Clinical Study Protocol: NAV3-30

Study Title:	An Evaluation of the Safety of Intravenous (IV) Tc 99m Tilmanocept and a Comparison of Localization to Tc 99m Sulfur Colloid by Planar and SPECT/CT Imaging in Subjects with Nonalcoholic Steatohepatitis (NASH) and Healthy Controls (HC)
Study Number:	NAV3-30
Study Phase:	1
Product Name:	Technetium Tc 99m tilmanocept
IND Number:	132943
No. of Subjects	Up to 12
Investigator(s):	Multicenter
Sponsor:	Navidea Biopharmaceuticals, Inc 4995 Bradenton Ave, Suite 240 Dublin, OH 43017
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	Date
Original Protocol:	06 October 2017
Amendment 1	23 April 2018

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

SYNOPSIS

Study title	An Evaluation of the Safety of Intravenous (IV) Tc 99m Tilmanocept and a Comparison of Localization to Tc 99m Sulfur Colloid by Planar and SPECT/CT Imaging in Subjects with Nonalcoholic Steatohepatitis (NASH) and Healthy Controls (HC)
Study phase	Phase 1
Study objective(s)	**Primary** • Determine the safety and tolerability of Tc 99m tilmanocept in subjects with NASH. **Secondary** • Determine the three dimensional tessellation localization of Tc 99m tilmanocept by planar and SPECT (single photon emission computed tomography)/CT (Computerized x-ray Tomography) imaging in subjects with NASH and asymptomatic controls. • Determine the three dimensional tessellation localization of unfiltered Tc 99m sulfur colloid by planar and SPECT/CT imaging in subjects with NASH and asymptomatic controls. • Comparison of liver localization distribution tessellation pattern intensity between Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid. **Exploratory** • Concordance of intrahepatic localization tessellation discrimination of Tc 99m tilmanocept (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography • Concordance of intrahepatic localization tessellation discrimination of Tc 99m sulfur colloid (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography
Duration of treatment	Up to 57 days
Study drug	Technetium Tc 99m tilmanocept Unfiltered technetium Tc 99m sulfur colloid

Dose(s) and Route of administration	**Route of Administration:** All administrations of Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid will occur through an intravenous (IV) route of administration. A single syringe will be used for each product and administered as a slow push into the IV. At the completion of each administration, a 10 cc sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein. **Doses:** On Day 0, subjects will receive an on label 8.0 mCi dose of unfiltered Tc 99m sulfur colloid via IV administration. On Day 3, subjects will receive 200 µg of Tc 99m tilmanocept at the specified radiolabel dose of Tc 99m via IV administration. The first two enrolled subjects will be NASH subjects who will receive an 8.0 mCi radiolabel dose of Tc 99m tilmanocept. A review meeting will be held following completion of the first two subjects to evaluate tilmanocept safety and dose performance. At the review: 1. If it is determined that a lower radiolabel dose of tilmanocept may produce images of better quality, three additional NASH subjects and three HCs will be injected and imaged with 200 µg of 2.5 mCi Tc 99m tilmanocept. After these six subjects have completed the trial, no additional subjects will be enrolled. 2. If the review shows no safety signals and acceptable quality of imaging, one additional NASH subject as well as three HCs will be injected and imaged with 200 µg of 8.0 mCi Tc 99m tilmanocept. Once a total of three NASH subjects and three HCs have completed the trial, a second review meeting will be held to compare results from NASH subjects to those of the HCs. If it is determined at the second review meeting that a lower radiolabel dose of tilmanocept may produce additional clinically meaningful results, 3 NASH subjects and 3 HCs will be dosed with 200 µg of 2.5 mCi Tc 99m tilmanocept. If it is determined that additional doses will not provide clinically meaningful results, the trial will be concluded.

Inclusion criteria	ALL SUBJECTS: 1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) authorization before the initiation of any study-related procedures. 2. The subject is ≥18 years of age at the time of consent. 3. The subject has a body mass index (BMI) between 18 and 45. CONTROL SUBJECTS: 4. The subject is deemed to be clinically free of any infectious/inflammatory disease(s) for at least 4 weeks prior to the consent date. 5. The subject has not taken any antibiotics for at least 4 weeks prior to the consent date. NASH SUBJECTS: 4. The subject has biopsy-confirmed NASH within 12 months prior to enrollment. 5. The subject has a Nonalcoholic Fatty Liver Disease (NAFLD) Activity Score (NAS) of ≥ 4, with a score of at least 1 for each steatosis, lobular inflammation, and hepatocyte ballooning. 6. The subject has fibrosis staging of F3-F4.
Exclusion criteria	ALL SUBJECTS: 1. The subject is pregnant or lactating. 2. The subject size or weight is not compatible with imaging per the investigator. 3. The subject has received radiation therapy or chemotherapy or has a previous diagnosis of cancer other than basal cell carcinoma. 4. The subject has renal insufficiency as demonstrated by a glomerular filtration rate (GFR) < 60 mL/min. 5. The subject has a chronic or persistent infection or has any condition that would, in the opinion of the examining physician, preclude their participation. 6. The subject has a known allergy to or has had an adverse reaction to dextran exposure. 7. The subject has received an investigational product within 30 days prior to the Tc 99m sulfur colloid administration.

	8. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m sulfur colloid. 9. The subject is HIV positive. 10. The subject has a history of alcohol abuse or currently consumes alcohol in excess of 3 drinks/day for men or 2 drinks/day for women. 11. The subject has hepatitis B or C. CONTROL SUBJECTS: 12. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than two times the upper limit of normal (ULN). 13. The subject has been diagnosed with NASH, NAFLD, or other chronic liver disease. 14. The subject has been diagnosed with metabolic syndrome or Type I or II diabetes. NASH SUBJECTS: 12. The subject has any chronic liver disease aside from NASH/NAFLD. 13. The subject has uncontrolled diabetes as indicated by an A1c >9% within the 3 months prior to enrollment.
Study design	Prospective, open-label, multicenter, safety, comparative study of injected Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid in the detection of and assessment of three dimensional tessellation localization to the liver in subjects with and without moderate to severe NASH by planar and SPECT/CT imaging. On Day 0, subjects will receive IV administration of 8.0 mCi of unfiltered Tc 99m sulfur colloid. Subjects will have planar imaging of the liver followed by SPECT/CT scanning of the abdomen, liver, and spleen at 20 minutes ± 5 minutes post-administration. On Day 3, subjects will receive IV administration of 200 µg of tilmanocept at the specified radiolabeled dose of Tc 99m. Subjects will have planar imaging of the liver followed by SPECT/CT scanning of the abdomen, liver, and spleen at three specified time points post-administration: 20 minutes ± 5 minutes, 75 minutes ± 15 minutes and 180 minutes ± 15 minutes.

Methodology	The study includes: screening, enrollment, pre- and post-injection assessments, administration of unfiltered Tc 99m sulfur colloid, administration of Tc 99m tilmanocept, imaging and follow-up. **Visit 1, Screening (Day -45 to Day -1):** The screening visit will include informed consent, review of study eligibility, demography, collection of medical history including medications, vital signs, ECG (electrocardiogram), physical exam including height and weight, clinical labs, urinalysis, a serum pregnancy test for subjects of childbearing potential, and a transient elastography. Adverse event monitoring will begin from Visit 1 Screening and continue through subject completion of the trial. **Visit 2, Day 0:** **Pre-Tc 99m Sulfur Colloid Administration:** • Before Administration (Day 0) o Urine pregnancy test will be performed for subjects of childbearing potential o Assessment of adverse events o Concomitant medication review o Vital Signs - within 15 minutes prior to administration **Tc 99m Sulfur Colloid Administration :** Subjects will receive the on label 8.0 mCi dose. **Post-Tc 99m Sulfur Colloid Administration:** • 20 ± 5 Minutes Post-Administration: o Assessment of adverse events o Vital Signs o Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen **Visit 3, Day 3:** **Pre-Tc 99m Tilmanocept Administration:** • Before administration (Day 3) o Urine pregnancy test will be performed for subjects of childbearing potential o Assessment of adverse events

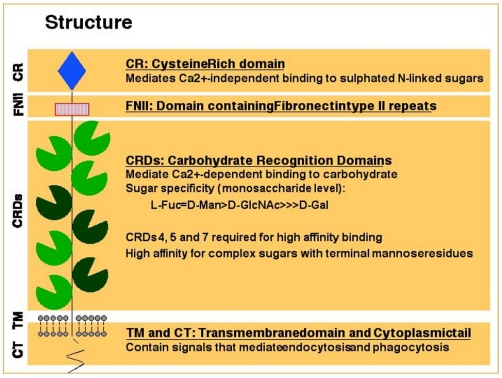
	○ Concomitant medication review ○ Vital Signs - within 15 minutes prior to administration **Tc 99m Tilmanocept Administration:** Subjects will receive the open label 200 µg at the specified radiolabeled dose. **Post-Tc 99m Tilmanocept Administration:** • 20 ± 5 Minutes Post-Administration: ○ Assessment of adverse events ○ ECG (completed before vital signs) ○ Vital Signs ○ Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen • 75 ± 15 Minutes Post-Administration: ○ Assessment of adverse events ○ Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen • 180 ± 15 Minutes Post-Administration: ○ Assessment of adverse events ○ Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen • After Completion of All Imaging: ○ Blood draw for hematology and chemistry testing ○ Urine collection for urinalysis **Day 8 ± 3:** **Safety Follow-Up Telephone Call 5 ± 3 Days Post-Injection of Tc 99m Tilmanocept:** • Concomitant medication review • Assessment of adverse events	
Planned study dates	**Start of study recruitment /** November 2017	**End of Recruitment/**June 2018 **End of Study/**September 2018
Planned number of study centers	Approximately 1-3 centers in the United States	
Number of subjects	Up to 12 evaluable subjects may be enrolled.	

