Clinical Trial Protocol: NAV3-32

Study Title:	A Comparison of Tc 99m Tilmanocept Quantitative Imaging with Immunohistochemical (IHC) Analysis of CD206 Expression in Synovial Tissue from Subjects Clinically Diagnosed with Rheumatoid Arthritis (RA)
Study Number:	NAV3-32
Study Phase:	2b
Product Name:	Tc 99m tilmanocept
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Investigators:	Multicenter, multinational
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SYNOPSIS

Study title	A Comparison of Tc 99m Tilmanocept Quantitative Imaging with Immunohistochemical (IHC) Analysis of CD206 Expression in Synovial Tissue from Subjects Clinically Diagnosed with Rheumatoid Arthritis (RA)
Study phase	Phase 2b

Study objective(s)	**Primary**
	• Assessment of the relationship between joint-specific tilmanocept uptake value (TUV_{joint}) and synovial anatomic pathology.
	• IHC assessment of macrophage expression of the CD206 receptor.
	Secondary
	• Assessments of CD68 and CD163 receptor expression levels through IHC microscopy.
	• Classification of synovial anatomic pathology as
	  • Lympho-myeloid
	  • Diffuse myeloid
	  • Pauci-immune fibroid
	Exploratory
	• Determine the relationship between TUV_{joint} and mRNA expression profiles of CD68, CD163, and CD206 as determined by RNA sequencing (RNA-seq).
	• Determine the relationship between TUV_{joint} and the number, size, and intensity of CD68, CD163, and CD206 as determined by (optional) flow cytometry.
	• Evaluate synovial expression of CD3, CD20, CD55, and TE-7 and CD206 in synovial tissue biopsy specimens.
	• Assessment of the relationship between global tilmanocept uptake value (TUV_{global}) and synovial anatomic pathology.
	Safety
	• Evaluate safety through the examination of adverse event (AE) incidence, physical examination findings, and changes over time in laboratory tests, electrocardiograms (ECGs), and vital signs.

Study duration	Up to 45 days
Study drug	Tc 99m tilmanocept
Dose(s) and Route of administration	**Dose:** Tilmanocept will be administered at a dose of 150-mcg radiolabeled with 10 mCi (370 MBq) Tc 99m. **Route of Administration:** Tc 99m tilmanocept will be administered through an intravenous (IV) route of administration in 3 mL using a single syringe, with the dose injected as a slow push into the IV catheter. The preferred site of IV placement will be the left or right antecubital vein. At the completion of Tc 99m tilmanocept administration, a 10-mL sterile normal saline flush will be administered.
Inclusion criteria	1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) or equivalent authorization before the initiation of any study-related procedures. 2. Women and men of reproductive potential must use adequate birth control measures (e.g., abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, or surgical sterilization) for the duration of the study. 3. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis. 4. The subject has RA as determined by the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria (score of ≥ 6/10 at or before screening). 5. The subject has a 28-joint disease activity score (DAS28) of ≥ 3.2 (includes the C-reactive protein [CRP] test and visual analog scale [VAS]). 6. Subjects receiving traditional DMARDs must have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the first imaging visit (Day 0). 7. If the subject is receiving biologic disease-modifying antirheumatic drug (bDMARD) or janus kinase (JAK) inhibitor therapy, they have been at a stable dose > 60 days prior to the imaging visit (Day 0). bDMARD therapy should not be administered less than 4 days prior to the imaging visit. 8. If the subject is receiving NSAIDs (nonsteroidal anti-inflammatory drug) or oral corticosteroids, the dose has been at a stable dose for ≥ 28 days prior to imaging. The

	corticosteroid dose should be ≤ 10 mg/day of prednisone or an equivalent steroid dose. 9. The subject has a hand or wrist joint with a minimum ultrasound gray-scale synovitis score of 2 (range 0 to 3).
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 is currently receiving radiation therapy or chemotherapy or has received radiation or chemotherapy within the past 5 years. 4. The subject has an active malignancy or a history of malignancy within the past 5 years. 5. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty. 6. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min. 7. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 3 times the upper limit of normal. 8. The subject has any severe, acute, or chronic medical conditions and/or psychiatric conditions and/or laboratory abnormalities that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration that would deem the subject inappropriate for study participation. 9. The subject has a known allergy to or has had an adverse reaction to dextran exposure. 10. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration (Day 0). 11. The subject has received injectable (e.g., intra-articular, intramuscular, etc.) corticosteroids ≤ 8 weeks prior to imaging (Day 0). 12. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to the administration of Tc 99m tilmanocept (Day 0). 13. The subject has an intolerance to anesthetic and antiseptic agents indicated for the synovial biopsy procedure.

	14. The subject is currently receiving anticoagulants (oral anti-platelet agents are permitted) or has a condition that is contraindicated with ultrasound-guided synovial biopsy e.g., needle phobia. 15. The subject has heart failure [New York Heart Association (NYHA) Class III-IV], a demyelinating disorder, or a chronic/latent infection [e.g., +Purified Protein Derivative (PPD) test, Human Immunodeficiency Virus (HIV), Hepatitis B].
Study design	This is a phase 2b, open-label, multi-center, multinational, non-randomized, single-dose study designed to assess the relationship between quantitative Tc 99m tilmanocept planar imaging and synovial histopathology in subjects clinically diagnosed with RA. Subjects will undergo an imaging assessment of the bilateral hands and wrists followed by an ultrasound-guided synovial biopsy of a select joint at a subsequent visit. Tissue samples from the synovial biopsy procedure will be used for 2 mandatory anatomic pathology evaluations, [1] IHC and [2] RNA-seq, and optional flow cytometry, to provide quantitative and semi-quantitative information regarding joint-specific disease activity. Results from each anatomic pathology evaluation will be correlated with TUV_{joint} on planar imaging to mechanistically assess the relationship between synovial anatomic pathology and TUV_{joint}. The quantitative characterization of Tc 99m tilmanocept uptake will be defined using the standardized computation of TUV_{joint} and TUV_{global} by a central imaging laboratory. TUV_{joint} and TUV_{global} metrics are further described in the NAV3-32 Imaging Manual and NAV3-32 Statistical Analysis Plan. Results from Phase 1 and 2 studies have revealed that TUV_{joint} is a sensitive and specific identifier of Tc 99m tilmanocept localization in joint regions with presumed inflammatory macrophage activity. Anatomic pathology evaluations, including IHC and RNA-seq, will be performed by a central pathology laboratory in accordance with standardized operating procedures (SOPs). Synovial immunofluorescence evaluations will be used to determine the degree of expression of various immune markers including CD3, CD20, CD55, TE-7, CD68, CD163, and CD206. These markers will be evaluated in tandem to observe trends in co-expression and will also be compared with corresponding TUV_{joint}(s). IHC evaluations will also be used to characterize the pathotype of the corresponding synovial specimens (as diffuse myeloid, lympho-myeloid, or pauci-

immune fibroid), which will then be evaluated against TUV_{joint} and TUV_{global} to assess the discriminatory capacity of TUV for pathotype designation. RNA-seq will be used for the exploratory quantitative assessment of CD68, CD163, and CD206 mRNA expression, which will then be evaluated against corresponding TUV_{joint}(s). Flow cytometry will be performed (optionally) by a local pathology laboratory in accordance with standardized SOPs for the exploratory analysis of TUV_{joint} and the number and size of CD68, CD163, and CD206.

All joints selected for biopsy will be required to have an ultrasound synovial thickness score of ≥ 2. In instances where more than 1 joint qualifies for biopsy, the candidate joint will be selected based on TUV_{joint} (as described in Table 1 below).

Table 1 Biopsy Selection Options

Option	Description
1	The subject has only 1 metacarpophalangeal (MCP) joint or wrist joint with an ultrasound gray-scale synovitis score of ≥ 2. This joint will be selected for biopsy.
2	The subject has 2 or more MCP and/or wrist joints with an ultrasound gray-scale synovitis score of ≥ 2. The joint for biopsy will be selected based on evaluation of corresponding TUV_{joint}. In the first subject with multiple qualifying joints, the joint with the highest TUV_{joint} will be selected; in the second subject with multiple qualifying joints, the joint with the lowest TUV_{joint} will be selected. Selection will proceed in this fashion, alternating between highest and lowest TUV_{joint}.

Visit #1 (Screening; Day -30 to Day -1)

- Informed consent
- Review of study eligibility
- Collection of medical history (including medications)
- Vital sign assessment
- Physical examination (including height and weight)
- Clinical labs

	• RA-specific labs • Urinalysis • Urine pregnancy for subjects of childbearing potential • 2010 ACR/EULAR score • DAS28 evaluation • Ultrasound synovitis assessment • AE assessment **Visit #2 (Day 0; Tc 99m Tilmanocept Administration and Imaging)** <u>Pre-Tc 99m Tilmanocept Administration</u> • Urine pregnancy test for women of child-bearing potential • ECG up to 30 minutes prior to drug administration • Post-ECG vital sign assessment • AE assessment • Review of concomitant medications <u>Tc 99m Tilmanocept Administration:</u> Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m. <u>0-30 Minutes Post-Tc 99m Tilmanocept Administration</u> • ECG • Post-ECG vital sign assessment • AE assessment <u>60 to 75 Minutes Post-Tc 99m Tilmanocept Administration</u> • AE assessment Image acquisition: Static gamma camera scan of bilateral hands and wrists • Clinical labs (after imaging) **Visit #3 (24-72 hours Post-Tc 99m Tilmanocept Administration; Follow-up Telephone Safety Assessment)** • Review of concomitant medications • AE assessment

	Visit #4 (Day 3-7; Synovial Tissue Biopsy) • Ultrasound assessment of synovitis • Synovial tissue biopsy of selected joint in accordance with Table 1 • AE assessment • Review of concomitant medications **Visit #5 (5 ± 2 days Post-Biopsy; Follow-up Telephone Safety Assessment)** • Review of concomitant medications • AE assessment	
Planned study dates	**Start of study recruitment / February 2021**	**End of Recruitment/ December 2023** **End of Study/ March 2024**
Planned number of study centers	Up to 10 centers which include sites in the US, UK, and EU	
Sample size	Approximately 12 to 24 joints (final number based on an interim analysis and adaptive design)	
Justification of sample size	The proposed sample size is sufficient to provide an acceptable standard error (approximately 0.15) on the Fisher Z scale and yield an acceptable confidence interval width on the population correlation.	
Primary endpoint	• The correlation between joint-specific tilmanocept uptake value (TUV_{joint}) and the number and area fraction of CD206 expression as determined by IHC assessment.	

Secondary endpoints	• The correlation between TUV_{joint} and the number and area fraction of CD68 and CD163 determined by IHC assessments.
	• Classification of synovial anatomic pathology into
	  • Lympho-myeloid
	  • Diffuse myeloid
	  • Pauci-immune fibroid
	  types as a function of CD68, CD163, CD206, CD3, CD20, CD55, and TE7 expression determined by IHC assessments using a multinomial logistic regression model with TUV_{joint} as a covariate.
	• The correlation between the expression of CD68, CD163, and CD206.	

