Clinical Trial Protocol: NAV3-35

Study Title:	Development of a Normative Database for Rheumatoid Arthritis (RA) Imaging with Tc 99m Tilmanocept
Study Number:	NAV3-35
Study Phase:	Phase 2b
Product Name:	Technetium Tc 99m tilmanocept
IND Number:	132943
Investigators:	Multicenter
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	Date
Original Protocol:	17 March 2021

SYNOPSIS

Study Title:
Development of a Normative Database for Rheumatoid Arthritis (RA) Imaging with Tc 99m Tilmanocept

Study Number:
NAV3-35

Study Phase: Phase 2b

Primary Objective(s):

- To establish mean and variance of joint-specific Tilmanocept Uptake Value (TUV_{joint}) in healthy controls (HCs) age-matched to RA population.
- To assess the feasibility of detecting Tc 99m tilmanocept anatomic localization using single photon emission computed tomography/computed tomography (SPECT/CT) imaging of hands and wrists in HCs and subjects with active RA.

Secondary Objective(s):

- To quantitate Tc 99m tilmanocept anatomic localization based on SPECT/CT imaging of hands and wrists in HCs and joints with RA-involved inflammation.

Safety Objective:

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

Inclusion Criteria:

ALL SUBJECTS

1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) authorization before the initiation of any study-related procedures.
2. The subject has agreed to not engage in any diet, lifestyle, or medication changes until study completion.

HEALTHY CONTROL SUBJECTS

3. The subject is 30 years of age or greater at the time of consent.
4. The subject is deemed to be clinically free of any inflammatory disease(s), auto-immune disease(s), or arthropathies and has not experienced joint pain for at least 28 days prior to the consent date.
5. The subject is not currently on anti-inflammatory drugs (including non-steroidal anti-inflammatory drugs [NSAIDs]) and has not taken any anti-inflammatories for at least 28 days prior to the consent date.
6. For all ongoing concomitant medications, the subject has maintained a stable dose for at least 28 days prior to the consent date.

RA SUBJECTS

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 moderate to severe RA as determined by the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria (score of ≥ 6/10).
5. The subject has a 28-joint disease activity score (DAS28) of ≥ 3.2 (includes the Erythrocyte Sedimentation Rate [ESR] 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 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).
8. If the subject is receiving NSAIDs or oral corticosteroids, the dose has been stable for ≥ 28 days prior to the imaging visit (Day 0). The corticosteroid dose must be ≤ 10 mg/day of prednisone or an equivalent steroid dose.

Exclusion Criteria:

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator.
3. The subject is currently receiving radiation therapy or chemotherapy or has received radiation therapy or chemotherapy in the past six months.
4. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty.
5. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.

6. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 2 times the upper limit of normal.
7. 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 or compromise the safety of the subject or the quality of the data.
8. The subject has any unstable medical illnesses, including hepatic, renal, gastroenterologic, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease.
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 Tc 99m tilmanocept administration (Day 0).
11. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to Tc 99m tilmanocept administration (Day 0).
12. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to Tc 99m tilmanocept administration (Day 0).
13. **Healthy Controls only:** The subject has a positive rheumatoid factor and an elevated ESR or CRP.

Study Design:
This is a prospective, open-label, multicenter, single-dose study designed to develop a normative database of TUV_{joint} in HCs and to assess the feasibility of qualitative and quantitative SPECT/CT assessments in HCs and subjects with active RA following Tc 99m tilmanocept administration.

Tilmanocept Uptake Value (TUV) is a quantitative imaging metric used to characterize Tc 99m tilmanocept uptake on planar imaging. Results from prior Phase 1 and 2 studies have demonstrated that TUV is a sensitive and specific predictor of visually interrogated Tc 99m tilmanocept localization in joint regions with presumed inflammatory macrophage activity. A per-joint TUV (TUV_{joint}) relative ratio will be calculated for the each of the 22 DAS-28 joints located in the hands and wrists. A subject-level global TUV (TUV_{global}) assessed across the 22 joints will be used as an indication of overall disease burden. TUV metrics are further described in the NAV3-35 Statistical Analysis Plan.

This study is stratified into 2 arms. Arm 1 is comprised of 120 HCs, and Arm 2 is comprised of 5 HCs and 10 clinically diagnosed RA subjects on stable treatment.

Rationale for Sample Size:
The study will enroll up to 135 evaluable subjects in Arms 1 and 2 allocated below, imaged at up to 8 study centers. Arm 1 will have N = 120 evaluable HC subjects, Arm 2 will have N = 15 (5 HCs and 10 RA) evaluable subjects. A subject is considered evaluable if he/she meets the criteria for the analysis population and has the data necessary for computing the primary endpoint.

The sample size for Arm 1 was determined to provide acceptable precision for the estimated 5 and 95 percentiles. These percentiles will be estimated with 95% nonparametric confidence intervals. Although the standard error formula for quantile estimators is known and quantile estimators obey a central limit theorem, relying on the asymptotic Normal distribution seems imprudent.

The sample will be stratified 3:1, females males to reflect RA incidence in the United States. The sex-specific samples will be further stratified by age to reflect the Census estimated US population in 2019. The target sample sizes are given below.

Age Range	Male	Female	Total
30 to 39 years	8	23	31
40 to 49 years	7	21	28
50 to 59 years	8	22	30
60 to 69 years	7	21	28
70 years and older[a]	6	21	27
Total	36	108	144

[a] An effort will be made to target enrollment in this group to include 2 men and 8 women 80 years old and over.

The existing data for HC subjects was used in a bootstrap procedure to evaluate the width of 95% confidence intervals for the 95 percentiles for various sample sizes. The sponsor has decided for its business purposes that knowing the percentile to within 0.05 of its true value is sufficient. A sample of size N = 144 HC results (this includes the subjects from Arm 1 of NAV3-31) in 95% confidence intervals with half-widths less than 0.05.

The sample size for Arm 2 was determined to be sufficient for the business purposes of the sponsor. The sample size for Arm 2 will be 5 HCs and 10 RA subjects.

Efficacy Assessments:

Primary Endpoint(s)

ARM 1

- The normal limits of TUV_{joint} (on a per joint basis) in HC subjects, which are defined as the 5 and 95 percentiles of TUV_{joint} of bilateral joints (i.e., bilateral wrists, metacarpophalangeal joint [MCPs], proximal interphalangeal [PIPs]). The normal range will be determined separately for each of the three blinded readers.