Primary Endpoint	• Proportion of subjects experiencing a noxious pharmacologic activity/adverse drug reaction (ADR)
Secondary Endpoints	• Determine the three dimensional tessellation localization of Tc 99m tilmanocept by planar and SPECT/CT imaging in subjects with NASH and asymptomatic controls. • Determine the three dimensional tessellation localization of unfiltered Tc 99m sulfur colloid by planar and SPECT/CT imaging in subjects with NASH and asymptomatic controls. • Comparison of liver localization distribution tessellation pattern intensities between Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid
Exploratory Endoints	• Concordance of intrahepatic localization tessellation discrimination of Tc 99m tilmanocept (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography • Concordance of intrahepatic localization tessellation discrimination of Tc 99m sulfur colloid (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography
Data Analyses	The following analysis population will be defined for the study: • **Intent-to-Diagnose (ITD) Population** - Subjects who are enrolled in the study, administered Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid, and are imaged will be included in the ITD analysis population. • **Safety Population** – All patients who are enrolled and administered Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid in the study 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 number and percentage of subjects experiencing noxious pharmacologic activity or ADRs will be tabulated by subject type, Tc 99m tilmanocept dose group (if applicable), and overall.

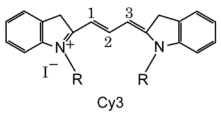
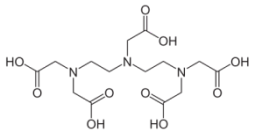
	Per subject localization rates and percentage will be calculated by time point, by subject type, Tc 99m tilmanocept dose group (if applicable), and overall. Localization intensities (tessellation) will be summarized by descriptive statistics (mean, median, standard deviation, minimum, maximum and range) at each time point. All results will be compared to statistics computed on the control group at each time point.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

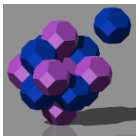
ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase (SGPT)
AST	aspartate aminotransferase (SGOT)
BMI	body mass index
BUN	blood urea nitrogen
CCL2	CC-chemokine ligand 2
CCR2	CC-chemokine receptor 2
CD206	Mannose-binding receptor (Ca2+-binding lectin)



CO_2	carbon dioxide
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
CSF1	Macrophage colony-stimulating factor 1
CSF1R	CSF1 receptor
CT	X-ray computed tomography
CX3CR1	CX3C-chemokine receptor 1
CXCL	CXC-chemokine ligand
CXCR	CXC-chemokine receptor

Cy3	Cy3 cyanine dyes fluoresce orange (~550 nm excitation, ~570 nm emission)  Cy3
DAMPs	Damage-associated molecular patterns
DICOM	Digital Imaging Communications in Medicine
DTPA	diethylene triamine pentaacetic acid 
ECG	electrocardiogram
EU	European Union
FASL	FAS ligand
FDA	Food and Drug Administration
FNR	false negative rate
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HC	Healthy controls
HCT	hematocrit
Hgb	hemoglobin
HIPAA	Health Information Portability and Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonization
$\%ID_{SN}$	percentage of Injected Dose in the Sentinel Node
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IL-6	Interleukin-6
ILM	intraoperative lymphatic mapping
IRB	Institutional Review Board
ISF	investigator site file

ITD	intent-to-diagnose population
IV	intravenous
K_D	Dissociation constant
kDa	kiloDalton (molecular weight designation)
LN	lymph node
LSEC	Liver sinusoidal endothelial cell
LY6C	Lymphocyte antigen 6 complex
M1	Macrophage 1
M2	Macrophage 2
mCi	milliCurie ($37x10^6$ becquerels; 37megabecquerels)
MedDRA	Medical Dictionary for Regulatory Activities
MoMFs	Monocyte-derived macrophages
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MSR1	Macrophage scavenger receptor 1
MTD	maximum tolerated dose
NAFLD	nonalcoholic fatty liver disease
NAS	nonalcoholic fatty liver disease activity score
NASH	nonalcoholic steatohepatitis
NHANES	National Health and Nutrition Examination Survey
NO	Nitric oxide
NOAEL	no-observed adverse-effect level
PGE2	Prostaglandin E2
PI	principal investigator
RA	Rheumatoid Arthritis
RBC	red blood cells
ROS	Reactive oxygen species
SAE	serious adverse event
SAP	statistical analysis plan

SC	Subcutaneous
SCC	squamous cell carcinoma
SLN	sentinel lymph node
SLNB	sentinel lymph node biopsy
SPECT	single photon emission computed tomography
SUSARs	Suspected, Unexpected, Serious Adverse Reactions
Tc 99m	technetium-99m metastable isotope; γ-emitting ($t_{½}$ = 6.02 h)
TcSC	Tc 99m Sulfur Colloid
Tessellation	Three dimensional diffential localization or heterogeneity of intrahepatic signal 
TGF-β	Transforming Growth Factor-β
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek®)
TMF	trial master file
TNF	Tumour necrosis factor
ULN	upper limit of normal
US	United States
VEGFA	Vascular endothelial growth factor A
VBD	vital blue dye
WBC	white blood cell
wt	weight

STUDY ADMINISTRATIVE STRUCTURE

The principal investigator (PI) must sign the protocol signature sheet before study 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 study
- Review of subject eligibility and medical records
- Clinical evaluations
- Safety assessments
- Injection and imaging
- On-site image analysis

Study 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

This clinical study provides a preliminary evaluation of Navidea's radiopharmaceutical Tc 99m tilmanocept as an imaging agent for the detection of CD206 (Cluster of Differentiation 206, the macrophage mannose receptor) expressing macrophages in the liver as a possible indicator of pathological processes related to nonalcoholic steatohepatitis (NASH). NASH is a chronic inflammatory disorder. Tc 99m tilmanocept contains mannose groups attached to a dextran backbone, enabling Tc 99m tilmanocept to bind strongly to CD206 expressed on the surface of several classes of activated macrophages. Elevated levels of activated macrophages are associated with liver inflammation raising the possibility that imaging Tc 99m tilmanocept localizations by single-photon emission computed tomography (SPECT) could be used alone or in combination with other diagnostic tests to identify persons with NASH.

NASH is an emerging public health concern affecting millions of persons. There is an urgent unmet need to develop noninvasive methods to augment and improve the accuracy of diagnostic evaluations of patients suspected of having NASH. Currently, liver biopsies in combination with various clinical findings are the most accurate means for diagnosing NASH [2]. However, such procedures are invasive and potentially challenging to deliver to a patient population as large as the one comprised of those suspected of having NASH. The primary objective of this study is to determine diagnostic feasibility and to explore a variety of imaging protocol parameters to provide a preliminary determination of an optimized imaging protocol for Tc 99m tilmanocept in NASH patients. Developing a non-invasive NASH diagnostic would greatly facilitate the delivery of new NASH therapeutics, many of which are currently in clinical trials [3-6].

Nonalcoholic fatty liver disease (NAFLD) is characterized by an accumulation of the fat in the liver cells. Histological evaluation of a liver biopsy revealing accumulation of fat without inflammation and associated damage is diagnosed as NAFLD. NAFLD is considered to be a hepatic manifestation of metabolic syndrome and develops in patients with non-significant alcohol usage. NAFLD progression leads to further accumulation of fat in the hepatocytes, inflammation, and damage to liver cells that is diagnosed NASH (see Figure 1). NASH is a silent disease. Further progression of NASH leads to NASH-associated liver fibrosis, cirrhosis and hepatocellular carcinoma. These NASH derived pathologies are the fastest growing indications for liver transplantation, while at the same time liver transplantation shortage is a major issue [8-10].

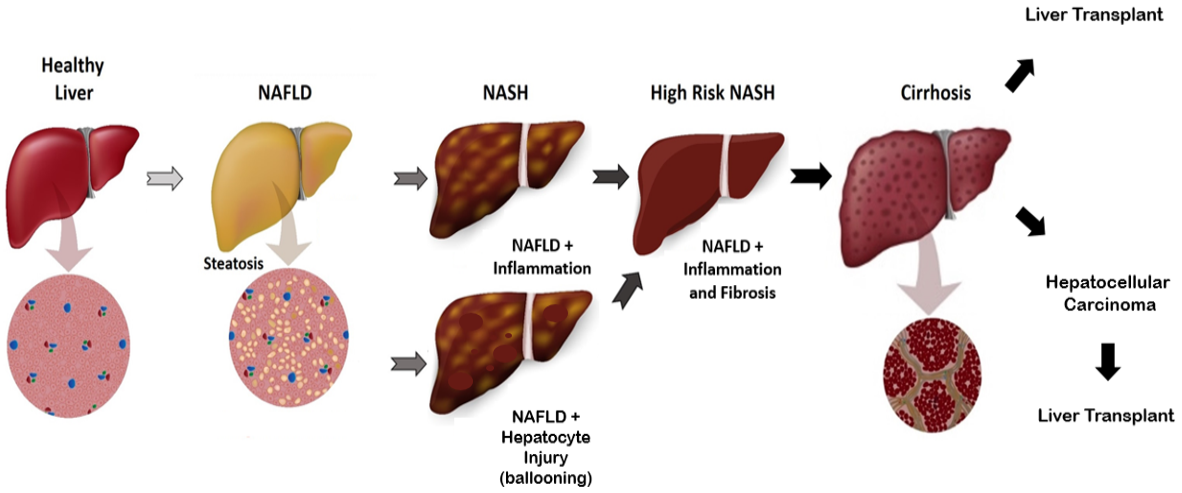


Figure 1. Nonalcoholic steatohepatitis (NASH) progression [7]

Changing diets and activity levels are causing a growing global obesity epidemic with the result that metabolic syndrome and its associated NAFLD are becoming more prevalent. Many of these individuals with NAFLD are progressing to NASH, exasperating the growing need for better and more assessable means to diagnose and manage this emerging patient population. Recent analysis of this epidemic showed global prevalence of NAFLD to be approximately 25% - 37% with the highest risk in the Middle East, South America and lowest in Africa [11, 12]. It is estimated that 30% of the US population has developed NAFLD with the prevalence of NASH currently at 5.7% [12]. Moreover, in 2010, 417,524 patients had NASH-associated cirrhosis, and 4,104,871 had NASH-associated advanced fibrosis based on National Health and Nutrition Examination Survey (NHANES) data [13].

