Clinical Trial Protocol: NAV3-25

Study Title:	An Exploratory Evaluation of Technetium Tc 99m Tilmanocept by Intravenous (IV) Injection in Subjects with Liver Metastases from Colorectal Carcinoma Patients using SPECT/CT Imaging Compared to FDG PET/CT Imaging.
Study Number:	NAV3-25
Study Phase:	1
Product Name:	Tilmanocept (Technetium Tc 99m tilmanocept)
IND Number:	132943
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Original Protocol:	13 March 2017
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Amendment 3:	20 September 2017

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SYNOPSIS

Study Title	An Exploratory Evaluation of Technetium Tc 99m Tilmanocept by Intravenous (IV) Injection in Subjects with Liver Metastases from Colorectal Carcinoma Patients using SPECT/CT Imaging Compared to FDG PET/CT Imaging.
Study Phase	Phase 1
Study Objectives	**Primary Objective(s)** • Estimation of the concordance of Tc 99m localization in liver metastases from colorectal carcinoma (CRC) using SPECT/CT (single photon emission computed tomography) imaging and abdominal FDG Positron Emission Tomography (PET)/CT imaging per subject. • Evaluation of safety including monitoring the incidence of adverse events and changes over time in laboratory tests, vital signs, and physical examination findings. **Secondary Objective(s)** • Estimation of the optimal dose of Technetium Tc 99m tilmanocept. • Evaluation of other areas of localization of Technetium Tc 99m tilmanocept.
Duration of Study Participation	Approximately 35 days
Study Drug	Tilmanocept (Technetium Tc 99m tilmanocept Injection)
Dose(s) and route of administration	2 Dose Cohorts: 1) IV injection: 2 mCi, 50 µg 2) IV injection: 2 mCi, 200 µg
Inclusion Criteria	1. The subject has provided written informed consent with Health Information Portability and Accountability Act (HIPAA) authorization before the initiation of any study-related procedures. 2. Subjects must be ≥18 years old; 3. The subject must have a diagnosis of adenocarcinoma of the colon and/or rectum with confirmed metastases to the liver; 4. The subjects must have an ECOG performance status of 0-3; 5. The subject must be at least 4 weeks past any major intraabdominal surgery, including surgery to the liver; 6. Subjects with prior malignancies other than colon and/or rectum cancer are allowed, provided they have been

	treated with curative intent, and have no evidence of recurrence of that malignancy; 7. If of childbearing potential, the subject has a negative urine pregnancy test within 48 hours before administration of Tc 99m Tilmanocept, has been surgically sterilized, or has been postmenopausal for at least 1 year
Exclusion Criteria	1. The subject is pregnant or lactating. 2. The subject has undergone any liver surgery, exclusive of a biopsy. 3. The subject has known sensitivity to dextran. 4. The subject has had preoperative chemotherapy, immunotherapy, or radiation therapy within the 10 days prior to Tc 99m Tilmanocept administration 5. Before the administration of Tc 99m Tilmanocept, has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical 6. Has received an investigational product within the 30 days prior to Tc 99m Tilmanocept administration
Study design	Prospective, open-label, single-center, comparator study of IV injected Tc 99m Tilmanocept in the localization and detection of liver metastases in subjects with confirmed CRC. The study will be divided into two cohorts as follows: Up to 12 subjects (up to 6 per cohort): 1) 50 µg tilmanocept radiolabeled with 2 mCi Tc99m at 1.0 mL through IV injection; n = up to 6 2) 200 µg tilmanocept radiolabeled with 2 mCi Tc99m at 1.0 mL through IV injection; n = up to 6
Methodology	The principal investigator (PI) will identify subjects diagnosed with CRC with confirmed metastasis to the liver Subjects will participate in 3 study visits (including screening) over the course of the study period. The screening visit will include an assessment of eligibility and informed consent. After consent has been obtained, subjects will undergo a clinical laboratory evaluation. **Visit 1, Screening (Day -29 to Day 0):** The screening visit will include informed consent, preliminary review of study eligibility, collection of medical history including medications, vital signs, physical exam including height and weight, clinical labs, urinalysis, and urine pregnancy test for subjects of childbearing potential. Subjects will undergo a FDG PET/CT Scan to identify hepatic lesions.

	Visit 2, Injection and Imaging Procedures (Day 1):
	Pre-Tc 99m Tilmanocept Injection:
	• Before injection (Day 1)
	  o Urine pregnancy test will be performed for subjects of childbearing potential
	  o Concomitant medication review
	  o Vital Signs - within 15 minutes prior to injection
	
	Tc 99m Tilmanocept Injection: Subjects will receive their open label dose assignment in accordance with the 2 dosing groups**.**
	
	Post-Tc 99m Tilmanocept Injection:
	• Vital signs - within 15 minutes post-injection
	• 4-6 hours post-injection:
	  o Assessment of adverse events
	  o SPECT/CT images will be obtained of the abdominal cavity (inclusive of entire liver).
	
	5±3 Days Post-injection Follow-Up Visit:
	• Vital Signs
	• Physical Exam
	• Clinical labs and urinalysis
	• Concomitant medication review
	• Assessment of adverse events
	
	General: Up to 12 subjects (2 cohorts as described above) will be injected intravenously with Tc99m (T½ = 6.02 hr).
	
	SPECT/CT Imaging
	Imaging and Acquisition of Imaging Data: Image acquisition of patients in this study will be via SPECT/CT scanning. Subjects will be imaged by SPECT/CT at 4-6 hours post injection.	