Exploratory endpoints	• The correlation of the expression of CD3, CD20, CD55, and TE7 with CD206 as measured through IHC. • The correlation of TUV_{joint} with the expression of CD206, CD163, and CD68 as measured through RNA-seq. • The number and size of CD206, CD163, and CD68 expressing macrophages as measured through (optional) flow cytometry and their correlation with TUV_{joint}. • The correlation between TUV_{global} and synovial anatomic pathology as determined by IHC assessments. • Classification of synovial anatomic pathology into Lympho-myeloid, Diffuse myeloid, and Pauci-immune fibroid types using a multinomial logistic regression model with TUV_{global} as a covariate.
Safety Evaluations	Adverse events, clinical laboratory results (hematology, serum chemistry, vital signs, ECG)
Plan for statistical analysis	The following populations will be defined for the study: • **Intent-to-Assay (ITA) population** – the ITA population includes all subjects who have been enrolled in the study, injected with Tc 99m tilmanocept, received all imaging procedures, and have been biopsied. • **Per Protocol (PP) population** – Consists of all members of the ITA population without major protocol violations. At least 4 evaluable synovial biopsy samples must be available for a subject to be included in the PP population. • **Safety population** – Consists of all subjects who are enrolled in the study and injected with Tc 99m tilmanocept regardless of imaging or biopsy status. All safety analyses will be conducted on the safety population. All efficacy analyses will be conducted on both the ITA population and PP population. Distributions of all baseline and demographic variables will be summarized for the safety population. Quantitative variables will be summarized by the mean, standard deviation, median, and range. Categorical variables will be summarized by counts and percentages.

	Primary Efficacy Analysis The primary endpoint will be analyzed by computing the Pearson (product-moment) and Spearman (rank) correlation between the TUV_{joint} for the biopsied joint and 2 measures of macrophage prevalence: • The fraction of CD206-expressing macrophages visible in the microscope field under 4x and 10x power magnification, measured as the area fraction (AF) $$AF = \frac{\text{Total Stained Area}}{\text{Total Field Area}} \times 100\%.$$ • The volume of synovial tissue in the biopsied joint derived from the ultrasound synovitis score (Vol). The final CD206-expressing macrophage count is defined as the product of Vol and AF defined above. A 95% confidence interval for the population correlation coefficient measuring the association between TUV and CD206 expression (as measured via IHC) will be computed using the Fisher's Z-transformation method ($Z = \tanh^{-1} r$) and its asymptotic Normal approximation. Statistical summaries of the marginal distributions of TUV_{joint} and CD206 macrophage count will be provided (mean, standard deviation, number of cases, median, and range). **Secondary Efficacy Analyses** The degree of co-expression of CD68, CD163, and/or CD206 macrophage types will be assessed by calculating the Pearson (product-moment) correlations and Spearman rank correlations among these variables. The marginal distributions will be summarized by calculating the mean, standard deviation, number of cases, median, and range for each variable. The ability of TUV_{joint} to discriminate among lympho-myeloid, diffuse myeloid, and pauci-immune fibroid types will be assessed by fitting a multinomial logistic regression model with RA pathotype as the response variable and TUV_{joint} as the explanatory variable. ROC curves will be generated for the multinomial logistic model using the lympho-myeloid type as the base class. No inferential statistics will be generated for this endpoint.

	Exploratory Efficacy Analyses The expression of CD68, CD163, and CD206 biomarkers as assessed using specific modules by RNA-seq in synovial tissue specimens will be measured by calculating the Pearson and Spearman correlation matrices for these variables with TUV_{joint}. Descriptive statistics for the variables (mean, number of complete cases, standard deviation) will be provided. The expression of CD68, CD163, and CD206 biomarkers as measured with optional flow cytometry in synovial tissue specimens will be measured by calculating the Pearson and Spearman correlation matrices for these variables with TUV_{joint}. Descriptive statistics for the variables (mean, number of complete cases, standard deviation) will be provided. The Pearson and Spearman correlation matrices for CD3, CD20, CD55, CD68, CD163, CD206, and TE7 biomarker expressions will be computed for each measurement method (IHC, RNA-seq, and optional flow cytometry). The marginal distributions of all variables will be summarized with descriptive statistics (number of complete data points, means, standard deviations, minima, medians, and maxima). The ability of TUV_{global} to discriminate among lympho-myeloid, diffuse myeloid, and pauci-immune fibroid types will be assessed by fitting a multinomial logistic regression model with RA pathotype as the response variable and TUV_{global} as the explanatory variable. ROC curves will be generated for the multinomial logistic model using the lympho-myeloid type as the base class. No inferential statistics will be generated for this endpoint.

	Safety Analyses The safety of Tc 99m tilmanocept will be summarized by tabulating the number and percentage of subjects experiencing AEs, treatment emergent AEs (TEAEs), and serious TEAEs. AEs, TEAEs and serious TEAEs will tabulated by system organ class (SOC) and preferred term (PT). Vital signs, clinical laboratory results, and ECG parameters will be summarized with descriptive statistics (mean, standard deviation, n, median, and range) for the observed values and the change from the baseline value.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

18F-FDG PET	^{18}F-labeled fluoro-2-deoxyglucose positron emission tomography
ACR	American College of Rheumatology
ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
ADR	Adverse drug reaction
AE	Adverse event
AF	Area fraction
ALT	Alanine aminotransferase
ACPA	Anti-citrullinated peptide antibody
AST	Aspartate aminotransferase (SGOT)
AUC	Area under the curve
BUN	Blood urea nitrogen
bDMARD	Biologic disease modifying antirheumatic drug
CD206	Mannose-binding receptor (Ca2+-binding lectin)
CI	Confidence interval
CRF	Case report form
CRP	C-reactive protein
CRA	Clinical research associate
CRO	Contract research organization
CT	Computed tomography
CV	Coefficient of variation
DAS28	Disease activity score used with the ACR/EULAR 2010 guidelines
DMARD	Disease-modifying antirheumatic drug
DTPA	diethylenetriaminepentaacetic acid
ESR	Erythrocyte sedimentation rate
ECG	Electrocardiogram
eCRF	Electronic case report form
EU	European Union

UK	United Kingdom
FDA	Food and Drug Administration
FDG	Fluoro-2-deoxyglucose
GCP	Good Clinical Practice
Hct	Hematocrit
Hgb	Hemoglobin
HIPAA	Health Information Portability and Accountability Act
HC	Healthy control
IAP	Interim analysis plan
ICF	Informed consent form
IEC	Independent ethics committee
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB	Institutional Review Board
ITA	Intent-to-assay
IV	Intravenous
JAK	Janus kinase
MedDRA	Medical Dictionary for Regulatory Activities
MCP	metacarpophalangeal joint
NSAID	Non-steroidal anti-inflammatory drug
PET	Positron emission tomography
PI	Principal investigator
PK	Pharmacokinetics
PP	Per protocol
PIP	Proximal interphalangeal
PT	Preferred term
QC	Quality control
QT	Interval from the Q wave to the end of the T wave

QTc	Corrected QT interval
RBC	Red blood cell (count)
ROI	Region of interest
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RR	Reference region
RSNA	Radiological Society of North America
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SGOT	Serum glutamic oxaloacetic transaminase (AST)
SGPT	Serum glutamic pyruvic transaminase (ALT)
SJC	Swollen joint count
SUV	Standard uptake value
SUSAR	Suspected unexpected serious adverse reactions
SOC	System organ class
SOP	Standardized operating procedures
$t_{1/2}$	Mean half-life
TEAE	Treatment emergent adverse event
TJC	Tender joint count
TMF	Trial Maser File
TNFα	Tumor necrosis factor alpha
TUV	Tilmanocept uptake value
TUV_{global}	Global tilmanocept uptake value
TUV_{joint}	Joint-specific tilmanocept uptake value
VAS	Visual analog scale
Vol	ultrasound synovial thickness score
US	United States
ULN	Upper limit of normal

WBC	White blood cell (count)
λ_z	Mean elimination rate constant

1 INTRODUCTION

1.1 Background and Significance

Worldwide, approximately 1 in 200 adults suffers from RA. In the US and UK, there are 1.3 million and 400,000 adults living with RA, respectively. Each year, about 130,000 Americans are newly diagnosed with RA. In the UK, the incidence of RA is 3.81/10,000 person-years (Abhishek 2017). Individuals with inadequately controlled RA have significantly shorter life expectancies and frequently become disabled, leading to reduced quality of life and severe adverse economic consequences (Lassere 2013, Uhlig 2014, Verstappen 2015, Michelsen 2018).

It has been realized for many years that RA patients who are placed on disease-modifying antirheumatic drugs (DMARDs) soon after they develop arthritis symptoms respond much more favorably to these therapies than do patients whose initiation of DMARD therapy is delayed (Demoruelle 2012). Many more of these early RA patients placed on therapy achieve disease remission than is observed in RA patients who do not initiate DMARD therapy until after they have been symptomatic for RA for 6 or more months. Furthermore, those early RA patients placed on timely therapy who do not achieve remission experience less severe disease (Anderson 2000, Nell 2004, van der Linden 2010). Indeed, the early diagnosis of RA affords a "window of opportunity" for the greatest probability of effective RA therapy and the possibility of disease remission (Cush 2007). This window closes 3 to 6 months after patients become symptomatic with RA. The problem is that only a portion of patients first presenting with arthritis have RA, and differentiating those patients who have RA from those who do not is challenging, leading frequently to delays in accurately identifying those patients with RA.

The realization that early diagnosis of RA is critical for delivering the most effective RA treatment led to a collaboration between the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) that resulted in 2010 in the publication of new criteria for diagnosing RA (Aletaha 2010). The intent of the ACR/EULAR 2010 criteria was to improve the diagnosis of early RA. This intent was only partially realized. There have been numerous publications reporting the results of studies evaluating the diagnostic accuracy of the ACR/EULAR 2010 criteria for identifying early RA patients. In a meta-analysis of this literature (Sakellariou 2013), it was shown that the ACR/EULAR 2010 criteria have a 73% sensitivity and a 74% specificity for correctly identifying early RA. If the ACR/EULAR 2010 criteria are used to decide who should receive DMARD therapy, this meta-analysis indicates that over a quarter of true early RA patients would not be provided with appropriate DMARD therapy during the critical window of opportunity for an optimal response. Furthermore, a significant portion of arthritis patients who do not have RA would be prescribed DMARD therapies for which they would not receive benefit and would be exposed to possible adverse side effects of the drugs. Clearly there remains an unmet need for a more accurate means to identify early RA patients, to improve aggregate outcomes for early RA patients, and to reduce adverse drug effects and healthcare costs associated with unproductive delivery of RA therapies to individuals who do not have RA.

Basic research on the pathobiology of RA has revealed that the inflammation observed in RA is the consequence of a self-perpetuating pathological alteration in the expression and downstream signaling of a network of cytokines (Olszewski 2001, Meyer 2010). Frequently, at the center of this cytokine network is the overexpression of tumor necrosis factor alpha (TNFα) (Leizer 1990, Keffer 1991, Choy 2001, Westra 2004). Recognition of the importance of disturbances in cytokine expression and especially that of TNFα formed the underlying rationale for the development of many antibody-based biologic therapies intended to block signaling by TNFα or one of the other various inflammatory cytokines involved in RA pathology (Kalden 2002, Chen 2006, Scott 2010, Vivar 2014). Many of these cytokine-directed RA biologic therapies have been granted regulatory approval and are currently commercially available. While many RA patients have benefited from recent advances in RA therapies, problems and deficiencies remain. Among these problems and deficiencies are:

- A significant portion of RA patients do not respond to RA therapies or respond insufficiently to RA therapies to achieve therapeutic goals (Furst 2011, Salliot 2011),
- All current RA therapies are associated with adverse effects, which can be common and/or severe (Tran 2013),
- Many current RA therapies, especially the biologic therapies, are exceedingly expensive, placing an imposing burden on healthcare costs (Hresko 2018) and affordability (Heidari 2018), and
- Nearly all RA therapies lack an adequate defined diagnostic element that can facilitate choosing an individual patient's therapeutic regimen that provides the highest probability of an effective treatment response.