NASH is an inflammatory syndrome in which immune cells infiltrate into and accumulate in liver tissue [14, 15]. Numerous among these immune cells are macrophages. There are two types of macrophages in the liver. The first type consists of Kupffer cells (aka stellate macrophages) that reside within the endothelial lining of blood vessels in the liver. The second type of macrophages in the liver consists of macrophages differentiated from bone marrow derived monocytes that have infiltrated into the liver and developed into activated macrophages in response to inflammatory signals originating in the liver [14]. It is hypothesized that Tc 99m tilmanocept, through its affinity to CD206, will bind to and accumulate in both types of liver macrophages permitting the degree and anatomical distribution of Tc 99m tilmanocept localization to be used as an indicator of the presence and intensity of NASH related hepatic inflammation. In addition, NASH results in the development of fibrotic lesions in the liver, basically scars resulting from resolved inflammation. These fibrotic lesions are depleted of macrophages, thus are expected to be regions of much reduced Tc 99m tilmanocept localization relative to regions where NASH related inflammation is ongoing [1, 16, 17]. In both cases of either increased Tc 99m tilmanocept localization due to active NASH mediated inflammation or decreased Tc 99m tilmanocept localization due to macrophage depletion due to fibrotic resolution of NASH mediated inflammation, the degree and distribution of Tc 99m tilmanocept localization is expected to significantly differ in NASH involved livers relative to the degree and distribution of Tc 99m tilmanocept localization

observed in livers which are not involved in NASH inflammation. This clinical study will provide a preliminary evaluation of the degree and distribution of Tc 99m tilmanocept localization in healthy livers and those involved in NASH inflammation. If this and follow on studies are successful in establishing that Tc 99m tilmanocept enabled imaging can identify areas of NASH involvement or resolution, then it may be possible to develop Tc 99m tilmanocept as a NASH imaging agent that can assist physicians to non-invasively diagnose and monitor NASH, thus satisfying the need for a non-invasive means to diagnose and assess NASH.

1.2 Liver Anatomy, Function, and Role of Kupffer cells

The liver is the largest internal organ, responsible for filtration of the blood coming from a digestive tract before passing it to the rest of the body (Figure 2) [19]. Liver is made up of microscopic lobules, including acinus. Acinus is constructed of interconnected hexagonal units packed with spokes-like internal canals transporting blood and bile ducts across tightly packed hepatocytes. Each acinus unit has three functional zones [20, 21]. Zone 1 (periportal) performs a majority of metabolic functions and contains the most oxygenated blood. Zone 2 (midzone) is situated between zones 1 and 3. Zone 3 (centrilobular) borders the central vein although it has the poorest oxygenation, and is the most susceptible to a restriction in blood supply to tissues resulting in ischemic damage, abnormal retention of lipids within the cell (steatosis), hepatocellular ballooning and hepatocyte death resulting in scar tissues (fibrosis) leading to possible progression to cirrhosis. Zone 3 is the anatomical location where NASH pathology and its consequences are most pronounced. In addition, activated Kupffer cells produce TGFβ and other cytokines that promote liver fibrosis [22]. Interestingly, during the process of liver inflammation and hepatocyte injury (ballooning), Kupffer cells have an ability to differentiate into activated macrophages, many of which highly express CD206 (see Figure 3) [23]. This observation further supports the hypothesis that Tc 99m tilmanocept binding to CD206 can facilitate imaging of NASH associated inflammation.

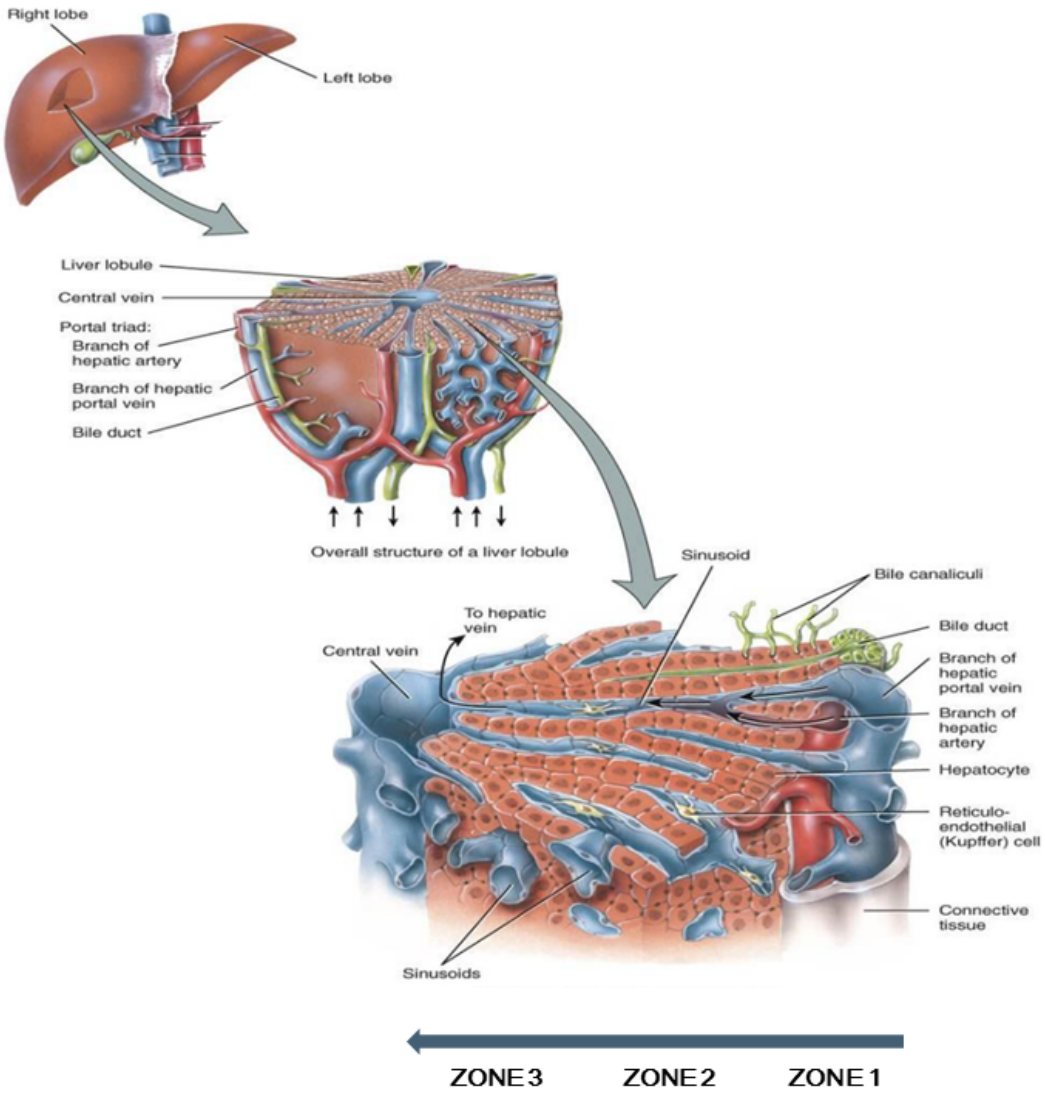


Figure 2. Liver anatomy and lobular zoning [18]

1.3 NASH Diagnosis and Other Imaging Modalities

Since NASH manifests as a chronic and gradually progressive inflammatory syndrome of the liver and since NASH-related liver inflammation is associated with large numbers of liver infiltrating CD206 expressing activated macrophages, Tc 99m tilmanocept enabled imaging of NASH inflamed livers could facilitate a diagnosis of NASH through observation of Tc 99m tilmanocept localization to areas of CD206-expressing macrophage aggregations associated with NASH inflammation.

Today, physicians use a variety of imaging modalities to inform a diagnosis of NASH [24-26]. These alternatives to Tc 99m tilmanocept based imaging for NASH include ultrasound (US), computerized tomography (CT), magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). All of these imaging modalities have their pros and cons, but most

importantly and unlike Tc 99m tilmanocept, none of these other imaging modalities directly image inflammatory processes [25]. Instead they measure either liver fat deposition or evidence of liver fibrosis [16, 24, 27]. NASH, especially in its earlier stages and before extensive tissue damage has occurred and when one would most likely want to initiate therapy, may not be associated with significant fibrosis. Thus, evaluating livers only for fibrosis introduces a diagnostic sensitivity issue into the diagnostic work up of patients suspected of having NASH. Conversely, evaluating a patient's liver for fat content can introduce a diagnostic specificity issue related to discriminating between NAFLD and NASH. Since, NAFLD is a very common condition with limited clinical consequences, confusing NAFLD and NASH is problematic. By contrast, Tc 99m tilmanocept may be capable of imaging NASH inflammation directly. As inflammation is central to the pathology of NASH [24], Tc 99m tilmanocept may localize conspicuously and specifically in the livers of patients with NASH.

