Clinical Study Protocol: NAV3-21

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and Skeletal Joint Imaging with SPECT in Subjects with Active Rheumatoid Arthritis (RA) and Healthy Controls
Study Number:	NAV3-21
Study Phase:	1 and 2
Product Name:	Technetium Tc 99m tilmanocept
IND Number:	132943
Investigator(s):	Multicenter
Sponsor:	Navidea Biopharmaceuticals, Inc 4995 Bradenton Avenue, Suite 240 Dublin, OH 43017
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	Date
Original Protocol:	23 May 2016
Amendment 1:	12 September 2016
Amendment 2:	22 December 2016
Amendment 3:	09 February 2017
Amendment 4:	23 August 2017
Amendment 5:	18 April 2018
Amendment 6:	19 June 2018

SYNOPSIS

Study title	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and Skeletal Joint Imaging with SPECT in Subjects with Active Rheumatoid Arthritis (RA) and Healthy Controls
Study phase	Phase 1 and 2
Study objective(s)	**Primary** • Determine the safety and tolerability of escalating doses of Tc 99m tilmanocept. **Secondary** • Determine the localization of Tc 99m tilmanocept by SPECT (single photon emission computed tomography) imaging in subjects with active RA and asymptomatic controls and concordance of localization with clinical symptomology. • Comparison of localization intensity between Tc 99m tilmanocept dose groups for optimal dose selection and determination of optimal imaging time. • To estimate the pharmacokinetics (PK) and dosimetry of Tc 99m tilmanocept in subjects with and without active RA.
Duration of treatment	Up to 53 days
Study drug	Technetium Tc 99m tilmanocept
Dose(s) and Route of administration	**Route of Administration:** All administrations of Tc 99m tilmanocept will occur through an intravenous (IV) route of injection. For the 50 and 200 µg doses a single syringe will be used and injected as a slow push into the Y-port. For the 400 µg dose 2 syringes will be used and injected sequentially as a slow push into the Y-port. At the completion of the injection(s), a 10 cc sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein unless both elbows are primary sites for RA imaging, and then the site for IV placement will be between the elbow and the wrist. **Doses:** Tc 99m tilmanocept will be received by subject through IV administration at one of 3 mass doses: 50 µg, 200 µg, or 400 µg. Within each mass dose group, subjects will receive Tc 99m tilmanocept labeled with one of 3 radiolabel doses: 1 mCi, 5 mCi, or 10 mCi.

<table>
<tr><td></td><td>
<table>
<tr><td></td><td colspan="3">Tilmanocept Mass Dose</td></tr>
<tr><td>Specific Radioactivity</td><td>50 µg</td><td>200 µg</td><td>400 µg</td></tr>
<tr><td>10 mCi</td><td>Group 1
n = 3 RA</td><td>Group 2
n = 3 RA</td><td>Group 3
n = 3 RA</td></tr>
<tr><td>5 mCi</td><td>Group 4
n = 3 RA</td><td>Group 5
n = 3 RA</td><td>Group 6
n = 3 RA</td></tr>
<tr><td>1 mCi</td><td>Group 7
n = 3 RA</td><td>Group 8
n = 3 RA</td><td>Group 9
n = 3 RA</td></tr>
</table>
* Group 10 will consist of 6 Healthy Controls (HC) (3 female subjects and 3 male subjects) and Group 11 will consist of 6 subjects with active RA (3 female subjects and 3 male subjects). Subjects in Groups 10 and 11 will be enrolled in parallel and will be dosed at the maximum tolerated dose (MTD) as determined from Groups 1-9.
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<tr><td>Inclusion criteria</td><td>
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. Has a negative urine drug screening for illicit or unprescribed drugs suggestive of drug abuse as determined by the investigator.
3. All subjects shall be ≥18 years of age at the time of consent.

CONTROL SUBJECTS:
4. The subject is deemed to be clinically free of any inflammatory disease (s) and has not experienced joint pain for at least 4 weeks prior to the consent date.

ACTIVE RHEUMATOID ARTHRITIS SUBJECTS:
4. The subject has moderate to severe RA as determined by the 2010 ACR/EULAR (score of ≥ 6/10) that includes an affected hand and at least one large jointing (elbow, knee or shoulder).
5. The subject has a DAS28 of ≥ 3.2 (includes the Erythrocyte Sedimentation Rate [ESR] test and Visual Analog Scale [VAS]) .
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	6. If the subject is receiving methotrexate, they have been at a stable dose for > 4 weeks prior to the Baseline Visit 2 (Day 1). 7. If the subject is receiving biologic therapy, they have been at a stable dose > 8 weeks prior to the Baseline Visit 2 (Day 1). 8. If the subject is receiving NSAIDS (nonsteroidal anti-inflammatory drug) or oral corticosteroids, the dose has been at a stable dose for > 4 weeks prior to the Baseline Visit 2 (Day 1). The corticosteroid dose should be ≤ 10mg/day of prednisone or an equivalent steroid dose.
Exclusion criteria	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 had or is currently receiving radiation therapy or chemotherapy for a condition other than rheumatoid arthritis. 4. The subject has renal insufficiency as demonstrated by serum creatinine clearance of < 60 mL/min. 5. 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. 6. The subject has a chronic or persistent infection or has any condition that would, in the opinion of the examining physician, preclude their participation. 7. The subject has a known allergy to or has had an adverse reaction to dextran exposure. 8. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration. 9. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m tilmanocept.
Study design	Prospective, open-label, multicenter, dose escalation, and safety with PK and dosimetry study of injected Tc 99m tilmanocept in the detection of and assessment of localization to skeletal joints in subjects with and without active RA by SPECT imaging. All subjects will receive IV administration at one of 3 mass doses: 50 µg, 200 µg, or 400 µg. Within each mass dose group, subjects will receive Tc 99m tilmanocept labeled with one of 3 radiolabel doses: 1 mCi, 5 mCi, or 10 mCi. All subjects will have a whole body planar SPECT scan. Subjects enrolled in Groups 1-9 will receive a whole body

	planar SPECT scan followed by a 3D SPECT or SPECT/CT scan on areas of interest at two specified time points post injection: 60 minutes ± 15 minutes and 180 minutes ± 15 minutes. Subjects enrolled in Groups 10-11 will receive a whole body planar SPECT scan performed at 4 specified time points post injection: 15 ± 5 minutes, 60 ± 15 minutes, 180 ± 15 minutes and 18-20 hours. Subjects enrolled in Groups 10-11 will also receive a planar SPECT hands scan at 60 ± 15 minutes and 180 ± 15 minutes post-injection. PK blood samplingwill be performed before injection (within 15 minutes), immediately following injection (within 5 minutes), and at each scanning timepoint. Dosimetry tests will be performed at each scanning timepoint. PK of urine will be assessed through counts of the bladder wall obtained from cumulative quantitative planar imaging from radiation dosimetry.
Methodology	The study includes: screening, enrollment, pre- and post-injection assessments, injection, imaging and follow-up. **<u>All Subjects:</u>** **Visit 1, Screening (Day -44 to Day 0):** The screening visit will include informed consent, preliminary review of study eligibility, collection of medical history including medications, urine drug screen, vital signs, ECG (electrocardiogram), physical exam including height and weight, clinical labs, RA specific labs, urinalysis, urine pregnancy test for subject of childbearing potential, 2010 ACR/EULAR score, DAS28 evaluation. **<u>Groups 1-9:</u>** **Visit 2, Baseline (Day 1):Pre-Tc 99m Tilmanocept Injection:** • Before injection (Day 1) o Urine pregnancy test will be performed for subjects of childbearing potential o Concomitant medication review o ECG - within 15 minutes prior to injection o Vital Signs - within 15 minutes prior to injection

	Tc 99m Tilmanocept Injection : Subjects will receive their open label serial dose assignment in accordance with Table 1. Adverse event monitoring will continue through completion of the trial.
	
	Post-Tc 99m Tilmanocept Injection:
	• 0 - 15 Minutes Post Injection:
	  ○ Assessment of adverse events
	  ○ ECG (completed before vital signs)
	  ○ Vital Signs
	• 60 ± 15 Minutes Post Injection:
	  ○ Assessment of adverse events
	  ○ Whole body planar imaging
	  ○ Planar image with both hands in the field of view (FOV)
	  ▪ Additional 3D SPECT or SPECT/CT will be obtained on identified joints of interest.
	• 180 ± 15 Minutes Post Injection
	  ○ Assessment of adverse events
	  ○ Whole body planar imaging
	  ○ Planar image with both hands in the FOV
	  ▪ Additional 3D SPECT or SPECT/CT will be obtained on identified joints of interest.
	5± 2 Days Post-injection Follow-Up Visit (Visit 3):
	• Vital Signs
	• ECG
	• Physical Exam, including body weight
	• Clinical labs and urinalysis
	• Concomitant medication review
	• Assessment of adverse event

	Groups 10-11: **Visit 2, Baseline (Day 1):** **Pre-Tc 99m Tilmanocept Injection:** • Before injection (Day 1) o Urine pregnancy test will be performed for subjects of childbearing potential o Concomitant medication review o ECG - within 15 minutes prior to injection o Vital Signs - within 15 minutes prior to injection o PK blood sample – within 15 minutes prior to injection **Tc 99m Tilmanocept Injection:** Subjects will receive the maximum tolerated dose, as determined from Table 1. Immediately following Tc 99m tilmanocept administration, a PK blood sample will be drawn. Adverse event monitoring will continue through completion of the trial. **Post-Tc 99m Tilmanocept Injection Procedures:** • 0 - 15 Minutes Post Injection: o Assessment of adverse events o ECG (completed before vital signs) o Vital Signs • 15 ± 5 Minutes Post Injection: o Whole body planar imaging o PK blood sampling o Assessment of adverse events • 60 ± 15 Minutes Post Injection: o Whole body planar imaging o Planar image with both hands in the FOV o PK blood sampling o Assessment of adverse events • 180 ± 15 Minutes Post Injection: o Whole body planar imaging o Planar image with both hands in the FOV o PK blood sampling o Assessment of adverse events

	Post-Tc 99m Tilmanocept Injection Procedures and Follow-Up Visit (Visit 3): • 18-20 Hours Post Injection: o Whole body planar imaging o PK blood sampling o Vital Signs o ECG o Physical Exam, including body weight o Clinical labs and urinalysis o Concomitant medication review o Assessment of Adverse Events	
Planned study dates	**Start of study recruitment** / August 2016	**End of Recruitment**/July 2018 **End of Study**/September 2018
Planned number of study centers	Approximately 1-3 centers in the United States	
Number of subjects	Up to 48 evaluable subjects may be enrolled. The dose escalation portion of this study is a modified 3+3 design with no dose de-escalation. The sequential cohort enrollment characteristics of this design do not allow a fixed computation of sample size. The parameters of the design that can be calculated are shown in Table 4. Based on an assumed vector of probabilities of no subjects experiencing pharmacologic activity or an Adverse Drug Reaction (ADR) in each dose group, the average sample size of the dose escalation phase of the study is n=26.3 patients and the probability that dose group 7 will be selected as the MTD (maximum tolerated dose) is 0.152.	
Primary endpoint	Proportion of subjects not experiencing pharmacologic activity or ADR in each dose group.	
Secondary endpoints	The secondary endpoints for this study are: • Per subject localization rate of Tc 99m tilmanocept by SPECT imaging • Per joint location clinically RA-identified joint localization rate of Tc 99m tilmanocept by SPECT imaging • Concordance of Tc 99m localization with anatomical areas of active RA defined by clinical symptomology. • Localization intensity for each clinically RA-defined joint (as determined by quantitative SPECT gamma counts)	