Planned Study Dates	**Start of Recruitment:** July 2017	**End of Recruitment**/April 2018 **End of Study/**July 2018
Planned number of study centers	1 center in the United States - University of Alabama at Birmingham	
Number of subjects	**Total:** Up to 12 evaluable subjects	
Primary Endpoint	• Estimation of the concordance of Tc 99m SPECT/CT imaging localization with FDG PET/CT imaging per subject	

	• Proportion of subjects not experiencing an adverse drug reaction (ADR) in each dose group
Secondary endpoints	• Estimation of the concordance of Tc 99m SPECT/CT imaging localization with FDG PET/CT imaging per lesion • Estimation of the concordance of FDG PET/CT imaging localization with Tc 99m SPECT/CT imaging per lesion • Incidence of other areas of localization
Plan for statistical analysis	Due to the exploratory nature of this study all analyses described in this protocol will be descriptive in nature and used in support of designing a larger study.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CD206	mannose-binding receptor (Ca2+ binding lectin)
CRC	colorectal carcinoma
CRF	case report form
CRA	clinical research associate
CRO	contract research organization
CT	X-ray computed tomography
DICOM	Digital Imaging Communications in Medicine
DTPA	pentetic acid
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FDG	fluorodeoxyglucose
GCP	Good Clinical Practice
Hgb	Hemoglobin
HIPAA	Health Information Portability and Accountability Act
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonization
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
IV	Intravenous
mCi	Millicurie
MedDRA	Medical Dictionary for Regulatory Activities
PET	positron emission tomography
PI	principal investigator
ROI	region of interest
SAE	serious adverse event
SD	study day
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SPECT	single photon emission computed tomography
SUSARs	Suspected, Unexpected, Serious Adverse Reactions
SUV	standardized uptake values
TAMs	Tumor-associated macrophages
Tc99m	technetium-99m metastable isotope; γ-emitting ($t_{½}$ = 6.02 h)
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek)
TMF	trial master file
WBC	white blood cell

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 Single Photon Emission Computer Tomography (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 BACKGROUND AND SIGNIFICANCE

1.1 The Unmet Needs: Need for earlier detection of hepatic metastases of colorectal primary tumors.

The liver is a common site of metastases for primary colorectal tumors [1] with between one third and one half of all patients diagnosed with colorectal cancer developing hepatic metastases [2]. Perhaps fortunately, in 30% to 40% of patients who develop advanced disease, the liver is the only site of metastatic disease [3]. As described in the next two sections, it is expected that 99mTc-tilmanocept will localize to hepatic metastases of colorectal tumors. If so, it should be possible to image such metastatic tumors using SPECT imaging (3D). There are currently available alternative imaging technologies, such as fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT), that are used to visualize hepatic tumors. There is a possibility that 99mTc-tilmanocept enabled imaging may have performance characteristics that would make it preferable to the alternatives for imaging of these tumors. If this is demonstrated to be the case, then 99mTc-tilmanocept would have utility as an imaging agent that could assist physicians to identify hepatic metastases, develop surgical plans and monitor patients for the efficacies of non-surgical therapies. This study will develop preliminary data relevant to the potential utility of 99mTc-tilmanocept as an imaging agent to identify and evaluate metastatic colorectal tumors in the liver.

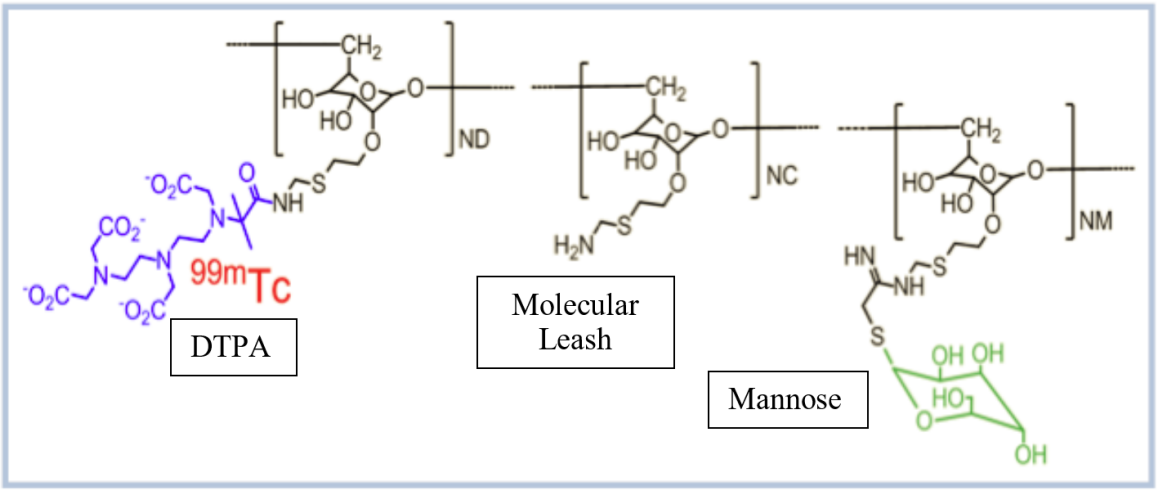
1.2 The Macrophage Mannose Receptor (CD206)

The Macrophage Mannose Receptor (CD206) is abundantly expressed on tumor associated macrophages (TAMs) and on macrophages that reside in regions immediately adjacent to tumors. CD206 is a C-type carbohydrate transmembrane, non-circulating binding protein that is expressed on the exterior surfaces of macrophages [4-6]. Each CD206 receptor has eight carbohydrate binding domains that bind to mannose (3 with mannose specificity) and 5 with recognition of fructose, galactose, and N-acetylglucosamine, in addition to mannose. An important feature of the macrophage mannose receptor is that multivalent binding is required for high affinity interaction between the receptor and its ligands [7]. It is also important to recognize that the level of CD206 expression on macrophages can vary greatly between macrophages. Macrophages respond to factors in their environments, such as the levels of various cytokines, cellular debris, pathogen derived materials and immune complexes, to alter their expression patterns of numerous genes and to acquire various activated states or phenotypes [8-10]. The level of CD206 expression is highly dependent on the activation state of a macrophage [11]. Our understanding of the full phenotypic plasticity of macrophages is still negligible and has as of yet to be fully elucidated.

