# NADALS STATISTICAL ANALYSIS PLAN (R-SAP)

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|---|---|
| **Title** | Neurodegenerative Alzheimer's Disease and Amyotrophic Lateral Sclerosis (NADALS) Basket Proof of Concept Trial including Asymptomatic Individuals using Baricitinib |
| **Regulatory Sponsor** | Mark Albers, MD, PhD |
| **Funding Sponsor:** | Department of Defense W81XWH2210293, Philanthropy |
| **Study Product:** | Baricitinib |
| **Protocol Short Title:** | NADALS Basket Trial |
| **IND Numbers:** | 154641, 154642 |
| **Protocol Version** | 10.0, 29 Apr 2024 |
| **SAP Version** | 1.0, 28 Jul 2025 |

SAP APPROVAL SIGNATURES

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Date: Jul 28, 2025 09:00:19 EDT

28-Jul-2025

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Date: Jul 28, 2025 09:09:16 EDT

28-Jul-2025

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Study Biostatistician

SAP REVISION HISTORY

| Version | Date | Description of Changes |
|---|---|---|
| 1.0 | 28 Jul 2025 | Initial version |

## Table of Contents

# 1. Governing Documents

This Statistical Analysis Plan (SAP) specifies the outcome measures, analysis samples, and planned analyses for the Neurodegenerative Alzheimer's Disease and Amyotrophic Lateral Sclerosis (NADALS) Basket Proof of Concept Trial including Asymptomatic Individuals using Baricitinib. This SAP supplements the trial protocol. Please refer to the protocol for the study design, eligibility criteria, conduct of the trial, clinical assessments and schedule of assessments, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. In case of discrepancies between the SAP and the protocol concerning matters of analysis, the SAP is authoritative. On all matters not related to analysis, the protocol is authoritative.

# 2. Study Design

## 2.1 Overview

The NADALS Basket Trial is a one-arm, open-label proof of concept trial of baricitinib in patients with Alzheimer's disease (AD), patients with amyotrophic lateral sclerosis (ALS), and asymptomatic carriers of an ALS-causative gene (AGC). Up to 20 eligible participants (10 AD and 10 ALS or AGC, with a limit of 5 AGC) will receive treatment with baricitinib 2 mg per day for 8 weeks, followed by baricitinib 4 mg per day for 16 weeks for a total of 24 weeks of treatment.

Interim analyses will be conducted to assess the ability of baricitinib to cross the blood-brain barrier and enter the cerebrospinal fluid (CSF) or to exert a pharmacodynamic effect on chemokine ligand 2 (CCL2) in the CSF. Enrollment will be suspended after 10 participants initiate treatment if no participant has yet met the criterion to continue the trial at that time, and the trial will be terminated if the continuation criterion is not met after the first 10 participants complete follow-up.

## 2.2 Study Objectives and Endpoints

Primary Objectives:

- To assess whether 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both achieve measurable levels of baricitinib in the CSF of patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.
- To determine whether 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both decrease levels of the inflammatory biomarker chemokine ligand 2 (CCL2) in the CSF of patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.

Secondary Objectives:

- To determine whether an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both decrease levels of the inflammatory biomarkers interleukin-6 (IL-6), phospho-protein kinase R (pPKR), PKR, pPKR / PKR ratio, C-X-C motif chemokine ligand 10 (CXCL10), or interferon gamma (IFNG) in the CSF of patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.
- To determine whether 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both decrease levels of neuronal death biomarkers of neurofilament light chain

(NfL), tau, or phospho-tau in CSF or TAR DNA-binding protein 43 (TDP-43) levels in the plasma in patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.
- To determine whether the measured concentration of baricitinib in the CSF or blood (pharmacokinetics) after 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both correlate with the changes in CSF biomarkers (pharmacodynamics).
- To determine whether 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both are safe and tolerable in patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.

Exploratory Objectives:

- To determine whether 8 weeks of an oral dosage of baricitinib 2 mg per day, 8 weeks or 16 weeks of baricitinib 4 mg per day, or both decrease levels of the inflammatory biomarkers CCL2, IL-6, pPKR, PKR, pPKR / PKR ratio, CXCL10, or IFNG in the plasma of patients with AD, ALS, and asymptomatic carriers of an ALS-causative gene.
- To determine whether changes in clinical outcomes correlate with CSF drug concentration or with changes in CSF biomarkers after 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both.
- To collect CSF for further biomarker discovery efforts via proteomics, dsRNA-seq, and other unbiased chemical profiling methods.
- To refine the CSF inflammatory signature for AD and ALS patients and identify factors that predict diminishment of the CSF inflammatory signature after 8 weeks of an oral dosage of baricitinib 2 mg per day, 4 mg per day, or both.

Primary Outcome Measures:

- Level of free baricitinib in the CSF.
- Change in the level of the inflammatory biomarker CCL2 in the CSF (from baseline to 8-weeks and to 16-weeks).

Secondary Outcome Measures:

- Changes in the inflammatory biomarkers pPKR, PKR, pPKR/PKR ratio, CXCL10, and IFNG in the CSF (from baseline to 8-weeks and to 16-weeks).
- Changes in the neuronal death biomarkers NfL, tau, and phospho-tau in the CSF and TDP-43 in plasma (from baseline to 8-weeks and to 16-weeks).
- Association between drug concentration(pharmacokinetics) and CSF biomarkers (pharmacodynamics).
- Treatment-emergent adverse events.
- Permanent discontinuation of study drug due to study drug-related AEs.

Exploratory Endpoints:

- Changes in the inflammatory biomarkers IL-6, CCL2, pPKR, PKR, pPKR/PKR ratio, CXCL10, and IFNG in the plasma (from baseline to 8-weeks, to 16-weeks and to 24-weeks).
- Changes in clinical outcomes (from baseline to 24-weeks) relative to previous trajectories, when available.

