Clinical Study Protocol: NAV3-33

Study Title:	Evaluation of Tc 99m Tilmanocept Imaging for the Early Prediction of Anti-TNFα Therapy Response in Patients with Moderate to Severe Active Rheumatoid Arthritis (RA)
Study Number:	NAV3-33
Study Phase:	3
Product Name:	Technetium Tc 99m tilmanocept
IND Number:	132943
Investigator(s):	Multicenter
Sponsor:	Navidea Biopharmaceuticals, Inc 4100 Horizons Dr., Suite 205 Columbus, OH 43220
Sponsor Contacts:	Simon Blackburn Director, Clinical Development 614-323-7034 Joanna Shuping Director, Clinical Operations 937-926-5252
Medical Monitor:	Michael Blue, MD Chief Medical Officer 614-571-4313

	Date
Original Protocol:	11 November 2021
Amendment #1:	18 January 2022
Amendment #2:	01 November 2022
Amendment #3	29 September 2023
Amendment #4	30 January 2024

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

SYNOPSIS

Study title	Evaluation of Tc 99m Tilmanocept Imaging for the Early Prediction of Anti-TNFα Therapy Response in Subjects with Active Moderate to Severe Rheumatoid Arthritis (RA)
Study phase	Phase 3
Study objective(s)	**Primary** • To demonstrate that global tilmanocept uptake values (TUV_{global}) obtained before initiation of anti-TNFα therapy ($TUV_{global[b]}$) and at 5 weeks ± 1 week following change in therapy ($TUV_{global[5w]}$) has a specificity of greater than 80% to correctly specify a clinical non-response to therapy at 24 weeks. • To demonstrate that TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following change in therapy has a sensitivity of greater than 65% to correctly classify a positive clinical response to therapy at 24 weeks. **Key Secondary** • To evaluate the negative predictive value (NPV) of TUV_{global} obtained before initiation of anti-TNFα therapy ($TUV_{global[b]}$) relative to a clinical non-response to therapy at 24 weeks. **Secondary** • To evaluate the sensitivity and specificity of TUV_{global} obtained before initiation of anti-TNFa therapy and at 5 weeks ± 1 week following change in therapy relative to a positive clinical response or a non-response to therapy at 12 weeks. • To evaluate the NPV of $TUV_{global[b]}$ relative to a clinical non-response to therapy at 12 weeks. • To evaluate the positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA) of TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week ($\Delta TUVglobal_{[5w]}$) following change in therapy relative to a positive clinical response or a non-response to therapy at 12 and/or 24 weeks.

	• To evaluate the qualitative assessment of Tc 99m tilmanocept imaging to predict clinical response or non-response following a change in anti-TNFα therapy. • To evaluate the additive effect of either quantitative or qualitative assessment of Tc 99m tilmanocept imaging to the other at baseline and change from baseline to 5 weeks ± 1 week to predict clinical response or non-response following a change in anti-TNFα therapy. • To evaluate the correlation of changes in TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following a change in therapy with changes in composite clinical assessments and their constituent parameters at 12 and/or 24 weeks. • To evaluate the correlation of changes in TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following a change in therapy with changes in clinical assessments at 12 and/or 24 weeks for specific anti-TNFα bDMARD agents. • To evaluate the correlation of changes with TUV_{global} and changes in clinical RA assessments as a means to monitor therapy. • To evaluate sensitivity and specificity in patient subgroups. • To evaluate the safety of IV-administered tilmanocept radiolabeled with Tc 99m.
Duration of treatment	24 ± 1 weeks; Tc 99m tilmanocept will be administered at baseline and at Week 5. Tilmanocept will also be administered to a subset of subjects at weeks 12 and 24 (those enrolled and completing Weeks 12 and 24 prior to activation of Amendment 4).
Study drug	Technetium Tc 99m tilmanocept
Dose(s) and Route of administration	**Dose:** Tilmanocept will be administered at a mass dose of 150 mcg radiolabeled with 10 mCi Tc 99m. **Route of Administration:** Tc 99m tilmanocept will be administered through an intravenous (IV) route of administration using a single syringe injected as a slow IV push. The preferred site of IV placement will be the left or right antecubital vein. At the completion of Tc 99m tilmanocept administration, a 10 mL sterile normal saline flush will be administered.

Inclusion criteria	1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) authorization before the initiation of any study-related procedures. 2. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis. 3. The subject is a candidate for initiation of, or change to, a new anti-TNFα bDMARD therapy. 4. The subject has RA as determined by the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria (score of ≥ 6/10). 5. The subject has moderate to severe RA as determined by 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 first imaging visit (Day 0). 7. Subjects receiving bDMARD or janus kinase (JAK) inhibitor therapy must have been at a stable dose > 60 days prior to the first imaging visit (Day 0). 8. If the subject is receiving NSAIDS (nonsteroidal anti-inflammatory drug) or oral corticosteroids, the dose must have been stable for > 28 days prior to the first 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 or chemotherapy within the past 5 years. 4. The subject has an active malignancy or a history of malignancy within the past 5 years. 5. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty. 6. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min. 7. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate

	aminotransferase [SGOT]) greater than 2 times the upper limit of normal. 8. The subject has any severe, acute, or chronic medical conditions and/or psychiatric conditions and/or laboratory abnormalities that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration that would deem the subject inappropriate for study participation. 9. The subject has a history of hypersensitivity reactions to TNF-inhibitors. 10. The subject has a known allergy to or has had an adverse reaction to dextran exposure. 11. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration at the first imaging visit (Day 0). 12. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to the first imaging visit (Day 0). 13. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to the administration of Tc 99m tilmanocept at the first imaging visit (Day 0). 14. The subject has heart failure [New York Heart Association (NYHA) Class III-IV], a demyelinating disorder, or a chronic/latent infection [e.g., +Purified Protein Derivative (PPD) test, Human Immunodeficiency Virus (HIV), Hepatitis B].
Study design	This is a prospective, open-label, multicenter study designed to evaluate the early predictive capacity of Tc 99m tilmanocept planar imaging for downstream clinical response(s) in individuals with moderate to severe RA who are candidates for change in anti-TNFα therapy. Temporal (Baseline to 5-week) differences in quantitative imaging will be correlated with longitudinal (Baseline to 12- and 24-week) assessments of clinical RA outcomes to evaluate the clinical utility of Tc 99m tilmanocept for the expedited evaluation of antirheumatic treatment efficacy when compared with longitudinal assessments in clinical practice. Prior to the initiation of a new anti-TNFα therapy, subjects will undergo baseline rheumatological evaluations of ACR/EULAR 2010 Classification Criteria, Clinical Disease Activity Index (CDAI), DAS28, and Health Assessment Questionnaire Disability Index (HAQ-DI©) for the characterization of disease activity. Following these evaluations, subjects will receive an IV dose of 150 mcg

	tilmanocept radiolabeled with 10 mCi of Tc 99m and undergo baseline planar imaging of the bilateral hands and wrists beginning 60-75 minutes following Tc 99m tilmanocept administration. Upon completion of baseline imaging procedures, subjects will commence a new anti-TNFα bDMARD treatment regimen and return to the clinic 5 ± 1 weeks later for a series of rheumatological evaluations (including CDAI, DAS28, ACR Response Criteria, and HAQ-DI©), drug administration, and bilateral hand/wrist imaging. The rheumatological evaluations will be repeated at 12 ± 1 weeks and 24 ± 1 weeks after commencement of the new therapy. Images will undergo centralized quantification for the derivation of joint-specific and global tilmanocept uptake values (TUV). At the joint level, TUV_{joint} is defined as the intra-patient ratio of the average pixel intensity of a joint to the average pixel intensity of the reference region. TUV_{global} is a per patient summary of TUV_{joint} values greater than the upper limit of the 95% prediction interval of the joint-and-view-specific TUV in the normative database representing RA-specific inflammation. TUV calculations are additionally described in the Statistical Analysis Plan. All images will be read by three nuclear medicine specialists trained in the reading of these images. Each reader will work independently of the other readers and will be blinded to clinical assessments. Each reader will provide both a qualitative read and map regions of interest for calculation of TUVs. Temporal differences in TUV_{global} from baseline to 5 ± 1 weeks of therapy ($\Delta TUV_{global[5w]}$) will be compared with longitudinal clinical outcomes at 12 ± 1 and 24 ± 1 weeks of therapy defined by changes in CDAI ($\Delta CDAI_{12/24w}$), changes in DAS28 ($\Delta DAS28_{[12/24w]}$), and assessment of ACR Response Criteria ($ACR_{12/24w}$) to evaluate the efficacy of TUV_{global} for the accelerated prediction of clinical response to antirheumatic drugs.	
Planned study dates	**Start of study recruitment**/February 2022	**End of Recruitment**/October 2024 **End of Study**/May 2025
Planned number of study centers	Up to 50 centers in the United States	

Number of subjects	198 to 672 evaluable subjects may be enrolled. Details of sample size calculations are further described in Statistical Methods below.
Efficacy Assessments	Primary endpoints: • Specificity of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at week 24. • Sensitivity of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at week 24. Key secondary endpoint: • NPV of $TUV_{global[b]}$ with respect to ACR50 at week 24. Secondary endpoints: • Sensitivity and specificity of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at week 12. • NPV, PPV, and OA of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at weeks 12 and 24. • NPV of $TUV_{global[b]}$ with respect to ACR50 at week 12 • $TUV_{global[b]}$ and response to new anti-TNFα bDMARD therapy defined by the change from baseline (CFB) of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively), by the CFB of DAS28 to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta DAS28_{12w}$ and $\Delta DAS28_{24w}$, respectively) and by the CFB in each of the ACR Response Criteria components at 12 ± 1 weeks and at 24 ± 1 weeks. • $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy defined by the CFB of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively). • Concordance of $\Delta TUV_{global[5w]}$ bucketing with clinical criteria, including ACR Response Criteria, CDAI, DAS28, and HAQ-DI©. Concordance between the predicted response or non-response status and the clinical criteria will be evaluated using NPV, PPV, sensitivity, specificity, and overall accuracy. • Concordance of $\Delta TUV_{global[12w]}$ bucketing with clinical criteria, including ACR Response Criteria, CDAI, DAS28, and HAQ-DI©. Concordance between the improvement classifications and the clinical criteria will be evaluated using NPV, PPV, sensitivity, specificity, and overall accuracy.

	• Response to new anti-TNFα bDMARD therapy defined by the CFB of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively).
	• The correlation of $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy from baseline to 24 ± 1 weeks defined by the changes from baseline in each of the ACR Response Criteria components.
	• Constituent parameters of $CDAI_{12w}$, $CDAI_{24w}$, $\Delta CDAI_{12w}$, $\Delta CDAI_{24w}$, ACR Response Criteria at 12 and 24 weeks, including:
	  • Tender joint count (TJC)
	  • Swollen joint count (SJC)
	  • Patient assessment of global disease activity
	  • Rheumatologist assessment of global disease activity
	  • Patient assessment of pain
	  • Patient assessment of physical function
	  • Acute-phase reactant value

Safety Assessments	Incidence of AEs and assessment of changes over time in clinical laboratory tests (hematology, serum chemistry, urinalysis, and RA panel), ECG parameters, and vital signs.
Statistical Methods	<u>Sample size</u> NAV3-33 is sized with respect to achieving specified goals in the co-primary endpoints of sensitivity and specificity of $\Delta TUV_{global[5w]}$ bucketing. Sensitivity and specificity require knowledge of the patient's clinical status at Week 24, and so hypothesis testing will be performed after classification. The value of $\Delta TUV_{global[5w]}$ necessary to predict response vs. non-response and the value of $TUV_{global[b]}$ at baseline necessary to predict non-response will be determined in advance. The classification procedure occurs in two stages, following the baseline image acquisition and (possibly) following the 5-week image acquisition and calculation of $\Delta TUV_{global[5w]}$. After the baseline imaging, patients whose $TUV_{global[b]}$ is < 5 are predicted to be non-responders. After $\Delta TUV_{global[5w]}$ is available, patients whose $TUV_{global[b]}$ is ≥ 5 and D% is $\leq -10\%$ are predicted to be responders. All other patients are predicted to be non-responders. The Week-24 ACR50 will be used as the primary determination of clinical response for the reference standard.