Quantitative assessment of CD206 positivity of inflamed synovia in RA patients is expected to remedy, at least partially, all 4 of these problems and deficiencies. Tc 99m tilmanocept is a synthetic radiopharmaceutical imaging agent that was purposefully designed to be a high affinity ligand for CD206. CD206 is highly upregulated on phenotypically activated macrophages that contribute mechanistically to the underlying pathobiology of RA. It has long been recognized that activated macrophages contribute significantly to RA pathology (Ishikawa 1976, Firestein 1990, Kinne 2007). Macrophages are common in inflamed synovial tissues when patients are first diagnosed with RA (Smolen 2018) and frequently become more numerous as the disease progresses. Activated macrophages produce most of the TNFα that, in a significant proportion of cases, drives and perpetuates the inflammatory cycle in RA (Choy 2001). In the synovial sublining of a joint affected by RA, activated macrophages are frequently the dominant cell type (Cutolo 1993, Kraan 2002, Kennedy 2011). Activated macrophages significantly contribute to the destruction of bone and cartilage through their secretion of proteases (Bresnihan 1999, Ma 2005). Furthermore, the densities of synovial membrane macrophages measured before treatment, and especially the densities of sublining macrophages, have been reported to predict future joint damage (Yanni 1994, Mulherin 1996, Vieira-Sousa 2011, Orr 2017). Not surprisingly, activated synovial macrophage numbers—but not the numbers of other immune cell types—correlate with radiographically determined joint destruction in RA (Yanni 1994, Mulherin 1996). *Thus, CD206 positivity of inflamed synovia in RA patients is expected to provide clinically significant prognostic information for RA*

patients. Another important finding is that activated macrophage numbers are reduced by effective RA therapy (Vieira-Sousa 2011), but do not significantly change over the course of at least months if a patient was given ineffective RA therapy (Baeten 2006). Also, importantly, reductions in activated synovial macrophages associated with effective RA therapy typically occur *before treatment mediated changes* in the severity of clinical symptoms can be observed (Filkova 2016). Thus, a change in activated synovial macrophage numbers is now recognized as a biomarker that provides an objective and early measure of responses to RA therapies (Smith 2001, Bresnihan 2009). In fact, a change in activated synovial macrophage numbers is considered a more accurate measure of treatment response than clinical assessments, which are highly subjective in nature and prone to observer error (Wijbrandts 2007, Bresnihan 2009, van de Sande 2012). Therefore, there is a possibility that future clinical studies may show that quantitative assessment of CD206 positivity of inflamed synovia in RA patients could be used to monitor the efficacy of RA therapies, providing physicians and patients with earlier and more objective criteria to abandon ineffective therapies and adopt alternative therapies that may be more effective. Navidea's current clinical development plan for Tc 99m tilmanocept includes Phase 2 and Phase 3 studies evaluating the safety and efficacy of Tc 99m tilmanocept imaging of RA patients initiating an anti-TNFα therapy and to provide an early indication of response to therapy in this patient population before clinical symptoms have improved. Success of this clinical trials program would provide physicians and RA patients with earlier and more objective criteria to abandon ineffective therapies and adopt alternative therapies that may be more effective.

In recent years, synovial biopsy-enabled studies have greatly increased our understanding of the pathological processes occurring within the inflamed joints of RA patients (Orr 2017). An important finding of these studies has been that the inflammatory cell compositions of RA inflamed joints can vary between patients (Townsend 2014, van de Sande 2016). The biopsy specimens obtained from different RA patients can have different numbers and densities of macrophages and monocytes, lymphocytes and lymphocyte containing structures, and fibroblast-like synoviocytes (Orr 2017). These differences in cellular composition suggest that RA inflammation can be divided into 3 pathotypes, referred to as diffuse myeloid, lympho-myeloid, and fibroid, respectively (Dennis 2014, Astorri 2015). High densities of macrophages can occur in both the myeloid and lymphoid pathotypes, whereas the fibroid pathotype is largely devoid of both macrophages and lymphoid cells. These pathotypes are not fully discrete, with some overlap occurring. However, they provide a strong basis for temporal or cytological compartmentalization in RA disease natural history.

Current studies utilizing IHC analyses of synovial biopsies are insufficient to determine the distribution of the various pathotypes in RA patients but suggest that the diffuse myeloid and lympho-myeloid pathotypes are about equally frequent in RA patients with the fibroid pathotype being less common. There is growing evidence that patients with different RA pathotypes respond differently to various therapies, holding out the possibility that determining the RA pathotype of an individual patient's RA can direct the choice of the most effective therapy for that patient (i.e., personalized RA therapy) (Dennis 2014). This is an area of ongoing active investigation in RA therapy research (Pitzalis 2013, Donlin 2018, Mandelin 2018). However, already there is significant evidence indicating that patients with a myeloid-

driven RA pathotype and/or with high densities of macrophages in their inflamed synovium respond best to anti-TNFα biologic therapy (Wijbrandts 2008, Dennis 2014), whereas patients with a fibroid pathotype do not respond significantly to anti-TNFα therapy. Although these results need to be confirmed and elaborated upon in further studies, they suggest that determination of the density of activated macrophages in the inflamed synovial membranes of patients with RA could facilitate identification of those RA patients who would most benefit from anti-TNFα therapy and/or those who would not receive benefit. Additional work in this field seeks to determine if similar associations between the efficacies of other treatments and synovial pathotypes can identify those treatments that are most effective in patients with lympho-myeloid and/or fibroid RA pathotypes. Such results, if attained, would provide a great benefit to RA patients by enabling personalized delivery of optimal treatments to all RA patients. The study to be conducted (NAV3-32) is designed to establish the correlation between Tc 99m tilmanocept localization to RA inflamed joints and the number and density of CD206 expressing cells (i.e., macrophages) in the inflamed joints as determined by IHC evaluations of synovial biopsy specimens. If this study is successful, Tc 99m tilmanocept imaging may be able to discriminate the fibroid pathotype from the myeloid and lymphoid pathotypes and may be able discriminate all 3 pathotypes from each other.

Previously generated results from clinical imaging studies conducted by Navidea and extensive peer reviewed scientific literature strongly indicate that Tc 99m tilmanocept can enable non-invasive imaging of aggregates of CD206-expressing cells associated with various pathologies using planar scintigraphy. While it has been suggested that synovial biopsy and IHC evaluations might be translatable to common rheumatological clinical practice, there are 5 reasons why quantitative assessment of CD206 positivity enabled by Tc 99m tilmanocept imaging may be preferred to synovial biopsies for evaluations of RA patients.

First, biopsy procedures usually sample a single joint. If variation exists between the pathotypes of different joints in the same patient, biopsy studies cannot detect or quantify this variation. Current RA therapies and new therapies in development are, by their designed targets, likely to be more effective against specific RA pathotypes. If pathotype variation occurs within individual RA patients, this could severely limit the ability of pathotype determination by biopsy to accurately predict treatment response. A key advantage of Tc 99m tilmanocept imaging over synovial biopsies is that Tc 99m tilmanocept imaging can provide a global quantitative assessment of all joints, providing Tc 99m tilmanocept imaging with the possibility of detecting pathotype variation without biopsies. Navidea's proposed studies will directly assess RA inflammatory variation within individual patients and provide evidence relevant to determining the extent to which pathotype variation exists within individual RA patients and the ability of Tc 99m tilmanocept imaging to detect this variation.

The second reason why Tc 99m tilmanocept imaging may be preferred to synovial biopsies for evaluations of RA patients is that synovial biopsies are only performed on patients with inflamed synovia that have expanded in volume beyond a certain grade, thereby enabling extraction of sufficient tissue to assess histologically. This could be a problem when evaluating patients in the early phase of symptomatic RA disease when high densities of activated macrophages have begun to aggregate into the inflamed synovial membrane, but the synovial

membrane may not yet have expanded (i.e., thickened) sufficiently to permit biopsy sampling. As discussed above, there is an urgent need to more accurately identify RA patients as early in the disease process as possible and place them on DMARD therapy immediately to provide these patients with their best possible therapy responses.

The third reason why Tc 99m tilmanocept imaging may be preferred to synovial biopsies for evaluations of RA patients is that, although synovial biopsy procedures typically extract 6 to 14 samples of tissue from each biopsied joint, in about 5% to 10% of cases, they do not provide tissue of sufficient quantity or quality to enable adequate histological evaluations of the inflamed synovial tissue (Kraan 2002, Pitzalis 2013). It is expected that Tc 99m tilmanocept imaging would not fail to quantitatively assess the aggregation of macrophages in RA inflamed joints at this frequency.

The fourth reason is that although more than one biopsy procedure can be performed on an individual joint, there are likely to be limitations on the number of times or how often a single joint can be biopsied. Furthermore, while not discussed in the literature, repeated biopsies may alter the inflammatory microenvironment in an inflamed synovial membrane and/or induce its own inflammation or wound healing response to trauma. In any event, Tc 99m tilmanocept imaging, being non-invasive and non-traumatic, is likely to be more amenable to repeated examination and would not affect synovial inflammation through repeated biopsy related trauma. These issues may be most significant when considering evaluations of the small joints of the hands where there is a limited quantity of inflammatory tissue.

The fifth and final reason why Tc 99m tilmanocept imaging will be preferred to synovial biopsies is that performing biopsies is challenging and requires extensive training (Mandelin 2018). Synovial biopsies have only been performed in research settings and until very recently, only in Europe where adequately trained and experienced investigators reside. Training and qualifying all physicians in the US who care for RA patients to perform synovial biopsies would be a significant barrier to adoption.

Tc 99m tilmanocept quantitative imaging reliably assesses all joints, is not dependent on synovial swelling, is non-invasive and non-traumatic, and does not require extensive practitioner training. Thus, for all these reasons, Tc 99m tilmanocept imaging is expected to provide clinically predictive information about the inflammatory status of inflamed joints in RA patients that is not obtainable from synovial biopsies or will be preferred over invasive and potentially risky synovial biopsies to evaluate RA patients.

