 minutes prior to injection

<u>Tc 99m Tilmanocept IV Injection:</u> Subjects will receive their open label serial IV dose assignment in accordance with Table 1. Adverse event monitoring will continue through completion of the trial.</td></tr>
</table>

Post-Tc 99m Tilmanocept Injection:

- Within 30 Minutes Post-Injection
  - ECG
  - Vital Signs
  - Assessment of Adverse Events
- 60-75 Minutes Post -Injection
  - Assessment of Adverse Events
  - Whole body planar imaging followed by optional SPECT/CT scan of region(s) of interest
- At Completion of Imaging:
  - Safety Labs
  - Biopsy of designated non-visceral KS lesion

7 ± 3 (4-10) Days Post Injection Telephone Follow-up:

- Assessment of Adverse Events
- Concomitant medication review

Cohort 3:

Visit 1, Screening (Day -29 to Day 0):

The screening visit will include an assessment of eligibility, informed consent, collection of medical history including medications, vital signs, ECG, physical exam including height and weight, assessment of KS lesions, photographs of all visible cutaneous KS lesions, assessment of edema/lymphedema, clinical labs, urinalysis, and serum pregnancy test for subjects of child bearing potential.

Visit 2 (Day 1):

Pre-Tc 99m Tilmanocept Injection

- Before Injection
  - Urine pregnancy test will be performed for subjects of child bearing potential within 48 hours of injection
  - Concomitant medication review
  - Vital signs—within 30 minutes prior to injection

Tc 99m Tilmanocept SC Injection: Subjects will receive their open label SC dose in accordance with Table 1. Adverse event monitoring will continue through completion of the trial.

Post-Tc 99m Tilmanocept Injection:

- Within 30 Minutes Post-Injection
  - ECG
  - Vital Signs
  - Assessment of Adverse Events
- 60-75 Minutes Post -Injection
  - Assessment of Adverse Events
  - Whole body planar imaging followed by optional SPECT/CT scan of region(s) of interest
- 4 – 6 Hours Post -Injection
  - Assessment of Adverse Events
  - Whole body planar imaging followed by optional SPECT/CT scan of region(s) of interest
- At Completion of Imaging:
  - Safety Labs

Visit 3 (Day 7 ± 3):

Pre-Tc 99m Tilmanocept Injection

- Before Injection
  - Follow-up assessment from SC injection including changes in concomitant medication and adverse events
  - Urine pregnancy test will be performed for subjects of child bearing potential within 48 hours of injection
  - Concomitant medication review
  - Vital signs—within 30 minutes prior to injection

Tc 99m Tilmanocept IV Injection: Subjects will receive their open label serial IV dose assignment in accordance with Table 1. Adverse event monitoring will continue through completion of the trial.

Post-Tc 99m Tilmanocept Injection:

- Within 30 Minutes Post-Injection
  - ECG
  - Vital Signs
  - Assessment of Adverse Events
- 60-75 Minutes Post -Injection
  - Assessment of Adverse Events
  - Whole body planar imaging followed by optional SPECT/CT scan of region(s) of interest
- At Completion of Imaging:

	○ Safety Labs ○ Biopsy of designated non-visceral KS lesion **<u>7 ± 3 Days Post-IV Injection Telephone Follow-up:</u>** • Assessment of Adverse Events • Concomitant medication review For determination of the secondary endpoints, the Tc 99m tilmanocept whole body planar and SPECT/CT images will be visually assessed for localization by a Nuclear Medicine Physician.	
Planned Study Dates	**Start of study recruitment** January 2018	**End of Recruitment:** June 2019 **End of Study**/ September 2019
Planned number of study centers	One (1) in the United States	
Number of subjects	**Total:** Up to 14 evaluable subjects may be enrolled, injected, and imaged. KS symptoms will be divided into three categories: a) confirmed cutaneous/oral lesions without edema, b) confirmed cutaneous/oral lesions with edema, or c) confirmed cutaneous/oral lesions with or without edema and suspected non-cutaneous KS due to clinical symptomology or confirmed non-cutaneous lesions. After the subjects are dosed and imaged in each cohort, safety and imaging data will be reviewed by the Co-PIs and Navidea for assessment of safety and preliminary localization.	
Primary Endpoint	Proportion of subjects experiencing noxious pharmacologic activity/Adverse Drug Reaction (ADR).	
Secondary Endpoints	The secondary endpoints for this study are: • Per subject localization rate of Tc 99m tilmanocept in at least one KS suspected or confirmed lesion by planar and/or SPECT/CT imaging • Per lesion/region concordance of Tc 99m localization with anatomical areas of active KS defined by confirmed diagnosis or clinical symptomology. • Localization intensity for each biopsied and clinically defined lesion as determined by quantitative SPECT gamma counts • Per biopsied lesion proportion of CD206-expressing cells and total CD206 as determined by histology and relative IHC fluorescence • Per lesion/region concordance of IV vs SC Tc 99m localization with anatomical areas of active KS defined by confirmed diagnosis or clinical symptomology. • Per subject localization rate of Tc 99m tilmanocept in areas other than KS by planar and/or SPECT/CT imaging	

	• Per area localization rate of Tc 99m tilmanocept in the most frequently identified areas other than KS by planar and/or SPECT/CT imaging
Exploratory Endpoint	Per biopsied lesion quantity of HHV8 as determined by qPCR
Data Analysis	The following analysis population will be defined for the study: • **Intent-to-Diagnose (ITD) Population** - Subjects who are enrolled in the study, injected with Tc 99m tilmanocept, and are imaged will be included in the ITD analysis population. • **Safety Population** – All patients who are enrolled in the study and injected with Tc 99m tilmanocept will be included in the safety population. All safety data analyses will be conducted on the safety population. All efficacy data analyses will be conducted on the ITD population. The distribution of each baseline and demographic variable of interest will be summarized by dose cohort. Continuous variables will be summarized via mean, median, standard deviation, and range. Categorical variables will be summarized via counts and percentages. For the primary safety endpoint, the number and percentage of subjects with ADRs will be tabulated by dose group and overall. The number and percentage of subjects with at least one biopsy-confirmed or clinically suspected KS lesion that has localized with Tc 99m tilmanocept by planar and/or SPECT/CT imaging will be computed. A 95% exact confidence interval on the per subject localization rate will be computed. This analysis will be performed by dose and overall. Per region lesion localization of Tc 99m tilmanocept by planar and/or SPECT/CT imaging will be computed across all biopsy-confirmed or clinically suspected KS lesions of all subjects. A 95% exact confidence interval on per region lesion localization will be calculated. This analysis will be performed by dose and overall. Additional analyses of efficacy will be performed as decribed in Section 9.5. Other efficacy analyses not addressed in this protocol will be described in the Statistical Analysis Plan (SAP) for the study.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADR	Adverse drug reaction
AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase (SGPT)
AST	aspartate aminotransferase (SGOT)
BUN	blood urea nitrogen
CD206	mannose binding receptors
CNS	central nervous system
CRA	clinical research associate
CRC	Colorectal cancer
CRF	case report form
CRO	contract research organization
CT	X-ray computed tomography
CVD	Cardiovascular disease
DICOM	Digital Imaging Communications in Medicine
DTPA	pentetic acid
ECG	electrocardiogram
EU	European Union
FDA	Food and Drug Administration
FFPE	formalin-fixed paraffin-embedded
FNR	false negative rate
FOV	field of view
GCP	Good Clinical Practice
GI	gastrointestinal
HCT	hematocrit
Hgb	hemoglobin
HHV8/KSHV	Kaposi sarcoma herpes virus
HIPAA	Health Information Portability and Accountability Act

HIV	human immunodeficiency virus infection
ICF	informed consent form
ICH	International Conference on Harmonization
ID	intradermal
$\%ID_{SN}$	percentage of Injected Dose in the Sentinel Node
IEC	Independent Ethics Committee
IHC	immunohistochemistry
ILM	intraoperative lymphatic mapping
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
ISF	investigator site file
ITD	intent to diagnose
IV	intravenous
KS	Kaposi Sarcoma
L_{SN}	primary sentinel lymph node
MBR	mannose binding receptor
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NASH	Nonalcoholic steatohepatitis
NOAEL	no-observed adverse-effect level
PI	principal investigator
PK	pharmacokinetics
PT	peritumoral
QC	quality control
qPCR	real-time polymerase chain reaction
RA	Rheumatoid arthrirtis
RBC	red blood cell
SAE	serious adverse event

SAP	statistical analysis plan
SC	subcutaneous
SCC	squamous cell carcinoma
SD	study day
SLNB	sentinel lymph node biopsy
SPECT	single photon emission computed tomography
SUSARs	Suspected, Unexpected, Serious Adverse Reactions
TAMs	Tumor-associated macrophages
TcSC	Tc 99m sulfur colloid
TEAE	treatment emergent adverse event
tilmanocept	Technetium Tc 99 tilmanocept
TMF	trial master file
US	United States
VBD	vital blue dye

TRIAL ADMINISTRATIVE STRUCTURE

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

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

In addition to the PI, there are additional onsite roles that may be performed by other sub-investigators:

- Subject referral to the trial
- Review of subject eligibility and medical records
- Safety assessments
- Injection and SPECT imaging

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

1 INTRODUCTION

1.1 Background

Tilmanocept (technetium Tc 99m tilmanocept) Injection was approved by the US (United States) Food and Drug Administration (FDA) for use in lymphatic mapping to assist in the localization of lymph nodes draining a primary tumor site in patients with solid tumors for which this procedure is a component of intraoperative management and for guiding sentinel lymph node biopsy (SLNB) in patients with clinically node negative squamous cell carcinoma (SCC) of the oral cavity, breast cancer, or melanoma.

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

Tilmanocept is a radiotracer that accumulates in lymphatic tissue by binding to a mannose binding receptor that resides on the surface of dendritic cells and macrophages within the first draining lymph nodes. Tilmanocept is a macromolecule consisting of multiple units of diethylenetriaminepentaacetic acid acid (DTPA) and mannose, each synthetically attached to a 10 kilodalton dextran backbone. The mannose acts as a substrate for the receptor, and the DTPA serves as a chelating agent for labeling with Tc-99m. Tilmanocept's small diameter permits rapid transit from the injection site (subcutaneous [SC] or intradermal [ID]) to the lymphatic channels and blood capillaries resulting in enhanced diffusion into lymph nodes.

The pathogenesis of cancer is a complex multistep process involving the acquisition of genetic abnormalities by cancer cells and tumor progression associated with an ongoing inflammatory process. The inflammation is generally driven by tumor-associated macrophages (TAMs), and these cells have been proven in a variety of systems to provide a supportive environment for tumor progression. In addition, there is increasing evidence that tumor metastasis is associated with tumor cells that co-express macrophage markers that allow more efficient metastasis. As metastasis is critical to the overall pathogenesis of cancer and survival in patients with cancer, both TAMs and tumor cells bearing macrophage markers would be important targets for quantitative imaging in the development of anti-cancer therapeutics. In this context, AIDS(acquired immunodeficiency syndrome)-associated Kaposi sarcoma (KS) is a form of cancer in which inflammation plays a critical role in tumor development. Because tilmanocept binds to CD206 (mannose binding receptor), it could have a significant role in the development of a new wave of diagnostic and anti-tumor agents directed against TAMs and metastatic tumor cells by imaging their metastatic pattern and response to therapy.

Macrophages are one of the most versatile cell types in the body, largely owing to their ability to fluctuate between activation states depending on their microenvironment. They function in both innate and adaptive immunity through the phagocytosis of foreign materials and pathogens, as well as by stimulating the activation of other immune cells. Macrophages make up a large portion of the inflammatory infiltrate for most cancer types, including KS, and may represent up to 80% of the total tumor mass. Monocyte recruitment is essential for tumor development as these cells are present during the early stages of tumor growth and promote progressive tumorigenesis. Blood monocytes are recruited from the vasculature into the tumor, in response to chemo-attractants released by tumor cells, where they differentiate into TAMs. TAMs are a heterogeneous mass of cells consisting of resident macrophages, early infiltrating perivascular macrophages, and monocytes/macrophages. Depending on the microenvironment, macrophages either become M1 (classically activated, MAC387-positive) in response to pro-inflammatory molecules and pathogens, or become M2 (alternatively activated,

CD163-positive). In KS, as well as many other cancer types, the primary infiltrating form of macrophage is the M2 subset [1-4].