	• Per subject localization rate of Tc 99m tilmanocept in areas other than RA-positive joints by SPECT imaging • The following PK parameters will be calculated, whenever possible, using Tc 99m tilmanocept total radioactivity in whole blood and urine: maximum observed concentration (C_{max}), time to C_{max} (t_{max}), area under the concentration-time curve (AUC) from Hour 0 to the last measureable concentration (AUC_{0-t}), AUC extrapolated to infinity ($AUC_{0-\infty}$), apparent terminal elimination rate constant (λ_Z), and apparent terminal elimination half-life ($t_{1/2}$). • Radiation dosimetry of Tc 99m tilmanocept will include radiation doses per organ (μGy/MBq), organs receiving the highest organ dose (HT), effective doses per organ (μSv/MBq), mean effective dose (μSv/MBq), effective dose resulting from a diagnostic dose ± 20%. Dosimetry will be based on target organ image counts and modeling per OLINDA/EXM® 2.0 (Organ Level INternal Dose Assessment/EXponential Modeling) software.
Data Analyses	The following analysis population will be defined for the study: • **Intent-to-Diagnose (ITD) Population** - Subjects who are enrolled in the study in Groups 1-9, injected with Tc 99m tilmanocept, and received whole body planar imaging will be included in the ITD analysis population. • **Radiopharmacokinetic (RPK) Population** – Subjects that have been enrolled in the study in Groups 10-11, injected with Tc 99m tilmanocept, and received whole body planar imaging will be included in the RPK analysis population. • **Safety Population** – All patients who are enrolled and injected with Tc 99m tilmanocept in the study will be included in the safety population. • **Per Protocol (PP) Population –** The PP population will include all safety subjects without major protocol violations. All safety data analyses will be conducted on the safety population. All efficacy data analyses will be conducted on the ITD and PP population with the ITD population being the primary analysis set. All analyses of RPK data will be carried out on the RPK population.

	The number and percentage of subjects experiencing pharmacologic activity or ADRs will be tabulated by dose group and overall. Per subject localization rates and percentage will be calculated by time point by dose group and overall. Per joint location localization rates and percentages will be calculated by time point by dose group and overall. Per location concordance of clinical diagnosis to nuclear medicine specialist illumination indication will be computed across all RA positive sites of all subjects at all time points. Localization intensities will be summarized by descriptive statistics (mean, median, standard deviation, minimum, maximum and range) by joint location at each time point. Radiopharmacokinetic (RPK) parameters will be estimated for each subject using noncompartmental models. Summary statistics (n, mean, std, CV, geometric mean) will be computed for each RPK parameter across all subjects by Group (10, 11). For dosimetry data, for each dosimetry parameter, summary statistics (n, mean, std, and a one standard deviation width confidence interval) will be computed by organ type and across all subjects by Group (10,11).

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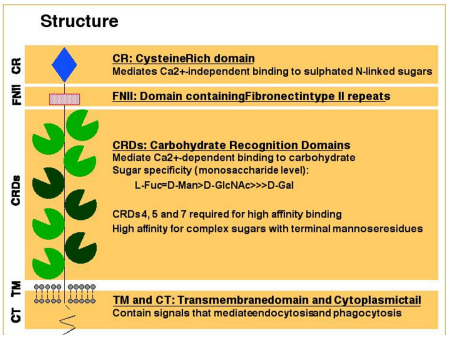
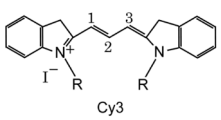
LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACR/EULAR	American College of Rheumatology/ European League Against Rheumatism
ACPA	Anti-citrullinated peptide antibody
ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase (SGPT)
AST	aspartate aminotransferase (SGOT)
AUC	area under the concentration-time curve
AUC_{0-t}	AUC from hour 0 to last measurable concentration
$AUC_{0-\infty}$	AUC extrapolated to infinity
BMI	body mass index
BUN	blood urea nitrogen
C_{max}	maximum observed concentration
CD206	Mannose-binding receptor (Ca2+-binding lectin)

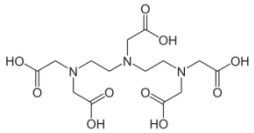


CO_2	carbon dioxide
CRF	case report form
CRA	clinical research associate
CRO	contract research organization
CRP	C-Reactive protein
CT	X-ray computed tomography
Cy3	Cy3 cyanine dyes fluoresce orange (~550 nm excitation, ~570 nm emission)



Cy3

DAS28	Disease activity score used with the ACR/EULAR 2010 RA guidelines
DICOM	Digital Imaging Communications in Medicine
DMARD(s)	disease-modifying antirheumatic drug(s)
DTPA	diethylene triamine pentaacetic acid



ECG	electrocardiogram
eCRFs	Electronic case report forms
ESR	Erythrocyte sedimentation rate
EU	European Union
FDA	Food and Drug Administration
FNR	false negative rate
FOV	field of view
GCP	Good Clinical Practice
HC	healthy controls
HCT	hematocrit
Hgb	hemoglobin
HIPAA	Health Information Portability and Accountability Act
HT	highest organ dose
ICF	informed consent form
ICH	International Conference on Harmonization
$\%ID_{SN}$	percentage of Injected Dose in the Sentinel Node
ILM	intraoperative lymphatic mapping
IRB	Institutional Review Board
ISF	investigator site file
ITD	intent-to-diagnose population
IV	intravenous
kDa	kiloDalton (molecular weight designation)
mCi	milliCurie ($37x10^6$ becquerels; 37megabecquerels)

MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NOAEL	no-observed adverse-effect level
NSAIDS	Nonsteroidal anti-inflammatory drug
OA	osteoarthritis
OLINDA/EXM®	Organ Level INternal Dose Assessment/EXponential Modeling
PI	principal investigator
PK	Pharmacokinetics
PP	per protocol
RA	Rheumatoid arthritis
RBC	red blood cells
RF	Rheumatoid Factor
RPK	Radiopharmacokinetic
SAE	serious adverse event
SAP	statistical analysis plan
SCC	squamous cell carcinoma
SD	study day
SJC	swollen joint count
SLN	sentinel lymph node
SLNB	sentinel lymph node biopsy
SPECT	single photon emission computed tomography
SUSARs	Suspected, Unexpected, Serious Adverse Reactions
$t_{1/2}$	apparent terminal elimination half life
t_{max}	time to C_{max}
Tc 99m	technetium-99m metastable isotope; γ-emitting (t½ = 6.02 h)
TcSC	Tc 99m sulfur colloid
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek)
TJC	tender joint count
TMF	trial master file

TNFα	Tumor necrosis factor alpha
ULN	upper limit of normal
US	United States
VAS	visual analog scale
VBD	vital blue dye
WBC	white blood cell
λ_z	apparent terminal elimination rate constant

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

Rheumatoid arthritis (RA) is a common, chronic, systemic, progressive, autoimmune disease causing inflammation and pathology throughout the body, but perhaps most noticeably in the peripheral joints of the skeleton (i.e. hands, feet, knees, hips, etc.). It has been estimated that 52.2 million U.S. adults suffer from arthritis of which 1.3 million adults suffer from RA (CDC, Helmick et al.). In the affected joints, RA is characterized by macrophage and lymphocyte infiltration, proliferation of synovial fibroblastic tissue (pannus), and joint destruction (Ma et al.). RA causes joint pain, stiffness and reduced mobility. If not successfully treated, joint inflammation and destruction in RA patients can lead to crippling loss of function, severe chronic pain, and disfigurement of the joints. Also, RA patients have significantly higher risk of coronary heart disease including acute myocardial infarction (odds ratio = 3.17) (Maradit-Kremers et al.). Patients with uncontrolled RA may also suffer a reduction of 3-10 years in their life expectancies (Carmona et al.). RA can strike anyone at any age, but is diagnosed most frequently in women in their 40s and 50s (Oliver et al.). Worldwide, about one adult in every 200 has RA. In the United States, approximately 1.3 million adults have RA (Helmick et al., Scott et al.). Because RA is a chronic disease, the prevalence of RA increases with age. Because of aging demographics in the United States and elsewhere, the number of patients with RA and the burden of RA on society are expected to increase in the coming decades (Hootman et al.). Therefore, there is a significant current and growing need to manage RA patients more effectively to limit the morbidity and mortality caused by RA.

Over the last decade or so, disease-modifying antirheumatic drugs (DMARDs) have transformed RA therapy. First line DMARD therapy is usually methotrexate (Scott et al.). In addition, a number of biologic DMARDs have been developed. Basic research on the pathobiology of RA revealed that the inflammation observed in RA is the consequence of a self-perpetuating disturbance in the expression and downstream signaling of a network of cytokines (Raza et al., Olszewski et al., Meyer et al.). At the center of this cytokine network is the overexpression of tumor necrosis factor alpha (TNFα) by synovial macrophages (Leizer et al., Westra et al., Hamilton et al., Keffer et al., Noack et al., Bugatti et al., Boissier et al., Tran et al., Kinne et al.). TNFα then induces IL-1 and other cytokines that together with TNFα mediate the inflammation and joint damage characteristic of RA (Leizer et al., Zwerina et al., Feldmann et al., Schett et al.). These findings led to the development of biologic therapies for RA that block TNFα (i.e., etanercept, infliximab, adalimumab, certolizumab pegol, and golimumab) (Scott et al., Kalden et al., Wiedmann et al., Chen et al.). In addition, other biologic therapies have been introduced that target other aspects of the immune system that are pathologically dysfunctional in RA. These include abatacept (targeting T-cell stimulation), rituximab (targeting B-cells), and tocilizumab (targeting the IL-6 receptor) (Vivar et al.). Generally, the biologic DMARDs are used as second line therapies for RA, either alone or with methotrexate, in patients who have failed methotrexate monotherapy (Wasserman et al., Saag et al.). All of the DMARDs can cause serious adverse side effects (Choy et al., Donahue et al.). Therefore, patients on DMARDs require close monitoring to manage and minimize their adverse side effect risks. Also, there is a need, not yet fully satisfied, to monitor the effectiveness of DMARD therapy to further minimize these risks and to optimize the benefits

of therapy. Conversely, the benefits of DMARD therapy can far outweigh its risks. Most patients on DMARDs receive a significant reduction in symptoms, and some patients experience a disease remission. In the past, the goals of RA therapy were to reduce the severity of symptoms and limit the rate of progression of the illness. Today, the goal is remission (Gramling et al.). Also, RA patients taking TNFα antagonists have a reduced risk for cardiovascular disease events (Barbhaiya et al.).