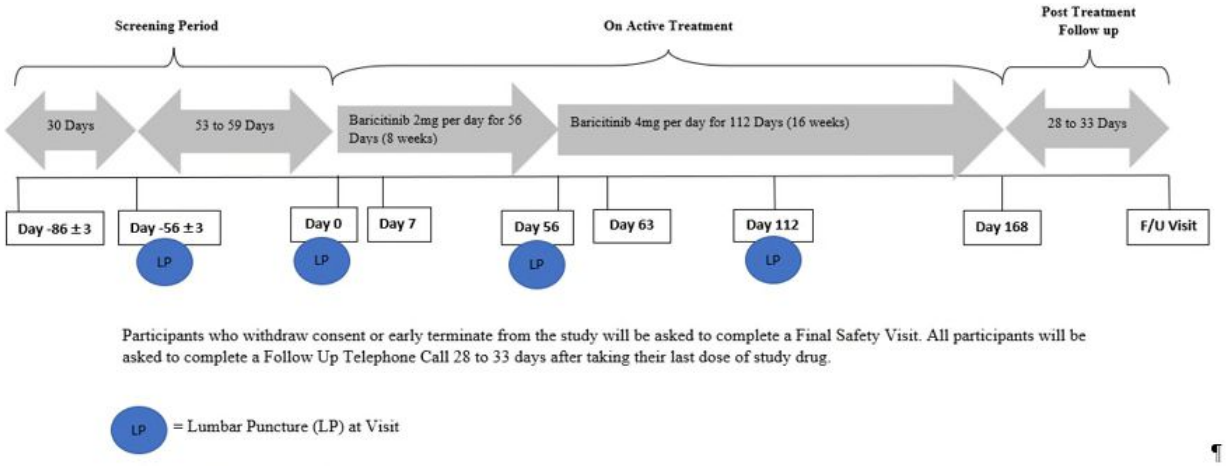
- Change in Accessible Remote Olfactory Mediated Health Assessments (AROMHA) olfactory battery score.

## 2.3 Study Population

Participants will be recruited from one site, Massachusetts General Hospital. Eligibility criteria include age 55 to 80 with a diagnosis of subjective cognitive decline (SCD), mild cognitive impairment (MCI), or possible or probable AD, age 18 to 80 with a diagnosis of sporadic or familial ALS, or age 18 to 80 and the individual is an asymptomatic carrier of an ALS-causative mutation, a CSF CCL2 level of 250 pg/mL or greater, and ability to undergo a lumbar puncture.

## 2.4 Participant Flow

Approximately 40 participants will be consented. Consenting individuals will be screened for eligibility, including collection of CSF by lumbar puncture (LP) and testing of CCL2 levels. Up to 20 eligible participants (10 AD and 10 ALS or AGC, with a limit of 5 AGC) will receive treatment with baricitinib 2 mg per day for 8 weeks, followed by baricitinib 4 mg per day for 16 weeks. Visits for participants who initiate treatment will occur at Baseline, Week 1, Week 8, Week 9, Week 16, and Week 24 or a Final Safety Visit. There will be a Follow-up Telephone Call 28 to 33 days after last dose of study drug. In addition to the LP at screening, participants who initiate treatment will undergo LPs at Baseline, Week 8, and Week 16. The schematic below describes the sequence of visits, LPs, and treatment.




## 2.5 Treatment Administration

Baricitinib (Olumiant®, Eli Lilly and Company) will be given orally, with or without food, at dosages of 2 mg per day and 4 mg per day. If needed, baricitinib tablets can be administered via a feeding tube. Baricitinib should be taken at roughly the same time every day, except for days when an LP will be performed. At study visits with LPs, participants will dose in clinic from home supply and time of dose will be recorded by the study team.

## 2.6 Allocation Concealment

The NADALS trial is an open-label trial. Study drug identity and dosage are not concealed either from the study team or from participants.

## 2.7 Schedule of Activities

| ACTIVITY | Screening | Study Drug Administration | | | | | | Follow-Up Telephone Call[15] |
|---|---|---|---|---|---|---|---|---|
| | | Baseline | Week 1 | Week 8 | Week 9 | Week 16 | Week 24 Final Safety Visit[14] | |
| | Visit 1[1] | Visit 2[1] | Visit 3[16] | Visit 4 | Visit 5[16] | Visit 6 | Visit 7 | Visit 8 |
| | Clinic | Clinic | Remote or Clinic | Clinic | Remote or Clinic | Clinic | Clinic | Phone |
| **Visit Window** | See Footnote 1 | Day 0 | Day 7 ±2 | Day 56 -4 or +1 | Day 63 +2 | Day 112 ±4 | Day 168 ±4 | 28 to 33 days |
| Written Informed Consent | X | | | | | | | |
| Eligibility Review | X | X | | | | | | |
| Medical History | X | | | | | | | |
| Demographics | X | | | | | | | |
| AD/ALS History and Diagnosis | X | | | | | | | |
| El Escorial Criteria (ALS only) | X | | | | | | | |
| RZV (Shingrix)[2] | X | | | X | | | | |
| Physical Examination | X | | | | | | X | |
| Neurological Exam | X | | | | | | X | |
| Vital Signs[3] & Weight | X | X | X | X | X | X | X | |
| Height | X | | | | | | | |
| 12-Lead ECG | X | | | | | | | |
| ALSFRS-R (ALS only) | X | X | | X | | | X | |
| SVC[4] (ALS only) | X | X | | X | | | X | |
| NIH Toolbox (AGC only) | | X | | | | | X | |
| NPI-Q (AD only) | | X | | | | | X | |
| ADAS-Cog (AD only) | | X | | | | | X | |
| MoCA (AD only) | X | | | | | | X | |
| AROMHA olfactory battery | | X | | | | | X | |
| Baseline C-SSRS | X | | | | | | | |
| Since Last Visit C-SSRS | | X | X | X | X | X | X | |
| End of Study Form | | | | | | | X | |
| GIC[5] | | | | | | | X | |
| Survival Assessment | | X | X | X | X | X | X | |
| Screening Labs (Blood & Urine)[6] | X | | | | | | | |
| Safety Labs (Blood & Urine)[7] | | X | X | X | X | X | X | |
| Blood Draw for PK analysis[8] | | X | | X | | X | X | |
| Blood Draw for Biomarkers[9] | X | X | | X | | X | X | |
| Blood Draw for Genetic Testing[10] | | X | | | | | | |
| Urine Sample for Future Research[11] | | X | | X | | X | X | |
| LP and CSF collection[12] | X[17,18] | X[18] | | X[18] | | X[18] | | |
| Concomitant Medication Review[19] | X | X | X | X | X | X | X | X |
| Adverse Event Review | X | X | X | X | X | X | X | X |
| Dispense Study Drug[13] | | X | | X | | X | | |
| Drug Accountability | | | | X | | X | X | |