TAMs are significant contributors to the cellularity of tumors. In established tumors, the majority of TAMs suppress anti-tumor immune responses and secrete various signaling molecules that promote tumor growth and progression [11-14]. This common and tumor promoting TAM phenotype includes the significant up regulation and abundant expression of CD206 [11, 15-17]. Prior to initiating this study, Navidea Biopharmaceuticals, through its academic collaborators, examined histological sections of surgical specimens obtained through resections of hepatic metastases of colorectal primary tumors. These sections were evaluated by immunohistochemical (IHC) staining using an anti-CD206 antibody. These studies confirmed, as has been observed in IHC evaluations using anti-CD206 antibodies of similar histological sections derived from a wide variety of cancer types, that macrophages that highly express CD206 are common components of the tumors. There were also much lesser but still elevated concentrations of CD206 expressing macrophages in anatomically normal tissue that was adjacent to the tumor. It is expected that the density of CD206 expressing macrophages will diminish to background levels as the distance from the tumor margin increases.

The study described in this protocol will evaluate the ability intravenously (IV) injected 99mTc-tilmanocept to localize to CD206 expressing TAMs and possibly also to CD206 expressing macrophages in tissues immediately adjacent to hepatic metastases of colorectal cancers. 99mTc-Tilmanocept is, as explained in the next section, a radiopharmaceutical specifically designed to bind to CD206. It is envisioned that 99mTc-tilmanocept that has localized to hepatic metastases of colorectal tumors can be detected prior to surgery using 3D SPECT imaging.Tilmanocept Structure99mTc-Tilmanocept is a synthetic radiopharmaceutical molecular construct that was designed to bind specifically and with high affinity to CD206 [18-21].

Figure 1: Structure of 99mTc-tilmanocept showing attachment of multiple units of DTPA and mannose to a backbone of dextran. The chelating agent, DTPA, is shown holding an atom of ^{99m}Technetium



Formally, 99mTc-tilmanocept is 99mTc-diethylenetriaminepentaacetic acid (DTPA)-mannosyl-dextran. Tilmanocept is synthesized beginning with a 10 kDa backbone of dextran to which multiple amine terminated molecular leashes have been attached. Through these molecular leashes, multiple moieties of the chelating agent, DTPA, and the sugar, mannose, are attached to the dextran backbone (**Figure 1**). Each tilmanocept molecule has 3-8 DTPA and 12-20 mannose moieties. DTPA permits tilmanocept to be efficiently labeled with ^{99m}Technetium (Tc99m). The final construct has an average molecular weight of 18.7 kDa, is highly water soluble and has an apparent particle diameter of 7 nm. The many mannoses on tilmanocept enable high affinity multivalent binding to CD206.

1.3 Current Approval

99mTc-Tilmanocept has already been approved by the Food and Drug Administration (FDA) and European regulators as a radiopharmaceutical imaging agent to detect sentinel lymph nodes (SLNs) before and during surgeries to remove solid tumor cancers [22]. Sentinel lymph nodes are those nodes first encountered in the lymphatic flow leading away from a tumor [23, 24]. As such, SLNs are the first lymph nodes to which a cancer will metastasize from a primary tumor. During a surgery to remove a primary tumor, it is important to identify and remove all lymph nodes that contain metastatic tumors. Lymph node metastases that are left in a patient's body after surgery can result in disease recurrences. In addition, a patient's lymph node status relative to the presence of nodal metastases provides important information that assists physicians in determining cancer patients' most effective and appropriate post-surgical therapies. In the past, cancer patients frequently underwent lymph node

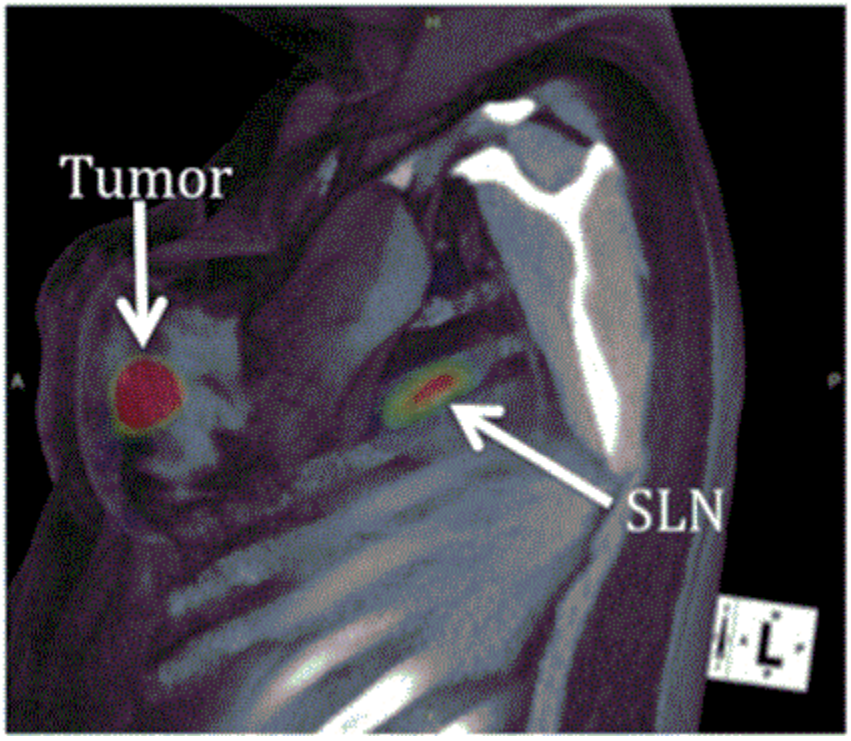
dissections (lymphadenectomies) that removed and examined many lymph nodes in the region near a tumor. Such lymph node dissections are traumatic, cause surgical recoveries of long durations, often lead to lifelong lymphedema [25] and are frequently disfiguring. In addition, in the majority of cases, none of a patient's lymph nodes are found to contain cancer, making the entire lymph node dissection unbeneficial and unnecessary. Alternatively and is currently in common practice, if the SLNs can be identified, examined and found to be free of cancer, it is extremely unlikely that cancer has spread to other lymph nodes further along the lymphatic flow [26]. Therefore, patients with cancer-free SLNs can be spared the trauma associated with removal of additional lymph nodes and the sequelae associated with lymphadenectomies [27].

