# Cover Page – Protocol

**Study Official Title:** A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS)

**NCT #:** NCT02469896

**Version Date of the Document:** May 30, 2017

# A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS)

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| **Regulatory Sponsor:** | Barrow Neurological Institute |
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| **Study Product:** | Tocilizumab (Actemra™) |
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| **Amended Version Number/Date:** | 8.0 / 30 May 2017 |

## STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and the applicable regulatory requirements, United States Code of Federal Regulations (CFR) Title 45 CFR Part 46 and Title 21 CFR Parts 50, 56, and 312.

**SIGNATURE PAGE**

I have read the attached protocol entitled, "**A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS)**" dated **30 May 2017 (Version 8.0)** and agree to abide by all described protocol procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable FDA regulations and guidelines identified in 21 CFR Parts 11, 50, 54, and 312, local Institutional Review Board (IRB) guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

Site Investigator: _____

Signed: _____ Date: _____

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADR | Adverse Drug Reaction |
| AE | Adverse Event/Adverse Experience |
| ALS | Amyotrophic Lateral Sclerosis |
| ALSFRS-R | Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised |
| ALT | Alanine Aminotransferase |
| ANC | Absolute Neutrophil Count |
| AST | Aspartate Aminotransferase |
| BiPAP | Bilevel Positive Airway Pressure |
| ºC | Degrees Celsius |
| CBC | Complete Blood Count |
| CC | Coordination Center |
| CFR | Code of Federal Regulations |
| CMP | Complete Metabolic Panel |
| CRF | Case Report Form |
| CSF | Cerebrospinal Fluid |
| CYP450 | Cytochrome P450 |
| DM | Data Management |
| DPS | Diaphragm Pacing System |
| DSMB | Data and Safety Monitoring Board |
| ECG | Electrocardiogram |
| EDC | Electronic Data Capture |
| EOS | End of Study |
| ºF | Degrees Fahrenheit |
| FDA | Food and Drug Administration |
| GCP | Good Clinical Practice |
| hCG | Human Chorionic Gonadotrophin |
| HHD | Handheld Dynamometry |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| hs- CRP | Highly sensitive C-reactive protein |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| IL-6 | Interleukin-6 |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |

| | |
|---|---|
| ITT | Intent to Treat |
| IV | Intravenous |
| LP | Lumbar Puncture |
| LFT | Liver Function Test |
| mg | Milligram |
| mg/kg | Milligram per kilogram |
| mL | Milliliter |
| $mm^3$ | Millimeter cubed |
| mmHg | Millimeter of mercury |
| MR-PET | Magnetic Resonance Imaging - PBR28 Positron Emission Tomography |
| N | Number (typically refers to subjects) |
| NEALS | Northeast ALS Consortium |
| NMDA | N-methyl-D-aspartate |
| OHRP | Office for Human Research Protections |
| pg/mL | Picogram per milliliter |
| PA | Posterioranterior |
| PBMC | Peripheral Blood Mononuclear Cell |
| PBR28 | Peripheral Benzodiazepine Receptor 28 |
| PET | Positron Emission Tomography |
| PLPH | Post-LP Headache |
| RNA | Ribonucleic Acid |
| SAE | Serious Adverse Event/Serious Adverse Experience |
| SDT | Source Document Templates |
| SI | Site Investigator |
| sIL-6r | Soluble IL-6 Receptor |
| SOD1 | Superoxide dismutase 1 |
| SVC | Slow Vital Capacity |
| TB | Tuberculosis |
| TSPO | Translocator Protein |
| ULN | Upper Limit of Normal |
| UMNB | Upper Motor Neuron Burden Scale |
| US | United States |
| µg/kg | Microgram per kilogram |
| WOCBP | Women of Childbearing Potential |

## PROTOCOL SUMMARY

**Study Title**

A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS)

**Version Number**

8.0

**Study Indication**

Amyotrophic Lateral Sclerosis (ALS)

**Phase of Development**

2

**Rationale for the Study**

Despite the aggressive nature of Amyotrophic Lateral Sclerosis (ALS) and decades of research there has been no cure or highly efficacious therapy. In 1995, the FDA approved the only treatment for ALS, riluzole (Rilutek®). Although the mechanism of action is not clear, it is felt to act upon multiple targets to reduce excitatory neurotoxicity in motor neurons. It may do this through inhibition of sodium channels, by inhibiting glutamate or other excitatory neurotransmitter release and by N-methyl-D-aspartate (NMDA) receptor antagonism [1]. Since riluzole's approval, various agents have been tested for efficacy and uniformly failed to demonstrate a survival benefit.

This failure has been attributed to several factors [2]. The first and primary reason is that the pathophysiology of the disease is poorly understood, especially with regard to early or precipitating cellular triggers. There is evidence that oxidative stress, excitoxicity, mitochondrial dysfunction, and aberrant ribonucleic acid (RNA) processing all contribute to eventual motor neuron death. The second reason is that current clinical trials lack a meaningful and robust surrogate marker of disease activity. This results in longer, more costly trials that are not able to properly stratify or randomize patients.

In this study, we hope to address these two issues by focusing on the pathophysiologic immune mechanisms involved in motor neuron death and by utilizing the peripheral blood mononuclear cells (PBMC) gene expression profile to quantify the immune response in ALS patients. If this profile is found to mirror disease progression, as our preliminary data suggests, this expression

profile could be used in a future phase 3 efficacy trial as a selection criteria as well as a surrogate marker of disease progression.

We will also measure the effect of tocilizumab in reducing glial activation as measured by PBR28 positron emission tomography (PET) in a subset of study participants.

**Study Design**

This is a multicenter, randomized, double-blind, placebo-controlled 16-week study evaluating the safety and tolerability of tocilizumab in subjects with sporadic ALS.

**Study Objectives and Endpoints**

The primary objective of the study is to determine the safety and tolerability of intravenous administration of 8 mg/kg of tocilizumab every 4 weeks vs. matched intravenous placebo administered every 4 weeks over an 8 week period.

The primary outcome measure will be safety and tolerability of tocilizumab.

The secondary objective of the study is to describe the expression of pro-inflammatory genes in PBMCs of sporadic ALS patients, to assess the ability of tocilizumab to reduce the expression of pro-inflammatory genes in PBMCs and pro-inflammatory cytokines in the cerebrospinal fluid (CSF) of patients with sporadic ALS and to assess the CSF penetration of tocilizumab.

Exploratory measures will include clinical measures of disease progression such as ALSFRS-R, forced vital capacity, and handheld dynamometry. In addition, genotyping of the IL-6 receptor gene will be performed to look for polymorphisms that might affect the inflammatory state mediated by the IL-6 pathway.

Subjects enrolled at Massachusetts General Hospital only will have an additional exploratory measure. A [$^{11}$C]PBR28-PET scan will be performed at the Baseline visit (Pre-dose), and once at Week 8 visit (Post-dose).

**Study Locations**

Approximately 5 Northeast ALS Consortium (NEALS) Centers in the US will participate in the study.

**Number of Planned Subjects**

Approximately 24 subjects will be randomized in the study.

**Study Population**

This study will be conducted in subjects who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS. At screening, eligible subjects must be at least 18 years old, must have a slow vital capacity (SVC) ≥ 40% of predicted capacity for age, height and gender, and must provide written informed consent prior to screening. Subjects on a stable dose of riluzole and those not taking riluzole, and women of child-bearing age at screening are eligible for inclusion as long as they meet specific protocol requirements. Detailed criteria are described in the body of the protocol.

**Treatment Groups**

Subjects will be randomly assigned in a 2:1 ratio to intravenous tocilizumab 8 mg/kg or matching placebo every 4 weeks over an 8 week period.

This research study protocol allows the subject to receive up to 3 infusions of Tocilizumab. Even if the treatment is shown to be of benefit, additional infusions of Tocilizumab beyond that allowed in the protocol cannot be given to the subject while she/he is participating in this study.

**Duration of Treatment and Follow-up**

Subjects will remain on randomized, placebo-controlled, double-blind treatment until the Week 8 visit. Each randomized subject will also have a Week 12 Follow-up visit and Week 16 End-of-Study visit to assess for adverse events (AEs), changes in concomitant medications, to administer the ALSFRS-R and selected study procedures.

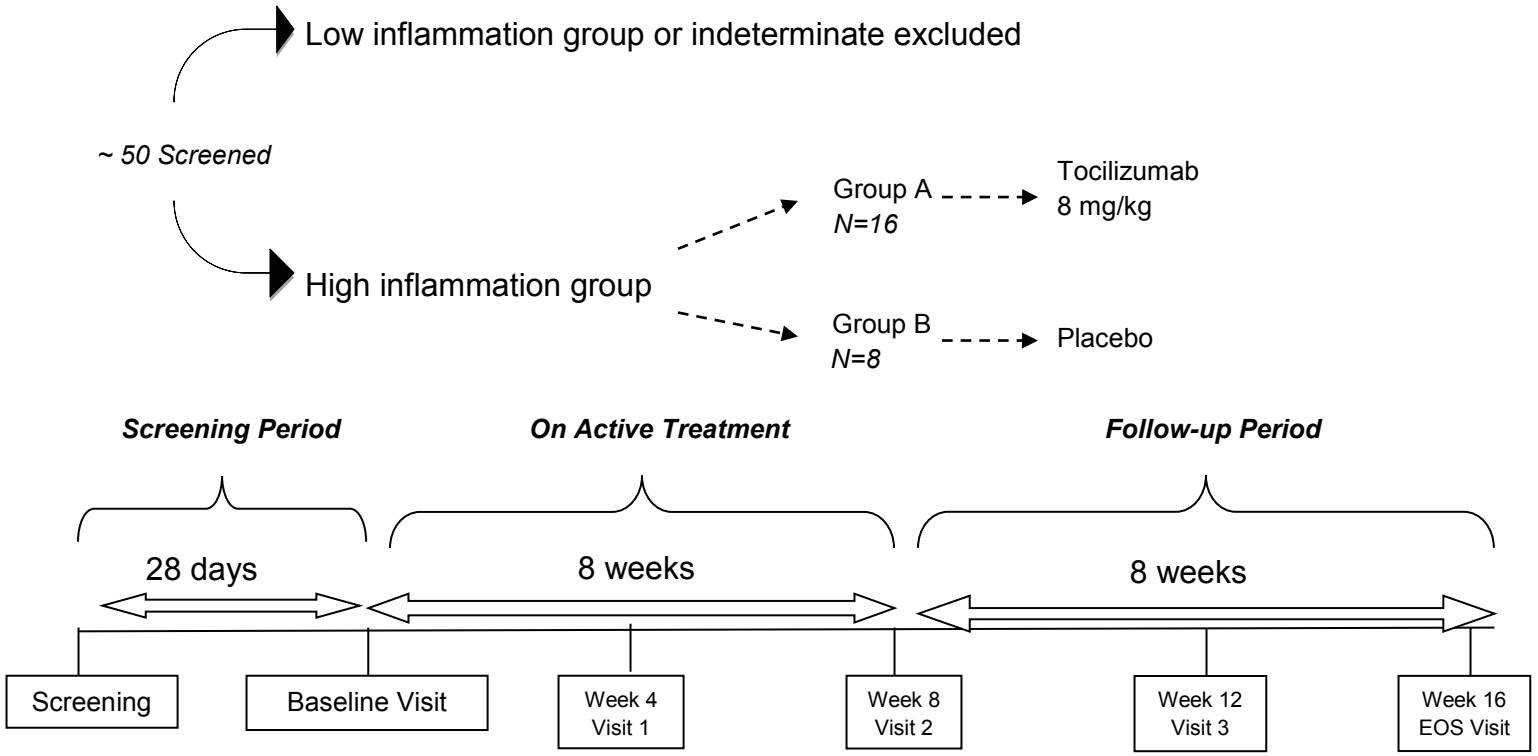
**SCHEDULE OF ACTIVITES**

| Activity | Screening Visit[1] | Baseline Visit | Week 4 | Week 8 | Week 12 | Week 16 |
|---|---|---|---|---|---|---|
| | | | Visit 1 | Visit 2 | Visit 3 | EOS Visit |
| Written Informed Consent | X | | | | | |
| Inclusion/Exclusion Review | X | X | | | | |
| Medical History | X | | | | | |
| Demographics | X | | | | | |
| ALS Diagnosis History | X | | | | | |
| Physical Examination | X | | | | | X |
| Neurological Exam | X | | | | | X |
| Columbia Suicide Severity Rating Scale (C-SSRS) | | X | X | X | X | X |
| Vital Signs[2] including Height[3] and Weight | X | X | X | X | X | X |
| Lumbar Puncture (LP)[4] | | X[4] | | X[4] | | |
| ALSFRS-R | | X | X | X | X | X |
| Slow Vital Capacity (SVC) | X | X | X | X | X | X |
| Hand Held Dynamometry (HHD) and Grip Strength | | X | | X | | X |
| Electrocardiogram (12-Lead ECG) | X | | | | | |
| PA and Lateral Chest X-Ray | X | | | | | |
| Quantiferon or T-SPOT TB Blood Test | X | | | | | |
| Safety Labs[5] | X | X | X | X | X | X |
| PBMC Collection | X[6] | X[7] | X | X | X | X |
| Plasma Biomarker Collection | | X | X | X | X | X |
| Blood Collection for IL-6R genotype, Genentech sample | | X | | | | |
| Concomitant Medication Review | X | X | X | X | X | X |
| Adverse Event Review[8] | X | X | X | X | X | X |
| Randomization[9] | | X | | | | |
| Administer Study Drug | | X | X | X | | |
| Exit Questionnaire | | | | | | X |
| [$^{11}$C]PBR28-PET | | X* | | X** | | |
| MRI Safety Questionnaire | X | X | | X | | |
| Upper Motor Neuron Burden Scale | X | X | | X | | |
| Blood draw for TSPO | X*** | | | | | |

[1] Screening procedures must be completed within 28 days prior to Baseline Visit.
[2] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.

[3] Height measured at Screening Visit only.
[4] Telephone call will be made 24-48 hours post lumbar puncture (LP) to assess for adverse events (AEs) related to the LP.
[5] Safety labs include Hematology (CBC with differential), ChemistryPanel, Hepatitis B panel, , Urinalysis and pregnancy test (WOCBP) as required throughout the study. Serum hCG will be collected at Screening Visit, and urine pregnancy tests will be collected at each subsequent visit for all WOCBP.
[6] Plasma Biomarkers will not be collected at the screening visit.
[7] Collection at Baseline to occur prior to infusion
[8]Adverse events that occur AFTER signing the informed consent form will be recorded.
[9] Randomization should occur immediately prior to the Baseline Visit.
*Pre-dose [$^{11}$C]PBR28-PET will be performed one time only at the Baseline visits. (-2 Weeks)
**Post-dose [$^{11}$C]PBR28-PET will be performed one time only at the Week 8 visit (- 2 Weeks).
*** Blood draw for TSPO affinity testing to occur at the Screening visit, if not previously determined.

## STUDY WORKFLOW




- Study drug infusion at Baseline, Visit 1 and Visit 2.
- Subjects who discontinue study drug early but agree to be followed will be asked to remain in the study and to attend all assigned visits until the Week 16 End-of- study visit.
- Subjects who discontinue study drug early and do not agree to be followed will be asked to return to the study site for a final safety assessment, and will be asked to have a final follow-up telephone call 28 days (+5 days) after taking their last dose of study drug.

# 1 ETHICS/PROTECTION OF HUMAN SUBJECTS

## 1.1 Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21 Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

## 1.2 Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonization (ICH) and the ethical principles of the Declaration of Helsinki.

## 1.3 Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subjects who wish to will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

# 2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

## 2.1 Background Information

Despite the aggressive nature of Amyotrophic Lateral Sclerosis (ALS) and decades of research, there has been no cure or highly efficacious therapy. In 1995, the FDA approved the only treatment for ALS, riluzole (Rilutek®). Although the mechanism of action is not clear, it is believed to act upon multiple targets to reduce excitatory neurotoxicity in motor neurons. It may do this through inhibition of sodium channels, by inhibiting glutamate or other excitatory neurotransmitter release, and by N-methyl-D-aspartate (NMDA) receptor antagonism [1]. Since riluzole's approval, various agents have been tested for efficacy and uniformly failed to demonstrate a survival benefit.

This failure has been attributed to several factors [2]. The primary reason is that the pathophysiology of the disease is poorly understood, especially with regard to early or precipitating cellular triggers. There is evidence that oxidative stress, excitoxicity, mitochondrial dysfunction, and aberrant ribonucleic acid (RNA) processing all contribute to eventual motor neuron death. The second reason is that current clinical trials lack a surrogate marker of disease activity. This results in longer, more costly trials that are not able to properly target patients for enrollment and do not advance understanding of ALS pathophysiology.

In this study, we hope to address these two issues by focusing on the pathophysiologic immune mechanisms involved in motor neuron death and by utilizing the peripheral blood mononuclear cells (PBMC) gene expression profile to select patients for enrollment and to quantify the immune response in ALS patients. If this profile is found to mirror disease progression, as our preliminary data suggests, this expression profile could be used in a future phase 3 efficacy trial as surrogate marker of disease progression.

## 2.2 Rationale

Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor distributed by Genentech, Inc. under the trade name Actemra®. It was FDA approved for treatment of refractory moderate to severe rheumatoid arthritis. It is delivered by intravenous infusion monthly at a standard dose of 4-8 mg/kg [3]. We propose that tocilizumab treatment will reduce inflammation in ALS patients by impeding both the classical and trans-signaling IL-6 pathways.

The anti-inflammatory approach to treat ALS by tocilizumab is based on basic science discoveries and information related to inflammatory signaling by the cytokine IL-6 [4-9]. IL-6 is one of the most important mediators of fever and acute phase responses, is capable of crossing the blood-brain barrier, and is secreted by macrophages in response to pathogens. IL-6 binds to cell surface IL-6 receptors (IL6-R) present on numerous cell types. In the spinal fluid and in the spinal cord of sporadic ALS patients, IL-6 may also transmit its inflammatory signal through a soluble IL6 receptor (sIL6-R) containing only the extracellular portion of the IL6-R, which then binds to a ubiquitous cell surface receptor called gp130 and activates numerous signaling pathways, including the JAK/STAT and Ras-Raf-MAPK pathways. This process is called IL-6 "trans-signaling" and can activate intracellular pathways in cell types that lack the cell surface IL6-R. Interestingly, many CNS cell types only respond to IL-6 via IL-6 trans-signaling [10, 11]. We have shown that the spinal fluid of ALS patients contains up to nanogram amounts of sIL6-R but only 100 pg/mL (mean) of IL-6 protein. However, macrophages infiltrating the ALS spinal cord strongly express IL-6 suggesting that IL-6 signaling may be occurring in the spinal cord through both trans-signaling via the sIL-6R and classical signaling through the membrane receptor. The significance of IL-6 signaling lies in the stimulation of phagocytosis of motor neurons by inflammatory macrophages expressing IL-6 [5].

The **study hypothesis** is that tocilizumab is safe in ALS patients, penetrates the blood brain barrier and reduces expression of pro-inflammatory genes in patients with ALS.

### 2.2.1 Rationale for Dose

The dosing rationale of tocilizumab in this study is based on the FDA labeling for its use in rheumatoid arthritis. In general, it is administered at a dose of 8 mg/kg as an intravenous infusion over 1 hour every 4 weeks with the drug mixed with normal saline to a total volume of 100 mL.

## 2.3 PRELIMINARY DATA

### 2.3.1 Gene expression of pro-inflammatory cytokines

Peripheral blood mononuclear cells (PBMCs) isolated from ALS patients and treated with aggregated SOD1 (2 μg/mL) exhibit increased gene expression and protein levels for multiple cytokines [4]. Addition of tocilizumab (10 μg/mL) inhibited the expression of pro-inflammatory cytokines induced by aggregated SOD1 (Figure 1).

We analyzed gene expression of activated PBMCs from 10 sporadic ALS patients, 5 of whom were treated with tocilizumab infusions, and four healthy controls [7]. Among our sample of sporadic ALS patients, the innate immune system was highly inflammatory in 50% of the patients (Group 1, mean age +/- S.E of 53.4 +/- 1.6 years) and nearly non-inflammatory in the

other 50% (Group 2, age 55.2 +/- 7.9 years) based on analysis of PBMC gene expression by RT-PCR (Figure 2). Group 1 patients were defined as those whose PBMCs show up-regulation (compared to healthy controls, mean age 62 +/- 10.2 years) of the transcription of inflammatory genes for cytokines/chemokines IL-1α, IL-1β, IL-6, IL-8, CXCL3, CXCL5, CCL20, MMP1, and COX-2 ($p$ <0.05 Group 1 vs. healthy controls), whereas the "Group 2" PBMCs show normal or low inflammatory activation except for elevated transcription of CXCL 9, 10, 11 when compared to healthy controls [4]. In one Group 1 patient, we observed a reduction over 12 weeks in inflammatory gene expression and inflammatory biomarkers, such as serum C-reactive protein, IL-6 and IL-1 cytokines, after tocilizumab infusion therapy, followed by recurrence from 4 to 5 months after start of therapy, and subsequent remission of inflammation. Serum level of tocilizumab in this patient was 12.3 μg/mL before infusion (trough level from prior infusion) and 109 μg/mL after infusion, with CSF levels of 0.18 μg/mL after infusion, demonstrating that drug was reaching the CNS. The patient's rate of progression by ALSFRS-R slowed from 2.6 points per month to 0.4 points per month. The patient discontinued tocilizumab after 8 months of therapy and the disease course accelerated by ALSFRS-R measurements. ALSFRS-R progression slowed from 1.3 points per month to 0.75 points per month in another Group 1 patients treated with tocilizumab for 2 months. In one Group 2 patient, the effect of tocilizumab infusion therapy on inflammatory gene expression was minimal, but the patient's rate of progression by ALSFRS-R reversed course, changing from a 0.7 point per month decline to a 1.0 point per month improvement over 4 months of treatment. One other Group 2 patient experienced an acceleration of progression during 5 months of tocilizumab treatment. The small number of patients in this pilot clinical study makes these findings difficult to generalize to the ALS population as a whole. However these clinical findings merit continued study of tocilizumab in a larger and more carefully controlled clinical trial. Specifically, an important goal of the proposed clinical trial will be to demonstrate that our gene expression profile biomarker of PBMCs can be used to demonstrate target engagement and a biological effect [4, 7].

