Statistical Analysis Plan – Part A (SAD) and Part B (MAD)

Final Version No.: 1.0

Study Title: A Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis and Confirmed Superoxide Dismutase 1 Mutation

Name of Study Treatment: BIIB067

Protocol No.: 233AS101/ NCT02623699

Study Phase: 1/2/3

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List of Abbreviations

ADM	abductor digit minimi
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSFRS-R	ALS Functional Rating Scale (revised)
[redacted]	
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
APB	abductor pollicis brevis
aPTT	Activated partial thromboplastin time
AST	aspartate aminotransferase
BB	bicep
BLQ	below limit quantitation
bpm	beats per minute
BP	bodily pain
BUN	Blood urea nitrogen
[redacted]	
CO2	Bicarbonate
CS	compound symmetry
CSF	Cerebral-spinal fluid
C-SSRS	Columbia suicide severity rating scale
DLT	dose limiting toxicity
ECG	Electrocardiogram
EDB	extensor digitorum
[redacted]	
EOS	end of study (visit)
[redacted]	
ET	early termination (visit)
FDIO	First dorsal interosseous
[redacted]	
GGT	gamma-glutamyl transferase
HHD	hand-held dynamometry
INR	International normalized ratio
ITT	intent-to-treat
LLOQ	lower limit of quantitation
LOCF	last observation carried over
LS	least square
LP	lumbar puncture
MAD	multiple ascending dose
[redacted]	
MedDRA	Medical Dictionary for Regulatory Activities

mg	milligram
mmHg	millimeters of mercury
MMRM	Mixed Model for Repeated Measures
MMSE	mini-mental state exam
[redacted]	
N	number of subjects or observations
NFH	neurofilament heavy chain
NFL	neurofilament light chain
PCS	Physical component score
PD	pharmacodynamics
[redacted]	
PK	pharmacokinetic(s)
p-NFH	phosphorylated axonal neurofilament heavy chain
PT	prothrombin time
QTc	interval between the start of the QRS complex and the end of the T wave, corrected for heart rate
QTcB	QTc interval using Bazett's formula
QTcF	QTc interval using Fridericia's formula
RBC	red blood cell (count)
TA	tibialis anterior
SAD	Single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
[redacted]	
SOC	System organ class
SOD	superoxide dismutase 1
SVC	slow vital capacity
ULN	Upper limit of normal
UN	unstructured
[redacted]	
vs.	versus
WBC	white blood cell (count)
WHO	World Health Organization
[redacted]	

1. Introduction

Study 233AS101 is a randomized, double-blind, placebo-controlled, 3-part study to examine the efficacy, safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of BIIB067, administered by intrathecal bolus injection to up to approximately 144 subjects with ALS and confirmed SOD1 mutation.

Parts A and B are single and multiple dose escalating parts, respectively, in which subjects will be randomized to receive BIIB067 or placebo in a 3:1 (active:placebo) ratio. In Part C, subjects will be randomized to receive BIIB067 100 mg or placebo in a 2:1 (active:placebo) ratio. This statistical analysis plan is prepared for clinical study report of Parts A and B; a separate statistical analysis plan will be prepared for Part C.

Part A will be a randomized, double-blind, placebo-controlled, single-ascending-dose (SAD) study of up to 4 dose levels (10, 20, 40 and 60 mg) of BIIB067 administered to up to 36 subjects with sporadic ALS or SOD1-ALS. Subjects within the 2 lower dose cohorts may re-enroll within the 2 higher dose cohorts, after a washout period. Each cohort will be randomized to receive BIIB067 or placebo in a 3:1 (active:placebo) ratio.

Part B will be a randomized, double-blind, placebo-controlled, multiple-ascending-dose (MAD) evaluation of up to 4 dose levels (20, 40, 60 and 100 mg) of BIIB067 administered up to 5 times to approximately 48 adults with SOD1-ALS. Each cohort will be randomized to receive BIIB067 or placebo in a 3:1 (active:placebo) ratio.

Unblinded interim analyses have previously been conducted on all subjects from all cohorts in Part A and Part B. At the time of the second interim analysis including all Part B cohorts, Cohort 8 subjects were ongoing in the study so the interim analysis included partial data from this cohort. These interim analyses were pre-specified in separate statistical analysis plans (Interim Statistical Analysis Plans V2.0 and V3.0).

1.1 Primary objective and endpoints

The primary objective of Parts A and B of this study is to evaluate the safety, tolerability, and PK of BIIB067 in adults with ALS and confirmed SOD1 mutation.

The primary endpoints are as follows:

- Incidence of adverse events and serious adverse events (SAEs)

- Incidence of abnormalities in clinical laboratory assessments, vital signs, physical and neurological examinations, and electrocardiograms (ECGs).
- PK measures, including plasma and cerebrospinal fluid (CSF) levels of BIIB067.

1.2 Secondary objectives and endpoints

The secondary objective is to evaluate the effects of BIIB067 on levels of SOD1 protein in the CSF.

- The secondary endpoint is the change from baseline in CSF levels of SOD1 protein.

1.3 Exploratory objective and endpoints

To evaluate the effect of BIIB067 [redacted] handheld dynamometry (HHD).

- The endpoints that relate to this objective are the changes from baseline in [redacted] HHD scores.

To evaluate the effect of BIIB067 on measures of clinical function. The endpoints that relate to this objective are changes from baseline in the following measures:

- ALS Functional Rating Scale - Revised (ALSFRS-R) scores
- Slow vital capacity (SVC)
- [redacted]

To explore possible relationships between [redacted] phosphorylated axonal neurofilament heavy chain (p-NFH), and neurofilament light chain (NFL).

- [redacted]

2. PART A: Single-Ascending-Dose (SAD) Evaluation

2.1 Study Design

Part A will be a randomized, double-blind, placebo-controlled, SAD study of up to 4 dose levels of BIIB067 administered to subjects with sporadic ALS or SOD1-ALS. The protocol was later amended to only allow subjects with SOD1-ALS. The dose levels will be evaluated sequentially. Final sample size for Part A of the study will be determined by the incidence of dose limiting toxicity (DLT). If no DLTs are encountered, a minimum of 20 subjects will be randomized. The maximum sample size for Part A is 36 subjects. The first 3 dose levels will be assessed in cohorts of 4 subjects each: 1 subject will be administered placebo, and 3 subjects will be administered BIIB067 at 10, 20, or 40 mg. The last dose level will be assessed in a cohort of 8 subjects: 2 subjects will be administered placebo, and 6 subjects will be administered BIIB067 at 60 mg. Up to 16 additional subjects may be enrolled into the selected dose levels (4 administered placebo and 12 administered BIIB067), based on the incidence of DLT.

If no DLTs are observed in the 2 lower dose cohorts, then subjects within these 2 lower dose cohorts may re-enroll, after a washout period of at least 5 times the t½ (~20-week; including the 8-week follow-up period), within the 2 higher dose cohorts. These subjects will repeat the screening assessments prior to re-enrolling, and will be rerandomized into either the BIIB067 or placebo group.

The duration of study participation for each subject enrolled in Part A will be up to approximately 15 weeks, which will include an up to 7-week screening period and an 8-week follow-up period. For subjects who choose to re-enroll in a higher dose cohort, the total duration will be approximately 35 weeks, which will include a washout period of 3 times the t½ (~12 weeks) after the 8-week follow-up visit (total washout of 5 times the t½, or ~20 weeks).

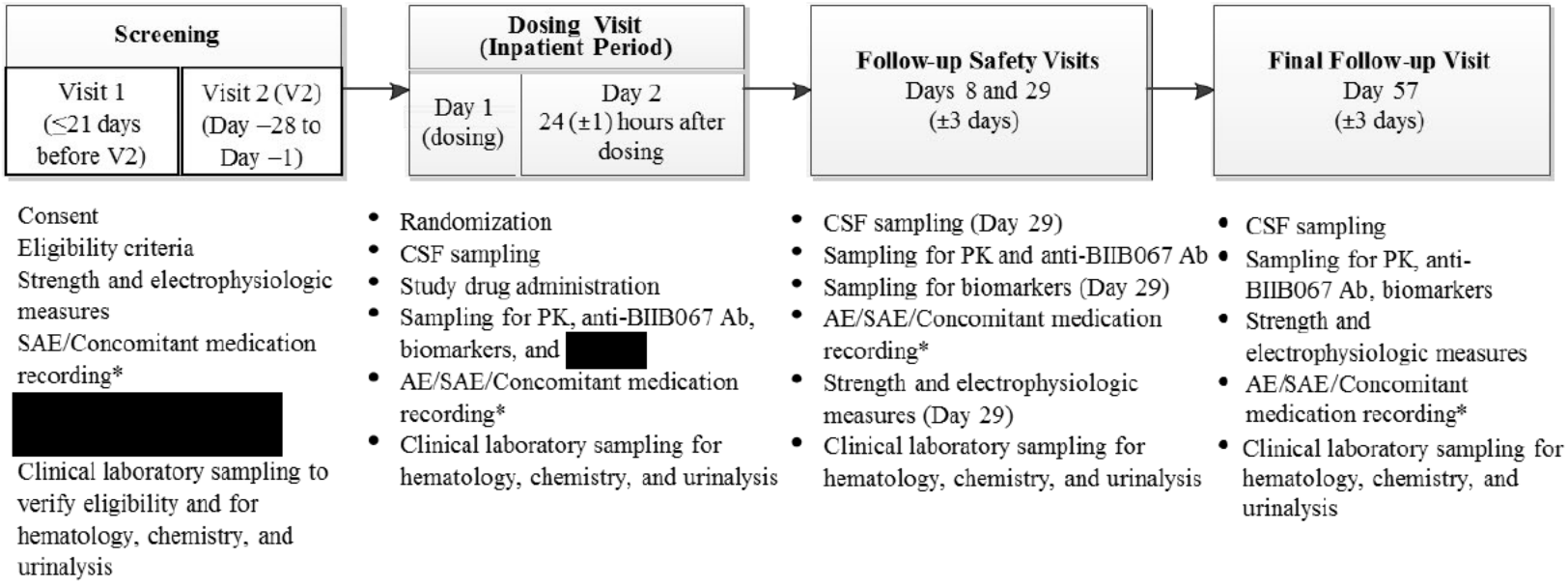
Subjects who re-enroll in a higher cohort will repeat Screening Visit 2 assessments, which can occur during the washout period.

The SAD portion of the study will be completed when any one of the following criteria is met.

- MTD or the highest planned dose has been given to up to 9 active subjects.
- A maximum of 36 subjects have been enrolled.

Figure 1 is the study schematic and Table 1 shows the schedule of assessments.

Figure 1: Study Design: Part A – Single Ascending Dose



Ab = antibody; AE = adverse event; CSF = cerebrospinal fluid; DNA = deoxyribonucleic acid; [redacted]
PK = pharmacokinetic; SAE = serious adverse event; V = visit

* SAE monitoring will be ongoing starting at the Screening Visit; AE and concomitant medication monitoring will be ongoing starting on Day 1.