ARM 2

- The qualitative evaluations of SPECT/CT in detecting localization within synovial spaces of the bilateral hands and wrists in HCs and RA subjects.

Secondary Endpoints

ARM 1

- Applicability of the Normal (Gaussian) distribution to TUV_{joint} data.

ARM 2

- Quantitative evaluations of Tc 99m tilmanocept localization from SPECT/CT within synovial spaces of the bilateral hands and wrists in HCs and RA subjects.
- Normative joint-specific standardized uptake value (SUV).
- Predictive value of planar scans for SPECT/CT scans.

Statistical Methods:

Primary Endpoint Arm 1

The distribution of TUV_{joint} in HC subjects will be summarized with descriptive statistics: mean, standard deviation, n, minimum, maximum, and median per reader, joint, and view. The 5 and 95 percentiles will be estimated with quantile regression fitting an intercept as a fixed term. The lower and upper limits of normal TUV_{joint} will be determined using non-parametric confidence intervals and kernel density-based confidence intervals.

Primary Endpoint Arm 2

Localization of tilmanocept in the hands and wrists will be summarized with frequency counts and percentages by reader and joint.

Secondary Endpoint Arm 1

Normal quantile plots will be provided per joint and reader, along with the *p*-value for the Shapiro-Wilk test of Normality.

Secondary Endpoints Arm 2

SUV will be calculated and summarized on both a per-joint and a total of all joints with summary statistics: mean, standard deviation, n, minimum, maximum, and median.

The predictive value of planar scans for SPECT/CT qualitative findings will be summarized per joint and reader with a crosstabulation and the uncertainty coefficients for row | column, column | row, and symmetric. The planar result will be deemed "positive" (i.e., inflamed) if TUV_{joint} > 95 percentile of the reader results for the same joint in Arm 1. Otherwise, the planar result will be deemed "negative".

Additional statistical methods are described in Section 9.5 and in the NAV3-35 Statistical Analysis Plan.

Date of Original Approved Protocol: 17 March 2021

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACPA	Anti-Citrullinated Peptide Antibody
ACR	American College of Rheumatology
$ACR_{12/24w}$	American College of Rheumatology response criteria at 12 ± 1 weeks / 24 ± 1 weeks of newly initiated bDMARD therapy and baseline
ACR20/50/70	American College of Rheumatology response criteria
ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
AE	Adverse event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
bDMARD	Biological disease-modifying antirheumatic drug
BMI	Body Mass Index
CD206	Mannose-binding receptor (Ca2+-binding lectin)
CDAI	Clinical disease activity index
CFB	Change from baseline
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CV	Coefficient of variation
DAS28	Disease activity score -28 for RA describing the severity of RA using clinical and laboratory data.
DMARD	Disease-modifying antirheumatic drug
DTPA	Diethylenetriaminepentaacetic Acid
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ESR	Erythrocyte Sedimentation Rate
GCP	Good Clinical Practice
HC	Healthy control

ICF	Informed Consent Form
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
ITD	Intent to Diagnose
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
MCP	Metacarpophalangeal joint
NSAID	Non-steroidal anti-inflammatory drug
PIP	Proximal Interphalangeal
PK	Pharmacokinetics
PP	Per Protocol
QT	Interval from the Q wave to the end of the T wave
QTc	Corrected QT Interval
RA	Rheumatoid arthritis
RF	Rheumatoid Factor
RMSD	Root mean square difference
ROC	Receiver Operating Characteristic
ROI	Region of interest
RR	Reference region
RSNA	Radiological Society of North America
SAP	Statistical Analysis Plan
SJC	Swollen joint count
SC	Sulfur Colloid
SOC	System Organ Class
SPECT	Single-Photon Emission Computerized Tomography
SPECT/CT	Combined Single-Photon Emission Computerized Tomography and computed tomography
SUV	Standardized Uptake Value
TJC	Tender joint count

TMF	Trial Master File
TNFα	Tumor necrosis factor alpha
TUV	Tilmanocept Uptake Value
TUV_{global}	Global Tilmanocept Uptake Value
TUV_{joint}	Joint-specific Tilmanocept Uptake Value
UC	Uncertainty Coefficient
ULN	Upper limit of normal
VAS	Visual Analog Scale
WB	Whole body

1 INTRODUCTION

1.1 Background

Worldwide, approximately 1 in 200 adults suffer from RA. In the US, 1.3 million adults are living with RA. Each year, about 130,000 Americans are newly diagnosed with RA. Persons with inadequately controlled RA have significantly shorter life expectancies and frequently become disabled, leading to reduced quality of life and severe adverse economic consequences [1-4].

It has been realized for many years that RA patients who are placed on DMARDs soon after they develop arthritis symptoms respond much more favorably to these therapies than do patients whose initiation of DMARD therapy is delayed [5]. 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 [6-8]. 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 [9]. 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 [10]. 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 [11], it was shown that the ACR/EULAR 2010 criteria has 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 [12, 13]. Frequently, at the center of this cytokine network is the overexpression of tumor necrosis factor alpha (TNFα) [14-17].

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 [18-21]. 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 [22, 23],
- All current RA therapies are associated with adverse effects, which can be common and/or severe [24],
- Many current RA therapies, especially the biologic therapies, are exceedingly expensive, placing an imposing burden on healthcare costs [25] and affordability [26], 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 [27-29]. Macrophages are common in inflamed synovial tissues when patients are first diagnosed with RA [30] 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 [14]. In the synovial sublining of a joint affected by RA, activated macrophages are frequently the dominant cell type [31-33]. Activated macrophages significantly contribute to the destruction of bone and cartilage through their secretion of proteases [34, 35]. 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 [36-39]. Not surprisingly, activated synovial macrophage numbers—but not the numbers of other immune cell types—correlate with radiographically determined joint destruction in RA [36, 37]. 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 [38], but do not significantly change over the course of at least months if a patient was given ineffective RA therapy [40]. 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 [41]. 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 [42, 43]. 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 [42, 44, 45]. 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.

In recent years, synovial biopsy-enabled studies have greatly increased our understanding of the pathological processes occurring within the inflamed joints of RA patients [39]. An important finding of these studies has been that the inflammatory cell compositions of RA inflamed joints can vary between patients [46, 47]. 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 [48]. 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 [49, 50]. Myeloid pathotype variants have the highest density of macrophages, whereas the fibroid pathotype is largely devoid of both macrophages and lymphoid cells. These pathotypes are not fully discreet 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 choice of the most effective therapy for that patient (i.e., personalized RA therapy) [50]. This is an area of ongoing active investigation in RA therapy research [51-53]. 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 [50, 54], 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 an optimal treatments to all RA patients.