AIDS-related KS is an aggressive, multifocal, angioproliferative neoplasm associated with Kaposi sarcoma herpes virus (HHV8/KSHV) infection. It involves cutaneous and non-cutaneous tissues, with later forms of disease associated with widespread organ involvement. It is the most common cancer in patients infected with HIV. Effective antiviral therapy has produced a decline in the incidence of AIDS-related KS, but HIV-infected individuals still have a 3,640-fold greater risk of developing KS than the uninfected population. In general, no imaging studies have been able to identify specific KS-involved tissues, apart from standard ultrasound and CT (computed tomography) imaging, in which therapy-associated changes are implied to be associated with KS lesion shrinkage [1-4].

KS lesions are comprised of KS spindle cells infected with HHV8/KSHV, as well as numerous macrophage antigen-expressing cells. Crucially, a large collection of both skin and visceral forms of KS were tested to determine whether the CD206 molecule would be present on both KS tumor cells and TAMs, allowing the potential for using Tc 99m tilmanocept as a tumor-specific imaging agent capable of identifying both tumor cells and TAMs in patients with KS.

Historically, no imaging platform has been able to identify KS-specific lesions in patients with KS. This has caused problems for the delivery of clinical care, as physicians are unable to appropriately stage patients with KS, other than by tracking skin lesions. KS is known to involve lymph nodes and organs, but to date no approach has been able to confirm tumor involvement beyond skin. Our investigations have confirmed that the majority of both TAMs and KS cells express the macrophage marker CD206, the receptor for the tilmanocept imaging agent.

The observation that tilmanocept binds specifically to CD206-positive macrophages has been extended to CD206-positive tissue from KS patients. This performance makes tilmanocept an ideal agent for use in patients with KS to stage and quantitatively image tumor-specific response to therapy. By extension, other classes of tumors may be similar hybrid-like cells and may be imaged with tilmanocept and clinically addressed using macrophage-targeted therapy[5-15].

1.2 Preliminary Data

As prelude to this clinical study, Navidea, in collaboration with Drs. Michael McGrath and Toby Maurer undertook an ex vivo assessment of the expression of macrophage markers in KS and the expression of CD206 and the determination of co-localization of these markers using a fluorescent congener of tilmanocept (Cy-3-tilmanocept; red fluorescence reporting). The results of this study are presented in Figure 1, Figure 2, and Figure 3[16]. In summary, Cy3-tilmanocept binds to the KS lesions with high specificity and sensitivity and co-localizes with markers for HHV8, CD68, and CD163. All of the evidence acquired in this assessment suggests that localization and imaging of not only primary KS lesions but disseminated KS disease will strongly localize Tc 99m tilmanocept. In addition to the ex vivo study, Dr. Maurer completed a Phase 2 study, which confirmed that Tc 99m tilmanocept administered through a subcutaneous injection localized in KS lesions as well as lymph nodes. Thus, this receptor targeting approach to CD206 presents itself as a novel portal for imaging and staging KS.

Figure 1. Macrophage CD206 Expression Permits Targeting of a Broad Range of Immuno-Inflammatory Disorders

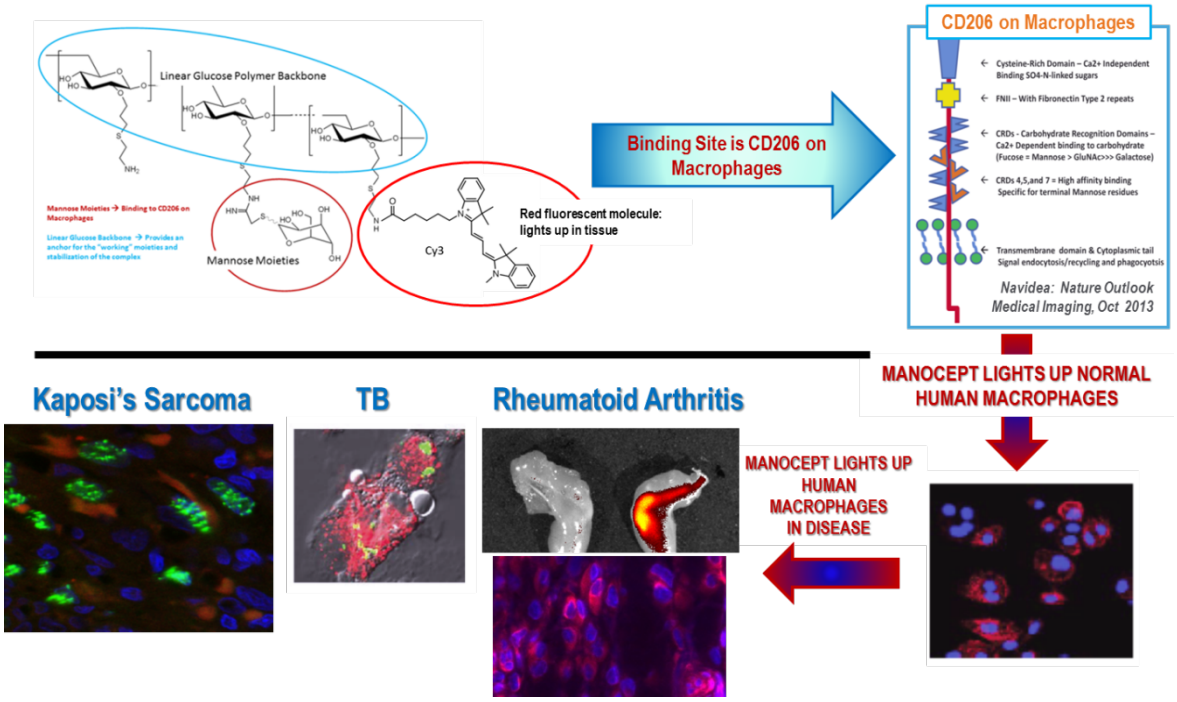


Figure 2. Kaposi Sarcoma Tumor and Macrophages Express CD206

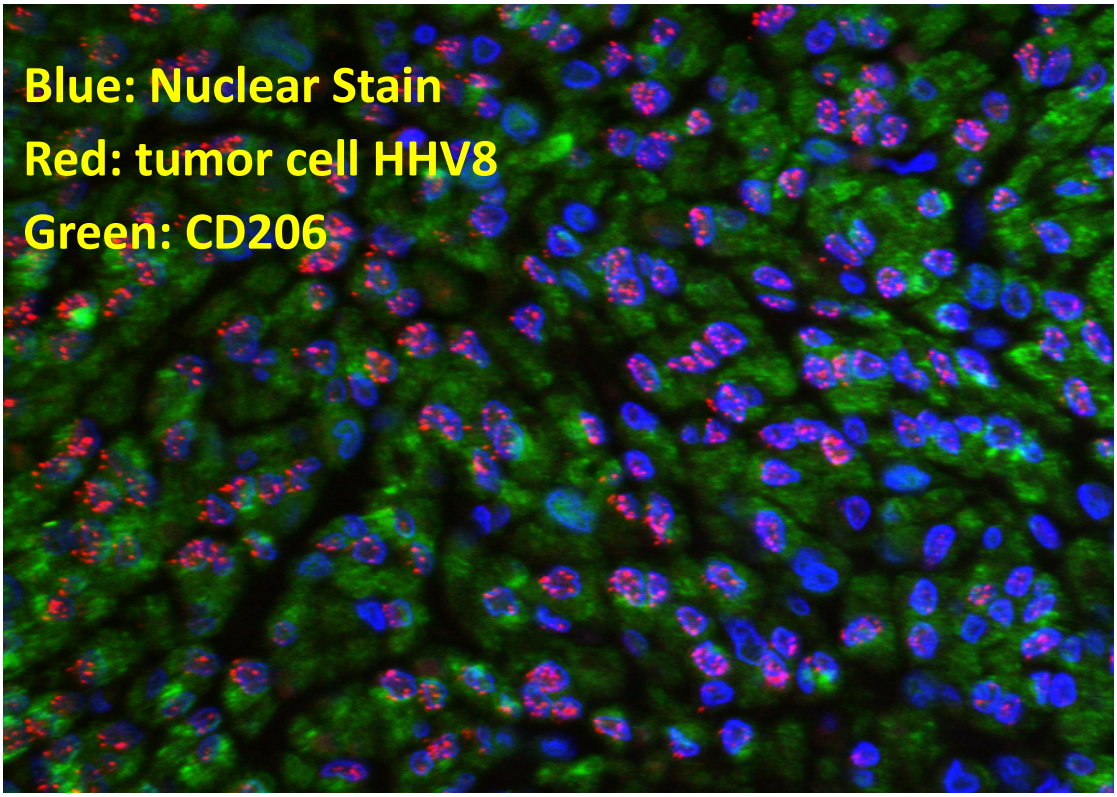
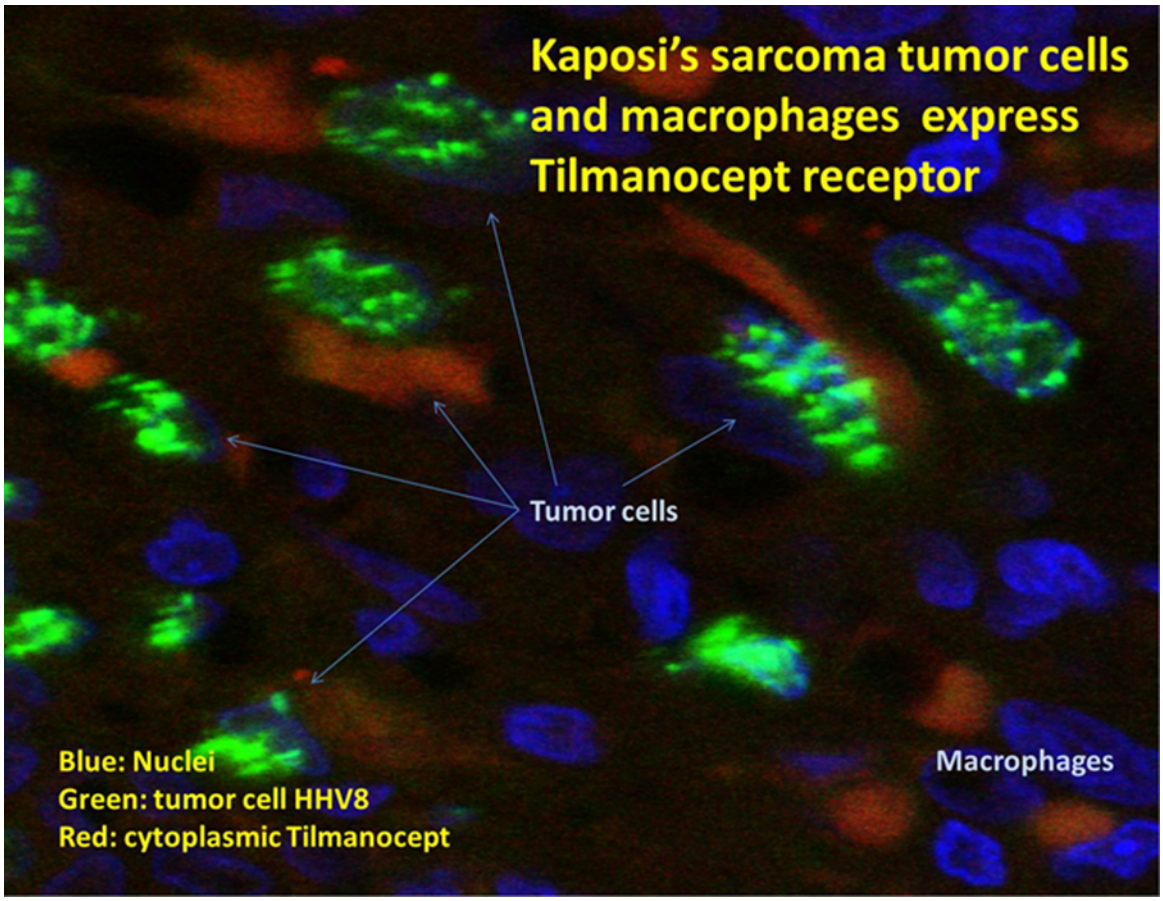