In diagnostic radiology, quantitative imaging provides a layer of clinically meaningful information beyond that of qualitative interrogation. The Radiological Society of North America (RSNA) defines quantitative imaging as “the extraction of quantifiable features from medical images for the assessment of normal or the severity, degree of change, or status of a disease, injury, or chronic condition relative to normal. Quantitative imaging includes the development, standardization, and optimization of anatomical, functional, and molecular imaging acquisition protocols, data analyses, display methods, and reporting structures. These features permit the validation of accurately and precisely obtained image-derived metrics with

anatomically and physiologically relevant parameters, including treatment response and outcome, and the use of such metrics in research and patient care." (RSNA 2018)

In nuclear medicine, the SUV (standard uptake value) is an established quantitative imaging metric for the assessment of disease-related activity across a variety of neurological, cardiovascular, oncological, and immunological conditions. For example, in 18F-labeled fluoro-2-deoxyglucose positron emission tomography (18F-FDG PET) imaging, SUV is used to measure the proliferative activity of malignant tumors in various cancers through the quantification of FDG uptake using the following parameters: r, the radioactivity activity concentration [kBq/mL] measured by the PET scanner within a region of interest (ROI), a', the decay-corrected amount of injected radiolabeled FDG [kBq], and w, the weight of the patient [g], such that such that $SUV = \frac{r}{(a'/w)}$. (Kinahan 2010)

Based on the clinical utility of SUV in 18F-FDG PET imaging, Navidea pursued the development of the TUV (tilmanocept uptake value) to quantify CD206 activity on planar gamma camera imaging. TUV considers the fundamental principles of SUV and introduces modifications to account for inter- and intra-patient variability and disease pathobiology. After the evaluation of several formula permutations, Navidea has established TUV as a metric for the measurement of joint-specific CD206 activity in RA through the quantification of Tc 99m tilmanocept uptake using the following parameters: $\bar{x}$, the average pixel intensity of a ROI, and B, the pixel intensity of an anatomically defined within-patient reference region, such that $TUV = \frac{\bar{x}}{RR} \times 100$. Thus, another purpose of this study will be to test the reliability and clinical utility of TUV.

1.2 Previous Nonclinical and Clinical Trial Experience in Tc 99m Tilmanocept

A detailed evaluation of nonclinical evaluations from subcutaneous (SC) and IV routes of administration, clinical pharmacokinetics (PK), clinical efficacy, and clinical safety of Tc 99m tilmanocept can be found in the accompanying Investigator's Brochure supplied by Navidea Biopharmaceuticals, Inc.

1.2.1 Clinical Pharmacokinetics (IV)

Pharmacokinetics was evaluated in IV administered Tc 99m tilmanocept in the Phase 1 and 2 trial NAV3-21 (NCT02865434). In this trial, 12 subjects (6 RA/6 healthy control [HC]) were administered the maximum dose of 400 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m, and urine and blood data were non-compartmentally modeled to assess potential differences in drug distribution and elimination by disease group (active RA vs. HC).

Subject-level whole blood PK parameters were assessed between HC subjects (n = 6) and subjects with active RA (n = 6) including mean maximum concentration (C_{max}), mean area under the concentration-time curve (AUC_{0-t}), mean area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), mean clearance, mean half-life ($t_{1/2}$), and mean elimination rate constant (λ_z) to evaluate potential differences between disease groups (Table 2). The

geometric mean of whole blood clearance was 26.5 mL/min for HC subjects and 24.8 mL/min for RA patients.

Table 2 Whole Blood PK Parameter Summaries by Group

Group	Statistic	Clearance (mL/min)	AUC(0-t) (min*nCi)	AUC(0-∞) (min*nCi)	Cmax (nCi)	T1/2 (min)
HC	n	6	6	6	6	6
	Mean	27.3	235258.0	370580.1	1244.2	759.0
	Std Dev	7.36	50010.28	92535.92	500.36	134.20
	CV%	26.9	21.3	25.0	40.2	17.7
	Geometric Mean	26.5	230853.1	360357.6	1155.5	749.3
	Lower 90% CI	21.26	193698.75	290005.04	809.55	648.36
	Upper 90% CI	33.04	275134.27	447777.17	1649.33	865.85
RA	n	6	6	6	6	6
	Mean	25.5	268110.7	396026.4	2043.8	719.1
	Std Dev	6.18	61667.51	101351.07	1211.86	138.10
	CV%	24.3	23.0	25.6	59.3	19.2
	Geometric Mean	24.8	262309.5	385984.3	1761.9	707.4
	Lower 90% CI	20.11	217187.41	315475.79	1078.28	598.97
	Upper 90% CI	30.59	316806.03	472251.44	2878.94	835.46

Similarly, subject-level urinary PK parameters including maximum rate, AUC_{0-t}, or percent recovered were assessed for HC subjects (n = 6) and subjects with active RA (n = 6) to evaluate potential differences between disease groups (Table 3). The geometric mean of urine percent recovered was 7.4% in HC subjects and 6.7% in RA subjects.

Table 3 Urine PK Parameter Summaries by Group

Group	Statistic	Percent Recovered [a]	AUC(0-t) (h*nCi)	Max Rate (nCi/h) [b]
HC	n	6	6	6
	Mean	7.6	1468788.9	949280.7
	Std Dev	2.07	432723.27	236916.28
	CV%	27.2	29.5	25.0
	Geometric Mean	7.4	1411786.8	924858.6
	Lower 90% CI	5.84	1088888.09	752742.08
	Upper 90% CI	9.28	1830437.75	1136329.94
RA	n	6	6	6
	Mean	6.9	1384642.0	841331.0
	Std Dev	1.74	313600.65	227308.94
	CV%	25.2	22.6	27.0
	Geometric Mean	6.7	1355137.9	817282.6
	Lower 90% CI	5.46	1123310.11	659111.71
	Upper 90% CI	8.33	1634810.07	1013410.74

[a] Percent recovered is the cumulative amount of radioactivity divided by the dose and multiplied by 100.
[b] Maximum observed excretion rate, calculated as (radioactivity*volume)/ (end time – start time).

A comparison of the PK parameters in HCs and subjects with active RA does not reveal any apparent differences in the elimination of radioactivity from the body.

1.2.2 Clinical Efficacy

1.2.2.1 NAV3-23 (SC)

This was an open-label, multicenter study of Tc 99m tilmanocept by SC administration in HCs and in subjects with active RA. Tilmanocept was administered SC at 1 of 2 mass doses: [1] 50 mcg (Cohorts 1 & 3), or [2] 200 mcg (Cohorts 2 & 4). Both mass doses were radiolabeled with 2 mCi of Tc 99m. A total of 18 subjects were enrolled and evaluated (9 active RA, 9 HC). Imaging was performed 60 ± 15 minutes and 180 ± 15 minutes post-injection. The following performance conclusions were drawn upon study completion:

- Based on data from this study and parallel pathology studies, Tc 99m tilmanocept localizes to activated macrophage-infiltrated joints at doses of 50 µg and 200 µg radiolabeled with 2.0 mCi (74.0 MBq) by SC administration.
- Across all combined RA subjects, swollen/tender joints demonstrating the highest proportions of localization include the wrists and knees.
- Based on qualitative image evaluation, Tc 99m tilmanocept does not show differences in localization between 2 to 3-hour and 4 to 6-hour planar imaging within dosing groups.
- Tc 99m tilmanocept demonstrates a greater frequency of localization to swollen/tender joints at 200 mcg/2.0 mCi than 50 mcg/2.0 mCi.

- There is an overall lack of concordance between qualitative observation of Tc 99m tilmanocept localization to swollen/tender joints identified in DAS28 joint count assessment. Swollen/tender joints did not appear to be reliable predictors of presumed abnormal activated macrophage infiltration and overall disease progression when used as an isolated diagnostic system.
- Increased tilmanocept mass dosing, increased Tc 99m specific activity, and other routes of administration may enhance localization and anatomic delineation in tilmanocept-positive joints of RA patients.
- The potential for using Tc 99m tilmanocept to delineate macrophage infiltration in RA-affected joints may allow for earlier RA-specific treatment beyond the current standard of care ACR/EULAR criteria.

1.2.2.2 NAV3-21 (IV)

This was an open-label, multicenter, dose-escalation safety with PK and dosimetry study of Tc 99m tilmanocept by IV administration in HCs and in subjects with active RA. Thirty-nine subjects were enrolled. A total of 27 subjects with active RA were enrolled to Groups 1 to 9 in the dose escalation phase. Group 10 consisted of 6 HCs (3 female and 3 male), and Group 11 consisted of 6 subjects with active RA (3 female and 3 male). Tilmanocept was administered IV at 1 of 3 mass doses: 50 mcg, 200 mcg, or 400 mcg. Within each mass dose group, tilmanocept was radiolabeled with 1 of 3 Tc 99m doses: 1 mCi, 5 mCi, or 10 mCi. Subjects in Groups 10 and 11 received maximum dose of 400 mcg/10 mCi. Imaging was performed 60 ± 15 minutes and 180 ± 15 minutes post-injection. Clinical efficacy conclusions for this study are still under review.

1.2.3 Clinical Safety

1.2.3.1 NAV3-23 (SC)

The NAV3-23 (NCT02683421) safety evaluation included all trial subjects injected with Tc 99m tilmanocept (N = 18). The AE monitoring was performed from the time of dose administration until completion of onsite safety assessment. There was 1 AE that was possibly related to, and 1 AE that was probably related to Tc 99m tilmanocept. However, there were no AEs that led to trial discontinuation, and no serious adverse events (SAEs) were observed. There were no deaths on trial.

1.2.3.2 NAV3-21 (IV)

The primary safety endpoint of the NAV3-21 study was evaluated by examining the incidence of AEs, changes over time in clinical laboratory tests, physical exams, electrocardiogram (ECG) parameters, and vital signs. The safety evaluation included all subjects who were enrolled in the study and administered Tc 99m tilmanocept (n =39). There were no Tc 99m tilmanocept related AEs. There were no deaths during the trial, no SAEs, and no AEs that led to discontinuation from the trial.

2 STUDY OBJECTIVES

2.1 Primary Objectives

The primary objectives of this study are:

- Assessment of the relationship between joint-specific tilmanocept uptake value (TUV_{joint}) and synovial anatomic pathology.
- IHC assessment of macrophage expression of the CD206 receptor.

2.2 Secondary Objectives

The secondary objectives of this study are:

- Assessments of CD68 and CD163 receptor expression levels through IHC microscopy.
- Classification of synovial anatomic pathology as
  - Lympho-myeloid
  - Diffuse myeloid
  - Pauci-immune fibroid

2.3 Exploratory Objectives

The exploratory objectives of this study are:

- Determine the relationship between TUV_{joint} and mRNA expression profiles of CD68, CD163, and CD206 as determined by RNA sequencing (RNA-seq).
- Determine the relationship between TUV_{joint} and the number, size, and intensity of CD68, CD163, and CD206 as determined by (optional) flow cytometry.
- Evaluate synovial expression of CD3, CD20, CD55, and TE-7 and CD206 in synovial tissue biopsy specimens.
- Assessment of the relationship between TUV_{global} and synovial anatomic pathology.

2.4 Safety Objective

The safety objective of this study is:

- Evaluate safety through the examination of adverse event (AE) incidence, physical examination findings, and changes over time in laboratory tests, electrocardiograms (ECGs), and vital signs.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a phase 2b, open-label, multicenter, multinational, non-randomized, single-dose study designed to assess the relationship between quantitative Tc 99m tilmanocept planar imaging and synovial histopathology in subjects clinically diagnosed with RA. This study will take place in up to 10 centers in the US, UK, and EU.

