Clinical Trial Protocol: NAV3-31

Study Title:	Evaluation of the Precision and Sensitivity of Tilmanocept Uptake Value (TUV) on Tc 99m Tilmanocept Planar Imaging
Study Number:	NAV3-31
Study Phase:	Phase 2b
Product Name:	Technetium Tc 99m tilmanocept
IND Number:	132943
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Amendment #2	19 April 2019
Amendment #3	24 April 2019
Amendment #4	02 February 2021

SYNOPSIS

Study Title:
Evaluation of the Precision and Sensitivity of Tilmanocept Uptake Value (TUV) on Tc 99m Tilmanocept Planar Imaging

Study Number:
NAV3-31

Study Phase: Phase 2b

Primary Objective(s):

- To assess the longitudinal precision of joint-specific (TUV_{joint}) and global (TUV_{global}) TUVs on planar imaging in subjects with clinically diagnosed active rheumatoid arthritis (RA) on stable anti-rheumatic therapy.
- To evaluate the camera-specific precision of TUV_{joint} and TUV_{global} on planar imaging in healthy controls (HCs) and in subjects with clinically diagnosed active RA using centralized and standardized imaging parameters.
- To assess the correlation of TUV and changes in TUV with changes in clinical assessments at multiple time points after initiation of a new anti-tumor necrosis factor alpha (TNFα) biologic disease-modifying antirheumatic drug (bDMARD) therapy.

Secondary Objective(s):

- To assess the temporal post-injection stability of TUV_{joint} and TUV_{global} at a dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m.
- To establish normal ranges for TUV_{joint} in HCs.
- To assess Tc 99m tilmanocept anatomic localization based on single photon emission computed tomography/ computed tomography (SPECT/CT) imaging of the synovial space in hands and wrists.

Safety Objective:

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

Inclusion Criteria:

ALL SUBJECTS

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

HEALTHY CONTROL SUBJECTS

3. The subject is between 18 and 80 years of age at the time of consent.
4. The subject is deemed to be clinically free of any inflammatory disease(s) and has not experienced joint pain for at least 28 days prior to the consent date.
5. The subject is not currently on anti-inflammatory drugs (including non-steroidal anti-inflammatory drugs [NSAIDs]) and has not taken any anti-inflammatories for at least 28 days prior to the consent date.
6. For all ongoing concomitant medications, the subject has maintained a stable dose for at least 28 days prior to the consent date.

RA SUBJECTS

3. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis.
4. The subject has moderate to severe RA as determined by the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria (score of ≥ 6/10).
5. The subject has a 28-joint disease activity score (DAS28) of ≥ 3.2 (includes the Erythrocyte Sedimentation Rate [ESR] test and Visual Analog Scale [VAS]).
6. Subjects receiving traditional DMARDs must have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the first imaging visit (Day 0).
7. If the subject is receiving bDMARD or janus kinase (JAK) inhibitor therapy, they have been at a stable dose > 180 days prior to the first imaging visit (Day 0).
8. If the subject is receiving NSAIDs or oral corticosteroids, the dose has been stable for > 28 days prior to first imaging visit (Day 0). The corticosteroid dose must be ≤ 10 mg/day of prednisone or an equivalent steroid dose.
9. **ARM 3 (only):** The subject is receiving anti-rheumatic treatment and is a candidate for initiation of, or change to, a new anti-TNFα bDMARD treatment.

Exclusion Criteria:

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator.
3. The subject has had or is currently receiving radiation therapy or chemotherapy.
4. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.

5. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 3 times the upper limit of normal.
6. 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.
7. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
8. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration (Day 0).
9. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to the first imaging visit (Day 0).
10. 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).

Study Design:
This is a prospective, open-label, multicenter, single and repeat-dose study designed to evaluate the reliability and sensitivity of TUV assessments in HCs and subjects with active RA.

TUV is a quantitative imaging metric used to characterize Tc 99m tilmanocept uptake on planar imaging. Results from a prior Phase 1 and 2 study have demonstrated that TUV is a sensitive and specific predictor of visually interrogated Tc 99m tilmanocept localization in joint regions with presumed inflammatory macrophage activity. A per-joint TUV (TUV_{joint}) relative ratio will be calculated for the each of the 22 DAS-28 joints located in the hands and wrists. A subject-level global TUV (TUV_{global}) assessed across the 22 joints will be used as an indication of overall disease burden. TUV metrics are further described in the NAV3-31 Statistical Analysis Plan.

This study is stratified into 3 arms. The first 2 arms, comprised of [1] disease-free HCs and [2] clinically diagnosed RA subjects on stable treatment, respectively, are designed to apprise the *image re-image* and/or *test re-test* (i.e., repeat dose) consistency of joint-specific and global TUVs across a variety of image acquisition intervals including:

- Consecutive (15-minute) acquisitions—for the assessment of TUV precision in relation to camera settings and standardization (*Study Arms 1 and 2*);
- Temporal (1-hour and 3-hour) acquisitions—for the assessment of TUV stability across a 2-hour time window that is conducive to variable patient workflow in clinical practice (*Study Arms 1 and 2*), and;
- Longitudinal (8 ± 1-day) acquisitions—for the assessment of TUV precision in the context of disease-specific Tc 99m tilmanocept pharmacodynamics (*Study Arm 2 only*).

The third arm is designed to assess the efficacy of TUV_{global} in clinically diagnosed subjects with active RA who are candidates for initiation of, or change to, a new anti-TNFα bDMARD therapy. Specific evaluations will include:

- *Sensitivity*—the capacity for TUV_{global} to detect changes in disease activity from baseline to 5 ± 1 weeks of new anti-TNFα bDMARD treatment ($\Delta TUV_{global[5w]}$), and;
- *Clinical concordance*—the correlation between the change in TUV_{global} from baseline to 5 ± 1 weeks post-therapy and change in established rheumatological measures of disease response from baseline to 12 ± 1 and 24 ± 1 weeks post-therapy including Clinical Disease Activity Index ($\Delta CDAI_{12/24w}$) and ACR Response ($ACR_{12/24w}$).

Methodology:

<u>Study Arm 1:</u> HC Subjects Free of Inflammatory Disease

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

- Informed consent
- Review of study eligibility
- Collection of medical history (including medications)
- Vital sign assessment
- Physical examination (including height and weight)
- Clinical labs
- RA-specific labs
- Urinalysis
- Urine pregnancy test for subjects of childbearing potential
- 2010 ACR/ EULAR score

Visit 2 (Tc 99m Tilmanocept Administration and Imaging; Day 0)
<u>Pre-Tc 99m Tilmanocept Administration</u>

- Urine pregnancy test for women of child-bearing potential
- Electrocardiogram (ECG) up to 30 minutes prior to drug administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

<u>Tc 99m Tilmanocept Administration:</u> Subjects will receive a single intravenous (IV) dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

<u>0-30 Minutes Post-Tc 99m Tilmanocept Administration</u>

- ECG
- Post-ECG vital sign assessment

- AE assessment

<u>60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration</u>

- AE assessment
- Image acquisition (in the following order)
    1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
    2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
    3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
    4. Consecutive planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint.

<u>180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration</u>

- AE assessment
- Image acquisition (in the following order)
    1. Planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
    2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
    3. Consecutive planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
    4. Consecutive planar scan of the bilateral hands and wrists
- Clinical labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- AE assessment

Study Arm 2: RA Subjects on Stable Therapy

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

- Informed consent
- Review of study eligibility
- Collection of medical history (including medications)
- Vital sign assessment
- Physical examination (including height and weight)
- Clinical labs
- RA-specific labs
- Urinalysis
- Urine pregnancy test for subjects of childbearing potential
- 2010 ACR/EULAR score
- DAS28 evaluation

Visit 2 (Tc 99m Tilmanocept Administration and Imaging; Day 0)
Pre-Tc 99m Tilmanocept Administration

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to drug administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- Adverse event (AE) assessment

Tc 99m Tilmanocept Administration: Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration:

- AE Assessment
- Image acquisition (in the following order):
    1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
    2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;

3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
4. Consecutive planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint.

<u>180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration</u>

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
    2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
    3. Consecutive planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
    4. Consecutive planar scan of the bilateral hands and wrists.
- Clinical labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- AE assessment

Visit 4 (Tc 99m Tilmanocept Administration and Imaging; Day 8 ± 1)
<u>Pre-Tc 99m Tilmanocept Administration</u>

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to drug administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

<u>Tc 99m Tilmanocept Administration:</u> Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

<u>0-30 Minutes Post-Tc 99m Tilmanocept Administration</u>

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration:

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition)
    2. Planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint

180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
    2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 15 minutes) for the subsequent SPECT/CT scan
    3. SPECT/CT scan of the bilateral hands and wrists.
- Clinical labs (after imaging)
- Urinalysis (after imaging)

Visit 5 (Follow-up Telephone Safety Assessment; 1-3 days following Visit 4)

- Review of concomitant medications
- AE assessment

Study Arm 3: RA Subjects who are Candidates for Initiation of, or Change to, a New Anti-TNFα bDMARD Therapy*

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

- Informed consent
- Review of study eligibility
- Collection of medical history (including medications)
- Vital sign assessment
- Physical examination (including height and weight)
- Clinical labs
- RA-specific labs
- Urinalysis
- Urine pregnancy test for women of childbearing potential
- 2010 ACR/EULAR score
- DAS28 evaluation
- Health Assessment Questionnaire Disability Index (HAQ-DI©)
- CDAI

- Widespread Pain Index (WPI)

Visit 2 (Baseline Tc 99m Tilmanocept Administration and Imaging; Day 0)
Pre-Tc 99m Tilmanocept Administration

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to drug administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

Tc 99m Tilmanocept Administration: Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of whole body
    2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- Urinalysis (after imaging)

Upon the completion of all Visit 2 procedures, subjects will initiate their new anti-TNFα bDMARD treatment regimen.