Abbreviations: AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale Cognitive Subscale; AGC, asymptomatic carriers of an ALS-causative gene; ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale Revised; AROMHA, Accessible Remote Olfactory Mediated Health Assessments; CSF, cerebrospinal fluid; C-SSRS, Columbia-Suicide Severity Rating Scale; ECG, electrocardiogram; GIC, Global Impression of Change; LP, lumbar puncture; MoCA, Montreal Cognitive Assessment; NPI-Q, Neuropsychiatric Inventory Questionnaire; PK, pharmacokinetic; RZV, Recombinant Zoster Vaccine; SVC, slow vital capacity.

[1] Screening Visit activities may be performed over a period of 30 days. The Baseline Visit will occur 53 to 59 days after the Screening LP.

[2] All study participants must receive at least one dose of RZV prior to treatment initiation. The first dose should be administered after all other eligibility criteria have been confirmed and at least 14 days prior to the Baseline Visit unless the participant has provided documentation of vaccination within 4 years prior. The second dose of RZV can be administered at the Week 8 Visit or any time within 2 to 6 months after the first dose per CDC guidelines.

[3] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature. Vital signs will not be collected at remote visits.

[4] SVC testing is required at the Screening Visit and Week 24 or Final Safety Visit but is optional at additional study visits. Every attempt should be made to perform SVC at required visits. SVC or Forced Vital Capacity (FVC) can be performed in a pulmonary function lab, if necessary.

[5] The study participant, study partner (for AD patients only), and investigator will complete a Global Impression of Change survey at the Week 24 or Final Safety Visit.

[6] Screening labs include Hematology (CBC with differential), Comprehensive metabolic panel (including liver function tests), Coagulation, B12, thyroid stimulating hormone (TSH), folate, HIV, Hepatitis B panel, Hepatitis C panel, Urinalysis, and Urine pregnancy test (for WOCBP). Participants will also be tested for Tuberculosis (TB) at the Screening Visit (see Protocol Section 8.1.2 Clinical Laboratory Assessments for additional information regarding TB testing).

[7] Safety labs include Hematology (CBC with differential), Comprehensive metabolic panel (including liver function tests), Coagulation, Urinalysis, and Urine pregnancy test (for WOCBP). A Lipid Panel will be collected at the Baseline, Week 8, and Week 24.

[8] All participants will provide a blood sample for PK analysis at the Baseline Visit (pre-dose). At Week 8, Week 16, and Week 24, samples will be collected 1 hour (±10 minutes) post-dose.

[9] All participants will provide a blood sample for biomarker testing and immune cell profiling as well as storage in a biorepository. At Week 8, Week 16, and Week 24, samples will be collected 1 hour (±10 minutes) post-dose.

[10] All participants will provide a blood sample for genetic testing for *C9ORF72* repeat expansion and *APOE* genotype. The sample will be sent to Prevention Genetics for analysis and results will be returned to participants.

[11] Participants will provide a urine sample for storage in a biorepository for use in future research.

[12] CSF will be collected for PK and biomarker analysis. The results of CSF examination from the Screening LP are needed to determine participant eligibility and before the Baseline Visit can occur. The Week 8 and Week 16 LPs will be performed 2 to 4 hours post-dose, ideally 2 hours post-dose.

[13] Administer the first dose of study drug AFTER all Baseline Visit procedures are completed and a final eligibility determination is made. At Week 8, Week 16, and Week 24, participants will bring all remaining study drug to clinic and dose in clinic from home supply at the beginning of the visit. Time of study drug administration will be recorded.

[14] Participants that withdraw consent or early terminate will be asked to complete a Final Safety Visit when they notify the site of their desire to end participation or as soon as possible thereafter.

[15] The Follow-up Telephone Call will be conducted 28 to 33 days after the participant takes their last dose of study drug (regardless of whether or not they have discontinued from the study).

[16] Visits may be conducted in clinic or remotely. Remote visits can occur via telemedicine or telephone with local safety lab collection.

[17] If a patient had an LP and CSF collected within 6 months of the Screening Visit, please contact the Coordination Center to confirm if this CSF sample could be utilized in place of the screening LP. Allowing CSF from a prior LP will be determined on case-by-case basis.

[18] Review of CBC and Coagulation results is required to confirm if LP can be performed. Please follow local policies for performing LPs and refer to Protocol Section 8.1.10 Lumbar Puncture for CSF collection.

[19] Routine vaccinations (e.g., influenza, COVID-19) received during the course of the trial should be administered at least 14 days prior to LP visits and will be recorded on the Concomitant Medication Log. Any live vaccinations are not recommended for participants taking baricitinib.

### 2.8 Interim Analyses

Interim analyses will be conducted by the chief biostatistician to assess the ability of baricitinib to cross the blood-brain barrier and enter the CSF or to exert a pharmacodynamic effect on CCL2 in the CSF. One of the following two PK and PD criteria must be met in at least one participant to continue enrollment beyond 10 participants:

1. PK criterion: free baricitinib concentration in the CSF > 0.1 μM from samples collected at the Week 8 or Week 16 LP, or
2. PD criterion: 25% reduction of CCL2 concentration in the CSF from baseline to the Week 8 or Week 16 LP.

Interim analyses will be conducted approximately monthly (as long as there are new results to review) after the first participant completes Week 8 until at least 1 participant meets the continuation criterion or until 10 participants have completed the Week 8 LP and none has met the continuation criterion. Enrollment will be suspended after 10 participants enroll if no participant has yet met the continuation criterion, including no fewer than 2 AD participants and no fewer than 2 ALS participants (of whom no more than 1 may be an AGC). No additional interim analyses will be conducted after the first participant meets the continuation criterion. Enrollment will be terminated if the continuation criterion has not been observed after 10 participants complete follow-up.