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 below, 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 6-14 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 [31, 51]. 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 1 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 repeat 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 [52]. 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.

Indeed, 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 as a means 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." [55]

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 $\mathrm{SUV} = \frac{\mathrm{r}}{(\mathrm{a}'/\mathrm{w})}$. [56]

Based on the clinical utility of SUV in 18F-FDG PET imaging, Navidea pursued the development of the TUV 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 an ROI, and B, the average pixel intensity of the whole hand and part of the forearm on the same view and side as the joint ROI (serving as an intra-patient reference region), such that $\mathrm{TUV} = \frac{\bar{\mathrm{x}}}{\mathrm{B}}$. The overall purpose of this

study will be to establish the mean and variance of healthy control joint uptake values for reference to be used to assess RA-inflamed joints in patients with RA.

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

A detailed evaluation of the 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 Nonclinical Evaluations –Subcutaneous Administration

Nonclinical studies of Tc 99m tilmanocept demonstrated that the drug selectively binds to its intended receptor (the CD206 mannose binding receptor), and is well tolerated by rats, rabbits, guinea pigs, and dogs.

PK data obtained from nonclinical studies demonstrated rapid absorption into the plasma. Urinary excretion was a major pathway of elimination. Tc 99m tilmanocept exhibited rapid clearance from the injection site, rapid uptake by the local lymph node, and low uptake by the remaining lymph nodes. Tilmanocept was well tolerated at all doses tested in nonclinical safety pharmacology studies and in single and repeated dose toxicology studies in rats, rabbits, and dogs. In some studies in rabbits and dogs, tilmanocept acted as a local irritant of the subcutis or skeletal muscle, and induced mild inflammation and tissue degeneration. The no-observed adverse-effect level (NOAEL) was 42µg/kg/day. Tilmanocept was not mutagenic or genotoxic in vitro or in vivo. No signs or symptoms of hypersensitivity were observed in a study in guinea pigs.

1.2.2 Nonclinical Evaluations – IV Administration

In preparation to initiate the IV route of administration, 11 preclinical tests were conducted to assess safety, toxicity, and interaction potential at doses hundreds to thousands of times the expected maximum human dose, as summarized in Table 1. The nonclinical evaluations yielded safety and pharmacokinetics profiles that were appropriate for initiation of IV dosing in clinical trials. Nonclinical study results can be found in the accompanying Investigator's Brochure.

Table 1 Preclinical Tests

Type of Study / Description	Test System	Method of Administration	Dosing
Central nervous system safety pharmacology	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Expanded single-dose toxicology (including toxicokinetics and local tolerance)	Rat	Intravenous	37, 190, and 380 µg/animal or equivalent 490X and 61X the anticipated study doses of 50 µg and 400 µg in humans
Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of Tilmanocept following Intravenous Bolus Injection in Male Rats	Rat	Intravenous	60, 120, and 300 µg/animal or equivalent 320X and 41X the anticipated study doses of 50 µg and 400 µg in humans
In Vitro Evaluation of Tilmanocept as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes	Human Liver Samples	In vitro	0.6 to 600 nM
In Vitro Evaluation of Tilmanocept as an Inhibitor of Human ABC and SLC Transporters	Human Liver Samples	In vitro	0.04, 0.4 µM
Pharmacokinetics, Excretion, and Distribution by Quantitative Whole-Body Autoradiography Following Intravenous Administration of 99mTc-Tilmanocept in Rats	Rat	Intravenous	25 µg in 0.5 mL with collection of blood, urine, feces, and carcasses for QWBA
Hemolysis and protein flocculation	Human blood samples	In vitro	2.5, 25, and 250 µg/mL whole human blood
Target profiling screen (K, Na, and Ca ion channels)	Ion Channel	In vitro	0.025 to 0.5 mg/mL

1.2.3 Clinical Pharmacokinetics (IV)

Clinical PK 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 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) to evaluate potential differences between 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 ($t_{1/2}$), or mean elimination rate constant (λ_z) across each disease group (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 patients.

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 subjects with active RA and HC subjects does not reveal any apparent differences in the elimination of radioactivity from the body.

1.2.4 Clinical Efficacy

1.2.4.1 NAV3-23 (SC)

This was an open-label, multicenter study of Tc 99m tilmanocept by subcutaneous injection in patients with active RA and healthy controls. 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 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 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.

1.2.4.2 NAV3-21 (IV)

This was an open-label, multicenter, dose-escalation safety with PK and dosimetry study of Tc 99m tilmanocept by IV injection in HCs and 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 the maximum dose of 400 mcg/10 mCi. Imaging was performed 60 ± 15 minutes p.i. The following clinical efficacy conclusions were drawn upon study completion:

- The $TUV_{brain(average)}$ readout provides an objective means of quantification of Tc 99m tilmanocept localization when administered at the calculated optimal dose of 150 mcg/10 mCi and planar images are acquired 60 to 180 minutes after drug administration.

- Joint-specific localization of activity and joint-specific clinical symptomology were not fully concordant. Within the spectrum of RA disease involvement, there may be a decoupling of the causal active immune process and subsequent clinical sequelae. We surmise that imaging and quantification of Tc 99m tilmanocept uptake may provide a fuller and more accurate, responsive, and objective measure of RA disease activity than can be obtained from clinical assessments alone. Future studies will examine whether the approach to image acquisition and calculation of TUV can be further refined.

1.2.4.3 NAV3-31 (IV)

This is an ongoing open-label, multi-center, single and repeat-dose study designed to evaluate the reliability and sensitivity of TUV assessments in HCs and subjects with active RA. One-hundred and five evaluable subjects will be enrolled. Tilmanocept is administered IV at a dose of 150 mcg tilmanocept radiolabeled with 10 mCi Tc 99m. Clinical efficacy conclusions for this study are still in progress.

1.2.5 Clinical Safety

1.2.5.1 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 SAEs were observed. There were no deaths on trial.

1.2.5.2 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. In addition, radiation exposure was within regulatory and safety limits at the doses evaluated. Radiation exposure and pharmacokinetics do not appear to differ in subjects with RA and healthy control subjects.

1.2.5.3 NAV3-31 (IV)

This is an ongoing open-label, multi-center, single and repeat-dose study designed to evaluate the reliability and sensitivity of TUV assessments in HCs and subjects with active RA. One-hundred and five evaluable subjects will be enrolled. Tilmanocept is administered IV at a dose of 150 mcg tilmanocept radiolabeled with 10 mCi Tc 99m. Clinical safety conclusions for this study are still in progress.