	The hypotheses for the co-primary endpoints to be tested are: $H_{O1}: \pi_{\mathrm{Sp}} \leq 0.8$ $H_{A1}: \pi_{\mathrm{Sp}} > 0.8;$ And $H_{O2}: \pi_{\mathrm{Se}} \leq 0.65$ $H_{A2}: \pi_{\mathrm{Se}} > 0.65.$ In the above π_{Sp} represents the true specificity of the classification procedure and π_{Se} represents the true sensitivity of the classification procedure when used to predict the outcome of the anti-TNFα bDMARD inhibitor therapy at 24 weeks. An overall two-tailed Type I error rate of 0.05 (one-tailed 0.025) was used without adjusting for multiple endpoints, as both co-primary endpoints must be achieved (i.e., at least the same two of three readers reject both null hypotheses) in order to have a successful study. If the actual specificity is 0.92 and the actual sensitivity is 0.8, and if an exact binomial test is used to test the above hypotheses, a total of 98 clinical non-responders and 100 clinical responders are needed for 90% power of the individual tests. That is, a minimum of 198 total patients is needed. This applies to a single reader. To evaluate the power characteristics of this process with 3 correlated readers where at least the same two of the three readers must reject both null hypotheses above, a simulation study was performed. Correlated binomial random variables were generated using a copula. The hypotheses were tested for each simulated reader using an exact binomial test, and the number of iterations where at least the same two of the three readers rejected both hypotheses was counted. Under the conditions above this procedure has power 0.93 when $\rho = 0.7$ and 0.89 when $\rho = 0.9$. In Arm 3 of the NAV3-31 study a ratio of 5 non-responders to 1 responder was observed. If this ratio is maintained in NAV3-33 for the $\Delta TUV_{global[5w]}$ bucketing predictions, a total of 600 patients is needed to achieve the desired power. That is, a total of 500 clinical non-responder patients is expected to be enrolled before reaching the required 100 clinical responders. Taking into account a 10% dropout rate, the anticipated total sample size for the study is approximately 672 patients (560 clinical non-responders and 112 clinical responders). That is:

- Clinical Non-Responders: 90% completion rate relative to 560 enrolled patients will result in 500 completed patients
- Clinical Responders: 90% completion rate relative to 112 enrolled patients will result in 100 completed patients

Because there is uncertainty about how responding and non-responding patients will enroll, enrollment will continue until at least 560 clinical non-responders and 112 clinical responders are participating in the study. The cumulative enrollment of clinical non-responders and responders will be tracked throughout the study in order to stop enrollment once the required number has been reached for each clinical category. This tracking of clinical response and non-response status will be performed by individuals without knowledge of the TUV data.

If the two co-primary endpoints are achieved (i.e., both null hypotheses are rejected for at least the same 2 out of 3 readers), then the following hypotheses will be tested at one-tailed 0.025 for the key secondary endpoint of NPV of $TUV_{global[b]}$ with respect to ACR50 at week 24:

$$H_{O3}: \pi_{\mathrm{NPV}} \leq 0.7$$
$$H_{A3}: \pi_{\mathrm{NPV}} > 0.7;$$

If the actual NPV is 0.85, and if an exact binomial test is used to test the above hypotheses, a total of 89 predicted non-responders using $TUV_{global[b]}$ are needed for 90% power and a total of 71 non-responders using $TUV_{global[b]}$ are needed for 80% power.

Analysis of Primary Efficacy Variables

The co-primary efficacy variables will be summarized by reader in a 2 by 2 contingency table displaying the cell frequencies and marginal totals at 24 weeks. The specificity of $\Delta TUV_{global[5w]}$ bucketing and the sensitivity of $\Delta TUV_{global[5w]}$ bucketing will be calculated for each reader. The 95% exact (Clopper-Pearson) confidence intervals will be provided for each of these parameters. Observed significance levels (p-values) for the exact binomial tests of specificity of $\Delta TUV_{global[5w]}$ bucketing greater than 0.8 and sensitivity of $\Delta TUV_{global[5w]}$ bucketing greater than 0.65 will be provided.

Sensitivity and specificity are defined according to the following cross-tabulation:

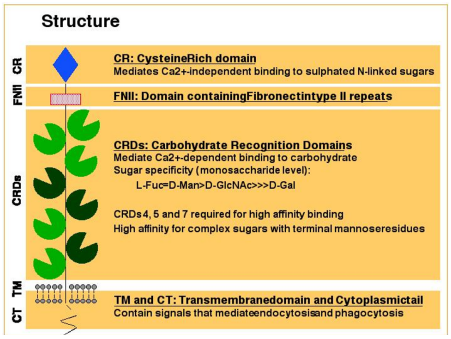
	Clinical Response Predicted Response / Responder / Non-Responder / Total Responder: A / B / T_1 Non-Responder: C / D / T_2 Total: T_3 / T_4 / N Sensitivity = A/T_3. Specificity = D/T_4. The above 2 by 2 contingency table will also be used to calculate the secondary efficacy endpoints of NPV, PPV, and overall accuracy (OA) as follows: NPV = D/T_2. PPV = A/T_1. Overall Accuracy = $(A + D)/N$. <u>Analysis of Key Secondary Efficacy Variables</u> NPV of $TUV_{global[b]}$ will be calculated for each reader. The 95% exact (Clopper-Pearson) confidence intervals will be provided. Observed significance levels (p-values) for the exact binomial tests of NPV of $TUV_{global[b]}$ greater than 0.7 will be provided. <u>Analysis of Secondary Efficacy Variables</u> All RA quantitative assessment variables (CDAI, ACR Response Criteria component scores, DAS28 score, and HAQ-DI©) will be summarized by computing the mean, standard deviation, number of observations, minimum, median, and maximum for the observed values at each time point and for the change from baseline for values collected after Day 0. The ACR Response Criteria will be summarized with a frequency table of the highest ACR Response level (None, ACR20, ACR50, ACR70) attained by that patient at that time point. That is, a patient who satisfies ACR50 also satisfies ACR20 but will not appear in the frequency count for ACR20. Concordance of improvement classification for $\Delta TUV_{global[5w]}$ bucketing with clinical criteria (ACR Response, CDAI, DAS28, and HAQ-DI©) will be analyzed as follows. Note that the concordance of $\Delta TUV_{global[5w]}$ bucketing with ACR50 is

	evaluated as co-primaries (sensitivity and specificity). For each of the RA improvement criteria, a cross-classification table will be provided. The PPV, NPV, sensitivity, specificity, NPV, PPV, and OA will be calculated, and 95% exact (Clopper-Pearson) confidence intervals will be provided. The Kendall rank correlation between $\Delta TUV_{global[5w]}$ with $\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$ will be computed and a 95% confidence interval for its value will be computed using Fisher's Z-transformation. Similarly, the Kendall rank correlation between $\Delta TUV_{global[5w]}$ with changes from baseline in DAS28 and in each of the ACR Response Criteria components at weeks 12 and 24 will be computed and a 95% confidence interval for its value will be computed using Fisher's Z-transformation. The marginal distributions of the variables will be characterized with the mean, standard deviation, and the number of data pairs. Additional efficacy analyses may be described in the study Statistical Analysis Plan (SAP). <u>Analysis of Safety</u> All safety analyses will be performed on the Safety Population. The safety analysis variables are defined as follows: • Adverse Events (AEs) • Clinical Laboratory Tests (hematology, serum chemistry, urinalysis, RA panel) • ECG Parameters • Vital Signs **Adverse Events** Adverse events will be observed for each patient from signing of informed consent until termination from the study. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®). A treatment-emergent AE (TEAE) is defined as an AE whose start date is on or after the initial procedure date. Based on the coded terms, frequencies of each TEAE will be summarized by MedDRA® preferred term within system organ class (SOC), by severity grade, and by relationship to Tc 99m tilmanocept. A summary of TEAEs will be constructed showing the following:

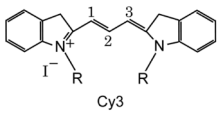
	• Number of patients with at least one TEAE • TEAEs by severity grade • TEAE by relationship to Tc 99m tilmanocept • TEAEs by relationship of TEAE to study procedure • Number of patients with at least one treatment emergent serious adverse event (TESAE) TESAEs will be tabulated by MedDRA® preferred term within SOC. A by-patient AE data listing of all AEs including verbatim term, coded term, grade, and relation to study drug will be provided. Vital signs, clinical laboratory results, and ECG parameters will be summarized with descriptive statistics (mean, standard deviation, n, median, and range) for the observed values and the change from the baseline value. Additional safety analyses may be described in the study Statistical Analysis Plan (SAP).

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

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

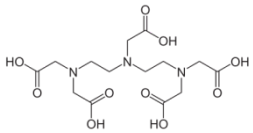


CO_2	carbon dioxide
CRF	case report form
CRA	clinical research associate
CRO	contract research organization
CRP	C-reactive protein
CT	X-ray computed tomography
Cy3	Cy3 cyanine dyes fluoresce orange (~550 nm excitation, ~570 nm emission)



Cy3

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



ECG	electrocardiogram
eCRFs	electronic case report forms
ESR	erythrocyte sedimentation rate
EU	European Union
FDA	Food and Drug Administration
FNR	false negative rate
GCP	Good Clinical Practice
HC	healthy controls
HCT	hematocrit
Hgb	hemoglobin
HIPAA	Health Information Portability and Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonization
ILM	intraoperative lymphatic mapping
IRB	Institutional Review Board
ISF	investigator site file
ITD	intent-to-diagnose population
IV	intravenous
kDa	kiloDalton (molecular weight designation)
mCi	milliCurie ($37x10^6$ becquerels; 37megabecquerels)
MedDRA	Medical Dictionary for Regulatory Activities

MTD	maximum tolerated dose
NOAEL	no-observed adverse-effect level
NSAIDS	nonsteroidal anti-inflammatory drug
OA	overall accuracy
OLINDA/EXM®	Organ Level INternal Dose Assessment/EXponential Modeling
PI	principal investigator
PK	pharmacokinetics
PP	per protocol
RA	rheumatoid arthritis
RBC	red blood cells
RF	rheumatoid factor
RPK	radiopharmacokinetic
SAE	serious adverse event
SAP	statistical analysis plan
SD	study day
SJC	swollen joint count
SPECT	single photon emission computed tomography
SUSARs	suspected, unexpected, serious adverse reactions
$t_{1/2}$	apparent terminal elimination half life
Tc 99m	technetium-99m metastable isotope; γ-emitting (t½ = 6.02 h)
TcSC	Tc 99m sulfur colloid
Tilmanocept	DTPA Mannosyl Dextran (the US Adopted Name for the drug substance of Lymphoseek)
TJC	tender joint count
TMF	trial master file
TNFα	tumor necrosis factor alpha
TUV	tilmanocept uptake value
ULN	upper limit of normal
US	United States
VAS	visual analog scale

WBC	white blood cell
λ_z	apparent terminal elimination rate constant

STUDY ADMINISTRATIVE STRUCTURE

The principal investigator (PI) must sign and date the protocol signature sheet before study participant recruitment may start. Likewise, all protocol amendments must be signed and dated by the PI before coming into effect.