Figure 3. Ex Vivo Tilmanocept Localization in Tumor Tissue from a KS Patient

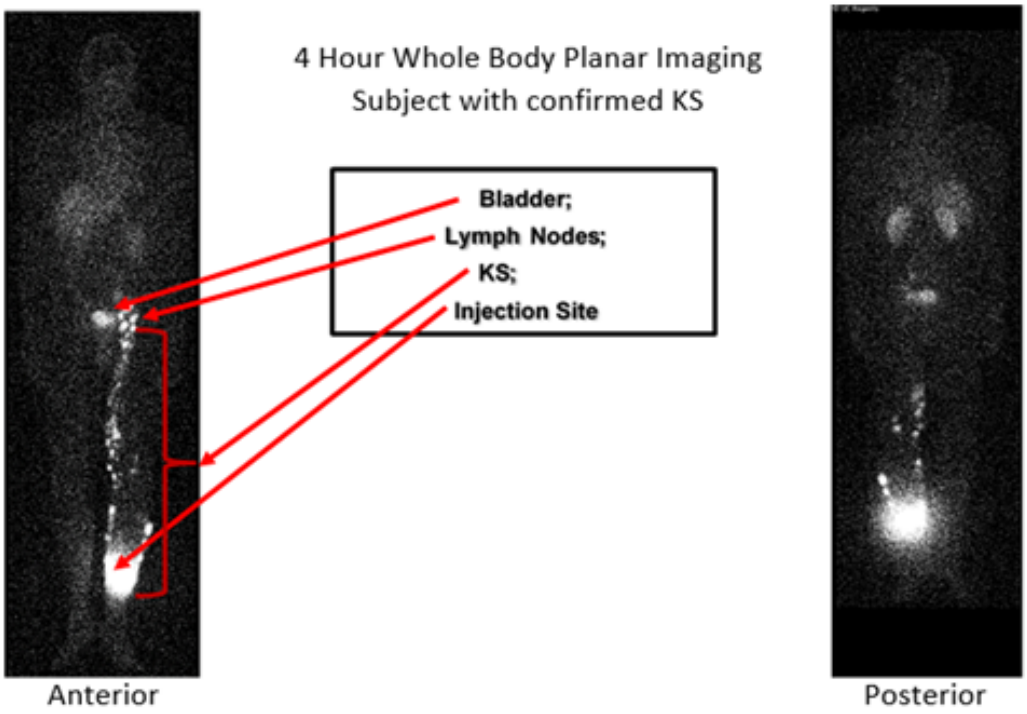


1.2.1 Prior Experience of Tilmanocept Injection in KS Patients

Imaging of KS with high specificity and sensitivity has been a remarkable challenge even with today's high resolution technology and available imaging agents[17-29]. In addition to the ex vivo study, Dr. Maurer completed a phase II clinical pilot study in KS patients at the University of California San Francisco (UCSF Protocol 14-13522). Five subjects received a subcutaneous injection of 50 µg tilmanocept radiolabeled with 2.0 mCi Tc 99m. No subjects experienced any SAEs. Three AEs were reported in two subjects, including peripheral edema (probably not related to study drug or procedures) and fatigue and nausea (possibly related to study drug or procedures). No interaction of tilmanocept with HAART therapy or other drugs utilized in the treatement of HIV or HHV8 was observed.

Tc 99m tilmanocept localized regionally in clinically confirmed KS lesions as well as lymph nodes, as shown in Figure 4. Thus, this receptor targeting approach to CD206 presents itself as a novel portal for imaging and staging KS. Based on this previous experience, it is anticipated that IV-administered Tc 99m tilmanocept will be able to systemically localize both cutaneous and non-cutaneous (i.e. visceral or CNS) KS lesions.

Figure 4. Localization of Tc 99m Tilmanocept in KS Lesions



1.3 Previous Experience with Tc 99m Tilmanocept

A detailed evaluation of safety and efficacy data from nonclinical and clinical studies can be found in the accompanying Investigator's Brochure supplied by Navidea Biopharmaceuticals, Inc.

1.3.1 Nonclinical Evaluations – IV and Intraperitoneal Administration

Nonclinical studies with tilmanocept demonstrated that the drug selectively binds to its intended receptor (the MBR), is appropriately distributed for radio detection of lymphatic tissue, and is well tolerated by rats, rabbits, guinea pigs, and dogs.

PK (pharmacokinetics) data obtained from SC repeated dose toxicity studies in rats and dogs demonstrated that absorption into the plasma was rapid in both species. Urinary excretion was a major pathway of elimination. A tissue distribution study demonstrated that tilmanocept exhibited rapid clearance from the injection site, rapid uptake by the local lymph node, and low uptake by the remaining lymph nodes. In addition, substantial accumulation was noted in the kidneys, bladder, and bladder contents by 3 hours post dose, providing additional evidence that urinary excretion occurs rapidly and is a major route of elimination. Tilmanocept was well tolerated at all doses tested in nonclinical safety pharmacology studies and in a number of single and repeated dose toxicology studies in rats, rabbits, and dogs. In some studies in rabbits and dogs, tilmanocept acted as a local irritant of the subcutis or skeletal muscle, and induced mild inflammation and tissue degeneration. The no-observed adverse-effect level (NOAEL) in the definitive repeated dose studies in rats and dogs was 42 µg/kg/day.

Tilmanocept was not mutagenic or genotoxic in vitro (mouse lymphoma test, Ames test) or in vivo (mouse micronucleus test). No signs or symptoms of hypersensitivity were observed in an in vivo study

in male guinea pigs. Most of the nonclinical safety studies used SC drug administration, a proposed route for administration to humans. The human dose for clinical studies is 50 µg Tilmanocept per procedure, equivalent to 0.714 µg/kg if a conservative estimate of 70 kg is used for human body weight. The single dose administration in animals ranged from 14 to 280 µg/kg in animals, which is approximately 20 to 390 times the human µg/kg dose. Repeated doses in animals ranged from 10.5 to 42 µg/kg/day in animals and are approximately 15 and 60 times the human µg/kg dose. These doses were all well tolerated in the nonclinical studies.

Dosing in Dogs, Guinea Pigs and Mice: Intravenous (IV) dosing nonclinical studies represents cumulative dosing in male and female beagle dogs [eight dogs (4/sex; wt females= 7.6 kg; wt males = 10.5 kg) given an IV injection of saline on Study Day (SD) 1 and 2 and DTPA-mannosyl-dextran at 0.084 mg/kg on SD 4, 0.42 mg/kg on SD 6, and 0.84 mg/kg on SD 8 and 10] 1,560X the 50 µg and 195X the 400 µg dose (female dogs) and 1706X the 50 µg dose and 213X the 400µg dose (male dogs). Based on the highest single dose administration (0.84 mg/kg) the dogs received 600X the 50 µg and 75X the 400 µg dose (female dogs) and 657X the 50 µg and 82X the 400 µg dose (male dogs). [NOTE: In a separate dog study the dose was 0.56 mg/kg in 2 each male and female dogs of similar weight/sex), no effect was noted in that study, consistent with the higher dose dog study].

With regard to the sensitization test (IV administration in guinea pigs, wt = 0.415 kg) the maximum dose was the equivalent of 96X of the anticipated high dose tilmanocept.

Lastly, although intraperitoneal administered in mice, based on the highest single dose administration (2000 mg/kg) the mice received 300,000 X the 50 µg and 37,500X the 400 µg dose (female mice, wt = 22 gm) and 340,000X the 50 µg and 42,500X the 400 µg dose (male mice, wt = 29 gm).

Type of Study / Description	Test System	Method of Administration	Dosing
Central nervous system safety pharmacology	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Expanded single-dose toxicology (including toxicokinetics and local tolerance)	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of Tilmanocept following Intravenous Bolus Injection in Male Rats	Rat	Intravenous	60, 120, and 300 µg/animal or equivalent 320X and 41X the anticipated study doses of 50 µg and 400 µg in humans
In Vitro Evaluation of Tilmanocept as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes	Human Liver Samples	In vitro	0.6 to 600 nM

Type of Study / Description	Test System	Method of Administration	Dosing
In Vitro Evaluation of Tilmanocept as an Inhibitor of Human ABC and SLC Transporters	Human Liver Samples	In vitro	0.04, 0.4 µM
Pharmacokinetics, Excretion, and Distribution by Quantitative Whole-Body Autoradiography Following Intravenous Administration of 99mTc-Tilmanocept in Rats	Rat	Intravenous	25 µg in 0.5 mL with collection of Blood, Urine, Feces, and Carcasses for QWBA
Hemolysis and protein flocculation	Human blood samples	In vitro	2.5, 25, and 250 µg/mL whole human blood
Target profiling screen	Ion Channel	In vitro	See Individual Tests Below
K Ion Channel	Ion Channel	The cardiac potassium channel, hERG, is responsible for a rapid delayed rectifier current (IKr) in human ventricles. This channel has been selected for evaluation because inhibition of IKr is the most common cause of cardiac action potential prolongation by non-cardiac drugs. In this assay, hERG potassium channels are expressed in a human embryonic kidney (HEK293) cell line that lacks endogenous IKr.	0.025, 0.05, 0.25, 0.5 mg/mL
Na Ion Channel	Ion Channel	Cloned hNav1.5 sodium channels (SCN5A gene expressed in CHO cells)	0.025, 0.05, 0.25, 0.5 mg/mL

Type of Study / Description	Test System	Method of Administration	Dosing
Ca Ion Channel	Ion Channel	1. Cloned L-type calcium channels (hCav1.2, encoded by the human CACNA1C gene and coexpressed with the β2 subunit, encoded by the human CACNB2 gene and the α2δ1 subunit encoded by the human CACNA2D1 gene in CHO cells), responsible for ICa,L, high threshold calcium current. 2. Cloned hNav1.5 sodium channels (SCN5A gene expressed in CHO cells).	0.025-0.5 mg/mL

Conclusions from these Tests:

CNS: In conclusion, a single IV administration of tilmanocept was well tolerated in rats at levels of 0.15, 0.75, and 1.50 mg/kg. Brief sedation shortly after dosing was observed at 0.75 and 1.50 mg/kg, which resolved by the time of the first functional observational battery assessments and was attributed to the mannosyl-dextran content of tilmanocept. There were no tilmanocept-related effects on functional observational battery parameters.

Single Bolus Toxicity: Tilmanocept-related clinical pathology changes were limited to minimally greater, dose-related, aspartate aminotransferase values for 0.75 and 1.5 mg/kg males and females that were likely caused by muscle or erythrocyte release as changes did not occur in other hepatobiliary-related clinical pathology parameters. This change had resolved at the end of the recovery phase.

At the Day 2 necropsy, dark focus in the glandular stomach was considered to be a potential test article-related gross pathology finding in males at ≥ 0.75 mg/kg. The gross finding of dark focus in the stomach correlated microscopically with focal erosion or minimal hemorrhage. Focal erosion in the stomach was test article related in males at ≥0.75 mg/kg, but was considered to be of little toxicological significance. No test article-related organ weight changes were noted. At the end of the recovery phase (Day 15), there were no test article-related findings in gross pathology, organ weights, or histopathology.

Respiratory: In conclusion, respiratory function was assessed in male Crl:CD(SD) rats given a single IV injection dose of vehicle control article or 0.150, 0.300, or 0.750 mg/kg of tilmanocept at a dose volume of 3 mL/kg. Administration of tilmanocept had no effect on mortality, but it was associated with severe abnormal clinical observations of hypoactivity, ataxia, labored or irregular respiration, and pale skin of entire body for animals given 0.750 mg/kg. Administration of >0.150 mg/kg tilmanocept had no direct effect on respiration rate, but it was associated with higher tidal volume (up to +26, +14, and +50% in animals given 0.150, 0.300, or 0.750 mg/kg, respectively) and higher minute volume (up to +18, +5, and +40% 0.5 through 1 hour post-dose in animals given 0.150, 0.300, or 0.750 mg/kg, respectively).

CYP: This study was designed to independently evaluate the in vitro inhibitory effect of tilmanocept on major CYP enzymes in human liver microsomes. Specifically, the inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 (utilizing two different substrates) by tilmanocept was assessed with the aim of ascertaining the potential of tilmanocept to inhibit the metabolism of concomitantly administered drugs.

To evaluate tilmanocept as a direct, time-dependent and metabolism-dependent inhibitor of CYP activity, human liver microsomes from a pool of 200 human donor sources were incubated with marker substrates in the presence or absence of tilmanocept. To distinguish between time-dependent and metabolism-dependent inhibition, tilmanocept was preincubated with human liver microsomes for 30 min without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrate. Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments.

Under these experimental conditions, there was little or no evidence of direct, time- or metabolism-dependent inhibition of any CYP enzyme by tilmanocept.

ABC and SLC transporters: It was found that tilmanocept was not an inhibitor of human transporters (namely P-gp, BRCP, OAT1B1, OATP1B3, OAT1, OAT3, and OCT2) under the conditions tested.

Hemolysis: No hemolysis and no flocculation were observed following in vitro treatment of human whole blood with tilmanocept at final whole blood concentrations of 2.5, 25 or 250 μg/mL.