In addition to the imaging modalities discussed in the previous paragraph, many investigators have evaluated the utility of using various ^{99m}technetium colloids as imaging liver agents [1, 16, 17, 27]. In fact, Tc 99m sulfur colloid will be evaluated as a comparator to Tc 99m tilmanocept for liver imaging in this protocol. Colloids are particles that are actively phagocytized by Kupffer cells. When injected into the blood circulation, Tc 99m sulfur colloid is rapidly cleared from the circulation by the Kupffer cells [26]. Tc 99m sulfur colloid localization to Kupffer cells enables SPECT imaging of livers. Tc 99m tilmanocept localization is expected to differ from Tc 99m sulfur colloid localization in two ways. First, colloidal particles are expected to penetrate poorly into the liver. Thus, Tc 99m sulfur colloid will be unable to localize to aggregates of activated macrophages that have penetrated into liver tissue. Second, Tc 99m sulfur colloid localization to Kupffer cells is independent of their cellular activation state and CD206 expression phenotype. Conversely, Tc 99m tilmanocept localization is CD206 dependent and the extent of localization is expected to vary relative to the level of CD206 expression and the density of CD206 expressing cells.

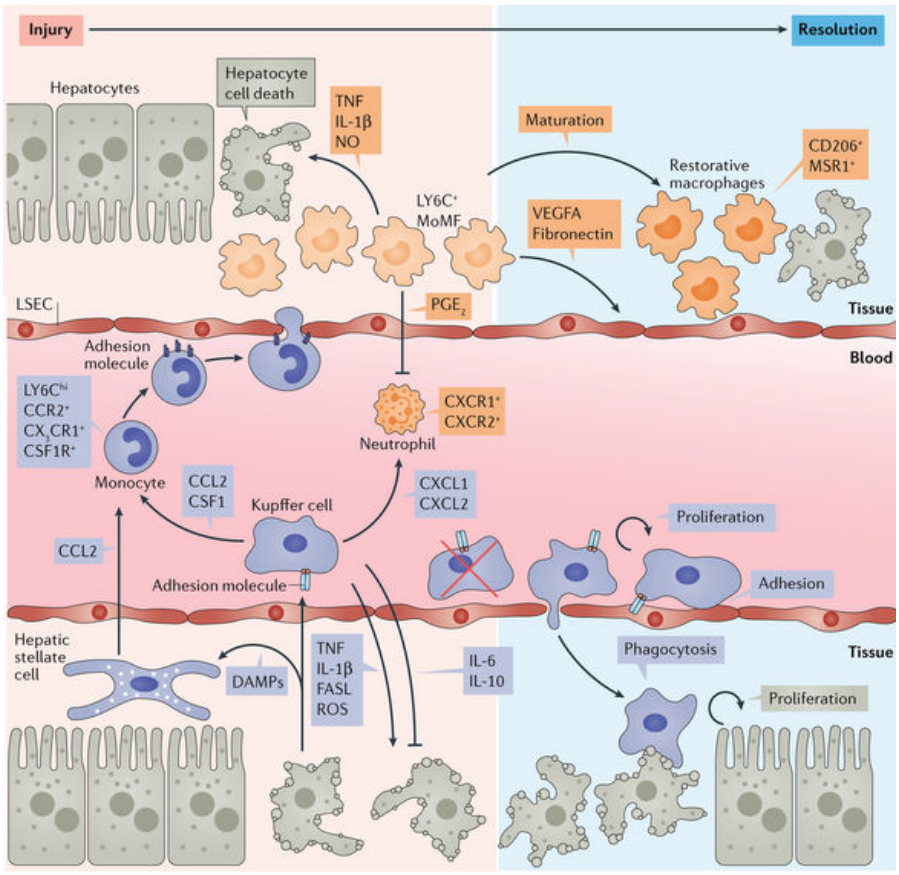


Figure 3. Involvement of the macrophages in the liver damage [15]

Abbreviations: CCL2, CC-chemokine ligand 2; CCR2, CC-chemokine receptor 2; CSF1, macrophage colony-stimulating factor 1; CSF1R, CSF1 receptor; CX3CR1, CX3C-chemokine receptor 1; CXCR, CXC-chemokine receptor; CXCL, CXC-chemokine ligand; DAMPs, damage-associated molecular patterns; FASL, FAS ligand; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; LSEC, liver sinusoidal endothelial cell; LY6C, lymphocyte antigen 6 complex; MoMFs, monocyte-derived macrophages; MSR1, macrophage scavenger receptor 1; NO, nitric oxide; PGE2, prostaglandin E2; ROS, reactive oxygen species; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A.

1.4 Preliminary Data

1.4.1 ^{99m}Tc-tilmanocept (Lymphoseek®)

Tc 99m tilmanocept is a radiopharmaceutical imaging agent that was originally designed to bind specifically and with high affinity to CD206 on macrophages that reside in lymph nodes (Figure 4). Tilmanocept is a macromolecule consisting of multiple units of DTPA (diethylenetriaminepentaacetic acid) and mannose, each synthetically attached to a 10 kDa dextran backbone via amine terminated molecular leashes. The mannose acts as a ligand for CD206 while the DTPA moieties serve as chelating agents for labeling with ^{99m}technetium (^{99m}Tc).

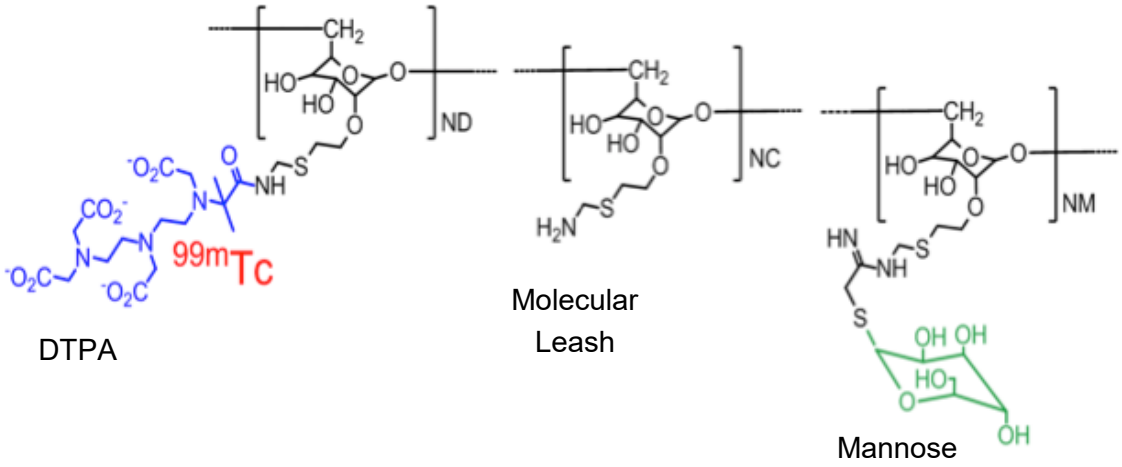


Figure 4. Structure of Tc99m-tilmanocept (Lymphoseek®)

Showing attachment of multiple units of DTPA and mannose to a backbone of dextran. The chelating agent, DTPA, is shown holding an atom of 99mTechnetium.

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. Tilmanocept's multiple mannose moieties permit it to bind multivalently with very high affinity (K_D = 2.7 x 10^{-11}M) to CD206 through CD206's 8 mannose binding domains. Multivalent binding is required for this high affinity interaction [28]. After binding to CD206, tilmanocept is internalized and accumulates in endosomes. Tilmanocept is protected by the U.S. patent #6,409,990.

Following various preclinical studies in both cells based assay systems and in various animal species and following numerous clinical studies (including three phase 3 trials) in human subjects with breast cancers, melanomas, and head and neck cancers [29-34], Lymphoseek® was approved by the United States Food and Drug Administration (FDA) in 2013 [35] for use as a lymphatic mapping agent in patients undergoing surgeries to remove melanomas and breast tumors without presurgical evidence of metastatic disease. Since then, the indications for which Lymphoseek® has been approved have been expanded to all 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). Additionally, Lymphoseek® is approved in the European Union (EU) for use in imaging and intraoperative detection of sentinel lymph nodes (SLNs) draining a primary tumor in adult patients with breast cancer, melanoma, or localized squamous cell carcinoma of the oral cavity. Lymphoseek® has an extraordinary safety record. Since its first regulatory approval in 2013, Lymphoseek® has been injected into over 170,000 cancer patients for various SLN related indications. To date, there have been no serious adverse events associated with these procedures that have been attributed to Lymphoseek®. Typical of how Lymphoseek® is used, Figure 5 shows a SPECT/CT image of a breast cancer patient injected peritumorally with Lymphoseek®. Localization to a SLN is clearly shown.

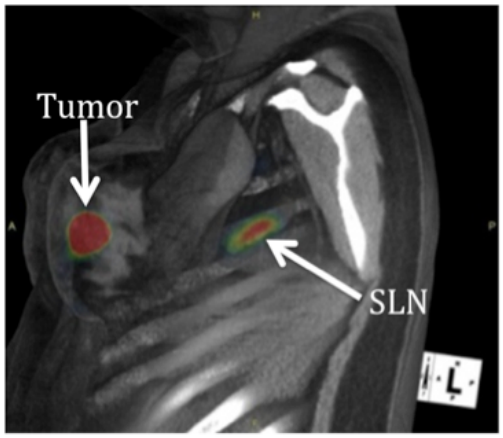


Figure 5. Pre-operative SPECT-CT image of a breast cancer patient who had been injected peritumorally with ^{99m}Tc-tilmanocept

Lymphatic transport and accumulation in the first encountered lymph node, the sentinel lymph node (SLN), is observed.

1.4.2 Transitioning from Subcutaneous and Intradermal Administration of ^{99m}Tc-Tilmanocept to IV Administration in Diagnostic Imaging indications

For all previously approved SLN and lymphatic mapping indications, the approved routes of administration were subcutaneous, intradermal or peritumoral injection. Intravenous (IV) injection, as is proposed in this protocol has not been approved as part of the label for Lymphoseek®. It is well known that CD206 expressing macrophages aggregate at sites of inflammation associated with a number of serious illnesses. Navidea wishes to expand the approved diagnostic indications for which ^{99m}Tc-Tilmanocept is approved to include several new diagnostic imaging indications that exploit the pathological aggregations of CD206 expressing macrophages associated with these illnesses. Most if not all of these alternative indications, including the one proposed in this protocol for imaging NASH inflammation, will require IV administration.