2 STUDY OBJECTIVES

2.1 Primary Objective(s)

- To establish mean and variance of TUV_{joint} in HCs age-matched to RA population.
- To assess the feasibility of detecting Tc 99m tilmanocept anatomic localization using SPECT/CT imaging of hands and wrists in HCs and subjects with active RA.

2.2 Secondary Objective(s)

- To quantitate Tc 99m tilmanocept anatomic localization based on SPECT/CT imaging of hands and wrists in HCs and joints with RA-involved inflammation.

2.3 Safety Objective(s)

- To evaluate safety through the examination of AE incidence and changes over time in laboratory tests, vital signs, and physical examination findings.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a prospective, open-label, multicenter, single-dose study designed to develop a normative database of TUV_{joint} in HCs and to assess the feasibility of qualitative and quantitative SPECT/CT assessments in HCs and subjects with active RA following Tc 99m tilmanocept administration. Subjects will be enrolled in 1 of 2 study arms (Table 4) with distinct study procedures in accordance with Arm-specific eligibility requirements.

Table 4 Study Arms

Arm	n	Subjects	Evaluation(s)
1	120 HCs	HC subjects clinically free of any inflammatory disease and/or joint pain	Planar image of bilateral hands/wrists
2	5 HCs 10 RA	HC subjects clinically free of any inflammatory disease and/or joint pain and subjects with clinically diagnosed active RA who have been on stable anti-rheumatic therapy	Planar image of bilateral hands/wrists SPECT/CT of bilateral hands/wrists

3.1.1 Arm 1: No Inflammatory and/or Joint Disease (HC Subjects)

3.1.1.1 Description of Patient Population

This arm will be comprised of 120 HCs who are deemed to be clinically free of inflammatory disease(s) and/or arthropathies and clinically free of joint pain for at least 28 days prior to the consent date. These subjects will not receive any anti-inflammatory treatment(s) (including NSAIDs) during study participation and will not have taken any such drugs at least 28 days prior to the consent date. All other concomitant medications (for non-inflammatory conditions) will have been maintained at a stable dose for at least 28 days prior to consent.

3.1.1.2 Overview of Study Procedures

Subjects in this arm will have a total of two on-site visits and one telephone safety assessment. The maximum possible study duration for these subjects will be 39 days.

3.1.1.3 Justification for Population

The reason for the inclusion of this study population is to establish mean and variance for TUV_{joint} values in HCs to develop a normative database to which the inflamed joints of subjects with active RA can be compared. By determining the upper limit of normal (ULN) for average anterior or posterior TUV_{joint} for right and left joints of the bilateral hands and wrists, these

assessments can be further investigated for their utility in providing clinically meaningful reference data for symptomatic individuals presenting to the clinic.

3.1.2 Arm 2: HCs and RA Subjects on Stable Anti-Rheumatic Therapy

3.1.2.1 Description of Patient Population

This arm will include five (5) HCs who are deemed to be clinically free of inflammatory disease(s) and/or arthropathies and clinically free of joint pain for at least 28 days prior to the consent date. These subjects will not receive any anti-inflammatory treatment(s) (including NSAIDs) during study participation and will not have taken any such drugs at least 28 days prior to the consent date. All other concomitant medications (for non-inflammatory conditions) will have been maintained at a stable dose for at least 28 days prior to consent.

This arm will also include ten (10) subjects with clinically diagnosed active RA on stable anti-inflammatory and/or anti-rheumatic therapy. All subjects' RA diagnoses will be moderate to severe in accordance with a 2010 ACR/EULAR score of 6 or higher. All 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; all subjects receiving bDMARDs must have been on therapy for at least 60 days prior to the first imaging visit; and all subjects receiving NSAIDs and/or oral corticosteroids must have been on therapy for at least 28 days prior to the first imaging visit (Table 7).

Table 5 Inclusion Criteria Treatment Windows for Recent or Concomitant RA-specific Treatments

Drug Class	Window
Traditional DMARDs	On therapy ≥ 90 days and at a stable dose ≥ 30 days prior to the imaging visit (Day 0)
bDMARDs	> 60 days prior to the imaging visit (Day 0)
JAK inhibitors	> 60 days prior to the imaging visit (Day 0)
NSAIDs/Corticosteroids	≥ 28 days prior to the imaging visit (Day 0)

3.1.2.2 Overview of Study Procedures

Subjects in this arm will have a total of two on-site visits and one telephone safety assessment. The maximum possible study duration for these subjects will be 39 days.

3.1.2.3 Justification for Population

The reason for the inclusion of this study population is to assess the feasibility of qualitative and quantitative SPECT/CT assessments following Tc 99m tilmanocept administration. HCs will provide control data to which data from RA subjects on stable therapy can be compared. Planar imaging from these subjects will be utilized for comparison to SPECT/CT results and will be pooled with data from this and previous Navidea RA trials for TUV evaluations.

3.2 Protocol Adherence

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

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

3.3 Study Duration(s)

The maximum possible study duration for Arm 1 or 2 subjects is 39 days.

4 STUDY POPULATION

4.1 Study Arms

The study population will be comprised of HCs and subjects with clinically diagnosed active RA on stable anti-rheumatic therapy. Subjects will be enrolled in 1 of 2 study arms (Table 6):

Table 6 Study Arms

Arm	n	Subjects	Evaluation(s)
1	120 HCs	HC subjects clinically free of any inflammatory disease and/or joint pain	Planar image of bilateral hands/wrists
2	5 HCs 10 RA	HC subjects clinically free of any inflammatory disease and/or joint pain and subjects with clinically diagnosed active RA who have been on stable anti-rheumatic therapy	Planar image of bilateral hands/wrists SPECT/CT of bilateral hands/wrists

4.2 Eligibility

All inclusion/exclusion criteria must be verified before any subject is considered eligible for enrollment and for administration of Tc 99m tilmanocept and imaging (Day 0 procedures). Subjects will be considered enrolled once injected with Tc 99m tilmanocept (Day 0). Written and dated (with time-noted) informed consent will be obtained from all subjects. A subject who withdraws consent prior to receiving Tc 99m tilmanocept injection on Day 0 will be considered a screen failure.

4.2.1 Inclusion Criteria

ALL SUBJECTS

1. The subject has provided written informed consent with HIPAA authorization before the initiation of any study-related procedures.
2. The subject has agreed to not engage in any diet, lifestyle, or medication changes until study completion.