Ion Channels (Na+, K+, Ca2+): Although there is a small dose-dependent effect that is fractional to the positive control, variability within concentration renders the median value observations not significantly different (Kruskal-Wallis).

1.3.2 Clinical Pharmacokinetics and Pharmacodynamics in Lymphatic Mapping Clinical Trials (Subcutaneous, Peritumoral and Intradermal administration)

After a single peritumoral (PT), ID, or SC dose for solid tumor lymphatic mapping, Tc 99m tilmanocept demonstrated significantly faster injection site clearance than Tc 99m sulfur colloid (TcSC, filtered and unfiltered), the standard imaging agent and comparator used in the Phase 1 trials.

The mean Tc 99m tilmanocept injection site clearance half-life was approximately 2 to 3 hours vs. approximately 15 to 27 hours for TcSC. Absolute uptake in the primary "sentinel" lymph node (L_{SN}) was dose-related for Tilmanocept, although relative nodal uptake (%ID_{SN}) overall was independent of dose and ranged from 0.05%ID_{SN} to 1.81%ID_{SN}, while the TcSC values ranged from 0.64%ID_{SN} to 3.66%ID_{SN}; differences between Tc 99m tilmanocept and TcSC were not statistically significant.

In the Phase 2 trial, Tc 99m tilmanocept was highly effective in identifying tumor-draining lymph nodes (i.e., overall, a "hot spot" from Tc 99m tilmanocept was identified in 93.0% of patients for whom preoperative lymphoscintigraphy was performed, and the per patient intraoperative localization rate was 96.2%). Diagnostic performance of in vivo Tc 99m tilmanocept findings relative to pathology assessment of tumor tissue indicated a high per tissue sensitivity estimate (overall, 92.0%). The overall false negative rate (FNR) for pathology was 8.0%, supporting the accuracy of Tc 99m tilmanocept in identifying lymph nodes with a high potential for containing tumor metastases in the lymphatics draining the tumor bed. The effect of time between Tc 99m tilmanocept injection and surgery on the localization rate was also evaluated, with surgeries done either on the same day as the Tc 99m tilmanocept injection or on the following day. In patients with melanoma, the time interval made no difference in the localization rate. In patients with breast cancer, the same day surgery group had a

95.5% (21 out of 22 patients) localization rate compared with 88.9% (8 of 9 patients) in the next-day surgery group, although the difference is not statistically significant (p = 0.5032, Fisher's exact test) and can be attributed to the small number of breast cancer patients in the next day surgery group, and not the time between injection and surgery.

1.3.3 Clinical Efficacy in Lymphatic Mapping/Sentinel Node Mapping Clinical Trials

Clinical efficacy was evaluated in two pivotal Phase 3 clinical trials in patients with breast cancer and melanoma, comparing lymph node detection of Tc 99m tilmanocept and with the FDA-approved intraoperative Tc 99m tilmanocept mapping (ILM) agent and standard-of-care, vital blue dye (VBD). Tc 99m tilmanocept demonstrated a statistically significant concordance rate with VBD (meta-analysis concordance rate = 99.99%, $p < 0.0001$).

The detection concordance between Tc 99m tilmanocept and VBD was similar among patients with melanoma and patients with breast cancer (meta-analysis concordance rate = 99.99% for both populations). Tc 99m tilmanocept also demonstrated a higher sensitivity for detecting tumor-positive (as confirmed by pathology) lymph nodes, corresponding to a decreased FNR when compared with VBD on a per node basis. The corresponding sensitivity rate in detection of lymph nodes most likely to be positive for tumor cells for Tc 99m tilmanocept was 99.99%, compared with 78.02% for VBD. The FNR for VBD (21.98%) was higher than the FNR for Tc 99m tilmanocept (0.01%). These findings suggest that, when VBD is used as the imaging agent, there is an increased risk of missing the detection of tumor-involved lymph nodes and incorrectly staging cancer patients. The Tc 99m tilmanocept-only findings (pathology-positive nodes that were hot/not blue) suggest that Tc 99m tilmanocept was markedly more effective in identifying lymph nodes that harbored disease than was VBD. Clinical safety has been evaluated in an integrated analysis of the pooled safety population (506 patients) from six completed trials and one ongoing clinical trial. No on-study deaths in Tc 99m tilmanocept -treated patients have occurred in this population. Twenty-seven serious adverse events (SAEs) have been reported for 26 patients across all Tilmanocept studies. None of the SAEs were attributed to the use of Tc 99m tilmanocept. No patients were withdrawn from any study due to an SAE or adverse event (AE). No clinically significant safety event associated with the use of Tc 99m tilmanocept has been reported in the clinical trials.

1.3.4 Clinical Safety using Intradermal, Subcutaneous, Peritumoral, and Intravenous Injections

Non-IV Experience - Well over 250,000 patients in clinical trials and US commercial use for intraoperative lymphatic mapping (ILM) with sentinel lymph node biopsy (SLNB) have been exposed to tilmanocept, and there have been no safety signals, no deaths due to drug, and no SAEs due to tilmanocept. There are no known drug interactions leading to contraindications with the use of tilmanocept. Post-marketing reports have shown less than 0.12% of subjects experiencing AEs, with the most common one being lack of node localization.

IV Experience - Navidea is currently conducting disease imaging studies in patients with rheumatoid arthritis (RA), nonalcoholic steatohepatitis (NASH), colorectal cancer (CRC) with metastases to the liver, and cardiovascular disease (CVD) utilizing Tc 99m tilmanocept administered IV. Dosages consist of 50, 100, 200 or 400 µg of tilmanocept labeled with 1, 2, 5, or 10 millicurie(s) (mCi) of ^{99m}Tc injected IV. At the time of this writing, sixty (60) subjects have received IV administration of Tc 99m tilmanocept. No subject has exhibited any adverse reaction to the drug that was related to the drug.

2 TRIAL OBJECTIVES

2.1 Primary Objective(s)

To determine the safety of escalating IV doses of Tc 99m tilmanocept in HIV subjects with biopsy-confirmed KS.

2.2 Secondary Objective(s)

- Concordance between clinical assessment/diagnosis and the localization of Tc 99m tilmanocept by planar and/or SPECT/CT imaging in cutaneous and non-cutaneous sites of KS.
- Qualify and quantify Tc 99m tilmanocept localization intensity on imaging with CD206 locale and quantity by histology and IHC in biopsied KS lesions to determine optimal IV dose.
- Concordance of localization of Tc 99m tilmanocept via IV and SC routes of administration by planar and/or SPECT/CT imaging in cutaneous and non-cutaneous sites of KS.

2.3 Exploratory Objective(s)

- Quantify HHV8 in biopsied KS lesions by qPCR

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Trial Design

This is a prospective, single-center, open-label, non-randomized, dose escalation, comparative, safety study of IV and SC injected Tc 99m tilmanocept in the localization and detection of cutaneous and non-cutaneous KS lesions in subjects with biopsy-confirmed KS. This study includes IV administration of 3 doses Tc 99m tilmanocept: 100µg/ 5 mCi, 100 µg/ 10 mCi and 200µg/ 5 mCi. All subjects will receive IV administration at one of these three doses. Cohort 3 will serve as a comparator arm, and Cohort 3 subjects will additionally receive a SC injection of Tc 99m tilmanocept at the Cohort 3 dose of 200µg/ 5 mCi . Following IV injection of Tc 99m tilmanocept, all subjects will have whole body planar imaging with optional SPECT/CT imaging beginning 60-75 minutes post-IV injection. Following SC injection of Tc 99m tilmanocept, Cohort 3 subjects will undergo whole body planar imaging with optional SPECT/CT imaging twice: first beginning 60-75 minutes after SC injection , and again 4-6 hours post-SC injection. The Schedule of Events, Appendix 1, contains a list of all study procedures and time-points. Study activities are described in detail in Section 7.

3.2 Justification for Study Design and Population

This study is designed to evaluate the safety and tolerability of escalating doses of IV Tc 99m tilmanocept and to compare results obtained from IV and SC administrations of Tc 99m tilmanocept in the same subjects. Whole body planar imaging will be utilized, as well as optional SPECT/CT to provide greater resolution of areas of Tc 99m tilmanocept localization.

This study is designed to evaluate the use of Tc 99m tilmanocept as an imaging agent in HIV-positive subjects with biopsy-confirmed KS by evaluating the localization in known and unknown cutaneous and non-cutaneous lesions.

No prior clinical trials have been conducted to evaluate IV administration of Tc 99m tilmanocept performance in subjects with KS. One previous clinical trial was conducted to evaluate SC administration of Tc 99m tilmanocept performance in subjects with KS. The rationale for evaluating Tc 99m tilmanocept in this subject population is discussed in Section 1.1 and Section 1.2.

3.3 Protocol Adherence

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

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

3.4 Trial Duration

Subjects will be “on trial” for up to 50 days.

4 STUDY POPULATION

The evaluation of Tc 99m tilmanocept in KS will involve up to 14 enrolled, injected, and imaged subjects.

4.1 Inclusion Criteria

Each subject must meet the following criteria to be enrolled in this study.

1. The subject has provided written informed consent with HIPAA authorization before the initiation of any study related procedures.
2. The subject is at least 18 years of age at the time of consent.
3. The subject is HIV positive.
4. The subject has a biopsy-confirmed diagnosis of KS and is classified into one of the categories below:
   a. Confirmed cutaneous KS/oral lesions without edema
   b. Confirmed cutaneous KS/oral lesions with edema
   c. Confirmed cutaneous KS/oral lesions with or without edema and suspected non-cutaneous KS due to clinical symptomology or confirmed non-cutaneous KS lesion(s).

4.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study.

1. The subject is pregnant or lactating.
2. The subject has received chemotherapy or radiation therapy to KS sites within six weeks of enrollment.
3. The subject has known sensitivity to dextran.
4. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration on Day 1.
5. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m tilmanocept on Day 1.
6. Any condition that, in the clinical judgment of the treating physician, is likely to prevent the subject from complying with any aspect of the protocol or that may put the subject at unacceptable risk.

4.3 Kaposi Sarcoma Classification

All subjects will have a biopsy-confirmed diagnosis of KS prior to screening. Subjects will be classified into 1 of 3 categories: a) confirmed cutaneous/oral lesions without edema, b) confirmed cutaneous/oral lesions with edema, or c) confirmed cutaneous/oral lesions with or without edema and suspected non-cutaneous KS due to clinical symptomology or confirmed non-cutaneous lesions.

4.4 Recruitment

Up to fourteen (14) evaluable subjects will be recruited from medical practice in accordance with the inclusion and exclusion criteria listed above. Potentially suitable subjects will be asked by the treating specialist about their willingness to participate in this trial. An assessment of safety and preliminary localization results will occur at the completion of each cohort. Up to fourteen (14) subjects will be enrolled, injected, and imaged.

4.5 Withdrawal

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

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

Should a subject withdraw after administration of the investigational product, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible.

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

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

4.6 Enrollment and Screen Failures

Subjects who sign an informed consent form (ICF) but are ultimately not enrolled in the trial will be considered screen failures. Subjects will be assigned a subject identification number at the time the ICF is signed. A subject will be considered enrolled once injected with technetium Tc 99m on Day 1. Demographic data and the reason for the screen failure will be collected for all screen failure subjects. Enrollment will continue until a total of up to 14 subjects have completed planar imaging.

Subject Identification

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

Digits 1 to 2: Trial number 24
Digits 3 to 4: Site number (e.g., "01")
Digits 5 to 7: Sequential subject number (e.g., "001", "002", "003")

For example, the first subject consented at site 01 is subject number “24-01-001.”

Subjects will maintain the same number given at screening for the entire trial. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Identification of Investigational Product

Tc 99m tilmanocept is a radiopharmaceutical that accumulates in KS lesions and lymphatic tissue by binding to mannose binding receptors (CD206) that reside on the surfaces of dendritic cells and macrophages.

5.2 Investigational Product Dosage and Administration

This study includes IV administration of 3 doses Tc 99m tilmanocept: 100µg/ 5 mCi, 100 µg/ 10 mCi, and 200 µg/ 5 mCi. All subjects will receive IV administration at one of these three doses. Cohort 3 will serve as a comparator arm, and Cohort 3 subjects will additionally receive a SC injection of Tc 99m tilmanocept at the Cohort 3 dose of 200 µg/ 5 mCi (see Table 1). At least one subject enrolled into each cohort must meet inclusion criteria 4c (suspected non-cutaneous or confirmed non-cutaneous KS), as described in Section 4.1.