Lymph nodes and especially SLNs are densely populated by macrophages that highly express CD206 [20]. In current clinical practice, 99mTc-tilmanocept is injected subcutaneously, intradermally or peritumorally near a tumor or the location from which a tumor was recently removed. From its injection site, 99mTc-tilmanocept rapidly enters the lymphatic drainage and is transported via lymph vessels to the first encountered lymph node(s), the SLN(s) where it binds to CD206 and accumulates in macrophages through receptor mediated endocytosis. The high density of CD206 expressing macrophages in SLNs binds and internalizes all of the 99mTc-tilmanocept delivered via the lymphatic flow, thus preventing 99mTc-tilmanocept from further transiting to higher echelon nodes [21, 28]. Accumulation of 99mTc-tilmanocept enables identification of SLNs prior to surgery by lymphoscintigraphy (**Figure 2**) or during surgery using a handheld gamma probe [29, 30].

99mTc-Tilmanocept has been evaluated for its safety and efficacy relative to various lymphatic mapping and SLN-related indications in numerous clinical studies including three phase 3 clinical studies in patients with melanomas, breast cancers and head and neck squamous cell carcinomas [31-33]. Based on the results of these trials, 99mTc-tilmanocept was first approved by the FDA for lymphatic mapping indications in March, 2013. The label has been expanded since then to specifically include SLN-related indications. 99mTc-Tilmanocept has also been approved for marketing in Europe. To date, 99mTc-tilmanocept has been used to identify SLNs in over 100,000 cancer patients.

Throughout this experience, there have been no observed serious adverse events that can be attributed to the administration of 99mTc-tilmanocept.

Figure 2. **Presurgical lymphoscintigraphy SPECT/CT (3D) image of breast cancer patient injected subdermally with 99mTc-tilmanocept. Imaging taken 1 hour after injection clearly shows localization of 99mTc-tilmanocept in a SLN.**



1.3.1 Nonclinical Evaluations – IV AND IP Administration

Dosing in Dogs, Guinea Pigs and Mice: IV dosing in investigational new drug (IND) submitted non-clinical studies represents, cumulative dosing in male and female beagle dogs [eight dogs (4/sex; wt ♀= 7.6 kg; wt ♂ = 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 ♂ and ♀ 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 investigational product (IP) administered in mice, based on the highest single dose administration (2000 mg/kg) the mice received 300,0000X the 50 µg and 37,500X the 400 µg dose (♀ mice, wt = 22 gm) and 340,000X the 50 µg and 42,500X the 400 µg dose (♂ mice, wt = 29 gm).

Dosing In Rats:

Type of Study / Description	Test System	Method of Administration	Dosing
Central nervous system safety pharmacology	Rat	IV	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	IV	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 IV Bolus Injection in Male Rats	Rat	IV	60, 120, and 300 µg/animal or equivalent 320X and 41X the anticipated study doses of 50 µg and 400 µg in humans
Pharmacokinetics, Excretion, and Distribution by Quantitative Whole-Body Autoradiography Following IV Administration of 99mTc-Tilmanocept in Rats	Rat	IV	25 µg in 0.5 mL with collection of Blood, Urine, Feces, and Carcasses for QWBA

Type of Study / Description	Test System	Method of Administration	Dosing
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
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 (AST) 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(Sprague Dawley) 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).

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).

Cytochrome P-450 (CYP) inhibition and drug transporter activity as modulated by tilmanocept exposure: Navidea has completed in vitro studies on the effects of tilmanocept on human hepatic Cytochrome P-450 isozymes and multi-drug transporters derived from human liver biopsies. Concentrations of tilmanocept utilized in these studies represent the equivalent of the injection 2,500,000 µg injected into the total blood pool of reference human (75kg). There were neither effects on any of the CYP isozymes nor on any of the multi-drug transporters. These data are consistent with, to date, the lack of any known drug interactions in >135,000 clinical experiences (administered via any route).

1.3.2 Clinical Safety

Non-IV Experience - Well over 150,000 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 serious adverse events (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 0.09% of subjects experiencing adverse events (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 utilizing ^{99m}Tc-tilmanocept administered IV. Dosages consist of 50, 200 and 400 µg of tilmanocept labeled with 1, 5, or 10 millicurie (mCi) of ^{99m}Tc injected IV. At the time of this writing, twenty-two subjects have been administered either 50 µg (9 subjects), 200 µg (7 subjects) or 400 µg (6 subjects) of tilmanocept with 1, 5, or 10 mCi of ^{99m}Tc. No subject has exhibited any adverse reaction to the drug that was related to the drug. This study, when complete, will provide data on 33 subjects with IV administration of ^{99m}Tc-tilmanocept (at each mass dose level with varied ^{99m}Tc-tilmanocept specific activity).