The name and address of the participating center, the investigators, and all required signature documents will be maintained in the trial master file (TMF).

In addition to the PI, there are additional onsite roles that may be performed by other sub-investigators:

- Subject referral to the study
- Review of subject eligibility and medical records
- Clinical evaluations
- Safety assessments
- Injection and imaging
- On-site image analysis

Study personnel not listed in this section are identified in a separate personnel list. This list will be updated as needed. The list of personnel will be available in the center's investigator site file (ISF).

1 INTRODUCTION

1.1 Background

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 (Lassere 2013, Michelsen 2018, Uhlig 2014, Verstappen 2015).

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

The realization that early diagnosis of RA is critical for delivering the most effective RA treatment led to a collaboration between the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) that resulted in 2010 in the publication of new criteria for diagnosing RA (Aletaha 2010). The intent of the ACR/EULAR 2010 criteria was to improve the diagnosis of early RA. This intent was only partially realized. There have been numerous publications reporting the results of studies evaluating the diagnostic accuracy of the ACR/EULAR 2010 criteria for identifying early RA patients. In a meta-analysis of this literature (Sakellariou 2013), it was shown that the ACR/EULAR 2010 criteria 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 (Meyer 2010, Olszewski 2001). Frequently,

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

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

Quantitative assessment of CD206 positivity of inflamed synovia in RA patients is expected to remedy, at least partially, all 4 of these problems and deficiencies. Tc 99m tilmanocept is a synthetic radiopharmaceutical imaging agent that was purposefully designed to be a high affinity ligand for CD206. CD206 is highly upregulated on phenotypically activated macrophages that contribute mechanistically to the underlying pathobiology of RA. It has long been recognized that activated macrophages contribute significantly to RA pathology (Firestein 1990, Ishikawa 1976, Kinne 2007). Macrophages are common in inflamed synovial tissues when patients are first diagnosed with RA (Smolen 2018) and frequently become more numerous as the disease progresses. Activated macrophages produce most of the TNFα that, in a significant proportion of cases, drives and perpetuates the inflammatory cycle in RA (Choy 2001). In the synovial sublining of a joint affected by RA, activated macrophages are frequently the dominant cell type (Cutolo 1993, Kennedy 2011, Kraan 2002). Activated macrophages significantly contribute to the destruction of bone and cartilage through their secretion of proteases (Bresnihan 1999, Ma 2005). Furthermore, the densities of synovial membrane macrophages measured before treatment, and especially the densities of sublining macrophages, have been reported to predict future joint damage (Mulherin 1996, Orr 2017, Vieira-Sousa 2011, Yanni 1994). Not surprisingly, activated synovial macrophage numbers—but not the numbers of other immune cell types—correlate with radiographically determined joint destruction in RA (Mulherin 1996, Yanni 1994). Thus, CD206 positivity of inflamed synovia in RA patients is expected to provide clinically significant prognostic information for RA patients. Another important finding is that activated macrophage numbers are reduced by effective RA therapy (Vieira-Sousa 2011), but do not significantly change over the course of at least months if a patient was given ineffective RA therapy (Baeten 2006). Also, importantly,

reductions in activated synovial macrophages associated with effective RA therapy typically occur *before treatment mediated changes* in the severity of clinical symptoms can be observed (Filkova 2016). Thus, a change in activated synovial macrophage numbers is now recognized as a biomarker that provides an objective and early measure of responses to RA therapies (Bresnihan 2009, Smith 2001). 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 (Bresnihan 2009, van de Sande 2012, Wijbrandts 2007). 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 (Orr 2017). An important finding of these studies has been that the inflammatory cell compositions of RA inflamed joints can vary between patients (Townsend 2014, van de Sande 2016). The biopsy specimens obtained from different RA patients can have different numbers and densities of macrophages and monocytes, lymphocytes and lymphocyte containing structures, and fibroblast-like synoviocytes (Orr 2017). These differences in cellular composition suggest that RA inflammation can be divided into 3 pathotypes, referred to as diffuse myeloid, lympho-myeloid, and fibroid respectively (Astorri 2015, Dennis 2014). 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) (Dennis 2014). This is an area of ongoing active investigation in RA therapy research (Donlin 2018, Mandelin 2018, Pitzalis 2013). 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 (Dennis 2014, Wijbrandts 2008), whereas patients with a fibroid pathotype do not respond significantly to anti-TNFα therapy. Although these results need to be confirmed and elaborated upon in further studies, they suggest that determination of the density of activated macrophages in the inflamed synovial membranes of patients with RA could facilitate identification of those RA patients who would most benefit from anti-TNFα therapy and/or those who would not receive benefit. Additional work in this field seeks to determine if similar associations between the efficacies of other treatments and synovial pathotypes can identify those treatments that are most effective in patients with lympho-

myeloid and/or fibroid RA pathotypes. Such results, if attained, would provide a great benefit to RA patients by enabling personalized delivery of optimal treatments to all RA patients.

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 to 14 samples of tissue from each biopsied joint, in about 5% to 10% of cases, they do not provide tissue of sufficient quantity or quality to enable adequate histological evaluations of the inflamed synovial tissue (Kraan 2002, Pitzalis 2013). It is expected that Tc 99m tilmanocept imaging would not fail to quantitatively assess the aggregation of macrophages in RA inflamed joints at this frequency.

The fourth reason is that although more than 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 (Mandelin 2018). Synovial biopsies have only been performed in research settings and until very recently, only in Europe where adequately trained and experienced investigators reside. Training and qualifying all physicians in the US who care for RA patients to perform synovial biopsies would be a significant barrier to adoption.

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.” (RSNA 2018)

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

Based on the clinical utility of SUV in 18F-FDG PET imaging, Navidea pursued the development of the TUV 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 $\text{TUV} = \frac{\bar{x}}{B}$. 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 With 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 (QWBA) 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 mcg and 200 mcg 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 after injection. 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 was an 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 sixteen evaluable subjects were enrolled. Tilmanocept was 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)

The primary safety endpoint of the NAV3-31 study was evaluated by examining the incidence of AEs, changes over time in clinical laboratory tests, electrocardiogram (ECG) parameters, and vital signs. The safety evaluation included all subjects who were enrolled in the study and administered Tc 99m tilmanocept (n = 116). There were no Tc 99m tilmanocept related AEs. There were no deaths during the trial; there were three SAEs that were not related to study drug or study procedures; and one AE that led to discontinuation from the trial. There were no Adverse Drug Reactions reported.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- To demonstrate that global tilmanocept uptake values (TUV_{global}) obtained before initiation of anti-TNFα therapy ($TUV_{global[b]}$) and at 5 weeks ± 1 week following change in therapy ($TUV_{global[5w]}$) has a specificity of greater than 80% to correctly specify a clinical non-response to therapy at 24 weeks.
- To demonstrate that TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following change in therapy has a sensitivity of greater than 65% to correctly classify a positive clinical response to therapy at 24 weeks.

2.2 Key Secondary Objective

- To evaluate the negative predictive value (NPV) of TUV_{global} obtained before initiation of anti-TNFα therapy ($TUV_{global[b]}$) relative to a clinical non-response to therapy at 24 weeks.

2.3 Secondary Objectives

- To evaluate the sensitivity and specificity of TUV_{global} obtained before initiation of anti-TNFa therapy and at 5 weeks ± 1 week following change in therapy relative to a positive clinical response or a non-response to therapy at 12 weeks.
- To evaluate the NPV of $TUV_{global[b]}$ relative to a clinical a non-response to therapy at 12 weeks.
- To evaluate the positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA) of TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week ($\Delta TUVglobal_{[5w]}$) following change in therapy relative to a positive clinical response or a non-response to therapy at 12 and/or 24 weeks.
- To evaluate the qualitative assessment of Tc 99m tilmanocept imaging to predict clinical response or non-response following a change in anti-TNFα therapy.
- To evaluate the additive effect of either quantitative or qualitative assessment of Tc 99m tilmanocept imaging to the other at baseline and change from baseline to 5 weeks ± 1 week to predict clinical response or non-response following a change in anti-TNFα therapy.
- To evaluate the correlation of changes in TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following a change in therapy with changes in composite clinical assessments and their constituent parameters at 12 and/or 24 weeks.
- To evaluate the correlation of changes in TUV_{global} obtained before initiation of anti-TNFα therapy and at 5 weeks ± 1 week following a change in therapy with changes in clinical assessments at 12 and/or 24 weeks for specific anti-TNFα bDMARD agents.
- To evaluate the correlation of changes in TUV_{global} and changes in clinical RA assessments as a means to monitor therapy.

- To evaluate sensitivity and specificity in patient subgroups.
- To evaluate safety of IV-administered tilmanocept radiolabeled with Tc 99m.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a prospective, open-label, multicenter study designed to evaluate the predictive capacity of Tc 99m tilmanocept planar imaging for downstream clinical response(s) in individuals with moderate to severe RA who are candidates for change in anti-TNFα therapy. Temporal (Baseline to 5-week) changes in quantitative imaging will be correlated with longitudinal (Baseline to 12- and 24-week) assessments of clinical RA outcomes to evaluate the clinical utility of Tc 99m tilmanocept for the expedited evaluation of antirheumatic treatment efficacy when compared with longitudinal assessments in clinical practice.

Prior to the initiation of a new therapy, subjects will undergo baseline rheumatological evaluations of ACR/EULAR 2010 Classification Criteria, CDAI, DAS28, HAQ-DI©, and a serological RA panel for the characterization of disease activity. Following these evaluations, subjects will receive an IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m and undergo baseline planar imaging of the bilateral hands and wrists beginning 60-75 minutes following Tc 99m tilmanocept administration. Upon completion of baseline imaging procedures, subjects will commence a new anti-TNFα bDMARD treatment regimen and return to the clinic 5 ± 1 weeks later for a series of rheumatological evaluations (including CDAI, DAS28, ACR Response Criteria, and HAQ-DI©), drug administration, and bilateral hand/wrist imaging. The rheumatological evaluations will be repeated at 12 ± 1 weeks and 24 ± 1 weeks after commencement of the new therapy.

Images will undergo centralized quantification for the derivation of joint-specific and global tilmanocept uptake values (TUV). At the joint level, TUV is defined as the intrasubject ratio of the average pixel intensity of a joint to the average pixel intensity of the reference region. TUV_{global} is a per subject summary of TUV_{joint} values greater than the upper 95th percent (2 standard deviations) of the normative database representing RA-specific inflammation. TUV calculations are additionally described in the Statistical Analysis Plan.

All images will be read by three nuclear medicine specialists trained in the reading of these images. Each reader will work independently of the other readers and will be blinded to clinical assessments. Each reader will provide both a qualitative read and map regions of interest for calculation of TUVs.

Temporal differences in TUV_{global} from baseline to 5 ± 1 weeks of therapy ($\Delta TUV_{global[5w]}$) will be compared with longitudinal clinical outcomes at 12 ± 1 and 24 ± 1 weeks of therapy defined by changes in CDAI ($\Delta CDAI_{12/24w}$), changes in DAS28 ($\Delta DAS28_{[12/24w]}$), and assessment of ACR Response Criteria ($ACR_{12/24w}$) to evaluate the efficacy of TUV_{global} for the accelerated prediction of clinical response to antirheumatic drugs.