1.4.3 Nonclinical Evaluations – IV Administration

Dosing In Rats: In preparation to initiate the IV route of administration, eleven (11) preclinical tests were conducted to assess safety, toxicity, and interaction potential at doses hundreds to thousands of times the expected maximum human dose, as summarized below:

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

Type of Study / Description	Test System	Method of Administration	Dosing
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
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

Type of Study / Description	Test System	Method of Administration	Dosing
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(SD) rats given a single intravenous 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 timedependent 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 metabolismdependent 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.

Drug Distribution: Drug-related radioactivity was widely distributed throughout the whole body. The highest mean concentrations of radioactivity were observed in urine > kidney cortex > liver > kidney > kidney medulla > bile > spleen > spleen red pulp > spleen white pulp > urinary bladder between 0.25 and 36 hours post-dose. The concentration of radioactivity in other tissues was low (<500 ng equivalents 99mTc-Tilmanocept/g) and maximum levels were generally observed between 0.25 and 2 hours post-dose. Concentrations declined over time and were not detectable in the majority of the tissue by 36 hours post-dose. The exceptions were the kidney, liver, spleen, and urine, in which concentrations were still detectable at 48 hours post-dose. By 72 hours post-dose levels of radioactivity in all tissues were not detectable.

The main route of elimination of drug-related radioactivity following an IV administration of 99mTc-Tilmanocept was via urine, with a mean 38.9% of the dose administered detected in urine over 0 to 120 hours post-dose. A mean of 10.0% of the administered dose was eliminated via feces. The total recoveries were a mean of 50.8 ± 4.87% over the measurable time period for technetium Tc99m detection (t0.5 = 6.02 hr).

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).

1.4.4 Clinical Evaluations – IV Administration

The first questions that needed to be addressed when initiating studies evaluating ^{99m}Tc-Tilmanocept against new diagnostic targets using a new route of administration are: Can ^{99m}Tc-Tilmanocept specifically and sufficiently localize to aggregations of pathology related CD206 expressing macrophages to enable diagnostic imaging of disease associated inflammation, and what is the optimal imaging protocol to facilitate this desired imaging capability. In February of 2017, Navidea initiated the first such study evaluating these questions in which IV injected ^{99m}Tc-Tilmanocept was evaluated for localization in skeletal joints of subjects with skeletal joint inflammation due to rheumatoid arthritis (RA). As of this writing, this study in patients with active RA is still ongoing but has already provided positive affirmation that IV injected ^{99m}Tc-Tilmanocept localizes specifically and sufficiently to CD206 macrophages in RA inflamed joints with enough intensity to enable diagnostic imaging.

The study (Clincaltrials.gov number NCT02865434) was designed to determine an optimal imaging protocol utilizing IV injected ^{99m}Tc-Tilmanocept to visualize aggregations of CD206 expressing macrophages in skeletal joints inflamed due to involvement of rheumatoid arthritis (RA) and to evaluate safety variables. The study is expected to have 39 participants: 33 with active RA and 6 (healthy controls) without joint inflammation. Twenty-seven of the participants will be divided into 9 cohorts comprised of three participants each. The participants in each cohort will receive a different combination of tilmanocept mass dose labels with a different specific activity of ^{99m}technetium (^{99m}Tc) as described in Table 1.

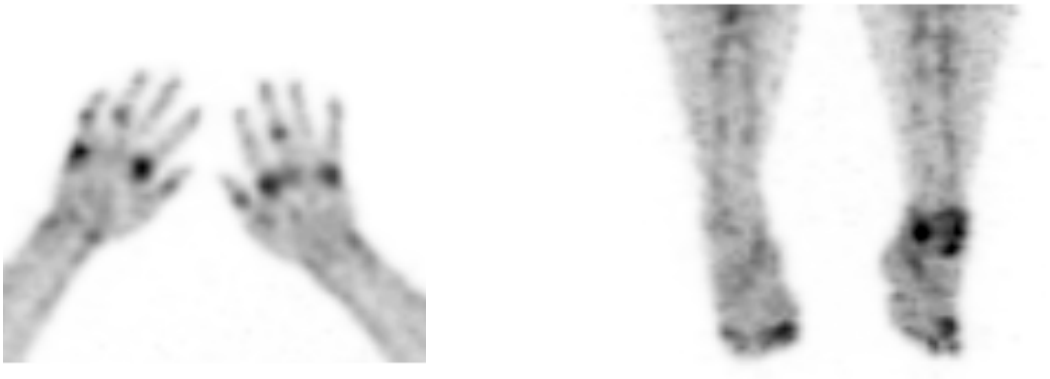
Table 1. The RA Dose Matrix of 99mTc-Tilmanocept administration

Dose Matrix	50 μg Tilmanocept	200 μg Tilmanocept	400 μg Tilmanocept
10 mCi ^{99m}Tc	Cohort 1	Cohort 2	Cohort 3
5 mCi ^{99m}Tc	Cohort 4	Cohort 5	Cohort 6
1.0 mCi ^{99m}Tc	Cohort 7	Cohort 8	Cohort 9

Two additional cohorts of 6 participants each will be recruited and imaged using the selected optimal dose. One cohort (Cohort 10) will be comprised of individuals with active RA, while the other cohort (Cohort 11) will consist of healthy individuals without joint disease. In addition to imaging of skeletal joints that may be involved in RA, participants in Cohorts 10 and 11 with be evaluated for ^{99m}Tc-Tilmanocept biodistribution, dosimetry and blood plasma half-life.

Three more subjects remain to be injected and imaged in each Cohort 10 and Cohort 11. To date, no serious adverse events have been observed and no adverse events have been attributed to tilmanocept in this study.

Figure 6A and Figure 6B show examples of the kinds of imaging results that are being obtained in this study. Figure 6A shows that hands of a participant with active RA, while Figure 6B shows an image of an RA involved ankle of a different individual. In both figures, localization of ^{99m}Tc-Tilmanocept to RA inflamed joints is clearly visible.



(A) **(B)**

Figure 6. IV Administered Tc 99m tilmanocept Localization to RA Joints

(A) ^{99m}Tc-Tilmanocept localization in hands (small joints' detection area after 200 µg/10 mCi dose of ^{99m}Tc-tilmanocept; picture on the left) and **(B)** in ankles and feet (50 µg/10 mCi dose of ^{99m}Tc-tilmanocept; picture on the right) after injections of ^{99m}Tc- tilmanocept administrated intravenously.

1.4.5 Clinical Safety using Intradermal, Subcutaneous, and Peritumoral Injections

Over 200,000 patients in clinical trials and commercial use in the U.S. for ILM with SLNB have been exposed to Tc 99m tilmanocept (Lymphoseek®), and there have been no safety signals, no deaths due to drug, and no Serious Adverse Events (SAEs) due to Lymphoseek®. There are no known drug interactions leading to contraindications with the use of Lymphoseek®. Post-marketing reports have shown less than 0.12% of subjects experiencing AEs, with the most common one being lack of node localization.

2 STUDY OBJECTIVES

2.1 Primary Objective(s)

- Determine the safety and tolerability of Tc 99m tilmanocept in subjects with NASH.

2.2 Secondary Objective(s)

- Determine the three dimensional tessellation localization of Tc 99m tilmanocept by planar and SPECT/CT imaging in subjects with NASH and asymptomatic controls.
- Determine the three dimensional tessellation localization of unfiltered Tc 99m sulfur colloid by planar and SPECT/CT imaging in subjects with NASH and asymptomatic controls.
- Comparison of liver localization distribution tessellation pattern intensity between Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid

2.3 Exploratory

- Concordance of intrahepatic localization tessellation discrimination of Tc 99m tilmanocept (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography

- Concordance of intrahepatic localization tessellation discrimination of Tc 99m sulfur colloid (intrahepatic disease heterogeneity) by SPECT/CT imaging and elastography

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Study Design

This is a prospective, open-label, multicenter, safety, comparative study of IV administered Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid in the detection of and assessment of three dimensional tessellation localization to the liver in subjects with and without moderate to severe NASH by planar and SPECT/CT imaging.

On Day 0, subjects will receive IV administration of 8.0 mCi of unfiltered Tc 99m sulfur colloid. Subjects will have planar imaging of the liver followed by SPECT/CT scanning of the abdomen, liver, and spleen at 20 minutes ± 5 minutes post-administration.

On Day 3, subjects will receive IV administration of 200 µg of tilmanocept at the specified radiolabeled dose of Tc 99m. Subjects will have planar imaging of the liver followed by SPECT/CT scanning of the abdomen, liver, and spleen at three specified time points post-administration: 20 minutes ± 5 minutes, 75 minutes ± 15 minutes, and 180 minutes ± 15 minutes.

The proposed study includes 3 visits and a safety follow-up telephone call: a screening visit for initial determination of eligibility and evaluation of clinical status ("Visit 1"), Day 0 ("Visit 2") the day of unfiltered Tc 99m sulfur colloid administration and imaging, Day 3 ("Visit 3") the day of Tc 99m tilmanocept administration and imaging, and a safety follow-up telephone call 5 ± 3 days following Tc 99m tilmanocept administration.

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.

Following completion of the first two enrolled NASH subjects, a review meeting will be held to evaluate tilmanocept safety and dose performance.

3.2 Justification for Study Design and Population

This feasibility study is designed to evaluate the safety of Tc 99m tilmanocept and to compare Tc 99m tilmanocept tessellation localization in the livers of subjects with moderate to severe NASH and healthy controls (HCs) to unfiltered Tc 99m sulfur colloid localization. Planar imaging of the liver using a SPECT camera will be combined with SPECT/CT imaging of the abdomen to provide greater resolution of areas of intrahepatic localization.