HEALTHY CONTROL SUBJECTS

3. The subject is 30 years of age or greater at the time of consent.
4. The subject is deemed to be clinically free of any inflammatory disease(s), auto-immune disease(s), or arthropathies and has not experienced joint pain for at least 28 days prior to the consent date.

5. The subject is not currently on anti-inflammatory drugs (including NSAIDs) and has not taken any anti-inflammatories for at least 28 days prior to the consent date.
6. For all ongoing concomitant medications, the subject has maintained a stable dose for at least 28 days prior to the consent date.

CLINICALLY DIAGNOSED ACTIVE RA SUBJECTS

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 moderate to severe RA as determined by the 2010 ACR/EULAR Classification Criteria (score of ≥ 6/10).
5. The subject has a DAS28 of ≥ 3.2 (includes the ESR test and VAS).
6. If the subject is receiving traditional DMARDs, they have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the imaging visit (Day 0).
7. If the subject is receiving bDMARD or JAK inhibitor therapy, they have been at a stable dose > 60 days prior to the imaging visit (Day 0).
8. If the subject is receiving NSAIDs or oral corticosteroids, the dose has been stable for ≥ 28 days prior to the imaging visit (Day 0). The corticosteroid dose must be ≤ 10mg/day of prednisone or an equivalent steroid dose.

4.2.2 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 therapy or chemotherapy in the past six months.
4. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty.
5. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.
6. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 2 times the upper limit of normal.
7. 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 or compromise the safety of the subject or the quality of the data.
8. The subject has any unstable medical illnesses, including hepatic, renal, gastroenterologic, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease.

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 Tc 99m tilmanocept administration (Day 0).
11. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to Tc 99m tilmanocept administration (Day 0).
12. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to Tc 99m tilmanocept administration (Day 0).
13. **Healthy Controls only:** The subject has a positive rheumatoid factor and an elevated ESR or CRP.

4.3 Recruitment

Healthy control subjects will be recruited via clinical research sites and clinically assessed for the "absence" of painful and/or swollen joints. RA 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.

4.4 Withdrawal

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

A subject who withdraws consent prior to receiving Tc 99m tilmanocept injection on Day 0 will be considered a screen failure.

Should a subject withdraw after administration of the investigational product, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. An explanation should be given of why the subject is withdrawing or being withdrawn from the study.

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

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

4.5 Replacement

Subjects will be replaced if they have not completed all study procedures necessary for the primary endpoint(s) relevant to their study arm or their data are determined to be not evaluable. A subject is considered evaluable if he/she meets the criteria for the analysis population and has the data necessary for computing the primary endpoint.

4.6 Screen Failures

Subjects who sign an informed consent form (ICF) but are ultimately not injected will be considered a screen failure. Subjects will be assigned a study identification number at the time the ICF is signed. 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 “35”

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 “35-01-001.”

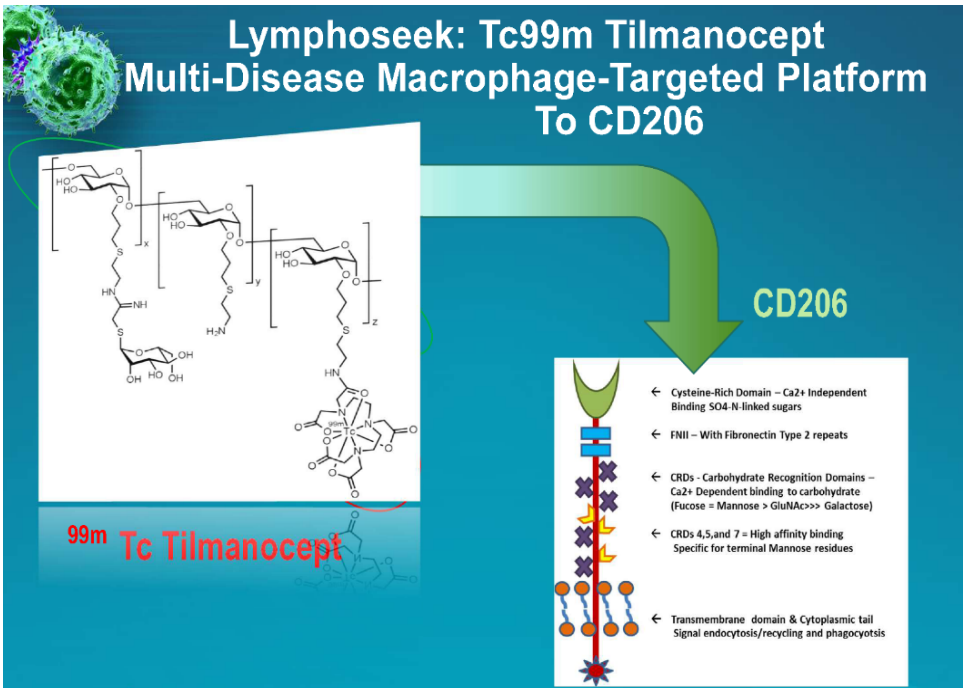
Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Description of Investigational Project

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 is comprised of multiple units of DTPA (diethylenetriaminepentaacetic acid) 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 1 syringe. The dose will be 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 between the left or right antecubital vein.

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

5.3 Timing and Frequency of Drug Administration

Subjects in either arm will receive the study-defined dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m on Day 0 Visit 2.

5.4 Packaging and Labeling

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 (kit) 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.

5.5 Drug Logistics and Investigational Product Accountability

The investigator (or designated personnel) will confirm receipt of the investigational product in writing and will use the investigational product only within the framework of this clinical study and in accordance with this study protocol. For each subject, he/she will keep a record of the investigational product dispensed and store all other forms that accompanied the delivery of the radiolabeled product to the clinical site. These documents are to be filed in the investigator site file. Overall drug accountability and reconciliation will be completed by the sponsor or its representative. A list of investigational product vials and other materials that were returned, or destroyed, must be recorded and signed by the PI or an appropriately qualified designee as documented in the study site responsibility sheet. An overall accountability and reconciliation form of the investigational product will be prepared and completed. If there are any discrepancies, they must be investigated and their resolution documented. All unused study kits will be destroyed in accordance with institutional destruction procedures.

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapies

All medications taken 30 days prior to Tc 99m tilmanocept injection through the post-injection safety follow-up must be documented and maintained at a stable dose according to the inclusion criteria. Subjects 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.

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

A schedule of evaluations is provided in the schedule of events (Appendix 1).