There are two barriers preventing more RA patients from achieving disease remission (Bykerk et al.). Both of these barriers may be remedied by tilmanocept. Firstly, DMARD therapy is much more effective when provided soon after the onset of RA symptoms and declines in efficacy if initiation of DMARD therapy is delayed (Demoruelle et al.). Currently, and as described in the next section, early diagnosis of RA is problematic. Secondly, even when DMARD therapy is provided soon after the onset of symptoms, a significant minority of patients do not respond or respond poorly, demonstrating the continuing need for new and better DMARD therapies (Sacre et al.). For example, in a study reported by Anderson et al. (Anderson et al.), the authors found that the time between the onset of RA symptoms and the initiation of methotrexate therapy was inversely associated with the response rate for therapy ($p = 0.001$). In another study of 598 RA patients who were followed for a number of years after their RA diagnosis (van der Linden et al.), patients who initiated therapy more than 12 weeks from the onset of symptoms experienced higher probability of failure to achieve DMARD-free remission (HR= 1.87) and experienced greater long term joint damage when compared with patients who initiated therapy within 12 weeks of symptom onset. These studies and the observations of others (Nell et al., Cush et al.) indicate that early diagnosis of RA affords a "window of opportunity" for the greatest probability of effective RA therapy and the possibility of disease remission. Delayed diagnosis increases the chance of DMARD treatment failure. Furthermore, even in the best of circumstances, the response rate for RA therapies is in the 50%-70% range (Choy et al., Bykerk et al., Sacre et al., Zwerina et al.),indicating the continued need for better DMARD therapies.

The growing realization that early diagnosis of RA can positively impact treatment efficacy and long term patient outcomes motivated the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) to collaborate on the development of new diagnostic criteria for RA. The results of this collaboration were published in 2010 as the ACR/EULAR 2010 criteria (Aletaha et al.). These new criteria replaced the older 1987 ACR criteria and were largely hailed as a "game changer" for the diagnosis and management of RA (Cohen et al.). Since its publication, the ACR/EULAR 2010 criteria have been evaluated in a number of studies to determine which patients presenting with polyarthralgia progress to RA (Humphreys et al.). For example, in one study 313 undifferentiated arthritis patients were evaluated at presentation and again after 1 year of follow up for a clinical diagnosis of RA and/or the initiation of methotrexate therapy (Berglin et al.). At baseline, 56% of patients fulfilled the 1987 ACR criteria, while 74% fulfilled the ACR/EULAR 2010 criteria for having RA. Compared with the diagnosis at follow up, the ACR/EULAR 2010 had a sensitivity and specificity for diagnosis of RA of 0.79 and 0.54, respectively. This compared with a sensitivity and specificity for the 1987 ACR criteria of 0.68 and 0.84, respectively. Other studies have made similar observations (Varache et al., Liao et al., Biliavska et al., de Hair et al., Alves et al., Britsemmer et al., Cader et al., Kaneko et al., van der Linden et al.). Sakellariou et al.

recently published a meta-analysis of this literature. They observed that the sensitivity and specificity of the ACR/EULAR 2010 criteria were 0.73 (CI: 0.64,0.80) and 0.74 (CI: 0.68,0.79) respectively. Clearly, there is significant risk of misclassification. As stated by Zeidler, "overdiagnosis and under-diagnosis may become important issues if the (ACR/EULAR 2010) criteria recommend" DMARD therapies. While the ACR/EULAR 2010 criteria may be a little better than the 1987 ACR criteria, the current situation is that diagnostic misclassification currently results in about a quarter of early RA patients not being given the DMARD therapies from which they could receive great benefit while a significant portion of arthritis patients without RA are being given DMARD therapy from which they will not benefit and which exposes them to risks from adverse side effects (Vonkeman et al.). Therefore, there is a critical unmet need for a more sensitive and specific diagnostic for RA that can differentiate patients first presenting with polyarthralgia and identify those with RA to facilitate the more efficacious delivery of DMARD therapies.

While many types of cells, including T-cells, B-cells, dendritic cells and activated synovial fibroblasts contribute significantly to the establishment and maintenance of the pathology of RA, (Ma et al., Noack et al., Bugatti et al., Boissier et al., Tran et al.) macrophages play a critical role in RA (Kinne et al.). They produce most of the TNFα that drives and perpetuates the inflammatory cycle in RA (Leizer et al., Westra et al., Hamilton et al., Keffer et al., Noack et al., Bugatti et al., Boissier et al., Tran et al., Kinne et al., Zwerina et al., Feldman et al., Schett et al.). In the synovial sublining of a joint affected by RA, macrophages are the dominant cell type (Kraan et al., Cutolo et al.). In the inflamed joint as a whole, macrophages in RA patients make up at least 30%-40% of all cells (Kennedy et al.). Furthermore, macrophages participate directly in the destruction of bone and cartilage (Ma et al.). Activated macrophage populations and synoviocytes are the predominant cell types at the interface between pannus and cartilage and secrete destructive proteases in abundance (Bresnihan et al.). As a result, it may not be surprising that synovial macrophage numbers—but not the numbers of other immune cell types—correlate with radiographically determined joint destruction in RA (Mulherin et al., Yanni et al.). While macrophages may play a role in other pathologies that cause joint pain and inflammation, the degree to which macrophages are involved in the pathological process of RA and the sheer mass or volume of macrophages that infiltrate the joints inflamed due to RA differentiates RA from other rheumatic diseases. Therefore, detection of the density or numbers of macrophages in inflamed joints may permit differentiation of patients with RA from those with other causes of arthritis. In addition, it is known that the RA pathology begins significantly before, perhaps years before, the onset of symptoms (i.e., joint pain and inflammation) and well before the beginning of bone destruction (Deane et al., El-Gabalawy et al.). Macrophage infiltration of synovial tissues precedes development of clinical signs of RA in animal studies (Kraan et al.). In humans, macrophage infiltrations of synovial tissues are present when RA patients first develop clinical symptoms (Demoruelle et al., van de Sande et al.). Therefore, detection of the density or numbers of macrophages in inflamed joints may facilitate more sensitive and specific identification of RA patients as soon as they present with symptoms and early in the course of their illnesses when DMARDs are likely to be most effective.

An interesting and important observation that has been made in many studies is that the number of macrophages in synovial tissue, and particularly in the synovial sublining, declines in RA

patients when they are given DMARD therapy (Hamilton et al.). Furthermore, the degree to which synovial macrophage numbers decline is correlated with the magnitude of the DMARD treatment response. One study compared changes in the 28 joint count Disease Activity Score (DAS28) with changes in sublining macrophage numbers as determined by biopsies and found a significant correlation between the change in the number of macrophages and the change in DAS28 (Pearson correlation 0.874, $p < 0.01$) (Haringman et al.). The authors of this study have confirmed these findings in two additional studies, which used slightly different methodologies (Bresnihan et al., Bresnihan et al.). This correlation between declining macrophage numbers and the efficacy of DMARD therapy appears to be largely independent of the kind of DMARD therapy being investigated (Hamilton et al., Kinne et al., Franz et al., Kraan et al., Catrina et al., Cunnane et al., Vieira-Sousa et al.). These findings indicate that assaying the number of macrophages in inflamed joints of patients with RA could be used as an objective measure of the efficacy of DMARD therapy. These findings further suggest that assaying the number of macrophages in inflamed joints of patients with RA could be used in clinical studies as a biomarker of clinical response for potential new RA therapeutics. The problem with current methodologies is that macrophage numbers and densities need to be determined with synovial biopsies. This is obviously an invasive procedure that samples only a small portion of the inflamed synovial tissue and is painful and unpleasant for the patient. What would be preferable and likely more accurate is an imaging protocol, such as the one proposed in this application, that can assay synovial macrophages more completely and less invasively.

1.2 Preliminary Data

Dr. Thomas Rosol, DVM PhD FACVP, Professor, Veterinary Biosciences investigated CD206 expression and tilmanocept binding to synovial macrophages in an anti-type II collagen monoclonal antibody induced mouse model of RA (Hutamekalin et al.). Evidence of arthritis in this model (joint swelling and redness) developed in 5-6 days, and on days 9 or 11, mice were imaged 1-2 hours after they had received an intravenous (IV) injection of Cy3-tilmanocept. The mice were then euthanized followed by limb dissection and reimaging. The primary result of this experiment was that Cy3-tilmanocept administered intravenously localized to synovial macrophages in the affected joints of arthritic mice but not control mice (Figure 1 and Figure 2).

Figure 1. Significantly increased Cy3-tilmanocept mediated joint fluorescence in arthritic joints (right) compared with control (left)

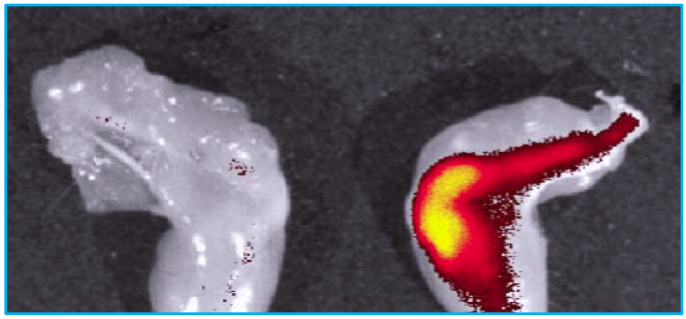
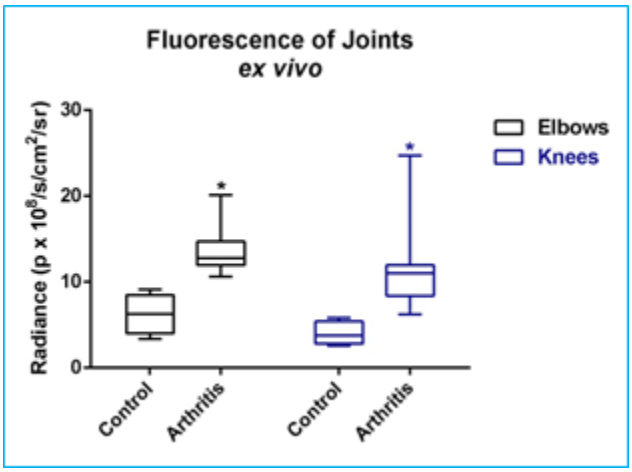
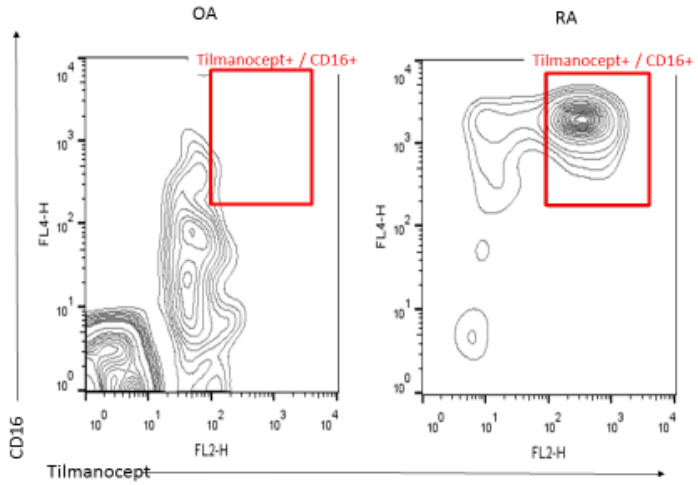


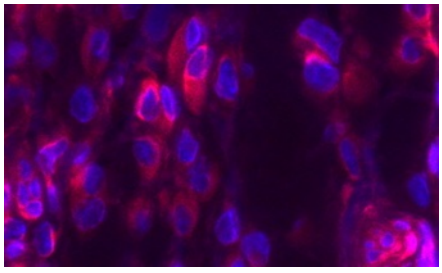
Figure 2. Significantly increased Cy3-tilmanocept mediated joint fluorescence in arthritic joints compared with controls



The success of these studies in mice strongly supports the feasibility and likely success of the proposed studies in humans. Further support for this notion could be derived from *ex vivo* experiments carried out that showed specific binding of Cy3-tilmanocept to *human* synovial macrophages obtained from patients with active RA but not osteoarthritis (OA) patients who were undergoing therapeutic/diagnostic arthrocentesis (Figure 3). In these experiments the synovial cells were isolated by a ficoll gradient and the cells stained with a variety of surface markers to identify the cell type that can stain with Cy3-tilmanocept. Flow cytometry contour plots from two representative RA and OA patients demonstrate a significant population of macrophages that stain with Cy3-tilmanocept in RA but is largely absent in OA (A) and staining of synovial tissue frozen sections from a patient with rheumatoid arthritis with Cy3-tilmanocept, a fluorescent version of the radio-tracer version, Tc99m tilmanocept.