Tc99 tilmanocept images with low levels of localization in the joints- and thus presumptively low numbers of synovial lining macrophages are thought to be less likely to respond to anti-TNFα therapies, and these cases will be classified as predicted non-responders using their baseline scan alone. The determination of low level of localization is done quantitatively by

applying a threshold TUV_{global} of 5 as the cutoff value. Patients whose baseline scans are determined to have TUVglobal below this are predicted to be non-responders. This value was determined in the completed Phase 2B trial NAV3-31. Patients whose TUV_{global} scores are 5 or above then have their baseline scans compared to their Week 5 scans to determine if they will be classified as predicted responders or non-responders. A quantitative analysis of their change in TUV_{global} score from baseline to Week 5 is performed to see if their levels of localization have changed significantly. All subjects in this trial will undergo baseline and Week 5 scans, including those whose baseline scans demonstrate TUV_{global} below 5, as an objective is to assess if any longitudinal changes in TUV_{global} from baseline to Week 5 can be predictive of clinical response or non-response. The process of dichotomizing subjects based on their baseline TUV_{global} scores is referred to as bucketing. Statistical analyses will be performed on bucketed data. In the bucketed analyses, subjects with baseline TUV_{global} below 5 are classified as predicted non-responders while those with baseline TUV_{global} above 5 are classified as either predicted responders or non-responders based on the change in TUV_{global} between baseline and Week 5.

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

3.2 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

Subjects will be “on study” for up to 213 days depending on the duration of the screening window (up to 30 days).

4 STUDY POPULATION

The study population will comprise subjects with clinically diagnosed active RA who are candidates for initiation of, or change to, a new anti-TNFα bDMARD regimen. Subjects who meet the eligibility criteria listed below will be eligible for enrollment without regard to other aspects of their disease, such as chronicity of their RA. That is, subjects with newly diagnosed disease, those with chronic disease, and those who have failed multiple therapies will all be eligible for enrollment if they meet the enumerated eligibility criteria.

4.1 Eligibility

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

4.1.1 Inclusion Criteria

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

1. The subject has provided written informed consent with HIPAA (Health Information Portability and Accountability Act) authorization before the initiation of any study-related procedures.
2. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis.
3. The subject is a candidate for initiation of, or change to, a new anti-TNFα bDMARD therapy.
4. The subject has RA as determined by the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria (score of ≥ 6/10).
5. The subject has moderate to severe RA as determined by 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 first imaging visit (Day 0).
7. Subjects receiving bDMARD or janus kinase (JAK) inhibitor therapy must have been at a stable dose > 60 days prior to the first imaging visit (Day 0).
8. If the subject is receiving NSAIDS (nonsteroidal anti-inflammatory drug) or oral corticosteroids, the dose has been stable for > 28 days prior to the first imaging visit (Day 0). The corticosteroid dose must be ≤ 10 mg/day of prednisone or an equivalent steroid dose.

4.1.2 Exclusion Criteria

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

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator
3. The subject is currently receiving radiation therapy or chemotherapy or has received radiation or chemotherapy within the past 5 years.
4. The subject has an active malignancy or a history of malignancy within the past 5 years.
5. The subject has had a finger, hand, and/or wrist amputation or hand or wrist joint arthroplasty.
6. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.
7. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 2 times the upper limit of normal.
8. The subject has any severe, acute, or chronic medical conditions and/or psychiatric conditions and/or laboratory abnormalities that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration that would deem the subject inappropriate for study participation.
9. The subject has a history of hypersensitivity reactions to TNF-inhibitors.
10. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
11. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration at the first imaging visit (Day 0).
12. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to the first imaging visit (Day 0).
13. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to the administration of Tc 99m tilmanocept at the first imaging visit (Day 0).
14. The subject has heart failure (NYHA Class III-IV), a demyelinating disorder, or a chronic/latent infection (e.g., +PPD, HIV, Hepatitis B).

4.2 Recruitment

Subjects will be recruited from rheumatology practices in accordance with the inclusion and exclusion criteria listed above. Potentially suitable subjects will be asked by their treating physician about their willingness to participate in this study. Subjects may also be identified through social media campaigns or advertising.

4.3 Withdrawal

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

A subject who withdraws consent prior to arrival at the study site on Day 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

4.4 Replacement

Subjects will be replaced under the following conditions:

- Subjects who did not receive study drug administration or did not proceed to imaging

4.5 Subject Identification

After the subject provides written informed consent, the site will assign the subject a 7-digit subject number. Subject numbers are to be assigned in a sequential manner using the following format:

Digits 1 to 2: Study number "33"
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 "33-01-001."

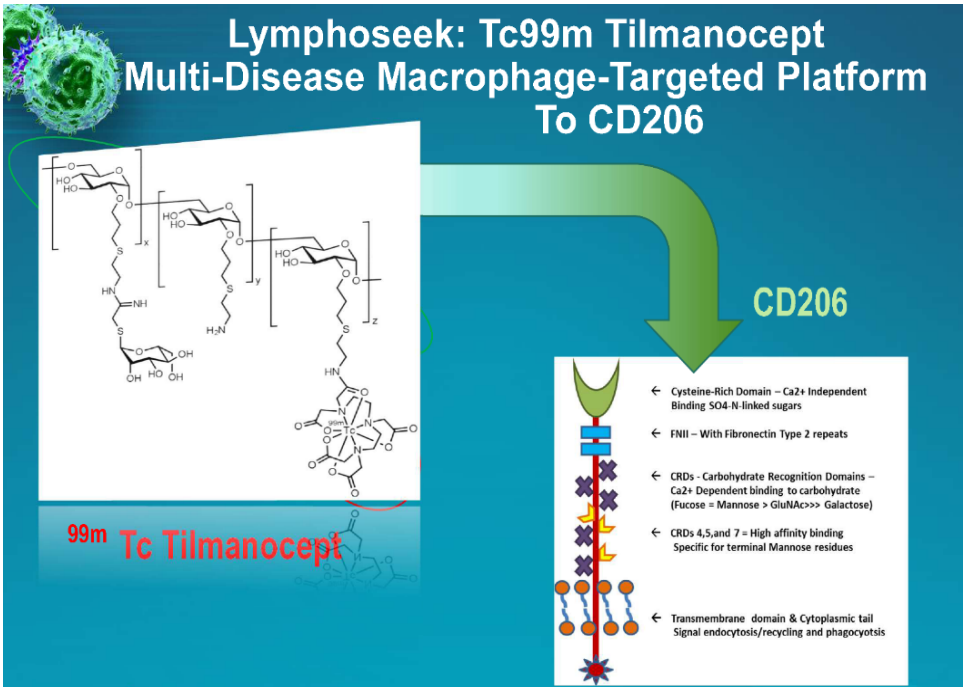
Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Identification of Investigational Product

Technetium Tc 99m tilmanocept was approved under the brand name Lymphoseek in the USA by the FDA in year 2013 NDA number 202207 as radio-inaging agent. Technetium Tc 99m tilmanocept is a scintigraphic imaging radiotracer that binds to CD206 (mannose 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 the left or right antecubital vein.

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

5.3 Treatment Assignment

Subjects will receive the study-defined dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc99m. Subjects will undergo drug administration and imaging at the following 4 timepoints:

1. Day 0 (Visit 2)
2. 5 ± 1 weeks after the initiation of new anti-TNFα bDMARD therapeutic regimen (Visit 4)

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 with instructions on how to radiolabel the vials and prepare the final tilmanocept product for injection. Quality Control worksheets will also be completed.

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. An overall accountability and reconciliation form of the investigational product will be prepared and completed. If there are any discrepancies, they must be investigated and their resolution documented. All unused study kits will be destroyed in accordance with institutional destruction procedures.

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapy

All medications taken 30 days prior to Tc 99m tilmanocept injection through the post-injection safety follow-up must be documented and maintained at a stable dose according to the inclusion criteria. The subject's complete history of RA treatments will also be collected.

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

7.1 Schedule of Evaluations

A schedule of evaluations is provided in the Schedule of Study Events (see Appendix 1). Visits 7 and 9 have been eliminated per Protocol Amendment 4. For continuity, visits have not been renumbered (i.e., Visit 6 is followed by Visit 8).

7.2 Visit Description

7.2.1 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, biological sex, race
- Medical and surgical history – all relevant prior medical and surgical conditions, which may include RA family history, and RA treatment history, 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 (Table 4)
- Urine collection for routine analysis
- Urine pregnancy test for women of childbearing potential. Females of childbearing 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 Classification Criteria and DAS28. A HAQDI© assessment will be completed to assess quality of life related to health. A CDAI assessment will be used to quantify and track disease activity. A review of the subject's RA history including previous treatments, date of symptom onset, and date of diagnosis will also be performed.

- Widespread Pain Index (WPI) Assessment

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

7.2.2 Visit 2, Baseline (Day 0; Drug administration/Imaging)

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

7.2.2.1 Before Administration of Tc 99m Tilmanocept

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

- A urine pregnancy test for women of childbearing potential. Females of childbearing 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

7.2.2.2 Administration of Tc 99m Tilmanocept

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

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

7.2.2.3 0 to 30 Minutes After 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)

7.2.2.4 60 to 75 Minutes After Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition: planar scan of bilateral hands and wrists
- Clinical labs (within 60 minutes after the completion of imaging)
- Urinalysis (within 60 minutes after the completion of imaging)

Upon the completion of all Visit 2 procedures, subjects will initiate their new anti-TNFα bDMARD treatment regimen. The exact agent, dose, start date, and planned dosing frequency will be documented. For each subsequent administration, the following details will be documented:

1. Administration start time
2. Administration end time
3. Total volume injected
4. Total dose administered

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

- Review of concomitant medications
- Assessment of adverse events

7.2.4 Visit 4 (Week 5 ± 1; Tc 99m Tilmanocept Administration, Imaging, and Clinical Assessments)

7.2.4.1 Before Tc 99m Tilmanocept Administration (0-7 days pre-injection)

The following procedures will be completed for all subjects within 7 days prior to the administration of Tc 99m tilmanocept:

- CDAI
- DAS28 (note: associated labs occur after imaging)
- HAQ-DI©
- WPI

7.2.4.2 Before Tc 99m Tilmanocept Administration (day of injection)

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 childbearing potential. Females of childbearing 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

7.2.4.3 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 a catheter for a slow push injection. At the completion of the injection, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

7.2.4.4 0 to 30 Minutes After 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)

7.2.4.5 60 to 75 Minutes After Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition: planar scan of bilateral hands and wrists
- Clinical labs (within 60 minutes after the completion of imaging)
- Urinalysis (within 60 minutes after the completion of imaging)
- RA labs (within 60 minutes after the completion of imaging)

7.2.5 Visit 5 (5 ± 3 Days After Visit 4; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- Assessment of adverse events

7.2.6 Visit 6 (Week 12 ± 1; Clinical Assessments)

The following procedures will be completed for all subjects at Week 12 ± 1:

- CDAI
- DAS28
- HAQ-DI©
- WPI
- RA labs
- Assessment of adverse events
- Concomitant medication review

7.2.7 Visit 8 (Week 24 ± 1; Clinical Assessments; note that Visit 7 will not be conducted per this amendment)

7.2.7.1 Before Tc 99m Tilmanocept Administration (0-7 days pre-injection)

The following procedures will be completed for all subjects at Week 24 ± 1:

- CDAI
- DAS28
- HAQ-DI©
- WPI
- RA labs
- Assessment of adverse events
- Concomitant medication review

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographic and Other Baseline Characteristics

Based on response to anti-TNFα bDMARD therapy, 198 to 672 evaluable subjects may be enrolled. Subjects will be men and women ≥ 18 years of age with evidence of active RA. 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. There will be no exclusions based on specific subgroups (e.g., subjects with various demographic characteristics; disease severity, duration, or response to prior therapies; co-existing conditions; genetic variants; concomitant medications; or prior treatment with non-TNF biologic therapies).