7.1 Schedule of Evaluations

7.1.1 Arm 1

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

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – date of birth, gender, race, ethnicity
- Medical surgical history – all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
- Concomitant medications (within 30 days before injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- Physical examination will include an assessment of height, weight, and examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (see Table 7)
- 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.
- 2010 ACR/EULAR: to confirm that the subject is clinically free of inflammatory disease and joint pain Swollen and tender joints will be identified and documented during physical examination as established by the 2010 ACR/EULAR.

Visit 2 (Tc 99m Tilmanocept Administration and Imaging; Day 0)
All subjects will be assessed for adverse events in an ongoing manner from the day of injection through the end of participation.

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 adverse events
- Concomitant medication review
- 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 (30-60 seconds). 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 adverse events.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate)

60 - 75 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition: Planar scan of the bilateral hands and wrists acquired per NAV3-35 Image Acquisition Guidelines

Visit 3 (Day 5 ± 3; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- Assessment of adverse events

7.1.2 Arm 2

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

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – date of birth, gender, race, ethnicity
- Medical surgical history – all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
- Concomitant medications (within 30 days before injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- 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 injection.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (Section 8.8)
- 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 will be identified and documented during physical examination as established by the 2010 ACR/EULAR for both HCs and RA subjects. RA subjects will also undergo a DAS28 evaluation and a review of RA history including previous treatments, date of symptom onset, and date of diagnosis.

Visit 2 (Day 0; Tc 99m Tilmanocept Administration and Imaging)
All subjects will be assessed for adverse events in an ongoing manner from the day of injection through the end of participation.

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 adverse events
- Concomitant medication review
- 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 (30-60 seconds). 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 adverse events.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate)

60 - 75 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition (in the following order):
  1. Planar scan of the bilateral hands and wrists acquired per NAV3-35 Image Acquisition Guidelines followed by preparation (up to 10 minutes) for the subsequent scan;
  2. SPECT/CT of the bilateral hands and wrists acquired per NAV3-35 Image Acquisition Guidelines

Visit 3 (Day 5 ± 3; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- Assessment of adverse events

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographics and Other Baseline Characteristics

One-hundred and thirty-five (135) evaluable subjects will be enrolled across the 2 study arms. Arm 1 will be comprised of 120 male or female HC subjects who are clinically free of any inflammatory and/or joint disease. Arm 2 will be comprised of 15 subjects. Ten (10) subjects in Arm 2 will be male or female individuals with clinically diagnosed active RA who are on stable anti-inflammatory and/or anti-rheumatic therapy. Five (5) subjects in Arm 2 will be male or female HCs who are clinically free of any inflammatory and/or joint disease. Enrolled HCs in either Arm will be categorized by age and sex to ensure intended distributions are met for the normative database, as described in Section 9.4.

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 follow-up safety visit will be documented. In addition to the summarization of prior RA-specific treatments, recent or concomitant treatments taken for RA in the last 6 months must be collected.

8.2 Tc 99m Tilmanocept Administration

Tc 99m tilmanocept must be ordered from the study-assigned radiopharmacy once the subject has been scheduled for IV administration and imaging. 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. Immediately after the completion of the injection, a 10-mL sterile normal saline flush will be administered. Injection of Tc 99m tilmanocept 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 as part of eligibility and inclusion (Aletaha et. al 2010). The 2010 ACR/EULAR classification criteria includes 4 components: number and site of involved joints, serologic abnormality, elevated acute-phase response and symptom duration. See Appendix 5 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".

8.3.2 DAS28

RA subjects in Arm 2 will receive the DAS28 evaluation (Prevoo et. al 1995) at screening as part of eligibility and inclusion. DAS28 is calculated from 4 components: tender joint count (TJC), swollen joint count (SJC), visual analogue scale (VAS) of the subject's global health, and the laboratory parameter erythrocyte sedimentation rate (ESR), such that:

$$DAS28 = 0.56\sqrt{TJC} + 0.28\sqrt{SJC} + 0.7\ln(ESR) + 0.014(VAS)$$

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 3 for details.

8.3.3 Other

8.3.3.1 28-joint Count (SJC and TJC)

The 28-joint count will be performed for swollen and/or tender joints in the following: shoulder, elbow, wrist, MCP and 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 [57]. This assessment will be used as an input parameter for 2010 ACR/EULAR score and DAS28 score.

8.3.3.2 Patient Assessment of Pain (VAS)

Patient assessment of pain will be evaluated using the visual analog scale (VAS). Using a ruler, the score will be determined by measuring the distance (mm) on the 10-cm line between the 'no pain' anchor and the patient's mark, providing a range of scores from 0 to 100. This assessment will be used as an input parameter for DAS28 score.

8.3.3.3 Acute-phase Reactant

ESR and CRP will be obtained in the RA-specific laboratory panel (see Table 7). This assessment will be used as an input parameter for 2010 ACR/EULAR score and DAS28 score.

8.4 Imaging

8.4.1 Image Acquisition

8.4.1.1 Planar Scintigraphy

Bilateral hand and wrist planar image acquisition occurs in Arm 1 and 2 subjects beginning 60-75 minutes following Tc 99m tilmanocept injection. Refer to the NAV3-35 Image Acquisition Guidelines for all required imaging technical specifications and acquisitions.

8.4.1.2 SPECT/CT

SPECT/CT is performed for Arm 2 subjects on the bilateral hands and wrists only following the planar scan. Refer to the NAV3-35 Image Acquisition Guidelines for all required imaging technical specifications and acquisitions. SUV will be calculated for DAS28 joints of interest according to standard methods. See the NAV3-35 Image Review Charter and Statistical Analysis Plan for more details.

8.4.2 Tilmanocept Uptake Value (TUV)

TUV is a quantitative imaging metric used to characterize the amount of CD206 activity on planar imaging. Results from prior Phase 1 and 2 studies have demonstrated that TUV is a sensitive and specific predictor of visually interrogated Tc 99m tilmanocept localization in joint regions with presumed inflammatory macrophage activity. A per-joint TUV (TUV_{joint}) relative ratio will be calculated for the each of the 22 DAS-28 joints located in the hands and wrists. A subject-level global TUV (TUV_{global}) assessed across the 22 joints will be used as an indication of overall disease burden.

For all subjects, delegated trained imaging scientists blinded to all clinical subject information will perform semi-automated ROI drawing on planar images of the bilateral hands and wrists to derive relevant count statistics, which are input parameters for TUV_{joint} and TUV_{global}. TUV metrics are further described in the NAV3-35 Statistical Analysis Plan.