This study is designed to evaluate the use of Tc 99m tilmanocept as an imaging agent in subjects with biopsy-confirmed moderate to severe NASH by evaluating intrahepatic localization tessellation.

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 IRB as applicable.

3.4 Study Duration

Subjects will be "on study" for up to 57 days depending on the duration of the screening window (up to 45 days).

4 STUDY POPULATION

4.1 Eligibility

Subjects who fulfill all respective inclusion and none of the respective exclusion criteria will be eligible for enrollment into the study. All inclusion/exclusion criteria must be verified before a subject may be considered eligible for administration of unfiltered Tc 99m sulfur colloid and imaging (Day 0 procedures). A subject will be considered enrolled in the study on the morning of study Day 0 when they arrive at the study site and receive Tc 99m sulfur colloid via IV administration. Written and dated (with time noted) informed consent will be obtained from all subjects. A subject who withdraws consent prior to arrival at the study site on Day 0 will be considered a screen failure.

4.1.1 Inclusion Criteria

ALL SUBJECTS:

1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) authorization before the initiation of any study-related procedures.
2. The subject is ≥18 years of age at the time of consent.
3. The subject has a body mass index (BMI) between 18 and 45.

CONTROL SUBJECTS:

4. The subject is deemed to be clinically free of any infectious/inflammatory disease(s) for at least 4 weeks prior to the consent date.
5. The subject has not taken any antibiotics for at least 4 weeks prior to the consent date.

NASH SUBJECTS:

4. The subject has biopsy-confirmed NASH within 12 months prior to enrollment.
5. The subject has a NAFLD Activity Score (NAS) of ≥ 4, with a score of at least 1 for each steatosis, lobular inflammation, and hepatocyte ballooning.
6. The subject has fibrosis staging of F3-F4.

4.1.2 Exclusion Criteria

ALL SUBJECTS:

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator.
3. The subject has received radiation therapy or chemotherapy or has a previous diagnosis of cancer other than basal cell carcinoma.
4. The subject has renal insufficiency as demonstrated by a GFR of < 60 mL/min.

5. The subject has a chronic or persistent infection or has any condition that would, in the opinion of the examining physician, preclude their participation.
6. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
7. The subject has received an investigational product within 30 days prior to the Tc 99m sulfur colloid administration.
8. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m sulfur colloid.
9. The subject is HIV positive.
10. The subject has a history of alcohol abuse or currently consumes alcohol in excess of 3 drinks/day for men or 2 drinks/day for women.
11. The subject has hepatitis B or C.

CONTROL SUBJECTS:

12. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than two times the upper limit of normal (ULN).
13. The subject has been diagnosed with NASH, NAFLD, or other chronic liver disease.
14. The subject has been diagnosed with metabolic syndrome or Type I or II diabetes.

NASH SUBJECTS:

12. The subject has any chronic liver disease aside from NASH/NAFLD.
13. The subject has uncontrolled diabetes as indicated by an A1c >9% within the 3 months prior to enrollment.

4.2 Recruitment

Subjects will be recruited from gastroenterology practices in accordance with the inclusion and exclusion criteria listed above. Potentially suitable subjects will be asked by their treating physician about their willingness to participate in this study. Healthy, "NASH-free" subjects (controls) may be recruited via IRB approved advertisements and clinically assessed for the "absence" of liver and inflammatory diseases.

4.3 Withdrawal

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

A subject who withdraws consent prior to administration of Tc 99m sulfur colloid at the study site on Day 0 will be considered a withdrawn subject.

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

possible. An explanation should be given of why the subject is withdrawing or being withdrawn from the study.

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

- A protocol violation occurs
- A serious or intolerable 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

4.4 Replacement

Subjects will be replaced under the following conditions:

- Subjects who did not receive both unfiltered Tc 99m sulfur colloid and Tc 99m tilmanocept administration or did not proceed to imaging following either administration.

4.5 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: Study number "30"
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 "30-01-001."

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

5 INVESTIGATIONAL PRODUCT

5.1 Identification of Investigational Product

Tc 99m tilmanocept is a radiopharmaceutical that binds to mannose binding receptors (CD206) that reside on the surfaces of dendritic cells and macrophages.

5.2 Investigational Product Dosage and Administration

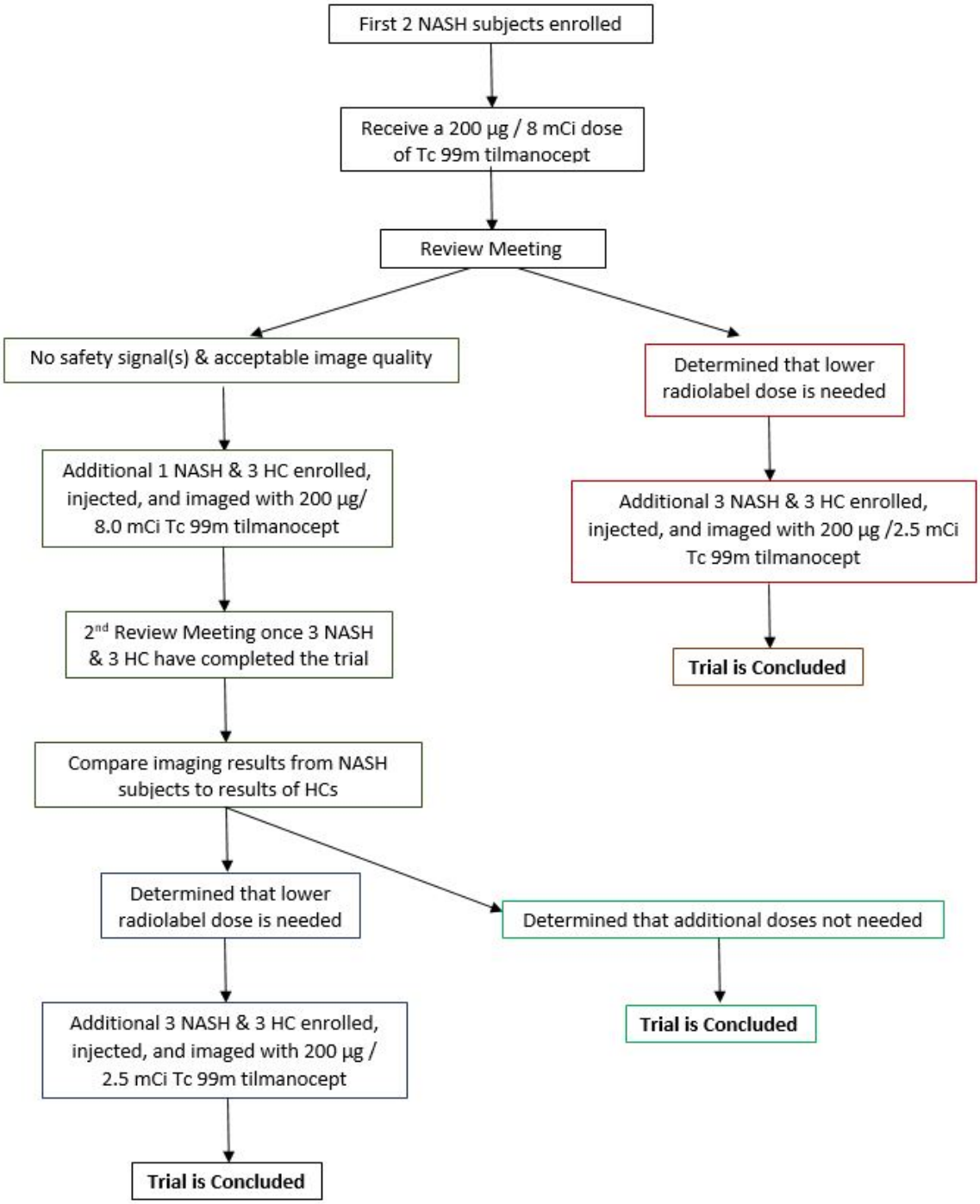
On Day 0, subjects will receive an on label 8.0 mCi dose of unfiltered Tc 99m sulfur colloid via IV administration. On Day 3, subjects will receive 200 µg tilmanocept via IV administration at the specified radiolabeled dose of Tc 99m. A single syringe will be used for each product and injected as a slow push into the IV. At the completion of each 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 tilmanocept dose will be ± 20% of the mass dose and radiolabel mCi dose assignment.

The first two enrolled subjects will be NASH subjects who will receive an 8.0 mCi radiolabel dose of Tc 99m tilmanocept. A review meeting will be held following completion of the first two subjects to evaluate tilmanocept safety and dose performance.

At the review (as diagrammed below):

1. If it is determined that a lower radiolabel dose of tilmanocept may produce images of better quality, three additional NASH subjects and three HCs will be injected and imaged with 200 µg of 2.5 mCi Tc 99m tilmanocept. After these six subjects have completed the trial, no additional subjects will be enrolled.

2. If the review shows no safety signals and acceptable quality of imaging, one additional NASH subject as well as three HCs will be injected and imaged with 200 µg of 8.0 mCi Tc 99m tilmanocept. Once a total of three NASH subjects and three HCs have completed the trial, a second review meeting will be held to compare results from NASH subjects to those of the HCs. If it is determined at the second review meeting that a lower radiolabel dose of tilmanocept may produce additional clinically meaningful results, 3 NASH subjects and 3 HCs will be dosed with 200 µg of 2.5 mCi Tc 99m tilmanocept. If it is determined that additional doses will not provide clinically meaningful results, the trial will be concluded.