8.1.2 Medical 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.

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 a 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 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 2 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 Evaluation

Subjects will be evaluated for the DAS28 (Prevoo 1995) at the following timepoints: screening (Day -30 to -1), Visit 4 (5 ± 1 weeks post-initiation of new anti-TNFα bDMARD), Visit 6 (12 ± 1 weeks post-initiation of new anti-TNFα bDMARD), and Visit 8 (24 ± 1 weeks post-

initiation of new anti-TNFα bDMARD). DAS28 evaluations may be completed up to 30 days before imaging Visit 2 and up to 7 days before imaging Visits 4, 6, and 8.

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 CDAI

Subjects will be evaluated for disease activity using the Clinical Disease Activity Index (Aletaha 2009). CDAI will be collected a total of 4 times per subject (screening, Visit 4, Visit 6, and Visit 8). The CDAI evaluation may be completed up to 30 days before imaging Visit 2 and up to 7 days before imaging Visits 4, 6, and 8. This metric is calculated as the sum of 4 components: TJC, SJC, patient global assessment of disease activity, and provider global assessment of disease activity. A CDAI score of 0.0 to 2.8 is indicative of disease remission; 2.9 to 10.0 of low activity, 10.1 to 22.0 of moderate activity, and 22.1 to 76.0 of high activity. See Appendix 4 for details.

8.3.4 ACR Response Criteria

Subjects will be evaluated using the ACR Response Criteria (Felson 1995). ACR Response is derived from 6 possible parameters: swollen/tender joint count, patient assessment, physician assessment, pain scale, disability/functionality questionnaire, and acute phase reactant (ESR/CRP). Response is reported as percent (%) improvement from baseline to a later timepoint. In this case, a total of 2 timepoints will be evaluated per subject (Visits 6 and 8).

Percent improvement is defined as a combination of reductions in swollen and tender joint counts as well as improvement in at least at least 3 of the other parameters (patient assessment, physician assessment, pain scale, disability/functionality questionnaire, and acute phase reactant [ESR]). An ACR20 indicates that 20% improvement is observed in tender and swollen joint counts as well as 20% improvement in at least 3 of the other 5 criteria. An ACR50 indicates that 50% improvement is observed in tender and swollen joint counts as well as 50% improvement in at least 3 of the other 5 criteria. An ACR70 indicates that 70% improvement is observed in tender and swollen joint counts as well as 20% improvement in at least 3 of the other 5 criteria. See Appendix 5 for details.

8.3.5 Other

8.3.5.1 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 (Scott 1996). This assessment will be used as an input parameter for 2010 ACR/EULAR Classification Criteria (Section 8.3.1), DAS28 score (Section 8.3.2), and CDAI score (Section 8.3.3).

Patient Global Assessment of Disease Activity

Patient global assessment of disease activity will be evaluated using a 10-point scale wherein a score of 0 is considered 'very well' and score of 10 is considered 'very poor'. Score intervals are 0.5 apart (yielding 20 possible options – 0, 0.5, 1.0, 1.5…20). This assessment will be used as an input parameter for CDAI score (Section 8.3.3) and ACR Response Criteria (Section 8.3.4).

Provider Global Assessment of Disease Activity

Provider global assessment of disease activity will be evaluated using a 10-point scale wherein a score of 0 is considered 'very well' and score of 10 is considered 'very poor'. Score intervals are 0.5 apart (yielding 20 possible options – 0, 0.5, 1.0, 1.5…20). This assessment will be used as an input parameter for CDAI score (Section 8.3.3) and ACR Response Criteria (Section 8.3.4).

8.3.5.2 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 (Section 8.3.2) and ACR Response Criteria (Section 8.3.4).

8.3.5.3 Patient Assessment of Physical Function (HAQ-DI©)

Patient assessment of physical function will be evaluated using the Health Assessment Questionnaire Disability Index (HAQ-DI©) at screening (up to 30 days before imaging Visit 2) and Visits 4, 6, and 8 (up to 7 days before imaging visits 4, 6, and 8). This evaluation includes questions about functional ability, fine movements of the upper extremity, locomotor activities of the lower extremity, and compound activities requiring both extremities. It also includes assessments of various functional activities such as dressing, rising, eating, walking, etc. Responses are scored on a 0 (no disability) to 3 (completely disabled) scale (Bruce 2003). See Appendix 6 for more information.

8.3.5.4 Acute-phase Reactant

ESR and CRP will be obtained in the RA-specific laboratory panel (see Table 4) at screening and Visits 4, 6, and 8. ESR is used in the calculation of the ACR response level.

8.3.5.5 Widespread Pain Index

Assessment of widespread pain will be evaluated using the widespread pain index (WPI) at screening (up to 30 days before imaging Visit 2) and Visits 4, 6, and 8 (up to 7 days before imaging visits 4, 6, and 8). Providers will assess 19 areas of widespread pain over the last 7 days and will add the total number of areas in pain for the WPI score (score range 0 to 19). This assessment will be used as an additional pain assessment tool to evaluate central pain.

8.4 Image Acquisition

8.4.1 Planar Scintigraphy

Planar spot views of the hands and wrists will be acquired beginning 60 to 75 minutes post injection at Visits 2 and 4. Images and results derived from the images are investigational and should not be used for clinical decision making or shared with the participant.

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 NAV3-33 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 E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this

treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

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

By definition for this study, all untoward medical occurrences beginning on the day of Visit 2 Baseline (Day 0) through the final visit (Visit 8) 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

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

Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 8.5.5.

Severity

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

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

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationship to investigational product

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

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

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

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

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

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

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

Causal relationship to study procedure

The investigator will use the following definitions to assess the relationship of the adverse event 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 of Tc 99m tilmanocept administration) will be recorded in the subject's medical history. Untoward medical events beginning on Visit 2 (day of Tc 99m tilmanocept administration) through the completion of Visit 8 will be reported as adverse

events. Thus, subjects should be closely observed by the investigator both during and after the evaluation.

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

The following information is required:

- The **date** and **time of onset** of any AE.
- The duration (the entire duration of an event or symptom, calculated from date of onset to date of end, if not recorded directly).
- The seriousness of the AE will be assessed by the investigator. If the investigator deems that an AE qualifies as an SAE, a special form provided by the sponsor should be completed and the event must immediately, and within one day (i.e., within 24 hours) of becoming aware of the SAE, be 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, involving 553 subjects, only 3 events (breast pain and injection site pain reported by subjects with breast cancer and injection site irritation reported by a subject with head and neck squamous cell cancer) were deemed definitely related to the administration of Lymphoseek by the investigator. The most common adverse reactions (incident < 1%) have been lack of effect (< 0.067%), injection site pain (< 0.02%) and rash (< 0.02%). Adverse events from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

In addition to the Lymphoseek pre-approval clinical studies, post-marketing surveillance shows that Lymphoseek has been administered to more than 500,000 patients. Routes of administration included: subcutaneous, intradermal, and peritumoral. The intended route of administration in this study is intravenous.

Precautionary Measures

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

Unexpected Adverse Events

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

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

8.5.5 Serious Adverse Events

Definition of Serious Adverse Events

Definition

The following SAE definition is based on ICH guidelines and the final rule issued by the Food and Drug Administration (FDA) and effective 06 Apr 1998 on Expedited Safety Reporting Requirements for Human Drug and Biological Products.

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

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

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

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

Actions and reporting obligations in case of serious adverse events

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

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

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

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

Notification of the IRB(s)

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

Notification of the authorities

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

Sponsor's notification of the investigators

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

8.6 Physical Examination

Complete physical examinations will be conducted at screening including height and weight assessments.

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

8.8 Clinical Laboratory Parameters for Screening and Safety

Clinical laboratory tests to be evaluated in this study include hematology, serum chemistry, and urinalysis. Clinical laboratory tests will include the following as defined in Table 4.

Table 4 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; this assay may also appear as cyclic citrullinated peptide [CCP])

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

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

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

Any clinically significant changes in laboratory values are to be followed up with repeated tests at appropriate intervals (as determined by the investigator) until the values return to baseline level or until the abnormality is explained by the investigator. The expected amount of blood to be drawn is shown in Table 5.

Table 5 Approximate Amount of Blood Drawn per Subject

Timepoint	Test (Sample Volume)	Total Blood Drawn at Timepoint
Visit 1 (Day -30 to -1)	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (3 teaspoons)
Visit 2 (Day 0)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
Visit 4 (5 ± 1 weeks after initiation of new anti-TNFα bDMARD therapy)	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (2.6 teaspoons)
Visit 6 (12 ± 1 weeks after initiation of new anti-TNFα bDMARD therapy	RA panel (4 mL)	4 mL (0.8 teaspoons)
Visit 8 (24 ± 1 weeks after initiation of new anti-TNFα bDMARD therapy)	RA panel (4 mL)	4 mL (0.8 teaspoons)
		Total: 43 mL (8.7 teaspoons)

9 STATISTICAL METHODS

This is a prospective, open-label, multicenter study designed to evaluate the early predictive capacity of quantitative Tc 99m tilmanocept planar imaging for downstream clinical response(s) in individuals who are candidates for change in anti-TNFα therapy. Temporal (Baseline to 5-week) differences in quantitative imaging will be correlated with longitudinal (Baseline to 12- and 24-week) assessments of clinical RA outcomes to evaluate the clinical utility of Tc 99m tilmanocept for the expedited evaluation of antirheumatic treatment efficacy when compared with longitudinal assessments in clinical practice.

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

9.3.1 Primary Efficacy Variables

The co-primary efficacy predictor variables are the response/non-response status at 24 weeks to new or changed anti-TNFa bDMARD therapy for rheumatoid arthritis based on the ACR50 criterion, and the predicted response/non-response based on $\Delta TUV_{global[5w]}$ bucketing.

The co-primary endpoints of the study are sensitivity and specificity of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at week 24.

9.3.2 Key Secondary Efficacy Variable

The key secondary efficacy predictor variable is the response/non-response status at 24 weeks to new or changed anti-TNFa bDMARD therapy for rheumatoid arthritis based on the ACR50 criterion, and the $TUV_{global[b]}$ value.

The key secondary endpoint of the study is the NPV of $TUV_{global[b]}$ with respect to ACR50 at Week 24.

9.3.3 Secondary Efficacy Variables

The secondary efficacy response variables are:

- CDAI score at baseline ($CDAI_b$), 12 ($CDAI_{12w}$) and 24 weeks ($CDAI_{24w}$)

- ACR Response Criteria (ACR20, ACR50, ACR70) at 12 and 24 weeks
- DAS28 Score at baseline, 12 weeks, and 24 weeks
- Constituent scores of the ACR Response Criteria at baseline, 12 weeks, and 24 weeks, including:
  - TJC
  - SJC
  - Patient assessment of global disease activity
  - Rheumatologist assessment of global disease activity
  - Patient assessment of pain
  - Patient assessment of physical function
  - Acute-phase reactant value.
- Constituent scores of the CDAI score at baseline, 12 weeks, and 24 weeks, including:
  - TJC
  - SJC
  - Patient Global Assessment
  - Physician Global Assessment

The secondary efficacy endpoints are as follows:

- Sensitivity and specificity of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at week 12.
- NPV, PPV, and OA of $\Delta TUV_{global[5w]}$ bucketing with respect to ACR50 at weeks 12 and 24.
- NPV of $TUV_{global[b]}$ with respect to ACR50 at week 12.
- $TUV_{global[b]}$ and response to new anti-TNFα bDMARD therapy defined by the change from baseline (CFB) of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively), by the CFB of DAS28 to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta DAS28_{12w}$ and $\Delta DAS28_{24w}$, respectively) and by the CFB in each of the ACR Response Criteria components at 12 ± 1) weeks and at 24 ± 1 weeks.
- $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy defined by the CFB of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively).
- Concordance of $\Delta TUV_{global[5w]}$ bucketing with clinical criteria, including ACR Response Criteria, CDAI, DAS28, and HAQ-DI. Concordance between the predicted response or non-response status and the clinical criteria will be evaluated using NPV, PPV, sensitivity, specificity, and OA. Note that sensitivity and specificity of

$\Delta TUV_{global[5w]}$ with bucketing with respect to ACR50 at weeks 12 and 24 are excluded from this set of secondary endpoints, as they are the co-primary.