Table 1. Dosing Cohorts

	Tc 99m tilmanocept Dose	Route of Administration	Number of Subjects
Cohort 1	100 µg/ 5 mCi	IV	n = 4
Cohort 2	100 µg/ 10 mCi	IV	n = 4
Cohort 3	200 µg/ 5 mCi	IV and SC	n = up to 6

All subjects will receive Tc 99m tilmanocept through an IV route of administration. A single syringe will be used and injected as a slow push into the venous catheter. The preferred site of catheter placement will be the left or right antecubital vein. Injection volume will be 1.0 mL in sterile normal saline. At the completion of the injection, a 10 cc sterile normal saline flush will be administered.

Cohort 3 subjects will also receive Tc 99m tilmanocept through a SC route of administration. Two syringes will be used and injected bilaterally as a slow push into the ankle(s) or the dorsal surface of the foot (feet). Total injection volume will be 1.0 mL (0.5 in each syringe) in sterile normal saline. Each syringe will contain half of the intended total dose of 200 µg/ 5 mCi (100 µg/ 2.5 mCi per syringe).

5.3 Treatment Assignment

In this prospective, single center, open-label, non-randomized, dose-escalation, comparative, safety study, Cohort 1 subjects will receive an IV administration of 100 µg/ 5 mCi Tc 99m tilmanocept. Cohort 2 subjects will receive an IV administration of 100 µg/ 10 mCi Tc 99m tilmanocept. Cohort 3 subjects will receive a SC and an IV administration of 200 µg/ 5 mCi Tc 99m tilmanocept approximately one week apart. Cohort 1 will be the starting dose group. Four subjects will be enrolled into Cohort 1.

If one of the four Cohort 1 subjects experiences a noxious pharmacologic activity/an ADR, two more subjects will be treated at the same tilmanocept mass dose and same radiolabel level as the subject with the ADR. If 2 out of 4 or 3 out of 6 subjects experiences a noxious pharmacologic activity/an ADR in

Cohort 1, the maximum tolerated dose (MTD) cannot be determined. If no additional noxious pharmacologic activities/ADRs are observed in the expanded cohort, the dose escalation will continue with Cohort 2. If one additional noxious pharmacologic activity/ADR is observed, the current dose will be deemed the MTD. If more than one additional pharmacologic activity or ADR is observed in the expanded cohort, the previous dose will be deemed the MTD.

If no noxious pharmacologic activities/ADRs are experienced in Cohort 1, Cohort 2 will be open for enrollment.

Dose escalation will continue until the MTD is found or until Cohort 3 is reached with only one ADR or without any ADRs. The maximum tolerated dose is defined as the highest dose level at which no more than 33% of subjects experienced an ADR. Visual representation of the dose escalation scheme may be found in Appendix 2.

NOTE: Adverse Drug Reaction

An Adverse Drug Reaction (ADR) includes all noxious and unintended responses to a medicinal product related to any dose or dose regimen. The phrase "responses to a medicinal product" means that a casual relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

5.4 Packaging and Labeling

Tilmanocept cartons ready for radiolabeling will be shipped and stored at the region-specific Cardinal Health radiopharmacy. Tilmanocept is provided in a vial. Vials are packaged as a kit. A carton contains five vials of tilmanocept. One kit, which is one tilmanocept vial, should be used for no more than one subject. The carton also contains five shield labels and 25 syringe labels. This package has been designed specifically for tilmanocept and protects the vials during shipment, handling, and storage. Navidea will provide a radiolabeling protocol and Quality Control worksheets. Cardinal Health will radiolabel tilmanocept with the protocol cohort specified tilmanocept dose and the cohort assigned millicurie dose Tc 99m in 1.0 mL and deliver one syringe for IV doses and two syringes (0.5 mL each) for SC doses to the clinical site that is/are ready for administration.

5.5 Investigational Product Accountability

The investigator (or designated personnel) will confirm receipt of the investigational product in writing and will use the investigational product only within the framework of this clinical trial and in accordance with this study protocol. For each subject, he/she will keep a record of the investigational product dispensed and all other accompanying forms to the investigational product. These documents are to be filed in the ISF.

Overall drug accountability and reconciliation will be completed by the sponsor or its representative. A list of investigational product vials and other materials that were returned, or destroyed, must be recorded and signed by the principal investigatior (PI) or an appropriately qualified designee as documented in the study site responsibility sheet. Each time the investigational product is dispensed to a subject, the following information should be recorded on the Drug Accountability Log: subject number, radiolabel date, and the date delivered to nuclear medicine. At the completion or termination of the trial, a final drug accountability review and reconciliation must be completed and any discrepencies must be investigated and their resolution documented. All unused trial kits will be destroyed in accordance with institutional destruction procedures.

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapy

All medications taken 30 days prior to Visit 2 (Day 1) Tc 99m tilmanocept injection through the Follow-Up Phone Call will be documented.

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

Cohorts 1, 2, and 3:

7.1 Screening Visit (within 30 days of Day 1 injection)

- Obtain signed informed consent for study participation
- Preliminary review of inclusion and exclusion criteria
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Interview including the following subject-specific characteristics:
  - Demographic data - date of birth, gender, race
  - Medical/surgical history - all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
  - Concomitant Medications (within 30 days before Day 1 injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position).
- Electrocardiogram (ECG); subjects will either have a normal ECG or an abnormal ECG deemed not clinically significant by the PI.
- Blood draw for routine hematology, chemistry, serum pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Urine collection for routine urinalysis.
- Physical examinations will include assessment of height and weight and an examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Assessment of edema/lymphedema, utilizing a pitting edema scale as provided in Appendix 3, will be performed.
- KS history and classification.
- KS Lesion Assessment:
  - Cutaneous KS evaluation: All cutaneous lesions will be identified, documented, and photographed during the screening exam. Photographs will be collected per the photography protocol currently in use within the Department of Dermatology at UCSF, as provided in Appendix 4.
  - Non-cutaneous KS evaluation: Confirmed based on specific appropriate tests or suspected based on clinical symptomology. Non-cutaneous lesions will be identified and documented.

Changes in health occurring after consent and prior to the day of injection will be added to the subject's medical history unless related to a study procedure.

Cohorts 1 and 2:

7.2 Day 1 (IV Injection)

7.2.1 Before Injection

- Assessment of adverse events
- Concomitant medication review
- A negative urine pregnancy test for women of child-bearing potential within 48 hours of injection.
- Final check of inclusion/exclusion criteria
- Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate within 30 minutes prior to investigational product injection, after at least 1 minute in a resting position)

7.2.2 IV Administrstion of Tc 99m tilmanocept

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be the left or right antecubital vein.

Tc 99m tilmanocept shall be administered through the catheter as a slow IV push followed by a 10 cc sterile normal saline flush. The injection will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician.

7.2.3 Post-Injection

The following procedures will be completed at the specified timepoints:

- 0-30 Minutes Post-Injection
    - ECG (completed before vital signs)
    - Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate) will be collected
    - Assessment of adverse events
- 60-75 Minutes Post-Injection
    - Assessment of adverse events
    - Whole body planar imaging
    - Optional SPECT/CT region(s) of interest imaging
- After the 60-75 Minute Imaging:
        - Clinical labs and urinalysis will be performed.
        - A KS tissue biopsy will be taken of an imaging-visualized non-visceral lesion

- If no lesion is visualized on imaging, a lesion of at least 5-6 mm in a clinically accessible area will be biopsied as chosen by the Investigator(s) who will record selection and rationale
- A photograph of the biopsy site with a measurement scale must be taken and stored with the subject's research chart

7.3 Follow-Up Telephone Call (7±3 days after Tc 99m tilmanocept injection)

- Concomitant medication review
- Assessment of adverse events

Cohort 3:

7.4 Day 1 (SC Injection)

7.4.1 Before Injection

- Assessment of adverse events
- Concomitant medication review
- A negative urine pregnancy test for women of child-bearing potential within 48 hours of injection.
- Final check of inclusion/exclusion criteria
- Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate within 30 minutes prior to investigational product injection, after at least 1 minute in a resting position)

7.4.2 SC Administrstion of Tc 99m tilmanocept

SC administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of SC injection placement will be the ankle(s) or the dorsal surface of the foot (feet).

The injection will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician.

7.4.3 Post-Injection

The following procedures will be completed at the specified timepoints:

- 0-30 Minutes Post-Injection
    - ECG (completed before vital signs)
    - Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate) will be collected
    - Assessment of adverse events
- 60-75 Minutes Post-Injection

  - Assessment of adverse events
  - Whole body planar imaging
  - Optional SPECT/CT region(s) of interest imaging
- 4-6 Hours Post-Injection
  - Assessment of adverse events
  - Whole body planar imaging
  - Optional SPECT/CT region(s) of interest imaging
- After the 4 – 6 Hour Imaging:
    - Clinical labs and urinalysis will be performed.

7.5 Day 7 ± 3 (IV Injection)

7.5.1 Before Injection

  - Follow-up assessment from SC injection including changes in concomitant medication and adverse events

- A negative urine pregnancy test for women of child-bearing potential within 48 hours of injection.
- Final check of inclusion/exclusion criteria
- Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate within 30 minutes prior to investigational product injection, after at least 1 minute in a resting position)

7.5.2 IV Administrstion of Tc 99m tilmanocept

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be the left or right antecubital vein.

Tc 99m tilmanocept shall be administered through the catheter as a slow IV push followed by a 10 cc sterile normal saline flush. The injection will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician.

7.5.3 Post-Injection

The following procedures will be completed at the specified timepoints:

- 0-30 Minutes Post-Injection
  - ECG (completed before vital signs)
  - Vital signs (body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate) will be collected
  - Assessment of adverse events
- 60-75 Minutes Post-Injection
  - Assessment of adverse events
  - Whole body planar imaging
  - Optional SPECT/CT region(s) of interest imaging

- After the 60-75 Minute Imaging:
    - Clinical labs and urinalysis will be performed.
    - A KS tissue biopsy will be taken of an imaging-visualized non-visceral lesion
        - If no lesion is visualized on imaging, a lesion of at least 5-6 mm in a clinically accessible area will be biopsied as chosen by the Investigator(s) who will record selection and rationale
        - A photograph of the biopsy site with a measurement scale must be taken and stored with the subject's research chart

7.6 Follow-Up Telephone Call (7±3 days after IV Tc 99m tilmanocept injection)

- Concomitant medication review
- Assessment of adverse events

7.7 Imaging and Acqusition of Imaging Data

Imaging will be a whole body planar scan followed by optional SPECT/CT region(s) of interest scan beginning at 60-75 minutes post-IV injection (all Cohorts) and 60-75 minutes and 4-6 hours post-SC injection (Cohort 3 only).

7.8 Image Acqusition

The same scanner should be used during the entire course of the study and any updates to hardware or software should be avoided. If changes occur, they must be reported to the sponsor. Whole body planar with optional SPECT/CT images will be obtained from each subject at the 60-75 minute post-injection timepoint following IV injection (all Cohorts) and at the 60-75 minute and 4-6 hours post-injection timepoints following SC injection (Cohort 3 only).

7.9 Evaluation of Planar and SPECT Images

Tc 99m tilmanocept localization in cutaneous and non-cutaneous lesions will be evaluated through image analysis by the onsite Nuclear Medicine physician. First, an assessment of the overall technical adequacy of the scan will be made. Following the technical assessment, an overall assessment of the scan will be made. For each subject, the de-identified planar images and SPECT/CT scans (in DICOM [digital imaging communications in medicine] format) will be forwarded to the sponsor.

7.10 End of Study

For the entire study, end of study is defined as the follow-up telephone call.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Up to fourteen (14) HIV-positive female or male subjects ≥ 18 years that have been diagnosed with biopsy-confirmed KS will be enrolled, injected, and imaged. There are no minimum or maximum numbers for gender in the cohorts.

8.1.2 Medical and Surgical History

Relevant medical and surgical histories, specifically for HIV and KS, will be obtained on all study subjects.

As part of the medical history, the date of the last spontaneous menstruation will be recorded, if childbearing potential is not excluded by surgical sterilization.

8.1.3 Prior and Concomitant Medication

All prior medications used within the last 30 days before Visit 2 (Day 1) Tc 99m tilmanocept administration through the Follow-Up Call will be documented.

8.2 Tc 99m Tilmanocept Preparation and Administration

Based on the dose cohort the subject is enrolled in, the dose of Tc 99m tilmanocept should be ordered from Cardinal Health once the subject has been scheduled for imaging.