1.3.3 Radiation Dosimetry

Table 1. The Lymphoseek radiation doses (mGy/MBq) to organs and tissues of an average patient (70 kg) with breast cancer or melanoma

Target Organ	Adults With Breast Cancer	Adults With Melanoma
brain	0.0002	0.0050
breast (injection site)	0.0897	0.0427
gall bladder wall	0.0019	0.0038
lower large intestine wall	0.0007	0.0031
small intestine	0.0005	0.0032
stomach	0.0010	0.0030
upper large intestine wall	0.0007	0.0031
kidney	0.0101	0.0150
liver	0.0018	0.0050
lungs	0.0020	0.0032
muscle	0.0005	0.0024
ovaries	0.0101	0.0162
red marrow	0.0007	0.0027
bone	0.0010	0.0047
spleen	0.0015	0.0032
testes	0.0027	0.0056
thymus	0.0063	0.0031
thyroid	0.0048	0.0025
urinary bladder	0.0032	0.0076
total body (blood)[c]	0.0011	0.0030
Effective Dose (males, mSv/MBq)	0.01600	0.01094
Effective Dose (females, mSv/MBq)	0.01785	0.01357

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

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

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

1.3.4 Radiation Exposure

Participants in this study will be exposed to radiation during both scanning procedures. The SPECT/CT scan will expose the participant to approximately 6.1 millisieverts of radiation. The FDG PET/CT scan will expose the participants to approximately 13-15 millsieverts.

2 TRIAL OBJECTIVES

2.1 Primary Objective(s)

- Estimation of the concordance of Tc99m localization in liver metastases from colorectal carcinoma (CRC) using SPECT/CT imaging and abdominal FDG PET/CT imaging per subject.
- Evaluation of safety including monitoring the incidence of adverse events and changes over time in laboratory tests, vital signs, and physical examination findings.

2.2 Secondary Objective(s)

- Estimation of the the optimal dose of Technetium Tc 99m tilmanocept
- Evaluation of other areas of localization of Technetium Tc 99m tilmanocept.

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Trial Design

This is a single-center, open-label, within-subject feasibility study of tilmanocept in the localization of liver metastasis from CRC. Up to 6 subjects will receive a single IV dose of 50 µg tilmanocept radiolabeled with 2 mCi Tc 99m and imaged at 4-6 hours post-injection. Up to 6 subjects will receive a single IV dose of 200 µg tilmanocept radiolabeled with 2 mCi of Tc99m and imaged at 4-6 hours post-injection.

See Appendix 1, Schedule of Events.

3.2 Justification for Study Design and Population

This study is designed to evaluate the safety and tolerability of a single dose of Tc 99m tilmanocept administered intravenously. SPECT/CT imaging of the subject's abdominal cavity (to include the liver) will be reviewed to establish concordance with images previously acquired with FDG PET/CT imaging.

This study is designed to evaluate the use of Tc 99m tilmanocept as an imaging agent in subjects with known active liver metastasis from CRC by comparing to FDG PET/CT images.

No clinical trials have been conducted to evaluate IV administration of Tc 99m tilmanocept performance in this population. 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 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 approximately 35 days.

4 STUDY POPULATION

The evaluation of tilmanocept in liver metastasis from CRC will involve up to 12 evaluable 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 Health Information Portability and Accountability Act (HIPAA) authorization before the initiation of any study-related procedures;
2. The subject must be ≥18 years old;
3. The subject must have a diagnosis of adenocarcinoma of the colon and/or rectum with confirmed metastases to the liver
4. The subject must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-3;
5. The subject must be at least 4 weeks past any major intraabdominal surgery, including surgery to the liver;
6. Subjects with prior malignancies other than colon and/or rectal cancer are allowed, provided they have been treated with curative intent, and have no evidence of recurrence of that malignancy;
7. If of childbearing potential, the subject has a negative urine pregnancy test within 48 hours before administration of Tc 99m Tilmanocept, has been surgically sterilized, or has been postmenopausal for at least 1 year

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 undergone any liver surgery, exclusive of a biopsy;
3. The subject has known sensitivity to dextran;
4. The subject has had preoperative chemotherapy, immunotherapy, or radiation therapy within the 10 days prior to Tc 99m Tilmanocept administration;
5. Before the administration of Tc 99m Tilmanocept, has received any radiopharmaceutical within 7 radioactive half-lives of that radiopharmaceutical;
6. Has received an investigational product within the 30 days prior to Tc 99m Tilmanocept administration

4.3 Recruitment

Up to 12 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. After 3 subjects have been enrolled into Cohort 1, the data will be reviewed by the PI and Navidea to determine if additional subjects should be enrolled and if additional timepoints should be considered. If it is determined that additional enrollment will not provide meaningful data, enrollment into Cohort 2 will begin. After 3 subjects have been enrolled into Cohort 2, the data will be reviewed by the PI and Navidea to determine if additional subjects should be enrolled and if additional timepoints should be considered.

4.4 Withdrawal

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

Should a subject withdraw after administration of the IP, 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

4.5 Enrollment and Screen Failures

Subjects who sign an informed consent form (ICF) but are ultimately not injected 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 12 subjects have completed SPECT/CT 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 (e.g., "25")
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 "25-01-001."

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

Tilmanocept is a radiotracer that accumulates in CD206-expressing tissue by binding to the CD206 mannose binding receptors that reside on the surface of dendritic cells and macrophages.

5.2 Investigational Product Dosage and Administration

Subjects will receive the administration of Tc 99m tilmanocept through an IV route of administration. Cohort 1 will receive an IV injection of 50µg tilmanocept radiolabeled with 2 mCi (74MBq) Tc99m in 1.0 mL from a single dose syringe. Cohort 2 will receive an IV injection of 200µg tilmanocept radiolabeled with 2mCi (74MBq) Tc 99m in 1.0mL from a single syringe. Tc 99m tilmanocept will be administered as a slow push through a catheter. At the completion of the injection, a 10 cc sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein.