- Concordance $\Delta TUV_{global[12w]}$ bucketing with clinical criteria, including ACR Response Criteria, CDAI, DAS28, and HAQ-DI.
- Response to new anti-TNFα bDMARD therapy defined by the CFB of CDAI to 12 ± 1 weeks and 24 ± 1 weeks ($\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$, respectively).
- The correlation of $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy from baseline to 24 ± 1 weeks defined by the changes from baseline in each of the ACR Response Criteria components.
- Constituent parameters of $CDAI_{12w}$, $CDAI_{24w}$, $\Delta CDAI_{12w}$, $\Delta CDAI_{24w}$, ACR Response Criteria at 12 and 24 weeks, including:
  - Tender joint count (TJC)
  - Swollen joint count (SJC)
  - Patient assessment of global disease activity
  - Rheumatologist assessment of global disease activity
  - Patient assessment of pain
  - Patient assessment of physical function
  - Acute-phase reactant value

9.4 Sample Size Justification

The study is sized with respect to achieving specified goals in the co-primary endpoints of sensitivity and specificity of $\Delta TUV_{global[5w]}$ bucketing. Sensitivity and specificity require knowledge of the patient's clinical status at Week 24, and so hypothesis testing will be performed after classification. The value of $\Delta TUV_{global[5w]}$ necessary to predict response vs. non-response and the value of $TUV_{global[b]}$ at baseline necessary to predict non-response will be determined in advance. The classification procedure occurs in two stages, following the baseline image acquisition and (possibly) following the 5-week image acquisition and calculation of $\Delta TUV_{global[5w]}$. After the baseline imaging, patients whose $TUV_{global[b]}$ is < 5 are predicted to be non-responders. After $\Delta TUV_{global[5w]}$ is available, patients whose $TUV_{global[b]}$ is ≥ 5 and Δ % is ≤ -10% are predicted to be responders. All other patients are predicted to be non-responders. See the $\Delta TUV_{global[5w]}$ bucketing algorithm specified in the Statistical Analysis Plan for details on this algorithm and for additional algorithms specified for the secondary analysis. The Week 24 ACR50 will be used as the primary determination of clinical response for the reference standard. The hypotheses for the co-primary endpoints to be tested are:

$$H_{O2}: \pi_{\mathrm{Sp}} \leq 0.8$$
$$H_{A2}: \pi_{\mathrm{Sp}} > 0.8;$$

And

$$H_{O1}: \pi_{\mathrm{Se}} \leq 0.65$$
$$H_{A1}: \pi_{\mathrm{Se}} > 0.65.$$

In the above π_{Sp} represents the true specificity of the classification procedure and π_{Se} represents the true sensitivity of the classification procedure when used to predict the outcome of the anti-TNFa bDMARD inhibitor therapy at 24 weeks. An overall two-tailed Type I error rate of 0.05 (one-tailed 0.025) was used without adjusting for multiple endpoints, as both co-primary endpoints must be achieved (i.e., at least the same two of three readers reject both null hypotheses) in order to have a successful study.

If the actual specificity is 0.92 and the actual sensitivity is 0.8, and if an exact binomial test is used to test the above hypotheses, a total of 98 clinical non-responders and 100 clinical responders are needed for 90% power of the individual tests. That is, a minimum of 198 total patients is needed. This applies to single reader.

To evaluate the power characteristics of this process with 3 correlated readers where at least the same two of the three readers must reject both null hypotheses above, a simulation study was performed. Correlated binomial random variables were generated using a copula. The hypotheses were tested for each simulated reader using an exact binomial test, and the number of iterations where at least the same two of the three readers rejected both hypotheses was counted. Under the conditions above this procedure has power 0.93 when $\rho = 0.7$ and 0.89 when $\rho = 0.9$.

In Arm 3 of the NAV3-31 study a ratio of 5 non-responders to 1 responder was observed. If this ratio is maintained in NAV3-33 for the $\Delta TUV_{global[5w]}$ bucketing predictions, a total of 654 patients is needed to achieve the desired power. That is, a total of 500 clinical non-responder patients is expected to be enrolled before reaching the required 100 clinical responders. Taking into account a 10% dropout rate, the anticipated total sample size for the study is approximately 672 patients (560 clinical non-responders and 112 clinical responders). That is:

- Clinical Non-Responders: 90% completion rate relative to 560 enrolled patients will result in 500 completed patients

- Clinical Responders: 90% completion rate relative to 112 enrolled patients will result in 100 completed patients

Because there is uncertainty about how responding and non-responding patients will enroll, enrollment will continue until at least 560 clinical non-responders and 112 clinical responders are participating in the study. The cumulative enrollment of clinical non-responders and responders will be tracked throughout the study in order to stop enrollment once the required number has been reached for each clinical category. This tracking of clinical response and non-response status will be performed by individuals without knowledge of the clinical response data.

If the two co-primary endpoints are achieved (i.e., both null hypotheses are rejected for at least the same 2 out of 3 readers), then the following hypotheses will be tested at one-tailed 0.025 for the key secondary endpoint of NPV of $TUV_{global[b]}$ with respect to ACR50 at week 24:

$$H_{O3}: \pi_{\mathrm{NPV}} \leq 0.7$$
$$H_{A3}: \pi_{\mathrm{NPV}} > 0.7;$$

If the actual NPV is 0.85, and if an exact binomial test is used to test the above hypotheses, a total of 89 predicted non-responders using $TUV_{global[b]}$ are needed for 90% power and a total of 71 non-responders using $TUV_{global[b]}$ are needed for 80% power.

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following populations are defined for this study:

Intent-to-Diagnose (ITD) Population – Patients in the safety population meeting the following criteria are members of the ITD population:

- Were injected with Tc 99m tilmanocept at Visit 2 (Day 0)
- Received planar imaging of the hands at Visit 2 (Day 0)

This population will include all patients for whom a prediction might be made, although some patients may be excluded from analysis under this definition if a prediction cannot be made due to missing Day 0 imaging data.

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 at least one dose of 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 (biological sex, race and ethnicity) will be summarized via counts and percentages.

Additionally, summaries of the agreement between the primary TUV prediction algorithm and all clinical outcomes will be performed in the following subgroups:

- Age (< 60 years, 60 years and older)

- Sex
- Race (white, non-white)
- Time from RA diagnosis (< 5 years, 5 years and longer)
- Disease severity at baseline (moderate, severe)
- Previous use of biologics (yes, no)
- Previous and current use of DMARDs (background methotrexate only, other conventional DMARDs only, combination of methotrexate and other conventional DMARDs)
- Specific anti-TNFα therapy taken during trial
- Prednisone use on-study (on a stable dose, no prednisone use)
- ACPA (or CCP) level at baseline (high/low: 85 and above / < 85)

The study will make every effort to enroll enough patients in each of these subgroups so that half-widths of the 95% confidence intervals of NPV and PPV are less than 20%. P-values on parameter estimates will not be included.

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 Analysis of Primary Efficacy Variables

The co-primary efficacy variables will be summarized by reader in a 2 by 2 contingency table displaying the cell frequencies and marginal totals at 24 weeks. The specificity of $\Delta TUV_{global[5w]}$ bucketing and the sensitivity of $\Delta TUV_{global[5w]}$ bucketing will be calculated for each reader. The 95% exact (Clopper-Pearson) confidence intervals will be provided for each of these parameters. Observed significance levels (p-values) for the exact binomial tests of specificity of $\Delta TUV_{global[5w]}$ bucketing greater than 0.8 and sensitivity of $\Delta TUV_{global[5w]}$ bucketing greater than 0.65 will be provided.

Specificity and sensitivity are defined according to the following cross-tabulation:

Predicted Reponse	Clinical Response: Responder	Clinical Response: Non-Responder	Total
Responder	A	B	T_1
Non-Responder	C	D	T_2
Total	T_3	T_4	N

$$\text{Sensitivity} = A/T_3$$

$$\text{Specificity} = D/T_4$$

The above 2 by 2 contingency table will also be used to calculate the secondary efficacy endpoints of NPV, PPV, and OA as follows:

$$\text{NPV} = D/T_2$$

$$\text{PPV} = A/T_1$$

$$\text{Overall Accuracy} = (A + D)/N.$$

9.5.3.2 Analysis of Key Secondary Efficacy Variables

NPV of $TUV_{global[b]}$ will be calculated for each reader. The 95% exact (Clopper-Pearson) confidence intervals will be provided. Observed significance levels (p-values) for the exact binomial tests of NPV of $TUV_{global[b]}$ greater than 0.7 will be provided.

9.5.3.3 Analysis of Secondary Efficacy Variables

All RA quantitative assessment variables (CDAI, ACR Response Criteria component scores, DAS28 score, and HAQ-DI) will be summarized by computing the mean, standard deviation, number of observations, minimum, median, and maximum for the observed values at each time point and for the change from baseline for values collected after Day 0. The ACR Response Criteria will be summarized with a frequency table of the highest ACR Response level (None, ACR20, ACR50, ACR70) attained by that patient at that time point. That is, a patient who satisfies ACR50 also satisfies ACR20 but will not appear in the frequency count for ACR20.

Concordance of improvement classification for $\Delta TUV_{global[5w]}$ bucketing with clinical criteria (ACR Response, CDAI, DAS28, and HAQ-DI) will be analyzed as follows. Note that the concordance of $\Delta TUV_{global[5w]}$ bucketing with ACR50 is evaluated as co-primaries (sensitivity and specificity). For each of the RA improvement criteria, a cross-classification table will be provided. The sensitivity, specificity, PPV, NPV, and OA will be calculated and 95% exact (Clopper-Pearson) confidence intervals will be provided.

The Kendall rank correlation between $\Delta TUV_{global[5w]}$ with $\Delta CDAI_{12w}$ and $\Delta CDAI_{24w}$ will be computed and a 95% confidence interval for its value will be computed using Fisher's Z transformation. Similarly, the Kendall rank correlation between $\Delta TUV_{global[5w]}$ with changes from baseline in DAS28 and in each of the ACR Response Criteria components at weeks 12 and 24 will be computed and a 95% confidence interval for its value will be computed using

Fisher's Z-transformation. The marginal distributions of the variables will be characterized with the mean, standard deviation, and the number of data pairs.

Additional efficacy analyses may be described in the NAV3-33 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, ECG parameters, and hematology, clinical chemistry and urinalysis parameters will be summarized using descriptive statistics (mean, standard deviation, median, and range) at each time point.

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

9.5.5 Handling of Missing Values

The data required for the statistical analysis of the primary efficacy endpoints are the predictions of responder status (i.e., based on TUV) and the 24-week determination of clinical response using ACR50. Handling of potential missing TUV and/or clinical data and imputation rules are discussed in the SAP.

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 one or more visits designed to clarify all prerequisites before the study starts. Interim monitoring visits will take place on a regular basis according to a schedule fixed by mutual agreement. During these visits, the CRA will check for completion of the entries on the CRFs, their compliance with the protocol and with GCP, and will compare the CRF entries with the source data.