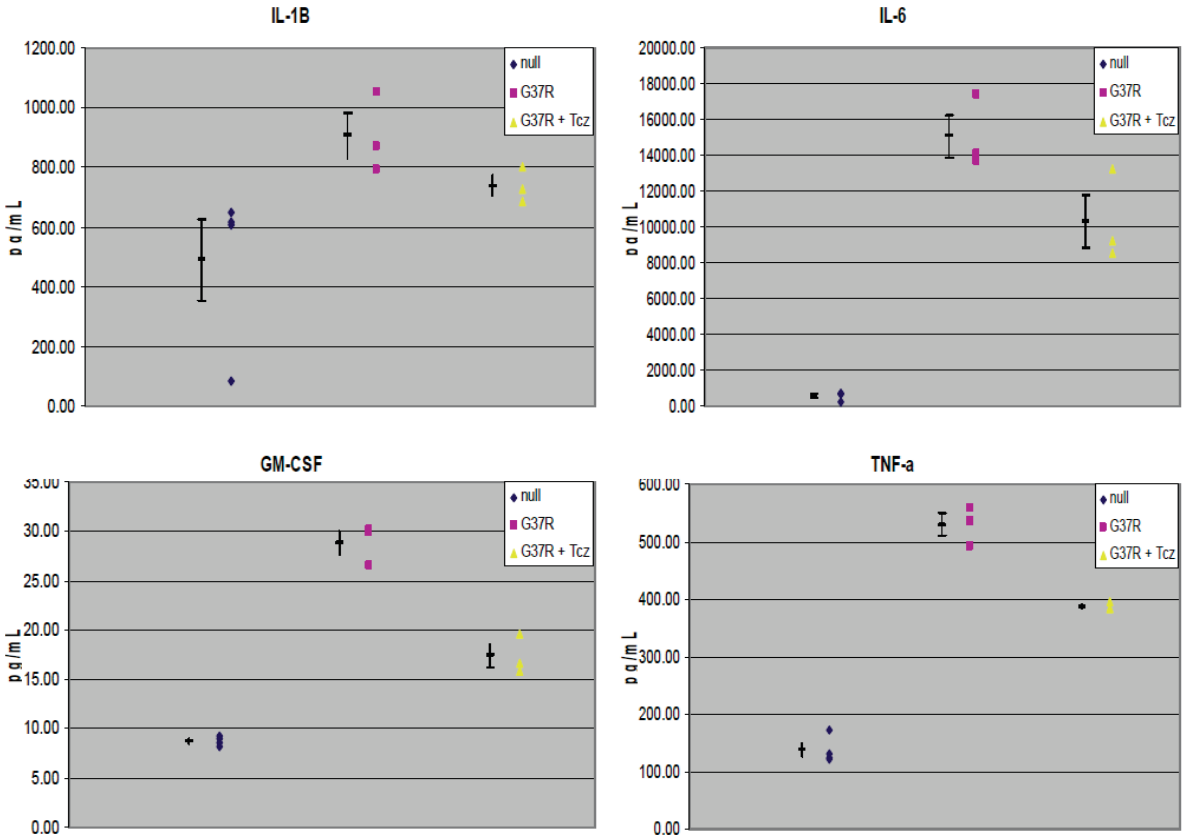



**FIGURE 1: Reduction of cytokine levels after tocilizumab treatment**

Levels of IL-1β, IL-6, GM-CSF and TNF-α in PBMC's from Group 1 ALS patients before (magenta) and after (yellow) overnight tocilizumab treatment, compared to healthy controls (black, null). Drug treatment led to significantly reduced levels of cytokines released from activated PBMCs.

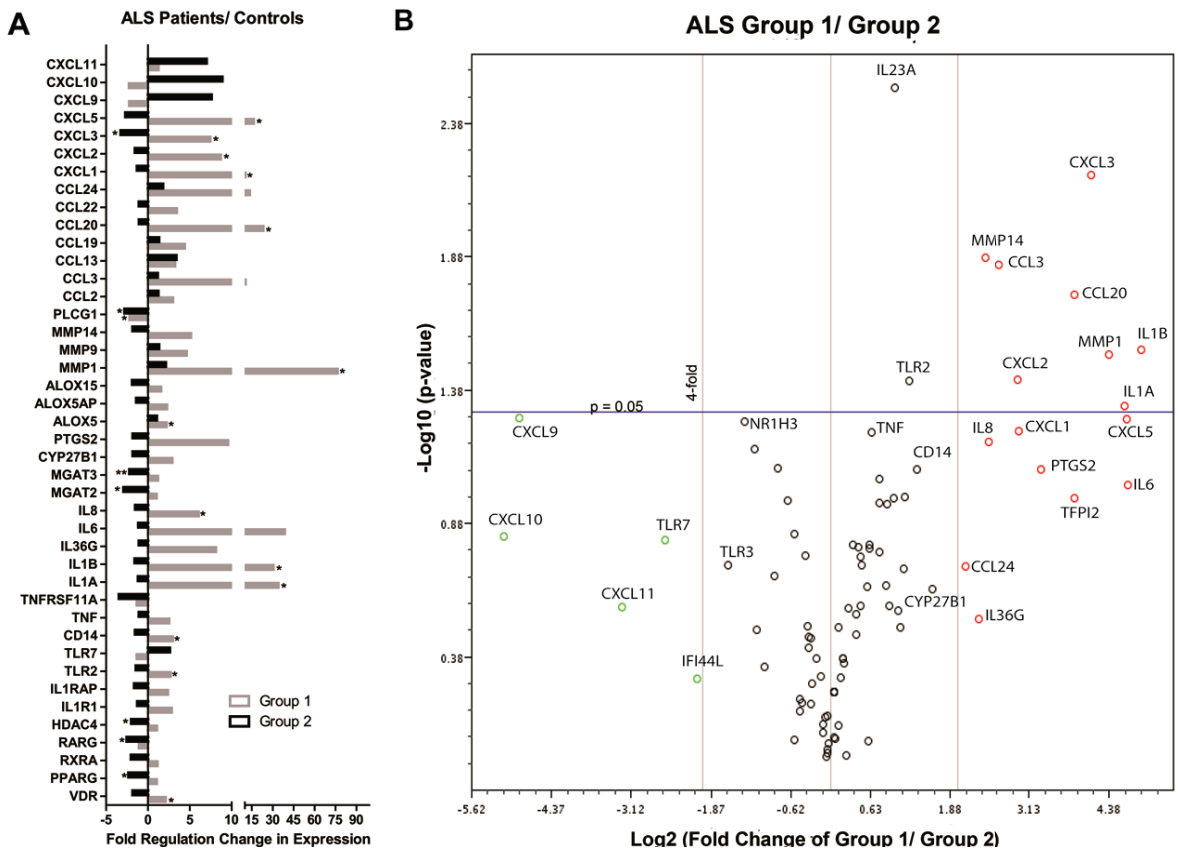



**FIGURE 2: Gene expression profiling identifies two inflammation groups in ALS**

### 2.3.2 PBR28 preliminary data

***Imaging Neuroinflammation using [$^{11}$C]PBR28-PET***

[$^{11}$C]PBR28 is a novel radiotracer of neuroinflammation. It binds to the 18 kDa translocator protein (TSPO), which is highly expressed in activated microglia and monocytes. We have shown that [$^{11}$C]PBR28 uptake is increased in the motor cortices of symptomatic ALS patients [12]. We have administered [$^{11}$C]PBR28 to more than 50 ALS participants and 25 healthy volunteers at MGH. Below is a summary of our main published and unpublished preliminary findings to date:

I. [$^{11}$C]PBR28 uptake is significantly increased in the motor cortices and subcortical regions in ALS participants compared to healthy controls [12]
**(Figure 3A,B,C).**

II. The anatomical distribution of increased [$^{11}$C]PBR28 uptake corresponds to ALS clinical presentation; ALS participants with limb-onset weakness had elevated [$^{11}$C]PBR28 retention in the motor cortices, whereas participants with bulbar-onset weakness had increased retention in the brainstem [12].

III. Increased [$^{11}$C]PBR-28 uptake strongly correlates with increased upper motor neuron dysfunction as measured by the Upper Motor Neuron Burden (UMNB) scale **(Figure 3D)** [12].

IV. Using our combined Positron emission tomography/magnetic resonance imaging MR/PET scanner, we demonstrated that [$^{11}$C]PBR-28 uptake (molecular measure of inflammation) co-localizes and correlates

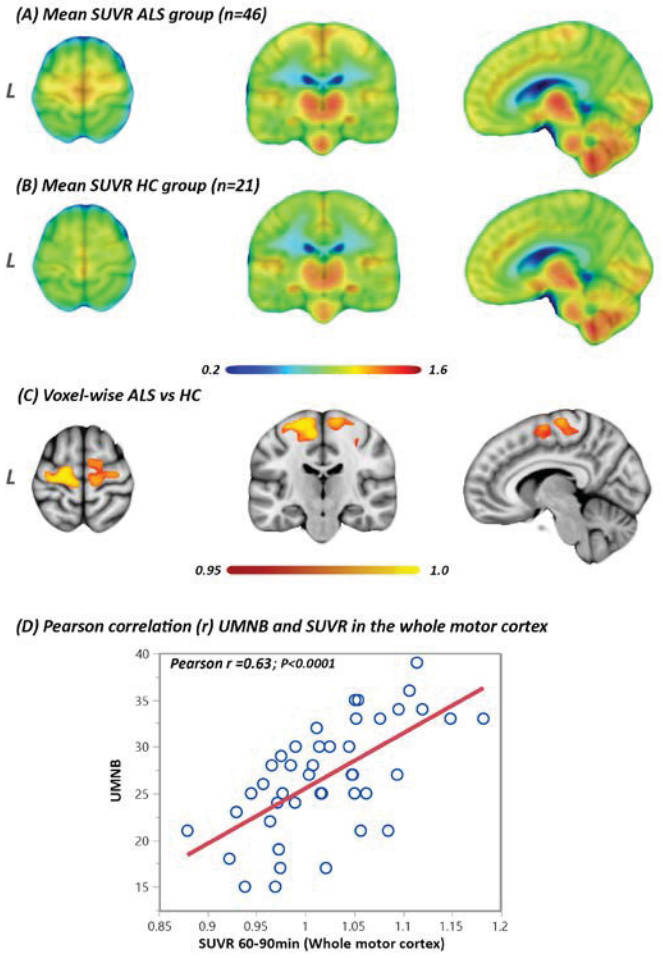



**Figure 3: PBR28 PET study in 46 ALS participants and 21 healthy volunteers *(unpublished data)*.** PBR28 uptake in the motor cortices is increased in ALS subjects **(A)** compared to healthy volunteers **(B).** Statistical maps with family-wise corrections for multiple testing show increased PBR28 uptake in the motor cortices in the ALS group **(C).** PBR28 uptake in the motor cortices is highly correlated with upper motor neuron burden (UMNB) **(D).**

with axonal loss (anatomical change) measured by fractional anisotropy FA and cortical thinning (anatomical change), providing unique *in vivo* evidence of the anatomical connection between motor neuron degeneration and the surrounding inflammation [13].

### 2.3.3 Tocilizumab

Tocilizumab (TCZ), formerly known as myeloma receptor antibody (MRA) is a recombinant humanized antihuman monoclonal antibody of the immunoglobulin IgG1 subclass directed against the IL-6R and produced by recombinant DNA technology. Clinical efficacy and safety studies of TCZ have been conducted or are ongoing in various disease areas, including adult-onset RA, systemic-onset juvenile idiopathic arthritis and polyarticular juvenile idiopathic arthritis.

The half-life of TCZ is approximately 7 days. The TCZ exposures were stable over 2- years of treatment. The observed mean (± SD) Ctrough at 8 mg/kg IV was 15.9 ± 12.0 at week 24 and 19.9 ± 17.0 at week 104. The observed mean (± SD) Ctrough at 4 mg/kg was 1.02 ± 6.14 at week 24 and 1.09 ± 2.77 at week 104.

The Roche clinical development program in adult RA, comprised five pivotal Phase 3 trials and two open-label, long-term treatment extension studies.

Further information on TCZ can be found in the Investigator's Brochure (IB).

## 2.4 POTENTIAL RISKS AND BENEFITS

### 2.4.1 Potential Risks

The main risks associated with the study relate to exposure to tocilizumab and lumbar puncture (LP). Tocilizumab has both short-term and long-term risks associated with it. As with any intravenous (IV) drug, there is a chance for injection reactions such as anaphylaxis. These reactions are usually mild and responsive to antipyretics, antihistamines, and analgesics. The experience with tocilizumab in rheumatoid arthritis patients suggests that serious reactions occur at a rate of 0.1%-0.2% [3]. The most common long-term AEs were upper respiratory tract infections (7%), nasopharyngitis (7%), headache (7%), hypertension (6%) and elevated liver functions tests (6%), reversible neutropenia, and elevated cholesterol (13%). Serious infections have been reported with tocilizumab use and include opportunistic infections such as tuberculosis. Finally, tocilizumab reverses inflammation-induced CYP450 enzyme inhibition, effectively increasing drug metabolism via this pathway and decreasing CYP450 metabolized drug exposure. Although this has been studied in the rheumatoid arthritis population, it is unclear

if ALS patients have clinically significant inflammation-induced CYP450 alterations [14, 15]. Unfortunately, no other anti IL-6 receptor agents are available as an alternative to tocilizumab. Given our hypothesis that IL-6 activity is central to the role of the inflammatory cascade in motor neuron death, exposure to tocilizumab is unavoidable if we wish to test the effect of altering IL-6 receptor activity.

Lumbar puncture poses short-term risks to patients unrelated to study drug exposure. As with any procedure, there is a low risk of bleeding or infection. Pain and post-lumbar puncture headache (PLPH) are more common risks that may impact quality of life. Pain is localized, short lived and responds to over-the-counter analgesics. PLPH occurs in 10-30% of cases depending on technique and patient population and may lead to hospitalization or the need for an autologous blood patch procedure [16]. This risk can be greatly reduced by the use of atraumatic needles. In a retrospective study of ALS patient receiving LPs for CSF biomarker studies, the use of atraumatic needles reduced the risk of PLPH by 50% compared to traditional cutting needles [17]. No patient in the atraumatic needle group required a blood patch procedure. A fundamental tenet of the proposed therapy is that the monoclonal antibody tocilizumab should cross the blood-brain barrier. There is no systematic data available to establish that the CSF contains sufficient concentrations of tocilizumab to be effective. A full characterization of the pharmacokinetics of tocilizumab would require serial sampling of blood and CSF over several weeks with a lumbar catheter. Instead, we propose to sample CSF from patients in this study prior to treatment and 2.5 hours after their third infusion to estimate maximal CSF concentration and change in cytokine levels and IL-6 receptor levels in the CSF. Given the critical importance of these data, LP is unavoidable.

To minimize the risk to patients, the protocol includes criteria and algorithms for interrupting or stopping therapy in patients with serious infections, low absolute neutrophil count (ANC), elevated liver enzymes, and thrombocytopenia. It will also include criteria to exclude patients at risk for opportunistic infections. To minimize risk of hypersensitivity, the protocol will utilize pre-treatment anti-histamines as described in the site SOP, and require all infusions to occur in a hospital or clinic-based infusion center with experienced infusion staff and the ability to rapidly treat anaphylaxis. We will exclude patients who concomitantly receive CYP450 metabolized drugs whose reduced exposure may pose a short-term risk. Finally, the proposed study will utilize atraumatic needles, standard LP procedures and exclude anticoagulated patients to minimize the risks of LPs.

No socioeconomic or psychological risks are associated with the study.

*Risk of Magnetic Resonance Imaging:* People who have electrically, magnetically or mechanically activated implants (such as heart pacemakers) or those who have clips on blood

vessels in their brain will not be allowed to participate in the study due to associated risks with MRI scanning. The MRI systems will be operated in a manner accepted by the Food and Drug Administration (FDA). The scanner makes loud knocking or beeping sounds as it takes images. Subjects will be given earplugs to help reduce this noise. If a subject notices any discomfort while in the MRI scanner, (s) he should notify the administrator immediately. If the discomfort cannot be stopped, the scanning session may be stopped.

All credit cards and other items with magnetic strips should also be kept out of the MRI room. The MRI has the potential, during normal routine use, to cause localized warming of the skin and the underlying tissues. Subjects should immediately inform the study staff if they experience discomfort due to warming of the skin and the procedure will be stopped.

The MRI can be stopped at any time at the subject's request. Subjects with severe claustrophobia are excluded from the study.

Imaging will be stopped should any untoward reaction be observed during the imaging session or if the subject so requests for whatever reason. Some subjects find it unpleasant or feel anxious when confined in the enclosed space of the scanner. If this happens, the subject will be unable to participate in the study.

*Risk of Positron Emission Tomography Imaging:* For the PET scan, no side effects from the radioligand [11C]PBR28. Subjects will be exposed to a small amount of radiation that will be significantly less than the yearly amount of radiation allowed for persons who work with radiation. The total amount of radiation exposure subjects will receive from participation in this study is equal to a whole body exposure of approximately ~3.7 milliSieverts (mSv). This amount of radiation is about the same as one would normally receive in 15 months from natural background sources from the earth and the sky.

The radiation exposure in this study will be small and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be asked to inform the investigators or study staff (by writing initials on the consent form verifying that they have not been exposed to other radiation in the past 12 months). If it is determined that their prior radiation exposure exceeds the current institutional guidelines, they will not be allowed to participate in this study.

Subjects will be required to lie still in the PET camera several times during an approximately 1 hour 45 minute period, and subjects may find it uncomfortable to remain still over this time. In order to insure that subjects do not move their heads during the imaging procedure cushions could be placed around the head. Subjects will be required to lie still on the imaging table with their heads in the scanner. This is usually well tolerated by most subjects.

We will use [$^{11}$C]PBR28 produced by the cyclotron/radiochemistry/radiopharmacy facility at the A.A. Martinos Center for Biomedical Imaging. We will follow the safety standards approved by the Radiation Safety Committee for the use of radioligands. The IV injection will be administered either by a physician or by a trained technician. Should there be an adverse event related to a study procedure, the principal investigator, co-investigator or study staff will be responsible for communicating with the IRB within the stipulated timeframe. At the time of continuing review a cumulative log of adverse events will be submitted, regardless of relatedness.

The PET scan can be stopped at any time at the subject's request. Subjects with severe claustrophobia are excluded from the study.

Imaging will be stopped should any untoward reaction be observed during the imaging session or if the subject so requests for whatever reason. Some subjects find it unpleasant or feel anxious when confined in the enclosed space of the scanner. If this happens, the subject will be unable to participate in the study.

### 2.4.2 Known Potential Benefits

There are no specific anticipated potential benefits to subjects for participating in this study, although subjects may benefit from the close follow-up and detailed medical care associated with study participation. It is not known whether treatment with tocilizumab will be helpful or harmful for ALS subjects. It is possible that results from the study may yield benefits to future ALS patients.

# 3 OBJECTIVES

## 3.1 Study Objectives

The **study objective** is to demonstrate that tocilizumab is safe and well tolerated in patients with sporadic ALS and determine its effect on immune markers and clinical progression in patients with sporadic ALS. Successful completion of this study will support further clinical investigation to test the efficacy of tocilizumab in patients with sporadic ALS and define patient enrichment criteria and biomarkers that could be used in subsequent clinical studies.

### 3.1.1 Primary Objective

**The primary objective of the study is** to assess the short-term safety and tolerability of tocilizumab in patients with sporadic ALS.

### 3.1.1 Secondary Objectives

The secondary objectives of this study are:

- To describe the inflammatory gene expression profiles in PBMCs of sporadic ALS patients and to assess the ability of tocilizumab to reduce expression of pro-inflammatory genes in PBMCs of patients with sporadic ALS and a high inflammation state at baseline.
- To assess whether tocilizumab achieves a concentration in the CSF sufficient to block IL-6 signaling.
- To gather preliminary data on the concentration of key inflammatory cytokines (IL-6, IL-8, IL-17, IL-1β, TNF-α, sIL-6 receptor, or hs-CRP) in the plasma and CSF before and after treatment with tocilizumab vs. placebo.
- To identify polymorphisms that might affect the inflammatory state mediated by the IL-6 receptor.
- Measure the effects of tocilizumab on reducing glial activation measured by PBR28 PET in a subset of trial participants.

## 3.2 Study Outcome Measures

### 3.2.1 Primary Outcome Measures

We will assess safety of tocilizumab treatment based on mortality, occurrence of SAEs, overall rates of AEs, clinically significant abnormal laboratory tests, and changes in vital signs. We will focus on events at least possibly related to study drug of moderate or severe intensity by

comparing participants receiving at least one dose of tocilizumab to those on placebo for time to mortality and time to first SAE by Cox regression, for overall rate of AEs or abnormal laboratory tests by negative binomial regression, and for rates of change in vital signs.

Tolerance of tocilizumab treatment will be judged on the proportion of participants remaining on study drug through all 3 doses and remaining on study and free from possibly drug-related and dose-limiting SAEs to the end of follow-up.