Table 1: Schedule of Activities: Part A – Single Ascending Dose

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Follow-Up**	
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) hours Postdose**	**Days 8 & 29 (±3 days)**	**Day 57 (±3 days)/ Early Termination Visit**
			Predose	**Dosing/ Postdose**			
Informed Consent (main)	X	X[3]					
[redacted]							
Medical History		X	X				
Clinical Laboratory Samples to Verify Eligibility[4]		X					
FVC		X					
Admission to Inpatient Facility			X				
Physical Examination		X	X		X[5]	X[5]	X[5]
Weight		X	X				X
Height		X					
Neurological Examination		X	X	X[6]	X	X	X
Vital Signs (temperature, blood pressure, pulse rate, respiratory rate)		X	X	X[7]	X	X	X
12-Lead ECG[8]		X	X	X[7]	X	X	X
C-SSRS Questionnaire			X				X[9]
Urine Pregnancy Test[10]		X	X				X
FSH Test[11]		X					

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Follow-Up**	
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) hours Postdose**	**Days 8 & 29 (±3 days)**	**Day 57 (±3 days)/ Early Termination Visit**
			Predose	**Dosing/ Postdose**			
Randomization			X				
Study Drug Administration				X			
SVC			X			X	X
ALSFRS-R		X	X			X	X
[redacted]							
HHD		X				X[12]	X
Clinical Laboratory Samples for Hematology, Chemistry, and Urinalysis		X	X		X	X	X
CSF Samples[13]			X			X[14]	X
[redacted]							
Blood Samples for Plasma anti-BIIB067 Ab			X			X	X
Blood Samples for Biomarkers			X			X[12]	X
[redacted]							
Blood Samples for Plasma PK			X	X[16]	X	X	X

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Follow-Up**	
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) hours Postdose**	**Days 8 & 29 (±3 days)**	**Day 57 (±3 days)/ Early Termination Visit**
			Predose	**Dosing/ Postdose**			
Discharge from Inpatient Facility					X		
AE and Concomitant Therapy and Procedures Recording			----------X (ongoing) ----------				
SAE Recording	----------X (ongoing) ----------						

Ab = antibody; AE = adverse event; ALS = amyotrophic lateral sclerosis; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; CSF = cerebrospinal fluid; C-SSRS = Columbia Suicide Severity Rating Scale; DNA = deoxyribonucleic acid; ECG = electrocardiogram, [redacted]; FSH = follicle-stimulating hormone; FVC=forced vital capacity; HHD = handheld dynamometry; [redacted] PK = pharmacokinetic(s); [redacted]; SAE = serious adverse event; SOD1 = superoxide dismutase 1; SVC = slow vital capacity; V = visit

[1] Required only for subjects without a prior documentation of SOD1 mutation. Results must be available before performing Screening Visit 2 assessments.

[2] Screening assessments can be performed over ~2 days (need not be consecutive) to minimize subject burden.

[3] Not required if collected during Screening Visit 1.

[4] Including blood samples for human immunodeficiency virus, hepatitis C virus, and hepatitis B virus tests, and platelet and coagulation tests. Coagulation tests may be repeated at the local laboratory once if, in the opinion of the Investigator, values of the initial tests are only slightly out of range. Subjects with nonclinically significant and stable out-of-range values for coagulation tests may be eligible to enroll in the study at the discretion of the Investigator, and after a consultation with the Sponsor.

[5] Limited physical examination will be conducted at the Investigator's discretion.

[6] To be assessed at 3 and 6 hours postdose.

[7] To be assessed within an hour prior to drawing PK blood samples at 2 and 4 hours postdose.

[8] Triplicate 12-lead (paper) ECGs will be obtained after the subject has rested in a supine position for at least 10 minutes. The first ECG will be interpreted, and the last 2 will be checked for consistency and quality.

[9] Use the "Since Last Visit" version of C-SSRS.

[10] For women of childbearing potential only.

[11] To confirm postmenopausal status in postmenopausal female subjects.

[12] Day 29 only.

[13] Lumbar puncture (LP) will be performed to collect CSF samples for PK, pharmacodynamic, safety, and biomarker analysis. The results of the most recent (i.e., obtained at Screening or other visit during the study) coagulation tests and platelet count must be reviewed before each LP can be performed. Should the

results suggest, in the opinion of the Investigator, that an LP may be safely performed, then no further laboratory values need to be reviewed. However, should, in the opinion of the Investigator, repeat coagulation and platelet tests be clinically indicated, then these tests may be done locally to facilitate timely review. Results of any repeat tests must be available before the LP can be performed.

[14]To be collected at Day 29 only. Subjects will remain under observation in the clinic for approximately 1 hour after the LP procedure and will receive a safety follow-up telephone call ~24 hours after the procedure.

[redacted]

[16]To be collected at 1, 2, 4, and 6 hours postdose.

2.2 Statistical Methods: General Considerations

Descriptive summary statistics will be presented for all primary, secondary and exploratory endpoints collected. Unless otherwise specified, for continuous endpoints, the summary statistics will generally include: number of subjects with data, mean, standard deviation, median, first and third quartiles and range. For categorical endpoints, the summary statistics will generally include: number of subjects with data, and the percentage of those with data in each category.

Placebo patients across cohorts 1 to 4 in Part A will be combined for Part A summaries. All summaries will be presented by dose group unless otherwise specified. Some summaries may also present the overall population combining both placebo and active dose groups.

The statistical software, SAS® version 9.4 or above, will be used for all summaries and statistical analyses.

As a general rule, any visit/dose group combination that only has data for 1 patient will not be presented in group plots. However, all data will be summarized, listed and included in spaghetti plots, unless otherwise specified.

2.2.1 Analysis Populations

2.2.1.1 ITT Population

The ITT (Intention-to-Treat) population is defined as all randomized subjects who receive at least 1 or a part of 1 dose of study treatment (BIIB067 or placebo) in Part A. In case of any subjects who are mis-randomized, the ITT analysis will be based on treatment assignment at the time of randomization.

2.2.1.2 Safety Population

The safety population is defined as the ITT population of subjects in Part A. This will be the primary population for the analysis of safety endpoints. In the case of any subjects who are mis-randomized, the any analyses based on the safety population will be based on actual treatment received. If a population is not specified otherwise for an analysis of safety, the safety population should be used.

2.2.1.3 Pharmacokinetic (PK) Population

The PK population is defined as the subset of the ITT population of subjects with at least 1 post-dose PK measurement in Part A.

2.2.1.4 Pharmacodynamic (PD) Population

The PD population is defined as the subset of the ITT population of subjects with at least 1 post-dose PD measurement in Part A.

2.2.1.5 Clinical Function Set

The clinical function set is defined as the subset of the ITT population of subjects who have at least 1 post-dose clinical function measurement in Part A. Subjects will be analyzed in the dose group to which they are randomized. This will be the population for the analysis of clinical function endpoints.

2.2.1.6 Immunogenicity Population

The analysis population for immunogenicity is defined as all subjects in the safety population who have at least 1 post-dose sample evaluated for immunogenicity in Part A.

2.2.2 Data from Early Withdrawal or Unscheduled Visits

For subjects who are early terminated (ET), EOS visit is defined as the scheduled follow-up visit after ET visit.

Any assessments of clinical functions at unscheduled visits will not be used in the analysis but will be listed.

2.3 Study Subjects

2.3.1 Subject Accountability

The number (and percentage) of subjects randomized; dosed; completed the treatment; completed the study; discontinued treatment and the reasons for discontinuation; and withdrew from study early and the reasons for withdrawal will be summarized in a table.

A listing of subjects who discontinued treatment/withdrew from study and the associated reasons for discontinuation/withdrawal will be presented.

The number (and percentage) of subjects randomized by country and site, the number (and percentage) of subjects who completed the study by country and site, and number (and percentage) of subjects by analysis population will be summarized by dose group and for all subjects.

2.3.2 Demography and Baseline Disease Characteristics

All demographics and ALS disease history data will be summarized for the ITT population.

Demographic data, including age (years), age category (18-<35,35-<50, 50-<65, ≥65), gender, ethnicity, race, height, weight and body mass index (BMI) will be summarized by dose group and for all subjects. BMI will be calculated as weight (kg) / height^2 (m^2).

ALS disease history will also be summarized by dose group and for all subjects using descriptive statistics. These include form of ALS, mutant genes among familial ALS, SOD1 mutation subtype (variant), time since ALS symptom onset, site of onset, time since ALS diagnosis, pre-randomization ALSFRS-R slope and riluzole usage/duration. Additionally, a listing of individual subjects with known SOD1 mutation types will be provided.

Time since ALS symptom onset will be calculated in months as (date of first dose received – date of ALS symptom onset)/30.4375. Time since ALS diagnosis will be calculated in months as (date of first dose received – date of ALS diagnosis)/30.4375. For the purpose of these calculations, partial dates for ALS symptom onset or ALS diagnosis will be imputed as follows: missing day will be imputed with 15th and missing month/day will be imputed with January 15th.

The pre-randomization ALSFRS-R slope will be calculated using the ALSFRS-R score at baseline (Day 1) i.e. Baseline ALSFRS-R score – maximum possible score of 48/duration of symptom onset, where duration of symptom onset will be calculated as (date of baseline ALSFRS-R score – date of symptom onset)/30.4375. Partial dates for symptom onset will be handled as defined above.

Medical history will be classified using MedDRA version 22.0. A summary of medical history by system organ class and preferred term will also be provided.

2.3.3 Extent of Exposure

Since all subjects are planned to receive a single dose of placebo or BIIB067, no additional summary of extent of exposure will be provided. Separate listings will be provided showing what subjects were randomized to as well as study drug administration which will include lot numbers.

Time on study will be summarized descriptively by dose group. Time on study will be calculated as (end of study date – first dose date + 1).

2.3.4 Concomitant Medications and Non-Drug Treatments/Procedures

Concomitant medications will be coded using the World Health Organization (WHO) dictionary WHODRUG (March 2019) and concomitant non-drug treatments/procedures using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0.

Medications and non-drug treatments are considered concomitant if they are taken during the study. This includes medications/treatment/procedures that were started prior to the date and time of first dose of study drug if their use continued on or after the date and time of first dosing. If the start date is available but start time is missing and the medication/non-drug treatment start date is the same as the first dose date, the medication/non-drug treatment will be considered concomitant. Similarly, if the stop date is available but stop time is missing and the medication/non-drug treatment stop date is the same as the first dose date, the medication/non-drug treatment will also be considered concomitant. Medications/therapies with missing start or stop dates and times, will also be considered as concomitant in the following situations:

- If both the start and stop dates and times of a medication/treatment/procedure are missing;
- If the start date and time of a medication/treatment/procedure is missing and the stop date and time of the medication/therapy occurred on or after the date and time of first dose of study drug;
- If the start date and time of a medication/treatment/procedure therapy occurred prior to the date and time of first dose of study drug and the stop date and time of the medication/treatment/procedure is missing and the medication/treatment/procedure is listed as ongoing;

If the start date and time of a medication/treatment/procedure occurred prior to the date and time of first dose of study drug and the stop date and time of the medication/treatment/procedure is missing and the medication/therapy is not listed as ongoing, then the medication/treatment/procedure will not be considered as concomitant.