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

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

10.3 Data Processing

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

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

10.4 Auditing

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

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

10.5 Archiving

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

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

The investigator site file is not to be destroyed without the sponsor's approval.

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

10.6 Premature Termination of the Study

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 4.3.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

The planning and conduct of this clinical study are subject to national laws. Only when all of the requirements of the appropriate regulatory authority have been fulfilled will the study begin. The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and the ICH-GCP Guidelines of 17 Jan 1997 including Integrated Addendum to ICH E6(R1) as well as the Guideline For Good Clinical Practice E6(R2) of 24 August 2018. 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.

12 REFERENCE LIST

1. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Annals of the rheumatic diseases.* 2010;69(9):1580-1588.
2. Aletaha D, Smolen JS. The Simplified Disease Activity Index and Clinical Disease Activity Index to monitor patients in standard clinical care. *Rheum Dis Clin North Am.* 2009;35(4):759-772, viii.
3. Anderson JJ, Wells G, Verhoeven AC, Felson DT. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. *Arthritis and rheumatism.* 2000;43(1):22-29.
4. Astorri E, Nerviani A, Bombardieri M, Pitzalis C. Towards a stratified targeted approach with biologic treatments in rheumatoid arthritis: role of synovial pathobiology. *Current pharmaceutical design.* 2015;21(17):2216-2224.
5. Baeten D, Houbiers J, Kruithof E, et al. Synovial inflammation does not change in the absence of effective treatment: implications for the use of synovial histopathology as biomarker in early phase clinical trials in rheumatoid arthritis. *Annals of the rheumatic diseases.* 2006;65(8):990-997.
6. Bresnihan B. Pathogenesis of joint damage in rheumatoid arthritis. *J Rheumatol.* 1999;26(3):717-719.
7. Bresnihan B, Pontifex E, Thurlings RM, et al. Synovial tissue sublining CD68 expression is a biomarker of therapeutic response in rheumatoid arthritis clinical trials: consistency across centers. *J Rheumatol.* 2009;36(8):1800-1802.
8. Bruce B, Fries JF. The Stanford Health Assessment Questionnaire: dimensions and practical applications. *Health Qual Life Outcomes.* 2003;1:20.
9. Chen YF, Jobanputra P, Barton P, et al. A systematic review of the effectiveness of adalimumab, etanercept and infliximab for the treatment of rheumatoid arthritis in adults and an economic evaluation of their cost-effectiveness. *Health technology assessment (Winchester, England).* 2006;10(42):iii-iv, xi-xiii, 1-229.
10. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *The New England journal of medicine.* 2001;344(12):907-916.
11. Cush JJ. Early rheumatoid arthritis -- is there a window of opportunity? *The Journal of rheumatology Supplement.* 2007;80:1-7.
12. Cutolo M, Sulli A, Barone A, Seriolo B, Accardo S. Macrophages, synovial tissue and rheumatoid arthritis. *Clin Exp Rheumatol.* 1993;11(3):331-339.
13. Demoruelle MK, Deane KD. Treatment strategies in early rheumatoid arthritis and prevention of rheumatoid arthritis. *Curr Rheumatol Rep.* 2012;14(5):472-480.
14. Dennis G, Jr., Holweg CT, Kummerfeld SK, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis Res Ther.* 2014;16(2):R90.
15. Donlin LT, Rao DA, Wei K, et al. High dimensional analyses of cells dissociated from cryopreserved synovial tissue. *bioRxiv.* 2018.
16. Felson DT, Anderson JJ, Boers M, et al. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum.* 1995;38(6):727-735.

17. Filkova M, Cope A, Mant T, Galloway J. Is there a role of synovial biopsy in drug development? *BMC Musculoskelet Disord.* 2016;17:172.
18. Firestein GS, Zvaifler NJ. How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum.* 1990;33(6):768-773.
19. Furst DE, Keystone EC, Braun J, et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2010. *Annals of the rheumatic diseases.* 2011;70 Suppl 1:i2-36.
20. Heidari P, Cross W, Crawford K. Do out-of-pocket costs affect medication adherence in adults with rheumatoid arthritis? A systematic review. *Seminars in arthritis and rheumatism.* 2018.
21. Hresko A, Lin J, Solomon DH. Medical Care Costs Associated with Rheumatoid Arthritis in the US: A Systematic Literature Review and Meta-analysis. *Arthritis care & research.* 2018.
22. Ishikawa H, Ziff M. Electron microscopic observations of immunoreactive cells in the rheumatoid synovial membrane. *Arthritis Rheum.* 1976;19(1):1-14.
23. Kalden JR. Emerging role of anti-tumor necrosis factor therapy in rheumatic diseases. *Arthritis research.* 2002;4 Suppl 2:S34-40.
24. Keffer J, Probert L, Cazlaris H, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* 1991;10(13):4025-4031.
25. Kennedy A, Fearon U, Veale DJ, Godson C. Macrophages in synovial inflammation. *Front Immunol.* 2011;2:52.
26. Kinahan PE, Fletcher JW. Positron emission tomography-computed tomography standardized uptake values in clinical practice and assessing response to therapy. *Semin Ultrasound CT MR.* 2010;31(6):496-505.
27. Kinne RW, Stuhlmuller B, Burmester GR. Cells of the synovium in rheumatoid arthritis. Macrophages. *Arthritis Res Ther.* 2007;9(6):224.
28. Kraan MC, Reece RJ, Smeets TJ, Veale DJ, Emery P, Tak PP. Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patients: Implications for pathogenesis and evaluation of treatment. *Arthritis Rheum.* 2002;46(8):2034-2038.
29. Lassere MN, Rappo J, Portek IJ, Sturgess A, Edmonds JP. How many life years are lost in patients with rheumatoid arthritis? Secular cause-specific and all-cause mortality in rheumatoid arthritis, and their predictors in a long-term Australian cohort study. *Intern Med J.* 2013;43(1):66-72.
30. Leizer T, Cebon J, Layton JE, Hamilton JA. Cytokine regulation of colony-stimulating factor production in cultured human synovial fibroblasts: I. Induction of GM-CSF and G-CSF production by interleukin-1 and tumor necrosis factor. *Blood.* 1990;76(10):1989-1996.
31. Ma Y, Pope RM. The role of macrophages in rheumatoid arthritis. *Current pharmaceutical design.* 2005;11(5):569-580.
32. Mandelin AM, 2nd, Homan PJ, Shaffer AM, et al. Transcriptional Profiling of Synovial Macrophages Using Minimally Invasive Ultrasound-Guided Synovial Biopsies in Rheumatoid Arthritis. *Arthritis & rheumatology (Hoboken, NJ).* 2018;70(6):841-854.

33. Meyer PW, Hodkinson B, Ally M, et al. Circulating cytokine profiles and their relationships with autoantibodies, acute phase reactants, and disease activity in patients with rheumatoid arthritis. *Mediators of inflammation.* 2010;2010:158514.
34. Michelsen B, Uhlig T, Sexton J, et al. Health-related quality of life in patients with psoriatic and rheumatoid arthritis: data from the prospective multicentre NOR-DMARD study compared with Norwegian general population controls. *Annals of the rheumatic diseases.* 2018;77(9):1290-1294.
35. Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. *Arthritis Rheum.* 1996;39(1):115-124.
36. Nell VP, Machold KP, Eberl G, Stamm TA, Uffmann M, Smolen JS. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. *Rheumatology (Oxford, England).* 2004;43(7):906-914.
37. Olszewski WL, Pazdur J, Kubasiewicz E, Zaleska M, Cooke CJ, Miller NE. Lymph draining from foot joints in rheumatoid arthritis provides insight into local cytokine and chemokine production and transport to lymph nodes. *Arthritis and rheumatism.* 2001;44(3):541-549.
38. Orr C, Vieira-Sousa E, Boyle DL, et al. Synovial tissue research: a state-of-the-art review. *Nature Reviews Rheumatology.* 2017;14:60.
39. Pitzalis C, Kelly S, Humby F. New learnings on the pathophysiology of RA from synovial biopsies. *Current opinion in rheumatology.* 2013;25(3):334-344.
40. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* 1995;38(1):44-48.
41. RSNA. Quantitative Imaging Biomarkers Alliance. Available at: http://www.rsna.org/QIBA.aspx. *Radiological Society of North America.* 2018.
42. Sakellariou G, Scire CA, Zambon A, Caporali R, Montecucco C. Performance of the 2010 classification criteria for rheumatoid arthritis: a systematic literature review and a meta-analysis. *PLoS One.* 2013;8(2):e56528.
43. Salliot C, Finckh A, Katchamart W, et al. Indirect comparisons of the efficacy of biological antirheumatic agents in rheumatoid arthritis in patients with an inadequate response to conventional disease-modifying antirheumatic drugs or to an anti-tumour necrosis factor agent: a meta-analysis. *Annals of the rheumatic diseases.* 2011;70(2):266-271.
44. Scott DL, Houssien DA. Joint assessment in rheumatoid arthritis. *Br J Rheumatol.* 1996;35 Suppl 2:14-18.
45. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet (London, England).* 2010;376(9746):1094-1108.
46. Smith MD, Kraan MC, Slavotinek J, et al. Treatment-induced remission in rheumatoid arthritis patients is characterized by a reduction in macrophage content of synovial biopsies. *Rheumatology (Oxford).* 2001;40(4):367-374.
47. Smolen JS, Aletaha D, Barton A, et al. Rheumatoid arthritis. *Nat Rev Dis Primers.* 2018;4:18001.

48. Townsend MJ. Molecular and cellular heterogeneity in the Rheumatoid Arthritis synovium: clinical correlates of synovitis. *Best practice & research Clinical rheumatology.* 2014;28(4):539-549.
49. Tran TN, Caspard H, Magrini F. Incidence density of serious infection, opportunistic infection, and tuberculosis associated with biologic treatment in patients with rheumatoid arthritis - a systematic evaluation of the literature. *Open Access Rheumatol.* 2013;5:21-32.
50. Uhlig T, Moe RH, Kvien TK. The burden of disease in rheumatoid arthritis. *Pharmacoeconomics.* 2014;32(9):841-851.
51. van de Sande MG, Baeten DL. Immunopathology of synovitis: from histology to molecular pathways. *Rheumatology (Oxford, England).* 2016;55(4):599-606.
52. van de Sande MG, de Hair MJ, Schuller Y, et al. The features of the synovium in early rheumatoid arthritis according to the 2010 ACR/EULAR classification criteria. *PLoS One.* 2012;7(5):e36668.
53. van der Linden MP, le Cessie S, Raza K, et al. Long-term impact of delay in assessment of patients with early arthritis. *Arthritis and rheumatism.* 2010;62(12):3537-3546.
54. Verstappen SM. Rheumatoid arthritis and work: The impact of rheumatoid arthritis on absenteeism and presenteeism. *Best Pract Res Clin Rheumatol.* 2015;29(3):495-511.
55. Vieira-Sousa E, Gerlag DM, Tak PP. Synovial tissue response to treatment in rheumatoid arthritis. *Open Rheumatol J.* 2011;5:115-122.
56. Vivar N, Van Vollenhoven RF. Advances in the treatment of rheumatoid arthritis. *F1000prime reports.* 2014;6:31.
57. Westra J, Doornbos-van der Meer B, de Boer P, van Leeuwen MA, van Rijswijk MH, Limburg PC. Strong inhibition of TNF-alpha production and inhibition of IL-8 and COX-2 mRNA expression in monocyte-derived macrophages by RWJ 67657, a p38 mitogen-activated protein kinase (MAPK) inhibitor. *Arthritis Res Ther.* 2004;6(4):R384-392.
58. Wijbrandts CA, Dijkgraaf MG, Kraan MC, et al. The clinical response to infliximab in rheumatoid arthritis is in part dependent on pretreatment tumour necrosis factor alpha expression in the synovium. *Annals of the rheumatic diseases.* 2008;67(8):1139-1144.
59. Wijbrandts CA, Vergunst CE, Haringman JJ, Gerlag DM, Smeets TJ, Tak PP. Absence of changes in the number of synovial sublining macrophages after ineffective treatment for rheumatoid arthritis: Implications for use of synovial sublining macrophages as a biomarker. *Arthritis Rheum.* 2007;56(11):3869-3871.
60. Yanni G, Whelan A, Feighery C, Bresnihan B. Synovial tissue macrophages and joint erosion in rheumatoid arthritis. *Annals of the rheumatic diseases.* 1994;53(1):39-44.