Subjects will undergo an imaging assessment of the bilateral hands and wrists followed by an ultrasound-guided synovial biopsy of a select joint at a subsequent visit. Tissue samples from the synovial biopsy procedure will be used for 2 mandatory anatomic pathology evaluations, [1] IHC and [2] RNA-seq, with optional flow cytometry, to provide quantitative and semi-quantitative information regarding joint-specific disease activity. Results from each of the 3 anatomic pathology evaluations will be correlated with TUV_{joint} on planar imaging to mechanistically assess the relationship between synovial anatomic pathology and TUV_{joint}.

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 correlation between TUV_{joint} and the number and area fraction of CD206 expression as determined by IHC assessment in the synovium of subjects clinically diagnosed with RA who are not receiving anti-rheumatic treatment or who are on stable anti-rheumatic treatment. The ability of Tc 99m tilmanocept imaging to detect and discriminate RA pathotypes is being evaluated in these subjects with the goal of supporting personalized therapy. A complete rationale for evaluating Tc 99m tilmanocept in this subject population is discussed in Section 1.1.

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)] or Independent Ethics Committee(s) [IEC(s)].

If an investigator has deviated from the protocol 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 or IEC as applicable.

3.4 Study Duration and Dates

Subjects will be “on study” for up to 45 days depending on the screening window (up to 30 days).

4 STUDY POPULATION SELECTION

4.1 Study Population

This study will evaluate subjects clinically diagnosed with RA who are on stable therapy.

4.2 Inclusion Criteria

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

1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) or equivalent authorization before the initiation of any study-related procedures.
2. Women and men of reproductive potential must use adequate birth control measures (e.g., abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, or surgical sterilization) for the duration of the study.
3. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis.
4. The subject has RA as determined by the 2010 ACR/EULAR Classification Criteria (score of ≥ 6/10 at or before screening).
5. The subject has a DAS28 of ≥ 3.2 (includes the C-reactive protein [CRP] test and visual analog scale [VAS]).
6. Subjects receiving traditional DMARDs must have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the first imaging visit (Day 0).
7. If the subject is receiving bDMARD or janus kinase (JAK) inhibitor therapy, they have been at a stable dose > 60 days prior to the imaging visit (Day 0). bDMARD therapy should not be administered less than 4 days prior to the imaging visit.
8. If the subject is receiving NSAIDs or oral corticosteroids, the dose has been at a stable dose for ≥ 28 days prior to imaging. The corticosteroid dose should be ≤ 10 mg/day of prednisone or an equivalent steroid dose.
9. The subject has a hand or wrist joint with a minimum ultrasound gray-scale synovitis score of 2 (range 0 to 3).

4.3 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 is currently receiving radiation therapy or chemotherapy or has received radiation or chemotherapy within the past 5 years.
4. The subject has an active malignancy or a history of malignancy within the past 5 years.

5. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty.
6. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.
7. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 3 times the upper limit of normal.
8. The subject has any severe, acute, or chronic medical conditions and/or psychiatric conditions and/or laboratory abnormalities that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration that would deem the subject inappropriate for study participation.
9. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
10. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration (Day 0).
11. The subject has received injectable (e.g., intra-articular, intramuscular, etc.) corticosteroids ≤ 8 weeks prior to imaging (Day 0).
12. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to the administration of Tc 99m tilmanocept (Day 0).
13. The subject has an intolerance to anesthetic and antiseptic agents indicated for the synovial biopsy procedure.
14. The subject is currently receiving anticoagulants (oral anti-platelet agents are permitted) or has a condition that is contraindicated with ultrasound-guided synovial biopsy, e.g., needle phobia.
15. The subject has heart failure (NYHA Class III-IV), a demyelinating disorder, or a chronic/latent infection (e.g., +PPD, HIV, Hepatitis B).

4.4 Recruitment

Subjects will be recruited from rheumatology practices in accordance with the inclusion and exclusion criteria listed above. Candidate subjects will be asked by their treating physician about their willingness to participate in the study.

4.5 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.

Should a subject withdraw after administration of Tc 99m tilmanocept, 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 if it is in the best interest of the subject
- The sponsor or investigator terminates the study
- The subject requests to be discontinued from the study

4.6 Screen Failures

Subjects who sign a patient informed consent form (ICF/PCF) but are ultimately not injected with Tc 99m tilmanocept will be considered a screen failure. eCRFs for informed consent, inclusion, exclusion, demographics, adverse events, and final disposition should be completed for all screen failure subjects.

4.7 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 "32"

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 "32-01-001."

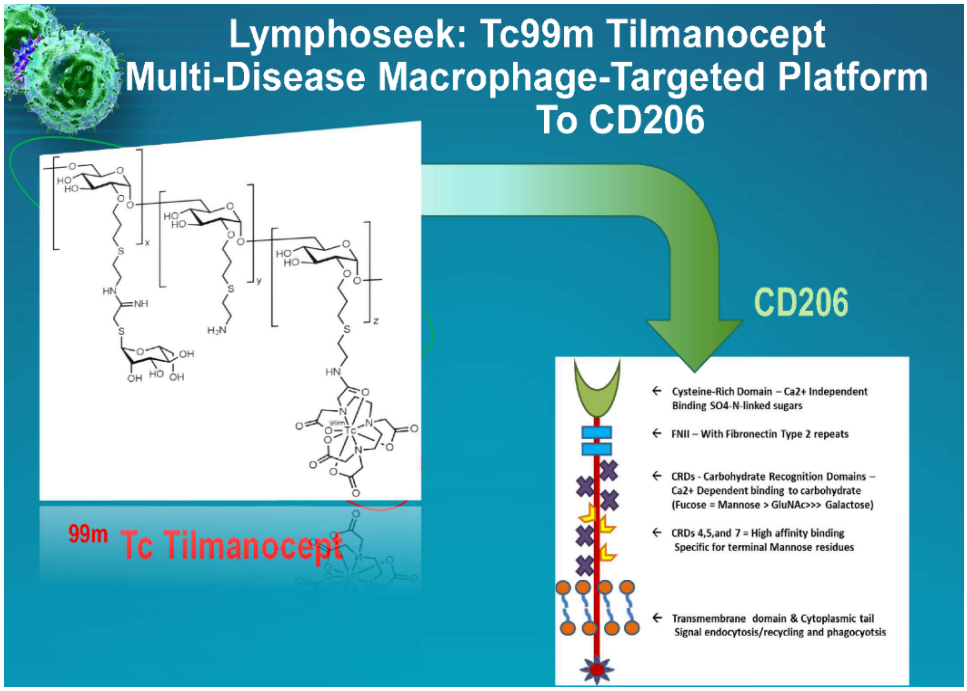
Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Description of Investigational Product

Technetium Tc 99m tilmanocept is a scintigraphic imaging radiotracer that binds to CD206 (mannose-binding receptor) on the surface of macrophages and other inflammatory cells. It comprises multiple units of diethylenetriaminepentaacetic acid (DTPA) and mannose, each synthetically attached to a 10 kDa dextran backbone (Figure 1). The mannose acts as a substrate for the receptor, and the DTPA serves as a chelating agent for labeling with Tc 99m. Tilmanocept has a diameter of about 7 nm, which permits enhanced diffusion into lymph nodes and blood capillaries.

Figure 1 Tc 99m tilmanocept and the Mannose Receptor



5.2 Investigational Product Dosage and Administration

Tc 99m tilmanocept will be administered through an IV route of injection. A 150 mcg dose containing 10 mCi of Tc 99m in 3 mL will be delivered using a single syringe, with the dose injected as a slow push into the IV catheter. At the completion of the injection, a 10-mL sterile normal saline flush will be administered. The preferred site of IV placement will be the left or right antecubital vein.

5.3 Timing and Frequency of Drug Administration

This is an open-label, non-randomized study in which all subjects will receive the study-defined dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m at a single occurrence on Day 0 (Visit 2).

Tilmanocept cartons ready for radiolabeling will be shipped and stored at the study-assigned radiopharmacy. Tilmanocept is provided in a vial. Vials are packaged as a kit. A carton contains 5 vials of tilmanocept. A detailed radiolabeling protocol will be provided to each radiopharmacy for instructions on how to radiolabel the vials and prepare the final tilmanocept product for injection. Quality Control worksheets will also be provided.

5.4 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 principal investigator (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 Therapies

All medications taken 30 days prior to Tc 99m tilmanocept administration through biopsy and post-biopsy telephone safety assessment must be documented, and a stable dose of the medication must be maintained for RA medications through the end of trial participation according to the inclusion criteria. Subjects who have received or are currently receiving radiation therapy or chemotherapy are not eligible for participation in the trial. If applicable, the subject's history of RA treatments for up to 6 months will also be collected.

Dosing of a bDMARD or injectable corticosteroid should be avoided particularly between Visit 2 and Visit 4 (i.e., between imaging and biopsy) for subjects on such therapies. Visit 2 should be scheduled at least 4 days following a subject's last dose of a bDMARD. Injectable corticosteroids (e.g., intra-articular, intramuscular, etc.) should be avoided for at least 8 weeks prior to imaging (Day 0).

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 Events (Appendix 1) and Study Work Flow (Appendix 2).

7.1.1 Visit 1, Screening (Day -30 to Day -1)

- Preliminary review of inclusion and exclusion criteria
- 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 and 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 (administered within 30 days prior to Tc 99m tilmanocept administration); RA concomitant medications administered within 6 months prior to Tc 99m tilmanocept administration will be recorded.
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- Physical examination will include an assessment of height, weight and an examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of Tc 99m tilmanocept administration.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (see Table 5)
- Urine collection for routine analysis
- 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.
- RA Evaluations:
  - Swollen and tender joints as established by the 2010 ACR/EULAR Classification Criteria and DAS28 will be identified and documented during physical examination.

- An ultrasound of the hands and wrist joints will be obtained to assess synovitis. Subjects must have at least 1 joint with a minimum gray-scale synovitis score of 2 (range 0 to 3) to be eligible for the study.
- Assessment of AEs

7.1.2 Visit 2, Tc 99m Tilmanocept Administration and Imaging (Day 0)

Pre-Tc 99m Tilmanocept Administration

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 AEs
- Concomitant medication review
- ECG (see Section 8.7.3) within 30 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 30 minutes prior to administration of Tc- 99m tilmanocept

Tc 99m Tilmanocept Administration

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. The filled syringe will be connected to the catheter for a slow push injection. At the completion of the injection, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the Nuclear Medicine Department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for AEs.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of AEs
- ECG (completed before vital signs)
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate)

60 to 75 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of AEs
- Image acquisition: Static gamma camera scan of the bilateral hands and wrists
- Clinical Labs (at conclusion of the imaging session)

7.1.3 Visit 3, Follow-up Telephone Safety Assessment (24 to 72 hours post-Tc 99m tilmanocept administration)

- Review of concomitant medications
- Assessment of AEs

7.1.4 Visit 4, Synovial Tissue Biopsy (Day 3 to 7 post-Tc 99m tilmanocept administration)

- Ultrasound assessment of synovitis
- Ultrasound-guided synovial tissue biopsy of selected joint in accordance with Table 4
- AE assessment
- Review of concomitant medications

7.1.5 Visit 5, Follow-up Telephone Safety Assessment (5 ± 2 days post-biopsy)

- Review of concomitant medications
- AE assessment

7.1.6 End of Study

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

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographics and Other Baseline Characteristics

Twelve (12) to 24 evaluable subjects may be enrolled. Subjects will be men and women aged ≥ 18 years with clinically diagnosed RA who are on stable therapy.