5.3 Treatment Assignment

In this prospective, open-label, non-randomized, multicenter, safety, comparative study, all subjects will receive IV administration of both 8.0 mCi unfiltered Tc 99m sulfur colloid and 200 µg tilmanocept at the specified radiolabeled Tc 99m dose . After the completion of two

NASH subjects at the 8.0 mCi radiolabel dose of Tc 99m tilmanocept, the tilmanocept dose will reviewed, as detailed in Section 5.2.

5.4 Packaging and Labeling

Unfiltered Tc 99m sulfur colloid will be prepared at an on label 8.0 mCi dose according to standard clinical practice by Nuclear Medicine technologists at the clinical site [36].

The dose of Tc 99m tilmanocept should be ordered from Cardinal Health once the subject has been scheduled for IV administration and imaging.

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 contains 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. The Cardinal Health radiopharmacy will radiolabel the specified tilmanocept dose at the assigned millicurie dose Tc 99m in 1.0 mL and deliver one syringe to the clinical site radiopharmacy that is ready for administration.

5.5 Drug Logistics and 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 study and in accordance with this study protocol. For each subject, he/she will keep a record of the investigational product dispensed and store all other forms that accompanied the delivery of the radiolabeled product to the clinical site. These documents are to be filed in the investigator site file. Overall drug accountability and reconciliation will be completed by the sponsor or its representative. A list of investigational product vials 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 investigational product 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 the first screening visit through the 5 ± 3 days post-tilmanocept injection (Day 8 ± 3) safety follow-up telephone call will be documented. Subjects receiving radiation therapy or chemotherapy within 12 months of tilmanocept injection or who have been diagnosed with cancer other than basal cell carcinoma are not eligible for participation in the trial. The subject's history of treatments for NASH will also be collected.

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

7.1 Schedule of Evaluations

A schedule of evaluations is provided in the Schedule of Study Events (see Appendix 1).

7.2 Visit Description

7.2.1 Visit 1, Screening (Day -45 to Day -1)

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – 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. The subject's history of NASH treatment will also be collected.
- Concomitant Medications (within 30 prior to screening visit).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- ECG – The electrocardiogram (ECG) reading will be assessed for clinical significance by the investigator, and any clinically significant abnormal findings will be noted on the subject's medical history.
- Physical examinations will include an assessment of height, weight and calculation of BMI and an examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, 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.
- Blood draw for routine hematology, chemistry, and serum pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy, tubal ligation, 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
- Transient elastography will be performed to assess liver fibrosis
- Final confirmatory review of inclusion and exclusion criteria.

AE monitoring will begin from Visit 1 Screening and continue through subject completion of the trial.

7.2.2 Visit 2 (Day 0) Unfiltered Tc 99m Sulfur Colloid Administration

7.2.2.1 Before Administration of Unfiltered Tc 99m Sulfur Colloid

The following will be completed on the day of administration prior to the administration of unfiltered Tc 99m sulfur colloid:

- A urine 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.
- Assessment of AEs
- Concomitant medication review
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 15 minutes prior to administration of unfiltered Tc 99m sulfur colloid

7.2.2.2 Administration of Unfiltered Tc 99m Sulfur Colloid

IV administration of unfiltered Tc 99m sulfur colloid will be at study time Day 0 Time 00:00. The preferred site of IV placement will be the left or right antecubital vein.

All subjects will receive an on label 8.0 mCi dose of unfiltered Tc 99m sulfur colloid. The filled syringe will be connected to the IV for a slow push injection. At the completion of the injection, a 10 cc sterile normal saline flush will be administered.

The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for AEs.

7.2.2.3 After Administration of Unfiltered Tc 99m Sulfur Colloid

The following procedures will be completed at the specified times:

- 20 ± 5 Minutes After Administration
    - Assessment of AEs
    - Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate)
    - Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen

7.2.3 Visit 3 (Day 3) Tc 99m Tilmanocept Administration

7.2.3.1 Before Administration of Tc 99m Tilmanocept

The following procedures will be completed on the day of injection prior to the administration of Tc 99m tilmanocept:

- A urine 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.
- Assessment of AEs
- Concomitant medication review
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 15 minutes prior to administration of Tc 99m tilmanocept

7.2.3.2 Administration of Tc 99m Tilmanocept

IV administration of Tc 99m tilmanocept will be at study time Day 3 Time 00:00. The preferred site of IV placement will be the left or right antecubital vein.

All subjects will receive an open label 200 µg dose of Tc 99m tilmanocept at the specified radiolabel dose. The filled syringe will be connected to the IV for a slow push injection. At the completion of the injection, a 10 cc sterile normal saline flush will be administered.

The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for AEs.

7.2.3.3 After Administration of Tc 99m Tilmanocept

The following procedures will be completed at the specified times:

- 20 ± 5 Minutes After Administration:
  - Assessment of AEs
  - ECG (completed before vital signs)
  - Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate)
  - Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen

- 75 ± 15 Minutes After Administration:
  - Assessment of AEs

- Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen

- 180 ±15 Minutes After Administration:
  - Assessment of AEs
  - Planar imaging of the liver/spleen followed by SPECT/CT of the liver/spleen and abdomen

- After Completion of All Imaging:
  - Blood draw for hematology and chemistry testing
  - Urine collection for urinalysis

7.2.4 Safety Follow Up Telephone Call (Day 8 ± 3)

A safety follow-up telephone call will be performed 5 ± 3 days after administration of Tc 99m tilmanocept (Day 8 ± 3):

- Concomitant medication review
- Assessment and review of AEs

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Up to six (6) female or male subjects with biopsy-confirmed moderate to severe NASH will be enrolled. Up to six (6) female or male subjects without evidence of any liver or inflammatory diseases will be enrolled. There are no minimum or maximum numbers for gender in the groups.

8.1.2 Medical and Surgical History

Relevant medical and surgical histories will be obtained on all study subjects. For NASH subjects, this will include a history of all NASH treatment.

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 the first screening examination through the 5 ± 3 day post-tilmanocept injection (Day 8 ± 3) follow-up safety telephone call will be documented.

8.2 SPECT/SPECT-CT Image Acquisition

The camera used to obtain the images should be a dual-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 tests as per the manufacturer's recommendation for that day's scan schedule.

Whenever possible, subjects should be asked to void after injection and prior to the first imaging session and again between imaging sessions.

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 taping around the ankles can be used to keep the legs together. A pillow under the knees is often very helpful for comfort.

A planar scan of the liver followed by SPECT/CT should be obtained at the following time points following administration of the specified product:

Unfiltered Tc 99m Sulfur Colloid:

- 20 ± 5 minutes post administration

Tc 99m Tilmanocept:

- 20 ± 5 minutes post administration
- 75 ± 15 minutes post administration
- 180 ± 15 minutes post administration

It is anticipated that the planar scan would be acquired for 25-30 minutes. Using state-of-the-art 2-headed cameras (nominal 20" x 15" FOV), a target of 5-7 million counts should be obtained for the summed view of both heads. For 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.

A simple measure of the subject's "thickness" (i.e. from a lateral visual view, the height of the subject's anterior-most chest/abdomen above the scan bed) should be documented. This will be used for the attenuation correction to be applied to the anterior/posterior views obtained from the planar scan.

Following completion of the the planar imaging session at the designated time points, a 3D SPECT/CT should be obtained on the liver, spleen, and abdomen, followed by a patient break.

It is anticipated that each planar or SPECT/CT scan would be acquired for 25-30 minutes (30 minutes preferred). A break between scans should address the comfort of the patient, encouraging the subject to void, consume fluids or food and to move or rest freely. It is very important that the subject remain motionless for the duration of each scan.

The SPECT transverse 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 planar imaging as well as the SPECT/CT imaging acquisitions.

De-identified DICOM (Digital Imaging Communications in Medicine) copies of all acquired images will be transmitted to Navidea Biopharmaceuticals. The areas of localization will be identified, documented and captured in the CRFs.

8.3 Pharmacokinetics

No pharmacokinetic investigation will be performed in this study.

8.4 Safety

8.4.1 Adverse Events

8.4.1.1 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 1 Screening (Days -45 to -1) through the assessment of the Safety Follow-up Telephone Call (Day 8 ± 3) are to be reported as AEs. AEs continuing after study completion will be followed to normalization or stabilization. SAEs will be reported from the time of consent through the end of participation.

8.4.1.2 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 8.4.1.5.

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 AE 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 AE to the study procedure:

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

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

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

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

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

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

8.4.1.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs for the duration of subject participation. Untoward medical events beginning on Visit 1 Screening (Days -45 to -1) through the completion of the Follow-up Telephone Call will be reported as AEs. 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 8.4.1.5.
- 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]).

8.4.1.4 Expected Adverse Events

Investigational Product-Related Risks

In all completed studies of Tc 99m tilmanocept, involving 575 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 Tc 99m tilmanocept by the investigator. The most commonly reported adverse reactions have been lack of effect (<0.067%), injection site pain (<0.02%) and rash (<0.02%). 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.

Post-marketing surveillance shows that Tc 99m tilmanocept (Lymphoseek®) has been administered to more than 200,000 patients with not a single drug-related SAE. Routes of administration have included: subcutaneous, intradermal, and peritumoral. The intended route of administration in this study is IV.

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 AE 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.

8.4.1.5 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 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 AEs 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 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 all investigators about reported relevant events (e.g., SAEs, SUSARs) according to all applicable regulations.

8.4.2 Further Safety Assessments

8.4.2.1 Physical Exam

Complete physical examinations will be conducted according to the Schedule of Study Events (see Appendix 1). Height and body weight will be collected at Visit 1 (Screening).

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)
- Musculoskeletal
- Nervous system

8.4.2.2 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, and respiration, 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, within 15 minutes before each product administration, and 20 ± 5 minutes after each product administration.

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.

8.4.2.3 Electrocardiogram

A standard 12-lead ECG will be obtained at screening and 20 ± 5 minutes following Tc 99m tilmanocept administration. 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 both the Fridericia and Bazett formulas.