The number and percentage of subjects taking any concomitant medications will be summarized by dose group for the safety population. The number and percentage of subjects taking any concomitant non-drug treatments will also be summarized by dose group for the safety population.

2.3.5 Protocol Deviations

Protocol deviations identified during site monitoring will be captured in a protocol deviation log. All protocol deviations will be listed and a summary of major protocol deviations by dose group will be presented.

2.4 Safety Data

The safety population will be used for the analyses of the safety data. Safety data will be summarized using descriptive statistics by dose group.

2.4.1 Adverse Events

All AEs will be classified using MedDRA version 22.0. All AEs will be listed but only treatment emergent AEs will be summarized, where treatment emergence will be relative to the first dose of study drug. A treatment emergent AE/SAE is defined as any AE/SAE with an onset date and time that is on or after the first dose of study drug or any pre-existing condition that has worsened in severity after the first dose of study drug. In case of missing dates, any AE/SAE with both a missing onset date and resolution date, or any AE/SAE with a missing onset date and a resolution date which is after the first dose of study drug, will be considered treatment emergent. If the onset date is available but onset time is missing and the AE onset date is the same as the first dose date the AE will be considered treatment emergent. The incidence of treatment emergent AEs will be summarized by dose group as follows:

- by MedDRA preferred term
- by primary system organ class (SOC) and MedDRA preferred term
- by maximum toxicity grade, primary SOC and MedDRA preferred term
- AEs related to lumbar puncture by primary SOC and MedDRA preferred term

For the AE summary by primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term. For the summary of AEs by maximum toxicity grade, primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term and will only be counted under the maximum toxicity grade.

Listings of the following events will be produced. If the number of events is large, the incidence of these events may also be summarized in tables:

- AEs related to study drug
- AEs led to discontinuation of study drug
- AEs led to withdrawal from study
- AEs leading to study drug interruption

If a summary is provided for related AEs and if the relationship for an event is missing then this will be summarized as unknown. Listing of deaths will be provided if applicable.

SAEs will also be summarized by primary MedDRA preferred term.

2.4.2 Laboratory Data

Laboratory data will be evaluated to determine the incidence of abnormalities that emerge during the course of the study. Changes in laboratory evaluations will be presented relative to baseline, which is defined as the closest visit prior to the subject starting treatment.

The following clinical laboratory parameters are assessed in the protocol:

- Hematology panel: complete blood count with differential (hematocrit, hemoglobin, platelets, red blood cell count [RBC], white blood cell count [WBC], basophils, eosinophils, lymphocytes, monocytes, neutrophils)
- Blood chemistry panel: albumin, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase (ALT) (SGPT), aspartate aminotransferase (AST) (SGOT), gamma-glutamyl transferase (GGT), sodium, potassium, calcium, chloride, phosphate, blood urea nitrogen (BUN), creatinine, uric acid, bicarbonate (CO2), glucose, total protein.
- Urinalysis: blood, glucose, protein, and microscopic examination if abnormal
- CSF analysis: RBC, WBC, protein, glucose.

If multiple samples are collected at the same visit, the samples collected at the earliest date/time will be analyzed. For CSF laboratory parameters, tube 2 results will be analyzed if available.

Baseline value is defined as data collected which are prior to and/or on the date of the first dose, usually also the same day as the Day 1 visit. If there is more than one value on or before Day 1, then the last non-missing value prior to (including on) the date of first dose will be used as the baseline value.

Each hematology, blood chemistry, and CSF laboratory parameter will be flagged as “low” or “high” relative to the parameter’s normal range or as “unknown” if no result is available. For each urinalysis laboratory parameter, the number and percentage of subjects experiencing post-dosing shifts to ‘high’ (positive) will be summarized. For each hematology, blood chemistry, and CSF parameter, the number and percentage of subjects experiencing post-dosing shifts to ‘low’ or ‘high’ will be summarized. In each summary, the denominator for the percentage is the number of subjects at risk for the shift. The number at risk for the shift to low is the number of subjects whose baseline value was not low and who had at least one post-baseline value. The number at risk for the shift to high is the number of subjects whose baseline value was not high and who had at least one-post baseline value. Subjects will be counted only once for each parameter and each shift regardless of how many post-dosing assessments had that type of shift.

Summary statistics for actual values and change from baseline in laboratory values will be summarized by dose group and visit. Line plots for chemistry, hematology and CSF showing mean value for each dose group at each visit will also be presented.

Listings of all chemistry, hematology, CSF, urinalysis values and urine pregnancy test will be provided. Abnormal values and potentially clinically significant values will be flagged.

A listing of lumbar puncture and CSF sample collection data other than CSF laboratory results (i.e. CSF volume collected, needle and gauge information, caffeine/smoking/alcohol use prior to CSF collection) will also be provided.

2.4.3 Vital Signs

Summary statistics for actual values and change from baseline will be presented for each vital sign parameter (temperature, weight, pulse, respiration, systolic BP and diastolic BP) by dose group and visit. A listing of vital sign data will also be provided.

Vital signs (temperature, pulse, systolic BP and diastolic BP) will also be examined to determine the incidence of potentially clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with potentially clinically relevant abnormalities will be presented. The criteria for potentially clinically relevant post-dosing abnormalities are shown in Table 2.

Table 2: Criteria to determine potentially clinically relevant abnormalities in vital signs

Vital Sign	Criteria for Abnormalities
Temperature	>38°C or an increase from baseline of ≥1°C
Pulse	>120 beats per minute (bpm) or an increase from baseline of >20 bpm <50 bpm or a decrease from baseline of >20 bpm
Systolic Blood Pressure	>180 mmHg or an increase from baseline of >40 mmHg <90 mmHg or a decrease from baseline of >30 mmHg
Diastolic Blood Pressure	>105 mmHg or an increase from baseline of >30 mmHg <50 mmHg or a decrease from baseline of >20 mmHg

2.4.4 ECG

The ECG test includes heart rate, PR interval, QRS interval, QT interval, QTc interval using Bazett's formula, QTc interval using Fridericia's formula, and RR interval. Summary statistics for actual values and change from baseline in each ECG parameter will be presented by dose group and visit. A listing of ECG data will be provided.

The number and percentage of subjects with shifts from normal to each of the categorical values denoting an abnormal scan (abnormal not AE, abnormal AE) will be summarized by dose group.

QTc (interval using Fridericia's formula) will also be examined to determine the incidence of clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with clinically relevant abnormalities will be presented. The criteria for clinically relevant post-dosing abnormalities are:

- Maximum increase from baseline QTcF > 30 to 60 ms
- Maximum increase from baseline QTcF > 60 ms
- Maximum post-baseline QTcF > 480 to 500 ms
- Maximum post-baseline QTcF > 500 ms

2.4.5 Limited neurological examinations

The number and percentages of the status in each assessment of general neurological examination (cranial nerves, coordination/cerebellar function, sensation upper/lower extremities), El Escorial, and each assessment of other reflexes (jaw jerk, Hoffman sign right/left, plantar response right/left) will be summarized by dose group and visit. The reflexes neurological examination of upper and lower extremities will be analyzed as continuous variables (0 = absent, 1 = trace, 2 = normal, 3 = brisk, 4 = clonus). The descriptive statistics will be summarized by dose group and visit. Plots of mean values by visit will be also generated.

The motor neurological examination will be analyzed as continuous variable (0 = no contraction or can't position limb; 1 = flicker or trace contraction, no movement; 2 = movement only with gravity eliminated; 3 = movement against gravity; 4 = movement against gravity and resistance; 5 = normal strength). The Ashworth spasticity scale will also be analyzed as continuous variable (1 = normal; 2 = slight increase in tone; 3 = more marked increase in tone; 4 = considerable increase in tone; 5 = affected part rigid, immobile). Summary statistics will be presented by dose group and visit. Plots of mean values by visit will also be generated.

Listings will also be provided with complete details for the neurological examinations.

2.4.6 Columbia Suicide Severity Rating Scale (C-SSRS)

The details of derivation and imputation for C-SSRS is described in Appendix A. The following analyses on C-SSRS measurements will be conducted:

- Descriptive summary of subjects who answered "Yes" to any question 1-12 as well as subjects who had suicidal ideation or suicidal behavior at baseline and at any post-baseline visit. The denominator for baseline summary is the number of subjects who were dosed and had baseline assessment; the denominator for post-baseline summary is the number of subjects who were dosed and had at least one post-baseline assessment for each question.

- Descriptive summary of subjects who had treatment-emergent suicidal ideation, subjects who had new suicidal ideation as well as subjects who had worsening suicidal ideation. The denominator is the number of dosed subjects with both baseline and at least one post-baseline suicidal ideation assessment.

- Descriptive summary of subjects who had treatment-emergent suicidal behavior. The denominator is the number of subjects who answered "No" to all suicidal behavior questions at baseline and had at least one post-baseline suicidal behavior assessment.

Listing of subjects having treatment-emergent suicidal ideation will be provided. Subjects who had new suicidal ideation and subjects who had worsening suicidal ideation will be flagged. The listing will display both baseline and post-baseline Suicidal Ideation Scores for each subject. Listing of subjects having treatment-emergent suicidal behavior will also be provided.

2.5 Pharmacokinetic Data

The PK population will be used for the analyses of the PK data.

Values below limit of quantitation (BLQ) are set to half of the lower limit of quantitation (LLOQ, 1 ng/mL) at day 1 pre-dose rather than zero so that geometric means can be calculated. Values that are BLQ at all other visits will also be set to half of LLOQ (1 ng/mL) in calculations.

Both plasma and CSF BIIB067 concentrations will be listed and summary statistics will be presented for each visit. Summary statistics will also include both the arithmetic and geometric means with corresponding standard errors. Plots of arithmetic and geometric mean concentrations of BIIB067 versus time will be provided, including standard error bars. Spaghetti plots will be presented for each BIIB067 group to show individual subjects throughout all visits. These will include both pre-dose and post-dose visits. Both the geometric mean and the median for the dose group will also be plotted on the spaghetti plots.

The BIIB067 concentrations will be used to calculate the following parameters at Day 1 using non-compartmental methods by the PK Scientist: AUC[0-24h], Cmax, Tmax. Summary statistics will be provided for these PK parameters.

2.6 Pharmacodynamic Data

The PD population will be used for the analyses of the PD/biomarker data.

The following PD biomarkers will be summarized: SOD1 protein in CSF and the neurofilament levels in both the CSF and plasma. For SOD1 protein, the overall protein level will be analyzed. For neurofilament, the phosphorylated axonal neurofilament heavy chain (p-NFH) will be analyzed. Values that are BLQ will be set to half of LLOQ (15.6 ng/mL for SOD1 and 7.46 pg/mL for pNFH).

Descriptive statistics for the PD data at each visit, and ratio to baseline will be summarized by dose group. The summary statistics will include arithmetic mean and SD for the ratio to baseline as well as the geometric mean ratio and corresponding standard error. The geometric mean ratio will be obtained by back-transforming change from baseline on log transformed data.

Plots of arithmetic mean ratio to baseline will be presented as well as plots of geometric mean ratio to baseline with standard error bars. Plots of the geometric mean and standard error at each visit will also be presented.