Figure 3. A, B – (A) Significant staining of a CD16 and CD206 concordance in cells in synovial fluid (RA (right) but not OA (left) patients) and (B) - Staining of synovial tissue frozen sections from a patient with rheumatoid arthritis.





B - Sections were stained with 4',6-diamidino-2-phenylindole (DAPI = DNA) and Cy3-tilmanocept (CD206) and examined under fluorescence microscopy. Image Key: Red, Cy3-tilmanocept; blue (DAPI).

Lymphoseek (technetium Tc 99m tilmanocept) is a radiotracer that accumulates in lymphatic tissue by binding to a mannose binding receptor that resides on the surface of dendritic cells and macrophages within the nodes. Tilmanocept is a macromolecule consisting of multiple units of DTPA (diethylenetriaminepentaacetic acid) and mannose, each synthetically attached to a 10 kDa dextran backbone. The mannose acts as a substrate for the receptor and the DTPA serves as a chelating agent for labeling with Tc 99m. Standard lymphoscintigraphy has been performed using Tc 99m sulfur colloid (TcSC) particles (50 to 800 nm in size) that are nonselectively removed by lymph node phagocytosis. Tilmanocept has a diameter of about 7 nm, which is substantially smaller than current radiolabeled agents used for targeting lymphoid tissue. Tilmanocept' s small diameter permits enhanced diffusion into lymph nodes and blood capillaries, resulting in a rapid injection site clearance. Lymphoseek has demonstrated less injection pain perception by patients, faster injection site transit, more successful localization of lymph nodes, and detection of more lymph nodes in the lymphatic drainage pathways in subsets of patients receiving Lymphoseek compared with other subsets having sulfur colloid.

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

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

1.3 Previous Nonclinical Research and Clinical Trial Experience Tilmanocept and Lymphoseek

A detailed evaluation of safety and efficacy data from nonclinical and clinical studies can be found in the accompanying Investigator's Brochure supplied by Navidea Biopharmaceuticals, Inc. In addition, the data described in this protocol are reported in peer-reviewed publications, including nonclinical studies (Hoh et al., 2003), the Phase 1 clinical trials (Ellner et al.; Wallace et al.; Wallace et al.; Wallace et al.; Wallace et al.), the Phase 2 clinical trial (Leong et al.) and the Phase 3 studies (Tokin et al.; Sondak et al.; Wallace et al.; Marcinow et al., Agrawal et al., 2015).

1.3.1 Nonclinical Evaluations – Injection Site Subcutaneous

Nonclinical studies with Lymphoseek or tilmanocept demonstrated that the drug selectively binds to its intended receptor (the CD206 mannose binding receptor), is appropriately distributed for radio-detection of lymphatic tissue, and is well tolerated by rats, rabbits, guinea pigs, and dogs.

Pharmacokinetics data obtained from nonclinical studies demonstrated rapid absorption into the plasma. Urinary excretion was a major pathway of elimination. Lymphoseek exhibited rapid clearance from the injection site, rapid uptake by the local lymph node, and low uptake by the remaining lymph nodes. Tilmanocept was well tolerated at all doses tested in nonclinical

safety pharmacology studies and in single and repeated dose toxicology studies in rats, rabbits, and dogs. In some studies in rabbits and dogs, tilmanocept acted as a local irritant of the subcutis or skeletal muscle, and induced mild inflammation and tissue degeneration. The no-observed adverse-effect level (NOAEL) was 42µg/kg/day. Tilmanocept was not mutagenic or genotoxic in vitro or in vivo. No signs or symptoms of hypersensitivity were observed in a study in guinea pigs.

1.3.2 Nonclinical Evaluations – IV AND IP Administration

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

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

Lastly, although IP administered in mice, based on the highest single dose administration (2000 mg/kg) the mice received 300,0000X the 50 µg and 37,500X the 400 µg dose (♀ mice, wt = 22 gm) and 340,000X the 50 µg and 42,500X the 400 µg dose (♂ mice, wt = 29 gm).

Dosing In Rats:

Type of Study / Description	Test System	Method of Administration	Dosing
Central nervous system safety pharmacology	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Expanded single-dose toxicology (including toxicokinetics and local tolerance)	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of Tilmanocept following Intravenous Bolus Injection in Male Rats	Rat	Intravenous	60, 120, and 300 µg/animal or equivalent 320X and 41X the anticipated study doses of 50 µg and 400 µg in humans

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

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

Conclusions from These Tests:

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

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

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

Respiratory: In conclusion, respiratory function was assessed in male Crl:CD(SD) rats given a single 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).

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

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

1.3.3 Clinical Pharmacokinetics and Pharmacodynamics

In Phase 1 clinical trials, Lymphoseek showed rapid injection site clearance (approximately 2 to 3 hours). Absolute uptake in the primary sentinel lymph node was dose-related for Lymphoseek, although relative nodal uptake (%ID_{SN}) overall was independent of dose and ranged from 0.05%ID_{SN} to 1.81%ID_{SN}.

In a Phase 2 trial, Lymphoseek was highly effective in identifying tumor-draining lymph nodes (i.e., overall, a "hot spot" from Lymphoseek was identified in 93.0% of patients for whom preoperative lymphoscintigraphy was performed, and the per-patient intraoperative localization rate was 96.2%). Diagnostic performance of in vivo Lymphoseek findings relative to pathology assessment of tumor tissue indicated a high per tissue sensitivity estimate (overall, 92.0%). The overall false negative rate (FNR) for pathology was 8.0%, supporting the accuracy of Lymphoseek in identifying lymph nodes with a high potential for containing tumor metastases in the lymphatics draining the tumor bed.

1.3.4 Clinical Efficacy

Clinical efficacy was evaluated in two pivotal Phase 3 clinical trials in subjects with breast cancer and melanoma, comparing lymph node detection of Lymphoseek and vital blue dye (VBD). Lymphoseek demonstrated a statistically significant concordance rate with VBD (meta-analysis concordance rate = 99.99%).

The detection concordance between Lymphoseek and VBD was similar among subjects with melanoma and subjects with breast cancer (meta-analysis concordance rate = 99.99% for both populations). Lymphoseek also demonstrated a higher sensitivity for detecting tumor-positive (as confirmed by pathology) lymph nodes, corresponding to a decreased FNR when compared with VBD on a per node basis. The corresponding sensitivity rate in detection of lymph nodes most likely to be positive for tumor cells for Lymphoseek was 99.99%, compared with 78.02% for VBD. The FNR for VBD (21.98%) was higher than the FNR for Lymphoseek (0.01%). These findings suggest that, when VBD is used as the imaging agent, there is an increased risk of missing the detection of tumor-involved lymph nodes and incorrectly staging cancer patients. The Lymphoseek-only findings (pathology-positive nodes that were hot/not blue) suggest that Lymphoseek was markedly more effective in identifying lymph nodes that harbored disease than was VBD.

A third pivotal Phase 3 clinical trial was conducted in subjects with oral cavity or cutaneous SCC. Subjects received an injection of Lymphoseek prior to surgery for excision of the primary tumor and Lymphoseek-guided sentinel lymph node (SLN) dissection followed by planned elective neck dissection. All excised lymph nodes (Lymphoseek-identified SLNs as well as non-SLNs) underwent histopathologic evaluation for presence of metastatic disease. The primary endpoint in this trial was the Lymphoseek FNR (subjects with pathology-positive lymph node(s) not identified by Lymphoseek). Thirty-nine subjects had at least one pathology-positive lymph node, and Lymphoseek detected nodes with positive pathology in all but one patient. The FNR in this trial was 2.56%.

1.3.5 Clinical Safety

Well over 180,000 patients in clinical trials and US commercial use for ILM (intraoperative lymphatic mapping) with SLNB have been exposed to Lymphoseek, and there have been no safety signals, no deaths due to drug, and no SAEs (Serious Adverse Events) 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 adverse events, with the most common one being lack of node localization.

2 STUDY OBJECTIVES

2.1 Primary Objective(s)

- Determine the safety and tolerability of escalating doses of Tc 99m tilmanocept

2.2 Secondary Objective(s)

- Determine the localization of Tc 99m tilmanocept by SPECT (single photon emission computed tomography) imaging in subjects with active RA and asymptomatic controls and concordance of localization with clinical symptomology.
- Comparison of localization intensity between the dose groups of Tc 99m tilmanocept by SPECT imaging for optimal dose selection and determination of optimal imaging time.
- To estimate the pharmacokinetics (PK) and dosimetry of Tc 99m tilmanocept in subjects with and without active RA.

3 OVERVIEW OF METHODOLOGY AND DESIGN

3.1 Overall Study Design

Prospective, open-label, multicenter, dose escalation, and safety with PK and dosimetry study of injected Tc 99m tilmanocept in the detection of and assessment of localization to skeletal joints in subjects with and without active RA by SPECT imaging. Subjects will receive IV administration at one of 3 mass doses: 50 µg, 200 µg, or 400 µg. Within each mass dose group, subjects will receive Tc 99m tilmanocept labeled with one of 3 radiolabel doses: 1 mCi, 5 mCi, or10 mCi. All subjects will have a whole body planar SPECT scan. Subjects enrolled in Groups 1-9 will receive a whole body planar SPECT scan and planar of hands at two specified time points: 60 minutes ± 15 minutes, and 180 minutes ± 15 minutes followed by a 3D SPECT or SPECT/CT scan on areas of interest. Subjects enrolled in Groups 10-11 will have a whole body planar SPECT scan with pharmacokinetic and dosimetry tests performed at 4 specified time points post injection: 15± 5 minutes, 60 ± 15 minutes, 180 ± 15 minutes, and 18-20 hours. Subjects enrolled in Groups 10-11 will also receive a planar SPECT hands scan at 60 ± 15 minutes and 180 ± 15 minutes post-injection. PK blood sampling will be performed before injection (within 15 minutes), immediately following injection (within 5 minutes),and at each scanning timepoint. Dosimetry tests will be performed at each scanning timepoint. PK of urine will be assessed through counts of the bladder wall obtained from cumulative quantitative planar imaging from radiation dosimetry.The proposed study includes 3 visits: A screening visit for initial determination of eligibility and evaluation of clinical status (Visit 1), a baseline: Day 1 (Visit 2) on the day of tilmanocept administration and imaging, and a safety follow-up (Visit 3).