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

- Review of concomitant medications
- AE assessment

Visit 4 (Week 5 ± 1 Tc 99m Tilmanocept Administration, Imaging, and Clinical Assessments)
Pre-Tc 99m Tilmanocept Administration (0-7 days pre-injection)

- CDAI
- DAS28
- HAQ-DI©
- WPI

Pre-Tc 99m Tilmanocept Administration (day of injection)

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to tilmanocept administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

Tc 99m Tilmanocept Administration: Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of whole body
    2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- RA-specific labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- AE assessment

Visit 6 (Week 12 ± 1 Tc 99m Tilmanocept Administration, Imaging, and Clinical Assessments*)

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

- CDAI
- DAS28
- HAQ-DI©
- WPI

Pre-Tc 99m Tilmanocept Administration (day of injection)

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to tilmanocept administration

- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

Tc 99m Tilmanocept Administration: Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of whole body
    2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- RA-specific labs (after imaging)
- Urinalysis (after imaging)

Visit 7 (Follow-up Telephone Safety Assessment; 5 ± 3 Days After Visit 6)

- Review of concomitant medications
- AE assessment

Visit 8 (Week 24 ± 1 Tc 99m Tilmanocept Administration, Imaging, and Clinical Assessments*)

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

- CDAI
- DAS28
- HAQ-DI©
- WPI

Pre-Tc 99m Tilmanocept Administration (day of injection)

- Urine pregnancy test for women of child-bearing potential
- ECG up to 30 minutes prior to tilmanocept administration
- Post-ECG vital sign assessment
- Review of concomitant medications
- AE assessment

Tc 99m Tilmanocept Administration: Subjects will receive a single IV dose of 150 mcg tilmanocept radiolabeled with 10 mCi (370 MBq) of Tc 99m.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

- ECG
- Post-ECG vital sign assessment
- AE assessment

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
    1. Planar scan of whole body
    2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- RA-specific labs (after imaging)
- Urinalysis (after imaging)

Visit 9 (Follow-up Telephone Safety Assessment; 5 ± 3 Days After Visit 8)

- Review of concomitant medications
- AE assessment

*If a subject in Arm 3 requires early exit from the study between Week 16 and Week 24, an attempt should be made to complete a final imaging visit and all clinical assessments.

Test Product, Dose, and Mode of Administration:

Tc 99m tilmanocept will be administered through an IV route of injection. A 150-mcg dose containing 10 mCi of Tc 99m will be injected as a slow push into the IV catheter. At the completion of the injection, a 10-mL sterile normal saline flush will be administered. The preferred site of IV placement will be between the elbow and the wrist.

Planned Number of Study Centers:

Up to 10 centers in the United States

Sample Size:

105 evaluable subjects in Arms 1, 2, and 3 as allocated below. Arms 1 and 2 will have N = 38 evaluable subjects per Arm, and Arm 3 will have N = 29 evaluable subjects. A subject is considered evaluable if he/she meets the criteria for the analysis population and has the data necessary for computing the primary endpoint.

Rationale for Sample Size:

The sample size for Arm 1 was determined to provide sufficient data to quantify the precision of TUV in HCs. The sample size of Arm 2 was determined to power a hypothesis test of

$$H_O: \pi \leq 0.80$$

$$H_A: \pi > 0.80$$

at 80%. Here, π represents the fraction of the absolute differences less than 7.5%. The sample size for Arm 3 was determined to set the length of the lower 97.5% confidence limit for Spearman's rank correlation to be no more than 0.15.

After the injection, imaging, and quantitation of a total of 4 subjects in Arms 1 and 2, an interim analysis (interim analysis 1) will be held for the review of quantitative TUV results and assessment of imaging parameters and logistics. A second interim analysis (interim analysis 2) will be held after a total of 30 subjects (≥ 15 RA) in Arms 1 and 2 are injected, imaged, and quantified for the review of intra-and inter-subject TUV variation to contribute to the power analysis of a future Phase 3 study and/or to terminate the study on grounds of data sufficiency.

After 15 subjects in Arm 3 have completed Visit 4, an interim analysis (interim analysis 3) will be performed to estimate the distribution of treatment effects on TUV and to optimize the TUV metric based on the interim data from all three Arms.

Efficacy Assessments:

Primary Endpoint(s)

ARMS 1-2

- The camera-specific precision of TUV_{joint} and TUV_{global} in HCs and subjects with active RA, which is defined as the Root Mean Square Difference (RMSD) between the consecutive 15-minute planar images.
- The stability of the mean/variance relationship, which is assessed by comparing the Coefficient of Variation (CV) of TUV_{joint} and TUV_{global} in HCs and subjects with active RA.

ARM 2

- The longitudinal (8-day) precision of TUV_{joint} and TUV_{global} in subjects with active RA, which is defined as the RMSD of images at the same time point.

ARM 3

- The correlation of $\Delta TUV_{global[5w]}$ 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). The correlation of $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy from baseline

to 12 ± 1 weeks and 24 ± 1 weeks defined by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).

Secondary Endpoint(s)

ARMS 1-2

- The temporal stability of the 150 mcg tilmanocept mass dose/10 mCi radiolabeling dose

ARM 1

- The normal ranges of TUV_{joint} in HC subjects, which is defined as the 5 and 95 percentiles of:
  - TUV_{joint} of bilateral joints (i.e., bilateral wrists, metacarpophalangeal joint [MCPs], proximal interphalangeal [PIPs], knees, elbows, shoulders)

ARM 2

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

ARM 3

- The correlation of the $TUVglobal_{[0week]}$ 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) and by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).
- The correlation of $\Delta TUV_{global[12w]}$ and $\Delta TUV_{global[24w]}$ 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) and by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).
- The correlation of $\Delta TUV_{global[5w]}$ and constituent parameters of $\Delta CDAI_{12/24w}$ and $ACR_{12/24w}$ including:
  1. Tender Joint Count (TJC)
  2. Swollen Joint Count (SJC)
  3. Patient assessment of global disease activity
  4. Rheumatologist assessment of global disease activity
  5. Patient assessment of pain
  6. Patient assessment of physical function
  7. Acute-phase reactant value

Statistical Methods:

ARMS 1-2

Longitudinal precision and camera-specific precision will be analyzed with confidence intervals for Root Mean Square Difference (RMSD) and the mean difference (i.e., the bias) between the relevant time points. Confidence intervals (95% confidence level)

will be calculated using Normal theory results (Lin 2002). As a sensitivity measure, the 80th and 90th percentiles of the distribution of the absolute differences will computed using quantile methods.

Stability of the mean/variance relationship will be assessed with a 95% confidence interval for the difference between the CV for RA and HC subjects at each time point. If the CV for both groups at an imaging time point is less than 0.30, a 95% confidence interval based on the methods outlined in (Forkman 2009) will be provided. Because Forkman's methods are based on Normal theory, a 95% bootstrap confidence interval will also be provided for all time points.

Temporal stability of the 150 mcg dose will be assessed with a 95% Student's T interval for the difference between the TUV at 60 minutes and at 180 minutes. As a sensitivity check, a signed-rank test of the hypotheses:

$$H_0: \mu_{180} - \mu_{60} = 0$$
$$H_1: \mu_{180} - \mu_{60} \neq 0$$

where μ_{180} represents the median TUV at 180 minutes and μ_{60} represents the median TUV at 60 minutes, will be provided.

ARM 1

Normal ranges in HC subjects will be estimated by pooling similar joints and computing the average TUV per joint per subject. The 5 and 95 percentiles will be estimated using quantile regression methods. Joints to be pooled include the following:

- Shoulders (bilateral, 2 per subject)
- Knees (bilateral, 2 per subject)
- Wrists (bilateral, 2 per subject)
- MCP (bilateral, 5 per hand, 10 per subject)
- PIP (bilateral, 5 per hand, 10 per subject)

ARM 2

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

ARM 3

The utility of $\Delta TUV_{global[5w]}$ to predict the subject's response to changed therapy will be assessed by computing the Kendall rank correlation between $\Delta TUV_{global[5w]}$ and $\Delta CDAI_{12w}$,$\Delta CDAI_{24w}$, ΔACR_{12w}, and ΔACR_{24w}. All times are relative to Study Day 0, and it is assumed that the new bDMARD therapy will begin after imaging on Day 0. A 95% confidence interval on the value of the rank correlation will be computed.

Date of Original Approved Protocol: 16 November 2018

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$\Delta CDAI_{12/24w}$	Change in Clinical Disease Activity Index from 12 ± 1 weeks / 24 ± 1 weeks of newly initiated bDMARD therapy and baseline
$\Delta TUV_{global[5w]}$	Difference (change) of TUV_{global} from 5 ± 1 weeks of newly initiated bDMARD therapy and baseline
$\Delta TUV_{global[12w]}$	Difference (change) of TUV_{global} from 12 ± 1 weeks of newly initiated bDMARD therapy and baseline
$\Delta TUV_{global[24w]}$	Difference (change) of TUV_{global} from 24 ± 1 weeks of newly initiated bDMARD therapy and baseline
ACPA	Anti-Citrullinated Peptide Antibody
ACR	American College of Rheumatology
$ACR_{12/24w}$	American College of Rheumatology response criteria at 12 ± 1 weeks / 24 ± 1 weeks of newly initiated bDMARD therapy and baseline
ACR20/50/70	American College of Rheumatology response criteria
ACR/EULAR	American College of Rheumatology/European League Against Rheumatism
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
bDMARD	Biological disease-modifying antirheumatic drug
BMI	Body mass index
CD206	Mannose-binding receptor (Ca2+-binding lectin)
CDAI	Clinical disease activity index
CFB	Change from baseline
CRF	Case report form
CRO	Contract research organization
CRP	C-reactive protein
CV	Coefficient of variation
DAS28	Disease activity score -28 for RA describing the severity of RA using clinical and laboratory data.
DMARD	Disease-modifying antirheumatic drug
DTPA	Diethylenetriaminepentaacetic Acid

ECG	Electrocardiogram
eCRF	Electronic case report form
ESR	Erythrocyte sedimentation rate
GCP	Good Clinical Practice
HAQ-DI©	Health Assessment Questionnaire - Disability Index
HC	Healthy control
ICF	Informed consent form
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
ITD	Intent to diagnose
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
MCP	Metacarpophalangeal joint
NSAID	Non-steroidal anti-inflammatory drug
p.i.	Post injection
PIP	Proximal interphalangeal
PK	Pharmacokinetics
PP	Per Protocol
QT	Interval from the Q wave to the end of the T wave
QTc	Corrected QT interval
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RMSD	Root mean square difference
ROC	Receiver operating characteristic
ROI	Region of interest
RR	Reference region
RSNA	Radiological Society of North America
SAP	Statistical analysis plan

SJC	Swollen joint count
SC	Sulfur colloid
SOC	System organ class
SPECT	Single-photon emission computerized tomography
SPECT/CT	Combined single-photon emission computerized tomography and computed tomography
TJC	Tender joint count
TMF	Trial master file
TNFα	Tumor necrosis factor alpha
TUV	Tilmanocept uptake value
TUV_{global}	Global tilmanocept uptake value
TUV_{joint}	Joint-specific tilmanocept uptake value
UC	Uncertainty coefficient
ULN	Upper limit of normal
VAS	Visual analog scale
WB	Whole body
WPI	Widespread pain index

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 2017a, 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 2017a). 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 2017b). 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 an optimal treatments to all RA patients.

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

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

The second reason why Tc 99m tilmanocept imaging may be preferred to synovial biopsies for evaluations of RA patients is that synovial biopsies are only performed on patients with inflamed synovia that have expanded in volume beyond a certain grade, thereby enabling extraction of sufficient tissue to assess histologically. This could be a problem when evaluating patients in the early phase of symptomatic RA disease when high densities of activated macrophages have begun to aggregate into the inflamed synovial membrane but the synovial membrane may not yet have expanded (i.e. thickened) sufficiently to permit biopsy sampling. As discussed below, there is an urgent need to more accurately identify RA patients as early in the disease process as possible and place them on DMARD therapy immediately to provide these patients with their best possible therapy responses.