Listings of individual data will also be provided.

Spaghetti plots of actual values and ratio to baseline will be presented for each treatment group to show individual subjects throughout all visits. Both the geometric mean and the median for the dose group will also be plotted on the spaghetti plots.

2.7 Immunogenicity Data

The immunogenicity population, as defined in Section 2.2.1, will be used for all analyses of immunogenicity data.

- For immunogenicity, the baseline value is defined as the latest immunogenicity data collected at any time prior to the first dose. If no immunogenicity data are collected prior to the first dose, the baseline value is missing and will be imputed as anti-drug antibody negative for immunogenicity analyses.

- Subjects with at least one confirmed post-treatment positive result will be considered positive for anti-drug antibodies if their baseline result is negative.

- Subjects where none of the post-treatment samples were positive for anti-drug antibodies will be considered negative regardless of their baseline result
- For subjects that are confirmed positive at baseline and have at least one post-treatment sample with a ≥ 2 fold increase in titer will be considered positive for anti-drug antibodies. Subjects that are positive at baseline, with subsequent post-treatment samples titers that are within 2 fold will be considered negative for anti-drug antibodies
- Numbers and percentage of subjects who develop antibodies will be determined and summarized by treatment groups and timepoint.

The same summary will be provided for subjects with positive baseline results and for subjects with negative or missing baseline results, separately.

2.8 Clinical Function Data

The clinical function set will be used for all analyses of clinical functions.

Unless stated otherwise, baseline data are defined as the data collected prior to the time and/or on the date of first dose, which is usually the same day as the Day1/Baseline visit. If there is more than one value on/before the date of the first dose, the non-missing value closest to and prior to the first dose will be used as the baseline value. For [redacted] HHD [redacted] data were collected at Screening and not Day 1 so baseline values for endpoints on these assessments will be taken from the Screening visit.

Descriptive statistics of clinical function endpoints will be presented for Part A based on observed data only; missing data at a visit level will not be imputed for Part A. When a placebo group is involved in an analysis, all subjects receiving placebo will be combined to form placebo control group. The derivation and imputation for each clinical function endpoint is described in Appendix B. Imputation will only be performed within an endpoint and visit for example if there are missing responses to some of the individual questions on a scale at a given visit, the endpoint may only be derived if there are a certain number of individual items/components with available data. Specific details are provided for each assessment/scale in Appendix B, as this differs across assessments/scales.

Any assessments of clinical functions at unscheduled visits will not be used in the analysis but will be listed.

The endpoints for clinical function data are given in Table 3.

For each endpoint listed, descriptive statistics of actual values and changes from baseline at each visit will be summarized by dose group based on observed data. Plots of mean values and mean

changes from baseline by visit will also be generated. Spaghetti plots of actual values and changes from baseline for ALSFRS-R total score, HHD overall megascore, and percent predicted SVC will be presented for each dose group to show individual subjects throughout all visits. The mutation subtype will also be indicated for each subject on the spaghetti plots. Listings of individual data on clinical functions will also be provided.

Table 3: Clinical function endpoints for Part A

Clinical Function	Endpoint
ALSFRS-R	- Total Score - Functional domain scores (bulbar function, fine motor skills, gross motor skills, respiratory function)
SVC	- Percent predicted SVC
[redacted]	
HHD	- HHD Megascore - Muscle strength for each of 16 muscles

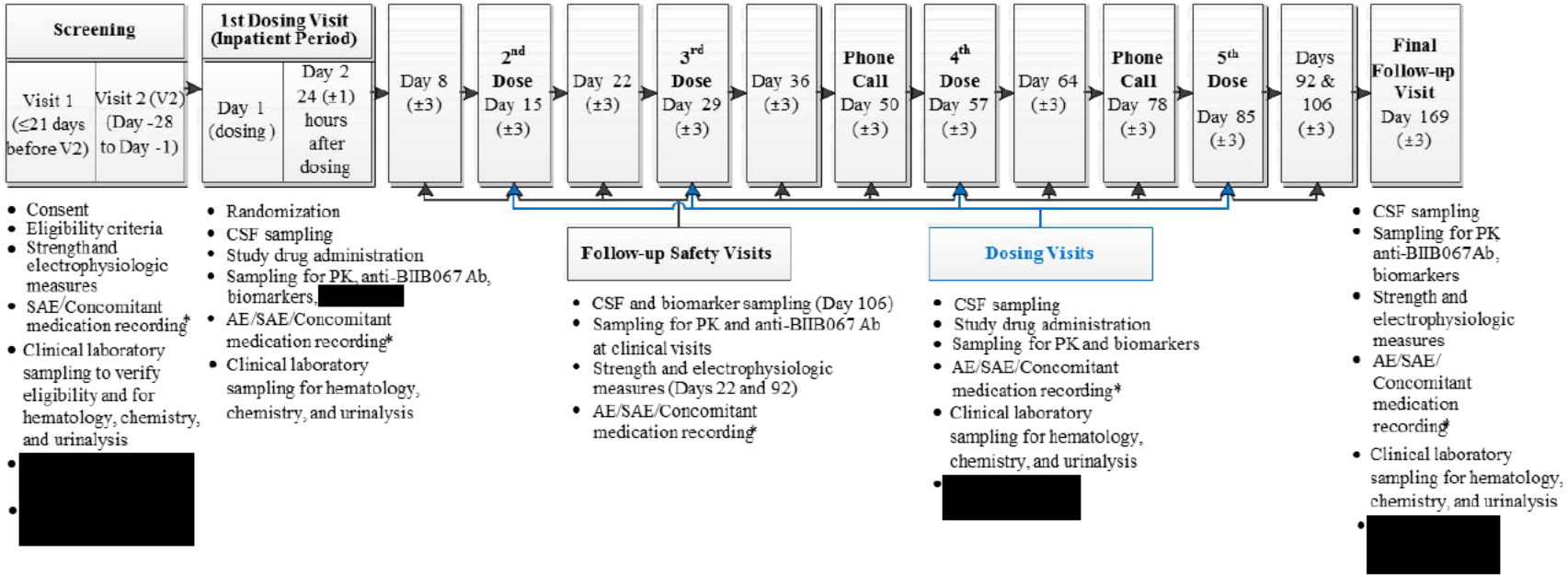
3. Part B: Multiple-Ascending-Dose (MAD) Evaluation

3.1 Study Design

Part B will be a randomized, double-blind, placebo-controlled, MAD evaluation of up to 4 dose levels of BIIB067 administered up to 5 times, over approximately 3 months, to up to approximately 48 subjects with SOD1-ALS. Each dose level will be assessed in cohorts of 12 subjects: 3 subjects will be administered placebo, and 9 subjects will be administered BIIB067 at 20, 40, 60 or 100 mg.

The duration of study participation for each subject enrolled in Part B will be up to approximately 31 weeks, which will include a screening period up to 7-weeks, a 12-week treatment period (consisting of 3 loading doses of BIIB067, administered approximately once every 2 weeks, followed by 2 maintenance doses of BIIB067, administered approximately once every 4 weeks), and a 12-week follow-up period. Figure 4 is the study schematic and Table 3 shows the schedule of assessments.

Figure 2: Study Design: Part B – Multiple Ascending Dose



Ab = antibody; AE = adverse event; CSF = cerebrospinal fluid; DNA = deoxyribonucleic acid;
PK = pharmacokinetic; SAE = serious adverse event; V = visit

* SAE monitoring will be ongoing starting at the Screening Visit; AE and concomitant medication monitoring will be ongoing starting on Day 1.

Table 4: Schedule of Activities: Part B – Multiple Ascending Dose

	Screening		**Dosing Inpatient Period**			**Dosing Days 15, 29, 57 & 85 (±3 days)**	**Follow-Up**		
			Day 1		**Day 2 24 (±1) Postdose**		**Days 8[3], 22, 36, 64, 92, and 106 (±3 days)**	**Days 50 & 78 via Telephone (±3 days)**	**Day 169 (±3 days) /Early Termination Visit**
Tests and Assessments	**V1[1] ≤21 days before V2**	**V2[2] Day -28 to Day -1**	**Predose**	**Dosing/ Postdose**					
Informed Consent (main)	X	X[4]							
[redacted]									
Medical History		X	X						
Clinical Laboratory Samples to Verify Eligibility[5]		X							
FVC		X							
Admission to Inpatient Facility			X						
Physical Examination		X	X		X[6]	X[6]	X[6]		X[6]
Weight		X	X						X
Height		X							
Limited Neurological Examination (including the MMSE)[7]		X	X	X[8]	X	X[9]	X		X

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Dosing Days 15, 29, 57 & 85 (±3 days)**	**Follow-Up**		
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) Postdose**		**Days 8[3], 22, 36, 64, 92, and 106 (±3 days)**	**Days 50 & 78 via Telephone (±3 days)**	**Day 169 (±3 days) /Early Termination Visit**
			Predose	**Dosing/ Postdose**					
Vital Signs (temperature, blood pressure, pulse rate, respiratory rate)		X	X	X[10]	X	X[11]	X		X
12-Lead ECG[12]		X	X	X[10]	X	X[11]	X		X
C-SSRS Questionnaire			X						X[13]
Urine Pregnancy Test[14]		X	X			X[15]			X
FSH Test[16]		X							
Randomization			X						
Study Drug Administration				X		X			
SVC			X			X[15]			X
ALSFRS-R		X	X			X	X[17]		X
[redacted]									

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Dosing Days 15, 29, 57 & 85 (±3 days)**	**Follow-Up**		
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) Postdose**		**Days 8[3], 22, 36, 64, 92, and 106 (±3 days)**	**Days 50 & 78 via Telephone (±3 days)**	**Day 169 (±3 days) /Early Termination Visit**
			Predose	**Dosing/ Postdose**					
[redacted]									
HHD		X					X[17]		X
Clinical Laboratory Samples for Hematology, Coagulation, Chemistry, and Urinalysis		X	X		X	X			X
CSF Samples[18]			X			X[19]	X[20]		X
[redacted]									
Blood Samples for Plasma anti-BIIB067 Ab			X				X		X
Blood Samples for Biomarkers			X			X	X[22]		X

Tests and Assessments	**Screening**		**Dosing Inpatient Period**			**Dosing Days 15, 29, 57 & 85 (±3 days)**	**Follow-Up**		
	V1[1] ≤21 days before V2	**V2[2] Day -28 to Day -1**	**Day 1**		**Day 2 24 (±1) Postdose**		**Days 8[3], 22, 36, 64, 92, and 106 (±3 days)**	**Days 50 & 78 via Telephone (±3 days)**	**Day 169 (±3 days) /Early Termination Visit**
			Predose	**Dosing/ Postdose**					
[redacted]									
Blood Samples for Plasma PK			X	X[23]	X	X[24]	X		X
Discharge from Inpatient Facility					X				
AE, and Concomitant Therapy and Procedures Recording			----------X (ongoing) ----------						
SAE Recording	----------X (ongoing)----------								

Ab = antibody; AE = adverse event; ALS = amyotrophic lateral sclerosis; [redacted]
ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; CSF = cerebrospinal fluid; C-SSRS = Columbia Suicide Severity Rating Scale; DNA = deoxyribonucleic acid; ECG = electrocardiogram, [redacted]; [redacted] FSH = follicle-stimulating hormone; FVC= forced vital capacity; HHD = handheld dynamometry; MMSE = Mini-Mental State Examination; [redacted]; PK = pharmacokinetic(s); RNA = ribonucleic acid; SAE = serious adverse event; [redacted] SOD1 = superoxide dismutase 1; SVC = slow vital capacity; V = visit; [redacted]

[1] Required only for subjects without a prior documentation of SOD1 mutation. Results must be available before performing Screening Visit 2 assessments.