## 3. General Considerations for Data Analysis

### 3.1 Statistical Software

Statistical analyses will be performed using SAS (SAS Institute, NC, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

### 3.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, efficacy endpoints, safety endpoints, and tolerability. Summary statistics for continuous variables will include the number of observations, the mean, median, standard deviation, inter-quartile range, and range. Summaries of categorical data will include counts, denominators, and percentages.

### 3.3 Precision

Results will generally be reported to 3 significant figures. Percentages will generally be reported to 0.1 percentage points. P-values will generally be reported to two decimal places when greater than or equal to 0.0995, to four decimal places when greater than or equal to 0.001 and less than 0.0995, and as <0.001 for smaller values.

### 3.4 Transformations

Clinical endpoints generally will not be transformed. Biomarker endpoints generally will be log-transformed unless the distributions are more skewed after transformation. Baseline

characteristics generally will be reported as medians and ranges when distributions have skewness > 3 and as means, standard deviations, and ranges otherwise.

### 3.5 Multiplicity Adjustments

The primary PK objective will be evaluated descriptively, without inferential testing. The primary PD objective will be tested at $p < 0.025$ to control the overall type 1 error rate at 5% when testing change from baseline to 8 weeks and from baseline to 16 weeks for all participants pooled without regard to diagnosis. Nominal comparison-wise p-values will also be reported for all primary, secondary, and exploratory analyses.

### 3.6 Missing Data

If baseline values of a given measure are missing, the last observed pre-treatment value will be used. Missing baseline covariates of specified analyses will be imputed using the mean of the respective covariate. For any baseline covariate that is transformed for analysis, means will be imputed after transformation.

For analyses that depend on visit-specific data, off-schedule post-baseline observations, e.g., those collected as part of an Early Termination Visit or an unscheduled visit, will be used in place of the closest missing scheduled visit that preserves the true visit sequence if the observation time is as close or closer to the missing scheduled visit than to any other post-baseline scheduled visit. Other observations will not be carried forward. For analyses that do not depend on visit-specific data, observations will be analyzed according to the actual time observed.

The planned mixed model analyses, where applied, yield estimates that are unbiased conditional on the observed values under a missing at random assumption.

## 4. Study Endpoints and Comparisons

### 4.1 Biomarker Endpoints

The following biomarker endpoints will be analyzed to evaluate pharmacokinetic and pharmacodynamic response:

| Vendor - Assay | Analyte | Matrix | Study Objective |
|---|---|---|---|
| NorthEastern BioLab - PK analysis | Baricitinib | Plasma | Secondary |
| | Baricitinib | CSF | Primary |
| Albers Lab - MSD assays | CCL2 | Plasma | Exploratory |
| | CXCL10 | Plasma | Exploratory |
| | IL6 | Plasma | Exploratory |
| | CCL2 | CSF | Primary |
| | CXCL10 | CSF | Secondary |
| | IL6 | CSF | Secondary |

| Vendor - Assay | Analyte | Matrix | Study Objective |
|---|---|---|---|
| Albers lab - western blot | pPKR | CSF | Secondary |
| | PKR | CSF | Secondary |
| Kivisäkk lab | NfL | Plasma | Exploratory |
| | Abeta40 | Plasma | Exploratory |
| | Abeta42 | Plasma | Exploratory |
| | Tau | Plasma | Exploratory |
| | Ptau 181 | Plasma | Exploratory |
| | Ptau 217 | Plasma | Exploratory |
| | IFNG | Plasma | Exploratory |
| | GFAP | Plasma | Exploratory |
| | GFAP | CSF | Exploratory |
| | NfL | CSF | Secondary |
| | Abeta40 | CSF | Secondary |
| | Abeta42 | CSF | Secondary |
| | Tau | CSF | Secondary |
| | Ptau 181 | CSF | Secondary |
| | Ptau 217 | CSF | Secondary |
| | TDP-43 | Plasma | Secondary |
| | IFNG | CSF | Secondary |

## 4.2 Clinical Efficacy Endpoints

The following clinical endpoints, depending on diagnosis, will be analyzed to evaluate efficacy:

| Assessment | Diagnosis | Data Provider | Study Objective |
|---|---|---|---|
| ADAS-Cog | AD only | EDC / Everest | Exploratory |
| MoCA | AD only | EDC / Everest | Exploratory |
| NPI-Q | AD only | EDC / Everest | Exploratory |
| ALSFRS-R | ALS only | EDC / Everest | Exploratory |
| SVC | ALS only | EDC / Everest | Exploratory |

| Assessment | Diagnosis | Data Provider | Study Objective |
|---|---|---|---|
| NIH Toolbox | AGC only | Everest | Exploratory |
| AROMHA<br>OPID-9<br>OPID-18<br>POEM<br>OD test | <br>All<br>All<br>All<br>All | REDCap | Exploratory |
| GIC<br>Participant<br>Partner<br>Investigator | <br>All<br>AD only<br>All | EDC / Everest | Exploratory |