In Nuclear Medicine, prior to IV injection and imaging, an indwelling catheter will be placed for venous access. The preferred site of IV placement should be the left or right antecubital vein. Other IV access sites are acceptable if access is problematic.

The filled syringe will be connected to the catheter for a slow push injection. Immediately after the completion of the injection, a 10 cc sterile normal saline flush will be administered. Injection of Tc 99m tilmanocept will be at study time 0:00.

For SC injection, the preferred site of placement is the ankle(s) or the dorsal surface of the foot (feet). Approximately 0.5 mL of the 1.0 mL prepared injection shall be administered to each the left and right leg. Injection of Tc 99m tilmanocept will be at study time 0:00.

8.3 SPECT/SPECT-CT Image Acquisition

The camera used to obtain the images should be a 2-headed SPECT or SPECT/CT camera equipped with a low-energy, high-resolution collimator with a 15% window (20% can be used if 15% setting not available) centered over a 140keV peak.

The camera must have passed the daily SPECT QC (quality control) tests as per the manufacturer's recommendation for that day's scan schedule.

Whenever possible, subject should be asked to void after injection and prior to the imaging session.

Subjects should be positioned supine with arms at their side. The subject's head, legs, hands, and hips should be in the true anterior position. The subject should be instructed to remain as motionless as possible during the scan and asked to breathe as shallowly as comfortable when the camera is passing over the chest. To help minimize leg motion, a light tape around the ankles can be used to keep the legs together. A pillow under the knees is often very helpful for comfort.

A whole body planar scan should be obtained at the following time points:

- 60-75 minutes post-IV/SC administration
- 4-6 hours post-SC injection

Optional SPECT/CT region(s) of interest scan(s) can be obtained at the following time points:

- 60-75 minutes post-IV/SC administration
- 4-6 hours post-SC injection

It is anticipated that the whole body planar scan would be acquired first for 25-30 minutes. Using a 2-headed camera (nominal 20" x 15" FOV [field of view]) a target of 5-7 million counts should be obtained for the summed view of both heads. For the higher activity injections, it may be possible to attain this target in a shorter time, allowing the subject more time off the bed between scans.

Following the whole body scan, optional SPECT/CT region(s) of interest scan(s) can be obtained. It is anticipated that a SPECT/CT scan would be acquired over 25-30minutes. The use of a calibration syringe for each SPECT scan is not necessary. It is very important that the subject remain motionless for the duration of the complete SPECT/CT scan.

SPECT/CT slices will be reconstructed using the manufacturer's recommended reconstruction algorithms including the recommended attenuation correction.

It is strongly recommended that each site should perform all imaging acquisitions per their camera manufacturer and model parameters and in accordance with their institutional practices for both the whole body scan as well as the SPECT/CT imaging.

De-identified DICOM copies of all acquired images will be transmitted to Navidea Biopharmaceuticals. The areas of localization will be identified, documented and captured in the eCRFs.

8.4 Post-Injection and Imaging Biopsy Acquisition

The purpose of the KS lesion biopsy will be to determine the proportion of CD206-expressing cells and quantitiy of CD206 (IHC fluorescence) in KS tissues in parallel with an analysis of KS imaged with Tc 99m tilmanocept. A non-visceral site of KS for biopsy post-imaging in each subject will be chosen by the Nuclear Medicine physician based on visualized localization and lesion size. If no lesion is determined to localize Tc 99m tilmanocept, a lesion of at least 5-6 mm in a clinically accessible area will be biopsied as chosen by the Investigator(s) who will record selection and rationale.

All subjects will receive the biopsy on the day of IV Tc 99m tilmanocept administration at the completion of imaging.

A biopsy up to 6mm of the designated lesion will be taken, and a photograph of the biopsy site with a measurement scale will be taken and stored with the subject's research chart. The tissue will be obtained at the completion of Tc 99m tilmanocept IV injection and imaging and delivered on ice to the Department of Pathology at Zuckerberg San Francisco General Hospital. The biopsy will be sectioned into a formalin-fixed paraffin-embedded (FFPE) segment and a fresh or frozen segment to be used for other analyses. FFPE tissue sections will be quantified for CD206 and LANA through a standardized quantitative image analysis program. HHV8 will be quantified by qPCR.

8.5 Pharmacokinetics

No pharmacokinetic investigation will be performed in this study.

8.6 Safety

8.6.1 Adverse Events

8.6.1.1 Definition of Adverse Event

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

Adverse Event (AE)

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

Any clinically significant change in a condition (worsening) from screening that results in a change in subject management will be considered an AE and will be recorded on the AE page of the CRF.

By definition for this study, all untoward medical occurrences beginning on the day of Visit 2 Baseline (Day 1) through the Follow-up Phone Call are to be reported as AEs. AEs continuing after study completion will be followed to normalization or stabilization. Additionally, untoward medical events occurring prior to the day of Tc 99m tilmanocept administration will be collected and added to the subject's medical history unless they are related to a study procedure, in which case the event will be recorded as an AE. SAEs will be reported from the time of consent through the end of participation.

8.6.1.2 Categories for Adverse Event Assessment

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

Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 8.6.1.5.

Severity

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

- Mild - The AE is transient and easily tolerated by the subject.
- Moderate - The AE causes the subject discomfort and interrupts the subject's usual activities.
- Severe - The AE causes considerable interference with the subject's usual activities and may be incapacitating or life-threatening.

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationship to investigational product

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

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

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

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

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

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

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

Causal relationship to study procedure

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

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

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

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

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

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

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

8.6.1.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs for the duration of subject participation. Events occurring prior to Visit 2 (day of Tc 99m tilmanocept administration) will be recorded in the subject's medical history unless determined to be related to the study procedure. Untoward medical events beginning on Visit 2 (day of Tc 99m tilmanocept administration) through the completion of the Follow-up Phone Call will be reported as adverse events. Thus, subjects should be closely observed by the investigator both during and after the evaluation.

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

The following information is required:

- The **date** and **time of onset** of any AE.
- The duration (the entire duration of an event or symptom, calculated from date of onset to date of end, if not recorded directly).
- The seriousness of the AE will be assessed by the investigator. If the investigator deems that an AE qualifies as an SAE, a special form provided by the sponsor should be completed and the event must be immediately reported to the sponsor. A definition of SAEs is provided in Section 8.6.1.5.
- The maximum intensity (mild, moderate, or severe).
- Whether drug treatment was administered for the event, any specific drug treatment must be documented.
- The relationship of the AE to the investigational product and to study conduct (for definitions, see above).

  The **outcome** of the AE (resolved, resolved with sequelae, not resolved, unknown, death).

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

8.6.1.4 Expected Adverse Events

Investigational Product-Related Risks

In all completed studies of Lymphoseek involving 553 subjects, only three events (breast pain and injection site pain reported by subjects with breast cancer and injection site irritation reported by a subject with head and neck squamous cell cancer) were deemed definitely related to the administration of Lymphoseek by the investigator. The most common adverse reactions (incident <1%) have been lack of effect (<0.067%), injection site pain (<0.02%) and rash (<0.02%).

In addition to the Lymphoseek pre-approval clinical studies, post-marketing surveillance shows that Lymphoseek has been administered to more than 250,000 patients with not a single drug-related SAE. Routes of administration include subcutaneous, intradermal, and peritumoral.

In Tc 99m tilmanocept trials for indications beyond lymphatic mapping and SLNB, there have been approximately 20 SC and 60 IV administrations of Tc 99m tilmanocept, and no SAEs or ADRs have been reported to date.

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

Precautionary Measures

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

Unexpected Adverse Events

An unexpected adverse event is defined as an adverse reaction that in nature and severity is not consistent with the applicable product information (e.g., Investigator's Brochure). Any adverse experience that is not listed in the current Investigator's Brochure or which is, with regard to the specificity or severity, not consistent with the risk information shall be regarded as unexpected.

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

8.6.1.5 Serious Adverse Events

Definition of Serious Adverse Events

Definition

The following SAE definition is based on ICH guidelines and the final rule issued by the Food and Drug Administration (FDA) and effective 06 Apr 1998.

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

- results in death, or

- is life threatening, or
- requires inpatient hospitalization or prolongation of existing hospitalization, or
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect, or
- is an important medical event (see paragraphs below).

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

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

Actions and reporting obligations in case of serious adverse events

The investigator should take appropriate diagnostic and therapeutic measures to minimize the risk to the subject.

If any SAE occurs over the course of the study, investigators or other site personnel will inform Navidea Biopharmaceutical representatives within one day (i.e., within 24 hours) of becoming aware of the SAE. Written notification of the SAE will be emailed to Navidea Biopharmaceuticals Pharmacovigilance at **safety@navidea.com**. For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately.

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

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

Notification of the IRB(s)

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

Notification of the authorities

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

Sponsor's notification of the investigators

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

8.6.2 Further Safety Assessments

8.6.2.1 Physical Exam

A complete physical examination at screening will be conducted according to the Schedule of Study Events (see Appendix 1). Height and body weight will be collected.

Physical examinations will be performed for the following body systems:

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

8.6.2.2 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be obtained at screening and within 30 minutes after investigational product injection. The ECG will be measured with the subject in a resting position for at least 1 minute. No continuous ECG monitoring will be required. At a minimum the heart rate, QRS, PR and QT intervals will be collected. QTc will be calculated using the Fridericia formula.

On-site investigator's responsibilities

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

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

8.6.2.3 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, respiration, and systolic and diastolic blood pressure. All measurements will be taken after the subject has been in a resting position for at least 1 minute.

Vital signs will be measured at screening and within 30 minutes before investigational product injection, and within 30 minutes post-injection.

Any clinically significant change from screening (worsening) that results in a change in subject management will be considered an AE and will be recorded on the AE page of the CRF.

8.6.2.4 Clinical Laboratory Parameters for Screening and Safety

Clinical laboratory tests to be evaluated in this study include hematology, serum chemistry, and urinalysis. Clinical laboratory tests will include the following as defined in Table 2.

Table 2. Clinical Laboratory Parameters

Hematology	Leukocytes, hemoglobin (Hgb), hematocrit (HCT), neutrophils, eosinophils, basophils, lymphocytes, monocytes, red blood cells (RBC), platelets
Serum chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, globulin, carbon dioxide (CO_2), blood urea nitrogen (BUN)
Urinalysis	pH, specific gravity

The site laboratory will provide the necessary supplies to collect the blood and urine samples.

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

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

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

Any clinically significant changes in laboratory values are to be followed up with repeated tests at appropriate intervals (as determined by the investigator) until the values return to baseline level or until the abnormality is explained by the investigator.

The expected amount of blood to be withdrawn is shown in Table 3.

Table 3. Approximate Amount of Blood Withdrawn

Time point of examination	Amount of blood taken
Laboratory examination (Screening)	10 mL
Laboratory examination (Post-IV Imaging)	10 mL
Laboratory examination (Post-SC Imaging: Cohort 3 only)	10 mL
Total	20 mL (Cohort 3: 30 mL)

9 STATISTICAL METHODS

The study is a prospective, open-label, single-center, dose escalation, comparative, safety study of injected Tc 99m tilmanocept in the detection of and assessment of localization to cutaneous KS or non-cutaneous focus (visceral or other, e.g. CNS [central nervous system]) in HIV-positive subjects with active KS by planar and SPECT/CT imaging. The dose escalation portion of the study is a 4+2 design with no dose de-escalation. The statistical objective of the study is to determine the maximum tolerated dose (MTD) of Tc 99m tilmanocept and to compare the capabilities of Tc 99m tilmanocept to localize in cutaneous and non-cutaneous sites of KS. A study center is defined as a treatment administration site or cohort of treatment administration sites under the control and supervision of the same PI.

9.1 Randomization Methods

The study is not randomized.

9.2 Safety Variables

The primary variable for this study is the presence or absence of an ADR during the subject's participation in the trial following Tc 99m tilmanocept injection. The primary safety endpoint will be computed as the proportion of subjects with an ADR.

9.3 Efficacy Variables

The efficacy variables for this study are as follows:

- Nuclear medicine specialist determination of presence/absence of illumination (i.e., localization positive/negative, anatomic site) relative to background from SPECT imaging results for each subject;
- Clinically identified presence/absence of KS (based on any previous diagnostic evaluations);
- Quantitative SPECT gamma counts for each biopsied lesion.