[2] Screening assessments can be performed over ~2 days (need not be consecutive) to minimize subject burden.

[3] Subjects may, at the discretion of the Investigator, have the option of home visits for non-dosing visits that do not require CSF collection or strength and electrophysiological measures, which are visits on Days 8, 36, and 64.

[4] Not required if collected during Screening Visit 1.

[5] Including blood samples for human immunodeficiency virus, hepatitis C virus, and hepatitis B virus tests, and platelet and coagulation tests. Coagulation tests may be repeated at the local laboratory once if, in the opinion of the Investigator, values of the initial tests are only slightly out of range. Subjects with nonclinically significant and stable out-of-range values for coagulation tests may be eligible to enroll in the study at the discretion of the Investigator, and after a consultation with the Sponsor.

[6] Limited physical examination will be conducted at the Investigator's discretion.

[7] The limited neurological examination pertains to cranial nerves, coordination/cerebellar function, reflexes, motor, and MMSE.

[8] To be assessed at 3 and 6 hours postdose.

[9] To be assessed predose, and 3 and 6 hours postdose.

[10] To be assessed just prior to drawing PK blood samples at 2 and 4 hours postdose.

[11] To be assessed just prior to drawing PK blood samples at predose, and 2 and 4 hours postdose.

[12] Triplicate 12-lead (paper) ECGs will be obtained after the subject has rested in a supine position for at least 10 minutes. The first ECG will be interpreted, and the last 2 will be checked for consistency and quality.

[13] Use "Since Last Visit" version of C-SSRS.

[14] For women of childbearing potential only.

[15] To be performed predose.

[16] To confirm postmenopausal status in postmenopausal female subjects.

[17] Day 22 and Day 92 for [redacted] HHD; Day 92 for ALSFRS-R [redacted]

[18] Lumbar puncture (LP) will be performed to collect CSF samples for PK, pharmacodynamic, safety, and biomarker analysis. The results of the most recent (i.e., obtained at Screening or other visit during the study) coagulation tests and platelet count must be reviewed before each LP can be performed. Should the results suggest, in the opinion of the Investigator, that an LP may be safely performed, then no further laboratory values need to be reviewed. However, should, in the opinion of the Investigator, repeat coagulation and platelet tests be clinically indicated, then these tests may be done locally to facilitate timely review. Results of any repeat tests must be available before the LP can be performed.

[19] To be collected predose. Subjects will remain under observation in the clinic for approximately 6 hours after the LP procedure and will receive a safety follow-up telephone call ~24 hours after the procedure.

[20] To be collected on Day 106 only. Subjects will remain under observation in the clinic for approximately 1 hour after the LP procedure and will receive a safety follow-up telephone call ~24 hours after the procedure.

[redacted]

[22]Day 106 only.

[23]To be collected at 1, 2, 4, and 6 hours postdose.

[24]To be collected predose at each visit; collected at 1, 2, 4, and 6 hours postdose for Day 85 only.

3.2 Statistical Methods: General Considerations

Descriptive summary statistics will be presented for all primary, secondary and exploratory endpoints collected. Unless otherwise specified, for continuous endpoints, the summary statistics will generally include: number of subjects with data, mean, standard deviation, median, first and third quartiles and range. For categorical endpoints, the summary statistics will generally include: number of subjects with data, and the percentage of those with data in each category.

Placebo patients across cohorts 5 to 8 in Part B will be combined for Part B summaries. All summaries will be presented by dose group unless otherwise specified. Some summaries may also present the overall population combining both placebo and active dose groups.

The statistical software, SAS® version 9.4 or above, will be used for all summaries and statistical analyses.

As a general rule, any visit/dose group combination that only has data for 1 patient will not be presented in group plots. The data collected at telephone follow-up visits after Day 169 visit (i.e. ALSFRS-R) at United Kingdom sites will be also excluded from group plots and spaghetti plots. However, all data will be summarized and listed, unless otherwise specified.

3.2.1 Analysis Populations

3.2.1.1 ITT Population

The ITT (Intention-to-Treat) population is defined as all randomized subjects who receive at least 1 or a part of 1 dose of study treatment (BIIB067 or placebo) in Part B. In case of any subjects who are mis-randomized, the ITT analysis will be based on treatment assignment at the time of randomization.

3.2.1.2 Safety Population

The safety population is defined as the ITT population of subjects in Part B. This will be the primary population for the analysis of safety endpoints. In the case of any subjects who are mis-randomized, the any analyses based on the safety population will be based on actual treatment received. If a population is not specified otherwise for an analysis of safety, the safety population should be used.

3.2.1.3 Pharmacokinetic (PK) Population

The PK population is defined as the subset of the ITT population of subjects with at least 1 post-dose PK measurement in Part B.

3.2.1.4 Pharmacodynamic (PD) Population

The PD population is defined as the subset of the ITT population of subjects with at least 1 post-dose PD measurement in Part B.

3.2.1.5 Clinical Function Set

The clinical function set is defined as the subset of ITT population of subjects who have at least 1 post-dose clinical function measurement in Part B. Subjects will be analyzed in the dose group to which they are randomized. This will be the population for the analysis of clinical function endpoints.

3.2.1.6 Immunogenicity Population

The analysis population for immunogenicity is defined as all subjects in the safety population who have at least 1 post-dose sample evaluated for immunogenicity in Part B.

3.2.2 Data from Early Withdrawal or Unscheduled Visits

For subjects who are early terminated (ET), EOS visit is defined as the scheduled follow-up visit after ET visit.

Any assessments of clinical functions at unscheduled visits will not be used in the analysis but will be listed.

3.3 Study Subjects

3.3.1 Subject Accountability

The number (and percentage) of subjects randomized; dosed; completed the treatment; completed the study; completed the study but missed one or more doses; discontinued treatment and the reasons for discontinuation; and withdrew from study early and the reasons for withdrawal will be summarized in a table. The number of subjects who enrolled and completed both Parts A and B will be summarized.

A listing of subjects who discontinued treatment/withdrew from study and the associated reasons for discontinuation/withdrawal will be presented.

The number (and percentage) of subjects randomized by country and site, the number (and percentage) of subjects who completed the study by country and site, and number (and percentage) of subjects by analysis population will be summarized by dose group and for all subjects.

3.3.2 Demography and Baseline Disease Characteristics

All demographics and ALS disease history data will be summarized for the ITT population.

Demographic data, including age (years), age category (18-<35,35-<50, 50-<65, ≥65), gender, ethnicity, race, height, weight and body mass index (BMI) will be summarized by dose group and for all subjects. BMI will be calculated as weight (kg) / height^2 (m^2).

ALS disease history will also be summarized by dose group and for all subjects using descriptive statistics. These include form of ALS, mutant genes among familial ALS, SOD1 mutation subtype (variant), time since ALS symptom onset, site of onset, time since ALS diagnosis, pre-randomization ALSFRS-R slope, disease progression type ("fast progressing disease" vs "other") and riluzole usage/duration. Additionally, a listing of individual subjects with known SOD1 mutation types will be provided.

Time since ALS symptom onset will be calculated in months as (date of first dose received – date of ALS symptom onset)/30.4375. Time since ALS diagnosis will be calculated in months as (date of first dose received – date of ALS diagnosis)/30.4375. For the purpose of these calculations, partial dates for ALS symptom onset or ALS diagnosis will be imputed as follows: missing day will be imputed with 15th and missing month/day will be imputed with January 15th.

The pre-randomization ALSFRS-R slope will be calculated using the ALSFRS-R score at baseline (Day 1) i.e. Baseline ALSFRS-R score – maximum possible score of 48/duration of symptom onset, where duration of symptom onset will be calculated as (date of baseline ALSFRS-R score – date of symptom onset)/30.4375. Partial dates for symptom onset will be handled as defined above.

For disease progression type, subjects are classified as "fast progressing disease" if they have one of the a priori-defined fast-progressing SOD1 mutations and a pre-randomization ALSFRS-R slope decline of at least 0.2 points per month; all other subjects are characterized as being "other". The list of fast-progressing SOD1 mutations was determined in a blinded fashion following the interim analysis of cohorts 1 to 7 but prior to the unblinding and interim analysis of Cohort 8. The mutations recorded for all subjects in Part B were reviewed to assess which were classified as fast-progressing SOD1 mutations through genetic and literature review: (identified fast-progressing SOD1 mutations from Part B cohorts 5 to 8: p.Ala5Val, p.Arg116Gly, p.Leu107Val).

Medical history will be classified using MedDRA version 22.0. A summary of medical history by system organ class and preferred term will also be provided.

3.3.3 Extent of Exposure

Extent of exposure to study treatment will be summarized for the safety populations by dose group.

Number of patients who received 1, 2, 3, 4, or 5 doses will be summarized by dose group, as will total cumulative dose in mg.

Study drug compliance percentage up to the last dose of study drug received will be defined as the number of doses actually received divided by the number of doses that the subject is expected to receive during the study period and will be summarized. For subjects who withdrew from study early, the number of expected doses is the planned number of doses before the time of withdrawal.

Separate listings will be provided showing what subjects were randomized to as well as study drug administration which will include lot numbers.

Duration of exposure and time on study will be summarized descriptively by dose group. Duration of exposure will be calculated as (last dose date – first dose date + 1) in days and time on study will be calculated as (end of study date – first dose date + 1) in days.

3.3.4 Concomitant Medications and Non-Drug Treatments/Procedures

Concomitant medications will be coded using the World Health Organization (WHO) dictionary WHODRUG (March 2019) and concomitant non-drug treatments/procedures using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0.

Medications and non-drug treatments are considered concomitant if they are taken during the study. This includes medications/treatment/procedures that were started prior to the date and time of first dose of study drug if their use continued on or after the date and time of first dosing. If the start date is available but start time is missing and the medication/non-drug treatment start date is the same as the first dose date, the medication/non-drug treatment will be considered concomitant. Similarly, if the stop date is available but stop time is missing and the medication/non-drug treatment stop date is the same as the first dose date, the medication/non-drug treatment will also be considered concomitant. Medications/therapies with missing start or stop dates and times, will also be considered as concomitant in the following situations:

- If both the start and stop dates and times of a medication/treatment/procedure are missing;

- If the start date and time of a medication/treatment/procedure is missing and the stop date and time of the medication/therapy occurred on or after the date and time of first dose of study drug;
- If the start date and time of a medication/treatment/procedure therapy occurred prior to the date and time of first dose of study drug and the stop date and time of the medication/treatment/procedure is missing and the medication/treatment/procedure is listed as ongoing;

If the start date and time of a medication/treatment/procedure occurred prior to the date and time of first dose of study drug and the stop date and time of the medication/treatment/procedure is missing and the medication/therapy is not listed as ongoing, then the medication/treatment/procedure will not be considered as concomitant.