The Schedule of Events (Appendix 1) contains a list of all study procedures and time points. Study activities are described in detail in Section 7.

3.2 Justification for Study Design and Population

This study is designed to evaluate the safety and tolerability of escalating doses of Tc 99m tilmanocept. Whole body planar as well as a planar scan of the hands using a SPECT camera will also be combined with 3D SPECT or SPECT/CT imaging to provide greater resolution of areas of Tc 99m tilmanocept localization. Additionally, the PK and dosimetry of the MTD of Tc 99m tilmanocept will be evaluated in twelve subjects from Groups 10 and 11. Planar gamma camera imaging will be used to assess dosimetry.

This study is designed to evaluate the use of Tc 99m tilmanocept as an imaging agent in subjects with known active rheumatoid arthritis by evaluating the localization in inflamed joints.

No clinical trials have been conducted to evaluate IV administration of Tc 99m tilmanocept performance in subjects with rheumatoid arthritis. The rationale for evaluating Tc 99m tilmanocept in this subject population is discussed in Section 1.1 and Section 1.2.

3.3 Protocol Adherence

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

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

3.4 Study Duration

Subjects will be "on study" for up to 53 days depending on the duration of the screening window (up to 45 days) and Group assignment.

4 STUDY POPULATION

4.1 Eligibility

Subjects who fulfill all respective inclusion and none of the 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 Tc 99m tilmanocept and imaging (Day 1 procedures). A subject will be considered enrolled in the study on the morning of study day 1 when they arrive at the study site. Written, 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 1 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. Has a negative urine drug screening for illicit or unprescribed drugs suggestive of drug abuse as determined by the investigator.
3. All subjects shall be ≥18 years of age at the time of consent.

CONTROL SUBJECTS:

4. The subject is deemed to be clinically free of any inflammatory disease (s) and has not experienced joint pain for at least 4 weeks prior to the consent date.

ACTIVE RHEUMATOID ARTHRITIS SUBJECTS:

4. The subject has moderate to severe RA as determined by the 2010 ACR/EULAR (score of ≥ 6/10) that includes an affected hand and at least one large jointing (elbow, knee or shoulder).
5. The subject has a DAS28 of ≥ 3.2 (includes the Erythrocyte Sedimentation Rate [ESR] test and Visual Analog Scale [VAS]) .
6. If the subject is receiving methotrexate, they have been at a stable dose for > 4 weeks prior to the Baseline Visit 2 (Day 1).
7. If the subject is receiving biologic therapy, they have been at a stable dose > 8 weeks prior to the Baseline Visit 2 (Day 1).
8. If the subject is receiving NSAIDS (nonsteroidal anti-inflammatory drug) or oral corticosteroids, the dose has been at a stable dose for > 4 weeks prior to the Baseline Visit 2 (Day 1). The corticosteroid dose should be ≤ 10mg/day of prednisone or an equivalent steroid dose.

4.1.2 Exclusion Criteria

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

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator.
3. The subject has had or is currently receiving radiation therapy or chemotherapy for a condition other than rheumatoid arthritis.
4. The subject has renal insufficiency as demonstrated by serum creatinine clearance of < 60 mL/min.
5. 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.
6. The subject has a chronic or persistent infection or has any condition that would, in the opinion of the examining physician, preclude their participation.
7. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
8. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration.
9. The subject has received any radiopharmaceutical within 7 days prior to the administration of Tc 99m tilmanocept.

4.2 Recruitment

Subjects will be recruited from rheumatology 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, "RA-free" subjects (controls) will be recruited via IRB approved advertisements and clinically assessed for the "absence" of painful and/or swollen joints.

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 arrival at the study site on Day 1 will be considered a screen failure.

Should a subject withdraw after administration of the investigational product, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. 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 study drug administration or did not proceed to imaging

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 "21"
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 "21-01-001."

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

Technetium 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

Subjects will receive the administration of Tc 99m tilmanocept through an intravenous (IV) route of administration. For the 50 and 200 µg doses a single syringe will be used and injected as a slow push into the Y-port. For the 400 µg dose 2 syringes will be used and injected sequentially as a slow push into the Y-port. At the completion of the injection(s), a 10 cc sterile normal saline flush will be administered. Injection volume will be between 0.5 - 4.0 ml in sterile normal saline based on the mass dose group assignment. The preferred site of IV placement will be the left or right antecubital vein unless both elbows are primary sites for RA imaging, and then the site for IV placement will be between the elbow and the wrist.

Tc 99m tilmanocept will be received by subjects through IV administration at one of 3 mass doses: 50 µg, 200 µg, or 400 µg. Within each mass dose group, subjects will receive Tc 99m tilmanocept labeled to one of 3 radiolabel doses: 1 mCi, 5 mCi, or 10 mCi (see Table 1).

Table 1. Dose Escalation Matrix

	Tilmanocept Mass Dose		
Specific Radioactivity Tc 99m	50 µg	200 µg	400 µg
10 mCi	Group 1 n = 3 RA	Group 2 n = 3 RA	Group 3 n = 3 RA
5 mCi	Group 4 n = 3 RA	Group 5 n = 3 RA	Group 6 n = 3 RA
1 mCi	Group 7 n = 3 RA	Group 8 n = 3 RA	Group 9 n = 3 RA

***Group 10 will consist of 6 HC (3 female subjects and 3 male subjects) and Group 11 will consist of 6 subjects (3 female subjects and 3 male subjects) with active RA. Subjects in Groups 10 and 11 will be enrolled in parallel and will be dosed at the MTDas determined from Groups 1-9.**

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

5.3 Treatment Assignment

In this open-label, non-randomized, multicenter, dose-escalation safety study all subjects will receive IV administration at one of 3 tilmanocept mass doses: 50 µg, 200 µg, or 400 µg. Within each mass dose group, subjects will receive tilmanocept labeled to one of 3 radiolabel Tc 99m doses: 1 mCi, 5 mCi, or10 mCi.

Group 1 will be the starting dose group. Three subjects will be enrolled into Group 1. If none of the 3 subjects experiences pharmacologic activity or an ADR, 3 subjects will be enrolled in Group 2. However, if one of the first three subjects experiences a pharmacologic activity or an ADR, three more subjects will be treated at the same dose level. If 2 out of 3 or 3 out of 6 subjects experiences a pharmacologic activity or an ADR in Group 1 the MTD cannot be determined. If no additional pharmacologic activity or ADRs are observed in the expanded group, the dose escalation will continue. If one additional pharmacologic activity or ADR is observed, the current dose will be deemed the maximum tolerated dose (MTD). If more than one additional pharmacologic activity or ADR is observed in the expanded group, the previous dose will be deemed the MTD. Dose escalation will continue until the MTD is found or until Group 9 is reached with only one ADR or without any ADRs. If no ADRs are experienced from Groups 1-9, then the MTD will be highest tilmanocept mass dose radiolabeled at the hightest level (400 µg/10 mCi).

Group 10 will consist of 6 HC subjects and Group 11 will consist of 6 subjects with active RA. Subjects enrolled in Groups 10 and 11 will be dosed at the dose level that is established as the MTD from Groups 1 through 9. The maximum tolerated dose is defined as the dose below where >33% of subjects experienced an ADR.

NOTE: Adverse Drug Reaction

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

In this multi-center trial, doses will be serially assigned in an open label fashion in accordance with Table 1. Investigators who have identified a subject for participation will receive the dose assignment from the sponsor and request that dose from the supporting Cardinal Health pharmacy.

5.4 Packaging and Labeling

Tilmanocept cartons ready for radiolabeling will be shipped and stored at the region-specific Cardinal Health radiopharmacy. Tilmanocept is provided in a vial. Vials are packaged as a kit. A carton contains five vials of tilmanocept. One kit, which is one tilmanocept vial, should be used for no more than one subject. The 400 µg dose groups will require two vials for each subject. The carton also contains five shield labels and 25 syringe labels. This package has been designed specifically for tilmanocept and protects the vials during shipment, handling, and storage. Navidea will provide a radiolabeling protocol and Quality Control worksheets.

Cardinal Health will radiolabel tilmanocept with the protocol group specified tilmanocept dose and the group assigned millicurie dose Tc 99m in 0.5 mL – 2.0 mL and deliver one syringe for the 50 and 200 µg doses and up to 2 syringes for the 400 µg dose 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 Tc 99m tilmanocept injection through the post-injection safety follow-up will be documented. Subjects receiving radiation therapy or chemotherapy for a condition other than rheumatoid arthritis are not eligible for participation in the trial. The subject's history of treatments for rheumatoid arthritis 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 -44 to Day 0)

All Subjects:

- 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.
- Concomitant Medications (within 30 days before injection).
- 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.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and RA panel (see Section 8.6.2.4).
- Urine collection for routine urinalysis
- 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.
- 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, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- RA Evaluation: The swollen and tender joints will be identified and documented during by physical examination as established by the 2010 ACR/EULAR, a DAS28 evaluation and a characterization of the subject's RA history including previous treatments, date of symptom onset and date of diagnosis.
- Final confirmatory review of inclusion and exclusion criteria

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

7.2.2 Visit 2, Baseline (Day 1)

All subjects will be assessed for adverse events in an ongoing manner from the day of injection through the end of participation.

7.2.2.1 Before Administration of Tc 99m Tilmanocept

The following procedures will be completed for all subjects 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 adverse events
- Concomitant medication review
- ECG within 15 minutes prior to administration of Tc 99m tilmanocept
- 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

A PK blood sample will be collected for Subjects in Groups 10 and 11 within 15 minutes prior to the administration of Tc 99m tilmanocept.

7.2.2.2 Administration of Tc 99m Tilmanocept

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be the left or right antecubital vein unless both elbows are primary sites for RA imaging, then the site for IV placement will be between the elbow and the wrist.

The filled syringe(s) will be connected to the Y-port for a slow push injection. At the completion of the injection(s), 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 adverse events during the Day 1 Baseline Visit.

7.2.2.3 After Administration of Tc 99m Tilmanocept

Groups 1-9

For subjects enrolled in Groups 1-9, the following procedures will be completed at the specified times:

- Within 15 Minutes (00:01 to 00:15) Post Injection:
    - Assessment of adverse events
    - 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)

- 60 ± 15 Minutes Post Injection:
    - Assessment of adverse events
    - Whole body planar imaging (anterior/posterior views)
    - Planar image with both hands in the FOV (5 min anterior/posterior views)
        - Additional 3D SPECT or SPECT/CT will be obtained on identified joints of interest.

- 180 ±15 Minutes Post Injection:
    - Assessment of adverse events
    - Whole body planar imaging (anterior/posterior views)
    - Planar image with both hands in the FOV (5 min anterior/posterior views)
        - Additional 3D SPECT or SPECT/CT will be obtained on identified joints of interest.