The final administered dose will be ± 20% of the tilmanocept mass dose and radiolabel mCi dose assignment.

Dose levels administered in this study were based on the safety and imaging results of Navidea studies NAV3-21, "An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and Skeletal Joint Imaging with SPECT in Subjects with Active Rheumatoid Arthritis (RA) and Healthy Controls" and NAV3-23, "Evaluation of Subcutaneously (SC) Injected Tc 99m Tilmanocept Localization in Active Rheumatoid Arthritis (RA) Subjects by SPECT and SPECT/CT Imaging." The safety and localization in this study is expected to be similar to the results collected in these studies.

5.3 Treatment Assignment

In this open-label, non-randomized, single center, study all subjects will receive either 50µg or 200µg tilmanocept radiolabeled with 2mCi (74MBq) Tc99m in 1.0mL. The clinical site will send a request to Navidea for subject cohort assignment. The Navidea project manager will confirm treatment group assignment and send authorization back to the investigator prior to placement of the dose order.

The investigator will receive the dose from the supporting Cardinal Health pharmacy.

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 either 50 µg or 200µg tilmanocept radiolabeled with 2mCi (74MBq) Tc99m in 1.0 mL and deliver one syringe to the clinical site radiopharmacy that is ready for administration.

The investigator (or designated personnel) will confirm receipt of the IP in writing and will use the IP only within the framework of this clinical study and in accordance with this study protocol. For each subject, he/she will keep a record of the IP dispensed and store all other forms that accompanied the delivery of the radiolabeled product to the clinical site. 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 IP vials and other materials that were returned, or destroyed, must be recorded and signed by the PI or an appropriately qualified designee as documented in the study site responsibility sheet. An overall accountability and reconciliation form of the IP will be prepared and completed. If there are any discrepancies, they must be investigated and their resolution documented. All unused study 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 Tc 99m tilmanocept injection through the 5 ± 3 days post-injection safety follow-up will be documented. Subjects must not have chemotherapy, immunotherapy, or radiation therapy within the 10 days prior to Tc 99m Tilmanocept administration

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

7.1 Screening Visit (Day -29 to Day 0)

- 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 form (CRF) and enrollment log
- Interview including the following subject-specific characteristics:
  - Demographic data
  - Medical/surgical history (including concomitant diseases)
  - Medication history
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate).
- Blood draw for routine hematology and chemistry
- Urine collection for routine urinalysis and for pregnancy testing 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.
- Physical examination including height and weight and a review of major body systems; any clinically relevant finding is to be documented as baseline finding.
- Subjects will undergo a FDG PET/CT scan to identify hepatic lesions.

7.2 Day 1 (Injection and Imaging Procedures)

7.2.1 Before Injection

- Assessment of AEs
- Concomitant medication review
- Final check of inclusion/exclusion criteria
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate) within 15 minutes

7.2.2 Injection of Tc 99m tilmanocept

Both cohorts will be injected from a single syringe in a single IV injection.

Table 2. Dose Cohort and Route of Administration Groups

Dosing Cohorts	
Cohort 1 IV Injection 50µg tilmanocept in 1.0 mL 2 mCi Tc99m	**Cohort 2** IV Injection 200µg tilmanocept in 1.0 mL 2 mCi Tc99m
Up to 6 subjects	Up to 6 subjects

Up to 12 subjects may be injected and imaged, divided into 2 cohorts of up to 6 subjects each.

Cohort 1 subjects will receive a Tc 99m tilmanocept IV injection in 1.0 mL and 2 mCi injected as a slow push into a catheter. At the completion of the injection, a 10 cc sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein.

Cohort 2 subjects will receive a Tc 99m tilmanocept IV injection in 1.0 mL and 2 mCi injected as a slow push into a catheter. At the completion of the injection, a 10 cc sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein.

Injection and imaging will begin with Cohort 1. After 3 subjects have been enrolled into Cohort 1, the imaging and safety data will be reviewed by the site staff and Navidea personnel to determine if additional subjects should be enrolled. If it is determined that additional enrollment will not provide meaningful data, for example no metatstic liver lesions were visualized by Tc 99m tilmanocept for any of the subjects in the cohort, enrollment into Cohort 2 will begin. After 3 subjects have been enrolled into Cohort 2, the data will be reviewed by the site staff and Navidea personnel to determine if additional subjects should be enrolled.

7.2.3 Post-Injection

The following procedures will be completed at the specified timepoints:

- 15 Minutes Post-Injection
    - Vital signs will be obtained (body temperature, heart rate, blood pressure, and respiratory rate)
- 4-6 Hours Post-Injection
    - Assessment of AEs
    - SPECT/CT will be obtained in the abdominal region (to include entire liver)
- 5 ± 3 Days Post-Injection
    - Assessment of AEs
    - Vital signs (body temperature, heart rate, blood pressure, and respiratory rate, after at least 5 minutes in a resting position)
    - Physical examination including a review of major body systems; any clinically relevant changes from screening are to be documented
    - Blood draw for routine hematology and chemistry
    - Urine collection for routine urinalysis

7.2.4 Imaging and Acquisition of Imaging Data

The camera used to obtain the images should be a 2 or 3-headed 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 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 first imaging session and again between imaging sessions.

Image acquisition of subjects in this study will be via SPECT/CT at 4-6 hours post-injection. The abdominal regional will be imaged and will include the entire liver.

7.2.5 Image Acquisition

The investigator will document the manufacturer and type of the SPECT or SPECT/CT scanner used in both the subject's file and CRF. 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.