8.1.2 Medical, Rheumatological, and Surgical History

Relevant medical, rheumatological, 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. Rheumatological history will include date of RA diagnosis as well as the timing, dose, administration frequency, and administration route (when available) of all RA-specific drugs taken in the last 6 months.

8.1.3 Prior and Concomitant Medication

All prior non-RA medications used up to 30 days before the first screening examination through the last follow-up telephone safety assessment (Visit 5) will be documented. All concomitant treatments for RA and RA-specific drugs taken within the last 6 months should be documented.

8.2 Tc 99m Tilmanocept Administration

The site representative will complete an investigational product order form to order Tc 99m tilmanocept from the local radiopharmacy once the subject has been scheduled for IV Tc 99m tilmanocept administration and imaging. The preferred site of IV placement will be the left or right antecubital vein. The filled syringe will be connected to a catheter for a slow push injection. Immediately after the Tc 99m tilmanocept administration, a 10-mL sterile normal saline flush will be injected. Tc 99m tilmanocept administration will be at study time 0:00.

8.3 Rheumatological Assessments

8.3.1 2010 ACR/EULAR Classification Criteria

All subjects will be evaluated at screening using the 2010 ACR/EULAR Classification Criteria (Aletaha 2010) as part of eligibility and inclusion. The 2010 ACR/EULAR classification criteria include 4 components: number and site of involved joints, serologic abnormality, elevated acute-phase response and symptom duration. See Appendix 3 for details. A total score of 6 or higher (out of a possible 10) combined with clinical synovitis not better explained by another disease confirms a diagnosis of “definite RA.” Eligibility for this trial requires that the subject has had a 2010 ACR/EULAR Classification Criteria score of ≥ 6 prior to or at screening.

8.3.2 DAS28

All subjects will be evaluated for the DAS28 (Prevoo 1995) at screening. DAS28 is calculated from 4 components: tender joint count (TJC), swollen joint count (SJC), VAS of the subject's global health, and the laboratory parameter CRP, such that:

$$DAS28 = 0.56\sqrt{TJC} + 0.28\sqrt{SJC} + 0.36\ln(CRP + 1) + 0.014(VAS) + 0.96$$

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 with a DAS28 lower than 2.6 is considered to be in remission. For consistent scoring, the following calculator should be utilized:

https://qxmd.com/calculate/

See Appendix 4 for details on classifying and calculating DAS28.

8.3.3 Other

8.3.3.1 28-joint Count (Swollen Joint Count [SJC] and Tender Joint Count [TJC])

The 28-joint count will be performed for swollen and/or tender joints in the following: shoulder, elbow, wrist, metacarpophalangeal (MCP), proximal interphalangeal (PIP), and knee. Joint swelling is defined as soft tissue swelling that is detectable along the joint margins. Joint tenderness is defined as the presence of pain in a joint at rest with pressure or on movement of the joint (Scott 1996). This assessment will be used as an input parameter for 2010 ACR/EULAR score (Section 8.3.1) and DAS28 score (Section 8.3.2).

8.3.3.2 Visual Analog Scale (VAS)

Patient assessment of global health will be evaluated using the VAS to self-assess disease activity on a 0-100 scale, where 100 represents maximal disease activity. Using a validated ruler, the score will be determined by measuring the distance (mm) on the 10-cm line between 0 and the subject's mark, providing a range of scores from 0 to 100. This assessment will be used as an input parameter for DAS28 score (Section 8.3.2).

8.3.3.3 Acute-phase Reactant

CRP and ESR will be obtained in the RA-specific laboratory panel (see Table 5). CRP will be used in the calculation of the DAS28 score.

8.4 Imaging

8.4.1 Planar Image Acquisition

All subjects will receive anterior and posterior static gamma camera views of the hands and wrists at 60 to 75 minutes post-Tc 99m tilmanocept administration.

Refer to the Imaging Manual for all required imaging technical specifications and acquisitions.

8.4.2 Image Evaluation

All images obtained for this study will be evaluated by a central image core lab. The image core lab will be responsible for image intake; processing, including overall assessment of the scan and QC; region of interest (ROI) drawings; and TUV calculations. All images will be de-identified and provided directly to the Image Core Lab by the site personnel via electronic or CD transfer. Refer to the Imaging Manual for details relating to image intake and analysis.

8.4.2.1 Tilmanocept Uptake Value (TUV)

TUV is a quantitative imaging metric used to characterize the amount of CD206 activity on planar imaging. Standardized TUV calculations are performed at a centralized core image lab in accordance with established image processing guidelines.

For all subjects, a delegated imaging scientist blinded to all subject information (including any clinical data and/or image acquisition data) will perform semi-automated ROI drawing on static images of the bilateral hands and wrists to derive relevant count statistics, which are input parameters for joint-specific (TUV_{joint}) TUVs.

TUV_{joint} is defined as the intrasubject ratio of the average pixel intensity of a joint to the average pixel intensity of the reference region, such that $TUV_{joint} = \frac{\bar{x}_{Joint\ ROI}}{\bar{x}_{RR}}$.

Timelines and further details on image intake, ROI drawing, and TUV calculations can be found in the NAV3-32 Imaging Manual and NAV3-32 Statistical Analysis Plan.

8.5 Synovial Biopsy

8.5.1 Joint Selection

All subjects will undergo a synovial biopsy of a selected hand or wrist joint. All joints selected for biopsy will be required to have an ultrasound gray-scale synovitis score of ≥ 2 (range 0 to 3). In instances where more than 1 joint is qualified for biopsy, the candidate joint will be selected based on TUV_{joint} (as described in Table 4).

Table 4 Biopsy Selection Options

Option	Description
1	The subject has only 1 MCP joint or wrist joint with an ultrasound gray-scale synovitis score of ≥ 2. This joint will be selected for biopsy.
2	The subject has 2 or more MCP and/or wrist joints with an ultrasound gray-scale synovitis score of ≥ 2. The joint for biopsy will be selected based on evaluation of corresponding TUV_{joint}. In the first subject with multiple qualifying joints, the joint with the highest TUV_{joint} will be selected; in the second subject with multiple qualifying joints, the joint with the lowest TUV_{joint} will be selected. Selection will proceed in this fashion, alternating between highest and lowest TUV_{joint}.

The ultrasound assessment for synovitis score should take place at screening and prior to biopsy at Visit 4 (Day 3 to 7 post-Tc 99m tilmanocept administration) in accordance with institutional standard operating procedures (SOPs).

8.5.2 Biopsy Procedure

Subjects will undergo a synovial biopsy of a single joint in the hand or wrist. The synovial biopsy will be performed by a trained member of the clinical study team. Biopsy samples (approximately 12) will be obtained via an 18- or 19-gauge ultrasound-guided quick core biopsy needle and immediately prepared for pathology assessment. Biopsy procedures including subject preparation, tissue harvest, and post-procedure care will be completed in accordance with institutional SOPs.

8.6 Pathology Evaluation of Synovial Tissue

Following the synovial biopsy, tissue will be collected following local SOPs, processed, and shipped to the central pathology lab in accordance with the NAV3-32 Pathology Manual. If fewer than 4 biopsy samples can be obtained for a given subject, all samples will be used for IHC analyses, but that subject's pathology data will be considered non-evaluable and will be excluded from the PP population. If 4 to 6 biopsy samples are obtained, all samples will be used for IHC analyses, and the subject's pathology data will be included in the PP population. When > 6 biopsy samples are obtained, 4 to 6 of the samples will be used for IHC and the remaining samples will be split approximately evenly to be used for RNA-seq and flow cytometry analyses, when applicable.

8.6.1 Preparation and Shipping of Synovial Tissue Samples to Central Laboratory

All viable tissue collected for IHC will be placed in formalin for processing at the central laboratory. Synovial tissue collected for RNA-seq will be placed into a tissue storage solution, such as RNAlater, for transfer and processing at the central pathology laboratory. All samples will be maintained by the central pathology laboratory until trial completion. Contact

information and additional preparation and shipping instructions can be found in the NAV3-32 Pathology Manual.

Shipments as well as questions regarding logistics, quality assurance, or documentation can be directed to the central pathology laboratory.

8.6.2 Central Pathology Laboratory Processing of Samples – IHC and RNA-seq

IHC

The central pathology laboratory will process approximately 4 to 6 samples with hematoxylin and eosin (H&E) and IHC stains for IHC analysis. Processing and semi-quantitative scoring of histological tissue samples will be followed in accordance with the detailed guidelines specified in the NAV3-32 Pathology Manual.

Central pathology laboratory results will be entered into the electronic CRFs (eCRFs) by designated central pathology laboratory staff.

The joint-level pathobiology of each synovial tissue will be classified as follows in accordance with the SOP of the central pathology laboratory:

A – diffuse myeloid

B - lympho-myeloid

C – pauci-immune fibroid

RNA-seq

The central pathology laboratory will process approximately 4 samples in a tissue storage solution (i.e., RNAlater) for RNA sequencing. Whole tissue processing for preparation of the RNA library and RNA-seq analysis will be performed in accordance with the guidelines specified in the NAV3-32 Pathology Manual.

8.6.3 Local Laboratory Processing of Samples – Flow Cytometry

Optional flow-cytometry analysis of tissues will be conducted on a selected subset of subjects enrolled and biopsied. Approximately 4 fresh samples will be placed into a MACS-buffered solution for tissue dissociation. Additional specifications for tissue preparation and cell sorting for flow cytometric analysis can be found in the NAV3-32 Pathology Manual.

8.7 Safety

8.7.1 Adverse Events

8.7.1.1 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).

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 drug related.

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.

For this study, all untoward medical occurrences beginning on the Visit 2 (Day 0) until the final Follow-up Telephone Safety Assessment (Visit 5) are to be reported as AEs. AEs continuing after study completion will be followed to normalization or stabilization. Additionally, untoward medical events occurring prior to the day of Tc 99m tilmanocept administration will be collected and added to the subject's medical history unless they are related to a study procedure in which case the event will be recorded as an AE. SAEs will be reported from the time of consent through the end of participation.

8.7.1.2 Categories for Adverse Event Assessment

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 administered for an AE will be documented.

Causal relationship to investigational product

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

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

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

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

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

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

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

Causal relationship to study procedure

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

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

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

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

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

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

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

8.7.1.3 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 0) will be recorded in the subject's medical history unless they are related to a study procedure in which case the event will be recorded as an AE. Untoward medical events beginning on Visit 2 (Day 0) through the completion of the Visit 5 Follow-up Telephone Safety Assessment will be reported as adverse events. SAEs will be reported from the time of consent through the end of participation.

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 8.7.1.5.
- The maximum **severity** (mild, moderate, or severe).
- If drug treatment was administered for the event, specific concomitant medication 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]).

8.7.1.4 Expected Adverse Events

Investigational Product-Related Risks

In all completed studies of Lymphoseek (Tc 99m tilmanocept), involving 553 subjects, only 3 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 300,000 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. There have been over 135IV administrations of Tc 99m tilmanocept and no SAEs or adverse drug reactions (ADRs) have been reported to date.