The third reason why Tc 99m tilmanocept imaging may be preferred to synovial biopsies for evaluations of RA patients is that, although synovial biopsy procedures typically extract 6-14 samples of tissue from each biopsied joint, in about 5% to 10% of cases, they do not provide tissue of sufficient quantity or quality to enable adequate histological evaluations of the inflamed synovial tissue (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 $\mathrm{SUV} = \frac{\mathrm{r}}{(\mathrm{a}'/\mathrm{w})}$. (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 decay-corrected average pixel intensity of a ROI, and B, the

decay-corrected pixel intensity of an anatomically defined area of the cerebral vasculature (serving as an intra-patient reference region), such that $\text{TUV} = \frac{\bar{x}}{B} \times 100$. Thus, the overall purpose of this study will be to test the reliability and clinical utility of TUV.

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

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

1.2.1 Nonclinical Evaluations –Subcutaneous Administration

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

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

1.2.2 Nonclinical Evaluations – IV Administration

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

Table 1 Preclinical Tests

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

1.2.3 Clinical Pharmacokinetics (IV)

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

Subject-level whole blood PK parameters were assessed between HC subjects (n = 6) and subjects with active RA (n = 6) to evaluate potential differences between mean maximum concentration (C_{max}), mean area under the concentration-time curve (AUC_{0-t}), mean area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), mean clearance, mean half-life ($t_{1/2}$), or mean elimination rate constant (λ_z) across each disease group (Table 2). The geometric mean of whole blood clearance was 26.5 mL/min for HC subjects and 24.8 mL/min for RA patients.

Table 2 Whole Blood PK Parameter Summaries by Group

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

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

Table 3 Urine PK Parameter Summaries by Group

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

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

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

1.2.4 Clinical Efficacy

1.2.4.1 NAV3-23 (SC)

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

- Based on data from this study and parallel pathology studies, Tc 99m tilmanocept localizes to activated macrophage-infiltrated joints at doses of 50 µg and 200 µg radiolabeled with 2.0 mCi (74.0 MBq) by SC administration.
- Across all combined RA subjects, swollen/tender joints demonstrating the highest proportions of localization include the wrists and knees.
- Based on qualitative image evaluation, Tc 99m tilmanocept does not show differences in localization between 2 to 3 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 and 180 ± 15 minutes p.i. Clinical efficacy conclusions for this study are still under review.

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, ECG parameters, and vital signs. The safety evaluation included all subjects who were enrolled in the study and administered Tc 99m tilmanocept (n = 39). There were no Tc 99m tilmanocept related AEs. There were no deaths during the trial; no SAEs; and no AEs that led to discontinuation from the trial.

2 STUDY OBJECTIVES

2.1 Primary Objective(s)

- To assess the longitudinal precision of TUV_{joint} and global TUV_{global} on planar imaging in subjects with clinically diagnosed active RA on stable anti-rheumatic therapy.
- To evaluate the camera-specific precision of TUV_{joint} and TUV_{global} on planar imaging in HCs and in subjects with clinically diagnosed active RA using centralized and standardized imaging parameters.
- To assess the correlation of TUV and changes in TUV with changes in clinical assessments at multiple time points after initiation of a new anti-tumor necrosis factor alpha (TNFα) biologic disease-modifying antirheumatic drug (bDMARD) therapy.

2.2 Secondary Objective(s)

- To assess the temporal post-injection stability of TUV_{joint} and TUV_{global} at a dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m.
- To establish normal ranges for TUV_{joint} in HCs.
- To assess Tc 99m tilmanocept anatomic localization based on SPECT/CT imaging of the synovial space in hands and wrists

2.3 Safety Objective(s)

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

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a prospective, open-label, multicenter, single and repeat-dose study designed to evaluate the reliability and sensitivity of TUV assessments in HCs and subjects with active RA. Subjects will be enrolled in 1 of 3 study arms (Table 4) with distinct timelines, study procedures, and study durations in accordance with Arm-specific eligibility requirements.

Table 4 Study Arms

Arm	Subjects	Evaluation(s)
1	HC subjects clinically free of any inflammatory disease and/or joint pain	Image Re-image
2	Subjects with clinically diagnosed active RA who have been on stable anti-rheumatic therapy	Image Re-image Test Re-test
3	Subjects with clinically diagnosed active RA who are candidates for initiation of, or change to, a new anti-TNFα bDMARD treatment.	Sensitivity Clinical Concordance

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

3.1.1.1 Description of Patient Population

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

3.1.1.2 Overview of Study Procedures

Refer to Section 7.1 for a detailed description of all study procedures. Refer to Appendix 2 for a schedule of events and Appendix 4 for a sequential diagram of study procedures.

3.1.1.3 Justification for Population

There are 2 reasons for the inclusion of this study population. The first is to evaluate the image re-image precision of TUV_{joint}. Identical image acquisitions performed up to 15 minutes apart (e.g., planar WB scan and planar bilateral hand/wrist scan followed by planar WB scan and planar bilateral hand/wrist scan 15 minutes later) will provide important information about potential differences in TUVs in the context of standardized imaging protocols. Identical image acquisitions performed 2 hours apart (e.g., planar WB scan at 60 ± 15 minutes post-drug administration and planar WB scan at 180 ± 15 minutes post-drug administration) will provide important information about any potential time-dependent pharmacokinetic effects of Tc 99m

tilmanocept on resultant TUVs. Both image re-image evaluations are ultimately designed to summarize the overall stability of TUV.

The second reason is to establish normal ranges for TUV_{joint}.. By determining the upper limit of normal (ULN) for average TUV_{joint} across pooled joints (e.g., MCPs 1-5) across patients, these assessments can be further investigated for their utility in providing clinically meaningful reference data for symptomatic individuals presenting to the clinic.

3.1.2 Arm 2: Stable Anti-Rheumatic Therapy (RA Subjects)

3.1.2.1 Description of Patient Population

This arm will be comprised of subjects with clinically diagnosed active RA on stable anti-inflammatory and/or anti-rheumatic therapy. All subjects' RA diagnoses will be moderate to severe in accordance with a 2010 ACR/EULAR score of 6 or higher. All subjects receiving traditional DMARDs must have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the first imaging visit; all subjects receiving bDMARDs or JAK inhibitors must have been on stable therapy for at least 180 days prior to the first imaging visit; and all subjects receiving NSAIDs and/or oral corticosteroids must have been on therapy for at least 28 days prior to the first imaging visit.

3.1.2.2 Overview of Study Procedures

Subjects in this arm will have a total of 3 on-site visits and 2 telephone safety assessments. The maximum possible study duration for these subjects will be 42 days.

Refer to Section 7.1 for a detailed description of all study procedures. Refer to Appendix 2 for a schedule of events and Appendix 4 for a sequential diagram of study procedures.

Justification for Population

There are 2 reasons for the inclusion of this study population. The first is to evaluate the image re-image precision of TUV_{joint} and TUV_{global}. Identical image acquisitions performed up to 15 minutes apart (e.g., planar WB scan and planar bilateral hand/wrist scan followed by planar WB scan and planar bilateral hand/wrist scan 15 minutes later) will provide important information about potential differences in TUVs in the context of standardized imaging protocols. Identical image acquisitions performed 2 hours apart (e.g., planar WB scan at 60 ± 15 minutes post-drug administration and planar WB scan at 180 ± 15 minutes post-drug administration) will provide important information about any potential time-dependent pharmacokinetic effects of Tc 99m tilmanocept on resultant TUVs.

The second reason is to determine the longitudinal test re-test precision of TUV_{joint} and TUV_{global}. Identical image acquisitions performed 8 ± 1 days apart (e.g., planar WB scan at 60 ± 15 minutes compared with planar WB scan at 60 ± 15 minutes 8 ± 1 days after the first planar WB scan) will provide important information about the pharmacodynamic stability of Tc 99m tilmanocept as it relates to standardized TUV derivations. The overall purpose of these evaluations will be to determine if longitudinally repeated injection and imaging can provide

reliable quantitative data with minimal variation (assuming stable disease and treatment). The results from these assessments will have significant translational importance for the clinical utility of TUVs in the stable monitoring of changes in disease activity over time.

3.1.3 Arm 3: Change in anti-TNFα bDMARD Therapy (RA Subjects)

3.1.3.1 Description of Patient Population

This arm will be comprised of subjects with clinically diagnosed active RA who are candidates for initiation of, or change to, a new anti-TNFα bDMARD treatment. All subjects' RA diagnoses will be moderate to severe in accordance with a 2010 ACR/EULAR score of 6or higher. All subjects receiving traditional DMARDs must have been on therapy for ≥ 90 days and at a stable dose for ≥ 30 days prior to the first imaging visit; all subjects receiving bDMARDs or JAK inhibitors must have been on stable therapy for at least 180 days prior to the first imaging visit; and all subjects receiving NSAIDs and/or oral corticosteroids must have been on therapy for at least 28 days prior to the first imaging visit. Upon completion of the first set of imaging assessments, all subjects will begin their new anti-TNFα bDMARD therapeutic regimen.

3.1.3.2 Overview of Study Procedures

Subjects in this arm will have a total of 5 on-site visits and 4 telephone safety assessments. The maximum possible study duration for these subjects will be 213 days.

Refer to Section 7.1 for a detailed description of all study procedures. Refer to Appendix 2 for a schedule of events and Appendix 4 for a sequential diagram of study procedures.

3.1.3.3 Justification for Population

There are 2 reasons for the inclusion of this study population. The first is to determine the sensitivity of TUV_{global} towards the detection of anti-TNFα bDMARD therapy-driven changes in CD206 macrophage activity after 5 ± 1 weeks of treatment. By establishing that TUV_{global} has sufficient sensitivity to detect such changes, the predictive utility of this metric for downstream clinical outcomes can be further investigated.

The second reason, which is strongly predicated on the results of the first reason, is to establish a preliminary understanding of how changes from baseline of TUV_{global} to 5 ± 1 weeks of a new anti-TNFα bDMARD treatment correlate with changes in clinical response parameters. Refer to Section 8.3 for an overview of all clinical parameters under investigation.

3.2 Protocol Adherence

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

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

3.3 Study Duration(s)

Study duration will be contingent upon study arm. The maximum possible study durations for Arms 1, 2, and 3 are 38 days, 42 days, and 213 days, respectively.

Table 5 Study Duration for Arms 1-3

Study Arm	Maximum Possible Study Duration
1	38 days
2	42 days
3	213 days

4 STUDY POPULATION

4.1 Study Arms

The study population will be comprised of HC subjects, subjects with clinically diagnosed active RA on stable anti-rheumatic therapy, and subjects with clinically diagnosed active RA who are candidates for initiation of, or change to, a new anti-TNFα bDMARD regimen (see Table 4).