The manufacturer's standard SPECT acquisition parameters for Hepatobiliary SPECT (HIDA SPECT) should be followed. This would typically result in a total acquisition time of 30 ± 10 minutes. For SPECT/CT cameras; the CT scan should be set for "low-dose" mode to obtain and use the transmission scan for attenuation correction. For non-CT coupled SPECT cameras, attenuation correction would be applied using standard ellipse-based Chang methods. Regardless of the number of heads on the SPECT camera, the emission data should be acquired with each camera head orbiting the full 360° around the patient with typically 90-120 projection views per head.

7.2.6 Evaluation of SPECT/CT Images

Tilmanocept localization in subjects with liver metastasis from a CRC will be evaluated through SPECT/CT image analysis by the onsite Nuclear Medicine physician. First, a "routine clinical" assessment of the overall technical adequacy of the scan will be made. It is anticipated that this would be done by viewing the projection data cine (allowing for an initial visual assessment of patient motion during the acquisition). This would be followed by a visual evaluation of the transverse reconstructed slices of the liver to assess whether hotspots could be attributed to expected metastases sites. The clinician reading the transverse SPECT liver slices will have access to the previously conducted PET scans and will report any concordance between uptake of tilmanocept with FDG at the metastases sites. The number of sites of concordance (between SPECT and PET) as well as the total number of diagnosed metastases sites for each subject imaged will be reported to the sponsor. Following the technical assessment, an overall assessment of the scan will be made. A de-identified copy of the Nuclear Medicine scan report will be provided by the site to the sponsor. For each subject the SPECT/CT scans and FDG PET/CT scans (in Digital Imaging Communications in Medicine [DICOM] format) will be forwarded to the sponsor.

All regions of interest (ROIs), standardized uptake values (SUVs), and quantitative analyses will be done by the sponsor.

Given that the imaging site has electronic transfer capabilities, each subject's scans will be transferred to the sponsors data server. If such an e-transfer is not possible, a standard "patient CD disc" will be generated and sent to the sponsor via ground mail.

7.2.7 End of Study

For the entire study, end of study is defined as the last subject visit.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Up to 12 subjects aged ≥ 18 years, with confirmed CRC with confirmed liver metastases will be included.

8.1.2 Medical and Surgical History

Relevant medical and surgical histories are to be recorded for the following body systems: head, ear, eye, nose, throat, respiratory, cardiovascular, gastrointestinal, genitourinary, musculoskeletal, endocrine, neurological, psychiatric, and skin/dermatological.

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 medications taken 30 days prior to injection through the 5 ± 3 day follow-up visit will be documented.

8.2 Safety

8.2.1 Adverse Events

8.2.1.1 Definition of Adverse Event

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

Adverse Event (AE)

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

By definition for this trial, all untoward medical occurrences beginning on the day of injection through the assessment 5 days post-injection are to be reported as AEs. Additionally, untoward medical events occurring prior to the day of injection and after the 5 ± 3 day post-injection follow-up will only be captured as AEs if they are related to a trial procedure or study drug. Any clinically significant change in 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. SAEs will be reported from the time of consent through the end of participation.

8.2.1.2 Categories for Adverse Event Assessment

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

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 AE to the use of IP:

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

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

Possibly related: Event may be explained by administration of the IP 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 IP.

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 IP.

Outcome

The outcome of the AE is to be documented as follows:

- Resolved
- Resolved with sequelae
- Ongoing

- Unknown
- Lost to follow-up
- Death

8.2.1.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs at all stages of the SPECT evaluation. Thus, the subject 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 below.
- The maximum **intensity** (mild, moderate, or severe).
- Specific drug treatment
- The **relationship** of the AE to the IP and to trial conduct (for definitions, see above).
- The **outcome** of the AE (resolved, resolved with sequelae, ongoing, unknown, lost to follow-up, death).

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

8.2.1.4 Expected Adverse Events

Expected Adverse Drug Reactions

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

Adverse Drug Reaction (ADR)

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

Investigational Product-Related Risks

In previous clinical trials with tilmanocept (n = 553 subjects injected subcutaneously, intradermally, subaereolarly, peritumorally), all individual AEs were experienced by less than ≤ 1% of all subjects enrolled in sponsor-initiated trials. The most commonly reported AEs in all subjects were injection site irritation, which occurred in 0.6% (n = 3), and pain, which occurred in 0.2% (n = 1) of all subjects. All other treatment-emergent AEs occurred in 0.2% or less of all subjects AEs from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

Precautionary Measures

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

Unexpected Adverse Drug Reactions

An unexpected ADR is defined as an adverse reaction that in nature and severity is not consistent with the applicable product information (e.g., Investigator's Brochure [IB]).

Any adverse experience that is not listed in the current IB or which is, with regard to the specificity or severity, not consistent with the risk information shall be regarded as unexpected.

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

8.2.1.5 Serious Adverse Events

Definition of Serious Adverse Events

The following SAE definition is based on ICH guidelines and the final rule issued by the 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 SAEs

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 trial, 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 on FRM-1084 of the SAE will be emailedto 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.

All SAEs must also be recorded on the AE CRFs.

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 the investigator about reported relevant events (e.g., SAEs, SUSARs) according to all applicable regulations.

8.2.2 Further Safety Assessments

8.2.2.1 Physical Examination

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

8.2.2.2 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, systolic and diastolic blood pressure, and respiratory rate All measurements will be taken after the subject has been in a resting position for at least 5 minutes. Measurements will be taken at Screening, Day 1 predose (15 minutes before injection), and postdose (within 15 minutes after 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.

8.3 Other Procedures and Variables

8.3.1 Clinical Laboratory Parameters for Screening and Safety

Clinical laboratory tests to be evaluated in this trial include hematology, serum chemistry and urinalysis. Blood and urine samples for safety will be obtained at screening.