Appendix 1 Schedule of Events

Evaluation	Visit #1 Screening (Day -30 to -1)	Visit #2 (Day 0)					Visit #3 Telephone (Day 5 ± 3)	Visit 4 (5 ± 1 Week Post-Treatment Change)				
		- 00:30 to - 00:01	00:00	00:01 to 00:30	60 to 75 min	After Imaging[a]		- 00:30 to - 00:01	00:00	00:01 to 00:30	60 to 75 min	After Imaging
Informed consent	x											
Entry criteria	x											
Medical History, RA History, Demography	x											
2010 ACR/EULAR	x											
Vital signs	x	x		x				x		x		
Physical Examination	x											
DAS28	x							x				
CDAI[b]	x							x				
HAQ-DI©[b]	x							x				
WPI[b]	x							x				
Clinical laboratory evaluation: chemistry, hematology, UA	x					x						x
RA panel	x											x
Urine pregnancy test WCBP	x	x						x				
Tc 99m tilmanocept administration			x						x			
Planar Imaging: bilateral hands and wrists					x						x	
Concomitant medications	x	x					x	x				
AE monitoring	x	x	x	x	x	x	x	x	x	x	x	x

[a] Initiation of anti-TNFα bDMARD therapy change will occur following Day 0 procedures; [b] May be completed up to 30 days prior to imaging Visit 2 and up to 7 days prior to imaging Visit 4;

Evaluation	Visit #5 Telephone (5 ± 3 days after Visit 4)	Visit #6 (12 ± 1 Week Post-Treatment Change)	Visit #8 (24 ± 1 Week Post Treatment Change)
Informed consent			
Entry criteria			
Medical History, RA History, Demography			
2010 ACR/EULAR			
Vital signs			
Physical Examination			
DAS28[b]		x	x
CDAI[b]		x	x
HAQ-DI© [b]		x	x
WPI[b]		x	x
Clinical laboratory evaluation: chemistry, hematology, UA			
RA panel		x	x
Urine pregnancy test WCBP			
Tc 99m tilmanocept administration			
Planar Imaging: bilateral hands and wrists			
Concomitant medications	x		
AE monitoring	x	x	x

Note: Visits 7 and 9 will not be conducted per this protocol amendment

Appendix 2 2010 ACR/ EULAR Classification Criteria

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

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

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

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

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

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

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

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

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

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

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

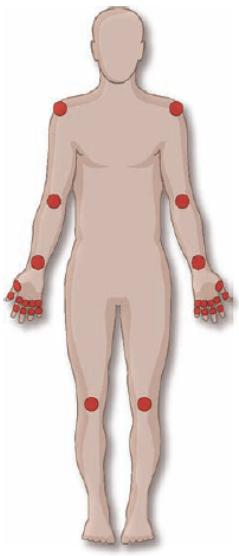
§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

Appendix 3 DAS28 Scale

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

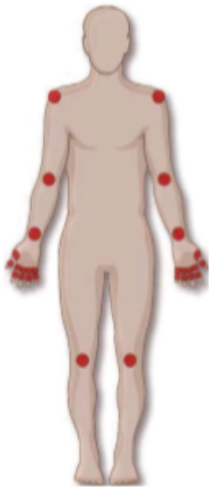
1. Perform a swollen and tender joint examination of your patient, noting each affected joint on Form A. When complete, add all of the swollen and tender joints and record the totals in the appropriate boxes on Form B.
2. Obtain and record the patient's erythrocyte sedimentation rate (ESR) in mm/h in 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 CDAI Scoring

Clinical Disease Activity Index (CDAI)

Joint	Left		Right	
	Tender	Swollen	Tender	Swollen
Shoulder				
Elbow				
Wrist				
MCP 1				
MCP 2				
MCP 3				
MCP 4				
MCP 5				
PIP 1				
PIP 2				
PIP 3				
PIP 4				
PIP 5				
Knee				
Total	Tender:		Swollen:	



Patient Global Assessment of Disease Activity

Considering all the ways your arthritis affects you, rate how well you are doing on the following scale:

Very Well 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10 Very Poor

Your Name_____ **Date of Birth** _____ **Today's Date** _____

Provider Global Assessment of Disease Activity

Very Well 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10 Very Poor

How to Score the CDAI

Variable	Range	Value
Tender joint score	(0-28)	
Swollen joint score	(0-28)	
Patient global score	(0-10)	
Provider global score	(0-10)	
Add the above values to calculate the CDAI score	**(0-76)**	

CDAI Score Interpretation	
0.0 – 2.8	Remission
2.9 – 10.0	Low Activity
10.1 – 22.0	Moderate Activity
22.1 – 76.0	High Activity

Appendix 5 ACR Response Criteria

ACR Response Criteria

The ACR (American College of Rheumatology) Criteria is a standard criteria to measure the effectiveness of various arthritis medications or treatments in clinical trials for Rheumatoid Arthritis. The ACR is used to maximally discrimate effective treatment for placebo treatment in clincal trials. The ACR criteria is indicated as ACR 20, ACR 50, or ACR 70. The ACR is reported as % improvement, comparing disease activity at two discrete time points (usually baseline and post-baseline comparison).

- **ACR20 is ≥ 20% improvement**
- **ACR50 is ≥ 50% improvement**
  - **ACR50 reponders include ACR20 responders**
- **ACR70 is ≥ 70% improvement**
  - **ACR70 reponders include ACR20 & ACR50 responders**

Definition

The ACR Criteria is a dichotomous variable with a positive (=responder) or negative (=non-reponder) outcome.

The ACR Criteria measures improvement in tender and swollen joint counts in improvement in at least three or the following parameters:

1. **Patient assessment**
2. **Physician assessment**
3. **Pain scale**
4. **Disability/functional questionnaire**
5. **Acute phase reactant (ESR or CRP)**

ACR 20 has a positive outcome if 20% improvement in tender and swollen joint counts were achieved as well as 20% improvement in at least three of the other five criteria.

ACR 50 has a positive outcome if 50% improvement in tender and swollen joint counts were achieved as well as 50% improvement in at least three of the other five criteria.

ACR 70 has a positive outcome if 70% improvement in tender and swollen joint counts were achieved as well as 70% improvement in at least three of the other five criteria.

Appendix 6 HAQ-DI© Questionnaire

HEALTH ASSESSMENT QUESTIONNAIRE (HAQ-DI)©

Name: _____ Date: _____

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
DRESSING & GROOMING				
Are you able to:				
Dress yourself, including shoelaces and buttons?	☐	☐	☐	☐
Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
Stand up from a straight chair?	☐	☐	☐	☐
Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
Cut your own meat?	☐	☐	☐	☐
Lift a full cup or glass to your mouth?	☐	☐	☐	☐
Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
Walk outdoors on flat ground?	☐	☐	☐	☐
Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Devices used for Dressing (button hook, zipper pull, etc.)
☐ Built up or special utensils
☐ Crutches
☐ Cane
☐ Wheelchair
☐ Special or built up chair
☐ Walker

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Dressing and grooming ☐ Arising ☐ Eating ☐ Walking

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
HYGIENE				
Are you able to:				
Wash and dry your body?	☐	☐	☐	☐
Take a tub bath?	☐	☐	☐	☐
Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
Reach and get down a 5 pound object (such as a bag of sugar) from above your head?	☐	☐	☐	☐
Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
Open car doors?	☐	☐	☐	☐
Open previously opened jars?	☐	☐	☐	☐
Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
Run errands and shop?	☐	☐	☐	☐
Get in and out of a car?	☐	☐	☐	☐
Do chores such as vacuuming or yard work?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Raised toilet seat ☐ Bathtub bar ☐ Long-handled appliances for reach

☐ Bathtub seat ☐ Long-handled appliances in bathroom ☐ Jar opener (for jars previously opened)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Hygiene ☐ Reach ☐ Gripping and opening things ☐ Errands and chores

Your ACTIVITIES: To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?

COMPLETELY	MOSTLY	MODERATELY	A LITTLE	NOT AT ALL
☐	☐	☐	☐	☐

Your PAIN: How much pain have you had IN THE PAST WEEK?
On a scale of 0 to 100 (where zero represents "no pain" and 100 represents "severe pain"), please record the number below.

☐☐☐

Your HEALTH: Please rate how well you are doing on a scale of 0 to 100 (0 represents "very well" and 100 represents "very poor" health), please record the number below.

☐☐☐

Courtesy of https://integrationacademy.ahrq.gov/center/default/files/-DI_0.pdf

Appendix 7 Sponsor Signatures

Study Title:	Evaluation of Tc 99m Tilmanocept Imaging for the Early Prediction of Anti-TNFα Therapy Response in Subjects with Moderate to Severe Active Rheumatoid Arthritis (RA)
Study Number:	NAV3-33
Original Protocol Date:	11 November 2021
Amendment 1 Date:	18 January 2022
Amendment 2 Date:	01 November 2022
Amendment 3 Date:	29 September 2023
Amendment 4 Date:	30 January 2024

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: DocuSigned by: Simon Blackburn — Date: 2/1/2024
Signer Name: Simon Blackburn
Signing Reason: I approve this document
Signing Time: 2/1/2024 | 10:18:58 AM PST
BB67D4AF1F9340EA8ABE937FC91BF60
Simon Blackburn
Director, Clinical Development
Navidea Biopharmaceuticals

Signed: DocuSigned by: Joanna Shuping — Date: 2/1/2024
Signer Name: Joanna Shuping
Signing Reason: I have reviewed this document
Signing Time: 2/1/2024 | 12:03:06 PM PST
Joanna Shuping
Director, Clinical Operations
Navidea Biopharmaceuticals

Signed: DocuSigned by: — Date: 2/1/2024
Signer Name: Michael Blue
Signing Reason: I approve this document
Signing Time: 2/1/2024 | 10:43:12 AM PST
Michael S. Blue, MD
Chief Medical Officer
Navidea Biopharmaceuticals

Signed: DocuSigned by: — Date: 2/2/2024
Signer Name: Richard McFerron
Signing Reason: I approve this document
Signing Time: 2/2/2024 | 7:39:59 AM PST
Rich McFerron
Sr. Director, Regulatory Affairs
Navidea Biopharmaceuticals

Appendix 8 Investigator's Signature

Study Title:	Evaluation of Tc 99m Tilmanocept Imaging for the Early Prediction of Anti-TNFα Therapy Response in Subjects with Moderate to Severe Active Rheumatoid Arthritis (RA)
Study Number:	NAV3-33
Original Protocol Date:	11 November 2021
Amendment 1 Date:	18 January 2022
Amendment #2:	01 November 2022
Amendment #3	29 September 2023
Amendment # 4:	30 January 2024

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:_____