Risks of Imaging Procedures

Radiation dose considerations from Tc 99m Tilmanocept planar scans – Subjects enrolled in this trial will have a hand/wrist planar gamma camera scan following Tc 99m tilmanocept injection.

The average effective radiation dose per 10 mCi Tc 99m tilmanocept injection is calculated to be 2.7 mSv (equivalent to about ten months of natural background radiation received in the US). Subjects enrolled in this study will receive one injection, equal to 2.7 mSv total. This is considered to be a minor to intermediate risk level corresponding to the benefit to the patient (category IIb based on the International Commission on Radiological Protection 62 (ICRP62)) and is balanced against the possible substantial societal benefit that can be gained from the trial (European Commission Radiation Protection 99, 1998 and ICRP 62, 1992). For further reference, the effective dose from a standard computed tomography (CT) abdomen and pelvis, with and without contrast, is up to 20 mSv.

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.

8.7.1.5 Serious Adverse Events

Definition of Serious Adverse Events

The following SAE definition is based on ICH guidelines and the final rule issued by the 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 1 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)/IEC(s)

The sponsor and/or the investigator will notify the IRB(s)/IEC(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.

8.7.2 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, respiration, and systolic and diastolic blood pressure. All measurements will be taken after the subject has been in a resting position for at least 1 minute. Vital signs will be measured at screening, within 30 minutes before investigational product administration, and within 30 minutes after investigational product administration (after the ECG assessment during this 30-minute post-Tc 99m tilmanocept interval). 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.

8.7.3 Electrocardiogram

A standard 12-lead ECG will be obtained up to 30 minutes before investigational product administration and within 30 minutes after investigational product administration. The ECG will be measured with the subject in a resting position for at least 1 minute. Continuous ECG monitoring is not required. At a minimum, the heart rate, QRS, PR, and QT intervals will be collected. QTc will be calculated using the Fridericia formula.

On-site investigator's responsibilities

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

8.7.4 Clinical Laboratory Parameters

Table 5 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)

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.

Only abnormal laboratory values will be collected in the electronic database. 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 that 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 drawn is shown in Table 6.

Table 6 Approximate Amount of Blood Drawn per Subject

Timepoint	**Test (Sample Volume)**	**Total Blood Drawn at Timepoint**
Visit 1 (Day -30 to -1)	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (2.6 teaspoons)
Visit 2 (Day 0)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
TOTAL		**22 mL (4.5 teaspoons)**

9 PLANNED STATISTICAL METHODS

This study is a Phase 2b, prospective, open-label, multicenter study of the patterns of association between quantitative Tc 99m tilmanocept planar imaging and synovial histopathology in subjects clinically diagnosed with RA.

Subjects will undergo an imaging assessment followed by an ultrasound-guided synovial biopsy of a selected joint in a subsequent visit. Tissue samples from the synovial biopsy procedure will be used for 2 mandatory anatomic pathology evaluations:

- IHC and immunofluorescence imaging targeting various immune markers associated with inflammatory disease; and,
- RNA-sequencing to provide transcriptional profiling of pathotype-specific gene expression profiles.

Optional flow cytometry will be performed by a local pathology laboratory with a subset of subjects for the exploratory analysis of TUV_{joint} and the number and size of CD68, CD163, and CD206.

Results from each of the 3 anatomic pathology evaluations will be correlated with TUVs on planar imaging to mechanistically assess the relationship between synovial anatomic pathology and TUV. These evaluations will provide quantitative and semi-quantitative information regarding joint-specific disease activity.

9.1 Randomization Methods

This study is not randomized.

9.2 Safety Variables

The safety analysis variables are defined as follows:

- Adverse Events (AEs)
- Clinical Laboratory Tests (hematology, serum chemistry, urinalysis
- ECG Parameters
- Vital Signs

9.3 Efficacy Variables

The primary efficacy variables for this study are defined below:

- Tc 99m tilmanocept uptake values (TUV_{joint}) for the biopsied joints;
- IHC assessment of macrophage expression of the CD206 receptor.

The secondary variables for this study are defined below:

- IHC and immunofluorescence assessment of macrophage expression and co-expression of the CD68 and CD163 receptors;
- Pathotype classification of RA disease as lympho-myeloid, diffuse myeloid, or pauci-immune fibroid, based on TUV_{joint}.

The exploratory efficacy variables for this study are defined below:

- IHC assessment of macrophage expression of the CD3, CD20, CD55, and TE7 biomarkers;
- mRNA levels in synovial tissue for the CD206, CD163, and CD68 biomarkers;
- Flow cytometry measurements of CD206, CD163, and CD68 biomarker expression and co-expression.
- IHC assessment of the expression of a biomarker (e.g., CD206, TE7) is determined from immunofluorescent microscopy, specifically:
  - The ratio of the total fluorescent stained area to the total field area under 4x and 10x power magnification.

All IHC assessments will be done at a single laboratory in accordance with the laboratory SOPs.

- Classification of synovial anatomic pathology into lympho-myeloid, diffuse myeloid, and pauci-immune fibroid types using a multinomial logistic regression model with TUV_{global} as a covariate.
- Tc 99m tilmanocept global uptake values (TUV_{global}) per subject.

It is presumed that the presence of radiotracer uptake for a joint indicates the presence of activated macrophages. The use of the term "localization" is synonymous with radiotracer uptake.

TUV is defined as:

$$\mathrm{TUV_{ROI}} = \frac{\overline{\mathrm{X}}_{\mathrm{joint}}}{\overline{\mathrm{X}}_{\mathrm{RR}}};$$

where

- $\overline{\mathrm{X}}_{\mathrm{joint}}$ is the average pixel intensity of a region of interest (ROI) of a subject at the time of imaging (nominally 60 minutes);
- $\overline{\mathrm{X}}_{\mathrm{RR}}$ is the average pixel intensity of the reference region (RR) of a subject at the time of imaging (nominally 60 minutes).

Calculation of TUV_{joint} and TUV_{global} are further described in the study statistical analysis plan (SAP).

9.4 Sample Size and Justification

The sample size is expected to be approximately 12 to 24 joints. The final sample size will be determined after the completion of an interim analysis including at least 4 subjects in each pathotype (diffuse myeloid, lympho-myeloid and pauci-immune fibroid). The final sample size will be chosen on the basis of the sponsor's business requirements regarding precision for the estimated correlation between CD206 expressing macrophage density and TUV_{joint}. The final sample size will be at least N = 12. If the rarest pathotype has 25% prevalence in the study patient pool, the expected number of subjects at the interim analysis is N = 16. These needs may be further increased because synovial biopsy fails to recover enough tissue for histological analysis in about 15% of cases (Wechalekar 2014).

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following populations are defined for this study:

Safety Population – The safety population includes all subjects who have been enrolled in the study and injected with Tc 99m tilmanocept regardless of imaging or biopsy status.

Intent-to-Assay (ITA) Population – the ITA population includes all subjects who have been enrolled in the study, injected with Tc 99m tilmanocept, received all imaging procedures, and have been biopsied.

Per-Protocol (PP) Population – the PP population consists of all ITA subjects without major protocol violations. At least 4 evaluable synovial biopsy samples must be available for a subject to be included in the PP population.

9.5.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics of the safety population will be summarized. Continuous variables (age, height, weight) will be summarized via mean, standard deviation, minimum, maximum, and number of non-missing responses. Categorical variables (gender, race and ethnicity) will be summarized via counts and percentages.

9.5.3 Analysis of Efficacy Variables

All efficacy analyses will be conducted on both the ITA and PP populations. The ITA population will be the primary analysis set. Additional efficacy analyses may be described in the study SAP.

9.5.3.1 Primary Endpoint

The primary endpoint will be analyzed by computing the Pearson (product-moment) and Spearman (rank) correlation coefficient between the TUV for the biopsied joint and the final CD206 expressing macrophage count. The CD206 expressing macrophage count is derived from IHC histopathology and ultrasound imaging:

- The fraction of CD206 expressing macrophages under 4x and 10x power magnification, defined as the ratio of the total fluorescent stained area to total field area. Specifically, area fraction (AF) is defined as

$$AF = \frac{Total\ Stained\ Area}{Total\ Field\ Area} \times 100\%.$$

- The volume of synovial tissue in the biopsied joint, defined as the ultrasound synovial thickness score (Vol). The CD206 macrophage count will be the product of these 2 variables (that is, CD206 = AF*Vol).

CD206 expressing macrophage fraction and TUV_{joint} will be summarized with descriptive statistics (number of data pairs, means, standard deviations, minima, medians, and maxima as well as the Pearson correlation between the variables). The ultrasound synovitis score will be summarized with a frequency table. A 95% confidence interval for the population correlation coefficient (ρ) based on the Fisher Z-transformation (the inverse hyperbolic tangent) and its normal approximation will be provided. The joint distribution of the CD206 macrophage count and TUV_{joint} will be summarized graphically with a scatter diagram of the data pairs with the least-squares regression line superimposed on the graph. The joint distribution of the component variables of CD206 macrophage count will be graphically summarized with box plots showing the conditional distribution of TUV_{joint} for each category of synovitis score.

9.5.3.2 Secondary Endpoints

Co-expression of the CD68, CD163, and CD206 markers will be analyzed by 2 methods. First, by providing the product-moment and Spearman rank correlation matrices of the IHC field measurements (that is, the fraction of macrophages expressing the markers will not be multiplied by the synovitis score). Summary statistics of the data triplets used to calculate the correlation matrices will be provided (number of data triplets, means, standard deviations, minima, medians, and maxima). In the second method, histological sections will undergo immunofluorescent staining for the 3 markers and DAPI. For each section evaluated, up to 50 cells from the synovial sublining will be identified for each of the 3 markers. For each identified cell (up to 150/section), the rate of co-expression of the 3 markers will be determined. For sections with sparse marker expressing cells, four microscope fields will be randomly selected. Co-expression of the markers will be quantified for any cell observed expressing any marker.

The ability of TUV_{joint} to discriminate among lympho-myeloid, diffuse myeloid, and pauci-immune fibroid RA disease types will be evaluated with a multinomial logistic regression

model with TUV_{joint} as a covariate. RA type will be the response variable and TUV_{joint} will be the explanatory variable. ROC curves will be generated using the lympho-myeloid type as the base class. The marginal distribution of disease type will be summarized with a frequency table, while the marginal distribution of TUV_{joint} will be summarized with descriptive statistics (number of data pairs, mean, standard deviation, minimum, median and maximum). The joint distribution will be graphically summarized with box plots showing the conditional distribution of TUV_{joint} for each disease type.

9.5.3.3 Exploratory Endpoints

Expression of CD3, CD20, CD55, CD68, CD163, CD206, and TE7 biomarkers and their association with TUV_{joint} will be assessed with the Pearson (product-moment) and Spearman (rank) correlation matrices calculated for each measurement of expression (IHC, RNA_{seq}, and [optional] flow cytometry). The marginal distributions will be summarized with descriptive statistics (number of complete data points, means, standard deviations, minima, medians, and maxima). The joint distribution will be presented graphically in a windowpane plot of the scatter diagrams.

Expression of CD206, CD163, and CD68 genes in mRNA will be measured with cDNA probes. The TUV_{joint} values concentrations will be regressed on the mRNA abundance as determined by RNA-seq values. The marginal distributions will be summarized with descriptive statistics (number of complete data points, means, standard deviations, minima, medians, and maxima). The joint distribution will be presented in a windowpane plot of the scatter diagrams. The regression coefficients will be presented with the estimated residual covariance matrix.