### 3.2.2 Secondary Outcome Measures

Secondary outcome measures will be descriptive and include measures of efficacy as well as measures of biochemical change. Efficacy outcome measures include change in the rate of change of ALSFRS-R, HHD, and SVC. Biochemical measures will include change in cytokine levels in the serum and CSF, change in PBMC pro-inflammatory gene expression, and change in CSF sIL-6 receptor concentrations. We will estimate the proportion of participants who experience a 2-fold or greater decline between baseline and the average of all follow-up assessments in at least 2 of the 3 pro-inflammatory genes. These measures will serve several purposes. First, they will be used to establish a correlation between the biochemical responses and drug exposure. Second, they will be used to identify the proportion of ALS patients who display a pro-inflammatory PBMC gene expression profile. This information will be useful when designing future trials, especially if an enrichment design is used. Finally, the measures will serve to demonstrate target engagement of the sIL-6 receptor. Mean PBR28 uptake will be measured in the motor cortices as regions of interest (ROIs), and will be compared between pre- and post-dose.

### 3.2.3 Safety Measures

The following laboratory tests will be performed for safety:

- Hematology with differential panel: complete blood count with differential (hematocrit, hemoglobin, platelet count, RBC indices, total RBC, total WBC, and WBC differential)

- Blood chemistry panel/Liver function tests (LFTs): alanine aminotransferase (ALT (SGPT)), aspartate aminotransferase (AST (SGOT)), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, magnesium, phosphate, potassium, sodium, total bilirubin, total protein and, carbon dioxide.

- Urinalysis: albumin, bilirubin, blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, urobilinogen and WBC screen

- Hepatitis B panel (collected only at Screening Visit)

- Serum human chorionic gonadotrophin (hCG) for women of childbearing potential (WOCBP) (collected only at Screening Visit). Urine pregnancy tests will be performed on all WOCBP at each subsequent visit throughout the study.

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The SI may order additional testing, if needed, to further assess an AE, or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

# 4 STUDY DESIGN

## 4.1 Overall Study Design and Plan

During the enrollment period, approximately 50 subjects will be screened from approximately 4 Northeast ALS Consortium (NEALS) Centers in the US. Twenty-four of these subjects (or more, if some randomized participants never initiated treatment) will be randomly assigned in a 2:1 ratio to intravenous tocilizumab 8 mg/kg vs. matching placebo every 4 weeks for 8 weeks (3 doses). After screening and randomization, subjects will undergo baseline measures, undergo LP and receive their first dose of study drug in clinic. Clinic visits will occur at 4 week intervals with total study duration of 16 weeks. A second LP will occur at the 8 week visit.

All visit windows are consecutive calendar days and are calculated from the day the participant starts study treatment (Day 0, the day of the Baseline Visit).

## 4.2 Study Centers

This study will be conducted at approximately 5 NEALS Centers in the US. Sites will be selected based on recruitment record from prior trials, compliance with prior study protocols and regulations, clinical research expertise and availability of necessary resources.

## 4.3 Study Duration

Subjects will remain on randomized, placebo-controlled, double-blind treatment until the Week 8 visit. Each randomized subject will also have a Week 12 Follow-up visit and Week 16 End-of-Study visit to assess for AEs, changes in concomitant medications and to administer selected study procedures.

## 4.4 Protocol Adherence

Each (SI) must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the Coordination Center (CC) or their representative prior to seeking approval from the site IRB. Each SI will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

# 5 STUDY ENROLLMENT AND WITHDRAWAL

## 5.1 Number of Study Subjects

Twenty-four (24) subjects will be randomized in a 2:1 ratio to intravenous tocilizumab or matching placebo. Additional subjects may be randomized if any randomized subjects withdraw from the study prior to initiating study drug.

## 5.2 Inclusion and Exclusion Criteria

### 5.2.1 Inclusion Criteria

Study subjects meeting all of the following criteria will be allowed to enroll in the study:

1. Participants with ALS diagnosed as possible, laboratory-supported probable, probable or definite according to the World Federation of Neurology El Escorial criteria.
2. Male or female, aged 18-75.
3. Capable of providing informed consent and complying with trial procedures.
4. High inflammatory profile of PBMC gene expression defined as having one of the following:
    i. A three-fold up-regulation (compared to pooled control levels) of IL-6 expression

    OR

    ii. A two-fold up-regulation (compared to pooled controls) of IL-6 and either IL-8 or MMP1 expression.

5. Upright SVC ≥40% of predicted value for gender, height and age at the Screening Visit and in the opinion of the investigator is able to comply with and complete the trial.
6. Women must not be able to become pregnant (e.g., post menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study.
7. Negative tuberculosis (TB) skin test, Quantiferon or T-SPOT TB blood test at the Screening Visit.
8. Not taking riluzole, or on a stable dosage for at least thirty (30) days prior to the Screening Visit.
   ***Riluzole.*** *Subjects taking concomitant riluzole at study entry must be on a stable dose for at least 30 days prior to the Screening Visit and must continue taking the same dosage throughout the study, unless the SI determines that riluzole should be discontinued for medical reasons.*

9. Subjects medically able to undergo lumbar puncture (LP) as determined by the investigator (i.e., no bleeding disorder, allergy to local anesthetics, a skin infection at or near the LP site, or evidence of high intracranial pressure).
10. Subjects must agree not to take live attenuated vaccines (including seasonal nasal flu vaccine, varicella vaccine for shingles or chickenpox, MMR or MMRV, oral polio vaccine and vaccines for yellow fever, measles, mumps or rubella) thirty (30) days before the Screening Visit, throughout the duration of the trial and for sixty (60) days following the subject's last dose of study drug.
11. Geographic accessibility to the study site.

**MR-PET Inclusion Criteria (MGH only):**

12. High or mixed affinity to bind TSPO protein (Ala/Ala or Ala/Thr) (see section 7.1)
13. Upper Motor Neuron Burden (UMNB) Scale Score ≥25 (out of 45) at the Screening Visit.
14. Able to safely undergo PET/MRI scans based on the opinion of the site investigator.

### 5.2.2 Subject Exclusion Criteria

Study subjects meeting any of the following criteria during screening evaluations will be excluded from entry into the study:

1. Prior use of:
    - Tocilizumab (Actemra®)
    - Cell-depleting therapies, including investigational agents or approved therapies (some examples are CAMPATH, anti-CD4, anti-CD5, anti¬CD3, anti-CD19 and anti-CD20)
    - Alkylating agents such as chlorambucil
    - Total lymphoid irradiation
    - Stem cell therapies (either investigational or approved therapies)
2. Presence of tracheostomy at Screening Visit.
3. Exposure to any anti-inflammatory agent currently under investigation for the treatment of patients with ALS (off-label use or investigational) within 30 days prior to the Screening Visit. Examples include NP001 or Lunasin. Medications that do not have an anti-inflammatory mechanism such as mexilitine or retigabine are allowed if on a stable dose for 30 days prior to the Screening Visit.
4. Treatment with a prohibited medication within 30 days of the ScreeningVisit:
    - Nuedexta
    - Theophylline
    - Warfarin
    - Cyclosporine

5. Treatment with intravenous gamma globulin, plasmapheresis or Prosorba column within 6 months of screening.
6. Presence of diaphragm pacing system (DPS) at Screening Visit.
7. Primary or secondary immunodeficiency (history of or currently active) unless related to primary disease under investigation.
8. History of or active diverticulitis, diverticulosis requiring antibiotic treatment, peptic ulcer disease, or GI tract perforation, or chronic ulcerative lower GI disease such as Crohn's disease, ulcerative colitis or other symptomatic lower GI conditions that might predispose to perforations.
9. Known active current or history of recurrent bacterial, viral, fungal, mycobacterial or other opportunistic infections (including but not limited to tuberculosis and atypical mycobacterial disease, Hepatitis B and C, EBV, CMV, and herpes zoster, but excluding fungal infections of nail beds).
10. History of severe allergic or anaphylactic reactions to human, humanized or murine monoclonal antibodies.
11. Presence of any of the following clinical conditions:
    - Pharmacologic or hereditary bleeding diathesis, or any other clinical condition that would, in the opinion of the investigator, place the patient at increased risk during LP.
    - Drug abuse or alcoholism within the past 12 months.
    - Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active infectious disease, including current or prior malignancy.
    - Rheumatic autoimmune disease, including Systemic lupus erythematsus, Mixed connective tissue disease, scleroderma, polymyositis, or significant systemic involvement secondary to RA (e.g., vasculitis, pulmonary fibrosis or Felty's syndrome).
    - Evidence of active malignant disease, malignancies diagnosed within the previous 5 years (including hematological malignancies and solid tumors, except basal and squamous cell carcinoma of the skin or carcinoma in situ of the cervix uteri that has been excised and cured, or Stage I uterine cancer), or breast cancer diagnosed within the previous 5 years.
    - Human immunodeficiency virus (HIV) infection or other immunodeficient state.
    - Uncontrolled hypertension defined as systolic blood pressure > 170 or diastolic blood pressure > 110.
    - Unstable psychiatric illness defined as psychosis or untreated major depression within 90 days of the Screening Visit.
12. Any major episode of infection requiring hospitalization or treatment with IV antibiotics within 4 weeks of screening
13. Laboratory values: Screening alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin > than 1.5 times the upper limit of normal, serum creatinine > 1.6

mg/dL (141 μmol/L) in female patients and > 1.9 mg/dL (168 μmol/L) in male patients (patients with serum creatinine values exceeding limits may be eligible for the study if their estimated glomerular filtration rates (GFR) are >30), hemoglobin < 85 g/L (8.5 g/dL; 5.3 mmol/L), white Blood Cells < 3.0 x $10^9$/L (3000/mm3), absolute neutrophil count of <2000/$mm^3$, absolute lymphocyte count < 0.5 x $10^9$/L (500/mm3), platelet concentration of <100,000/$mm^3$, positive Hepatitis B surface antigen (HBsAg).

14. Pregnant women or women currently breastfeeding.
15. No history of chicken pox infection by self report and no history of varicella zoster vaccination
16. Any reason in the opinion of the investigator that the patient may not be able to comply with study procedures, complete the study or is unsuitable for immunosuppressive therapy.

**MR-PET Exclusion Criteria (MGH only):**

17. Any contraindication to undergo MRI studies such as
    - History of a cardiac pacemaker or pacemaker wires
    - Metallic particles in the body
    - Vascular clips in the head
    - Prosthetic heart valves
    - Claustrophobia
18. Radiation exposure that exceeds the site's current guidelines
19. Current use of tobacco products including cigarettes, e-cigarettes, cigars, snuff and chewing tobacco, or nicotine replacement products such as gum, or patch
20. Taking any other anti-inflammatory or immune modulating medications except for over the counter NSAIDs
21. Unwilling or unable to discontinue benzodiazepine usage (other than Lorazepam, Clonazepam, or Zolpidem) for one day prior to scanning

### 5.2.3 Immunization during TCZ therapy

Live/attenuated vaccines should not be given within 4 weeks prior to baseline and during the study as clinical safety has not been established. No data are available on the secondary transmission of infection from persons receiving live vaccines to patients receiving Tocilizumab. No data are available on the effectiveness of vaccination in patients receiving Tocilizumab. Because IL-6 inhibition may interfere with the normal immune response to new antigens, patients should be brought up to date on all recommended vaccinations, except for live vaccines, prior to initiation of therapy with Tocilizumab.

## 5.3. Treatment Assignment Procedures

### 5.3.1 Randomization Procedures

Each subject who meets all eligibility criteria and signs an informed consent form will be randomized to receive either 8 mg/kg of tocilizumab every 4 weeks or matching placebo for 8 weeks.

The randomization scheme will be developed by the study statistician and will indicate the group assignment and the subject numbers to be used by each site. Subjects will be randomized in a 2:1 ratio to intravenous tocilizumab 8 mg/kg or matching placebo using permuted random blocks stratified by site. If a randomized subject withdraws from the study prior to initiating study drug, their assignment will be released and re-used.

### 5.3.2 Reasons for Withdrawal

A study subject will be discontinued from participation in the study if:

- Any clinical AE (AE), laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject. Note, however, that any subject who has initiated treatment should only discontinue study drug and remain on study if the safety concern relates to study drug and not to study procedures.

Subjects are free to withdraw from participation in the study at any time upon request.

### 5.3.3 Handling of Withdrawals

A subject may choose to discontinue participation in the study at any time. However, the SI or designee will encourage subjects to continue with follow-up visits, regardless of their compliance with study drug. If the SI or designee is concerned about the use of a prohibited medication or other safety issues, then study drug may be modified or discontinued. If a subject who initiated study drug permanently discontinues study drug, the SI or designee should still encourage subjects to follow the study protocol under the modified intent to treat principle (ITT). These subjects will be encouraged to follow the study visits, off drug, up to the Week 16 EOS Visit. Loss to follow-up should be prevented whenever possible.

Any subject who is on study drug and needs to begin the use of any prohibited medication, must immediately discontinue use of study drug and should not begin use of the prohibited medication before an appropriate wash-out period occurs. Subjects who permanently discontinue study drug should complete early study drug discontinuation procedures without any study drug unblinding,

if possible. Subjects who must permanently discontinue study drug may continue in the ITT portion of the study, per protocol.

Early termination occurs when a subject withdraws consent, i.e., withdrawing his or her participation in future study procedures. The subject will be asked to return to the study site for a final safety visit. At that visit, the subject will be asked to have a final telephone call 28 days (+ 5 days) after taking their last dose of study drug. Subjects who withdraw from the study due to AEs will be followed for outcome measures under the ITT protocol as noted above.

### 5.3.4 Termination of Study

This study may be prematurely terminated if, in the opinion of the principle investigator, DSMB, or the sponsor, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the PI by the terminating party.

Circumstances that may warrant termination include, but are not limited to:

1. Determination of unexpected, significant, or unacceptable risk to subjects.
2. Enrollment is unsatisfactory.
3. Insufficient adherence to protocol requirements.
4. Data that are not sufficiently complete and/or evaluable.
5. Plans to modify, suspend or discontinue the development of the study drug.

If the study is prematurely terminated or suspended, the sponsor will promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the PI/institution, as specified by the applicable regulatory requirement(s).

# 6 TREATMENTS ADMINISTERED

## 6.1 Treatments

### 6.1.1 Study Product Description

Tocilizumab (Actemra®) is a recombinant humanized anti-human interleukin 6 (IL-6) receptor monoclonal antibody of the immunoglobulin IgG1κ (gamma 1, kappa) subclass with a typical H2L2 polypeptide structure. Each light chain and heavy chain consists of 214 and 448 amino acids, respectively. The four polypeptide chains are linked intra- and inter-molecularly by disulfide bonds. Tocilizumab has a molecular weight of approximately 148 kDa.

It is supplied as a sterile, preservative-free solution for intravenous (IV) infusion at a concentration of 20 mg per mL. It is a colorless to pale yellow liquid, with a pH of about 6.5. Single-use vials are available containing 80 mg per 4 mL, 200 mg per 10 mL, or 400 mg per 20 mL of tocilizumab. Injectable solutions are formulated in an aqueous solution containing disodium phosphate dodecahydrate and sodium dihydrogen phosphate dehydrate (as a 15 mmol per L phosphate buffer), polysorbate 80 (0.5 mg per mL), and sucrose (50 mg per mL).

Tocilizumab will be provided free of charge by Genentech. The Sponsor or designee of the study will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all study drug in accordance with 21 Code of Federal Regulations (C.F.R.), Part 312.57 and 312.62 and Genentech requirements.

### 6.1.2 Placebo

A matched placebo will be used to maintain the dosage-blind. The site pharmacy will prepare equivolume doses of normal saline as placebo. Administration of matching placebo will be the same as for subjects in the treatment group.

## 6.2 Acquisition

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be

documented in the study files. The SI must notify study sponsor of any damaged or unusable study treatments that were supplied to the SI's site.

### 6.2.1 Formulation, Packaging, and Labeling

The study drug is prepackaged in vials containing 80 mg, 200 mg or 400 mg that will be mixed with normal saline to a total volume of 100 mL by the site research pharmacist. The Investigational Pharmacist has the responsibility to ensure that the integrity of packaged study drug is not jeopardized prior to dispensing. Each individual subject's vials must be dispensed as provided with no further repackaging or labeling done at the investigational site, unless required by the institution per institutional polices.

### 6.2.2 Product Storage and Stability

Tocilizumab must be refrigerated at 2°C to 8°C (36°F to 46°F). It should not be frozen and should be protected from light by storage in the original package until time of use. Vials should be inspected visually for particulate matter and discoloration prior to administration. If visibly opaque particles, discoloration or other foreign particles are observed, the solution should not be used.

The SI must ensure that all investigational drug supplies are kept in a locked, safe area at controlled temperature (2°C to 8°C) with access limited to those directly involved in the study. Investigational drug supplies should not be repackaged in any way.

## 6.3 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Tocilizumab concentrate for intravenous infusion should be diluted to 100 mL by the research pharmacist using aseptic technique as follows:

- Withdraw the required amount of tocilizumab for a dose of 8 mg/kg (Equivalent to 0.4 mL/kg).
- Withdraw the same amount of sterile, non-pyrogenic 0.9% w/v sodium chloride solution from the infusion bag and discard.
- Slowly add the withdrawn tocilizumab concentrate into the infusion bag. The final volume in the infusion bag should total 100 mL.
- To mix the solution, gently invert the bag to avoid foaming.
- Allow the fully diluted tocilizumab solution to reach room temperature prior to infusion.
- The infusion should be administered over 60 minutes, and must be administered

with an infusion set. Do not administer as an intravenous push or bolus.

Subjects should be weighed prior to each infusion, and dosage should be adjusted accordingly. One vial containing 400 mg tocilizumab or two vials containing 200 mg tocilizumab will be required for each 50 kg body weight to achieve an 8 mg/kg dose. The number of vials to be used depends on the patient's body weight as follows:

1. One 400-mg vial (or two 200-mg vials) is used for patients with a body weight ≤50 kg.
2. Two 400-mg vials (or four 200-mg vials) are used for patients with a body weight >50 kg combination of the 400-mg and 200-mg vials may be used but the total dose should not exceed 800 mg

For individuals whose body weight is more than 100 kg, doses exceeding 800 mg per infusion are not recommended .

Tocilizumab solutions do not contain preservatives; therefore, unused product remaining in the vials should not be used.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration by the site research pharmacist, whenever solution and container permit. Tocilizumab is a colorless to pale, yellow liquid. If particulates or discolorations are noted, the product should not be used.

Fully diluted solutions are compatible with polypropylene, polyethylene and polyvinyl chloride infusion bags and polypropylene, polyethylene and glass infusion bottles. Tocilizumab should not be infused concomitantly in the same intravenous line with other drugs. No physical or biochemical compatibility studies have been conducted to evaluate the co-administration of tocilizumab with other drugs.

### 6.3.1 Tocilizumab Storage

Tocilizumab should not be used after the expiry date (EXP) shown on the pack.

For vials: Store between 2ºC – 8ºC, do not freeze. Keep the container in the outer carton in order to protect from light.

For prepared infusion solution: Tocilizumab does not contain preservatives, therefore reconstitution and dilution of the product should be performed under aseptic conditions. The prepared infusion solution of tocilizumab is physically and chemically stable in 0.9% w/v sodium chloride solution **at** 30ºC for 24 hours.

From a microbiological point of view, the prepared infusion should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not be longer than 24 hours at 2ºC – 8ºC, unless dilution has taken place in controlled and validated aseptic conditions.

### 6.3.2 Tocilizumab Overdosage

There are limited data available on overdoses with Tocilizumab. One case of accidental overdose was reported in which a patient with multiple myeloma received a dose of 40 mg/kg. No adverse drug reactions were observed. No serious adverse drug reactions were observed in healthy volunteers who received single doses of up to 28 mg/kg, although all 5 patients at the highest dose of 28 mg/kg developed dose-limiting neutropenia.
In case of an overdose, it is recommended that the patient be monitored for signs and symptoms of adverse reactions. Patients who develop adverse reactions should receive appropriate symptomatic treatment.

## 6.4 Modification of Study Intervention/Investigational Product for a Subject

Any dosage adjustment, including the reason for and dates of adjustment, will be documented in the CRF for each subject requiring this manipulation. In consultation with the Medical Monitor, the SI or licensed physician Sub-Investigator may reduce the dosage of study drug or discontinue the study drug in its entirety for AEs (AEs) thought to be related to the study drug or for other reason during the trial (the reason for, and dates of suspension or dose reduction must be documented).

**Opportunistic Infections and Serious Infections**
Tociluzimab should not be administered in patients with active infection. The effects of TCZ on CRP, neutrophils, and the signs and symptoms of infection should be considered when evaluating a patient for a potential infection.

Vigilance for timely detection of serious infection is recommended for patients receiving biologic agents for treatment of moderate to severe RA as signs and symptoms of acute inflammation may be lessened due to suppression of the acute phase reaction. Patients must be instructed to contact their physician immediately when any symptoms suggesting infection appear, in order to assure rapid evaluation and appropriate treatment.

If a patient develops a serious infection, administration of TCZ is to be interrupted until the infection is controlled. The clinician should consider the benefit-risk before resuming treatment with tocilizumab.

**Gastrointestinal Perforations**
Timely diagnosis and appropriate treatment may reduce the potential for complications of diverticulitis and thus reduce the risk of GI perforations. Therefore, patients should be made aware of the symptomatology potentially indicative of diverticular disease, and they should be instructed to alert their healthcare provider as soon as possible if these symptoms arise. Discontinuation of TCZ is recommended for patients who develop GI perforations.

**Demyelinating Disorders**
The impact of treatment with TCZ on demyelinating disorders is not known; events were rarely reported. Patients should be closely monitored for signs and symptoms potentially indicative of central demyelinating disorders. Physicians should exercise caution in considering the use of TCZ in patients with pre-existing or recent onset demyelinating disorders. Treatment with tocilizumab should be interrupted during assessment of a potential demyelination event and only resumed if the benefit of continuing study drug is favorable.