### 4.3 Clinical Safety Endpoints

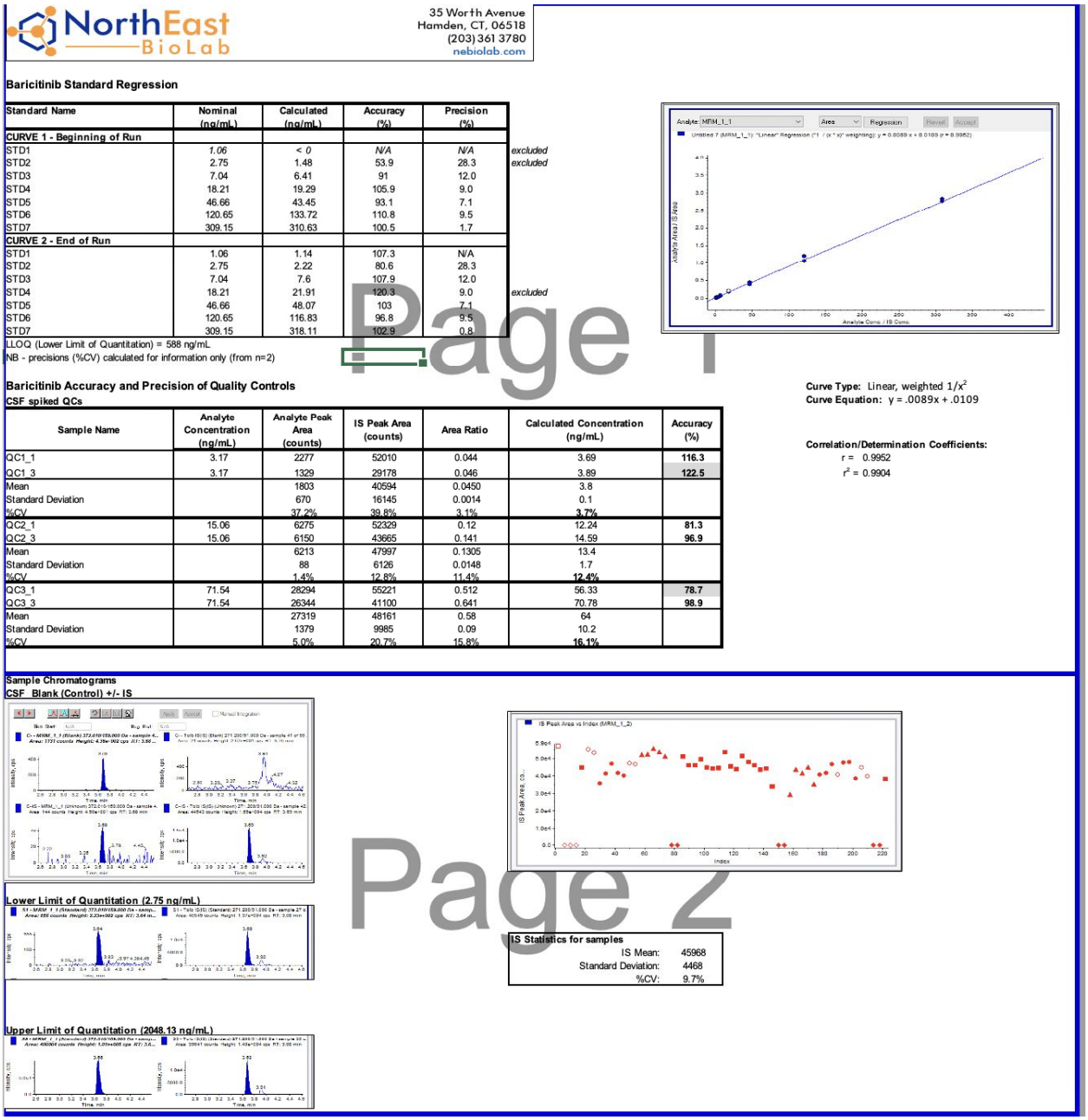
The following safety endpoints will be summarized to describe safety:

- Treatment-emergent adverse and serious adverse events, including clinically significant abnormalities in clinical and laboratory values.

## 5. Measurement Definitions

### 5.1 Pharmacokinetic Concentrations

Baricitinib concentration in plasma and CSF will be quantified by tandem high-performance liquid chromatography coupled with mass spectrometry (NorthEast BioLab, Hamden, CT). Baricitinib 99% purity diluted in control CSF will be used to conduct the standard curve. Ruxolitinib will be used as the internal standard. Samples will be run in duplicate for each combination of participant x visit x matrix.



NorthEast BioLab
35 Worth Avenue
Hamden, CT, 06518
(203) 361 3780
nebiolab.com

**Baricitinib Standard Regression**

| Standard Name | Nominal (ng/mL) | Calculated (ng/mL) | Accuracy (%) | Precision (%) | |
|---|---|---|---|---|---|
| **CURVE 1 - Beginning of Run** | | | | | |
| STD1 | *1.06* | *< 0* | *N/A* | *N/A* | *excluded* |
| STD2 | 2.75 | 1.48 | 53.9 | 28.3 | *excluded* |
| STD3 | 7.04 | 6.41 | 91 | 12.0 | |
| STD4 | 18.21 | 19.29 | 105.9 | 9.0 | |
| STD5 | 46.66 | 43.45 | 93.1 | 7.1 | |
| STD6 | 120.65 | 133.72 | 110.8 | 9.5 | |
| STD7 | 309.15 | 310.63 | 100.5 | 1.7 | |
| **CURVE 2 - End of Run** | | | | | |
| STD1 | 1.06 | 1.14 | 107.3 | N/A | |
| STD2 | 2.75 | 2.22 | 80.6 | 28.3 | |
| STD3 | 7.04 | 7.6 | 107.9 | 12.0 | |
| STD4 | 18.21 | 21.91 | 120.3 | 9.0 | *excluded* |
| STD5 | 46.66 | 48.07 | 103 | 7.1 | |
| STD6 | 120.65 | 116.83 | 96.8 | 9.5 | |
| STD7 | 309.15 | 318.11 | 102.9 | 0.8 | |

LLOQ (Lower Limit of Quantitation) = 588 ng/mL
NB - precisions (%CV) calculated for information only (from n=2)

**Curve Type:** Linear, weighted $1/x^2$
**Curve Equation:** y = .0089x + .0109

**Correlation/Determination Coefficients:**
r = 0.9952
$r^2$ = 0.9904

**Baricitinib Accuracy and Precision of Quality Controls**
**CSF spiked QCs**

| Sample Name | Analyte Concentration (ng/mL) | Analyte Peak Area (counts) | IS Peak Area (counts) | Area Ratio | Calculated Concentration (ng/mL) | Accuracy (%) |
|---|---|---|---|---|---|---|
| QC1_1 | 3.17 | 2277 | 52010 | 0.044 | 3.69 | **116.3** |
| QC1_3 | 3.17 | 1329 | 29178 | 0.046 | 3.89 | **122.5** |
| Mean | | 1803 | 40594 | 0.0450 | 3.8 | |
| Standard Deviation | | 670 | 16145 | 0.0014 | 0.1 | |
| %CV | | 37.2% | 39.8% | 3.1% | **3.7%** | |
| QC2_1 | 15.06 | 6275 | 52329 | 0.12 | 12.24 | **81.3** |
| QC2_3 | 15.06 | 6150 | 43665 | 0.141 | 14.59 | **96.9** |
| Mean | | 6213 | 47997 | 0.1305 | 13.4 | |
| Standard Deviation | | 88 | 6126 | 0.0148 | 1.7 | |
| %CV | | 1.4% | 12.8% | 11.4% | **12.4%** | |
| QC3_1 | 71.54 | 28294 | 55221 | 0.512 | 56.33 | **78.7** |
| QC3_3 | 71.54 | 26344 | 41100 | 0.641 | 70.78 | **98.9** |
| Mean | | 27319 | 48161 | 0.58 | 64 | |
| Standard Deviation | | 1379 | 9985 | 0.09 | 10.2 | |
| %CV | | 5.0% | 20.7% | 15.8% | **16.1%** | |