The number, size, and co-expression of CD68, CD163, and CD206 expressing macrophages measured by flow cytometry and their relationship with TUV_{joint} will be assessed with the Pearson (product-moment) and Spearman (rank) correlation matrices. The marginal distributions of all variables will be summarized by descriptive statistics (number of complete data points, means, standard deviations, minima, medians, and maxima). The joint distributions will be presented graphically in a windowpane plot of the scatter diagrams.

The ability of TUV_{global} to discriminate among lympho-myeloid, diffuse myeloid, and pauci-immune fibroid RA disease types will be evaluated with a multinomial logistic regression model with TUV_{global} as a covariate. RA type will be the response variable and TUV_{global} will be the explanatory variable. ROC curves will be generated using the lympho-myeloid type as the base class. The marginal distribution of disease type will be summarized with a frequency table, while the marginal distribution of TUV_{global} will be summarized with descriptive statistics (number of data pairs, mean, standard deviation, minimum, median and maximum). The distribution will be graphically summarized with box plots showing the conditional distribution of TUV_{global} for each disease type.

9.5.4 Analysis of Safety Variables

All safety analyses will be conducted on the safety population.

All adverse events (AEs) will be observed for each subject from the time of signing of informed consent until study completion. A treatment-emergent AE (TEAE) is defined as an AE whose start date is on or after the date of the first tilmanocept injection. If the first injection date or the AE start date is missing, the AE will be considered treatment emergent.

Prior to analysis all AEs will be coded using the MedDRA coding dictionary. Based on the coded terms, TEAEs will be summarized as follows:

- By system organ class (SOC) and preferred term (PT);
- By SOC and PT and relation to the study drug;
- By SOC and PT and severity.

Observed and change from baseline for vital sign parameters, ECG parameters, hematology, clinical chemistry, and urinalysis parameters will be summarized using descriptive statistics (n, mean, standard deviation, minima, median, and maximum) at each time point.

A data listing will be prepared that reflects the occurrence of TEAEs associated with each concomitant medication or class of medications to examine whether a drug interaction signal is detectable.

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

9.5.5 Handling of Missing Values

The analysis of the efficacy and safety variables will be carried out on the observed data, i.e., a complete case analysis. For the regression and correlation analyses, a subject must have valid values for all variables to be used in the analysis.

9.5.6 Interim Analysis

An interim analysis will be performed after 4 subjects in each of the 3 pathotypes (diffuse myeloid, lympho-myeloid, and pauci-immune fibroid) have been imaged. Specific analyses will be described in the study SAP (or interim analysis plan [IAP]) and will include computation of the product-moment and rank correlations between TUV_{joint} and CD206 expressing macrophage count (defined in Section 9.5.3.1). Additional analyses may be described in the study IAP (if written) or SAP (if no IAP is written). The final sample size will be recalculated after completion of the interim analysis.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

Data required according to this protocol are captured in the subject's source documentation and are to be entered onto the eCRFs (provided by the sponsor) as soon as possible.

10.1.1 CRF Design

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 trial master file (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 1 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/IEC and the regulatory authority and, if appropriate, from the radiation protection authorities. In addition, the CRA will determine whether all AEs or 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)/IEC(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

10.6.1 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

10.6.2 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)/IEC(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.

10.6.3 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/IEC of the termination or suspension and the reason for that.

10.6.4 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.

10.6.5 Study Participant

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

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

The planning and conduct of this clinical study are subject to national laws. Only when all 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)/IEC(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)/IEC(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/IEC has been obtained. Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the 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/IEC's approval/favorable opinion in advance of use.

11.3 Financing/Financial Disclosure

Each investigator (including principal and/or any sub-investigators; as well as their spouses and dependent children) who is directly involved in the treatment or evaluation of research subjects must 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.

12 REFERENCE LIST

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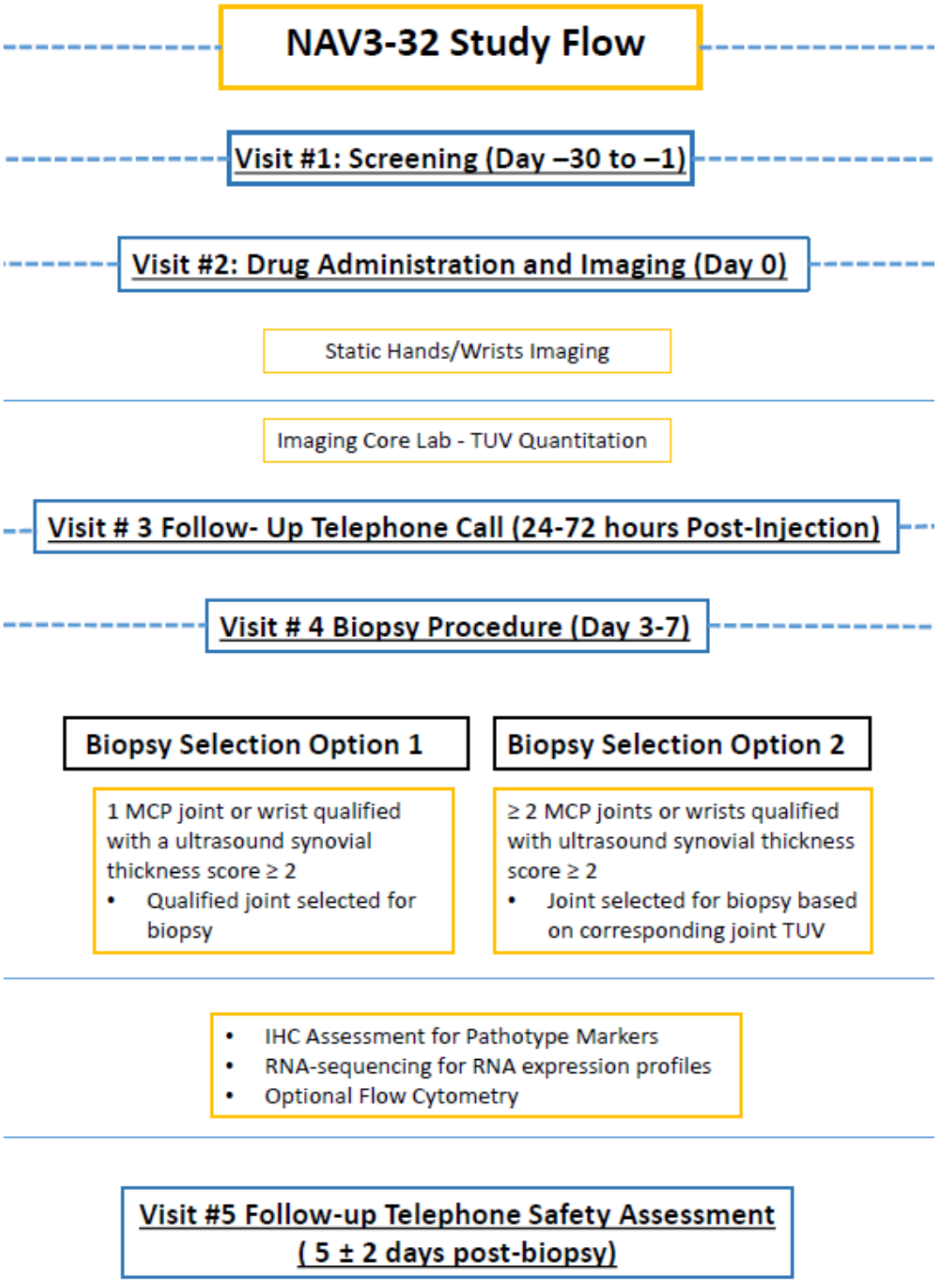
Appendix 1 Schedule of Events

Evaluation	Visit #1 (Screening)	Visit #2 (Tc 99m tilmanocept administration and Imaging Day 0)				Visit #3 Follow-up Safety Telephone Call	Visit #4 Synovial Tissue Biopsy	Visit #5 Follow-up Safety Telephone Call
	Days -30 to -1	-00:30 to 00:00	00:00	00:01 to 00:30	60 to 75 minutes	24 to 72 hours post-administration	Day 3-7 post-administration	5 ± 2 days post-biopsy
Informed Consent	X							
Eligibility Review	X							
Medical /Surgical /RA History, Demographics	X							
Physical Examination	X							
Clinical Labs: Chemistry, Hematology, Urinalysis	X				X[c]			
RA Panel (ESR, CRP, RF, ACPA)	X							
ACR/EULAR 2010 Classification	X							
DAS28 Assessment	X							
28 joint count assessment for DAS28	X							
VAS questionnaire for DAS28	X							
Ultrasound Assessment of Synovitis	X						X	
Vital Sign Assessment[b]	X	X		X				
Urine Pregnancy Test	X	X[a]						
Tc 99m tilmanocept Administration			X					
Imaging: Static Hands/Wrists					X			
ECG		X		X				
Adverse Event Monitoring	X	X	X	X	X	X	X	X
Concomitant Medications Review	X	X				X	X	X
Synovial Tissue Biopsy							X	

[a]Urine Pregnancy test must be completed and determined to be negative in women of childbearing potential within 48 hours of Tc 99m tilmanocept administration.
[b]Obtained post-ECG at Visit 2
[c]Labs will be completed at the end of the 60 to 75 minutes imaging session

Appendix 2 NAV3-32 Study Workflow



Appendix 3 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.

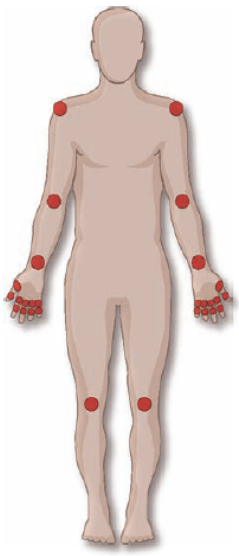
Appendix 4 DAS28 Scoring

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 5 Sponsor Signatures

Study Title:	A Comparison of Tc 99m Tilmanocept Quantitative Imaging with Immunohistochemical (IHC) Analysis of CD206 Expression in Synovial Tissue from Subjects Clinically Diagnosed with Rheumatoid Arthritis (RA)
Study Number:	NAV3-32
Original Date:	12 June 2019
Amendment 1 Date	28 October 2019
Amendment 2 Date	11 February 2020
Amendment 3 Date	29 April 2020
Amendment 4 Date	17 September 2020
Amendment 5 Date	07 April 2021
Amendment 6 Date	24 June 2021
Amendment 7 Date	25 October 2022

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: OCT 31, 2022

Michael Rosol, PhD
Chief Medical Officer
Navidea Biopharmaceuticals, Inc.

Signed: _____ Date: 31 October 2022

Michael Blue, MD, FACEP
Senior Medical Director
Navidea Biopharmaceuticals, Inc.

Appendix 6 Investigator's Signature

Study Title:	A Comparison of Tc 99m Tilmanocept Quantitative Imaging with Immunohistochemical (IHC) Analysis of CD206 Expression in Synovial Tissue from Subjects Clinically Diagnosed with Rheumatoid Arthritis (RA)
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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:_____