**Hematologic Abnormalities and Bleeding Events**
Decreases in neutrophil and platelet counts have been observed following treatment with TCZ in combination with MTX. In addition, there may be an increased risk of neutropenia in patients who have previously been treated with a TNF antagonist.

The risk mitigation strategies for neutropenia and thrombocytopenia are summarized below. For patients with concomitant medications associated with hematologic toxicity, the reduction or interruption of the suspected medication is recommended prior to modifying TCZ.

| **Low Absolute Neutrophil Count (ANC)** | |
|---|---|
| **(cells per $mm^3$)** | **Recommendation** |
| ANC greater than 1000 | Maintain dose |
| ANC 500 to 1000 | Interrupt study drug dosing and check Complete Blood Count (CBC) weekly. When ANC greater than 1000 cells per $mm^3$ resume tocilizumab at 4 mg per kg and increase to 8 mg per kg the next infusion if ANC remains greater than 1000. |
| ANC less than 500 | Discontinue study drug |

*Patients withdrawn from tocilizumab treatment due to a reduced neutrophil count should be monitored for signs of infection, with treatment as deemed appropriate by the sponsor or designee, and should have a repeat white blood cell count with differential performed weekly*

*until the ANC is above 1000 cells/mm$^3$ (1.0 x 10$^9$/L). If the ANC does not return to above 1000 cells/mm$^3$ (1.0 x 10$^9$/L) within 2 months (or sooner if deemed necessary by the sponsor or designee), a hematology referral is recommended.*

| **Low Platelet Count** | |
|---|---|
| **Lab Value (cells per mm$^3$)** | **Recommendation** |
| Greater than 100,000 | Maintain dose |
| 50,000 to 100,000 | Interrupt tocilizumabdosing and check CBC weekly. When platelet count is greater than 100,000 cells per mm$^3$ resume study drug at 4 mg per kg and increase to 8 mg per kg if platelets remain greater than 100,000 |
| Less than 50,000 | Discontinue tocilizumab |

*Patients withdrawn from tocilizumab treatment due to a reduced platelet count should have a repeat platelet count performed weekly until the count is above 100,000 cells/mm$^3$ (100 x 10$^9$/L). If the platelets do not return to above 100,000 cells/mm$^3$ (100 x 10$^9$/L) within 2 months (or sooner if deemed necessary by the sponsor or designee), a hematology referral is recommended.*

**Elevated Liver Enzymes and Hepatic Events**

Elevations in ALT and AST have been observed during treatment with the study medications

| **Liver Enzyme Abnormalities** | |
|---|---|
| **Lab Value** | **Recommendation** |
| Greater than 1 to 3x Upper limit of normal (ULN) | Dose modify concomitant disease modifying anti-rheumatic drugs (DMARD)if appropriate<br><br>For persistent increases in this range, reduce tocilizumab dose to 4 mg/kg or interrupt tocilizumab until ALT/AST have normalized. Check Complete Metabolic Panel (CMP) every 2 weeks until ALT/AST normal.<br><br>Restart with 4 mg/kg or 8 mg/kg, once ALT/AST normal and after discussion with medical monitor. |
| Greater than 3 to 5x ULN (confirmed by repeat testing) | Check CMP every 2 weeks until normal. If still greater than 3x ULN after 4 weeks, discontinue study drug |
| Greater than 5x ULN | Discontinue study drug |

*Patients withdrawn from tocilizumab treatment due to elevated liver function tests should have repeat tests performed, as clinically appropriate, until levels return to baseline. If the patient's liver function tests have not returned to baseline within 6 months (or sooner, if deemed necessary by the sponsor or designee), an ultrasound and/or liver biopsy should be considered.*

**Cardiovascular Events and Elevated Lipids**

Patients with RA have an increased risk for cardiovascular disorders, therefore, risk factors for cardiovascular disease (eg, hypertension, hyperlipidemia) should be managed as part of their standard of care. See section on Drug Interactions.

For patients with LDL cholesterol ≥160 mg/dL, it is strongly recommended that investigators advise therapeutic lifestyle changes that may include initiation lipid-lowering agents. Lipid-lowering agents should also be considered for patients with lower LDL cholesterol levels as part of their therapeutic lifestyle changes depending on their overall risk as defined in NCEP ATP III or other national guidelines.

**Malignancies**

The impact of immunosuppression on the development of malignancies is not known, however an increased rate of some malignancies, notably lymphoma, has been observed in RA patients. Although no imbalance of malignancies was observed in controlled clinical trials of TCZ, malignancies have been identified as a concern for other biologics. It is recognized that identification of such events in TCZ-treated patients may require a longer period of surveillance. TCZ should be discontinued in patients with malignancies (with the exception of local basal or squamous cell carcinoma of the skin that is completely excised with free margins).

**Hypersensitivity or Anaphylaxis**

An infusion/dose reaction is defined as an adverse event occurring during and within 24 hours after the infusion or subcutaneous injection of tocilizumab. This may include hypersensitivity reactions or anaphylactic reactions.

Signs of a possible hypersensitivity reaction include but are not limited to:

- fever, chills, pruritus, urticaria, angioedema, and skin rash.
- cardiopulmonary reactions, including chest pain, dyspnea, hypotension or hypertension.

Healthcare professionals administering TCZ infusions should be trained in the appropriate administrative procedures, be able to recognize the symptoms associated with potential anaphylactic or hypersensitivity reactions, and have the appropriate medication available for immediate use in case of anaphylaxis or hypersensitivity reaction during or after administration

of TCZ. Healthcare professionals should also instruct patients to seek medical attention if they experience symptoms of a hypersensitivity reaction outside of the clinic.

If a patient has symptoms of anaphylaxis or serious hypersensitivity, or requires an interruption of the study drug because of symptoms of anaphylaxis or hypersensitivity, administration of TCZ must be discontinued permanently and the patient should be withdrawn from the study. The patient should be treated according to the standard of care for management of the hypersensitivity reaction. A blood sample for the presence of anti-tocilizumab antibodies should be obtained at time of event and at least 6 weeks after the last dose*.

*6 weeks for the RA indication. For other indications consultation with the clinical pharmacology group will be required to assess how many weeks after the last dose testing should be conducted.

**Viral Reactivation**
Though rarely reported within the TCZ program due to exclusion criteria at study entry, reactivation of viral and other serious infections (e.g. EBV or TB) has been observed with biologic therapies for RA, including TCZ.

**Drug Interaction**
The formation of CYP450 enzymes may be suppressed by increased levels of cytokines (eg, IL-6) during chronic inflammation. Therefore, it is expected that for molecules that antagonize cytokine activity, such as TCZ, the formation of CYP450 enzymes could be normalized. Given its long elimination half-life (t1/2), the effect of tocilizumab on CYP450 enzyme activity may persist for several weeks after stopping therapy.

### 6.4.1. Dosage Discontinuation

Subjects who meet the following criteria should discontinue study medication:

#### 6.4.1.1. Tocilizumab-Specific Criteria

- Anaphylaxis or hypersensitivity reaction or requires an interruption of the study drug because of symptoms of anaphylaxis or hypersensitivity (tocilizumab should be permanently discontinued from these patients)
- ALT or AST value > 5X ULN or persistent elevation > 3X ULN
- Platelet count (cells/$mm^3$) < 50,000
- ANC (cells/$mm^3$) < 500

#### 6.4.1.2. General Criteria

- AE
- Sponsor termination
- Protocol deviation/Lost to follow-up
- Patient request
- Death
- Inability of subject to comply with study requirements
- Determination by the Medical Monitor or Site Investigator that it is no longer safe for the subject to continue therapy

All serious AEs (SAEs) that occur in a subject who has discontinued early must be recorded and reported to the CC within 24-hours of awareness.

Study subjects who discontinue study drug prematurely (early termination from study) and decide to not remain in the ITT portion of the study will be encouraged to return for a Final Safety Visit and participate in a Follow-Up Telephone Call 28 days (+ 5 days) after the last dose of study drug.

All subjects who discontinue study drug early and choose to remain in the ITT portion of the study will be encouraged to follow the study visits, off drug, up through the last visit (Week 16 EOS Visit).

SAEs will be followed for resolution for 28 days (+5 days) after a subject's last dose of study drug, regardless if they prematurely discontinued study drug or completed 4 weeks of treatment.

### 6.5 Accountability Procedures for tocilizumab

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented

prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

## 6.6 Prior and Concomitant Therapy

Throughout the study, SIs may prescribe concomitant medications or treatments including edaravone (Radicava®), deemed necessary to provide adequate supportive care provided that the medications are licensed in the United States. Study subjects should not receive other experimental agents during the study. This includes marketed agents at experimental dosages that are being tested for the treatment of ALS and experimental procedures such as stem cell therapies or diaphragm pacing devices. All concomitant medications and/or treatments and significant non-drug therapies including supplements and assistive devices, received by a subject should be recorded on the appropriate source document and eCRF at each study visit.

Any investigational pharmacotherapy being used or evaluated for the treatment of ALS is prohibited beginning 30 days prior to the Screening Visit and throughout the study. This includes, but is not limited to, the following:

- Pioglitazone
- Arimoclomol
- Olanzapine
- Nuedexta
- NP001
- Ibudilast
- Fingolimod
- GSK 1223249
- Rasagiline
- Memantine
- Ezogabine

### 6.6.1 Prohibited Medications and Contraindications

Prohibited Medications

Throughout the course of the trial, study subjects should not be treated with the following medications. If an investigator learns that a patient has begun therapy with any of these medications, this should be reported to the CC immediately and action should be taken to discontinue the prohibited medication. If, for safety or health reasons, or by subject choice, the prohibited medication cannot be stopped, then the study medication should be stopped.

Prohibited medications include:

- Nuedexta
- Theophylline
- Cyclosporine
- Warfarin
- Live vaccines

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Subjects or partners of male subjects should not become pregnant during the study or within 6 months after stopping study drug. If a female subject becomes pregnant, study treatment must be discontinued immediately.

It is not known whether tocilizumab is excreted in human milk or absorbed systemically after ingestion. Caution should be exercised; therefore, no subject should nurse her infant while participating in this study.

**MR-PET (MGH only):** Additionally, subjects should withhold taking benzodiazepines for 24 hours prior to scanning. Lorazepam, Clonazepam, and Zolpidem are allowed under the protocol within this timeframe.

## 6.7 Blinding of Study Drug

The Randomization ID will be used to identify the subject's electronic case report forms (eCRFs), laboratory tests, study medication and all communications.

Study Subjects, Site Investigators, Coordinators, Clinical Evaluators (and all other study site staff), Study Monitors, Project Management, Data Management personnel, and the Sponsor will be blinded to treatment group assignment throughout the study.

Only the Biostatistician who develops the randomization schedule, the Research Pharmacists at the Central Pharmacy and the Site Pharmacist (if applicable) storing and distributing the study drug at each site will be unblinded to the individual drug assignments in this study.

### 6.7.1. Emergency Unblinding

An emergency unblinding procedure will allow the Site Investigator (SI) the option of learning the treatment assignment for an individual subject if clinical circumstances require it. For unblinding, the SI must contact the Medical Monitor (MM). The SI should attempt to contact the MGH Coordination Center (CC) prior to completing emergency unblinding if time allows. If this

is not possible, the site must inform the MGH CC as soon as possible regarding the emergency unblinding and the circumstances surrounding it. The SI must document the reason for unblinding in the subject's source documents.

Rarely should such an extreme action be taken. Experimental medications can usually be withdrawn without the need for unblinding. Sites should take care to unblind only those study staff member(s) and other persons whose knowledge of the treatment assignment is necessary for the clinical safety of the subject. Sites should take all reasonable measures to keep study staff (especially those completing any Final Safety Visit procedures), MGH CC staff and the subject blinded to treatment assignment if possible.

In the event that emergency disclosure of treatment assignment is necessary, the study subject will be withdrawn from further participation in the trial. All adverse events (AEs) resulting in emergency unblinding will be followed for resolution.

# 7 STUDY SCHEDULE

No study procedures should be performed prior to the signing of the informed consent form (ICF). All subjects will sign an ICF prior to undergoing any study tests or procedures. The slow vital capacity (SVC) should be performed first at the visits so as not to fatigue the subject with other testing, however the order of testing will be at the discretion of each SI.

Visit windows are consecutive calendar days and the target visit dates are calculated from the Baseline Visit.

## 7.1 Screening Visit

The following procedures will be performed at an office visit to determine the subject's eligibility for the study:

1. Obtain written informed consent from subject
2. Assess inclusion and exclusion criteria
3. Obtain medical history and demographics
4. Obtain ALS diagnosis history
5. Measure vital signs including height and weight
6. Perform neurological examination
7. Perform physical examination
8. Perform slow vital capacity (SVC)
9. Perform 12-lead electrocardiogram (ECG)
10. PA and Lateral Chest X-Ray
11. Collect blood samples for baseline clinical laboratory assessments (CBC with differential and chemistry panel), Quantiferon or T-SPOT TB test (or TB skin test), Hepatitis B panel and serum pregnancy test (for woman of childbearing potential [WOCBP])
12. Collect urine sample for urinalysis
13. Collect blood samples for PBMC collection
14. Review and document concomitant medications and therapies
15. Assess and document AEs (AEs) after subject signs informed consent form (ICF)
16. Tentatively schedule the Baseline Visit

***Pregnancy Test:*** Serum pregnancy test will be done at the Screening Visit for women of child bearing potential (WOCBP). Thereafter, urine pregnancy testing will be performed on an as needed basis throughout the study.

**MR-PET (MGH only):**

17. Blood Draw for TSPO Binding Affinity Test (**If not previously determined)

18. Upper Motor Neuron Burden Scale.
19. MRI Safety Questionnaire

*Binding affinity:* Venous blood will be collected and sent to the Partners Healthcare Center for Personalized Genetic Medicine to genotype for the Ala147Thr TSPO polymorphism in the TSPO gene (rs6971) [18] . About 10% of human subjects show low binding to PBR28. High and mixed affinity binders (Ala/Ala or Ala/Thr) will be eligible, whereas the low affinity binders (Thr/Thr) will be ineligible to enroll.

### 7.1.1 Screen Failures

Any subject who signs consent will be considered enrolled in the study. If a subject fails screening, *at a minimum*, the following information should be captured and entered in the Electronic Data Capture (EDC) System:

- Inclusion and Exclusion Criteria
- Demographics
- Reason for screen failure

## 7.2 Baseline Visit

This visit will take place within 28 days of the Screening Visit. The following procedures will be performed:

1. Randomize subject (approximately 24 hours prior to the subject's Baseline visit)
2. Review inclusion and exclusion criteria
3. Measure vital signs including weight
4. Administer C-SSRS
5. Administer ALSFRS-R questionnaire
6. Perform SVC [Note: Baseline Visit SVC is not exclusionary, even if below 40%]
7. Perform Hand-held Dynamometry (HHD) and Grip Strength
8. Collect blood samples for clinical laboratory assessments
9. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
10. Collect blood sample for IL-6 receptor genotyping
11. Collect a blood sample for Genentech
12. Collect urine sample for urinalysis
13. Review and document concomitant medications and therapies
14. Assess and document AEs
15. Perform LP for CSF collection
16. Schedule next Study Visit
17. Administer 1$^{st}$ dose of study drug after all baseline study procedures are complete. The subject should be observed at the site for 60 minutes after the infusion by an appropriate

healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.

**Post-Lumbar Puncture Follow-up Telephone Call**
Subjects will receive a phone call 24-48 hours after their LP to assess and document AEs.

MR-PET (MGH only) (* -2 Week window for these procedures to be conducted):

1. *Pre*-dose neuroimaging study (MRI/PET) will be performed one time
2. Upper Motor Neuron Burden Scale.
3. MRI Safety Questionnaire

## 7.3 Week 4 (Visit 1)

This visit will take place 28 ± 3 days after the Baseline Visit. The following procedures will be performed:

1. Measure vital signs including weight
2. Administer C-SSRS
3. Administer ALSFRS-R questionnaire
4. Perform SVC
5. Collect blood samples for clinical laboratory assessments
6. Collect urine sample for urinalysis
7. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
8. Review and document concomitant medications and therapies
9. Assess and document AEs
10. Administer 2nd dose of study drug. The subject should be observed at the site for 30 minutes after the infusion by an appropriate healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
11. Schedule next Study Visit

## 7.4 Week 8 (Visit 2)

This visit will take place 56 ± 3 days after the Baseline Visit. The following procedures will be performed:

1. Measure vital signs including weight
2. Administer C-SSRS
3. Administer ALSFRS-R questionnaire
4. Perform SVC
5. Perform HHD and Grip Strength

6. Collect blood samples for clinical laboratory assessments
7. Collect urine sample for urinalysis
8. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
9. Review and document concomitant medications and therapies
10. Assess and document AEs
11. Administer 3rd dose of study drug. The subject should be observed at the site for 30 minutes after the infusion by an appropriate healthcare staff member according to the site's institutional/state regulations to assess medical status and any immediate reaction to the study drug.
12. Perform 2nd LP CSF collection 3 hours +/- 30 minutes after the start of study drug infusion.
13. Schedule next Study Visit

**Post-Lumbar Puncture Follow-up Telephone Call**

Subjects will receive a phone call 24-48 hours after their LP to assess and document AEs that may be directly related to the LP.

MR-PET (MGH only) (* -2 Week window for these procedures to be conducted):

1. *Post*-dose neuroimaging study (MRI/PET) will be performed one time
2. Upper Motor Neuron Burden Scale.
3. MRI Safety Questionnaire

## 7.5 Week 12 (Visit 3)

This visit will take place 84 ± 3 days after the Baseline Visit. The following procedures will be performed:

1. Measure vital signs including weight
2. Administer C-SSRS
3. Administer ALSFRS-R questionnaire
4. Perform SVC
5. Collect blood samples for clinical laboratory assessments
6. Collect urine sample for urinalysis
7. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
8. Review and document concomitant medications and therapies
9. Assess and document AEs
10. Schedule next Study Visit

## 7.6 Week 16 (EOS Visit)

This visit will take place 112 ± 3 days after the Baseline Visit. The following procedures will be performed:

1. Perform neurological examination
2. Perform physical examination
3. Measure vital signs including weight
4. Administer C-SSRS
5. Administer ALSFRS-R questionnaire
6. Perform SVC
7. Perform HHD and Grip Strength
8. Collect blood samples for clinical laboratory assessments
9. Collect urine sample for urinalysis
10. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
11. Review and document concomitant medications and therapies
12. Assess and document AEs
13. Exit questionnaire

## 7.7 Final Safety Visit & Telephone Call

Subjects who withdraw consent and do not agree to be followed for ITT will be asked to come in for a Final Safety Visit and will be asked to have a final Follow-Up Telephone Call 28 (+ 5) days after the subject's last dose of study drug.

1. The final visit should be scheduled as soon as possible. The following will be performed:
2. Perform neurological examination
3. Perform physical examination
4. Measure vital signs including weight
5. Administer C-SSRS
6. Administer ALSFRS-R questionnaire
7. Perform SVC
8. Perform HHD and Grip Strength
9. Collect blood samples for clinical laboratory assessments
10. Collect urine sample for urinalysis
11. Collect blood samples for cytokine levels, hs-CRP concentration and PBMC collection
12. Review and document concomitant medications and therapies
13. Assess and document AEs

This phone call will take place 28 days after the subject's last dose of study drug. The following procedures will be performed:

14. Review and document concomitant medications and therapies
15. Assess and document AEs

## 7.8 Protocol Deviations

A protocol deviation is any non-compliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on

the part of the subject, the SI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All deviations from the protocol must be addressed in the subject's source documents. Protocol deviations must be sent to the local IRB per their guidelines and entered in the Protocol Deviations Log in the Electronic Data Capture (EDC) System.

### 7.9 Missed Visits and Procedures

Missed visits and any procedures not performed (not attempted) for reasons other than illness, injury or progressive disability (i.e. subject is physically unable to perform test) will be reported as protocol deviations.

Procedures or visits not performed due to illness, injury or disability, including procedures that were attempted but failed (i.e. blood samples unable to be drawn after multiple attempts, or weight unable to be obtained due to subject immobility) will not be reported as protocol deviations unless there is a patient safety concern.

In the event of illness, injury or disability, every attempt will be made to complete each visit. In the event that the visit is not completed, a phone visit will be performed to review concomitant medications, perform the ALSFRS-R, and record AEs.

Study drug compliance that is outside the limits set in the study operations manual will be reported as a protocol deviation.

Details and specific instructions regarding protocol deviations, including any exceptions to this standard procedure, are found in the study operations manual.

# 8 CLINICAL ASSESSMENTS AND OUTCOME MEASURES

## 8.1 Clinical Variables

Assessments will be performed at designated time-points throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, past medical history, including ALS, as well as concomitant medication usage.

### 8.1.1 Vital Signs, Height & Weight

Vital signs will be obtained after the subject has been in a seated position for several minutes. Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, temperature and weight will be assessed at specified visits. Height will be measured and recorded at the Screening Visit only.

### 8.1.2 PBMC Blood Collection, hs-CRP concentration and Cytokine Levels

Blood will be collected to measure the expression of inflammatory genes in peripheral blood mononuclear cells (PBMCs). Plasma cytokine levels, including IL-6, IL-17A, IL-1β, IL-8, MMP1, TNF-α, sIL-6 receptor and hs-CRP, will also be assessed. These samples will be sent to Dr. Robert Bowser's laboratory at the Barrow Neurological Institute (BNI) for analysis.

In addition, a blood sample will be sent to Genentech, Inc. in California at baseline and again at the time of a hypersensitivity, anaphylaxis or drug discontinuation event. All samples will be labeled with a code. The code will not include any identifiable information.