Groups 10-11

For subjects enrolled in Groups 10-11, the following procedures will be completed at the specified times:

- Immediately following administration of Tc 99m Tilmanocept (00:00), a PK blood sample will be drawn.
- Within 15 Minutes (00:01 to 00:15) Post Injection:
    - 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)
    - Assessment of adverse events
- 15 ± 5 Minutes Post Injection:
    - Assessment of adverse events
    - PK blood sampling
    - Whole body planar imaging (anterior/posterior views)

- 60 ± 15 Minutes Post Injection:
    - Assessment of adverse events
    - PK blood sampling
    - Whole body planar imaging (anterior/posterior views)
    - Planar image with both hands in the FOV (5 min anterior/posterior views)

- 180 ±15 Minutes Post Injection:
    - Assessment of adverse events
    - PK blood sampling
    - Whole body planar imaging (anterior/posterior views)
    - Planar image with both hands in the FOV (5 min anterior/posterior views)

7.2.3 Visit 3 Follow Up and End of Study

18-20 Hours Post-Injection (Groups 10-11):

The following procedures will be conducted 18-20 hours after administration of Tc 99m tilmanocept at the final end of study visit for subjects enrolled in Groups 10-11:

- PK blood sampling
- Whole body planar imaging (anterior/posterior views)
- Vital signs
- ECG
- Physical Exam, including body weight
- Clinical Labs
- Urinalysis
- Concomitant medication review
- Assessment and review of adverse events

5± 2 Days Post-injection (Groups 1-9):

The following procedures will be conducted 3 to 7 days after administration of Tc 99m tilmanocept and the final end of study visit for Subjects in Groups 1-9:

- Vital signs
- ECG
- Physical Exam, including body weight
- Clinical Labs
- Urinalysis
- Concomitant medication review
- Assessment and review of adverse events

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Up to 48 evaluable subjects may be enrolled. Up to thirty-six (36) female or male subjects ≥18 years of age with evidence of active RA will be enrolled in Groups 1-9. Three (3) female and three (3) male subjects aged ≥18 without evidence of active RA (HC) will be enrolled in Group 10. Three (3) female and three (3) male subjects aged ≥18 with evidence of active RA will be enrolled in Group 11.

8.1.2 Medical and Surgical History

Relevant medical and surgical histories will be obtained on all study subjects.

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

8.1.3 DAS28 Evaluation

All subjects will be evaluated a single time at screening (Visit 1) for the Disease Activity Score (DAS) 28 (Prevoo et al, 1995) assessment.

The DAS28 will be calculated from four components: tender joint count (TJC), swollen joint count (SJC), Visual analogue scale (VAS) of the subject's global health, and the laboratory parameter erythrocyte sedimentation rate (ESR). The formula used to calculate the DAS28 score is: DAS28 = 0.56*√(TJC) + 0.28*√(SJC) + 0.70*LN(ESR) + 0.014*VAS). See Appendix 5 for details.

A DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. A subject is considered to be in remission if they have a DAS28 lower than 2.6

8.1.4 2010 ACR/EULAR Classification Criteria

All subjects will be evaluated at screening using the 2010 ACR/EULAR Classification Criteria as part of eligibility and inclusion.

The 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria includes four components: number and site of involved joints, serologic abnormality, elevated acute-phase response and symptom duration. See Appendix 4 for details.

A total score of 6 or higher (out of a possible ten) combined with clinical synovitis not better explained by another disease confirms a diagnosis of "definite RA" (Aletaha et al).

8.1.5 Prior and Concomitant Medication

All prior medications used within the last 30 days before the first screening examination through the follow-up safety visit will be documented. An assessment of RA treatments will also be collected.

8.2 Tc 99m Tilmanocept Preparation and Administration

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

In Nuclear Medicine, prior to initial imaging, an indwelling catheter will be placed for venous access. The preferred site of IV placement will be the left or right antecubital vein unless both elbows are primary sites for RA imaging, and then the site for IV placement will be between the elbow and the wrist.

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

8.3 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 whole body (head to toe) planar scan using auto-contour should be obtained at the following time points:

Groups 1-9 (see Appendix 2):

- 60 ± 15 minutes post administration
- 180 ± 15 minutes post administration

Groups 10-11 (see Appendix 3):

- 15 ± 5 minutes post administration
- 60 ± 15 minutes post administration
- 180 ± 15 minutes post administration
- 18-20 hours post administration

It is anticipated that the whole body 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 the higher activity injections, it may be possible to attain this target in a shorter time, allowing the subject more time off the bed between scans.

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 whole body scan.

Following the whole body acquisition, a planar image with both hands in the FOV should be acquired for ~ 5-7 minutes in both the anterior and posterior view for subjects in Groups 1-9 and subjects in Groups 10 and 11 at 60 ± 15 and 180 ± 15 minutes post-administration .

For subjects enrolled in Groups 1-9: Following completion of the the planar imaging session at 60 minutes, a 3D SPECT or SPECT/CT should be obtained on Joints A and B, followed by a patient break. Following the 180 minute whole body scan, and planar image of the hands, 3D SPECT or SPECT/CT will be repeated for joint A and completed for joint B and/or C in accordance with the prioritization below. No greater than 3 SPECT or SPECT/CT imaging sessions will be obtained after the whole body planar imaging session.

In the event that multiple joints of interest are identified during whole body planar imaging, and the planar image of the hands, the following prioritization will be applied for the selection of joints for SPECT or SPECT/CT imaging. Prioritization for joint selection may be revised after review of the first group of images. Any changes in the prioritization for joint selection will be communicated to investigators prior to the fulfillment of additional doses. Images will be obtained with bilateral joints in the field of view, including both hands, shoulders, elbows, knees.

"Greatest interest" will be defined as the joint of highest Tc99m localization on imaging and as guided by concordance with DAS 28 finding.

1. Joint A will be a the joint of greatest interest as defined above from the planar imaging session. In the event a determination could not be made due to equivalent localization in multiple joints, the hands will be selected as joint A. 3D SPECT or SPECT/CT of Joint A will occur after the 60 and 180 minute whole body scan.
2. Joint B will be the joint of second greatest interest (knee or elbow or shoulder) or the hands if not already imaged as Joint A. 3D SPECT or SPECT/CT of Joint B will follow the 180 minute whole body scan.

3. Joint C would be imaged in the event that Joints A or B were no longer identified as a joint of interest from the 180 minutes whole body imaging. Joint C would be determined by the interest and the discretion of the investigator.

In the event all DAS 28 joints are negative for joint localization on whole body imaging, SPECT/CT may be performed on other areas of localization. A visual representation of the Visit 2, Day 1 imaging procedures for subjects enrolled in Groups 1-9 can be found in Appendix 2.

It is anticipated that each SPECT or SPECT/CT scan would be acquired (at one bed position) for 25-30 minutes (30 minutes preferred). A break should address the comfort of the patient, encouraging the subject to void, consume fluids or food and to move or rest freely. For areas of low activity uptake, the SPECT scan may need to be increased. It is very important that the subject remain motionless for the duration of the 3D SPECT 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 whole body scan, planar imaging, as well as the SPECT and 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 eCRFs (electronic case report forms).

8.4 Pharmacokinetics

Blood will be collected in the contralateral arm that study drug was administered for the purpose of PK analysis for subjects enrolled in Groups 10 and 11 at the timepoints described below:

- Within 15 minutes prior to administration of Tc 99m tilmanocept
- Immediately following administration of Tc 99m Tilmanocept (00:00-00:05)
- 15 ± 5 minutes post injection
- 60 ± 15 minutes post injection
- 180 ±15 minutes post injection
- 18-20 hours post injection

Use of an intravenous catheter is not required for PK blood sampling. If an IV catheter is used, at least 3 mL of blood must be drawn and discareded prior to collecting the PK blood sample. Blood samples will be be collected in 5.5 mL heparinized vacutainer tubes.

Radioactivity in approximately 2 mL of whole blood will be quantitated in a calibrated well counter and decay corrected to the time of study drug administration. The radioactivity in urine will be obtained from cumulative quantitative planar imaging counts of the bladder wall obtained from radiation dosimetry.

8.4.1 Sample Processing

Whole blood samples will be processed in a well counter immediately following collection.

8.5 Dosimetry

Gamma camera planar images will undergo dosimetry analysis of all target organs. Dosimetry will be based on target organ image counts and modeling per OLINDA/EXM®2.0 (Organ Level INternal Dose Assessment/EXponential Modeling) software supplied by Hermes Medical Solutions (https://www.hermesmedical.com/products/oncology-dosimetry-olinda-theranostics/).

8.6 Safety

8.6.1 Adverse Events

8.6.1.1 Definition of Adverse Event

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

Adverse Event (AE)

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

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

By definition for this study, all untoward medical occurrences beginning on the day of Visit 2 Baseline (Day 1) through the assessment of Visit 3 Follow-up () are to be reported as AEs. AEs continuing after study completion will be followed to normalization or stabilization. Additionally, untoward medical events occurring prior to the day of Tc 99m tilmanocept administration will be collect and added to the subject's medical history unless they are related to a study procedure in which case the event will be recorded as an AE. SAEs will be reported from the time of consent through the end of participation.

8.6.1.2 Categories for Adverse Event Assessment

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

Seriousness

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

Severity

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

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

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationship to investigational product

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

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

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

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

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

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

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

Causal relationship to study procedure

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

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

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

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

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

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

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

8.6.1.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs for the duration of subject participation. Events occurring prior to Visit 2 (day of Tc 99m tilmanocept administration) will be recorded in the subject's medical history. Untoward medical events beginning on Visit 2 (day of Tc 99m tilmanocept administration) through the completion of the Visit 3 Follow-up will be reported as adverse events. Thus, subjects should be closely observed by the investigator both during and after the evaluation.

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

The following information is required:

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

- The relationship of the AE to the investigational product and to study conduct (for definitions, see above).

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

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

8.6.1.4 Expected Adverse Events

Investigational Product-Related Risks

In all completed studies of Lymphoseek, involving 553 subjects, only three events (breast pain and injection site pain reported by subjects with breast cancer and injection site irritation reported by a subject with head and neck squamous cell cancer) were deemed definitely related to the administration of Lymphoseek by the investigator. The most common adverse reactions (incident <1%) have been lack of effect (<0.067%), injection site pain (<0.02%) and rash (<0.02%). Adverse events from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

In addition to the Lymphoseek pre-approval clinical studies, post-marketing surveillance shows that Lymphoseek has been administered to more than 180,000 patients with not a single drug-related SAE. Routes of administration included: subcutaneous, intradermal, and peritumoral. The intended route of administration in this study is intravenous.

Precautionary Measures

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

Unexpected Adverse Events

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

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

8.6.1.5 Serious Adverse Events

Definition of Serious Adverse Events

Definition

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

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

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

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

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

Actions and reporting obligations in case of serious adverse events

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

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

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

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

Notification of the IRB(s)

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

Notification of the authorities

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

Sponsor's notification of the investigators

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

8.6.2 Further Safety Assessments

8.6.2.1 Physical Exam

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). Only body weight will be repeating at the Visit 3 (Follow-Up).

Physical examinations will be performed for the following body systems:

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

8.6.2.2 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, 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 investigational product injection, within 15 minutes post injection, and at the follow-up safety visit.

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

8.6.2.3 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be obtained at screening, as well as 15 minutes before investigational product injection, within 15 minutes after investigational product injection, and at the follow-up safety visit. 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.