4.2 Eligibility

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

4.2.1 Inclusion Criteria

ALL SUBJECTS

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

HEALTHY CONTROL SUBJECTS

3. The subject is between 18 and 80 years of age at the time of consent.
4. The subject is deemed to be clinically free of any inflammatory disease(s) and has not experienced joint pain for at least 28 days prior to the consent date.
5. The subject is not currently on anti-inflammatory drugs (including NSAIDs) and has not taken anti-inflammatories for at least 28 days prior to the consent date.
6. For all ongoing concomitant medications, the subject has maintained a stable dose for at least 28 days prior to the consent date.

CLINICALLY DIAGNOSED ACTIVE RA SUBJECTS

3. The subject is at least 18 years of age and was ≥ 18 years of age at the time of RA diagnosis.
4. The subject has moderate to severe RA as determined by the 2010 ACR/EULAR (score of ≥ 6/10) classification criteria.

5. The subject has a 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. If the subject is receiving bDMARD or JAK inhibitor therapy, they have been at a stable dose > 180 days prior to the first imaging visit (Day 0).
8. If the subject is receiving NSAIDs (nonsteroidal anti-inflammatory drugs) or oral corticosteroids, the dose has been stable for > 28 days prior to first imaging visit (Day 1). The corticosteroid dose must be ≤ 10mg/day of prednisone or an equivalent steroid dose.
9. **ARM 3 (only):** The subject is receiving anti-rheumatic treatment and is a candidate for initiation of, or change to, a new anti-TNFα bDMARD therapy.

4.2.2 Exclusion Criteria

1. The subject is pregnant or lactating.
2. The subject size or weight is not compatible with imaging per the investigator.
3. The subject has had or is currently receiving radiation therapy or chemotherapy.
4. The subject has renal insufficiency as demonstrated by a glomerular filtration rate of < 60 mL/min.
5. The subject has hepatic insufficiency as demonstrated by ALT (alanine aminotransferase [SGPT]) or AST (aspartate aminotransferase [SGOT]) greater than 3 times the upper limit of normal.
6. 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.
7. The subject has a known allergy to or has had an adverse reaction to dextran exposure.
8. The subject has received an investigational product within 30 days prior to the Tc 99m tilmanocept administration.
9. The subject has received intra-articular corticosteroid injections ≤ 8 weeks prior to the first imaging visit (Day 0).
10. The subject has received any radiopharmaceutical within 7 days or 10 half-lives prior to the administration of Tc 99m tilmanocept.

4.3 Recruitment

Subjects will be recruited from rheumatology practices in accordance with the inclusion and exclusion criteria listed above. Candidate subjects will be asked by their treating physician about their willingness to participate in the study. Healthy control subjects will be recruited via IRB-approved advertisements and clinically assessed for the “absence” of painful and/or swollen joints.

4.4 Withdrawal

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

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

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

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

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

4.5 Replacement

Subjects will be replaced only under the condition that they have not been administered the study drug or did not proceed to imaging.

4.6 Screen Failures

Subjects who sign an informed consent form (ICF) but are ultimately not injected will be considered a screen failure. Subjects will be assigned a study identification number at the time the ICF is signed. eCRFs for Inclusion, Exclusion, Demographics, Adverse Events, and final disposition should be completed for all screen failure subjects.

4.7 Subject Identification

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

Digits 1 to 2: Study number "31"

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 “31-01-001.”

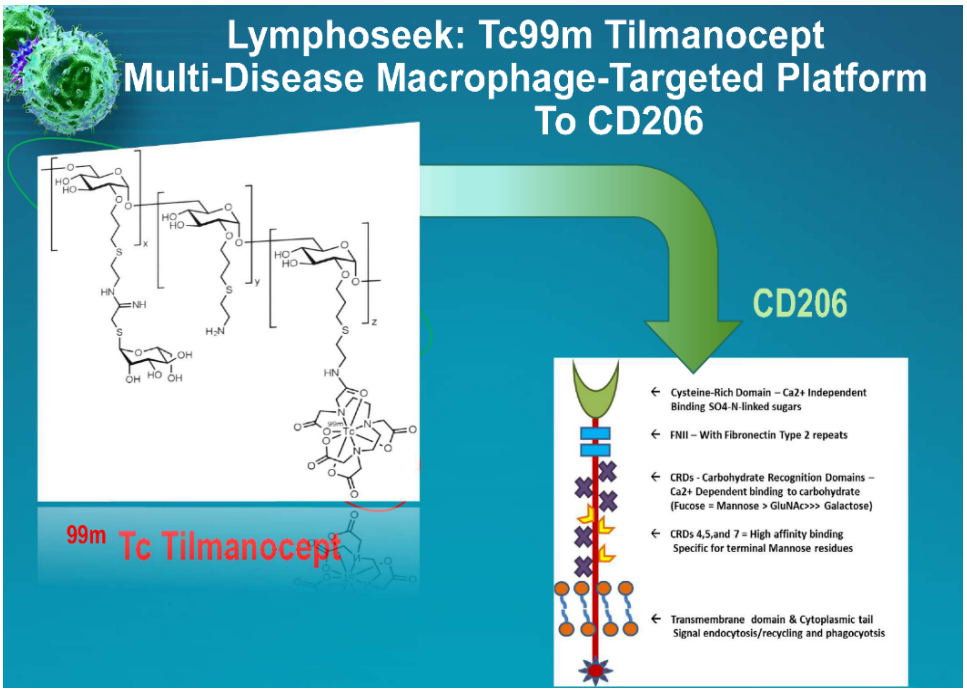
Subjects will maintain the same number given at screening for the entire study. If a subject is a screen failure, the number will not be used for any other subject.

5 INVESTIGATIONAL PRODUCT

5.1 Description of Investigational Product

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

Figure 1 Tc 99m tilmanocept and the Mannose Receptor



5.2 Investigational Product Dosage and Administration

Tc 99m tilmanocept will be administered through an IV route of injection. A 150-mcg dose containing 10 mCi of Tc 99m in 3 mL will be delivered using 1 syringe. The dose will be injected as a slow push into the IV catheter. At the completion of the injection, a 10-mL sterile normal saline flush will be administered. The preferred site of IV placement will be between the elbow and the wrist.

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

5.3 Timing and Frequency of Drug Administration

Subjects in all 3 arms will receive the study-defined dose of 150 mcg tilmanocept radiolabeled with 10 mCi of Tc 99m. The timing and frequency (i.e., repetition) of drug administration will be contingent upon arm assignment as described below and summarized in Table 6.

- Subjects enrolled in Arm 1, defined as those who are healthy controls clinically free of inflammatory disease and/or joint pain, will undergo drug administration at the Day 0 (Visit 1) only.
- Subjects enrolled in Arm 2, defined as those who are clinically diagnosed with active RA and are on a stable anti-rheumatic therapy, will undergo drug administration at the following two timepoints:
    1. Day 0 (Visit 2)
    2. Day 8 ± 1 (Visit 4)
- Subjects enrolled in Arm 3, defined as those who are clinically diagnosed with active RA and are candidates for initiation of, or change to, a new anti-TNFα bDMARD treatment, 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)
    3. 12 ± 1 weeks after the initiation of new anti-TNFα bDMARD therapeutic regimen (Visit 6)
    4. 24 ± 1 weeks after the initiation of new anti-TNFα bDMARD therapeutic regimen (Visit 8)

Table 6 Summary of Tc 99m Tilmanocept Administration Timepoints by Study Arm

Arm	Subjects	Administrations	Day(s)/Visit
1	HCs	1	[1] Day 0 (Visit 1)
2	Active RA on stable anti-rheumatic treatment	2	[1] Day 0 (Visit 1) [2] Day 8 ± 1 (Visit 4)
3	Active RA with initiation of, or change to, a new anti-TNFα bDMARD therapy	4	[1] Day 0 (Visit 2) [2] 5 ± 1 weeks after new anti-TNFα bDMARD [3] 12 ± 1 weeks after new anti-TNFα bDMARD [4] 24 ± 1 weeks after new anti-TNFα bDMARD

5.4 Packaging and Labeling

Tilmanocept cartons ready for radiolabeling will be shipped and stored at the study-assigned radiopharmacy. Tilmanocept is provided in a vial. Vials are packaged as a kit. A carton (kit) contains 5 vials of tilmanocept.

A detailed Radiolabeling protocol will be provided to each radiopharmacy for instructions on how to radiolabel the vials and prepare the final tilmanocept product for injection. Quality Control worksheets will also be provided.

5.5 Drug Logistics and Investigational Product Accountability

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

6 THERAPIES OTHER THAN INVESTIGATIONAL PRODUCT

6.1 Prior and Concomitant Therapies

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

6.2 Post-Study Therapy

There are no post-study therapy restrictions.

7 STUDY PROCEDURES

A schedule of evaluations is provided in the schedule of events (Appendices 1 and 3) and study workflow diagram (Appendices 2 and 4).

7.1 Schedule of Evaluations

7.1.1 Arm 1 (HC Subjects)

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

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – date of birth, gender, race
- Medical surgical history – all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
- Concomitant medications (within 30 days before injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- Physical examination will include an assessment of height, weight, and examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (see Table 12)
- Urine collection for routine analysis
- Urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- 2010 ACR/EULAR: to confirm that the subject is clinically free of inflammatory disease and joint pain Swollen and tender joints will be identified and documented during physical examination as established by the 2010 ACR/EULAR.

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

Pre-Tc 99m Tilmanocept Administration

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

- A urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG (see Section 8.8) within 30 minutes prior to administration of Tc 99m tilmanocept
- Vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition (in the following order);
  1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
  3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);

4. Consecutive planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint.

<u>180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration</u>

- Assessment of adverse events
- Image acquisition (in the following order):
  1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
  3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  4. Consecutive planar scan of the bilateral hands and wrists
- Clinical labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- Assessment of adverse events

7.1.2 Arm 2 (RA Subjects on Stable Therapy)

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

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – date of birth, gender, race
- Medical surgical history – all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
- Concomitant medications (within 30 days before injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- Physical examination will include an assessment of height, weight, and an examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (see Table 11)

- Urine collection for routine analysis
- Urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- RA Evaluations: Swollen and tender joints will be identified and documented during physical examination as established by the 2010 ACR/EULAR and DAS28. A review of the subject's RA history including previous treatments, date of symptom onset, and date of diagnosis will also be performed.

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

Pre-Tc 99m Tilmanocept Administration

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

- A urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept
- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition (in the following order):
  1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
  3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  4. Consecutive planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint.