### 8.1.4 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed at the Screening Visit only. Tracings will be reviewed by a site cardiologist and a copy of the tracings will be kept on site as part of the source documents.

### 8.1.5 Chest X-Ray

A standard PA and Lateral Chest X-Ray will be performed at the Screening Visit to assess for evidence of active pulmonary tuberculosis (TB). A copy of the report will be kept on site as part of the source documents.

### 8.1.6 Physical Examination

A physical examination will be performed and recorded at the Baseline Visit. The following systems will be examined: head/neck, eyes, ears, nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, central nervous system, extremities, and skin.

### 8.1.7 Neurological Examination

A complete neurological examination will be performed and recorded at the Baseline Visit.

### 8.1.8 Columbia Suicide Severity Rating Scale (C-SSRS)

The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA)[19]. The C-CASA was developed to assist the FDA in coding suicidality data accumulated during the conduct of clinical trials of antidepressant drugs. One such assessment instrument is the Columbia Suicide Severity Rating Scale (C-SSRS). The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior.

At the Baseline Visit, the C-SSRS Baseline version will be administered. This version is used to assess suicidality over the subject's lifetime and specifically for the previous 6 month time period.

At all other study visits, the Since Last Visit version of the C-SSRS will be administered. This version of the scale assesses suicidality since the subject's last visit.

### 8.1.9 IL-6 Genotyping

Blood will be collected and sent to the Hawkins lab at Wake Forest. DNA will be isolated and the IL-6 receptor gene will be sequenced. Common polymorphisms, including one previously described in patients with asthma, will be identified and be entered into the EDC record of each patient.

### 8.1.10 Lumbar Puncture

Lumbar punctures will be performed and CSF will be collected at the Baseline Visit and Visit 2. All patients will receive the Baseline Visit LP prior to receiving the first dose of study drug. Patients will receive the visit 2 LP 2 hours +/- 30 minutes after completion of the Visit 2 study drug infusion administration**.** The SI will discuss all potential LP risks to the subjects including:

- Local pain at injection site;
- Reaction to anesthetic agents;
- Bleeding at needle entrance site;

- Infection at needle entrance site; and,
- Post-LP low-pressure headache.

Extensive experience with research LP in Alzheimer's disease reveals a very low incidence of complication, including the incidence of PLPH[3]. Fewer than 2.6% of patients in a memory disorder clinic developed PLPH, and only a single patient in a cohort of over 1000 had a headache lasting more than 5 days [20]. No other local or generalized complications occurred.

The procedure must be performed by the SI or another licensed practitioner with experience and training in performing LPs, and who is listed on the site delegation log. LPs will be performed with an atraumatic Sprotte needle to reduce the risk of PLPH. CSF samples will be sent to Dr. Bowser's laboratory at BNI to determine the concentration of inflammatory cytokines (IL-6, IL-1β and sIL-6 receptor) in the CSF before and after treatment with tocilizumab vs. placebo. In addition, CSF will be sent to the Hawkins lab at Wake Forest for measurement of sIL-6 receptor levels.

Remaining CSF samples will be stored in a sample repository. These samples will be used for future research in motor neuron diseases. All samples will be labeled with a code. The code will not include any identifiable information.

Any research performed on the samples is for research purposes only. Although genetic information may be analyzed, no genetic information will be given to the subject nor will the information that may be obtained be placed in a subject's medical records.

There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decayed or otherwise unfit for analysis. Subjects have the option of declining participation in this portion of the study at any time by withdrawing their consent to have their sample used. However, it will not be possible to destroy samples that may have already been used.

### 8.1.11 Adverse Events

Adverse events will be documented at each study visit, including the Screening Visit once the informed consent form has been signed by the subject, and at all study visits. Information on adverse effects of study medication and on inter-current events will be determined at each visit by direct questioning of the subjects, review of concomitant medications, and vital sign results.

### 8.1.12 Exit Questionnaire

An exit questionnaire will be completed by subjects and Site Investigators at the End Of Study Visit (Week 16). This will include questions regarding blindedness, LP and infusion experiences, and overall views of the quality of the trial.

## 8.2 Outcome Measure

### 8.2.1 ALSFRS-R

The ALSFRS-R is a quickly administered (5 minutes) instrument used to determine subjects' assessment of their capability and independence in 12 functional activities, each rated on an ordinal scale (ratings 0-4). All 12 activities are relevant in ALS. Initial validity was established by documenting that in ALS patients, change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival. The test-retest reliability is greater than 0.88 for all test items. The advantages of the ALSFRS-R are that the categories are relevant to ALS, it is a sensitive and reliable tool for assessing activities of daily living function in those with ALS, and it is quickly administered. With appropriate training the ALSFRS-R can be administered with high inter-rater reliability and test-retest reliability. The ALSFRS-R can be administered by phone with good inter-rater and test-retest reliability. The equivalency of phone versus in-person testing, and the equivalency of study subject versus caregiver responses have also recently been established. Therefore, if necessary, the ALSFRS-R may be given to the study subject over the phone. All ALSFRS-R evaluators must be NEALS certified.

### 8.2.2 Slow Vital Capacity (SVC) Testing

The vital capacity (VC) (percent of predicted normal) will be determined, using the upright slow VC method. The VC can be measured using conventional spirometers that have had a calibration check prior to subject testing. A printout from the spirometer of all VC trials will be retained. All VC evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials with variability being calculated as [(Highest VC-Second highest VC)/ Highest VC] x 100. Only the 3 best trials are recorded on the CRF. The highest VC recorded is utilized for eligibility.

### 8.2.3 Hand-held Dynamometry (HHD) and Grip Strength

Hand Held Dynamometry (HHD): Hand held dynamometry (HHD) will be used as a quantitative measure of muscle strength for this study. Six proximal muscle groups will be examined bilaterally in both upper and lower extremities (shoulder flexion, elbow flexion, elbow

extension, hip flexion, knee flexion, and knee extension), all of which have been validated against maximum voluntary isometric contraction (MVIC) testing [21]. In addition, wrist extension, first dorsal interosseous contraction and ankle dorsiflexion will be measured bilaterally; these muscles are often affected in ALS. Mean and standard deviation for each muscle group will be established from the initial values for each subject in this trial, so that strength determinations can be converted to Z scores and averaged to provide an HHD megascore for both upper and lower extremities.

Grip Strength: Bilateral hand grip will be measured using a study approved dynamometer to test the maximum isometric strength of the hand and forearm muscles.

### 8.2.4 Training and Validation

All evaluators must be NEALS certified to perform the ALSFRS-R, SVC and HHD; specific certification requirements are outlined in the study operations manual. Repeat NEALS certification will be required on a bi-annual basis for all outcome measures. It is strongly preferred that a single evaluator performs all measures throughout the study, if possible. NEALS certification is required for all evaluators prior to performing any study tests.

### 8.2.5 Neuroimaging

MGH Subjects will undergo Magnetic Resonance Imaging (MRI) / PBR28 Positron Emission Tomography (PET) twice during the course of the study. The goal of these scans is to measure activated microglia in study participants before treatment and after several weeks of treatment. The first scan will occur at the Baseline visit (*Pre-dose*), after subject eligibility has been confirmed by TSPO affinity binding assay. The second scan will occur at the Week 8 (Post-dose). Neuroimaging will be performed at the MGH Martinos Center for Biomedical Imaging. Subjects will be asked to lie still in a supine position for the duration of the study, which will take approximately 90 min.

Each PET scan will include one administration of up to 15 mCi of [$^{11}$C]PBR28 (which is equivalent to ~3.7 mSv), injected intravenously with a slow bolus over a 30 s period. The catheter will be flushed post-injection with 0.9% saline solution. Dynamic data will be collected over approximately 90 minutes in list mode, and framed post-collection.

#### 8.2.5.1 Imaging Data Acquisition and Proccessing (MGH Only)

*Simultaneous PET-MRI Data Acquisition*

The proposed studies will benefit from our experience using a unique PET-MRI scanner called BrainPET**.** This scanner consists of a dedicated brain avalanche photodiode-based PET scanner

that operates in the bore of a 3T whole body MR scanner. Our center has over seven years' experience using this scanner and has successfully completed over 200 human studies. Our data suggest that high quality PET data can be acquired fully simultaneously with conventional and advanced MRI data without substantial artifacts. The simultaneous acquisition of PET and MRI data reduces scan time, which minimizes the discomfort experienced by our ALS patient population. In addition, the simultaneous PET/MRI acquisition allows us to apply our novel MRI-based methods for generating attenuation correction maps, which enable PET motion correction and improve image quality [22, 23].

*PET Data*

Up to 15 mCi of [$^{11}$C]PBR28 or ~5 mCi (4.8 ± 0.4 mCi) of [$^{11}$C]PBR28 will be injected intravenously with a slow bolus over a 30 second period [24]. The catheter will be flushed post-injection with 0.9% saline solution. Dynamic data will be collected over approximately 90 minutes in list mode, and framed post-collection. The PET list-mode data will first be sorted in the line-of-response (LOR) space and motion corrected using MRI-derived motion estimates for each individual frame [22, 23]. Next, the data will be rebinned in the sinogram space, generating prompt and random coincidences sinograms. The head attenuation map (μ-map) will be obtained using a recently implemented MR-based attenuation correction method [22, 23]. The scatter sinogram will be obtained using a calculated method based on the single scatter estimation method[25]. The images will be reconstructed using the Ordinary Poisson Ordered Subset Expectation Maximization (OP-OSEM) 3D algorithm from prompt and expected random coincidences, normalization, attenuation and scatter coincidences sinograms using 16 subsets and six iterations [26]. The reconstructed volume consists of 153 slices with 256×256 pixels (1.25×1.25×1.25 mm$^3$). Regional uptake of the tracer will be estimated as described below.

*MRI Data*

A high resolution structural volume will be collected for the purposes of anatomical localization, ROI definition, and structural analyses. A multi-echo MPRAGE pulse sequence will be utilized, with an isotropic voxel of 1 mm. Cortical reconstruction and volumetric segmentation will be performed with the Freesurfer image analysis suite (*http://surfer.nmr.mgh.harvard.edu/*). A dual-echo ultrashort echo time MRI sequence (DUTE) will be collected to determine attenuation correction for PET data. Other routine exploratory MRI sequences will be acquired including: a) Magnetic Resonance Spectroscopy (MRS) to measure NAA, Choline, Creatine, and MyoInositol in the motor cortices; and b) Diffusion tensors imaging (DTI) to detect changes in the corticospinal tract in correlation with PBR28 uptake.

#### 8.2.5.2 Imaging Analysis Plan

Region of interest (ROI) analyses will be conducted to detect differences in brain uptake of PBR28 (Pre-dose compared to Post-dose) as described in [12, 13].

Standard uptake volumes (SUV) images for PBR28 will be created for radioactivity in the field of view 60–90 minutes post-radioligand injection. To account for motion that may have occurred between MP-RAGE acquisition and the 60–90 minute post-injection time point corresponding to the PET frame of interest, $SUV_{60–90\ min}$ will be generated in a two-step procedure. First, a $SUV_{60–90\ min}$ image will be created for each subject using an attenuation correction map computed from the native MP-RAGE. Subsequently, a new attenuation map will be created based on the registration of the native MP-RAGE with the $SUV_{60–90\ min}$ image obtained in the first reconstruction using FreeSurfer3s spmregister. A final $SUV_{60–90\ min}$ will then be reconstructed based on this new attenuation correction image well registered with the 60–90 min PET data. Individual $SUV_{60–90\ min}$ images will then be registered to MNI (Montreal Neuroogical Institute) space, spatially smoothed (6 mm full width at half maximum), and intensity-normalized to a mean of 1 ($SUVR_{60–90\ min}$) in order to account for differences in global signal across subjects, as previously described [12]. Average $SUVR_{60–90\ min}$ in predetermined ROIs for the left and right precentral gyri for both the gray matter and the underlying white matter will be derived from the automatic T1 segmentation in Freesurfer (20) will be estimated from [$^{11}$C]-PBR28 SUVR in the ROIs.

### 8.2.6 Upper Motor Neuron Burden (UMNB) Scale

The Upper Motor Neuron-Burden (UMNB) Scale assesses pathological UMN signs on examination. The UMNB measures the following deep tendon (scores 0–4) and pathological reflexes (present — 1 or absent — 0): biceps, brachioradialis, triceps, knee jerk, ankle jerk, Hoffman, Babinski, and jaw jerk. The total UMNB score ranges from 0 to 45, with 0 representing no reflex involvement, and 45 maximal abnormal UMNB

# 9 SAFETY AND ADVERSE EVENTS

The AE definitions and reporting procedures provided in this protocol comply with all applicable United States Food and Drug Administration (FDA) regulations and International Conference on Harmonization (ICH) guidelines. The SI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. It is also important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

## 9.1 Definitions of AEs, Suspected Adverse Drug Reactions & SAEs

### 9.1.1 Adverse Event and Suspected Adverse Drug Reactions

An AE is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there is a possible causal relationship to the medicinal product.

Examples of AEs include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (e.g., skin rash, peripheral edema), or clinically significant abnormal test results (e.g., lab values or vital signs), with the exception of outcome measure results, which are not being recorded as AEs in this trial (they are being collected, but analyzed separately). Stable chronic conditions (e.g., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered AEs. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity, would be considered as worsened and therefore would be recorded as AEs.

AEs are generally detected in two ways:

Clinical → symptoms reported by the subject or signs detected on examination.

Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

For the purposes of this study, symptoms of progression/worsening of ALS, including 'normal' progression, will be recorded as AEs.

The following measures of disease progression will not be recorded as AEs even if they worsen (they are being recorded and analyzed separately): vital capacity results and ALSFRS-R results.

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the SI and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the SI to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the SI.

Subjects will be monitored for AEs from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, early study termination for other reasons, or following completion of the entire study).

An unexpected AE is any AE, the specificity or severity of which is not consistent with the current Investigator's Brochure. An unexpected, suspected adverse drug reaction is any unexpected AE for which, in the opinion of the SI or Sponsor, there is a reasonable possibility that the investigational product caused the event.

### 9.1.2 Adverse Events of Special Interest

Adverse events of special interest (non-serious and serious) are required to be reported by the Investigator to the CC within 24 hours after learning of the event. The CC must forward this information to Genentech Drug safety within 24 hours of receipt (see Section 9.3 for reporting instructions**). Non-serious and serious AEs** of special interest for this study include the following:

- Infections including all opportunistic infections and non-serious infections as defined by those treated with IV anti-infectives.
- Suspected transmission of an infectious agent by the medicinal product (STIAMP). Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious

agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

- Myocardial infarction/acute coronary syndrome.
- GI perforation and related events.
- Malignancies.
- Hypersensitivity reactions.
- Demyelinating disorders.
- Stroke.
- Bleeding events.
- Hepatic events.

Guided questionnaires have been prepared for the AEs of special interest.

The notification of AEs of special interest (including non-serious events of special interest) will follow the established procedures for AEs and SAEs in the study (i.e., documented and reported to the CC within 24 hoursGuided questionnaires have been prepared for the AEs of special interest and will be sent to the investigator(s) to obtain more detailed information, as necessary. The documentation and reporting requirements for those AEs of special interest will be further described in a separate document (Actemra Events of Special Interest Guidance Document).

### 9.1.3 Serious Adverse Events

A SAE is defined as an AE that meets any of the following criteria:

- Results in death.
- Is life threatening: that is, poses an immediate risk of death as the event occurred.
    - This criterion applies if the study subject, in the view of the SI or Sponsor, is at immediate risk of death from the AE as it occurred. It does not apply if an AE that hypothetically might cause death if it were more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
    - Hospitalization for an elective procedure (including elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled “procedure” or a “treatment” is not an untoward medical occurrence.
- Results in persistent or significant disability or incapacity.
    - This criterion applies if the “disability” caused by the reported AE results in a substantial disruption of the subject’s ability to carry out normal life functions.
- Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).

- Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include serious infections, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical AE may meet criteria for "seriousness" but is not an adverse experience, and will therefore, not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

A serious, suspected adverse drug reaction is an SAE for which, in the opinion of the SI or Sponsor, there is a reasonable possibility that the investigational product caused the event.

The SI is responsible for classifying AEs as serious or non-serious.

## 9.2 Assessment and Recording of Adverse Events

The SI will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in the electronic data capture (EDC) system and compiled into reports for monthlyreviewing by the Medical Monitor. The Medical Monitor shall promptly review all information relevant to the safety of the investigational product, including all SAEs. Special attention will be paid to those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

### 9.2.1 Assessment of Adverse Events

At each visit (including telephone interviews), the subject will be asked if they have had any problems or symptoms since their last visit in order to determine the occurrence of AEs. If the subject reports an AE, the Investigator will probe further to determine:

- Type of event
- Date of onset and resolution (duration)
- Severity (mild, moderate, severe)
- Seriousness (does the event meet the above definition for an SAE)
- Causality, relation to investigational product and disease

- Action taken regarding investigational product
- Outcome

### 9.2.2 Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product should be specified by the SI, using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.

2. Unlikely: The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.

3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (Suspected ADR)

4. Probably Related: The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (Suspected ADR)

5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; follows a known or expected response pattern to the investigational product; and is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (Suspected ADR)

### 9.2.3 Recording of Adverse Events

All clinical AEs are recorded in the AE Log in the subject's study binder. The site should fill out the AE Log and enter the AE information into the Electronic Data Capture (EDC) system within 48 hours of the site learning of a new AE or receiving an update on an existing AE.

Please Note: SAEs must be reported to the CC within 24 hours of the site learning of the SAE. This applies regardless of whether the subject is taking study drug or not. Infusion reactions, gastrointestinal perforations, tuberculosis, invasive fungal infections, bacterial, viral and other infections due to opportunistic pathogens, elevated liver enzymes, neutropenia or thrombocytopenia, whether classified as SAEs or not, must also be reported within 24 hours.
Entries on the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event.

## 9.3 Adverse Events and Serious Adverse Events - Reportable Events

The following are considered reportable events and must be reported to the CC within 24 hours of the site being notified of the event. The CC must then report the events to Genentech Drug safety within 24 hours of learning of the event, regardless of relationship to study drug:

- SAEs.
- Non-serious and serious AEs of special interest.
- Pregnancies.

The Investigator must report new significant follow-up information for these events to the CC within 1 working day after becoming aware of the information. The CC must send this information to Genentech Drug Safety within 1 working day of receipt. New significant information includes the following:

- New signs or symptoms or a change in the diagnosis.
- Significant new diagnostic test results.
- Change in causality based on new information.
- Change in the event's outcome, including recovery.
- Additional narrative information on the clinical course of the event.

Investigators must also comply with local requirements for reporting SAEs to the local health authority and IRB/EC.

All serious adverse events (SAEs) for which there is a reasonable possibility the experience may have been caused by Tocilizumab (this applies to both expected and unexpected events) should

be recorded on a MedWatch 3500A Form and emailed to the NCRI Project Manager and the Medical Monitor (contact information found in the study manual) .

The Coordination Center will then forward this form within 24 hours of receipt to:

Genentech Drug Safety
Tel: (888) 835-2555
Fax: (650) 225-4682 or (650) 225-4683

The Coordination Center will use the safety reporting fax sheet in Appendix V.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above.

The following are also considered reportable events and must be reported to the CC within 24 hours of the site being notified of the event.

- Dosage Changes (Dose Management)
  - Investigational Product Suspension, Reduction or Re-challenge
  - Investigational Product Discontinuation

- Key Study Events:
  - Subject Final Disposition
  - Feeding Tube Placement
  - Permanent Assisted Ventilation (PAV)*
  - Tracheostomy
  - Mortality
  - Pregnancy
  - Diaphragm Pacing System (DPS) device implantation

* Permanent Assisted Ventilation (PAV) is defined as more than 22 hours daily of non-invasive mechanical ventilation for more than one week (7 days). The date of onset of PAV is the first day of the seven days.

**STUDY CLOSE-OUT**

Any study report submitted to the FDA by the Sponsor or designee should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech.

# 10 DATA AND SAFETY MONITORING AND STATISTICAL ANALYSIS PLAN

## 10.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be assembled for the trial. The DSMB receives the blinded and unblinded summary reports of the frequency of all clinical AEs and safety laboratory tests for planned periodic meetings approximately every 3 months throughout the study. The DSMB can ask to receive the SAE reports more frequently. Meetings will be held via teleconference. In addition, the DSMB Chair may call ad hoc meetings.

Summaries of SAEs and enrollment will be provided approximately monthly to the DSMB by the Study Biostatistician. Serious infusion reactions or deaths occurring within 24 hours of dosing, infusion reactions, tuberculosis, invasive fungal infections, bacterial, viral and other infections due to opportunistic pathogens, elevated liver enzymes, neutropenia or thrombocytopenia, and any severe unexpected SAEs are considered events of interest and will be reported to the DSMB prior to their meeting on a quarterly basis. As necessary, the DSMB can review the frequencies of clinical and laboratory abnormalities. Recommendations for modification or termination of the trial based on safety data will be made by the DSMB to the PIs and Steering Committee. The DSMB will review safety data throughout the trial and may stop the trial for safety if they determine that there is a significant difference in the rate of a particular AE that would indicate a risk that is greater than the possible benefit of the study drug. A notable increase in the frequency of any AE should be examined by the DSMB although it may not lead to a recommendation by the DSMB. There will not be early stopping for efficacy in this trial.

Prior to each DSMB meeting, the CC will provide an update to the DSMB on enrollment, data quality (missing data) and protocol adherence. The CC will be responsible for communication with the DSMB.

Complete information can be found in the Data and Safety Monitoring Board Charter.