8.6.2.4 Clinical Laboratory Parameters for Screening and Safety

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

Table 2. Clinical Laboratory Parameters

Hematology	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, potassium, sodium, total protein, albumin, carbon dioxide (CO_2)/bicarbonate, blood urea nitrogen (BUN), glucose
Urinalysis	pH, specific gravity
Rheumatoid Panel*	Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), Rheumatoid Factor (RF); Anti-Citrullinated Peptide Antibody (ACPA)

* Only at screening; The site laboratory will provide the necessary supplies to collect the blood and urine samples.

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

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

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

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

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

Table 3. Approximate Amount of Blood Withdrawn

Time point of examination		**Blood Volume**
Screening: Laboratory examination	Chemistry 5 mLs Hematology 4 mLs RA Panel 4 mLs	13 mL (~2.6 tsp)
Pre-Injection	PK Sample	5.5 mL (~1.1 tsp)
Post-Injection	PK Samples	26 mL (~5.3 tsp)
Follow-up	Chemistry 5 mLs Hematology 4 mLs	9 mL (~2 tsp)
TOTAL:		54 mL (~11 tsp)

9 STATISTICAL METHODS

The study is a prospective, open-label, multicenter, dose escalation, and safety with PK and dosimetry study of injected Tc 99m tilmanocept in the detection of and assessment of localization to skeletal joints in subjects with and without active RA by SPECT and SPECT/CT imaging. The dose escalation portion of the study is a modified 3+3 design with no dose de-escalation as described in Chevret (2006). The statistical objective of the study is to determine the maximum tolerated dose (MTD) of Tc 99m tilmanocept and to compare the capabilities of Tc99m tilmanocept between subjects with and without RA.

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

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 pharmacologic activity or an 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.

9.3 Efficacy Variables

The efficacy variables for this study are as follows:

- Nuclear medicine specialist qualitative determination of presence/absence of radiotracer uptake (i.e., Tc 99m tilmanocept localization positive/negative) relative to background from SPECT imaging results for each DAS28 joint location for each subject (deemed the "gold standard" result, 28 joint locations per subject)
  - Clinically identified presence/absence of RA using the DAS28 instrument (28 joint locations per subject)
- Quantitative planar imaging intensity of radiotracer uptake for each joint location

A location/joint would be considered clinically identified as RA positive if it is either swollen or tender as measured by the DAS28 instrument. The presence of radiotracer uptake relative to background as determined by the nuclear medicine specialist at a location would indicate the location is RA positive.

The efficacy endpoints for this study are as follows:

- Per subject localization rate of Tc 99m tilmanocept by SPECT imaging
- Per joint location clinically RA-identified joint localization rate of Tc 99m tilmanocept by SPECT imaging

- Concordance of Tc 99m localization with anatomical areas of active RA defined by clinical symptomology.
- Localization intensity for each clinically RA-defined joint (as determined by quantitative SPECT gamma counts)
- Per subject localization rate of Tc 99m tilmanocept in areas other than RA-positive joints by SPECT imaging
- The following PK parameters will be calculated for each subject, whenever possible, using Tc 99m tilmanocept total radioactivity in whole blood and urine: maximum observed concentration (C_{max}), time to C_{max} (t_{max}), area under the concentration-time curve (AUC) from Hour 0 to the last measureable concentration (AUC_{0-t}), AUC extrapolated to infinity ($AUC_{0-\infty}$), apparent terminal elimination rate constant (λ_Z), and apparent terminal elimination half-life ($t_{1/2}$).
- Radiation dosimetry of Tc 99m tilmanocept will include radiation doses per organ (µGy/MBq), organs receiving the highest organ dose (HT), effective doses per organ (µSv/MBq), mean effective dose (µSv/MBq), effective dose resulting from a diagnostic dose ± 20%. Dosimetry will be based on target organ image counts and modeling per OLINDA/EXM® 2.0 software.

Per subject localization of Tc99m by SPECT imaging is defined as the illumination of at least one site relative to background in a subject as determined by the nuclear medicine specialist.

Per joint location RA-identified localization rate is the number of joint locations that are clinically RA positive and SPECT illuminated divided by the total number of joints.

Per joint location concordance of Tc99m localization to clinically positive RA joints is the number of joint locations that are clinically RA positive and SPECT illuminated divided by the number of clinically RA positive joints.

Per subject localization rate of Tc99m by SPECT imaging in areas other than RA-positive joints is defined as the illumination of at least one joint location relative to background that is clinically RA negative in a subject divided by the number of subjects.

9.4 Sample Size Justification

The dose escalation portion of this study is a modified 3+3 design with no dose de-escalation. The sequential cohort enrollment characteristics of this design do not allow a fixed computation of sample size. The parameters of the design that can be calculated are shown in Table 4. Based on an assumed vector of probabilities of any pharmacologic activity or an ADR for a subject, the probability of each dose being the MTD is shown in the table. The average sample size of the dose escalation phase of the study is n=26.3 patients for the assumed vector of probabilities of any pharmacologic activity or an ADR.

Table 4. Design Characteristics for the Study Design

Dose Group	1	2	3	4	5	6	7	8	9	Total
Assumed Probability of any pharmacologic activity or **a ADR for a subject**	.01	.02	.05	.08	.10	.13	.16	.19	.22	---
Probability of the Dose Being the MTD	.002	.012	.039	.071	.103	.136	.152	.148	.065	---
Average Sample Size for Each Dose and Total	3.1	3.2	3.4	3.5	3.3	3.2	2.8	2.2	1.6	26.3

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 in Groups 1-9, injected with Tc 99m tilmanocept, and received whole body planar imagingwill be included in the ITD analysis population.
- **Radiopharmacokinetic (RPK) Population** – Subjects that have been enrolled in the study in Groups 10-11, injected with Tc 99m tilmanocept, and received whole body planar imaging will be included in the RPK analysis population.
- **Safety Population** – All patients who are enrolled and injected with Tc 99m tilmanocept in the study will be included in the safety population.
- **Per Protocol (PP) Population –** The PP population will include all safety subjects without major protocol violations.

All safety data analyses will be conducted on the safety population. All efficacy data analyses will be conducted on the ITD and PP population with the ITD population being the primary analysis set. All analyses of RPK data will be carried out on the RPK 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

Per subject localization rates and percentage will be calculated by imaging time point by dose group and overall.

Per joint location localization rates and percentages will be calculated by time point by dose group and overall.

Per location concordance of clinical diagnosis to nuclear medicine specialist illumination indication will be computed across all RA positive sites of all subjects at all time points.

Localization intensities will be summarized by descriptive statistics (mean, median, standard deviation, minimum, maximum and range) by joint location at each time point.

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

9.5.4 Analysis of RPK and Dosimetry Variables

The RPK variables for Groups 10 and 11 are the whole blood and urine radioactivity levels per time point. The radioactivity in whole blood will be measured directly using the blood samples. The radioactivity of urine will be obtained from counts of the bladder wall obtained from radiation dosimetry. RPK parameters will be estimated for each subject using noncompartmental models. Summary statistics (n, mean, std, CV, geometric mean) will be computed for each RPK parameter across all subjects by Group (10, 11).

For dosimetry data, for each dosimetry parameter, summary statistics (n, mean, std, and a one standard deviation width confidence interval) will be computed by organ type and overall across all subjects by Group (10, 11). Analysis of Safety Variables

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

All adverse events (AEs) will be observed for each subject from the time of signing of informed consent until exit from the study. A treatment emergent adverse event is defined as an adverse event 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 adverse events (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 Statistical Analysis Plan (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. For a specific location/joint, the patient must have data for the both the SPECT response value and the clinical evaluation of RA in order to be used in the statistical 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 entered onto the electronic CRFs (provided by the sponsor) as soon as possible.

10.1.1 CRF Design

Electronic CRFs (eCRFs) 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 investigator site file 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 trial master file and the investigator site file, 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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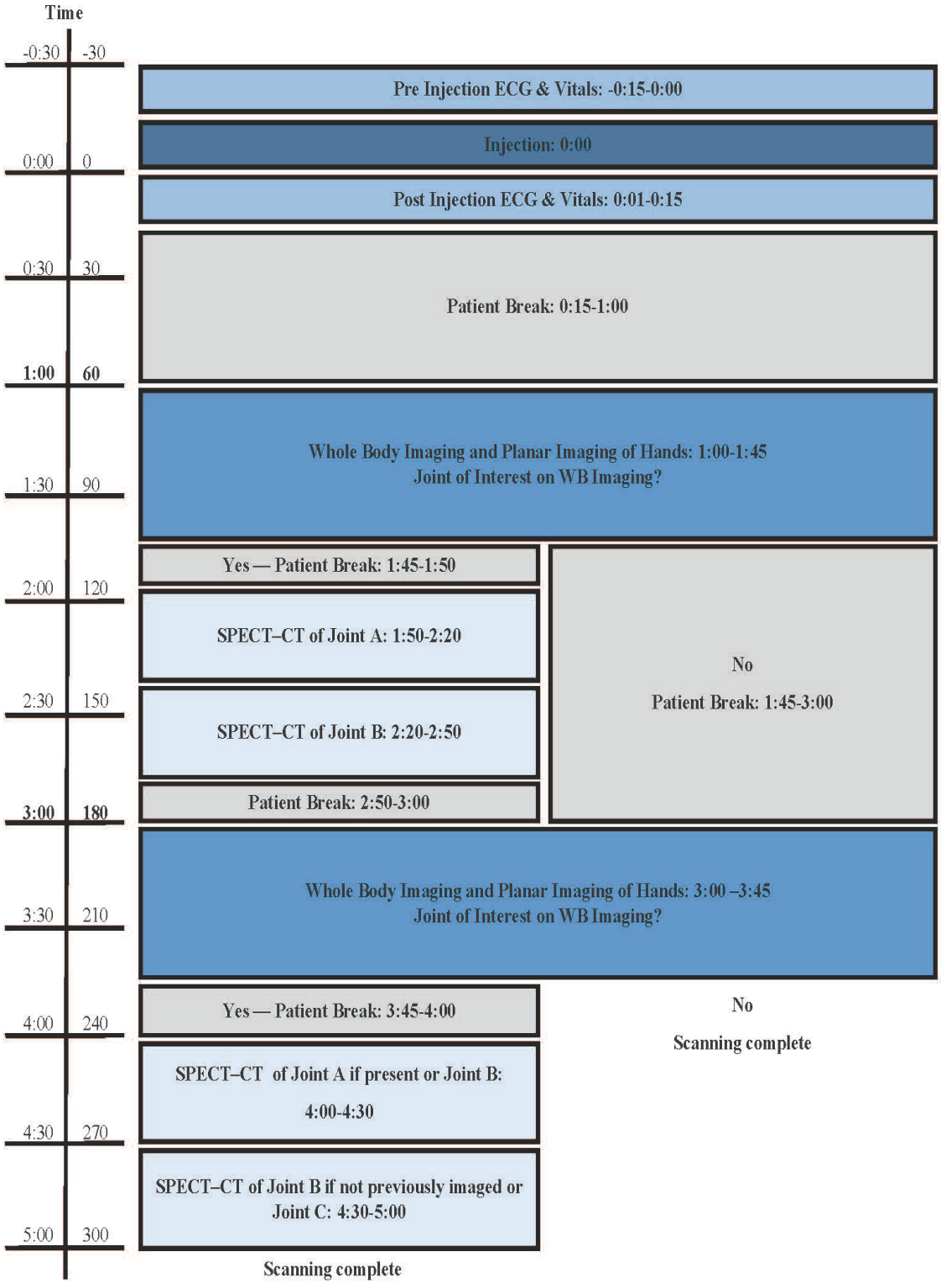
Appendix 1 Schedule of Events