The efficacy endpoints for this study are as follows:

- Per subject localization rate of Tc 99m tilmanocept in at least one KS suspected or confirmed lesion by planar and/or SPECT/CT imaging
- Per lesion/region concordance of Tc 99m localization with anatomical areas of active KS defined by confirmed diagnosis or clinical symptomology
- Localization intensity for each biopsied and clinically defined lesion as determined by quantitative SPECT gamma counts
- Per biopsied lesion proportion of CD206-expressing cells and total CD206 as determined by histology and relative IHC fluorescence
- Per lesion/region concordance of IV vs SC Tc 99m localization with anatomical areas of active KS defined by confirmed diagnosis or clinical symptomology.
- Per subject localization rate of Tc 99m tilmanocept in areas other than KS by planar and/or SPECT/CT imaging

- Per area localization rate of Tc 99m tilmanocept in the most frequently identified areas other than KS by planar and/or SPECT/CT imaging

A localized subject is one for whom Tc 99m by planar and/or SPECT/CT imaging results in the illumination of at least one site relative to background as determined by the nuclear medicine specialist.

A localized KS lesion is a lesion that is both clinically identified or suspected KS and planar or SPECT/CT illuminated.

Localization in areas other than KS will also be defined per subject and per area, where these areas are clinically negative KS locations based on clinical symptomology.

9.4 Sample Size Justification

The dose escalation portion of this study is a design with no dose de-escalation. The sequential cohort enrollment characteristics of this design do not allow a fixed computation of sample size. In a previous study with subjects receiving a 200 μg dose radiolabeled at 5 mCi (i.e., the maximum dose planned for this study), zero (0) subjects had an ADR. Therefore, it is expected that no subjects will have an ADR in this study and that each cohort will enroll the planned sample size of subjects. With 2 cohorts of 4 subjects and one cohort of up to 6 subject, it is anticpated that the study will enroll up to n=14 subjects.

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following analysis populations will be defined for the study:

- **Intent-to-Diagnose (ITD) Population** - Subjects who are enrolled in the study, injected with Tc 99m tilmanocept, and are imaged will be included in the ITD analysis population.
- **Safety Population** – All subjects who are enrolled in the study and are injected with Tc 99m tilmanocept will be included in the safety population.

All safety data analyses will be conducted on the safety population. All efficacy data analyses will be conducted on the ITD population.

9.5.2 Analysis of Baseline and Demographic Characteristics

The distribution of each baseline and demographic variable of interest will be summarized by dose cohort. Continuous variables will be summarized via mean, median, standard deviation, and range. Categorical variables will be summarized via counts and percentages. This analysis will be performed on the safety population.

9.5.3 Analysis of Efficacy Variables

The number and percentage of subjects with at least one biopsy-confirmed or clinically suspected KS lesion that has localized with Tc 99m tilmanocept by planar and/or SPECT/CT imaging will be

computed. A 95% exact confidence interval on the per subject localization rate will be computed. This analysis will be performed by dose.

Per region lesion localization of Tc 99m tilmanocept by planar and/or SPECT/CT imaging will be computed across all biopsy-confirmed or clinically suspected KS lesions of all subjects. A 95% exact confidence interval on per region lesion localization will be calculated. This analysis will be performed by dose.

Localization intensities of biopsied lesions will be summarized by descriptive statistics (mean, median, standard deviation, minimum, maximum and range) for each dose.

Per biopsied lesion proportion of CD206-expressing cells and quantity of CD206 as determined by histology and IHC fluorescence will be summarized by descriptive statistics (mean, median, standard deviation, minimum, maximum and range) for each dose.

Concordance between between IV and SC tilmanocept in biopsy-confirmed or clinically suspected KS lesion sites will assessed with a cross-tabulation including the joint probability distribution. The site will be the sampling unit for this analysis. An exact version of McNemar's test will be performed, testing the null hypothesis

$$H_0: \pi_{IV} = \pi_{SC}$$

$$H_A: \pi_{IV} \neq \pi_{SC}$$

whereπ_{IV} andπ_{SC} represent the probabilities of localization with IV (only) tilmanocept and localization with SC tilmanocept only respectively.

In addition, descriptive statistics will be generated relative to the concordance and reverse concordance of observed sites of discrete localization of Tc 99m tilmanocept following IV and SC administration.

The number and percentage of subjects with at least one anatomical site other than KS that has been localized with Tc 99m tilmanocept by SPECT imaging will be computed. A 95% exact confidence interval on the per subject localization rate will be computed. This analysis will be performed by dose and overall.

Locations showing localization of Tc 99m tilmanocept by planar and/or SPECT/CT imaging that are not locations of biopsy-confirmed or clinically suspected KS sites will be computed across all sites in all subjects. A 95% exact confidence interval on per location localization will be calculated. This analysis will be performed by dose and overall.

Other efficacy analyses will be described in the Statistical Analysis Plan (SAP) for the study.

9.5.4 Analysis of Safety Variables

The number and percentage of subjects with ADRs will be tabulated by dose group and overall.

All AEs will be observed for each subject from the time of signing of informed consent until exit from the study. A treatment emergent adverse event (TEAE) is defined as an adverse event whose start date is on or after the Tc 99m tilmanocept injection date and time. If the injection date and time and/or the AE start date are missing, the AE will be considered treatment emergent.

Prior to analysis, all AEs will be coded using the MedDRA coding dictionary. Based on these coded terms, treatment emergent AEs will be summarized by dose group and overall as follows:

- by system organ class and preferred terms
- by system organ class and preferred terms and relation to study drug
- by system organ class and preferred terms and severity

Observed and change from baseline vital sign parameters, ECG parameters and hematology, clinical chemistry and urinalysis parameters will be summarized using descriptive statistics (mean, median, standard deviation, minimum, maximum and range) by dose group and overall.

Other safety analyses will be described in the SAP for the study.

9.5.5 Handling Missing Values

The analysis of the primary efficacy variables will be carried out on the observed data, i.e., a complete case analysis.

9.5.6 Interim Analysis

There are no interim analyses planned for this study.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

Data required according to this protocol are to be entered into the CRFs (provided by the sponsor) as soon as possible.

10.1.1 eCRF Design

Electronic CRFs (eCRFs) will be used for collecting all data generated during the trial. eCRF completion details will be documented in a separate document that will be provided by the sponsor and maintained in the TMF.

10.2 Monitoring

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

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

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

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

10.3 Data Processing

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

For data coding (e.g., AEs, medication, medical/surgical history), internationally recognized and accepted dictionaries will be used.

10.4 Auditing

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

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

10.5 Archiving

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

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

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

The ISF is not to be destroyed without the sponsor's approval.

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

10.6 Premature Termination of the Trial

Termination by the Sponsor

The Sponsor may terminate the trial at any time for any of the following reasons:

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

Termination by the Investigator

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

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

10.6.1 Trial as a Whole

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

At the discretion of the sponsor, the entire trial may be canceled for medical reasons. In addition, the sponsor retains the right to end the trial at any time if the study cannot be carried out as agreed upon in the protocol.

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

10.6.2 Center

At any time, the trial may be terminated at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.6.3 Study Participant

Individual subjects may be withdrawn from the trial according to the criteria specified in Section 4.5.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

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

11.2 Subject Information and Consent

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

Based on this consent form and, if required, the subject information sheet, the investigator will explain all relevant aspects of the trial to each subject, before his/her entry into the trial (i.e., before examinations and procedures associated with selection for the trial are performed).

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

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

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

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

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

The informed consent form and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol which necessitates a change to the content of the subject information and/or the written informed consent form. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her participation in the trial by

signing the revised informed consent form. Any revised written informed consent form and written information must receive the IRB/IEC's approval/favorable opinion in advance of use.

11.3 Financing/Financial Disclosure

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

11.4 Publication Policy

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

11.5 Subject Injury

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

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Appendix 1 Schedule of Events – Cohorts 1 and 2

Evaluation	VISIT 1 Screening Days -29 to 0	VISIT 2 Pre- and Post-IV Injection Day 1		Follow-up Phone Call (Day 7±3)
		0:00	60-75 Minutes	
Informed Consent	x			
Entry Criteria	x			
Medical History	x			
Vital Sign Assessment	x	x[be]		
ECG	x	x[e]		
Physical Examination	x[c]			
Assessment of Edema/Lymphedema	x			
Photography of all KS Cutaneous Lesions	x			
Review of Medications	x	x		x
Clinical Laboratory Evaluation: Chemistry, Hematology, UA	x		x[d]	
Serum Pregnancy Test	x	x[a]		
Tilmanocept Administration		x		
Whole Body Planar and Whole Body SPECT/CT Imaging			x	
Biopsy of Non-Visceral Lesion			x[d]	
Adverse Event Monitoring	x	x	x	x

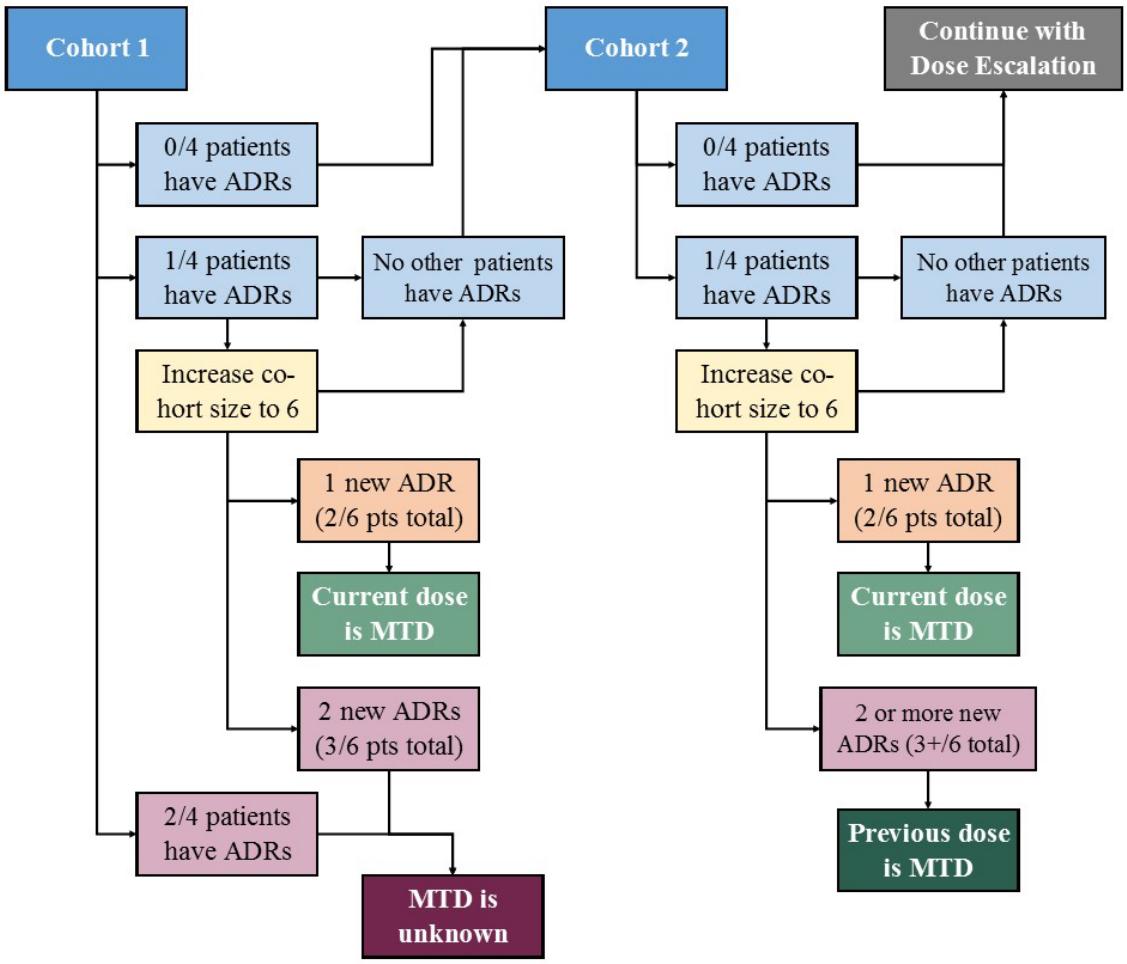
a. Urine pregnancy test must be completed and determined to be negative in women of childbearing potential within 48 hours of injection.
b. Time point 0:00 is before Tilmanocept administration (a 30 minute pre- and post injection window is permitted for vital signs).
c. Physical examinations done within 30 days of injection may be used even if conducted prior to obtaining informed consent if they are ordered and conducted at the direction of the Investigator as part of his/her standard of care.
d. Labs and biopsy will be completed at the conclusion of the 60-75 minute post-injection scan.
e. ECG and vital signs must be within 30 minutes post-injection.