180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- Assessment of adverse events
- Image acquisition
  1. Planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 10 minutes) for the subsequent scan;
  3. Consecutive planar scan of the whole body followed by up to a 15-minute *pause* (includes patient preparation for the subsequent acquisition);
  4. Consecutive planar scan of the bilateral hands and wrists.
- Clinical labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- Assessment of adverse events

Visit 4 (Day 8 ± 1; Tc 99m Tilmanocept Administration and Imaging)
Pre-Tc 99m Tilmanocept Administration

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

- A urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept

- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration:

- AE assessment
- Image acquisition (in the following order):
  1. Planar scan of the whole body followed by up to a 15--minute pause (includes patient preparation for the subsequent acquisition)
  2. Planar scan of the bilateral hands and wrists followed by a *pause* until the 180 ± 15-minute imaging timepoint

180 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition
  1. Planar scan of the whole body followed by up to a 15-minute pause (includes patient preparation for the subsequent acquisition);
  2. Planar scan of the bilateral hands and wrists followed by *immediate* preparation (up to 15 minutes) for the subsequent SPECT/CT scan
  3. SPECT/CT scan of the bilateral hands and wrists.
- Clinical labs (after imaging)
- Urinalysis (after imaging)

Visit 5 (Visit 4 + 1-3 days; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- AE assessment

7.1.3 Arm 3 (RA Patients Initiating Change in Anti-TNFα bDMARD Therapy)*

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

- Preliminary review of inclusion and exclusion criteria
- Obtain signed informed consent for study participation
- Allocation of unique subject number; this number will be used to document the subject data in the case report forms (CRFs) and enrollment log
- Demography – date of birth, gender, race
- Medical surgical history – all relevant prior medical and surgical conditions will be recorded in the CRF. Documented medical conditions will also note the month and year of onset if the condition is still active.
- Concomitant medications (within 30 days before injection).
- Vital signs (body temperature, heart rate, blood pressure, and respiratory rate after at least 1 minute in a resting position)
- Physical examination will include an assessment of height, weight, and an examination of general appearance, skin, eyes, ears, nose, throat, head and neck (including thyroid), lungs, heart, abdomen, lymph nodes, musculoskeletal, and nervous system. Any clinically relevant finding is to be documented as a baseline finding. Physical exams that are conducted as standard of care prior to signing informed consent may be used if they are performed within 30 days of injection.
- Clinical laboratory tests – study subjects will have blood obtained for hematology, chemistry, and an RA panel (Table 11)
- Urine collection for routine analysis
- Urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- RA Evaluations: Swollen and tender joints will be identified and documented during physical examination as established by the 2010 ACR/EULAR and DAS28. A HAQ-DI© 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.
- WPI Assessment

Visit 2 (Day 0;Drug Administration/Imaging)

Pre-Tc 99m Tilmanocept Administration

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

- A urine pregnancy test for women of child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept
- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
  1. Planar scan of whole body
  2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- Urinalysis (after 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

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

- Review of concomitant medications
- AE assessment

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

Pre-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

Pre-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 child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept
- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the

nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0 to 30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
- Planar scan of whole body
- Planar scan of bilateral hands and wrists
- Clinical Labs (after imaging)
- RA labs (after imaging)
- Urinalysis (after imaging)

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

- Review of concomitant medications
- AE assessment

Visit 6 (Week 12 ± 1; Tc 99m Tilmanocept Administration, Imaging, and Clinical Assessments)*
Pre-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
- HAQ-DI©
- WPI

Pre-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 child-bearing potential. Females of child bearing potential are defined as women who are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.

- Assessment of AEs
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept
- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (n the following order):
  1. Planar scan of whole body
  2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)
- RA labs (after imaging)
- Urinalysis (after imaging)

Visit 7 (5 ± 3 Days After Visit 6; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- AE assessment

Visit 8 (Week 24 ± 1; Post-Tc 99m Tilmanocept Administration, Imaging, Clinical Assessments)

Pre-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
- HAQ-DI©
- WPI

Pre-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 child-bearing potential. Females of child bearing potential are defined as women that are not surgically sterile (hysterectomy or bilateral oophorectomy) nor postmenopausal for at least 1 year prior to screening. Women who are not of childbearing potential will not require a pregnancy test.
- Assessment of adverse events
- Concomitant medication review
- ECG within 30 minutes prior to administration of Tc 99m tilmanocept
- Post-ECG vital signs after at least 1 minute in a resting position (body temperature, heart rate, blood pressure, and respiratory rate) within 30 minutes prior to administration of Tc 99m tilmanocept

Tc 99m Tilmanocept Administration

IV administration of Tc 99m tilmanocept will be at study time 00:00. The preferred site of IV placement will be between the elbow and the wrist. The filled syringes will be connected to the catheter for a slow push injection. At the completion of the injections, a 10-mL sterile normal saline flush will be administered. The IV administration will be performed in the nuclear medicine department by an onsite Certified Nuclear Medicine Technologist or Nuclear Medicine Physician. Subjects will be continuously monitored for adverse events.

0-30 Minutes Post-Tc 99m Tilmanocept Administration

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

60 ± 15 Minutes Post-Tc 99m Tilmanocept Administration

- AE assessment
- Image acquisition (in the following order):
  1. Planar scan of whole body
  2. Planar scan of bilateral hands and wrists
- Clinical labs (after imaging)

- RA labs (after imaging)
- Urinalysis (after imaging)

Visit 9 (5 ± 3 Days After Visit 7; Follow-up Telephone Safety Assessment)

- Review of concomitant medications
- AE assessment

***If a subject in Arm 3 requires early exit from the study between Week 16 and Week 24, an attempt should be made to complete a final imaging visit and all clinical assessments. If possible, this should include all Visit 8 (Week 24 ± 1; Post-Tc 99m Tilmanocept Administration, Imaging, Clinical Assessments) procedures.**

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.

8 PROCEDURES AND VARIABLES

8.1 Population Characteristics

8.1.1 Demographics and Other Baseline Characteristics

One-hundred and five (105) evaluable subjects will be enrolled across all 3 study arms. Arms 1 and 2 will each contain 38 male or female subjects, and Arm 3 will contain 29 subjects. Subjects in Arm 1 will be male or female HCs who are clinically free of any inflammatory and/or joint disease. Subjects in Arm 2 will be male or female individuals with clinically diagnosed active RA who are on stable anti-inflammatory and/or anti-rheumatic therapy. Subjects in Arm 3 will be male or female individuals who are candidates for initiation of, or change to, a new anti-TNFα bDMARD treatment.

8.1.2 Medical, Rheumatological, and Surgical History

Relevant medical, rheumatological, and surgical histories will be obtained on all study subjects. As part of the medical history, the date of the last spontaneous menstruation will be recorded if childbearing potential is not excluded by surgical sterilization. Rheumatological history will include date of RA diagnosis as well as the timing, dose, administration frequency, and administration route (when available) of all RA-specific drugs taken in the last 6 months.

8.1.3 Prior and Concomitant Medication

All prior non-RA medications used up to 30 days before the first screening examination through the follow-up safety visit will be documented. In addition to the summarization of prior RA-specific treatments (Section 8.1.2), recent or concomitant treatments taken for RA in the last 6 months must be collected in accordance with the following time windows listed in Table 7.

Table 7 Documentation Window for Recent or Concomitant RA-specific Treatments

Drug Class	Documentation Window
Traditional DMARDs	On therapy ≥ 90 days and at a stable dose ≥ 30 days prior to the first imaging visit (Day 0)
bDMARDs	>180 days prior to the first imaging visit (Day 0)
JAK inhibitors	> 180 days prior to the first imaging visit (Day 0)
NSAIDs/Corticosteroids	≥ 28 days prior to the first imaging visit (Day 0)

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 between the elbow and the wrist. The filled syringe will be connected to the catheter for a

slow push injection. Immediately after the completion of the injection, a 10-mL sterile normal saline flush will be administered. Injection of Tc 99m tilmanocept will be at study time 0:00.

8.3 Rheumatological Assessments

8.3.1 2010 ACR/EULAR Classification Criteria

All subjects will be evaluated at screening using the 2010 ACR/EULAR Classification Criteria as part of eligibility and inclusion (Aletaha 2010). The 2010 ACR/EULAR classification criteria includes 4 components: number and site of involved joints, serologic abnormality, elevated acute-phase response and symptom duration. See Appendix 5 for details. A total score of 6 or higher (out of a possible 10) combined with clinical synovitis not better explained by another disease confirms a diagnosis of "definite RA".

8.3.2 DAS28

Subjects assigned to Arms 2 and 3 will be evaluated for the DAS28 (Prevoo 1995) at screening. Additional time intervals for DAS28 assessment will vary by study arm (Table 8). DAS28 is calculated from 4 components: TJC, 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 6 for details.

Table 8 Timing of DAS28 Evaluations by Study Arm

Arm	Visit (Day)
1	*Not performed*
2	Visit 1 (Day -30 to -1)
3	Visit 1 (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) Visit 8 (24 ± 1 Weeks post-initiation of new anti-TNFα bDMARD)

8.3.3 CDAI

Subjects in Arm 3 will be evaluated for disease activity using the Clinical Disease Activity Index (CDAI) (Aletaha 2009). CDAI will be collected a total of 4 times per subject (Table 9). 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 7 for details.

Table 9 Timing of CDAI Evaluations by Study Arm

Arm	Visit (Day)
1	*Not performed*
2	*Not performed*
3	Visit 1 (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) Visit 8 (24 ± 1 Weeks post-initiation of new anti-TNFα bDMARD)

8.3.4 ACR Response Criteria

Subjects in Arm 3 will be evaluated using the ACR Response Criteria (Ward 2014). 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). Response is reported as percent (%) improvement from baseline to a later timepoint. In this case, a total of 3 timepoints will be evaluated per subject (Table 10).

Percent improvement is defined as a combination of reductions in swollen or 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 or 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 or 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 or swollen joint counts as well as 20% improvement in at least 3 of the other 5 criteria. See Appendix 7 for details.

Table 10 Timing of ACR Response Evaluations by Study Arm

Arm	Visit (Day)
1	*Not performed*
2	*Not performed*
3	Visit 4 (5 ± 1 Weeks post-initiation of new anti-TNFα bDMARD) Visit 6 (12 ± 1 Weeks post-initiation of new anti-TNFα bDMARD) Visit 8 (24 ± 1 Weeks post-initiation of new anti-TNFα bDMARD)

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 score (Section 8.3.1), DAS28 score (Section 8.3.2), CDAI score (Section 8.3.3), and ACR Response (Section 8.3.4).

8.3.5.2 Global Assessment of Disease Activity

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 (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 (Section 8.3.4).

8.3.5.3 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 (Section 8.3.4).

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

Patient assessment of physical function will be evaluated using the HAQ Disability Index (HAQ-DI©) questionnaire. 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 9 for more information.

8.3.5.5 Acute-phase Reactant

ESR will be obtained in the RA-specific laboratory panel (see Table 12).

8.3.5.6 Widespread Pain Index

Assessment of widespread pain will be evaluated using the widespread pain index (WPI). 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 1 to 19). This assessment will be used as an additional pain assessment tool to evaluate central pain.

8.4 Imaging

8.4.1 Image Acquisition

8.4.1.1 Planar Scintigraphy

Planar image acquisition occurs in all subjects. The frequency of planar imaging is contingent upon study arm assignment (Table 11).

Whole Body and Anterior and Posterior Spot View of the Hands and Wrists will also be obtained based on study arm assignment.