The number and percentage of subjects taking any concomitant medications will be summarized by dose group for the safety population. The number and percentage of subjects taking any concomitant non-drug treatments will also be summarized by dose group for the safety population.

3.3.5 Protocol Deviations

Protocol deviations identified during site monitoring will be captured in a protocol deviation log. All protocol deviations will be listed and a summary of major protocol deviations by dose group will also be provided.

3.4 Safety Data

The safety population will be used for the analyses of the safety data. Safety data will be summarized using descriptive statistics by dose group.

3.4.1 Adverse Events

All AEs will be classified using MedDRA version 22.0. All AEs will be listed but only treatment emergent AEs will be summarized, where treatment emergence will be relative to the first dose of study drug. A treatment emergent AE/SAE is defined as any AE/SAE with an onset date and time that is on or after the first dose of study drug or any pre-existing condition that has worsened in severity after the first dose of study drug. In case of missing dates, any AE/SAE with both a missing onset date and resolution date, or any AE/SAE with a missing onset date and a resolution date which is after the first dose of study drug, will be considered treatment emergent. If the onset date is available but onset time is missing and the AE onset date is the same as the first dose date the AE will be considered treatment emergent. The incidence of treatment emergent AEs will be summarized by dose group as follows:

- by MedDRA preferred term
- by primary system organ class (SOC) and MedDRA preferred term
- by maximum toxicity grade, primary SOC and MedDRA preferred term
- related AEs by primary SOC and MedDRA preferred term
- AEs related to lumbar puncture by primary SOC and MedDRA preferred term
- AEs led to discontinuation of study drug by primary SOC and MedDRA preferred term
- AEs led to withdrawal from study by primary SOC and MedDRA preferred term
- AEs led to drug interrupted by primary SOC and MedDRA preferred term

For the AE summary by primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term. For the summary of AEs by maximum toxicity grade, primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term and will only be counted under the maximum toxicity grade. For the summary of related AEs, if the relationship is missing then this will be summarized as unknown.

The most common treatment emergent AEs (occurring in 3 or more subjects in any treatment group; occurring in 2 or more subjects in any active treatment group compared to placebo) will be presented by SOC and MedDRA preferred term.

Listings of the following events will be produced.

- AEs led to discontinuation of study drug
- AEs led to withdrawal from study
- AEs led to drug interrupted

Listing of deaths will be provided if applicable.

SAEs will also be listed and summarized by primary SOC and MedDRA preferred term.

3.4.2 Laboratory Data

Laboratory data will be evaluated to determine the incidence of abnormalities that emerge during the course of the study. Changes in laboratory evaluations will be presented relative to baseline, which is defined as the closest visit prior to the subject starting treatment.

The following clinical laboratory parameters are assessed in the protocol:

- Hematology panel: complete blood count with differential (hematocrit, hemoglobin, platelets, red blood cell count [RBC], white blood cell count [WBC], basophils, eosinophils, lymphocytes, monocytes, neutrophils)
- Blood chemistry panel: albumin, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase (ALT) (SGPT), aspartate aminotransferase (AST) (SGOT), gamma-glutamyl transferase (GGT), sodium, potassium, calcium, chloride, phosphate, blood urea nitrogen (BUN), creatinine, uric acid, bicarbonate (CO2), glucose, total protein.
- Urinalysis: blood, glucose, protein, and microscopic examination if abnormal
- CSF analysis: RBC, WBC, protein, glucose.
- Coagulation: PT (prothrombin time), aPTT (activated partial thromboplastin time), and INR (international normalized ratio).

If multiple samples are collected at the same visit, the samples collected at the earliest date/time will be analyzed. For CSF laboratory parameters, tube 2 results will be analyzed if available.

Baseline value is defined as data collected which are prior to and/or on the date of the first dose, usually also the same day as the Day 1 visit. If there is more than one value on or before Day 1, then the last non-missing value prior to (including on) the date of first dose will be used as the baseline value.

Each hematology, blood chemistry, coagulation and CSF laboratory parameter will be flagged as "low" or "high" relative to the parameter's normal range or as "unknown" if no result is available. For each urinalysis laboratory parameter, the number and percentage of subjects experiencing post-dosing shifts to 'high' (positive) will be summarized. For each hematology, blood chemistry, coagulation and CSF parameter, the number and percentage of subjects experiencing post-dosing shifts to 'low' or 'high' will be summarized. In each summary, the denominator for the percentage is the number of subjects at risk for the shift. The number at risk for the shift to low is the number of subjects whose baseline value was not low and who had at least one post-baseline value. The number at risk for the shift to high is the number of subjects whose baseline value was not high and who had at least one-post baseline value. Subjects will be counted only once for each parameter and each shift regardless of how many post-dosing assessments had that type of shift.

Summary statistics for actual values and change from baseline in laboratory values will be summarized by dose group and visit. Line plots for chemistry, hematology and CSF showing mean value for each dose group at each visit will also be presented.

To evaluate potential serious hepatotoxicity subjects with a post-baseline AST and/or ALT value ≥3 times the upper limit of normal (ULN) and a post-baseline bilirubin value >2 times ULN at any time, not necessarily concurrent, will be listed with their values. In addition, a plot will be presented with each subject's maximum post-baseline AST or ALT value relative to the ULN against the subject's maximum post-baseline bilirubin value relative to the ULN; values do not have to be concurrent.

Listings of all chemistry, hematology, coagulation, CSF, urinalysis values and urine pregnancy test will be provided. Abnormal values and potentially clinically significant values will be flagged.

Subjects with at least one post-baseline CSF WBC values >5 will be plotted in a spaghetti plot. A similar plot will be provided for subjects who have a post-baseline CSF WBC value >10. Corresponding listings will also be presented with AEs that occurred at any time on or after the subject had a post-baseline CSF WBC value >5 and any time on or after the subject had a post-baseline CSF WBC value >10. Further investigation may be performed to look at the relationship between elevation in CSF WBC values and AEs.

A summary table and listing of lumbar puncture and CSF sample collection data other than CSF laboratory results (i.e. lumbar puncture position, number of attempts, interspaces, additional guidance used, CSF volume collected, needle and gauge information, caffeine/smoking/alcohol use prior to CSF collection) will also be provided.

3.4.3 Vital Signs

Summary statistics for actual values and change from baseline will be presented for each vital sign parameter (temperature, weight, pulse, respiration, systolic and diastolic blood pressure) by dose group and visit. A listing of vital sign data will also be provided.

Vital signs (temperature, pulse, systolic and diastolic blood pressure) will also be examined to determine the incidence of potentially clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with potentially clinically relevant abnormalities will be presented. The criteria for potentially clinically relevant post-dosing abnormalities are shown in Table 2 section 2.4.3.

3.4.4 ECG

The ECG test includes heart rate, PR interval, QRS interval, QT interval, QTc interval using Bazett's formula, QTc interval using Fridericia's formula, and RR interval. Summary statistics for actual values and change from baseline in each ECG parameter will be presented by dose group and visit. A listing of ECG data will be provided.

The number and percentage of subjects with shifts from normal to each of the categorical values denoting an abnormal scan (abnormal not AE, abnormal AE) will be summarized by dose group.

QTc (interval using Fridericia's formula) will also be examined to determine the incidence of clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with clinically relevant abnormalities will be presented. The criteria for clinically relevant post-dosing abnormalities are:

- Maximum increase from baseline QTcF > 30 to 60 ms
- Maximum increase from baseline QTcF > 60 ms
- Maximum post-baseline QTcF > 480 to 500 ms
- Maximum post-baseline QTcF > 500 ms

3.4.5 Mini-Mental State Exam (MMSE)

MMSE is collected for Part B subjects only. Summary statistics for actual values and change from baseline of the total score of MMSE will be presented for each visit by dose group. When there are multiple assessments during a single visit, the minimal value will be taken as the value for that visit. A listing of MMSE data for individual patients will also be provided.

3.4.6 Limited neurological examinations

The number and percentages of the status in each assessment of general neurological examination (cranial nerves, coordination/cerebellar function, sensation upper/lower extremities), El Escorial, and each assessment of other reflexes (jaw jerk, Hoffman sign right/left, plantar response right/left will be summarized by dose group and visit. The reflexes neurological examination of upper and lower extremities will be analyzed as continuous variables (0 = absent, 1 = trace, 2 = normal, 3 = brisk, 4 = clonus). The descriptive statistics will be summarized by dose group and visit. Plots of mean values by visit will be also generated.

The motor neurological examination will be analyzed as continuous variable (0 = no contraction or can't position limb; 1 = flicker or trace contraction, no movement; 2 = movement only with gravity eliminated; 3 = movement against gravity; 4 = movement against gravity and resistance; 5 = normal strength). The Ashworth spasticity scale will also be analyzed as continuous variable (1 = normal; 2 = slight increase in tone; 3 = more marked increase in tone; 4 = considerable increase in tone; 5 = affected part rigid, immobile). Summary statistics will be presented by dose group and visit. Plots of mean values by visit will also be generated.

Baseline values will be taken from Day 1 predose unless an entire section of the neurological exam (i.e. general, reflexes, motor) is missing or not done, in which case for that specific section baseline will be taken from the Screening visit for all items within that section of the exam.

Listings will also be provided with complete details for the neurological examinations.

3.4.7 Columbia Suicide Severity Rating Scale (C-SSRS)

The details of derivation and imputation for C-SSRS is described in Appendix A. The following analyses on C-SSRS measurements will be conducted:

- Descriptive summary of subjects who answered "Yes" to any question 1-12 as well as subjects who had suicidal ideation or suicidal behavior at baseline and at any post-baseline visit. The denominator for baseline summary is the number of subjects who were dosed and had baseline assessment; the denominator for post-baseline summary is the number of subjects who were dosed and had at least one post-baseline assessment for each question.

- Descriptive summary of subjects who had treatment-emergent suicidal ideation, subjects who had new suicidal ideation as well as subjects who had worsening suicidal ideation. The denominator is the number of dosed subjects with both baseline and at least one post-baseline suicidal ideation assessment.

- Descriptive summary of subjects who had treatment-emergent suicidal behavior. The denominator is the number of subjects who answered "No" to all suicidal behavior questions at baseline and had at least one post-baseline suicidal behavior assessment.

Listing of subjects having treatment-emergent suicidal ideation will be provided. Subjects who had new suicidal ideation and subjects who had worsening suicidal ideation will be flagged. The listing will display both baseline and post-baseline Suicidal Ideation Scores for each subject. Listing of subjects having treatment-emergent suicidal behavior will also be provided.

3.5 Pharmacokinetic Data

The PK population will be used for the analyses of the PK data.

Values below limit of quantitation (BLQ) are set to half of the lower limit of quantitation (LLOQ, 1 ng/mL) at day 1 pre-dose rather than zero so that geometric means can be calculated. Values that are BLQ at all other visits will also be set to half of LLOQ (1 ng/mL) in calculations.