Subjects will be in a seated or supine position during blood collection. Clinical laboratory tests will include the following:

Table 3. Clinical Laboratory Parameters

Hematology	hemoglobin (Hgb), platelets, white blood cell (WBC)
Serum chemistry	creatinine, chloride, potassium, sodium, carbon dioxide /bicarbonate, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST)
Urinalysis	pH, specific gravity

The investigator will provide a list of normal ranges of the local laboratory prior to the start of the study. The University of Alabama Birhmingham laboratory will provide the necessary supplies to collect the blood and urine samples.

All laboratory reports must be promptly reviewed by the investigator, and upon review, initialed and dated by the investigator. Change(s) in postdose test values considered clinically significant, which would require either additional control or therapy, must be specified in the source documentation and, in case of disturbing or influencing factor(s) on values/samples, details of the appropriate value(s) and the source of disturbance or influence (e.g., quality of sample, co-medication) are to be recorded. GCP would suggest that a copy of the laboratory results also be provided to the subject's referring physician.

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

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

8.3.2 Blood Sampling

The total amount of blood withdrawn during the trial will be approximately 20 mL over a period of approximately 2 weeks as shown in Table 4.

Table 4. Amount of Blood Withdrawn

Time point of examination	Amount of blood taken
Laboratory examination (Screening)	10 mL
Follow-up	10 mL
Total	20 mL

9 STATISTICAL METHODS

Statistics will be descriptive in nature. The sample size was chosen in order to provide a reasonable amount of data to assess the localization performance of Tc 99m tilmanocept in this population across the administered doses.

Demographics and baseline characteristics will be summarized for all subjects injected with tilmanocept using either descriptive statistics (sample size, mean, standard deviation, minimum, median, maximum) or frequency counts and percentages.

Primary and secondary endpoint will be determined as follows:

- The count and percentage of subjects that had at least one liver lesion identified by Tc99m tilmanocept that was also identified by FDG PET/CT.
- The count and percentage of subjects that had at least one liver lesion identified by FDG PET/CT that was also identified by Tc99m tilmanocept.
- The count and percentage of the number of liver lesions identified by Tc99m tilmanocept that were also identified by FDG PET/CT.
- The count and percentage of the number of liver lesions identified by FDG PET/CT that were also identified by Tc99m tilmanocept.
- Incidence of AEs reported for each cohort
- Other lesion localization (count and percentage of subjects with $\geq$ 1 localized lesion by tilmanocept).
- Exploratory statistics may be conducted for other localization of tilmanocept and quantitative uptake (radioisotope) in lesion(s) or lymph node(s).

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

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

10.1.1 CRF Design

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

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

The CRA will verify the correct use of the IP. The IP will not be supplied to the investigator site prior to a favorable opinion from the IRB/IEC 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 independent ethics committee(s) (IEC(s)/ 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 IP
6. Suspected lack of efficacy of the IP
7. Administrative decision

Termination by the Investigator

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

- Return all unused IP 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 PI/sponsor will promptly inform the investigator/institutions, regulatory authorities, and IRB of the termination or suspension and the reason for that.

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 4.4.

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 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 subject consent form and any required 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 ICF and has done so, may he/she enter the trial. Additionally, the investigator or his 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 ICF 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 ICF. 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 ICF form. Any revised written ICF 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 IP but not due to medical negligence on the part of the PI 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

Evaluation	VISIT 1 Screening Days -29 to 0	VISIT 2 Injection and Imaging Procedures Day 1		VISIT 3 Follow-up Day 5±3
		0:00	4-6 hrs	
Informed Consent	x			
Entry Criteria	x			
Medical History	x			
Vital Sign Assessment	x	x[bd]	x	x
Physical Examination	x[c]			x
Review of Medications	x	x		x
Clinical Laboratory Evaluation	x			x
Urine Pregnancy Test	x	x[a]		
Tc99m tilmanocept Administration		x		
FDG PET/CT Imaging	x			
SPECT/CT Imaging			x	
Adverse event Monitoring	x	x	x	x

a. Urine dipstick 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 injection (a 15 minute pre-injection window is permitted).
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. Timepoint is within 15 minutes post-injection.

Appendix 2 ECOG Performance Status

Grade	Eastern Cooperative Oncology Group
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Appendix 3 Sponsor Signatures

Study Title:	An Exploratory Evaluation of Technetium Tc 99m Tilmanocept by Intravenous (IV) Injection in Subjects with Liver Metastases from Colorectal Carcinoma Patients using SPECT Imaging Compared to FDG PET/CT Imaging.
Study Number:	NAV3-25
Original Protocol Date:	13 March 2017
Amendment 1 Date:	26 April 2017
Amendment 2 Date:	08 June 2017
Amendment 3 Date:	20 September 2017

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: 29 Sept 2017

Fredrick O. Cope, PhD, FACN, CNS
Senior VP and Chief Scientific Officer
Navidea Biopharmaceuticals

Signed:_____ Date: 9.29.17

Michael Blue, MD
Senior Medical Director
Navidea Biopharmaceuticals

Signed:_____ Date: 9.29.17

William J. Regan
Senior VP, Global Regulatory Affairs and Quality
Navidea Biopharmaceuticals

Appendix 4 Investigator's Signature

Study Title:	An Exploratory Evaluation of Technetium Tc 99m Tilmanocept by Intravenous (IV) Injection in Subjects with Liver Metastases from Colorectal Carcinoma Patients using SPECT Imaging Compared to FDG PET/CT Imaging.
Study Number:	NAV3-25
Original Protocol Date:	13 March 2017
Amendment 1 Date:	26 April 2017
Amendment 2 Date:	08 June 2017
Amendment 3 Date:	20 September 2017

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

Signed:_____ Date:_____

Pradeep Bhambhvani, MD
Associate Professor
Division of Molecular Imaging and Therapeutics
The University of Alabama at Birmingham