**Sample Chromatograms**
**CSF Blank (Control) +/- IS**

**Lower Limit of Quantitation (2.75 ng/mL)**

**Upper Limit of Quantitation (2048.13 ng/mL)**

**IS Statistics for samples**

| | |
|---|---|
| IS Mean: | 45968 |
| Standard Deviation: | 4468 |
| %CV: | 9.7% |



If both duplicate estimates are below the limit of quantitation (BLQ), then the result will be reported as BLQ. If both estimates are quantified, then the geometric mean concentration will be used for analysis. If one estimate is BLQ and one is quantified, then the geometric mean of the calculated concentrations will be used for analysis.

## 5.2 Pharmacodynamic Biomarkers

Levels of chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 10 (CXCL10), and interleukin-6 (IL-6) in plasma and CSF will be quantified by electrochemiluminescence immunoassay using the MSD platform (Meso Scale Diagnostics, Rockville, MD). The following kits will be used: CCL2 (K151UGK**,** U-PLEX Human MCP-1 assay), CXCL10 (K151UFK-2, U-PLEX Human IP-10 assay), IL-6 (K151TXK-2, U-PLEX Human IL-6 assay). Optical density

will be quantified using a MSD QuickPlex SQ 120mm plate reader (Meso Scale Diagnostics, Rockville, MD).

For each combination of participant x visit x matrix, three samples will be run on separate plates with each sample run in two duplicate wells on a given plate. The geometric mean of all six replicates will be used for analysis.

Levels of phospho-protein kinase R (pPKR) and protein kinase R (PKR) will be quantified by western blot following the protocol described in Rodriguez et al, Science Transl. Med., 2021. Gels will be probed using the following primary antibodies: pPKRT446 (Abcam, Ab32036), PKR (Thermo Fisher 23H52L96).

Optical density will be quantified using a Fujifilm LAS-3000 series or newer gel reader (Fujifilm, Tokyo, Japan).

For each combination of participant x visit x matrix, 2 samples will be run on separate gels with each sample run in 2 duplicate lanes on a given gel. The geometric mean of all 4 replicates will be used for analysis.

The CSF neurodegenerative biomarkers (Ab40, Ab42, tau-P217, NfL) will be performed on the Quanterix Simoa platform (Quanterix Corporation, Lexington, MA). The plasma neuro-inflammatory biomarker (GFAP) and neurodegenerative markers (tau-P217 and NfL) will be run on the Fujirebio platform (Fujirebio Diagnostics, Malvern, PA) in the lab of Dr. Steve Arnold. QC analyses will be performed, and each sample will be run in duplicate. Samples with duplicate coefficient of variation above 20% will be re-run. The average value from the final run will be used for analysis.

## 5.3 Clinical Instruments

### 5.3.1 ADAS-Cog

The Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog; Rosen et al. 1984) is an 11-item instrument completed by clinician interview for assessing participants' cognitive function in four domains: memory, language, praxis, and orientation. The ordered commands, constructional praxis, naming, ideational praxis, remembering instructions, comprehension of language, word finding, and spoken language tasks are scored from 0 to 5. The orientation task is scored from 0 to 8. The delayed recall task is scored from 0 to 10. And the word recognition task is scored from 0 to 12. In each case, higher scores indicate greater cognitive impairment. The ADAS-Cog total score is the sum of all items (range 0 to 70). A missing item results in a missing value for the total score.

### 5.3.2 MoCA

The Montreal Cognitive Assessment (MoCA; Nasreddine et al. 2005) is an 8-item instrument completed by clinician interview for assessing participants' cognitive function in 8 domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. One point is awarded for correct completion of each item of the visuospatial/executive function task (5 items), naming task (3 items), digit vigilance and tapping items of the attention task (3 items), the sentence repetition items of the language task (2 items), abstraction task (2 items), delayed recall task (5 items), and orientation task (6 items). One point is awarded for naming 11 or more words during the fluency item of the language task. Zero (none correct) to 3 (4 or more correct) points are awarded based on the

number of correct subtractions by 7 starting at 100 in the attention task. One point is awarded if the participant has 12 years or less of education unless the score is already 30. The MoCA total score is the sum of all items (range 0 to 30). Higher scores indicate greater cognitive capacity. A missing item results in a missing value for the total score.

### 5.3.3 NPI-Q

The Neuropsychiatric Inventory Questionnaire (NPI-Q; Cummings et al. 1994, Cummings 1997, Kaufer et al. 1998) is a caregiver-completed scale designed to evaluate 12 subdomains of behavioral functioning: delusions, hallucinations, agitation, dysphoria, anxiety, apathy, irritability, euphoria, disinhibition, aberrant motor behavior, night-time behavior disturbances, and appetite and eating abnormalities. Each domain has a screening question that assesses the presence of the specific disturbance. If present, a series of 7 to 9 more detailed Yes/No subquestions are asked, the frequency is rated on a scale from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously), the severity is rated on a scale from 1 (mild) to 3 (marked), and caregiver emotional distress is rated on a scale from 0 (not at all [distressed]) to 5 (very severely or extremely [distressed]). The total score for each domain (range 0 to 12) is calculated by multiplying the frequency by the severity or given a value of zero if presence of the disturbance for that domain was not endorsed. A total NPI score is calculated by adding all domain-specific total scores together (range 0 to 144) with higher scores indicating worse neuropsychiatric disturbance. A total caregiver distress score (NPI-D, range 0 to 60) is calculated by adding all domain-specific caregiver distress scores.