If 2 or more serious AEs are deemed to be definitely, probably, or possibly related to study drug occur, the DSMB will review these events promptly and make recommendations about potential changes to the study including possible changes to protocol, updates to the informed consent form, or even ending the study early.

## 10.2 Safety Monitoring

### 10.2.1 Safety Management Resource Team

The CC Safety Management Resource Team (SMaRT) is comprised of physicians, Project Managers, Data Managers, and Systems personnel.

On a periodic basis, the SMaRT group will review blinded reports of AEs, SAEs, abnormal laboratory measurements and protocol deviations as detailed in the study's Safety Management Plan**.**

### 10.2.2 Medical Monitoring

The designated Medical Monitor for the study is identified in the study operations manual. Site personnel should contact the Medical Monitor for assistance with the following:

- Medically-related protocol questions
- Safety concerns, including AEs and SAEs
- Protocol deviations
- Unblinding questions
- Protocol eligibilty questions
- Dose changes

## 10.3 Statistical Considerations

### 10.3.1 Statistical Methods

Given the difficulty with generalizing the safety and tolerability of tocilizumab to ALS patients, it is important to assess this as a primary outcome measure if a future phase 3 trial is to be considered. We will assess safety of tocilizumab treatment based on mortality, occurrence of SAEs, overall rates of AEs, clinically significant abnormal laboratory tests, and changes in vital signs. We will focus on events at least possibly related to study drug of moderate or severe intensity by comparing participants receiving at least one dose of tocilizumab to those on placebo for time to mortality and time to first SAE by Cox regression, for overall rate of AEs or abnormal laboratory tests by negative binomial regression, and for rates of change in vital signs. Tests will be two-tailed at alpha = 0.10 to increase power. With 16 participants randomized to tocilizumab and followed for safety outcomes, the study will have 80% power to detect adverse events expected to occur in at least 10% of patients. Although under-powered for comparing rates of rare events, the study will have 80% power to detect treatment differences in events expected to occur, for example, in 50% of placebo participants if tocilizumab increases the hazard 4.2-fold.

Tolerance of tocilizumab treatment will be judged on the proportion of participants remaining on study drug through all 3 doses and remaining on study and free from possibly drug-related and dose-limiting SAEs to the end of follow-up. The study will have 80% power to declare tocilizumab treatment tolerable based on non-inferiority to 50% tolerance with a one-sided 95% exact confidence bound if the true tolerance rate is at least 81%

We will assess successful target engagement by comparing rates of qualitative gene expression response in PBMCs between the two treatment groups. We will estimate the proportion of participants who experience a 2-fold or greater decline between baseline and the average of all follow-up assessments in at least 2 of the 3 pro-inflammatory genes. The study will have 80% power for this comparison if no more than 5% of placebo participants experience such a change and at least 30% of tocilizumab participants do. Assuming that at least 80% of participants are truly in a high inflammatory state given our enrollment criteria, that would correspond to roughly 6% vs. 38% response among susceptible patients randomized to placebo or tocilizumab, respectively. We will have greater power to detect quantitative changes in expression of pro-inflammatory genes. Serial measures of pro-inflammatory gene expression levels will be log-transformed and analyzed in repeated-measures ANOVAs with unstructured covariance over time. With an expectation of rapid change in gene expression, we will compare treatments on the basis of change from a shared baseline to the mean of all follow-up assessments using linear contrasts. The study will have 80% power to detect a difference in expression equal to an effect size of 1.11 based on two-tailed testing at or an effect size of 1.50 based on two-tailed tests at alpha = 0.017, applying a Bonferroni correction for multiple comparisons over 3 inflammatory genes.

Clinical responses to tocilizumab treatment, measured as change in ALSFRS-R, SVC, and HHD megascores will be analyzed in linear mixed models with shared baseline and random participant-specific intercepts and slopes. If we conservatively estimate power for detecting treatment-dependent differences in 16-wk change from a simple two-group t-test, the study will have 80% power for effect sizes of 0.76. With a standard deviation for ALSFRS-R rate of change equal to 1.58 units/month based on the follow-up schedule of this trial and variance component estimates from the Ceftriaxone trial, the study would have 80% power if tocilizumab reduces decline in ALSFRS-R by 2.0 units/month based on a two-tailed at alpha = 0.05. Power will be greater using the proposed linear mixed model.

Change in average [$^{11}$C]-PBR28 $SUV_{60\text{-}90\ min}$ in pre-defined regions of interest focused on the left and right precentral gyri for both the gray matter and the underlying white matter will be compared between participants receiving tocilizumab vs. placebo in a shared-baseline repeated-measures ANOVA with unstructured within-person covariance.

### 10.3.2 Analysis Populations

The primary analyses, whether for safety, tolerability, target engagement, efficacy, or PET/MRI, will use an as-treated sample that includes all participants classified according to the treatment actually received. This choice of an analysis sample reflects our interest in assessing mechanisms of action and potential efficacy rather than clinical effectiveness. For the purposes of planning future trials, a secondary efficacy analysis will use an intent-to-treat (ITT) sample that includes all randomized participants grouped according to their randomized treatment assignment.

## 10.4 Missing Data

Analyses will attempt to avoid bias due to intolerance by including all participants who initiate treatment regardless of their compliance with their assigned treatment. Every effort will be made to obtain follow-up information for all subjects whether or not they continue on treatment.

### 10.4.1 Stopping Rules

The DSMB will review safety data throughout the trial and may stop the trial for safety. Any unexpected death or death unrelated to disease progression will lead to prompt review by the DSMB. Two or more of the same SAE deemed probably or definitely related to study drug by SIs will lead to prompt review by the DSMB. In addition, if there is one death in the treatment group deemed probably or definitely related to study drug by the DMSB, the study will be stopped immediately.

# 11 DATA COLLECTION, MANAGEMENT AND MONITORING

## 11.1 Role of Data Management

Data Management (DM) is the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with NEALS policies, and applicable Sponsor and regulatory requirements. Site personnel will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and other forms used to report, track and record clinical research data. Clinical sites will be monitored to ensure compliance with data management requirements and Good Clinical Practices. DM is responsible for developing, testing, and managing clinical data management activities.

### 11.1.1 Data Entry and Checks

The site personnel are instructed to enter information into the Electronic Data Capture (EDC) System within 5 days of a visit. Data collection is the responsibility of the staff at the site under the supervision of the SI. During the study, the SI must maintain complete and accurate documentation for the study.

The EDC includes password protection. An edit checking and data clarification process will be put in place to ensure accuracy and completeness of the database. Logic and range checks as well as more sophisticated rules will be built into the EDC to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing or not calculated correctly. The sites will only have access to the queries concerning their subjects.

### 11.1.2 Data Lock Process

The application will have the ability to lock the database to prevent any modification of data once the study is closed. Once this option is activated, every user will have Read-Only access to the data. The database can only be locked after each SI has signed off on their subjects and all queries have been resolved.

### 11.1.3 Quality Assurance

Protocol procedures are reviewed with the SI and associated personnel prior to the study to ensure the accuracy and reliability of data. Each SI must adhere to the protocol detailed in this document and agree that any changes to the protocol must be approved by the CC prior to seeking approval from the site IRB. Each SI will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

## 11.2 Clinical Monitoring

Study Monitors will visit each study site to review source documentation materials, informed consent forms, and confirm entered data and that data queries have been accurately completed, and again at a study close-out visit. Study Monitors will also verify that SAEs and protocol deviations have been reported appropriately, as required. The Study Monitors will also review clinical facilities, resources and procedures for evaluating study subjects and study drug dispensing. Subsequently, the Study Monitors will provide monitoring reports to the Project Manager and, if requested, will provide reports of protocol compliance to the Study Principal Investigator and the Steering Committee. Completed informed consent forms from each subject must be available in the subject's file and verified for proper documentation. A document outlining the monitoring plan is provided to each Study Monitor.

## 11.3 Data Handling and Record Keeping

The SI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Dark ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Source document templates (SDTs) will be provided for use and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained. The CC will provide guidance to SIs on making corrections to the source documents and eCRFs.

### 11.3.1 Confidentiality

Study subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel

responsible for his or her welfare. All local and federal guidelines and regulations regarding maintaining study subject confidentiality of data will be adhered to.

Data generated by this study must be available for inspection by representatives of the Office for Human Research Protections (OHRP), the sponsor, all pertinent national and local health and regulatory authorities, the CC or their representative, Study Monitoring personnel, and the IRBs.

### 11.3.2 Study Discontinuation

The study can be terminated at any time. Reasons for terminating the study may include the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to study subjects.
- Study subject enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Sponsor withdraws funding.

### 11.3.3 Retention of Records

US FDA regulations (21 CFR 312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs (if applicable), consent forms, laboratory test results, and medical inventory records, must be retained by the Site Investigator (SI) for two years after marketing application approval. If no application is filed, these records must be kept for two years after the investigation is discontinued and the US FDA and the applicable national and local health authorities are notified. The Coordination Center or their representative will notify the Site Investigators of these events. The Site Investigators should retain all study documents and records until they are notified in writing by the Sponsor or their representative.

### 11.3.4 Publications

The Principal Investigator, Shafeeq Ladha, MD, and the study Steering Committee will be responsible for publications of results from this trial. Their responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

## 12 LITERATURE REFERENCES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# 13 APPENDICES

## 13.1 APPENDIX I: EL ESCORIAL WORLD FEDERATION OF NEUROLOGY CRITERIA FOR THE DIAGNOSIS OF ALS

Information obtained from the web site: ***www.wfnals.org***.

The diagnosis of Amyotrophic Lateral Sclerosis [ALS] requires:
A - The presence of:
(A:1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,
(A:2) evidence of upper motor neuron (UMN) degeneration by clinical examination, and
(A:3) progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination, together with
B - The absence of:
(B:1) electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and
(B:2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

**CLINICAL STUDIES IN THE DIAGNOSIS OF ALS**
A careful history, physical and neurological examination must search for clinical evidence of UMN and LMN signs in four regions [brainstem, cervical, thoracic, or lumbosacral spinal cord] (see Table A1) of the central nervous system [CNS]. Ancillary tests should be reasonably applied, as clinically indicated, to exclude other disease processes. These should include electrodiagnostic, neurophysiological, neuroimaging and clinical laboratory studies. Clinical evidence of LMN and UMN degeneration is required for the diagnosis of ALS. The clinical diagnosis of ALS, without pathological confirmation, may be categorized into various levels of certainty by clinical assessment alone depending on the presence of UMN and LMN signs together in the same topographical anatomic region in either the brainstem [bulbar cranial motor neurons], cervical, thoracic, or lumbosacral spinal cord [anterior horn motor neurons]. The terms Clinical Definite ALS and Clinically Probable ALS are used to describe these categories of clinical diagnostic certainty on clinical criteria alone:

A. Clinically Definite ALS is defined on clinical evidence alone by the presence of UMN, as well as LMN signs, in three regions.

B. Clinically Probable ALS is defined on clinical evidence alone by UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.

C. Clinically Probable ALS - Laboratory-supported is defined when clinical signs of UMN and LMN dysfunction are in only one region, or when UMN signs alone are present in one region, and LMN signs defined by EMG criteria are present in at least two limbs, with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.

D. Clinically Possible ALS is defined when clinical signs of UMN and LMN dysfunction are found together in only one region or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of Clinically Probable - Laboratory-supported ALS cannot be proven by evidence on clinical grounds in conjunction with electrodiagnostic, neurophysiologic, neuroimaging or clinical laboratory studies. Other diagnoses must have been excluded to accept a diagnosis of Clinically Possible ALS.

**Table A1**

| | Brainstem | Cervical | Thoracic | Lumbosacral |
|---|---|---|---|---|
| Lower motor neuron signs<br>weakness,<br>atrophy,<br>fasciculations | jaw, face,<br>palate,<br>tongue, larynx | neck, arm, hand,<br>diaphragm | back,<br>abdomen | back, abdomen,<br>leg, foot |
| Upper motor neuron signs<br>pathologic spread of reflexes, clonus, etc. | clonic jaw<br>gag reflex exaggerated<br>snout reflex<br>pseudobulbar features<br>forced yawning<br>pathologic DTRs<br>spastic tone | clonic DTRs<br>Hoffman reflex<br>pathologic DTRs<br>spastic tone<br><br>preserved reflex in weak wasted limb | loss of superficial abdominal reflexes<br>pathologic DTRs<br>spastic tone | clonic DTRs - extensor plantar response<br>pathologic DTRs<br>spastic tone<br><br>preserved reflex in weak wasted limb |

## 13.2 APPENDIX II: ALS FUNCTIONAL RATING SCALE – REVISED (ALSFRS-R)

**ALSFRS-R**

**QUESTIONS:** **SCORE:**

1. Speech
4 = Normal speech processes
3 = Detectable speech disturbances
2 = Intelligible with repeating
1 = Speech combined with nonvocal communication
0 = Loss of useful speech

☐

2. Salivation
4 = Normal
3 = Slight but definite excess of saliva in mouth; may have nighttime drooling
2 = Moderately excessive saliva; may have minimal drooling
1 = Marked excess of saliva with some drooling
0 = Marked drooling; requires constant tissue or handkerchief

☐

3. Swallowing
4 = Normal eating habits
3 = Early eating problems – occasional choking
2 = Dietary consistency changes
1 = Needs supplemental tube feeding
0 = NPO (exclusively parenteral or enteral feeding)

4. Handwriting ☐
4 = Normal
3 = Slow or sloppy; all words are legible
2 = Not all words are legible
1 = No words are legible but can still grip a pen
0 = Unable to grip pen

5a. Cutting Food and Handling Utensils (patients without gastrostomy) ☐
4 = Normal
3 = Somewhat slow and clumsy, but no help needed
2 = Can cut most foods, although clumsy and slow; some help needed
1 = Food must be cut by someone, but can still feed slowly
0 = Needs to be fed

5b. Cutting Food and Handling Utensils (alternate scale for patients with gastrostomy) ☐
4 = Normal
3 = Clumsy, but able to perform all manipulations independently
2 = Some help needed with closures and fasteners
1 = Provides minimal assistance to caregivers
0 = Unable to perform any aspect of task

6. Dressing and Hygiene ☐
4 = Normal function
3 = Independent, can complete self-care with effort or decreased efficiency
2 = Intermittent assistance or substitute methods
1 = Needs attendant for self-care
0 = Total dependence

7. Turning in Bed and Adjusting Bed Clothes ☐
4 = Normal function
3 = Somewhat slow and clumsy, but no help needed
2 = Can turn alone, or adjust sheets, but with great difficulty
1 = Can initiate, but not turn or adjust sheets alone
0 = Helpless

☐
8. Walking
4 = Normal
3 = Early ambulation difficulties
2 = Walks with assistance
1 = Nonambulatory functional movement only
0 = No purposeful leg movement

9. Climbing Stairs ☐
4 = Normal
3 = Slow
2 = Mild unsteadiness or fatigue
1 = Needs assistance
0 = Cannot do

R-1. Dyspnea ☐
4 = None
3 = Occurs when walking
2 = Occurs with one or more of the following: eating, bathing, dressing
1 = Occurs at rest, difficulty breathing when either sitting or lying
0 = Significant difficulty, considering using mechanical respiratory support

R-2 Orthopnea ☐
4 = None
3 = Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2 = Needs extra pillow in order to sleep (more than two)
1 = Can only sleep sitting up
0 = Unable to sleep without mechanical assistance

R-3 Respiratory Insufficiency ☐
4 = None
3 = Intermittent use of BiPAP
2 = Continuous use of BiPAP during the night
1 = Continuous use of BiPAP during the night and day
0 = Invasive mechanical ventilation by intubation or tracheostomy

Evaluator's Initials: _____ _____ _____

## 13.3 APPENDIX III: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) BASELINE VERSION

| Information obtained from: http://www.cssrs.columbia.edu/***SUICIDAL IDEATION*** | |
|---|---|
| *Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.* | Lifetime: Time He/She Felt Most Suicidal |
| **1. Wish to be Dead**<br>Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.<br>***Have you wished you were dead or wished you could go to sleep and not wake up?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| **2. Non-Specific Active Suicidal Thoughts**<br>General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan.<br>***Have you actually had any thoughts of killing yourself?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| **3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act**<br>Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."*<br>***Have you been thinking about how you might do this?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| **4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan**<br>Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*<br>***Have you had these thoughts and had some intention of acting on them?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| **5. Active Suicidal Ideation with Specific Plan and Intent**<br>Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.<br>***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| ***INTENSITY OF IDEATION*** | |
| *The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal.*<br>____ _____<br>***Most Severe Ideation:***<br>***Type # (1-5) Description of Ideation*** | Most Severe |
| **Frequency**<br>***How many times have you had these thoughts?***<br>(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day | ____ |
| **Duration**<br>***When you have the thoughts, how long do they last?***<br>(1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day<br>(2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous<br>(3) 1-4 hours/a lot of time | ____ |

| | |
|---|---|
| **Controllability**<br>***Could/can you stop thinking about killing yourself or wanting to die if you want to?***<br>(1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty<br>(2) Can control thoughts with little difficulty (5) Unable to control thoughts<br>(3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts | ____ |
| **Deterrents**<br>***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?***<br>(1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you<br>(2) Deterrents probably stopped you (5) Deterrents definitely did not stop you<br>(3) Uncertain that deterrents stopped you (0) Does not apply | ____ |
| **Reasons for Ideation**<br>***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?***<br>(1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on<br>(2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling)<br>(3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on<br>and to end/stop the pain. living with the pain or how you were feeling)<br>(0) Does not apply | ____ |

| ***SUICIDAL BEHAVIOR***<br>*(Check all that apply, so long as these are separate events; must ask about all types)* | **Lifetime** |
|---|---|
| **Actual Attempt:**<br>A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt.<br>Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred.<br>***Have you made a suicide attempt?***<br>***Have you done anything to harm yourself?***<br>***Have you done anything dangerous where you could have died?***<br>***What did you do?***<br>***Did you_____ as a way to end your life?***<br>***Did you want to die (even a little) when you_____?***<br>***Were you trying to end your life when you _____?***<br>***Or did you think it was possible you could have died from_____?***<br>***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent)<br>If yes, describe:<br>**Has subject engaged in Non-Suicidal Self-Injurious Behavior?** | **Yes No**<br>☐ ☐<br>Total # of Attempts<br>_____<br>**Yes No**<br>☐ ☐ |

| | | | |
|---|---|---|---|
| **Interrupted Attempt:**<br>When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*.<br>Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so.<br>***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?***<br>If yes, describe: | | | **Yes No**<br>☐ ☐<br>Total # of interrupted<br>_____ |
| **Aborted Attempt:**<br>When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else.<br>***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?***<br>If yes, describe: | | | **Yes No**<br>☐ ☐<br>Total # of aborted<br>_____ |
| **Preparatory Acts or Behavior:**<br>Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note).<br>***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?***<br>If yes, describe: | | | **Yes No**<br>☐ ☐ |
| **Suicidal Behavior:**<br>Suicidal behavior was present during the assessment period? | | | **Yes No**<br>☐ ☐ |
| ***Answer for Actual Attempts Only*** | Most Recent Attempt Date: | Most Lethal Attempt Date: | Initial/First Attempt Date: |
| **Actual Lethality/Medical Damage:**<br>0. No physical damage or very minor physical damage (e.g., surface scratches).<br>1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains).<br>2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel).<br>3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures).<br>4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area).<br>5. Death | *Enter Code*<br>_____ | *Enter Code*<br>_____ | *Enter Code*<br>_____ |
| **Potential Lethality: Only Answer if Actual Lethality=0**<br>Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over).<br>0 = Behavior not likely to result in injury<br>1 = Behavior likely to result in injury but not likely to cause death<br>2 = Behavior likely to result in death despite available medical care | *Enter Code*<br>_____ | *Enter Code*<br>_____ | *Enter Code*<br>_____ |