Appendix 2 Schedule of Events – Cohort 3

Evaluation	VISIT 1 Screening Days -29 to 0	VISIT 2 Pre- and Post-SC Injection Day 1			VISIT 3 Pre- and Post-IV Injection Day 7 ± 3		7 ± 3 Day Post-IV Follow-up Phone Call
		0:00	60-75 Minutes	4-6 Hours	0:00	60-75 Minutes	
Informed Consent	x						
Entry Criteria	x						
Medical History	x						
Vital Sign Assessment	x	x[be]			x[be]		
ECG	x	x[e]			x[e]		
Physical Examination	x[c]						
Assessment of Edema/Lymphedema	x						
Photography of all KS Cutaneous Lesions	x						
Review of Medications	x	x			x		x
Clinical Laboratory Evaluation	x			x[d]		x[d]	
Serum Pregnancy Test	x	x[a]			x[a]		
Tilmanocept Administration		x			x		
Whole Body Planar and Optional SPECT/CT Imaging			x	x		x	
Biopsy of Non-Visceral Lesion						x[d]	
Adverse Event Monitoring	x	x	x	x	x	x	x

a. Urine pregnancy test must be completed and determined to be negative in women of childbearing potential within 48 hours of injection.
b. Time point 0:00 is Tilmanocept administration (a 30 minute pre- and post-injection window is permitted for vital signs).
c. Physical examinations done within 30 days of injection may be used even if conducted prior to obtaining informed consent if they are ordered and conducted at the direction of the Investigator as part of his/her standard of care.
d. Labs and biopsy will be completed at the conclusion of the 60-75 minute post-injection scan.
e. ECG and vital signs must be within 30 minutes post-injection.

Appendix 3 Dose Escalation Diagram



Appendix 4 Edema Scale

Grade	Description	Physical Characteristics
0	None	N/A
1+	Trace	Slight pitting, no visible change in the shape of the extremity; depth of indentation 0-¼" (<6mm)
2+	Mild	No marked change in the shape of the extremity; depth of indentation ¼-½" (6-12mm); disappears in 10 to 15 seconds
3+	Moderate	Noticeably deep pitting, swollen extremity; depth of pitting ½ -1" (1-2.5 cm); duration 1 to 2 minutes
4+	Severe	Very swollen, distorted extremity; depth of pitting >1" (>2.5cm); duration 2 to 5 minutes

Appendix 5 KS Lesion Assessment/ Photography Protocol

INTERNATIONAL COLLABORATIVE	**Participant ID Number**	**Interviewer Initials**	**Date of Visit**	**Visit Number**
	☐☐☐☐☐☐	☐☐☐	☐☐/☐☐☐/☐☐☐☐ Day Month Year	☐☐
		Reviewer Initials	**Date of Review**	**Interim Visit Number**
		☐☐☐	☐☐/☐☐☐/☐☐☐☐ Day Month Year	☐☐

KS LESION ASSESSMENT

A "KS-suspicious" lesion is defined as a lesion that is either known to be KS (by virtue of biopsy) or clinically suspected to be KS. In the questions below, when asked about KS-suspicious lesions, consider only those which are **hyperpigmented** with the usual characteristics of KS, regardless if they are flat or raised. Do not count lesions which have subcutaneous swelling alone, without hyperpigmentation, as KS-suspicious.

A. Overall Cutaneous Do not count oral cavity lesions.

1. Overall inventory: How many KS-suspicious lesions are present? ○ 1-49 ○ ≥ 50

2. Indicate if KS-suspicious lesions are absent or present in each of these sites.

Site #	Absent	Present	Site #	Absent	Present	Site #	Absent	Present
12 – Head	○	○	17 – Right arm	○	○	22 – Left hand	○	○
13 – Neck	○	○	18 – Left arm	○	○	23 – Right foot	○	○
14 – Chest	○	○	19 – Right leg	○	○	24 – Left foot	○	○
15 – Abdomen	○	○	20 – Left leg	○	○	25 – Genital	○	○
16 – Back	○	○	21 – Right hand	○	○	26 – Gluteal	○	○

Lesion Counts

Include only KS-suspicious lesions (do not include lesions with subcutaneous swelling alone).

If ≥ 50 lesions overall, select three sites that are **most representative** of the total body in terms of lesion characteristics and which have a combined lesion count of at least 50. If three such areas cannot be found which total at least 50 lesions, choose 3 areas with the largest combined count. Indicate the sites using the corresponding codes above. Do not use the mouth. If lesions are only present in the mouth, go to question 4.

If < 50 lesions overall, enter code 11 ("total body") as the representative area.

Definition of a flat lesion: Area of skin that is hyperpigmented but not raised (i.e., the borders are not palpable; e.g., macule or patch are examples of flat lesions).

Definition of a raised lesion: any lesion that protrudes from skin surface and is palpable (e.g., plaque, nodule, or tumor)

3.

	Representative site (Site Code)	**Number of FLAT lesions (macule/patch)**	**Number of RAISED lesions (plaque/nodule/tumor)**
I.	☐☐	☐☐☐	☐☐☐

II. ☐☐ ☐☐☐ ☐☐☐
III. ☐☐ ☐☐☐ ☐☐☐

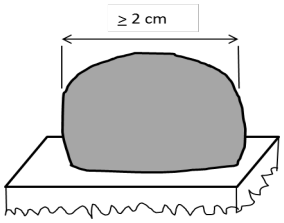
Questions 4-6 relate to lesions anywhere on the body:

4. Identity the KS-suspicious lesion with the **largest diameter** in its footprint on the skin. **This can be a flat or raised lesion.** Record, in mm, this diameter as well as the diameter that is perpendicular to the largest diameter (as in the image shown).

☐☐☐ x ☐☐☐ **mm**

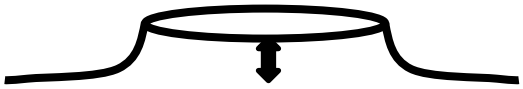
5. How many KS-suspicious lesions are present which meet the definition a tumor? A tumor is substantially raised above the surface of the skin, the height is nearly the same or greater than the footprint on the skin, and at least one diameter on the footprint of the skin is at least 20 mm (2 cm).

☐☐☐ **tumors**



6. What is the vertical height (in mm, as in image shown) of the tallest KS-suspicious lesion?

☐☐ **mm**



B. Feet

7. Are there KS-suspicious lesions present on either foot (below a horizontal transection at the ankle)? ○ Yes ○ No → ***Skip to 9***

8. How many flat or raised lesions are on each foot?

	Right	**Left**
Flat	☐☐☐	☐☐☐
Raised (plaque/ nodular/tumor)	☐☐☐	☐☐☐

C. Oral Cavity

9. Are there any KS-suspicious lesions in the oral cavity? ○ Yes ○ No → ***Skip to 11***

10. Evaluation of oral cavity by site.

	KS Lesions		**Mark all that apply**	
Oral Site	Absent	Present	Raised	Flat
Gingiva/gums	○	○	☐	☐
Hard palate	○	○	☐	☐
Soft palate	○	○	☐	☐
Buccal mucosa	○	○	☐	☐
Tongue	○	○	☐	☐
Floor of mouth	○	○	☐	☐
Tonsils	○	○	☐	☐
Uvula	○	○	☐	☐

Posterior pharynx	○	○	☐	☐

D. Edema

11. Is there any edema present? ○ Yes ○ No → ***Skip to 14***

12. Evaluation of edema by site.

	Edema	
Edema Site	Absent	Present
Face (Including Periorbital)	○	○
Genital	○	○
Right arm	○	○
Left arm	○	○
Right hand	○	○
Left hand	○	○
Right leg	○	○
Left leg	○	○
Right foot	○	○
Left foot	○	○

13. Measurement of extremity circumferences **(in cm).**

	Right (cm)	**Left (cm)**
Foot: At one-half the length of the foot.	☐☐☐.☐	☐☐☐.☐
Lower leg: One-third of the way from ankle to mid-knee joint (closer to ankle).	☐☐☐.☐	☐☐☐.☐
Thigh: Half-way between mid-knee joint and iliac crest.	☐☐☐.☐	☐☐☐.☐

E. Ulceration and Superinfection Oral cavity lesions may be considered.

14. Is there evidence of ulceration on any KS-suspicious lesions? ○ Yes ○ No → ***Skip to 15***

Choose the two lesions with greatest surface area of ulceration.
For each area of ulceration, use Area Site Codes and then provide a brief description of the specific location.
Extent of ulceration: Do not measure entire lesion; only measure ulcerated area.

For the diameters, use the 2 perpendicular diameters at 90 degrees to each other that cover the area with greatest involvement of ulceration.

Site Code	**Specify Location (e.g., below right eye; medial aspect of knee)**	**Diameters of ulceration, in mm**
a. ☐☐		☐☐☐ **x** ☐☐☐ **mm**
b. ☐☐		☐☐☐ **x** ☐☐☐ **mm**

15. Is there evidence of superinfection on any KS-suspicious lesions? ○ Yes ○ No → ***Skip to 16***

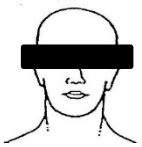
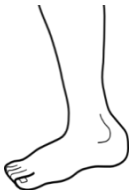
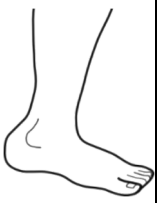
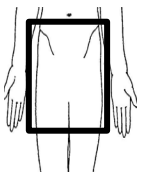
Superinfections is defined as having evidence of infection. Signs may include pus, crust, or odor.

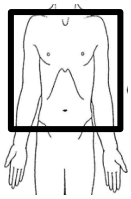
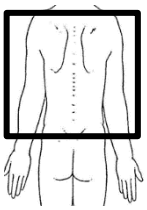
Choose the two areas with greatest surface area of involvement. For each area of superinfection, use Area Site Codes and then provide a brief description of the specific location.

Extent of superinfection: Do not measure entire lesion; only measure superinfected area. As above, for diameters, use the 2 perpendicular diameters at 90 degrees to each other that cover the area with greatest involvement of superinfection.

	Site Code	Specify Location (e.g., below right eye; medial aspect of knee)	Diameters of superinfection, in mm
a.	☐☐	_____	☐☐☐ x ☐☐☐ mm
b.	☐☐	_____	☐☐☐ x ☐☐☐ mm

16. Following the given photographic cues, take 9 photos of the participant from the angles specified.

○	**Take a photo of patient's ID number.**	Photo # ☐☐☐☐☐		○	**Take a photo of face with black cloth over eyes.**		Photo # ☐☐☐☐☐
○	**Take a photo of the bottom of the <u>right</u> foot.**		Photo # ☐☐☐☐☐	○	**Take a photo of the bottom of the <u>left</u> foot.**		Photo # ☐☐☐☐☐
○	**Take a photo of the inside of the <u>right</u> lower leg.**		Photo # ☐☐☐☐☐	○	**Take a photo of the inside of the <u>left</u> lower leg.**		Photo # ☐☐☐☐☐
○	**Take a photo of the front of both legs together.**		Photo # ☐☐☐☐☐	○	**Take a photo of the groin, taking care to include inguinal lymph nodes. Minimal underwear can be worn.**		Photo # ☐☐☐☐☐

○	**Take a photo of the chest and arms, with the palms of the hands facing forward.**		Photo # ☐☐☐☐☐	○	**Take a photo of the back and arms, including backs of the hands.**		Photo # ☐☐☐☐☐

Appendix 6 Sponsor Signatures

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and a Comparison to Subcutaneous (SC) Injection in Human Immunodeficiency Virus (HIV) Subjects Diagnosed with Kaposi Sarcoma (KS)
Study Number:	NAV3-24
Original Protocol Date:	29 August 2016
Amendment 1 Date:	26 June 2017
Amendment 2 Date:	01 May 2018
Amendment 3 Date:	30 January 2019

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ Date: 2-1-2019
Michael Rosol, PhD
Chief Medical Officer
Navidea Biopharmaceuticals

Signed: _____ Date: 2-1-2019
Michael Blue, MD
Senior Medical Director
Navidea Biopharmaceuticals

Signed: _____ Date: 2-1-2019
William J. Regan
Senior VP, Global Regulatory Affairs and Quality
Navidea Biopharmaceuticals

Appendix 7 Investigators' Signature

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and a Comparison to Subcutaneous (SC) Injection in Human Immunodeficiency Virus (HIV) Subjects Diagnosed with Kaposi Sarcoma (KS)
Study Number:	NAV3-24
Original Protocol Date:	29 August 2016
Amendment 1 Date:	26 June 2017
Amendment 2 Date:	01 May 2018
Amendment 3 Date:	30 January 2019

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

Signed:_____ Date:_____

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