8.5 Adverse Events

8.5.1 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 related to the medicinal (investigational) product.

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

By definition for this study, all untoward medical occurrences beginning on the Visit 2 (Day 0) until the final visit (variable per arm) 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.

8.5.2 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 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.

8.5.3 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 related to study procedure, in which case the event will be recorded as an AE. Untoward medical events beginning on Visit 2 (Day 0) until the final visit (variable per arm) will be reported as adverse events.

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 8.5.5.

- The maximum intensity (mild, moderate, or severe).
- Whether drug treatment was administered for the event, any specific drug treatment must be documented.
- The relationship of the AE to the investigational product and to study conduct (for definitions, see above).
- The **outcome** of the AE (resolved, resolved with sequelae, not resolved, unknown, death).

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

8.5.4 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. Routes of administration included: subcutaneous, intradermal, and peritumoral.

In addition to the Lymphoseek pre-approval clinical studies, post-marketing surveillance shows that Lymphoseek has been administered to more than 450,000 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 approximately 280 IV administrations of Tc 99m tilmanocept to date, and no SAEs or AEs related to the product have been reported to date.

Risks of Imaging Procedures

Radiation dose considerations from Tc 99m Tilmanocept planar and SPEC/CT scans – Patients enrolled in this trial will have hand/wrist planar gamma camera scans following Tc 99m Tilmanocept injection, with subjects enrolled in Arm 2 also receiving one hand/wrist SPECT/CT scan.

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 Arm 1 will receive one injection, equal to 2.7 mSv total; subjects enrolled in Arm 2 will receive one injection plus one SPECT/CT scan- with an estimated effective dose of 1 mSv for the CT portion- amounting to 3.7 mSv total. Therefore, the maximum possible dose of ~3.7 mSv for any enrolled subject (no one will cross over into more

than one study arm) 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 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.

8.5.5 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)

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

Notification of the authorities

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

Sponsor's notification of the investigators

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

8.6 Physical Examination

Complete physical examinations will be conducted at screening including height and weight assessments. A physical exam that has been conducted as standard of care prior to signing informed consent may be used if it was performed within 30 days of injection.

Physical examinations will be performed for the following body systems:

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

8.7 Vital Signs

Vital signs comprise the measurement of body temperature, heart rate, respiration, systolic and diastolic blood pressure. All measurements will be taken after the subject has been in a resting position for at least 1 minute. Vital signs will be measured at screening, within 30 minutes before investigational product injection, and within 30 minutes post injection. Any clinically significant change from screening (worsening) that results in a change in subject management will be considered an AE and will be recorded on the AE page of the CRF.

8.8 Clinical Laboratory Parameters

Table 7 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 that are evaluated as being clinically significant 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, which are not the result of laboratory error, are to be recorded as AEs.

Any clinically significant changes in laboratory values are to be followed up with repeated tests at appropriate intervals (as determined by the investigator) until the values return to baseline level or until the abnormality is explained by the investigator. The expected amount of blood to be drawn is shown in Table 8.

Table 8 Approximate Amount of Blood Drawn

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 STATISTICAL METHODS

This is a prospective, open-label, multicenter study of the patterns of variation in planar imaging using IV injected Tc 99m tilmanocept for the quantitative detection of disease activity in the skeletal joints of HC subjects and subjects with active RA. In addition to quantitative assessments, this study will also include qualitative (i.e., visual) evaluations of SPECT/CT in detecting localization within synovial spaces of the bilateral hands and wrists.

The study is stratified into 2 groups. Arm 1 consists of HC subjects. Arm 2 consists of HC subjects and RA subjects on stable therapy.

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, and RA panel)
- Vital signs

9.3 Efficacy Variables

The efficacy variables for this study are defined below:

- Quantitative determination of TUV_{joint} and TUV_{global}
- Qualitative (i.e., visual) determination of tilmanocept localization in planar and SPECT/CT images of the bilateral hands and wrists;
- Quantitative determination of tilmanocept localization in planar and SPECT/CT images of the bilateral hands and wrists.

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.

The efficacy endpoints for this study, by arm, are as follows:

Primary Endpoint(s)

ARM 1

- The normal limits of TUV_{joint} (on a per joint basis) in HC subjects, which are defined as the 5 and 95 percentiles of TUV_{joint} of bilateral joints (i.e., bilateral wrists, metacarpophalangeal joint [MCPs], proximal interphalangeal [PIPs]). The normal range will be determined separately for each of the three blinded readers.

ARM 2

- The qualitative evaluations of SPECT/CT in detecting localization within synovial spaces of the bilateral hands and wrists in HCs and RA subjects.

Secondary Endpoints

ARM 1

- Applicability of the Normal (Gaussian) distribution to TUV_{joint} data.

ARM 2

- Quantitative evaluations of Tc 99m tilmanocept localization from SPECT/CT within synovial spaces of the bilateral hands and wrists in HCs and RA subjects.
- Normative joint-specific SUV.
- Predictive value of planar scans for SPECT/CT scans.

9.4 Sample Size Justification

The study will enroll up to 135 evaluable subjects in Arms 1 and 2 allocated below, imaged at up to 8 study centers. Arm 1 will have N = 120 evaluable HC subjects, Arm 2 will have N = 15 (5 HCs and 10 RA) evaluable subjects. A subject is considered evaluable if he/she meets the criteria for the analysis population and has the data necessary for computing the primary endpoint.

The sample size for Arm 1 was determined to provide acceptable precision for the estimated 5 and 95 percentiles. These percentiles will be estimated with 95% nonparametric confidence intervals. Although the standard error formula for quantile estimators is known and quantile estimators obey a central limit theorem, relying on the asymptotic Normal distribution seems imprudent.

The sample will be stratified 3:1, females:males to reflect RA incidence in the United States [58, 59]. The sex-specific samples will be further stratified by age to reflect the Census estimated US population in 2019 (U.S. Census Bureau, 2020 [60]). The target sample sizes are given below.

Age Range	Male	Female	Total
30 to 39 years	8	23	31
40 to 49 years	7	21	28
50 to 59 years	8	22	30
60 to 69 years	7	21	28
70 years and older[a]	6	21	27
Total	36	108	144

[a] An effort will be made to target enrollment in this group to include 2 men and 8 women 80 years old and over.

The existing data for HC subjects was used in a bootstrap procedure to evaluate the width of 95% confidence intervals for the 95 percentiles for various sample sizes. The sponsor has decided for its business purposes that knowing the percentile to within 0.05 of its true value is sufficient. A sample of size N = 144 HC results (this includes the subjects from Arm 1 of NAV3-31) in 95% confidence intervals with half-widths less than 0.05.