## 13.4 APPENDIX IV: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) SINCE LAST VISIT VERSION

Information obtained from: http://www.cssrs.columbia.edu/

| ***SUICIDAL IDEATION*** | |
|---|---|
| *Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.* | Since Last Visit |
| **1. Wish to be Dead**<br>Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.<br>***Have you wished you were dead or wished you could go to sleep and not wake up?***<br>If yes, describe: | **Yes No**<br>□ □ |
| **2. Non-Specific Active Suicidal Thoughts**<br>General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period.<br>***Have you actually had any thoughts of killing yourself?***<br>If yes, describe: | **Yes No**<br>□ □ |
| **3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act**<br>Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."*<br>***Have you been thinking about how you might do this?***<br>If yes, describe: | **Yes No**<br>□ □ |
| **4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan**<br>Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*<br>***Have you had these thoughts and had some intention of acting on them?***<br>If yes, describe: | **Yes No**<br>□ □ |
| **5. Active Suicidal Ideation with Specific Plan and Intent**<br>Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.<br>***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?***<br>If yes, describe: | **Yes No**<br>□ □ |
| ***INTENSITY OF IDEATION*** | |
| *The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe).*<br>***Most Severe Ideation: _____ _____***<br>***Type # (1-5) Description of Ideation*** | Most Severe |
| **Frequency**<br>***How many times have you had these thoughts?***<br>(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day | ____ |
| **Duration**<br>***When you have the thoughts, how long do they last?***<br>(1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day<br>(2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous<br>(3) 1-4 hours/a lot of time | ____ |

| | |
|---|---|
| **Controllability**<br>***Could/can you stop thinking about killing yourself or wanting to die if you want to?***<br>(1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty<br>(2) Can control thoughts with little difficulty (5) Unable to control thoughts<br>(3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts | ____ |
| **Deterrents**<br>***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?***<br>(1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you<br>(2) Deterrents probably stopped you (5) Deterrents definitely did not stop you<br>(3) Uncertain that deterrents stopped you (0) Does not apply | ____ |
| **Reasons for Ideation**<br>***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?***<br>(1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on<br>(2) Mostly to get attention, revenge or a reaction from others living with the pain or how you were feeling)<br>(3) Equally to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on<br>and to end/stop the pain living with the pain or how you were feeling)<br>(0) Does not apply | ____ |

| ***SUICIDAL BEHAVIOR***<br>*(Check all that apply, so long as these are separate events; must ask about all types)* | Since Last Visit |
|---|---|
| **Actual Attempt:**<br>A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt.<br>Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred.<br>***Have you made a suicide attempt?***<br>***Have you done anything to harm yourself?***<br>***Have you done anything dangerous where you could have died?***<br>***What did you do?***<br>***Did you_____ as a way to end your life?***<br>***Did you want to die (even a little) when you_____?***<br>***Were you trying to end your life when you _____?***<br>***Or did you think it was possible you could have died from_____?***<br>***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)? (***Self-Injurious Behavior without suicidal intent)<br>If yes, describe:<br>**Has subject engaged in Non-Suicidal Self-Injurious Behavior?** | **Yes No**<br>☐ ☐<br>Total # of Attempts<br>_____<br>**Yes No**<br>☐ ☐ |
| **Interrupted Attempt:**<br>When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).*<br>Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so.<br>***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?***<br>If yes, describe: | **Yes No**<br>☐ ☐<br>Total # of interrupted<br>_____ |

| | |
|---|---|
| **Aborted Attempt:**<br>When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else.<br>***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?***<br>If yes, describe: | **Yes No**<br>☐ ☐<br>Total # of aborted<br>_____ |
| **Preparatory Acts or Behavior:**<br>Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note).<br>***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?***<br>If yes, describe: | **Yes No**<br>☐ ☐ |
| **Suicidal Behavior:**<br>Suicidal behavior was present during the assessment period? | **Yes No**<br>☐ ☐ |
| **Suicide:** | **Yes No**<br>☐ ☐ |
| ***Answer for Actual Attempts Only*** | Most Lethal Attempt Date: |
| **Actual Lethality/Medical Damage:**<br>0. No physical damage or very minor physical damage (e.g., surface scratches).<br>1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains).<br>2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel).<br>3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures).<br>4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area).<br>5. Death | *Enter Code*<br>_____ |
| **Potential Lethality: Only Answer if Actual Lethality=0**<br>Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over).<br>0 = Behavior not likely to result in injury<br>1 = Behavior likely to result in injury but not likely to cause death<br>2 = Behavior likely to result in death despite available medical care | *Enter Code*<br>_____ |

## 13.6 APPENDIX V: SAFETY REPORTING FAX COVER SHEET

Genentech
*A Member of the Roche Group*

**SAFETY REPORTING FAX COVER SHEET**
Investigator Sponsored Trials

**SAE FAX No: (650) 225-4682**
**Alternate Fax No: (650) 225-4683**

| | |
|---|---|
| **Study Number (Genentech study number)** | |
| **Principal Investigator** | |
| **Site Name** | |
| **Reporter name** | |
| **Reporter Telephone #** | |
| **Reporter Fax #** | |

| | |
|---|---|
| **Initial Report Date (DD/MON/YYYY)** | ____ / _____ /_____ |
| **Follow-up Report Date (DD/MON/YYYY)** | ____ / _____ /_____ |

| | |
|---|---|
| **Subject Initials (Please enter a dash if the patient has no middle name)** | ____ - ____ -____ |

PLEASE PLACE MEDWATCH REPORT or IND SAFETY REPORT BEHIND THIS COVER SHEET

**Please contact Genentech Safety for any questions regarding SAE or IND Safety reporting at (888) 835-2555**

***Page 1 of ___***

# Cover Page – Statistical Analysis Plan

**Study Official Title:** A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS)

**NCT #:** NCT02469896

**Version Date of the Document:** May 30, 2017

# STATISTICAL ANALYSIS PLAN (SAP)

| | |
|---|---|
| **Title** | A Phase 2 Randomized, Placebo-Controlled Trial of Tocilizumab in Amyotrophic Lateral Sclerosis (ALS) |
| **Regulatory Sponsor** | Shafeeq Ladha, MD |
| **Current Protocol Version** | 8.0 |
| **Current Protocol Date** | 30 May 2017 |
| **Statistical Analysis Plan Version** | 1.0 |
| **Statistical Analysis Plan Date** | 01 Mar 2019 |

SAP APPROVAL SIGNATURES

3/11/2019

Shafeeq Ladha, MD
Principle Investigator and Sponsor

Date

Eric A. Macklin

Digitally signed by Eric A. Macklin
DN: cn=Eric A. Macklin, o=Massachusetts General Hospital, ou=MGH Biostatistics Center, email=emacklin@mgh.harvard.edu, c=US
Date: 2019.03.14 23:14:34 -04'00'

Eric A. Macklin, PhD
Study Biostatistician

Date

SAP REVISION HISTORY

| Version | Date | Description of Changes |
|---|---|---|
| 1.0 | 01Mar2019 | Initial version |

**Table of Contents**

# 1. Introduction

This statistical analysis plan (SAP) defines the outcome measures and analysis samples and specifies the planned analyses of data from Dr. Shafeeq Ladha's Tocilizumab trial. The SAP supplements the clinical protocol. Please refer to the clinical protocol for details on the rationale for the intervention, eligibility criteria, conduct of the trial, clinical assessments and the timing of their use in the trial, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. In case of discrepancies between the SAP and the clinical protocol concerning matters of data analysis, the SAP is authoritative. On all other matters, the clinical protocol is authoritative.

# 2. Study Design

## 2.1 Overview

This is a multicenter, double-blind, placebo-controlled, two-arm, parallel-group, phase 2 randomized controlled trial to examine the safety and tolerability of tocilizumab in sporadic ALS patients. Trial participation includes a screening visit, randomization to 8 mg/kg tocilizumab or placebo administered every 4 weeks by intravenous infusions, 8 weeks of follow-up on study drug, and 4 weeks of follow-up after last dose of study drug. A subset of participants receive pre- and post-treatment MR-PET scanning to measure glial activation. The trial is registered at Clinicaltrials.gov as study NCT02469896 (see https://clinicaltrials.gov/ct2/show/NCT02469896).

## 2.2 Study Objectives

The primary objective of the trial is to determine whether 8 mg/kg tocilizumab administered every 4 weeks by intravenous infusions for 8 weeks is safe and tolerable. Safety will be assessed by the occurrence of severe adverse events (SAEs), rates of adverse events (AEs) classified to MedDRA system organ class and preferred term, frequency of clinically significant abnormal laboratory tests, and changes in vital signs. Tolerability will be assessed by the proportion of participants who complete all three infusions and remain on study and free from any possibly or definitely drug-related and dose-limiting AEs to the week 16 visit.

Secondary objectives include assessing the ability of tocilizumab to reduce the expression of pro-inflammatory genes in peripheral blood mononuclear cells (PBMCs), to reduce concentrations of pro-inflammatory cytokines in plasma and cerebrospinal fluid (CSF), to reduce concentration of soluble IL-6 receptor (sIL-6R) in the CSF, to reduce glial activation measured by [$^{11}$C]-PBR28 PET, and to slow progression of clinical measures of function and strength. The trial also aims to test whether the Asp358Ala polymorphism of the *IL6R* gene (SNP rs2228145) modifies effects of tocilizumab on expression of pro-inflammatory genes in PBMCs and cytokine and C-reactive protein (CRP) levels in the plasma and CSF.

## 2.3 Study Populations

Individuals eligible for trial participation as an ALS participant are men or women age 18 to 75 years who meet the El Escorial criteria of possible, laboratory-supported probable, probable, or definite criteria for a diagnosis of ALS, have either a 3-fold up-regulation of IL-6 gene expression or a 2-fold up-regulation of both IL-6 and either IL-8 or MMP1 in PBMCs relative to a pooled healthy control sample, have a slow vital capacity (SVC) at least 40% of that expected

based on age, sex, and height, and are free of conditions that contraindicate use of tocilizumab. Detailed inclusion and exclusion criteria are specified in the clinical protocol.

Participants will be recruited from approximately 5 clinical sites in the Northeast ALS (NEALS) Consortium located across the US.

## 2.4 Participant Flow and Site Approval

After a site receives local IRB approval and is activated, site staff may recruit participants. Individuals with sporadic ALS must provide written informed consent to initiate screening. After being determined eligible, approximately 24 ALS participants will be randomized to receive 8 mg/kg tocilizumab or placebo in a 2:1 ratio, stratified by clinical site. The first dose of study drug is administered by intravenous infusion at the baseline visit. If tolerant, participants receive two additional study drug infusions at 4-week intervals over 8 weeks. After baseline, participants complete in-clinic evaluations at weeks 4, 8, 12, and 16. Participants are contacted by telephone 24 to 48 hours after the lumbar punctures (LPs) at baseline and week 8 to assess for AEs. Participants who discontinue study drug should remain on study following the normal schedule of assessments. Patients withdrawing consent are asked to delay withdrawing consent until after they return for a final safety visit and after a final telephone call scheduled 28 days after their last dose of study drug.

Detailed descriptions of study procedures and timing are specified in the clinical protocol.

## 2.5 Treatment Allocation

Prior to the baseline visit, eligible ALS participants are randomly allocated to receive 8 mg/kg tocilizumab or placebo in a 2:1 ratio according to a permuted-block randomization schedule, stratified by site. The randomization schedule was prepared by computer program by the unblinded study statistician.

## 2.6 Treatment Administration

Study drug will be administered as a 100 mL intravenous infusion over 60 min. An unblinded research pharmacist at each site will prepare study drug infusion bags. Tocilizumab will be mixed in 0.9% sodium chloride solution at 8 mg/kg, up to a maximum 800 mg. Placebo participants will receive 0.9% sodium chloride solution.

## 2.7 Allocation Concealment

The randomization schedule is known only by the unblinded study statistician who generated the schedule and by unblinded research pharmacists at each site. Concealment of the true treatment allocation of specific participants is facilitated by the equivalent appearance of infusion bags containing tocilizumab (active arm) and those containing only saline (placebo arm). Plasma and CSF sIL-6R levels will be determined by a central lab not otherwise involved in the trial and sent only to the unblinded statistician prior to locking the trial database. Although no other biomarkers are expected to be unblinding, levels of CRP and pro-inflammatory cytokines in plasma and CSF will be handled similarly. Clinical members of the Steering Committee, site investigators and other site staff, clinical coordination and data management staff, the medical monitor, and all participants are blinded to participant treatment allocations. The Data and Safety Monitoring Board (DSMB) members are provided treatment-specific information in order to

monitor the trial but such information is masked by use of coded values to identify the treatment groups. The DSMB may request the true treatment identities.

## 2.8 Schedule of Assessments

| Activity | Screening Visit[1] | Baseline Visit | Week 4 Visit 1 | Week 8 Visit 2 | Week 12 Visit 3 | Week 16 EOS Visit |
|---|---|---|---|---|---|---|
| Written Informed Consent | X | | | | | |
| Inclusion/Exclusion Review | X | X | | | | |
| Medical History | X | | | | | |
| Demographics | X | | | | | |
| ALS Diagnosis History | X | | | | | |
| Physical Examination | X | | | | | X |
| Neurological Exam | X | | | | | X |
| Columbia Suicide Severity Rating Scale (C-SSRS) | | X | X | X | X | X |
| Vital Signs[2] including Height[3] and Weight | X | X | X | X | X | X |
| Lumbar Puncture (LP) | | X[4] | | X[4] | | |
| ALSFRS-R | | X | X | X | X | X |
| Slow Vital Capacity (SVC) | X | X | X | X | X | X |
| Hand Held Dynamometry (HHD) and Grip Strength | | X | | X | | X |
| Electrocardiogram (12-Lead ECG) | X | | | | | |
| PA and Lateral Chest X-Ray | X | | | | | |
| Quantiferon or T-SPOT TB Blood Test | X | | | | | |
| Safety Labs[5] | X | X | X | X | X | X |
| PBMC Collection | X | X[6] | X | X | X | X |
| Plasma Biomarker Collection | | X | X | X | X | X |
| Blood Collection for IL-6R genotype, Genentech sample | | X | | | | |
| Concomitant Medication Review | X | X | X | X | X | X |
| Adverse Event Review[7] | X | X | X | X | X | X |
| Randomization[8] | | X | | | | |
| Administer Study Drug | | X | X | X | | |
| Exit Questionnaire | | | | | | X |
| [$^{11}$C]-PBR28 PET | | X[9] | | X[10] | | |
| MRI Safety Questionnaire | X | X | | X | | |
| Upper Motor Neuron Burden Scale | X | X | | X | | |
| Blood draw for TSPO | X[11] | | | | | |

[1] Screening procedures must be completed within 28 days prior to Baseline Visit.
[2] Vital signs include systolic and diastolic pressure in mmHg, respiratory rate/minute, heart rate/minute and temperature.
[3] Height measured at Screening Visit only.
[4] Telephone call will be made 24-48 hours post lumbar puncture (LP) to assess for adverse events (AEs) related to the LP. Baseline LP occurs prior to the first infusion. Week 8 LP should occur 2.5 to 3.5 hours post-infusion.
[5] Safety labs include hematology (CBC with differential), chemistry panel, hepatitis B panel, urinalysis. For women of child-bearing potential, pregnancy will be tested by serum hCG at the Screening Visit and by urine pregnancy test at each subsequent visit.
[6] Collection at Baseline to occur prior to infusion
[7] Adverse events that occur after signing the informed consent form will be recorded.
[8] Randomization should occur immediately prior to the Baseline Visit.

[9] Pre-dose [$^{11}$C]-PBR28 PET will be performed one time only at the Baseline visits. (-2 Weeks)
[10] Post-dose [$^{11}$C]-PBR28 PET will be performed one time only at the Week 8 visit (- 2 Weeks).
[11] Blood draw for TSPO affinity testing to occur at the Screening visit, if not previously determined.

## 3. General Statistical Considerations

### 3.1 Statistical Software

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

### 3.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, safety outcomes, tolerability, and efficacy outcomes. Summary statistics for continuous variables will include the number of subjects, the mean, median, standard deviation, and range. For categorical data, summaries will include counts 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 be reported to two digits when greater than or equal to 0.095, to three digits when greater than or equal to 0.00095 and less than 0.095, and as <0.001 for all smaller values.

## 4. Analysis Samples

The following analysis samples will be used for testing safety, tolerability, and efficacy endpoints:

- Safety and Tolerability (ST) Sample: Participants who are eligible, randomized, and initiate at least the first infusion of study drug, classified according to the actual treatment received. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study.
- Efficacy As-treated (AT) Sample: Participants who are eligible, randomized, and initiate at least the first infusion of study drug, classified according to the actual treatment received. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study.
- Efficacy Modified Intent-to-treat (mITT) Sample: Participants who are eligible, randomized, and initiate at least the first infusion of study drug, classified according to their randomized treatment assignment. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study.

## 5. Study Endpoints

### 5.1 Safety Endpoints

The following safety endpoints will be evaluated:

- Time to mortality

- Time to first treatment-emergent SAE
- Proportion of participants experiencing and number of unique events of each type of treatment-emergent adverse event (TEAE) and serious TEAE classified by MedDRA system organ class and preferred term
- Proportion of participants experiencing and number of unique events of TEAEs classified by seriousness, severity, relatedness to study drug, action taken with study drug, and outcome, summarized across all MedDRA terms
- Proportion of participants experiencing and number of unique events of each type of treatment-emergent adverse event (TEAE) and serious TEAE classified by MedDRA system organ class and preferred term
- Instances of treatment-emergent laboratory abnormalities judged to be clinically significant by assay
- Mean change from baseline and percent change from baseline in weight, systolic blood pressure, and diastolic blood pressure
- Proportion of participants reporting any treatment-emergent suicidal ideation
- Maximum post-baseline suicidal ideation planning, frequency, duration, controllability, deterrents, and reasons
- Proportion of participants reporting any post-baseline suicidal behavior, actual attempts, non-suicidal self-injurious behavior, interrupted attempts, aborted attempts, preparatory acts or behaviors, or completed suicide
- Maximum post-baseline suicidal behavior lethality and potential lethality

Reported proportions will use as their denominator all participants in the ST sample.

## 5.2 Tolerability Endpoint

Participants will be judged tolerant of study drug if they complete all three infusions and remain on study and free from any possibly or definitely drug-related and dose-limiting AEs to the week 16 visit. Tolerability will be summarized as the proportion of participants in a treatment group who are tolerant of study drug. Reported proportions will use as their denominator all participants in the ST sample.

## 5.3 Efficacy Endpoints

Secondary efficacy endpoints include measures of target engagement and measures of clinical progression. Assessments of target engagement will focus on changes from baseline to the week 12 visit, the end of the study drug exposure interval. Assessments of possible effects on clinical progression will focus on mean rates of change over the 16-week follow-up interval.

Target engagement measures:

- proportion of participants who experience a 2-fold or greater decline between baseline and the average of all follow-up assessments in relative expression of at least 2 of the following 3 pro-inflammatory genes measured in PBMCs: IL-6, IL-8, and MMP1 (primary target engagement measure)

- relative expression levels of the following pro-inflammatory genes in PBMCs by Applied Biosystems quantitative polymerase chain reaction (qPCR): IL-6, IL-8, and MMP1
- concentrations of CRP in plasma and CSF by R&D Systems enzyme-linked immunosorbent assay (ELISA)
- concentrations of the following pro-inflammatory cytokines in plasma and CSF by Meso Scale Discovery (MSD) V-PLEX multiplexed sandwich immunoassay: IL-1β, IL-6, IL-8, IL-17A/F, and TNF-α
- concentrations of the following analytes in plasma and CSF by R&D Systems sandwich ELISA: sIL-6R, IL-6, and gp130
- brain glial activation measured by [$^{11}$C]-PBR28 PET

Clinical progression measures:

- ALS Functional Rating Scale-Revised (ALSFRS-R) total score
- Slow vital capacity (SVC) as percent of predicted normal volume
- Handheld dynamometry (HHD) of the following maneuvers: left and right shoulder flexion, left and right elbow flexion and extension, left and right wrist extension, left and right first dorsal interosseous, left and right hip flexion, left and right knee flexion and extension, and left and right ankle dorsiflexion
- Grip strength of left and right hands

### 5.4 Effect Modifiers

*IL6R* genotype for the Asp358Ala polymorphism (SNP rs2228145, A > C) will be investigated as a potential modifier of tocilizumab target engagement. *IL6R* genotype will be analyzed using an additive model for penetrance, with participants carrying more copies of the C allele expected to have higher sIL-6R levels and greater sensitivity to treatment with tocilizumab.

## 6. Measurement Definitions

### 6.1 Target Engagement Measures

#### 6.1.1 Expression of pro-inflammatory genes in PBMCs

PBMCs will be separated from whole blood using BD Vacutainer Cell Preparation Tubes (CPT Sodium Citrate tubes, catalog #362761). Sites will ship CPT tubes on ice to Dr. Robert Bowser's laboratory at Barrow Neurological Institute (BNI) for analysis on the day collected. PBMCs be isolated and frozen -80°C. RNA will be extracted from isolated PBMCs using a Qiagen RNeasy extraction kit (catalog #74106) using the manufacturer's protocol.

Expression levels of RNA for three pro-inflammatory genes (IL-6, IL-8, and MMP1) and one reference gene (GAPDH) will be estimated by quantitative real-time polymerase chain reaction (qPCR). The analysis will use an Applied Biosystems Quantstudio 6 qPCR system, Thermo Fisher TaqMan Gene Expression Assay system reverse transcription reagent and amplification solution (catalog #4331182) and TaqMan Fast Advanced Master Mix (catalog # 4444556). The primer and probe set used for each gene are as follows (Thermo Fisher catalog #4453320):

| Gene | Primer and Probe Set |
|---|---|
| IL-6 | Assay ID Hs00985639_m1 |
| IL-8 | Assay ID Hs00174103_m1 |
| MMP1 | Assay ID Hs00899658_m1 |
| GAPDH | Assay ID Hs99999905_m1 |

Each run of study samples will include RNA from pooled PBMCs from healthy controls for reference. Each study sample and the pooled control sample will be run in triplicate on each plate. A No Template control reaction will also be included in each run where nuclease-free water replaces the cDNA template. Complementary DNA (cDNA) templates will be synthesized for each sample from 0.5 μg of total RNA using a Thermo Fisher High-Capacity RNA-to-cDNA Kit (catalog #4387406).

For each qPCR run, the reaction set up and volume for each well of a 96-well plate will include:

| Component | Volume |
|---|---|
| 2X TaqMan Fast Advanced Master Mix | 10 μL |
| 20X TaqMan Gene Expression Assay | 1 μL |
| Nuclease-free water | 7 μL |
| cDNA template from each sample | 2 μL |

The TaqMan Fast qPCR protocol specifications will be:

| qPCR Step | Temp | Time |
|---|---|---|
| Incubation with uracil-N-glycosylase | 50°C | 120 s |
| Polymerase activation | 95°C | 20 s |
| PCR denaturation | 95°C | 1 s |
| PCR annealing and extension | 60°C | 20 s |

A quantification cycle ($C_q$) value will be obtained from each well to determine relative expression using standard ΔΔ Ct, log2-fold change calculations. Specifically, replicate $C_q$ values will be averaged for each gene, and the difference of each pro-inflammatory gene mean $C_q$ from the reference gene mean $C_q$ will be calculated separately for study samples and the pooled control sample ($\Delta C_q$). The negative difference of differences ($-\Delta\Delta C_q$) calculated as the pooled control $\Delta C_q$ minus the sample $\Delta C_q$, the log-base 2 of the fold-change between study and control samples, will be the measure of gene expression used for analysis. Higher values indicate greater relative expression of pro-inflammatory genes.