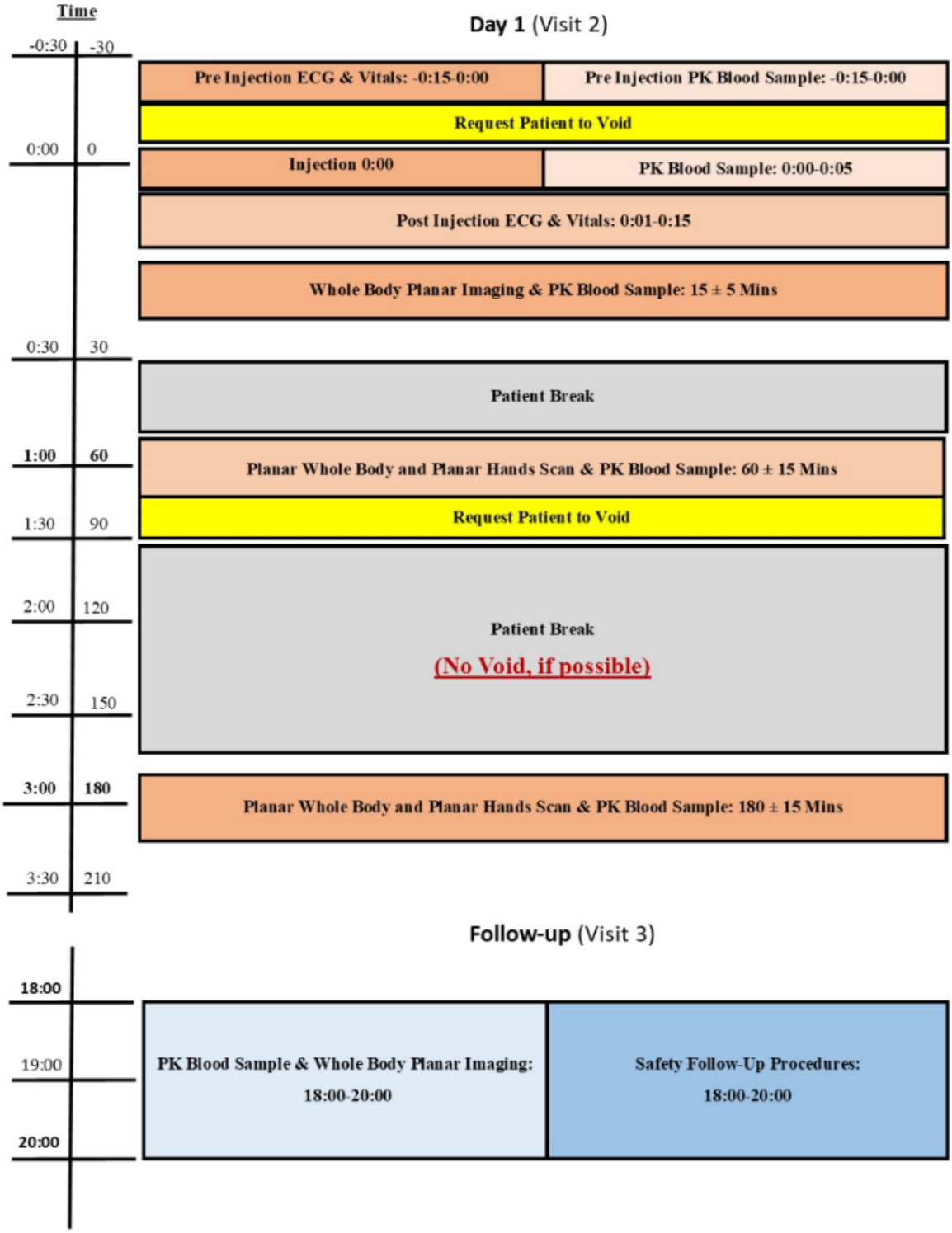
Evaluation	Visit 1 Screening		Visit 2 Baseline: Day 1						Visit 3 Follow-up	
	Days -44 to 0	Day 1 Prior to Injection	- 00:15 to -00:01	00:00-00:05	00:01 to 00:15	15± 5 Mins	60 ± 15 Mins	180 ± 15 Mins	18-20 Hours Post Injection[b]	Day 5 ± 2[a]
Informed Consent	x									
Entry Criteria	x									
Medical History, RA History and Demography	x									
Urine Drug Screen	x									
ACR/EULAR 2010 Classification	x									
Vital Sign Assessment	x		x		x				x	x
ECG	x		x		x				x	x
Physical Examination	x								x	x
DAS-28	x									
Clinical Laboratory Evaluation: Chemistry, Hematology, UA	x								x	x
Rheumatoid Arthritis Panel	x									
Urine Pregnancy Test	x	x								
Tilmanocept Administration				x						
Whole Body Planar and Optional 3D SPECT or SPECT/CT Image[a]							x[a]	x[a]		
Whole Body Planar Image for Dosimetry Evaluation[b]						x[b]	x[b]	x[b]	x	
Planar SPECT Imaging of Hands							x	x		
Blood Collection for PK Analysis[b]			x[b]	x[b]		x[b]	x[b]	x[b]	x	
Concomitant Medications	x	x							x	x
Adverse Event Monitoring	x	x	x	x	x	x	x	x	x	x

[a] Groups 1-9 only
[b] Groups 10 and 11 only

Appendix 2 Groups 1-9 Visit 2, Day 1 Diagram (example)



Appendix 3 Groups 10-11 Visits 2 and 3 Diagram (example)



Appendix 4 2010 ACR/ EULAR Classification Criteria

	Score
Target population (Who should be tested?): Patients who	
1) Have at least 1 joint with definite clinical synovitis (swelling)*	
2) With the synovitis not better explained by another disease†	
Classification criteria for RA (score-based algorithm: add score of categories A-D;	
a score of ≥ 6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement§	
1 large joint	0
2 – 10 large joints	1
1 – 3 small joints (with or without involvement of large joints)#	2
4- 10 small joints (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)**	5
B. Serology (at least 1 test result is needed for classification)‡‡	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification) ‡‡	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
< 6 weeks	0
≥ 6 weeks	1

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

† Differential diagnoses vary among patients with different presentations, but may include conditions such as systemic lupus erythematosus, psoriatic arthritis, and gout. If it is unclear about the relevant differential diagnoses to consider, an expert rheumatologist should be consulted.

‡ Although patients with a score of < 6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment.

Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

"Large joints" refers to shoulders, elbows, hips, knees, and ankles.

"Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤ 3times the ULN for the laboratory and assay; high-positive refers to IU values that are > 3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA = anti-citrullinated protein antibody.

‡‡ Normal/abnormal is determined by local laboratory standards. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

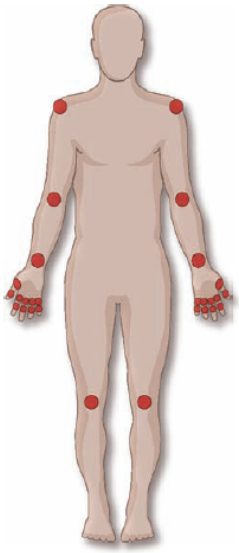
Appendix 5 DAS-28 Scale

DAS28

DISEASE ACTIVITY SCORE IN 28 JOINTS (DAS28)

The DAS28 is a frequent outcome measure used in therapeutic trials and is also used to guide treatment decisions and describe disease activity across populations. It is the basis for several other RA measurement tools, including the EULAR response criteria.

FORM A		LEFT		RIGHT	
		SWOLLEN	TENDER	SWOLLEN	TENDER
Shoulder					
Elbow					
Wrist					
Metacarpophalangeal (MCP)	1				
	2				
	3				
	4				
	5				
Proximal Interphalangeal (PIP)	1				
	2				
	3				
	4				
	5				
Knee					
Subtotal					
TOTAL		SWOLLEN		TENDER	



FORM B	
Swollen (0–28)	
Tender (0–28)	
ESR (or CRP)	
VAS disease activity (0–100mm)	
DAS28= 0.56*√(TENDER JOINTS) + 0.28* √(SWOLLEN JOINTS) + 0.70*LN(ESR/CRP) + 0.014*VAS	

Source: DAS-Score.nl. Available at http://www.das-score.nl/www.das-score.nl/index.html. Accessed February 5, 2009.

By comparing a patient's DAS28 score over multiple time points, you can substantiate his/her improvement or response. The EULAR response criteria are defined as follows:

PRESENT DAS28	DAS28 IMPROVEMENT OVER TIME POINTS		
	>1.2	0.6–1.2	<0.6
<3.2	good response	moderate response	no response
3.2–5.1	moderate response	moderate response	no response
>5.1	moderate response	no response	no response

HOW TO CALCULATE A DAS28 SCORE

1. Perform a swollen and tender joint examination of your patient, noting each affected joint on Form A. When complete, add all of the swollen and tender joints and record the totals in the appropriate boxes on Form B.
2. Obtain and record the patient's erythrocyte sedimentation rate (ESR) in mm/h in the appropriate box on Form B. Note: C-reactive protein (CRP) levels may be used as a substitute for an ESR.
3. Obtain and record the patient's general health on a Visual Analog Scale (VAS) of 100 mm in the appropriate box on Form B. Note: DAS28 calculations may be performed without a VAS measurement.
4. Plug the appropriate values into the formula at the bottom of Form B (many online calculators are available to compute this value including **http://www.das-score.nl/www.das-score.nl/dasculators.html**).
5. A DAS28 score of higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 3.2 indicates low disease activity. A patient is considered to be in remission if they have a DAS28 lower than 2.6.

Courtesy of http://www.iche.edu/newsletter/DAS28.pdf

Appendix 6 Sponsor Signatures

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and Skeletal Joint Imaging with SPECT in Subjects with Active Rheumatoid Arthritis (RA) and Healthy Controls
Study Number:	NAV3-21
Original Protocol Date:	23 May 2016
Amendment 1 Date:	12 September 2016
Amendment 2 Date:	22 December 2016
Amendment 3 Date:	09 February 2017
Amendment 4 Date:	23 August 2017
Amendment 5 Date:	18 April 2018
Amendment 6 Date:	19 June 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: 21 Jun 2018

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

Signed: _____ Date: 21-Jun-2018

Michael S. Blue, MD
Senior Medical Director
Navidea Biopharmaceuticals

Signed: _____ Date: 21 Jun 2018

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

Appendix 7 Investigator's Signature

Study Title:	An Evaluation of the Safety of Escalating Doses of Tc 99m Tilmanocept by Intravenous (IV) Injection and Skeletal Joint Imaging with SPECT in Subjects with Active Rheumatoid Arthritis (RA) and Healthy Controls
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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:_____