Table 11 Planar Image Acquisition by Study Arm

Arm	Whole Body [a]	Bilateral Hands and Wrists [b]	Total
1	Visit 2 60 ± 15 min. p.i. 60 ± 15 min. p.i. (consecutive) 180 ± 15 min. p.i. 180 ± 15 min. p.i. (consecutive)	Visit 2 60 ± 15 min. p.i. 60 ± 15 min. p.i. (consecutive) 180 ± 15 min. p.i. 180 ± 15 min. p.i. (consecutive)	8
2	Visit 2 60 ± 15 min. p.i. 60 ± 15 min. p.i. (consecutive) 180 ± 15 min. p.i. 180 ± 15 min. p.i. (consecutive) Visit 4 60 ± 15 min. p.i. 180 ± 15 min. p.i.	Visit 2 60 ± 15 min. p.i. 60 ± 15 min. p.i. (consecutive) 180 ± 15 min. p.i. 180 ± 15 min. p.i. (consecutive) Visit 4 60 ± 15 min. p.i. 180 ± 15 min. p.i.	12

Table 11 Planar Image Acquisition by Study Arm

Arm	Whole Body [a]	Bilateral Hands and Wrists [b]	Total
3	Visit 2 60 ± 15 min. p.i. Visit 4 60 ± 15 min. p.i. Visit 6 60 ± 15 min. p.i. Visit 8 60 ± 15 min. p.i.	Visit 2 60 ± 15 min. p.i. Visit 4 60 ± 15 min. p.i. Visit 6 60 ± 15 min. p.i. Visit 8 60 ± 15 min. p.i.	8

p.i. = post injection

[a] Whole body scan always precedes planar scans of bilateral hands/wrists.

[b] This scan always follows whole body planar scans.

8.4.1.2 SPECT/CT

SPECT/CT images are acquired in Arm 2 on Visit 4 (Day 8 ± 1) after the completion of all planar imaging at the 180 ± 15-minute imaging timepoint. SPECT/CT is performed on the bilateral hands and wrists only. Refer to the Image Acquisition Guidelines for all required imaging technical specifications and acquisitions.

8.4.1.3 Tilmanocept Uptake Value (TUV)

TUV is a quantitative imaging metric used to characterize the amount of CD206 activity on planar imaging. Results from a prior Phase 1 and 2 study 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 whole body planar images and static images of the bilateral hands and wrists to derive relevant count statistics, which are input parameters for joint-specific (TUV_{joint}) and global (TUV_{global}) TUVs. TUV metrics are further described in the NAV3-31 Statistical Analysis Plan.

8.5 Adverse Events

8.5.1 Definition of Adverse Event

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

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

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

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

8.5.2 Categories for Adverse Event Assessment

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

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

Specific drug treatment

Any specific drug treatment will be documented.

Causal relationship to investigational product

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

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

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

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

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

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

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

Causal relationship to study procedure

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

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

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

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

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

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

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

8.5.3 Assessments and Documentation of Adverse Events

Attention shall be paid to the occurrence of AEs for the duration of subject participation. Events occurring prior to Visit 2 (Day 0) will be recorded in the subject's medical history unless related to study procedure, in which case the event will be recorded as an AE. Untoward medical events beginning on Visit 2 (Day 0) until the final visit (variable per arm) will be reported as adverse events.

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

The following information is required:

- The **date** and **time of onset** of any AE.
- The duration (the entire duration of an event or symptom, calculated from date of onset to date of end, if not recorded directly).
- The seriousness of the AE will be assessed by the investigator. If the investigator deems that an AE qualifies as an SAE, a special form provided by the sponsor should be completed and the event must be immediately reported to the sponsor. A definition of serious adverse events is provided in Section 8.5.5.
- The maximum intensity (mild, moderate, or severe).
- Whether drug treatment was administered for the event, any specific drug treatment must be documented.
- The relationship of the AE to the investigational product and to study conduct (for definitions, see above).
- The **outcome** of the AE (resolved, resolved with sequelae, not resolved, unknown, death).

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

8.5.4 Expected Adverse Events

Investigational Product-Related Risks

In all completed studies of Lymphoseek (Tc 99m tilmanocept), involving 553 subjects, only 3 events (breast pain and injection site pain reported by subjects with breast cancer and injection site irritation reported by a subject with head and neck squamous cell cancer) were deemed definitely related to the administration of Lymphoseek by the investigator. The most common adverse reactions (incident < 1%) have been lack of effect (< 0.067%), injection site pain (< 0.02%) and rash (< 0.02%). Adverse events from the radioactive dose are not expected, since the applied radiation doses are far below doses that can cause acute effects in human tissues.

In addition to the Lymphoseek pre-approval clinical studies, post-marketing surveillance shows that Lymphoseek has been administered to more than 250,000 patients with not a single drug-related SAE. Routes of administration included: subcutaneous, intradermal, and peritumoral.

The intended route of administration in this study is intravenous. There have been approximately 60 IV administrations of Tc 99m tilmanocept and no SAEs or ADRs have been reported to date.

Risks of Imaging Procedures

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

The average effective radiation dose per 10 mCi Tc 99m Tilmanocept injection is calculated to be 2.7 mSv (equivalent to about ten months of natural background radiation received in the US). Subjects enrolled in Arm 1 will receive one injection, equal to 2.7 mSv total; subjects enrolled in Arm 2 will receive a maximum of two injections plus one SPECT/CT scan- with an estimated effective dose of 1 mSv for the CT portion- amounting to 6.4 mSv total; subjects enrolled in Arm 3 will receive a maximum of four injections, equal to 10.8 mSv total. The maximum possible dose of ~10.8 mSv for any enrolled subject (no one will cross over into more than one study arm) is considered to be a moderate risk level corresponding to the benefit to the patient (category III based on the International Commission on Radiological Protection 62 (ICRP62)) and is balanced against the possible substantial societal benefit that can be gained from the trial (European Commission Radiation Protection 99, 1998 and ICRP 62, 1992). For further reference, the effective dose from a standard CT abdomen and pelvis, with and without contrast, is up to 20 mSv.

Precautionary Measures

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

Unexpected Adverse Events

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

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

8.5.5 Serious Adverse Events

Definition of Serious Adverse Events

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

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

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

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

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

Actions and reporting obligations in case of serious adverse events

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

If any SAE occurs over the course of the study, investigators or other site personnel will inform Navidea Biopharmaceutical representatives within 1 day (i.e., within 24 hours) of becoming aware of the SAE. Written notification of the SAE will be emailed to Navidea Biopharmaceuticals Pharmacovigilance at safety@navidea.com. For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately.

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

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

Notification of the IRB(s)

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

Notification of the authorities

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

Sponsor's notification of the investigators

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

8.6 Physical Examination

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

Physical examinations will be performed for the following body systems:

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

8.7 Vital Signs

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

8.8 Electrocardiogram

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

On-site investigator's responsibilities

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

8.9 Clinical Laboratory Parameters

Table 12 Clinical Laboratory Parameters

Hematology	Hemoglobin (Hgb), hematocrit (HCT), platelets, neutrophils, basophils, lymphocytes, monocytes, red blood cells (RBC), white blood cells (WBC)
Serum chemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, carbon dioxide (CO_2)/bicarbonate, blood urea nitrogen (BUN), glucose
Urinalysis	pH, specific gravity
Rheumatoid Panel	Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF); anti-citrullinated peptide antibody (ACPA)

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

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

Only abnormal laboratory values will be collected in the electronic database. Any change in a laboratory value, which results in a change in subject management (additional controls or treatment required), will be reported as a clinically significant change. Clinically significant changes in laboratory parameters, 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 13.

Table 13 Approximate Amount of Blood Drawn per Subject by Study Arm

Arm	Timepoint	Test (Sample Volume)	Total Blood Drawn at Timepoint
1	Visit 1 (Day -30 to -1)	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (2.6 teaspoons)
	Visit 2 (Day 0)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
		Total (Arm 1)	**22 mL (4.5 teaspoons)**
2	Visit 1 (Day -30 to -1)	Chemistry (5 mL) Hematology (4 mL) RA Panel (4 mL)	13 mL (2.6 teaspoons)
	Visit 2 (Day 0)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
	Visit 4 (Day 8 ± 1)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
		Total (Arm 2)	**31 mL (6.3 teaspoons)**
3	Visit 1 (Day -30 to -1)	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (2.6 teaspoons)
	Visit 2 (Day 0)	Chemistry (5 mL) Hematology (4 mL)	9 mL (1.8 teaspoons)
	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	Chemistry (5 mL) Hematology (4 mL) RA panel (4 mL)	13 mL (2.6 teaspoons)
	Visit 8 (24 ± 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)
		Total (Arm 3)	**61 mL (12.4 teaspoons)**

9 STATISTICAL METHODS

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

The study is stratified into 3 groups. Arm 1 consists of HC subjects. Arm 2 consists of RA patients on stable therapy. Arm 3 consists of RA patients who are candidates for initiation of, or change to, a new anti-TNFα bDMARD treatment.

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)
- ECG parameters
- Vital signs

9.3 Efficacy Variables

The efficacy variables for this study are defined below:

- Quantitative determination of TUV_{joint} and TUV_{global}
- Qualitative (i.e., visual) evaluation of SPECT/CT in detecting localization within synovial spaces of the bilateral hands and wrists
- Subject RA status as summarized in the CDAI and ACR Response Criteria overall score and their constituent sub-scores

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

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

Primary Endpoint(s)

ARMS 1-2

- The camera-specific precision of TUV_{joint} and TUV_{global} in subjects with active RAand HCs, which is defined as the Root Mean Square Difference (RMSD) between the consecutive 15-minute planar images.

- The stability of the mean/variance relationship, which is assessed by comparing the Coefficient of Variation (CV) of TUV_{joint} and TUV_{global} in subjects with active RA and HCs.

ARM 2

- The longitudinal (8-day) precision of TUV_{joint} and TUV_{global} in subjects with active RA, which is defined as the RMSD of images at the same time point.

ARM 3

- The correlation of $\Delta TUV_{global[5w]}$ 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). The correlation of $\Delta TUV_{global[5w]}$ and response to new anti-TNFα bDMARD therapy from baseline to 12 ± 1 weeks and 24 ± 1 weeks defined by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).

Secondary Endpoint(s)

ARMS 1-2

- The temporal stability of the 150 mcg tilmanocept mass dose/10 mCi radiolabeling dose.

ARM 1

- The normal ranges of TUV_{joint} in HC subjects, which is defined as the 5 and 95 percentiles of:
  - TUV_{joint} of bilateral joints (i.e., bilateral wrists, MCPs, PIPs, knees, elbows, shoulders)

ARM 2

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

ARM 3

- The correlation of the $TUV_{global[0week]}$ 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) and by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).
- The correlation of $\Delta TUV_{global[12w]}$ and $\Delta TUV_{global[24w]}$ 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) and by ACR Response Criteria (ACR_{12w} and ACR_{24w}, respectively).
- The correlation of $\Delta TUV_{global[5w]}$ and constituent parameters of $\Delta CDAI_{12/24w}$ and $ACR_{12/24w}$ including:
  1. Tender Joint Count (TJC)
  2. Swollen Joint Count (SJC)

3. Patient assessment of global disease activity
4. Rheumatologist assessment of global disease activity
5. Patient assessment of pain
6. Patient assessment of physical function
7. Acute-phase reactant value

9.4 Sample Size Justification

The study will enroll up to 105 evaluable subjects in Arms 1, 2, 3 allocated below, imaged at up to 10 study centers. Arm 1 will have N = 38 evaluable subjects, Arm 2 will have N = 38 evaluable subjects, and Arm 3 will have N = evaluable 29 subjects. A subject is considered evaluable if he/she meets the criteria for the analysis population and has the data necessary for computing the primary endpoint.