The sample size for Arm 2 was determined to be sufficient for the business purposes of the sponsor. The sample size for Arm 2 will be 5 HCs and 10 RA subjects.

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following populations are defined for this study:

Intent-to-Diagnose (ITD) Population – the ITD population includes all subjects who have been enrolled in the study, injected with Tc 99m tilmanocept, and received all imaging and evaluations necessary for the primary endpoint(s) appropriate to their respective arm.

Per-Protocol (PP) Population – the PP population consists of all ITD subjects without major protocol violations.

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

9.5.2 Analysis of Baseline and Demographic Characteristics

Baseline and demographic characteristics of the safety population will be summarized by subject status and overall. Continuous variables (age, height, and 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 ITD and PP populations. The ITD population will be the primary analysis set.

9.5.3.1 Primary Endpoint Arm 1

The distribution of TUV_{joint} in HC subjects will be summarized with descriptive statistics: mean, standard deviation, n, minimum, maximum, and median per reader, joint, and view. The 5 and 95 percentiles will be estimated with quantile regression fitting an intercept as a fixed term. The lower and upper limits of normal TUV_{joint} will be determined using non-parametric confidence intervals and kernel density-based confidence intervals.

9.5.3.2 Primary Endpoint Arm 2

Localization of tilmanocept in the hands and wrists will be summarized with frequency counts and percentages by reader and joint.

9.5.3.3 Secondary Endpoint Arm 1

Normal quantile plots will be provided per joint and reader, along with the *p*-value for the Shapiro-Wilk test of Normality.

9.5.3.4 Secondary Endpoints Arm 2

SUV will be calculated and summarized on both a per-joint and a total of all joints with summary statistics: mean, standard deviation, n, minimum, maximum, and median.

The predictive value of planar scans for SPECT/CT qualitative findings will be summarized per joint and reader with a crosstabulation and the uncertainty coefficients for row | column, column | row, and symmetric. The planar result will be deemed "positive" (i.e., inflamed) if TUV_{joint} > 95 percentile of the reader results for the same joint in Arm 1. Otherwise, the planar result will be deemed "negative".

Additional efficacy analyses may be described in the NAV3-35 Statistical Analysis Plan (SAP).

9.5.4 Analysis of Safety Variables

All safety analyses will be conducted on the safety population.

All 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 initial procedure date. If the procedure 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 summarized by cohort and overall 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 vital sign parameters and hematology, clinical chemistry and urinalysis parameters will be summarized using descriptive statistics (mean, standard deviation, median, and range) for each Arm and overall at each time point.

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

9.5.5 Handling of Missing Values

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

9.5.6 Interim Analyses

No interim analyses will be performed for this study.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

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

10.1.1 CRF Design

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

10.2 Monitoring

This study will be monitored regularly by a clinical research associate (CRA) from the sponsor or a contract research organization (CRO). Monitoring procedures include 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 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) 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.

Imaging data will be archived by the central imaging core lab according to the NAV3-35 WorldCare Project Plan (WorldCare Clinical, Boston, MA).

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) 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 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 4.4.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

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

11.2 Subject Information and Consent

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

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

The investigator will also mention that written approval of the IRB has been obtained. Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Following this informative discussion, the subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the subject voluntarily agrees to sign the ICF and has done so, may he/she enter the study. Additionally, the investigator or his/her designee will personally sign and date the form. The subject will receive a duplicate of the signed and dated form.

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

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

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

11.3 Financing/Financial Disclosure

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

11.4 Publication Policy

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

11.5 Subject Injury

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

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Appendix 1 Schedule of Events

Evaluation	**Visit 1** Screening (Day -30 to -1)	**Visit 2** (Day 0)					**Visit 3** Telephone (Day 5 ± 3)
		- 00:30 to - 00:01	00:00 Injection	00:01 to 00:30	60 - 75 min	After Imaging	
Informed consent	1, 2						
Entry criteria	1, 2						
Medical History, RA history, demography	1, 2						
Physical examination	1, 2						
Clinical laboratory evaluation: chemistry, hematology, urinalysis	1, 2					1, 2	
RA panel	1, 2						
Urine pregnancy test (women of CBP only)	1, 2	1, 2					
2010 ACR/EULAR	1, 2						
DAS28 (RA subjects only)	2						
Vital signs	1,2	1, 2		1, 2			
Tc 99m tilmanocept administration			1, 2				
Planar scan of bilateral hands/wrists					1, 2		
SPECT/CT of bilateral hands/wrists					2		
Concomitant medications	1, 2	1, 2					1, 2
AE monitoring	1, 2	1, 2	1, 2	1, 2	1, 2		1, 2

[1] Arm 1
[2] Arm 2
[1,2] Arms 1 and 2

Appendix 2 2010 ACR/EULAR 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.

Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569-2581.

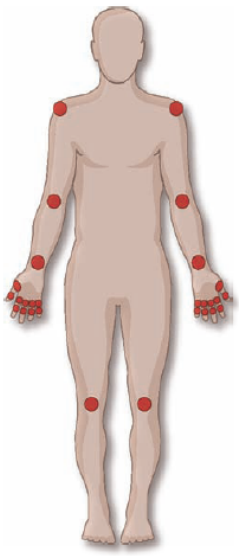
Appendix 3 DAS28 Scoring

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 4 Sponsor Signatures

Study Title:	Development of a Normative Database for Rheumatoid Arthritis (RA) Imaging with Tc99m Tilmanocept.
Study Number:	NAV3-35
Original Date:	17 March 2021

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: 23 Mar 2021
William Regan
Chief Compliance Officer
Navidea Biopharmaceuticals, Inc.

Signed: _____ Date: 23 Mar 2021
Michael Blue, MD, FACEP
Senior Medical Director
Navidea Biopharmaceuticals, Inc.

Signed: Bonnie Abbruzzese Date: 3/23/2021
Bonnie Abbruzzese, MS, CCRA
Senior Director, Clinical Research & Development
Navidea Biopharmaceuticals, Inc.

Signed: _____ Date: 3/23/21
Michael Rosol, PhD
Chief Medical Officer
Navidea Biopharmaceuticals, Inc.

Appendix 5 Investigator's Signature

Study Title:	Development of a Normative Database for Rheumatoid Arthritis (RA) Imaging with Tc99m Tilmanocept
Study Number:	NAV3-35
Original Date:	17 March 2021

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:_____