### 6.1.2 Concentration of CRP by R&D Systems ELISA

Plasma and CSF samples will be frozen and stored at sites at -70°C or colder prior to being shipped to Dr. Robert Bowser's laboratory at BNI for analysis. At the end of the study, all samples will be assayed as a single batch. Measurement of CRP will be performed using the R&D Systems Human C-Reactive Protein DuoSet ELISA kit (catalog #DY1707) following the

manufacturer's instructions. Briefly, plasma will be diluted 1:200 in Tris-buffered saline (TBS). CSF will be diluted 1:100 in TBS. Diluted samples will be incubated in the 96-well plate previously coated with 2.0 µg/mL capture antibody for 1.5 hrs. After washes, 100 µL of 90 ng/mL detection antibody will be added per well for 1.5 hrs, wells washed, streptavidin-HRP added for 20 min, and tetramethylbenzidine reaction product generated for 15 min. Standard curves will be generated using purified CRP protein in the following dilution series:

| Specimen | Standard concentration sequence (ng/mL) |
|---|---|
| Plasma | 25, 8.33, 2.78, 0.926, 0.309, 0.103, 0.034,0 |
| CSF | 1.0, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0 |

The geometric mean of replicate concentration estimates will be used for analysis. Concentrations of CRP in plasma and CSF will be reported as µg/mL and ng/mL, respectively.

### 6.1.3 Concentration of pro-inflammatory cytokines by MSD V-PLEX

Plasma and CSF samples will be frozen and stored at sites at -70°C or colder prior to being shipped to Dr. Robert Bowser's laboratory at BNI for analysis. At the end of the study, all samples will be assayed as a single batch. The concentration of the following cytokines will be assayed: IL-1β, IL-6, IL-8, IL-17A/F, and TNF-α. Measurements for IL-1β, IL-6, IL-8, and TNF-α will be performed using an MSD V-PLEX Proinflammatory kit (catalog #K15053). Measurements for IL-17 will be performed using an MSD V-PLEX IL-17A/F kit (catalog # K151WN). Plasma samples will be diluted 1:2. CSF samples will be used undiluted. A total sample volume of 50 µl will be assayed per well. Samples will be analyzed in duplicate. Standard curves will be generated using the following concentrations for each pure protein:

| Cytokine | Standard concentration sequence (pg/mL) |
|---|---|
| IL-1β | 576, 144, 36, 9, 2.25, 0.563, 0.141, 0 |
| IL-6 | 764, 191, 47.8, 11.9, 2.98, 0.746, 0.187, 0 |
| IL-8 | 621, 155, 38.8, 9.7, 2.43, 0.606, 0.152, 0 |
| IL-17 | 5430, 1360, 339, 84.8, 21.2, 5.3, 1.33, 0 |
| TNF-α | 369, 92.3, 23.1, 5.77, 1.44, 0.36, 0.0901, 0 |

Lower limit of detection (LLOD) ranges and lower limits of quantitation (LLOQ) are the following:

| Cytokine | LLOD (pg/mL) | LLOQ (pg/mL) |
|---|---|---|
| IL-1β | 0.01-0.17 | 0.646 |
| IL-6 | 0.05-0.09 | 0.633 |
| IL-8 | 0.03-0.14 | 0.591 |
| IL-17 | 0.930 | 7.57 |
| TNF-α | 0.01-0.13 | 0.690 |

The geometric mean of replicate concentration estimates will be used for analysis. Concentrations will be reported as pg/mL.

### 6.1.4 Concentration of sIL-6R, IL-6, and gp130 by R&D Systems ELISA

Plasma and CSF samples will be frozen and stored at sites at -70°C or colder prior to being shipped to BNI, inventoried, and sent to Dr. Carolanne Milligan's laboratory at Wake Forest School of Medicine for analysis. At the end of the study, all samples will be assayed as a single batch. The concentrations of the following proteins will be assayed by R&D Systems sandwich ELISA: sIL-6R, IL-6, and gp130.

| Protein | Assay | Control(s) |
|---|---|---|
| sIL-6R | Catalog #DR600<br>lot #P185493 (first six plates)<br>lot #P191955 (last two plates) | Catalog #QC05<br>lot #1279937 (low control)<br>lot #1279938 (medium control)<br>lot #1279939 (high control) |
| IL-6 | Catalog #Q6000B<br>lot #P186912 | Catalog #QC193<br>lot #732-180404 |
| gp130 | Catalog #DGP00<br>lot #P161984 | Catalog #QC23<br>lot #P180985 |

All samples, controls, and standards will be run in triplicate following kit instructions. For sIL-6R, plasma samples will be diluted 1:100 or more as needed and CSF sample will be assayed undiluted. For IL-6, all plasma and CSF samples will be assayed undiluted. For gp130, plasma samples will be diluted 1:100 and CSF sample will be diluted 1:9. The following standard concentration series will be used for IL-6: 0, 0.75, 1.5, 3, 6, 12, and 30 pg/mL.

For sIL-6R and gp130, plates will be read five minutes after the stop solution is added on a BioTek Epoch plate reader (BioTek Instruments, Winooski, VT) set to 450 nm, with wavelength correction at 570 nm. For IL-6, relative light units (RLUs) will be determined using a Victor 3 plate reader (PerkinElmer Life Science, Boston, MA) with the luminometer set for a lag time of 1.0 sec and a read time for wells of 0.5 sec.

If the three replicates have a coefficient of variation (CV) greater than 15%, the most disparate value will be eliminated, and the average of the remaining two wells will be used. If the CV is still above 15% after removing any outlier, then the sample will be re-run.

Concentrations will be estimated from OD values fit to a four-parameter logistic curve for sIL-6R and gp130 assays and from RLU values fit to a quadratic curve for IL-6 assay using PRISM software (GraphPad Software, San Diego, CA). The mean of replicate concentration estimates will be used for analysis.

### 6.1.5 *IL6R* genotyping for Asp358Ala polymorphism

Blood for determining *IL6R* genotype will be frozen and stored at sites at -70°C or colder prior to being shipped to BNI, inventoried, and sent to Dr. Carolanne Milligan's laboratory at Wake Forest School of Medicine for analysis. At the end of the study, all samples will be assayed as a single batch.

DNA will be purified from whole blood using Qiagen chemistry on the Gentra AutoPure platform. Absorption ratio at 260/280 nm will be used to determine DNA concentration and purity. A 9 ng sample of DNA will be genotyped according to manufacturer's instructions using a commercially available and validated Applied Biosystems TaqMan SNP genotyping assay (catalog # 4351379) for the *IL6R* Asp358Ala polymorphism (SNP rs2228145 A>C, assay ID C 16170664_10, lot #P160407-000 C10). The genotype at that locus will be read using an allelic discrimination protocol on an Applied Biosystems qPCR (model 7300, Foster City, CA). Each gene expression plate will be run with three control samples that had been fully sequenced and are known to carry one of the three genotypes, and three no-template controls.

Each participant will be scored for the *IL6R* Asp358Ala polymorphism as the count of the minor C allele, with CC homozygous participants expected to be more sensitive to treatment with tocilizumab.

### 6.1.6 Brain glial activation measured by [$^{11}$C]-PBR28 PET

Glial activation will be estimated in a subset of participants by magnetic resonance positron emission tomography (MR-PET) using the [$^{11}$C]-PBR28 ligand. The subset excludes individuals homozygous for the T/T allele of the Ala147Thr TSPO polymorphism (rs6971) associated with low affinity for [$^{11}$C]-PBR28. Glial activation will be quantified as a mean standardized uptake value (SUV) using PET images acquired from 60 to 90 minutes post-injection of approximately 430 MBq [$^{11}$C]-PBR28. FreeSurfer v6.0 (https://surfer.nmr.mgh.harvard.edu) will be employed to circumscribe a region of interest (ROI) defined anatomically as the precentral gyrus and the anterior portion of the paracentral lobule, bilaterally. SUV of the ROI will be normalized to the SUV of the whole brain and expressed as a SUV ratio (SUVR) to control for inter-individual variability in the global [$^{11}$C]-PBR28 PET signal.

## 6.2 Clinical Progression Measures

### 6.2.1 ALSFRS-R

The ALSFRS-R (Cedarbaum et al. 1999) is a 12-item clinician-completed interview scale 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).

### 6.2.2 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 an EasyOne Plus spirometer (ndd Medical Technologies, Inc., Andover, MA). Assessments will be analyzed if at least two acceptable maneuvers are recorded. The maximum volume expired is converted to percent of predicted normal. Normal values are calculated based on sex, age, and height using equations published by Knudsen et al. (1983). Higher values indicate better pulmonary function.

### 6.2.3 Handheld dynamometry

Handheld dynamometry (HHD) will be used by trained technicians to estimate isometric strength using a MicroFET2 HHD (Hoggan Scientific, Salt Lake City, UT). Nine upper and lower extremity muscles or muscle groups will be examined: shoulder flexion, elbow flexion, elbow extension, wrist extension, first dorsal interosseous contraction, hip flexion, knee flexion, knee

extension, and ankle dorsiflexion. Each muscle or muscle group will be measured twice or three times bilaterally. The average of the two highest measurements will be analyzed.

Two measures will be derived from HHD data: (1) upper and lower extremity megascores at each visit, and (2) time from baseline to first loss of measurable strength in any of the 18 muscles or muscle groups.

To calculate megascores, the mean and standard deviation of each muscle or muscle group, without regard to laterality, will be calculated from the baseline assessment of all participants in the AT sample. Maneuvers that cannot be completed by a participant due to weakness will be scored as zero kg. Strength estimates of each bilateral muscle or muscle group will be converted to Z scores by subtracting the relevant mean and dividing by the relevant standard deviation. Z scores for all upper extremity measurements (shoulder flexion, elbow flexion, elbow extension, wrist extension, and first dorsal interosseous contraction) and all lower extremity measurements (hip flexion, knee flexion, knee extension, and ankle dorsiflexion) will be averaged to yield upper and lower extremity megascores. Larger values indicate greater strength.

### 6.2.4 Grip strength

Grip strength of the left and right hand will be measured using a study-approved Jamar hand dynamometer (Performance Health, Warrenville, IL or Lafayette Instrument Company, Lafayette, IN). At each assessment, two trials will be completed with each hand. The average isometric strength recording of the stronger hand, measured in kg of force, will be analyzed.

## 7. Power Calculations

With 16 participants randomized to tocilizumab and followed for safety outcomes, the study will have 80% power to detect adverse events expected to occur in at least 10% of patients. Although under-powered for comparing rates of rare events, the study will have 80% power to detect treatment differences in events expected to occur, for example, in 50% of placebo participants if tocilizumab increases the hazard 4.2-fold.

The study will have 80% power to declare tocilizumab treatment tolerable based on non-inferiority to 50% tolerance with a one-sided 95% exact confidence bound if the true tolerance rate is at least 81%

The study will have 80% power to detect a significant treatment-dependent difference in the proportion of participants who experience a 2-fold or greater decline between baseline and the average of all follow-up assessments in at least 2 of the 3 pro-inflammatory genes if no more than 5% of placebo participants experience such a change and at least 30% of tocilizumab participants do. Assuming that at least 80% of participants are truly in a high inflammatory state given our enrollment criteria, this condition would correspond to roughly 6% vs. 38% response among susceptible patients randomized to placebo or tocilizumab, respectively. We will have greater power to detect quantitative changes in expression of pro-inflammatory genes. The study will have 80% power to detect a significant difference in expression equal to an effect size of 1.50 based on two-tailed tests at alpha = 0.017, applying a Bonferroni correction for multiple comparisons over 3 inflammatory genes.

If we conservatively estimate power for detecting treatment-dependent differences in 16-wk change in ALSFRS-R, SVC, and HHD megascores using a simple two-group t-test, the study will have 80% power for effect sizes of 0.76. With a standard deviation for ALSFRS-R rate of

change equal to 1.58 units/month based on the follow-up schedule of this trial and variance component estimates from the Ceftriaxone trial, the study would have 80% power if tocilizumab reduces decline in ALSFRS-R by 2.0 units/month based on a two-tailed at alpha = 0.05.

# 8. Participant Characterization

## 8.1 Baseline Characteristics

Each analysis sample will be summarized overall and by treatment group for the following characteristics: randomization site; age, sex, race, ethnicity, El Escorial diagnosis, years since ALS symptom onset, delay between symptom onset and ALS diagnosis, site of ALS symptom onset, use of riluzole at baseline, and baseline levels of IL-6, IL-8, and MMP1 gene expression, CRP and cytokines in plasma and CSF by MSD V-PLEX, sIL-6R, IL-6, and gp130 in plasma and CSF by R&D System ELISA, ALSFRS-R total score, SVC, HHD upper and lower extremity megascores, and grip strength.

## 8.2 Participant Disposition

The number of participants who consented and were screened, randomized, completed scheduled follow up, and prematurely withdrew will be summarized overall and by treatment group. Reasons for screen failure and for withdrawal from study will be tabulated and quantified as a percentage of consented and randomized participants, respectively.

# 9. Statistical Analyses

## 9.1 Safety Measures

### 9.1.1 Mortality

If any mortality is observed, time to mortality will be estimated starting at date of treatment initiation by Kaplan-Meier product-limit estimates in the ST sample. Association with treatment will be tested by log-rank test. The hazard ratio for treatment with tocilizumab vs. placebo will be estimated by Cox regression with 90% confidence bounds.

### 9.1.2 Treatment-emergent Adverse Events

Time to first treatment-emergent SAE will be estimated starting at date of treatment initiation by Kaplan-Meier product-limit estimates in the ST sample. Association with treatment will be tested by log-rank test. The hazard ratio for treatment with tocilizumab vs. placebo will be estimated by Cox regression with 90% confidence bounds.

The incidence of TEAEs and serious TEAEs will be summarized for the ST sample by the number of events of a given classification experienced by participants in each treatment group and by the number and proportion of participants experiencing such an event in each treatment group in the ST sample. TEAEs will be summarized in aggregate across all MedDRA terms and separately by MedDRA system organ class and preferred term. For each type of TEAE or serious TEAE, the association with tocilizumab will be tested by Poisson regression for counts of events per participant and by Fisher's exact test for the proportion of participants experiencing the given type of event. Association of tocilizumab with the overall TEAE rate will be tested by negative binomial regression.

TEAEs will also be summarized by tabulating treatment-specific frequency distributions for the following characteristics: (a) seriousness, (b) severity, (c) relatedness to study drug, (d) action taken with study drug, and (g) outcome. For each level of a given TEAE characteristic, summaries will include the number of events of a given classification and the number and proportion of participants for which that level of a characteristic was the worst they experienced (treating any unknown characteristic as not worst). For each characteristic, the association with tocilizumab will be tested by exact Cochrane-Armitage test for the proportion of participants experiencing a given level of the characteristic

### 9.1.3 Suicidality

The proportion of participants in the ST sample who report any post-baseline suicidal ideation or any post-baseline suicidal behavior will be summarized by treatment group. Suicidal behaviors will include: actual attempts, non-suicidal self-injurious behavior, interrupted attempts, aborted attempts, preparatory acts or behaviors, and completed suicide. The most severe ideation, maximal frequency, maximal duration, minimal controllability, minimal deterrents, maximal reasons for ideation, maximal actual lethality or medical damage, and maximal potential lethality will be summarized as means, standard deviations, medians, and ranges by treatment group.

### 9.1.4 Safety Labs

The absolute level and the absolute change from baseline for each safety laboratory assay will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group among participants in the ST sample. The proportion of participants with abnormal and clinically significant safety lab levels will be summarized by treatment group by visit and at any post-baseline visit. For each safety lab, the association of clinically significant safety lab abnormalities with tocilizumab will be tested by Fisher's exact test.

### 9.1.5 Anthropometrics and Vital Signs

The absolute level and the percent change from baseline for weight, systolic blood pressure, and diastolic blood pressure will be summarized as means, standard deviations, medians, and ranges at each visit by treatment group among participants in the ST sample. Associations with tocilizumab will be tested in a shared-baseline, linear mixed model with fixed terms for month since baseline and treatment x month interaction and random participant-specific intercepts and slopes with unstructured covariance.

## 9.2 Study Drug Tolerance

The number of completed infusions of study drug among participants in the ST sample will be summarized by treatment group. The proportion of participants classified as tolerant of study drug will be summarized for each treatment group. Associations with tocilizumab will be tested by Fisher's exact test. The relative risk of intolerance will be estimated with exact confidence bounds.

## 9.3 Target Engagement Measures

### 9.3.1 Primary Analysis

The primary analysis of target engagement measures will compare the proportion of participants in the AT sample who experience a 2-fold or greater decline between baseline and the average of all follow-up assessments in at least 2 of the 3 pro-inflammatory genes. Participants lost to

follow-up prior to any follow-up blood collection will be assumed to have not experienced a 2-fold or greater decline. Associations with tocilizumab will be tested by Fisher's exact test. The relative risk of intolerance will be estimated with exact confidence bounds. The following SAS code specifies the analysis, with trt a 0/1 indicator variable indicating treatment with 4/mg/kg tocilizumab and response a 0/1 indicator variable indicating a 2-fold or greater decline in at least 2 of the 3 pro-inflammatory genes:

```
proc freq data=xxx;
 tables trt * response / fisher;
 exact relrisk(method=score column=1);
```

A relative risk greater than 1 significant at two-tailed $p < 0.05$ would indicate successful target engagement.

### 9.3.2 Secondary Analysis

Secondary analysis of target engagement measures will use data from the AT sample in a shared-baseline, repeated-measures mixed model of log-transformed gene expression, CRP, cytokine, sIL-6R, or gp130 levels with fixed terms for visit (5 levels: baseline and weeks 4, 8, 12, and 16), treatment group (2 levels: placebo and 4 mg/kg tocilizumab) x post-baseline visit (4 levels) and random participant-specific visits with unstructured covariance. The interaction between fixed terms for treatment group and visit will be restricted to post-baseline visits by including a numeric indicator variable (0 pre-treatment, 1 post-treatment) in the interaction. Data from any early termination visits will be assigned to the next scheduled post-baseline visit. The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
 class id trt visit;
 model Value = visit post*trt*visit;
 random visit / subject=id type=un;
```

Treatment-dependent differences in the change from baseline to the average of all follow-up visits and to week 16 specifically will be estimated by linear contrasts and tested using a two-tailed Wald-test at alpha = 0.05. The following SAS code specifies the linear contrasts (with the sort order for treatment group being placebo first and 4 mg/kg tocilizumab second, and the sort order for visit being chronological):

```
 estimate "3|TCZ vs PLB|dWeek 16" post*trt*visit 0 0 0 0 -1  0 0 0 0 1 / cl;
 estimate "3|TCZ vs PLB|dWk 4-16" post*trt*visit 0 -1 -1 -1 -1  0 1 1 1 1 / cl divisor=4;
```

The estimates and their 95% confidence bounds will be back-transformed for reporting as a treatment-dependent ratio of post-baseline and week 16 concentration relative to baseline. Additional contrasts will be used to estimate treatment- and visit-specific concentrations with back-transformation for reporting on their original scale of measurement.

### 9.3.3 Subgroup Analyses

Differences in the effect of tocilizumab on target engagement measures dependent on *IL6R* genotype will be estimated by adding minor allele count, minor allele count x visit, and minor allele count x treatment x post-baseline visit interaction terms to the primary target engagement model.

## 9.4 Efficacy Measures

### 9.4.1 Primary Analysis

The primary analysis of clinical efficacy measures will use data from the mITT sample in a shared-baseline, random-slopes mixed model with fixed terms for month from treatment initiation and treatment group (2 levels: placebo and 4 mg/kg tocilizumab) x month from treatment initiation interaction and random participant-specific intercepts and slopes with unstructured covariance. Data from any early termination visits will be at their observed follow-up time. The following SAS code specifies the model and the linear contrast used to estimate the treatment-dependent difference in rate of change per month:

```
proc mixed data=xxx method=reml;
 class id trt;
 model Value = month trt*month;
 random intercept month / subject=id type=un;
 estimate "3|TCZ vs PLB|Slope/mn" intercept 0 month 0 trt*month -1 1 / cl;
```

### 9.4.2 Secondary Analysis

A secondary analysis of clinical efficacy measures will use the same shared-baseline, repeated-measures mixed model described for secondary analysis of target engagement measures with modification of the visit term to reflect the schedule of assessment for each measure (ALSFRS-R and SVC = 5 levels [baseline and weeks 4, 8, 12, and 16]; HHD and grip strength = 3 levels [baseline and weeks 8 and 16]). The same estimates of treatment- and visit-specific changes from baseline and treatment-dependent differences in change from baseline will be used.

## 9.5 Additional Considerations

### 9.5.1 Multiplicity Adjustments

Tests of treatment-dependent differences in time to mortality, time to first treatment-emergent SAE, overall TEAE rate, and distribution of TEAE characteristics will be declared significant based on two-tailed $p < 0.10$ without adjustment for multiple comparison. Tests of treatment-dependent differences in other safety outcomes will be declared significant based on two-tailed $p < 0.05$ without adjustment for multiple comparison. These criteria increase the probability of type 1 errors but also increase power for detecting true differences should any exist. Both unadjusted and step-down Bonferroni-adjusted p-values will be reported for secondary analyses of target engagement measures. Both unadjusted and step-down Bonferroni-adjusted p-values will be reported for clinical efficacy measures.

### 9.5.2 Missing Data

Baseline values for target engagement and clinical efficacy endpoints will be determined from the last non-missing data collected prior to the first dose of study medication. The planned mixed models yield estimates that are unbiased conditional on the observed scores under a missing at random assumption. Sensitivity analyses may be pursued to impute missing values or otherwise construct models for unobserved outcomes if more than 20% of participants are missing follow-up data for any reason.

## 10. References

Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999 Oct 31;169(1-2):13-21.

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