Both plasma and CSF BIIB067 concentrations will be listed and summary statistics will be presented for each visit. Summary statistics will also include both the arithmetic and geometric means with corresponding standard errors. Plots of arithmetic and geometric mean concentrations of BIIB067 versus time will be provided, including standard error bars. The plots will be repeated for plasma BIIB067 concentrations separately for all timepoints at Day 1 and Day 85. Spaghetti plots will be presented for each BIIB067 group to show individual subjects

throughout all visits. These will include both pre-dose and post-dose visits. Both the geometric mean and the median for the dose group will also be plotted on the spaghetti plots.

The BIIB067 concentrations will be used to calculate the following parameters at Day 1 and Day 85 using non-compartmental methods by the PK Scientist: AUC[0-24h], Cmax, Tmax. Summary statistics will be provided for these PK parameters.

A summary of missing plasma and CSF BIIB067 concentrations will also be provided.

3.6 Pharmacodynamic Data

The PD population will be used for the analyses of the PD/biomarker data.

The following PD biomarkers will be summarized:

- SOD1 protein in CSF. For SOD1 protein, the overall protein level will be analyzed.
- Neurofilament levels in both the CSF and plasma. For neurofilament, the phosphorylated axonal neurofilament heavy chain (p-NFH) will be analyzed.

Values that are BLQ will be set to half of LLOQ (15.6 ng/mL for SOD1 and 7.46 pg/mL for pNFH).

Missing Data

A summary of missing data for each PD biomarker will be presented.

For both CSF SOD1 and p-NFH measurements, any post-baseline missing up to and including Day 85 will be imputed using 2 methods. Baseline and data beyond Day 85 will not be imputed.

Primary method of imputation: Mixed Model for Repeated Measures (MMRM)

The MMRM model will be used to impute missing values and to obtain a complete dataset for actual values for each PD biomarker. The MMRM model for actual values will be used for the purpose of presenting descriptive statistics only. Missing values will be replaced by predicted values from the model. The model for actual values will be performed on the log scale (i.e. log (post-baseline)). The model will include dose group, visit, treatment-by-visit interaction, baseline score i.e. log value, and baseline score-by-visit interaction terms, and adjust for the following covariate: disease progression type. Visits to Day 85 only will be included in the model.

The MMRM model for log ratio to baseline will be used to impute missing values as well as for inference, analyzing longitudinal assessment on change from baseline values on log scale ((i.e log(post-baseline) – log(baseline)) up to Day 85 for each PD biomarker. The model will include dose group, visit, treatment-by-visit interaction, baseline score i.e. log value, and baseline score-by-visit interaction terms, and adjust for the following covariate: disease progression type.

For the MMRM model, an unstructured covariance (UN) matrix will be used to model the within-subject variability. Model convergence will be checked. If the unstructured covariance model fails to converge with the default Newton-Rhapson algorithm, the Fisher scoring algorithm will be used to provide initial values of the covariance parameters. In the event that none of the above methods yield convergence, a structured covariance such as the heterogeneous first-order autoregressive (ARH(1)) structure will be used.

The MMRM models specified above for actual values and ratio to baseline will also be applied on the subgroup analysis of disease progression type (other, fast). The model will be similar to that specified above for each subgroup level except disease progression type will not be included in the model.

Secondary method of imputation: Last observation carried forward (LOCF)

As a sensitivity analysis the LOCF method will be used as a conservative estimate. In the LOCF approach since baseline data will not be carried forward, if the first post-baseline visit is missing, it will remain missing after applying LOCF imputation. LOCF will be applied on actual values at each visit to provide a complete dataset.

Descriptive statistics for imputed datasets

Descriptive statistics for each PD biomarker at each visit and ratio to baseline will be summarized by dose group. The summary statistics will include arithmetic mean and SD for the ratio to baseline as well as the geometric mean ratio and corresponding standard error. The geometric mean ratio will be obtained by back-transforming change from baseline on log transformed data. Arithmetic means and geometric means will be presented for the actual values at each visit. The descriptive summaries will be presented overall and by disease progression type based on both MMRM imputed data as well as LOCF imputed data.

The following plots over time will be presented for each PD biomarker for both sets of LOCF and MMRM imputed datasets (overall and by disease progression type):

- Arithmetic mean ratio to baseline with standard error bars
- Geometric mean ratio to baseline with standard error bars.
- Geometric mean and standard error

Listings of individual data will also be provided.

Statistical analysis

The statistical analysis of PD biomarkers will be performed for Part B only. All statistical tests will be conducted at the nominal significance level of 0.1. CIs will be constructed at the traditional 95% confidence level. No adjustment for multiple testing will be performed. Nominal p-values will be presented.

For both CSF SOD1 and p-NFH measurements, a 2-sample Wilcoxon rank sum test will be performed on the log scale for ratio to baseline values at Day 85 comparing each BIIB067 group vs. placebo group overall and for each disease progression type. This will be the primary method of analysis based on the MMRM imputed dataset.

As sensitivity analysis the MMRM model for ratio to baseline will also be used for inference: For each PD biomarker least-squares (LS) means of each dose level at each visit as well as treatment differences between each dose level vs. placebo at each visit will be presented, along with 95% CI and p-values. The back-transformed LS means here represent geometric mean ratios compared to baseline, and the back-transformed LS means differences represent geometric mean ratios of the ratios compared to baseline between each BIIB067 dose level vs. placebo. Results will be presented overall, and by disease progression type. These geometric mean ratios will also be plotted overall and by disease progression type.

Similarly, a MMRM model will be performed on the LOCF imputed dataset for each PD biomarker. The model will be specified as detailed above for overall and by disease progression type. Results will be tabulated but not plotted.

In addition, relationships between PK and PD biomarkers and between different PD biomarkers will be examined using scatter plots, including the list below. The correlation coefficient (Spearman) will also be generated by dose group and for overall. The scatter plots and correlation coefficient tables will be repeated for disease progression type subgroups. These will be based on MMRM imputed data. Time points other than Day 85 may also be explored.

- CSF BIIB067 concentration at Day 85 vs ratio to baseline in CSF SOD1 at Day 85
- CSF BIIB067 concentration at Day 85 vs ratio to baseline in CSF pNFH at Day 85
- Plasma pNFH at Day 1 vs CSF pNFH at Day 1
- Plasma pNFH at Day 85 vs CSF pNFH at Day 85

Completers to Day 169

For subjects who completed the study to Day 169 and with data available at all visits through to Day 169, descriptive statistics of actual values and ratio to baseline will be presented by dose group for each PD biomarker. The summary statistics will include arithmetic mean and SD for the change from baseline as well as the geometric mean ratio and corresponding standard error. The geometric mean ratio will be obtained by back-transforming change from baseline on log transformed data. Arithmetic means and geometric means will be presented for the actual values at each visit. The descriptive summaries will be presented for all completers and by disease progression type.

Plots of geometric means and geometric mean ratio to baseline with standard error bars will also be presented for all completers. These plots may also be presented by disease progression type.

Observed data

Descriptive statistics of actual values and ratio to baseline will be presented by dose group for each PD biomarker at all visits through to Day 169 for all patients. The descriptive summaries will be presented overall and by disease progression type.

Spaghetti plots of actual values and ratio to baseline will be presented for each dose group to show individual subjects throughout all visits based on observed data to Day 169. Both the geometric mean and the median for the dose group will also be plotted on the spaghetti plots. Separate spaghetti plots of subjects who were fast progressors will also be presented.

3.7 Immunogenicity Data

The immunogenicity population, as defined in Section 3.2.1, will be used for all analyses of immunogenicity data.

- For immunogenicity, the baseline value is defined as the latest immunogenicity data collected at any time prior to the first dose. If no immunogenicity data are collected prior to the first dose, the baseline value is missing and will be imputed as anti-drug antibody negative for immunogenicity analyses.

- Subjects with at least one confirmed post-treatment positive result will be considered positive for anti-drug antibodies if their baseline result is negative.

- Subjects where none of the post-treatment samples were positive for anti-drug antibodies will be considered negative regardless of their baseline result

- For subjects that are confirmed positive at baseline and have at least one post-treatment sample with a ≥ 2-fold increase in titer will be considered positive for anti-drug

antibodies. Subjects that are positive at baseline, with subsequent post-treatment samples titers that are within 2-fold will be considered negative for anti-drug antibodies

- Numbers and percentage of subjects who develop antibodies will be determined and summarized by dose groups and timepoint.

The same summary will be provided for subjects with positive baseline results and for subjects with negative or missing baseline results, separately.

3.8 Clinical Function Data

The clinical function set will be used for all analyses of clinical functions.

Unless stated otherwise, baseline data are defined as the data collected prior to the time and/or on the date of first dose, which is usually the same day as the Day1/Baseline visit. If there is more than one value on/before the date of the first dose, the non-missing value closest to and prior to the first dose will be used as the baseline value. For [redacted] HHD and [redacted] data were collected at Screening and not Day 1 so baseline values for endpoints on these assessments will be taken from the Screening visit.

Any assessments of clinical functions at unscheduled visits will not be used in the analysis but will be listed. For some subjects in Cohort 5 of 233AS101 MAD study the [redacted] was not being collected until after the protocol was amended and approved (Version 2.0). Therefore, as these subjects in this cohort do not have a baseline [redacted], subjects in cohort 5 will not be included in the analysis models. These data will be listed and included under observed descriptive summaries. Similarly for ALSFRS-R Day 92, [redacted] HHD Day 22, these assessments were only collected after the protocol was amended and approved (Version 2.0) so were not collected for Cohort 5 subjects, so this timepoint cannot be used for analysis for Cohort 5 subjects.

[redacted]

[redacted]

For clinical function measurements, any post-baseline missing up to and including Day 85 or Day 92 will be imputed using 2 methods. Data beyond Day 85 (for [redacted] SVC) or Day 92 (for ALSFRS-R, [redacted] HHD, [redacted]) will not be imputed.

[redacted]

Primary method of imputation: MMRM

The MMRM model will be used to impute missing values and to obtain a complete dataset for actual values for each endpoint. The MMRM model for actual values will be used for the purpose of presenting descriptive statistics only. Missing values will be replaced by predicted values from the model. The model will include dose group, visit, treatment-by-visit interaction, baseline score and baseline score-by-visit interaction terms, and adjust for the following covariate: disease progression type. Visits to Day 85 (or Day 92) only will be included in the model.

The MMRM model for change from baseline will be used to impute missing values as well as for inference, analyzing longitudinal assessment on change from baseline values up to Day 85 (or Day 92) for each endpoint. The model will include dose group, visit, treatment-by-visit interaction, baseline score, and baseline score-by-visit interaction terms, and adjust for the following covariate: disease progression type.

For the MMRM model, an unstructured covariance (UN) matrix will be used to model the within-subject variability. Model convergence will be checked. If the unstructured covariance model fails to converge with the default Newton-Rhapson algorithm, the Fisher scoring algorithm will be used to provide initial values of the covariance parameters. In the event that none of the above methods yield convergence, a structured covariance such as the heterogeneous first-order autoregressive (ARH(1)) structure will be used.

The MMRM models specified above for actual values and change from baseline to Day 85 (or Day 92) will also be applied on the subgroup analysis of disease progression type (other, fast). The model will be similar to that specified above for each subgroup level except disease progression type will not be included in the model.