### 5.3.4 ALSFRS-R

The ALS Functional Rating Scale Revised (ALSFRS-R; Cedarbaum et al. 1999) is a 12-item instrument completed by clinician interview for assessing participants' function in four domains: bulbar, fine motor, gross motor, and respiratory. Each item is scored from 0 to 4 with higher scores indicating greater function. The ALSFRS-R total score is the sum of all items (range 0 to 48). A missing item results in a missing value for the total score.

### 5.3.5 SVC

Slow vital capacity (SVC) is the maximum volume of air that can be slowly exhaled after slow, maximal inhalation. Trained technicians coach participants through 3 to 5 maneuvers using a spirometer. A minimum of 2 completed SVC maneuvers are required to calculate an estimate of maximum SVC for a given visit. The maximum volume expired is converted to percent of predicted normal using normal values for forced vital capacity (FVC). FVC normal values are calculated based on sex, age, height, and race using equations published by the Global Lung Function Initiative (GLI, Quanjer et al. 2012). Higher values indicate greater respiratory function.

Age will be calculated as number of days from date of birth to the date of a given SVC assessment divided by 365.25. Age and height will be rounded to 0.1 units during calculation of normative volumes. The following correspondence between self-identified race and race defined by GLI classification will be used:

| Self-identified Race | GLI-defined Race |
|---|---|
| American Indian or Alaska Native | Mixed/Other |

| Self-identified Race | GLI-defined Race |
|---|---|
| Asian | South and East Asian |
| Black or African American | African American |
| Native Hawaiian or Other Pacific Islander | Mixed/Other |
| White | Caucasian |
| Unknown | Caucasian |
| Not reported | Caucasian |
| More than one race indicated | Mixed/Other |

### 5.3.6 AROMHA

The Accessible Remote Olfactory Mediated Health Assessments (AROMHA) olfactory battery is administered by a trained research coordinator to measure cognitive processing of odor stimuli in three domains: odor percept identification (OPID-9; OPID-18), episodic memory of odor percepts (POEM), and odor discrimination (OD).

The OPID-9 assesses recognition of 9 odors, each assessed using a multiple choice test with 4 options, one of which is correct. The OPID-9 is scored as the number of correctly identified odors (range 0 to 9) with higher scores indicating better odor precept identification.

The OPID-18 assesses both recognition of 18 odors and episodic memory of odors presented in the OPID-9 assessment. The participant is asked to recall whether they previously smelled each odor (yes/no, 9 of 18 were previously challenged) and then to identify the odor using a multiple choice test with 4 options, one of which is correct. The POEM is scored as the proportion of OPID-18 odors that were correctly identified as having been previously challenged or not previously challenged in the OPID-9 (range 0 to 1) with higher scores indicating better episodic memory of odor precepts. The OPID-18 is scored as the number of correctly identified odors (range 0 to 18) with higher scores indicating better odor precept identification.

The OD test assesses discrimination of 10 pairs of odors. The participant is asked to identify whether the two odors in a pair are the same or different (5 of 10 are the same). The OD is scored as the number of correctly discriminated pairs of odors (range 0 to 10) with higher scores indicating better odor discrimination.

### 5.3.7 GIC

The Global Impression of Change (GIC) will be completed by the study participant, study partner (for AD participants only), and the treating investigator. Each respondent will rate change from baseline on a 7-point scale (1 = Very Much Improved, 2 = Much improved, 3 = Minimally improved, 4 = No change, 5 = Minimally worse, 6 = Much worse, 7 = Very much worse) with higher scores indicating greater disease progression.

# 6. Statistical Methodology

## 6.1 Analysis Sets

The following analysis data sets will be used for testing pharmacokinetics, pharmacodynamics, clinical efficacy endpoints, and clinical safety endpoints:

- Biomarker (BM) Data Set: Non-missing values for a given pharmacokinetic or pharmacodynamic biomarker measured from samples collected at screening, baseline, and all post-baseline visits during which the participant was still taking baricitinib and classified according to the dosage of baricitinib (0 mg, 2 mg, or 4 mg) taken the day before sample collection.
- Clinical Efficacy (CE) Data Set: Non-missing values for a given clinical efficacy endpoint measured at all scheduled visits and classified according to the scheduled dosage of baricitinib (0 mg, 2 mg, or 4 mg).
- Clinical Safety (CS) Data Set: All adverse events and serious adverse events reported after the first dose of baricitinib.

## 6.2 Baseline Characterization

The following baseline characteristics will be summarized for all participants who initiated baricitinib treatment: age, sex, race, ethnicity, OPID-9, OPID-18, POEM, OD test, APOE genotype, all immunologic and neurodegenerative biomarkers.

The following baseline characteristics will be summarized for AD participants: diagnosis as SCD, MCI, or AD; biomarker evidence of AD pathophysiology; acute or insidious onset; slow, rapid, or stepwise progression; time from symptom onset; diagnostic delay; family history among first degree relatives; family history among other relatives; use of rivastigmine, galantamine, donepezil, memantine, aducanumab, and lecanemab; ADAS-Cog total score; MoCA total score; NPI-Q total score.

The following baseline characteristics will be summarized for ALS participants: site of symptom onset; time from symptom onset; diagnostic delay; El Escorial criteria; family history of ALS; C9orf72 repeated expansion; use of riluzole, edaravone, and Relyvrio; ALSFRS-R total score; SVC.

The following baseline characteristics will be summarized for AGC participants: C9orf72 repeated expansion.

## 6.3 Pharmacokinetics

At each visit, the proportion of participants with concentrations above the lower limit of quantitation and the geometric mean concentration, coefficient of variation, and concentration range in each matrix will be reported.

## 6.4 Pharmacodynamics

Change in over time in PD biomarkers will be estimated separately for each biomarker x matrix in the BM data set using a mixed model repeated-measures analysis with log-transformed concentration as the dependent variable, diagnostic group, visit, and their interaction as fixed effects, and unstructured participant-level covariance among repeated assessments. Satterthwaite's approximation will be used to estimate denominator degrees of freedom.