The sample size for Arm 1 was determined to assess temporal stability of the 150 mcg tilmanocept mass dose/10 mCi radiolabeling dose. The sample size for Arm 2 was determined to power the hypothesis test at 0.80. The sample size for Arm 3 was determined to provide a lower 97.5% confidence limit for the rank correlation of at most 0.15 when rho = 0.70, using Fisher's Z-transformation methods.

9.5 Statistical Analyses

9.5.1 Analysis Populations

The following populations are defined for this study:

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

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

Safety Population – The safety population includes all subjects who have been enrolled in the study and injected with at least 1 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 (gender, race and ethnicity) will be summarized via counts and percentages.

9.5.3 Analysis of Efficacy Variables

All efficacy analyses will be conducted on both the ITD and PP populations. The ITD population will be the primary analysis set.

9.5.3.1 Primary Endpoints Arms 1- 2

Precision endpoints will be assessed estimating the mean, standard deviation, n, minimum, median, and maximum of the signed differences, calculated as change from the initial measurement. The RMSD will be calculated and a 95% confidence interval on its value will be provided.

Stability of the mean/variance relationship will be assessed by the coefficient of variation of the differences for active RA subjects and HC subjects at 60 minutes and 180 minutes. 95% confidence intervals for the difference in CVs following the methods of (Forkman 2009) and from the bootstrap sampling distribution of the difference of the CVs will be provided based on N = 5000 bootstrap samples.

9.5.3.2 Secondary Endpoints Arms 1- 2

The normal ranges of TUV_{joint} will be estimated from data collected from HC subjects (Arm 1 only) at 60 minutes and 180 minutes post-injection. The mean, standard deviation, n (number of joints), and quantile regression estimates of the 5 and 95 percentiles will be provided for shoulder, elbow, wrist, MCP, PIP, and knee.

9.5.3.3 Primary endpoint Arm 3

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 ΔACR_{12w} and ΔACR_{24w} (overall scores only) will be computed and a 95% confidence interval for its value will be computed using Fisher's Z-transformation.

9.5.3.4 Secondary endpoint Arm 3

The Kendall rank correlations between $\Delta TUV_{global[5w]}$ with the subscores of ΔACR_{12w} and ΔACR_{24w} (including tender joint count, swollen joint count, 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) will be computed and a 95% confidence interval for its value will be computed using Fisher's Z-transformation.

The Kendall rank correlations between $TUV_{global[0w]}$, $\Delta TUV_{global[12w]}$, $\Delta TUV_{global[24w]}$ with the $\Delta CDAI_{12w}$, $\Delta CDAI_{24w}$, ΔACR_{12w}, ΔACR_{24w} will be computed and a 95% confidence interval for their values will be computed using Fisher's Z-transformation.

Additional efficacy analyses may be described in the Statistical Analysis Plan (SAP) for the study.

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) for each Arm and overall at each time point.

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

9.5.5 Handling of Missing Values

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

9.5.6 Interim Analyses

After the injection, imaging, and quantitation of a total of 4 subjects in Arms 1 and 2 combined, an interim analysis (interim analysis 1) will be held for the review of quantitative TUV results and assessment of imaging parameters and logistics. A second interim analysis will be held after a total of 30 subjects (≥ 15 RA) in Arms 1 and 2 are injected, imaged, and quantified for the review of intra-and inter-subject TUV variation to contribute to the power analysis of a future Phase 3 study and/or to terminate the study on grounds of data sufficiency.

After 15 subjects in Arm 3 have completed Visit 4, an interim analysis (interim analysis 3) will be performed to estimate the distribution of treatment effects on TUV and to optimize the TUV metric based on the interim data from all three Arms.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Data Recording

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

10.1.1 CRF Design

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

10.2 Monitoring

This study will be monitored regularly by a clinical research associate (CRA) from the sponsor or a contract research organization (CRO). Monitoring procedures include 1 or more visits designed to clarify all prerequisites before the study starts. Interim monitoring visits will take place on a regular basis according to a schedule fixed by mutual agreement. During these visits, the CRA will check for completion of the entries on the CRFs, their compliance with the protocol and with GCP, and will compare the CRF entries with the source data.

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

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

10.3 Data Processing

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

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

10.4 Auditing

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

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

10.5 Archiving

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

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

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

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

10.6 Premature Termination of the Study

10.6.1 Termination by the Sponsor

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

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

10.6.2 Termination by the Investigator

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

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

10.6.3 Study as a Whole

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

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

10.6.4 Center

At any time, the study may be terminated at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.6.5 Study Participant

Individual subjects may be withdrawn from the study according to the criteria specified in Section 4.4.

11 ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

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

11.2 Subject Information and Consent

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

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

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

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

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

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

11.3 Financing/Financial Disclosure

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

11.4 Publication Policy

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

11.5 Subject Injury

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

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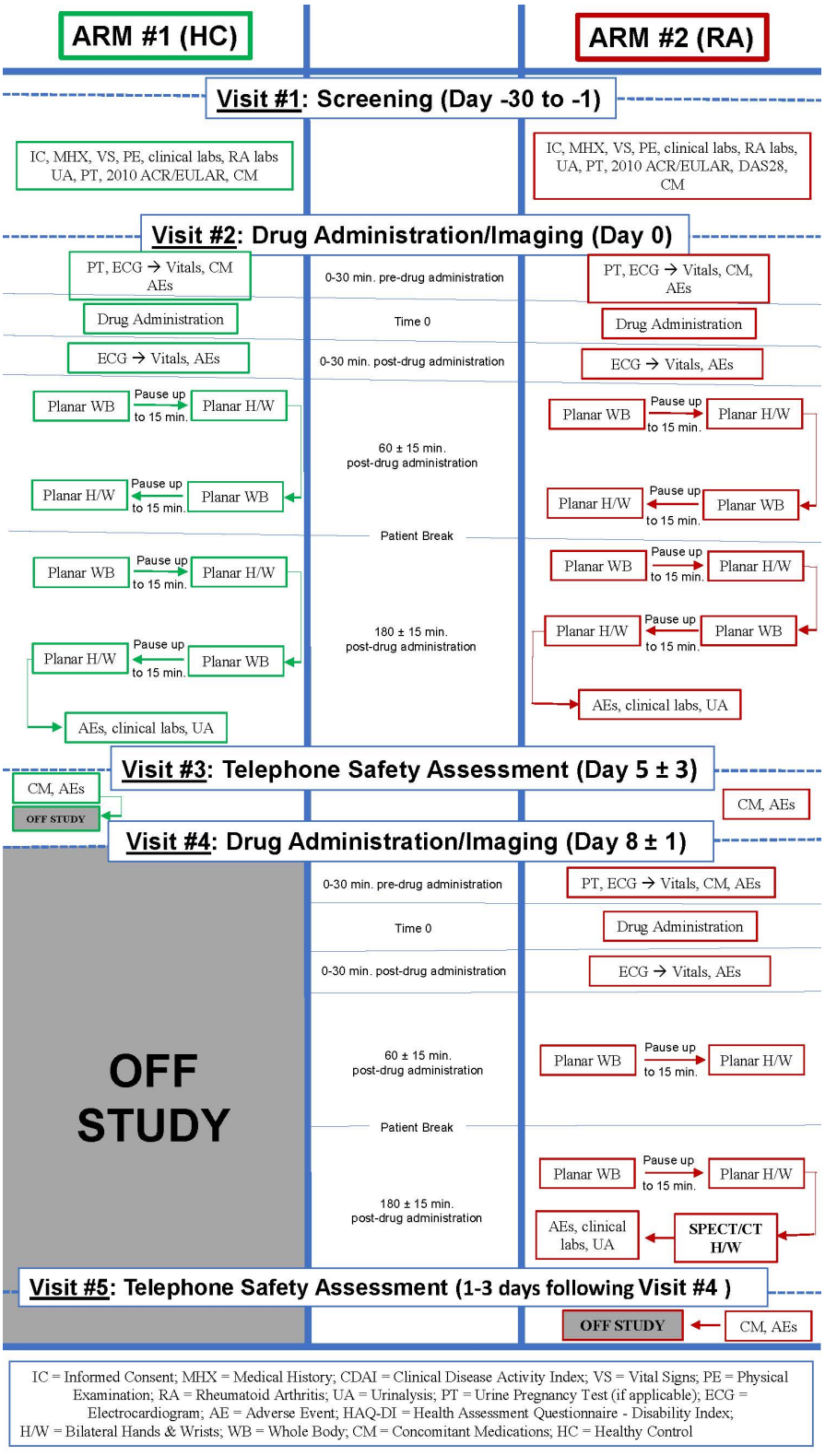
Appendix 1 Schedule of Events (Arms 1-2)

Evaluation	**Visit 1** Screening (Day -30 to -1)	**Visit 2** (Day 0)						**Visit 3** Telephone (Day 5 ± 3)	**Visit 4** (Day 8 ± 1)						**Visit 5** Telephone (Visit 4 + 1-3 days)
		- 00:30 to - 00:01	00:00 Injection	00:01 to 00:30	60 ± 15 min	180 ± 15 min	**After Imaging**		- 00:30 to - 00:01	00:00 Injection	00:01 to 00:30	60 ± 15 min	180 ± 15 min	**After Imaging**	
Informed consent	1, 2														
Entry criteria	1, 2														
Medical History, RA history, demography	1, 2														
Physical examination	1, 2														
Clinical laboratory evaluation: chemistry, hematology, urinalysis	1, 2						1, 2							2	
RA panel	1, 2														
Urine pregnancy test	1, 2	1, 2							2						
2010 ACR/EULAR	1, 2														
DAS28	2														
ECG		1, 2		1, 2					2		2				
Vital signs (obtained post-ECG at Visits 2 and 4)	1,2	1, 2		1, 2					2		2				
Tc 99m tilmanocept administration			1, 2							2					
Whole body planar scan					1, 2	1, 2						2	2		
Planar scan of bilateral hands/wrists					1, 2	1, 2						2	2		
Consecutive whole body planar scan					1, 2	1, 2									
Consecutive planar scan of bilateral hands/wrists					1, 2	1, 2									
SPECT/CT of bilateral hands/wrists													2		
Concomitant medications	1, 2	1, 2						1, 2	2						2
AE monitoring	1, 2	1, 2	1, 2	1, 2	1, 2	1, 2		1, 2	2	2	2	2	2		2

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

Appendix 2 Workflow Diagram (Arms 1-2)