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.

8.4.2.4 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	Hemoglobin (Hgb), hematocrit (HCT), platelets, neutrophils, basophils, lymphocytes, monocytes, red blood cells (RBC), white blood cells (WBC)
Serum chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, creatinine, chloride, hemoglobin A1c (screening only), potassium, sodium, total protein, albumin, carbon dioxide (CO_2)/bicarbonate, blood urea nitrogen (BUN), glucose
Urinalysis	pH, specific gravity

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		**Blood Volume**
Screening: Laboratory examination	Chemistry 5 mLs Hematology 4 mLs	9 mL (~2 tsp)
Post Tilmanocept Injection and Imaging: Laboratory examination	Chemistry 5 mLs Hematology 4 mLs	9 mL (~2 tsp)
TOTAL:		18 mL (~4 tsp)

9 STATISTICAL METHODS

This is a prospective, open-label, multicenter, safety, comparative study of injected Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid in the detection of and assessment of localization (tessellation discrimination) to the liver in subjects with and without moderate to severe NASH by planar and SPECT/CT imaging. The statistical objective of the study is to determine the safety of Tc 99m tilmanocept and to compare Tc 99m tilmanocept localization (tessellation characteristics) to unfiltered Tc 99m sulfur colloid between subjects with and without NASH.

Exploratory contrasts with available elastography and Tc99m tilmanocept images may be completed.

A study center is defined as a treatment administration site or group of treatment administration sites under the control and supervision the same PI.

9.1 Randomization Methods

The study is not randomized.

9.2 Safety Variables

The primary variable for this study is whether a subject observes any noxious pharmacologic activity/adverse drug reaction (ADR) during their enrollment in the trial. This is a safety endpoint. The pharmacologic activity or ADR must be observed after the dose of Tc99m tilmanocept is administered.

NOTE: Adverse Drug Reaction

An 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 AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

9.3 Efficacy Variables

The efficacy variables for this study are as follows:

- Liver uptake of unfiltered Tc99m-labeled sulfur colloid and tilmanocept; and,
- Intrahepatic distribution (tessellation) of Tc99m-labeled sulfur colloid and tilmanocept

9.4 Sample Size Justification

The sample size of N=12 subjects is deemed sufficient to accomplish the Sponsor's objectives of determining the safety of IV tilmanocept and localization patterns of tilmanocept and sulfur colloid in NASH and asymptomatic subjects.

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following analysis population will be defined for the study:

- **Intent-to-Diagnose (ITD) Population** - Subjects who are enrolled in the study, administered Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid, and are imaged will be included in the ITD analysis population.
- **Safety Population** – All patients who are enrolled and administered Tc 99m tilmanocept and unfiltered Tc 99m sulfur colloid in the study 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 group. Continuous variables will be summarized via mean, median, standard deviation, and range. Categorical variables will be summarized via counts and percentages.

9.5.3 Analysis of Efficacy Variables

Co-location of tilmanocept and sulfur colloid intrahepatic tessellation localization (Figure 7) will be assessed through frequency tables derived by creating virtual slices of the liver.. These scans will be false-colored and overlaid, matching landmarks (e.g. large blood vessels) for image registration. The resulting voxel colors will be classified as binding tilamanocept, binding sulfur colloid, or binding both. No formal hypothesis tests are planned. Frequency tables will created for individual subjects and overall.

The voxel radiation intensities will be used to create non-parametric density estimates of ^{99m}Tc gamma radiation intensities for both Tc 99m tilmanocept and Tc 99m sulfur colloid. Density estimates will be created for both individual subjects and for NASH subjects and healthy controls pooled separately.

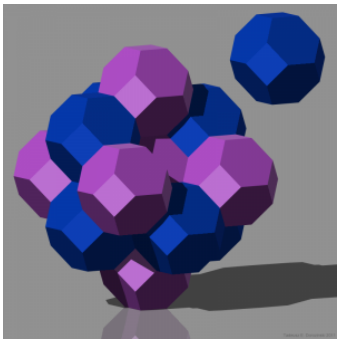


Figure 7. Diagrmatic representation of anticipated tessellation

Complete analyses and details 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 any noxious pharmacologic activity or 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 AE is defined as an AE whose start date is on or after the procedure date. If the procedure date 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) at each time point 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 is captured in the subject's source documentation and are to be recorded in the CRFs (provided by the sponsor) as soon as possible.

10.1.1 CRF Design

Paper 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 investigational product. The investigational product will not be supplied to the investigator site prior to a favorable opinion from the IRB and the regulatory authority and, if appropriate, from the radiation protection authorities. In addition, the CRA will determine whether all AEs and SAEs have been appropriately reported (including adherence to the time periods required for SAEs).

10.3 Data Processing

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

For data coding (e.g., AEs, medication, medical/surgical history), internationally recognized and accepted dictionaries will be used. These and the processes used for coding will be specified in the data management plan.

10.4 Auditing

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

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

10.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request. Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution, or private practice. Where the archiving procedures do not meet the minimum timelines required by the sponsor, alternative arrangements must be made to ensure the availability of the source documents for the required period.

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

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

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

10.6 Premature Termination of the Study

Termination by the Sponsor

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

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

Termination by the Investigator

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

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

10.6.1 Study as a Whole

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

At the discretion of the sponsor, the entire study may be canceled for medical reasons. In addition, the sponsor retains the right to end the study at any time if the study cannot be carried out as agreed upon in the protocol. In case of early termination or suspension of the study, the principal investigator/sponsor will promptly inform the investigator/institutions, regulatory authorities, and IRB of the termination or suspension and the reason for that.

10.6.2 Center

At any time, the study 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 study according to the criteria specified in Section 4.3.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

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

11.2 Subject Information and Consent

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

Based on this subject ICF, the investigator will explain all relevant aspects of the study to each subject, before entry into the study (i.e., before examinations and procedures associated with selection for the study are performed).

The investigator will also mention that written approval of the IRB has been obtained. Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Following this informative discussion, the subject 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 study. Additionally, the investigator or his/her designee will personally sign and date the form. The subject will receive a duplicate of the signed and dated form.

The investigator will record in the source documentation the consent process including the time and date of obtaining informed consent. In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

The 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 study by signing

the revised ICF. Any revised written ICF and written information must receive the IRB's approval/favorable opinion in advance of use.

11.3 Financing/Financial Disclosure

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

11.4 Publication Policy

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

11.5 Subject Injury

In general, if a subject is injured as a direct result of the investigational product but not due to medical negligence on the part of the principal investigator or study staff, the sponsor will pay for reasonable and necessary medical treatment for the injury, to the extent the expenses are not covered by the subject's medical insurance, a government program, or other responsible third party. If laws or regulations of the locality in which the study 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	Visit 2 Day 0 Tc 99m sulfur colloid Administration			
	Days -45 to -1	Day 0 Prior to Administration	- 00:15 to -00:01 Mins	00:00	20 ± 5 Mins
Informed Consent	x				
Entry Criteria	x				
Medical History, Demography	x				
Vital Sign Assessment	x		x		x
ECG	x				
Physical Examination	x				
Clinical Laboratory Evaluation: Chemistry, Hematology and UA	x				
Hemoglobin A1c Evaluation	x				
Pregnancy Test	x[a]	x[a]			
Transient Elastography	x				
Sulfur Colloid Administration				x	
Tilmanocept Administration					
Planar and SPECT/CT Liver Imaging					x
Concomitant Medications	x	x			
Adverse Event Monitoring	x	x	x	x	x

a. Pregnancy test at screening will be serum; Day 0 and Day 3 pregnancy tests will be urine.
b. Collection of samples for laboratory evaluation will occur after completion of all imaging procedures.

Evaluation	Visit 3 Day 3 Tc 99m tilmanocept Administration						Follow-up Telephone Call
	Day 3 Prior to Administration	- 00:15 to -00:01 Mins	00:00	20 ± 5 Mins	75 ± 15 Mins	180 ± 15 Mins	Day 8 ± 3
Informed Consent							
Entry Criteria							
Medical History, Demography							
Vital Sign Assessment		x		x			
ECG				x			
Physical Examination							
Clinical Laboratory Evaluation: Chemistry, Hematology and UA						x[b]	
Pregnancy Test	x[a]						
Transient Elastography							
Sulfur Colloid Administration							
Tilmanocept Administration			x				
Planar and SPECT/CT Liver Imaging				x	x	x	
Concomitant Medications	x						x
Adverse Event Monitoring	x	x	x	x	x	x	x

a. Pregnancy test at screening will be serum; Day 0 and Day 3 pregnancy tests will be urine.
b. Collection of samples for laboratory evaluation will occur after completion of all imaging procedures.

Appendix 2 Sponsor Signatures

Study Title:	An Evaluation of the Safety of Intravenous (IV) Tc 99m Tilmanocept and a Comparison of Localization to Tc 99m Sulfur Colloid by Planar and SPECT/CT Imaging in Subjects with Nonalcoholic Steatohepatitis (NASH) and Healthy Controls (HC)
Study Number:	NAV3-30
Original Protocol Date:	06 October 2017
Amendment 1 Date:	23 April 2018

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: 26 Apr 2018

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

Signed:_____ Date: 26-April 2018

Michael S. Blue, MD
Senior Medical Director
Navidea Biopharmaceuticals

Signed:_____ Date: 27-APR-2018

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

Appendix 3 Investigator's Signature

Study Title:	An Evaluation of the Safety of Intravenous (IV) Tc 99m Tilmanocept and a Comparison of Localization to Tc 99m Sulfur Colloid by Planar and SPECT/CT Imaging in Subjects with Nonalcoholic Steatohepatitis (NASH) and Healthy Controls (HC)
Study Number:	NAV3-30
Original Protocol Date:	06 October 2017
Amendment 1 Date:	23 April 2018

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:_____