The following equation describes the model:

$$Y_{ij} = \gamma_{1,D} d_i + \gamma_{2,j} v_j + \gamma_{3,D,j} d_i v_j + \epsilon_{ij}$$
$$\boldsymbol{\epsilon_{i\cdot}} \sim N(\mathbf{0}, \boldsymbol{R}) \quad \text{(eqn. 1)}$$

where $Y_{ij}$ is a given log-transformed biomarker level measured for participant $i$ at visit $j$, $d_i$ is an indicator variable for diagnostic group $i$, $v_j$ is an indicator variable for visit $j$, $\gamma_{1,D}$, $\gamma_{2,j}$, and $\gamma_{3,D,j}$, are estimated parameters for the fixed effects, and $\epsilon_{ij}$ is the residual for participant $i$ at visit $j$. The vector of residuals for a given participant are normally distributed with mean **0** and an unstructured covariance matrix **R**.

Visit-specific mean levels with 95% confidence bounds and changes from screening to baseline and from baseline to Week 8, Week 16, and Week 24 will be estimates across all participants at their enrolled frequency, across AD and ALS participants at equal frequency, and within each diagnosis separately. Pair-wise differences between diagnostic groups will be estimated.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class DX SUBJID AVISIT;
  model AVALLN = DX | AVISIT / solution cl ddfm=sat;
  repeated AVISIT / subject=SUBJID(DX) type=un;
```

where `DX` is the diagnosis identifier, `SUBJID` is a participant identifier, `AVISIT` is the visit identifier, and `AVALLN` is a log-transformed biomarker level for a given participant at a given visit.

### 6.5 Clinical Efficacy

Change over time in clinical efficacy endpoints will be estimated separately for each endpoint in the CE data set using a linear mixed model analysis with the clinical endpoint value as the dependent variable, diagnostic group, visit, and their interaction as fixed effects, and participant-level random intercepts and slopes with unstructured covariance. Satterthwaite's approximation will be used to estimate denominator degrees of freedom.

The following equation describes the model:

$$Y_{ij} = \gamma_{1,D} d_i + \gamma_{2,j} v_j + \gamma_{3,D,j} d_i v_j + b_k^0 + b_k^1 t_{ij} + \epsilon_{ij}$$
$$\{b_k^0, b_k^1\} \sim N\left(\mathbf{0}, \boldsymbol{\Sigma_p}\right), \epsilon_{ij} \sim N(0, \sigma_\epsilon^2), \text{and } \mathrm{Cov}(\boldsymbol{b_k}, \boldsymbol{\epsilon_{i\cdot}}) = \mathbf{0} \quad \text{(eqn. 3)}$$

where $b^0_i$ and $b^1_i$ are the random intercept and slope for participant $i$, $t_{ij}$ is the time from baseline to observation $j$ for participant $i$ in months calculated as days x 12 / 365.25, $\gamma_{1,D}$, $\gamma_{2,j}$, and $\gamma_{3,D,j}$, are estimated parameters for the fixed effects, and , and $\epsilon_{ij}$ is the residual for participant $i$ at visit $j$. The participant-specific random effects are normally distributed with mean **0** and unstructured covariance matrix $\boldsymbol{\Sigma_p}$. The residuals for a given participant are normally distributed with mean 0 and variance $\sigma^2_\epsilon$. The participant-specific random effects and residuals are uncorrelated.

Visit-specific mean levels with 95% confidence bounds and changes from screening to baseline and from baseline to Week 8, Week 16, and Week 24 will be estimated across all participants at

their enrolled frequency, across AD and ALS participants at equal frequency, and within each diagnosis separately. Pair-wise differences between diagnostic groups will be estimated.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class DX SUBJID AVISIT;
  model AVAL = DX | AVISIT / solution cl ddfm=sat;
  random intercept AMONTH / subject=SUBJID(DX) type=un;
```

where `AVAL` is the value of a clinical endpoint for a given participant at a given visit, `AMONTH` is time in months from the Baseline Visit (assuming 12 months in an average of 365.25 days per year), and other fields are the same as identified above in Section 6.4 above.

### 6.6 Prognostics

Prognostics of differential change from baseline to Week 8 and from baseline to Week 16 will be evaluated by correlating baseline values of potential prognostics vs. participant-specific 8-week or 16-week changes estimated from the models described in Section 6.4 and 6.5. Potential prognostics will include plasma and CSF baricitinib concentrations at Week 8 and Week 16, baseline values of PD biomarkers, age, and sex. A parsimonious signature of baseline prognostics will be identified by least-angle regression of estimated 8-week and 16-week change vs. the full list of potential baseline prognostics.

### 6.7 Correlated Changes

Participant-specific 16-week changes estimated from the models described in Section 6.4 and 6.5 will be cross-correlated to identify whether any PD biomarker changes best predict changes in clinical efficacy endpoints.

### 6.8 Pre-intervention Change

Mean change from screening to baseline with 95% confidence bounds will be estimated for all PD biomarkers and clinical efficacy endpoints in the models described in Section 6.4 and 6.5. Consistency of measurements prior to baricitinib treatment will also be quantified as intra-class correlations from analysis of the screening and baseline data alone in a heteroscedastic mixed model with fixed effects of diagnosis, visit, and their interaction and random participant-specific intercepts and residuals stratified by diagnosis.

### 6.9 Safety Analyses

Treatment-emergent adverse events (TEAEs) and serious TEAEs will be summarized in the CS data set by MedDRA system organ class, high-level term, and preferred term. Summaries will include the count of events and the number and proportion of participants who experienced at least one instance of each classified type of TEAE separately by diagnosis and overall.

### 6.10 Participant Disposition

Participant disposition will be summarized according to the number of participants consented, determined eligible, treated with baricitinib 2 mg per day, discontinued treatment, early terminated, or withdrew consent after initiating baricitinib 2 mg per day, treated with baricitinib 4 mg per day, discontinued treatment, early terminated, or withdrew consent after initiating baricitinib 4 mg per day, and completed 24 week follow-up. Reasons for early discontinued treatment, early terminated, or withdrew consent after initiating baricitinib will be summarized.

The number of participants who received a vaccination within 14 days of their Baseline Visit will be tabulated.

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