IC = Informed Consent; MHX = Medical History; CDAI = Clinical Disease Activity Index; VS = Vital Signs; PE = Physical Examination; RA = Rheumatoid Arthritis; UA = Urinalysis; PT = Urine Pregnancy Test (if applicable); ECG = Electrocardiogram; AE = Adverse Event; HAQ-DI = Health Assessment Questionnaire - Disability Index; H/W = Bilateral Hands & Wrists; WB = Whole Body; CM = Concomitant Medications; HC = Healthy Control

Appendix 3 Schedule of Events (Arm 3)

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 ± 15 min	After Imaging [a]		- 00:30 to - 00:01	00:00	00:01 to 00:30	60 ± 15 min	After Imaging
Informed consent	x											
Entry criteria	x											
Medical History, RA History, Demography	x											
2010 ACR/EULAR	x											
Vital signs (obtained post-ECG at Visits 2 and 4)	x	x		x				x		x		
ECG		x		x				x		x		
Physical Examination	x											
DAS28	x							x[b]				
CDAI	x							x[b]				
HAQ-DI©	x							x[b]				
WPI	x							x[b]				
Clinical laboratory evaluation: chemistry, hematology, UA	x					x						x
RA panel	x											x
Urine pregnancy test	x	x						x				
Tc 99m tilmanocept administration			x						x			
Planar Imaging: whole body followed by 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

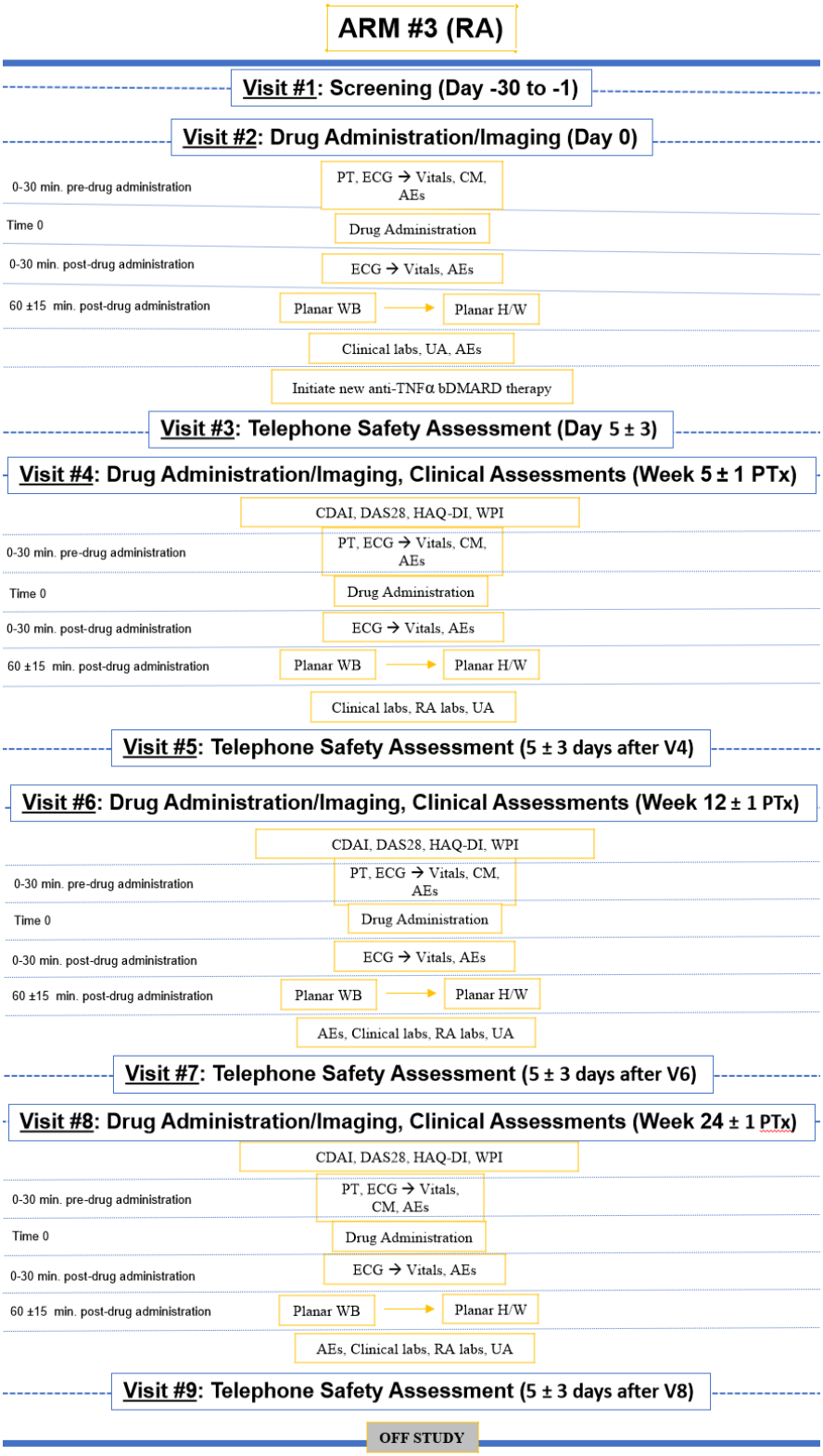
[b] DAS28, CDAI, HAQ-DI©, and WPI assessments are to be performed within seven days prior to injection

Evaluation	Visit 5 Telephone (5 ± 3 days after Visit 4)	Visit 6 (12 ± 1 Week Post-Treatment Change)					Visit 7 Telephone (5 ± 3 days after Visit 6)	Visit 8 (24 ± 1 Week Post Treatment Change)					Visit 9 Telephone (5 ± 3 days after Visit 8)
		- 00:30 to - 00:01	00:00	00:01 to 00:30	60 ± 15 min	After Imaging		- 00:30 to - 00:01	00:00	00:01 to 00:30	60 ± 15 min	After Imaging	
Informed consent													
Entry criteria													
Medical History, RA History, Demography													
2010 ACR/EULAR													
Vital signs (obtained post-ECG at Visits 6 and 8)		x		x				x		x			
ECG		x		x				x		x			
Physical Examination													
DAS28		x[b]						x[b]					
CDAI		x[b]						x[b]					
HAQ-DI©		x[b]						x[b]					
WPI		x[b]						x[b]					
Clinical laboratory evaluation: chemistry, hematology, UA						x						x	
RA panel						x						x	
Urine pregnancy test		x						x					
Tc 99m tilmanocept administration			x						x				
Planar Imaging: whole body followed by bilateral hands and wrists					x						x		
Concomitant medications	x	x					x	x					x
AE monitoring	x	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] DAS28, CDAI, HAQ-DI©, and WPI assessments are to be performed within seven days prior to injection

Appendix 4 Workflow Diagram (Arms 3)



Appendix 5 2010 ACR/EULAR Criteria

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

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

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

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

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

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

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

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

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

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

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

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

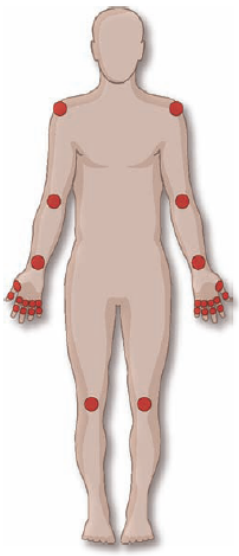
Appendix 6 DAS28 Scoring

DAS28

DISEASE ACTIVITY SCORE IN 28 JOINTS (DAS28)

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

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



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

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

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

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

HOW TO CALCULATE A DAS28 SCORE

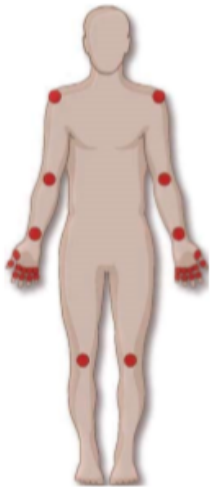
1. Perform a swollen and tender joint examination of your patient, noting each affected joint on Form A. When complete, add all of the swollen and tender joints and record the totals in the appropriate boxes on Form B.
2. Obtain and record the patient's erythrocyte sedimentation rate (ESR) in mm/h in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

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

Appendix 7 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	O 0	O 0.5	O 1.0	O 1.5	O 2.0	O 2.5	O 3.0	O 3.5	O 4.0	O 4.5	O 5.0	O 5.5	O 6.0	O 6.5	O 7.0	O 7.5	O 8.0	O 8.5	O 9.0	O 9.5	O 10	Very Poor

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

Provider Global Assessment of Disease Activity

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

Courtesy of https://www.rheumatology.org/Portals/0/Files/CDAI%20Form.pdf

Appendix 8 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 discriminate effective treatment from placebo treatment in clinical 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 responders include ACR20 responders
- ACR70 is ≥ 70% improvement
  - ACR70 responders include ACR20 & ACR50 responders

Definition

The ACR Criteria is a dichotomous variable with a positive (=responder) or negative (=non-responder) outcome.
The ACR Criteria measures improvement in tender or swollen joint counts in improvement in at least three of 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 or swollen joint counts were achieved as well as a 20% improvement in at least three of the other five criteria.
ACR 50 has a positive outcome if 50% improvement in tender or swollen joint counts were achieved as well as a 50% improvement in at least three of the other five criteria.
ACR 70 has a positive outcome if 70% improvement in tender or swollen joint counts were achieved as well as a 70% improvement in at least three of the other five criteria.

Courtesy of https://www.phusewiki.org/wiki/index.php?title=ACR_Response_Criteria

Appendix 9 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 10 Sponsor Signatures

Study Title:	Evaluation of the Precision and Sensitivity of Tilmanocept Uptake Value (TUV) on Tc 99m Tilmanocept Planar Imaging
Study Number:	NAV3-31
Original Date:	16 November 2018
Amendment 1 Date:	28 February 2019
Amendment 2 Date:	19 April 2019
Amendment 3 Date	24 April 2019
Amendment 4 Date	02 February 2021

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ Date: 11 February 2021
William Regan
Chief Compliance Officer
Navidea Biopharmaceuticals, Inc.

Signed: _____ Date: 11 February 2021
Michael Blue, MD, FACEP
Senior Medical Director
Navidea Biopharmaceuticals, Inc.

Signed: Bonnie Abbruzzese Date: 11 Feb 2021
Bonnie Abbruzzese, MS, RDN, CCRA
Senior Director, Clinical Research & Development
Navidea Biopharmaceuticals, Inc.

Signed: _____ Date: 11 Feb 2021
Michael Rosol, PhD
Chief Medical Officer
Navidea Biopharmaceuticals, Inc.

Appendix 11 Investigator's Signature

Study Title:	Evaluation of the Precision and Sensitivity of Tilmanocept Uptake Value (TUV) on Tc 99m Tilmanocept Planar Imaging
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Original Date:	16 November 2018
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Amendment 3 Date:	24 April 2019
Amendment 4 Date:	02 February 2021

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____