Predicted values that are outside of the range of a scale will be reset: eg if the predicted value is negative and the scale does not allow for a negative score then the value will be set to the

minimum possible score for that scale. Similarly if the predicted value is above the maximum possible score of the scale then the value will be set to the maximum possible score.

Secondary method of imputation: LOCF

As a sensitivity analysis the LOCF method will be used as a conservative estimate for some key endpoints. In the LOCF approach since baseline data will not be carried forward, if the first post-baseline visit is missing, it will remain missing after applying LOCF imputation. LOCF will be applied on actual values at each visit to provide a complete dataset.

The endpoints for clinical function data are given in Table 5 which also indicates whether imputation will be performed and which imputation methods.

Table 5: Clinical function endpoints for Part B

Clinical function	Endpoint	MMRM	LOCF	No imputed dataset
ALSFRS-R	- Total Score	Y	Y	N
	- Functional domain scores (bulbar function, fine motor skills, gross motor skills, respiratory function)	Y	N	N
SVC	- Percent predicted SVC	Y	Y	N
[redacted]				

[redacted]

HHD	- HHD Megascore	Y	Y	N
	- Muscle strength for each of 16 muscles	N	N	Y
	- Proportion of subjects with zero muscle strengths	N	N	Y

[redacted]

[redacted]

ALSFRS-R: single imputation analysis imputing score after death to a lower score

For ALSFRS-R total score only, this imputation method will be determined for sensitivity analysis.

- For those subjects who died determine what the last visit is;
- Based on this visit number calculate the slope for all placebo patients with available data at this and Day 92 (using only the data at these 2 timepoints).
- Of these placebo patients select the 50% with the worst slope (i.e. greatest decline).
- From this 50% use the median slope to impute Day 92 observation for the patient who died.
- Apply MMRM model as specified previously

For example, subject A died with last visit with last ALSFRS-R assessment at Day 29. Then we can calculate slope from Day 29 to Day 92 (using only Day 29 and Day 92 data) for all placebo patients. We can, pick the median slope of the worst 50% placebo patients to linearly impute Day 92 (and only Day 92) observation for subject A. Then we can use MMRM to analyze the data. This is consistent with treating death as worst outcome. The MMRM model will be run overall and by disease progression type.

[redacted]

[redacted]

[redacted]

[redacted]

Descriptive statistics to Day 85 (or Day 92)

For each endpoint listed in Table 5, descriptive statistics of actual values and change from baseline at each visit will be summarized for continuous variables by dose group based on the imputed datasets as per Table 5 i.e. MMRM only, or MMRM and LOCF, [redacted] (LOCF after death). The descriptive summaries will be presented overall and by disease progression type based

on both MMRM imputed data as well as LOCF imputed data. For ALSFRS-R total score the MMRM sensitivity model using single imputation after death will also be summarized.

For those endpoints where MMRM or LOCF imputed datasets are not generated the later section on observed data will cover these.

Plots of mean values and mean changes from baseline by visit will also be generated overall and by disease progression type for each clinical function based on MMRM imputed data (and LOCF data where applicable). These plots will also be presented for the MMRM sensitivity model using imputation after death for ALSFRS-R total score.

Listings of individual data will also be provided. For individual questions/items within questionnaires, stacked bar charts may also be presented to show any shifts in responses.

Statistical analysis

Statistical analyses of clinical function endpoints will be performed for Part B with imputed data. When a placebo group is involved in an analysis, all subjects receiving placebo will be combined to form placebo control group. The statistical analysis of clinical function endpoints will be performed for Part B only. All statistical tests will be conducted at the nominal significance level of 0.1. CIs will be constructed at the traditional 95% confidence level. No adjustment for multiple testing will be performed. Nominal p-values will be presented.

As shown in Table 6 for the specified continuous clinical function endpoints, a 2-sample Wilcoxon rank sum test will be performed for change from baseline values at Day 85 (for ALSFRS-R, [redacted] SVC) or Day 92 (HHD, [redacted]) each visit comparing each BIIB067 group vs. placebo group based on the MMRM imputed datasets. This will be the primary method of analysis based on the MMRM imputed dataset (as well as the sensitivity MMRM model with imputation after death for ALSFRS-R total score). These will be overall and by disease progression type.

As sensitivity analysis the MMRM model for change from baseline will also be used for inference: For each clinical function endpoint as specified in Table 6 least-squares (LS) means of each dose level at each visit as well as treatment differences between each dose level vs. placebo at each visit will be presented, along with 95% CI and p-values. Results will be presented overall, and by disease progression type. The LSmean changes will also be plotted overall and by disease progression type. These will also be presented from the sensitivity MMRM model with imputation after death for ALSFRS-R total score.

Similarly a MMRM model will be performed on the LOCF imputed dataset for those clinical function endpoints listed in Table 5. The model will be specified as detailed above for overall and by disease progression type. Results will be tabulated but not plotted.

Table 6: Clinical function endpoint statistical analysis for Part B

Clinical function	Endpoint	Statistical Analysis	Wilcoxon rank sum test at Day 85 (or 92)	LSMeans MMRM model	LSMeans LOCF imputed MMRM model
ALSFRS-R	- Total Score	Y	Y	Y	Y
	- Functional domain scores (bulbar function, fine motor skills, gross motor skills, respiratory function)	Y	Y	Y	N
SVC	- Percent predicted SVC	Y	Y	Y	Y
[redacted]					

HHD	- HHD Megascore	Y	Y	Y	Y
	- Muscle strength for each of 16 muscles - Proportion of subjects with zero muscle strengths	N	N	N	N

[redacted]

In addition, relationships between ALSFRS-R and PD biomarkers and selected clinical function endpoints will be examined using scatter plots, including the list below. The correlation coefficient (Spearman) will also be generated by dose group and for overall. The scatter plots and correlation coefficient tables will be repeated for disease progression type subgroups. These

will be based on MMRM imputed data. Time points other than Day 85 may also be explored. Visits may also be pooled through to Day 85 to look at the overall relationship.

- Ratio to baseline in Plasma pNFH at Day 85 vs ALSFRS-R at Day 85
- Ratio to baseline in Plasma pNFH at Day 85 vs change from baseline in ALSFRS-R to Day 85
- Ratio to baseline in CSF pNFH at Day 85 vs ALSFRS-R at Day 85
- Ratio to baseline in CSF pNFH at Day 85 vs change from baseline in ALSFRS-R to Day 85
- Ratio to baseline in CSF-SOD1 at Day 85 vs ALSFRS-R at Day 85
- Ratio to baseline in CSF-SOD1 at Day 85 vs change from baseline in ALSFRS-R to Day 85
- [redacted]
- [redacted]
- EQ-5D utility index at each visit with ALSFRS-R total score
- [redacted] with change from baseline in ALSFRS-R total score

Further exploratory analyses may be performed looking at the relationship between the reduction in CSF-SOD1 or pNFH at selected timepoints with ALSFRS-R. Baseline characteristics and their relationship with ALSFRS-R may also be performed.

Completers to Day 169

For subjects who completed the study to Day 169 and with data available at all visits through to Day 169, descriptive statistics of actual values and changes from baseline will be presented by dose group for each continuous clinical function endpoint listed in Table 5. The descriptive summaries will be presented for all completers and by disease progression type. For the following non-continuous endpoints in Table 5, frequencies and percentages will be presented for each item/question/endpoint by visit: [redacted]

Plots of changes from baseline with standard error bars will also be presented for all completers. These plots may also be presented by disease progression type.

Observed data

Descriptive statistics of actual values and changes from baseline will be presented by dose group for each continuous clinical function endpoint listed in Table 5 at all visits through to Day 169 for all patients. The descriptive summaries will be presented overall and by disease progression

type. For the following non-continuous endpoints in Table 5, frequencies and percentages will be presented for each item/question/endpoint by visit: [redacted]

Spaghetti plots of actual values and changes from baseline will be presented for each treatment group to show individual subjects throughout all visits based on observed data to Day 169. See Table 7. Separate spaghetti plots of subjects who were fast progressors will also be presented.

The mutation subtype will also be indicated for each subject on the spaghetti plots. Listings of individual data on clinical functions will also be provided.

Table 7: Clinical function endpoints with spaghetti plots

Clinical function	Endpoint	Spaghetti plot
ALSFRS-R	- Total Score	Y
	- Functional domain scores (bulbar function, fine motor skills, gross motor skills, respiratory function)	N
SVC	- Percent predicted SVC	Y
[redacted]		

HHD	- HHD Megascore	Y
	- Muscle strength for each of 16 muscles - Proportion of subjects with zero muscle strengths	N

[redacted]

Post-randomization slope in ALSFRS-R total score will be estimated by fitting a regression model on change from baseline over time (study day from Day 1 in months) for each subject's rate of change. Two separate slopes will be estimated, one includes data from Day 1 and one includes data from on or after day 29. Data up to and including Day 92 will be included in the slope; data from Day 169 or any additional follow-up data from telephone calls for UK sites will not be included. Only observed data will be used to estimate the slopes. Descriptive statistics for the pre-randomization and both post-randomization slopes in ALSFRS-R total score will be summarized by dose group. A scatter plot of pre-randomization slope vs post-randomization

slope from Day 29 will be generated. The correlation coefficient (Spearman) will also be generated. The scatter plot will be repeated for disease progression type subgroups.

To explore riluzole use in subjects, descriptive statistics for each of ALSFRS-R total score and percent predicted SVC will be presented by riluzole use (yes/no) for all subjects and also within each disease progression type. These will be based on observed data due to the small numbers of subjects and will be presented by visit from baseline through to Day 85. Similarly, descriptive statistics will also be presented for changes from baseline in ALSFRS-R total score and percent predicted SVC by visit to Day 85.

4. Sample Size Justification

The maximum sample size of 36 subjects for Part A of the study (SAD) is based on clinical rather than statistical considerations. Subjects will be randomized to receive BIIB067 or placebo in a 3:1 ratio in each cohort. Each cohort will have a minimum of 4 subjects (3 active and 1 placebo), and a maximum of 12 subjects (9 active and 3 placebo). The final number for each cohort will be dependent on the DLT profile. If no DLT occurs in any dose level, then a total of 20 subjects (15 active and 5 placebo) will be randomized. This design will allow for adequate evaluation of the safety of BIIB067, should DLT be encountered, as well as minimize subject exposure at subtherapeutic single doses.

For Part B (MAD), up to 48 subjects will be enrolled in up to 4 cohorts, with up to 9 subjects randomized to receive BIIB067 and 3 subjects randomized to receive placebo for each cohort. This design will provide approximately 80% power to detect a difference in SOD1 reduction between 25% from the BIIB067 group and 12% from the placebo group at a 10% significance level. The sample size calculation assumes the same 10.5% SD in the 2 treatment groups.

5. Changes to Planned Analyses

The following analyses were specified in the protocol but not performed as they were not required.

- For the summary of AEs by relationship, this was amended to a summary of related AEs by system organ class and preferred term
- The percent change from baseline in clinical functions and PD parameters. The PD parameters were summarized using geometric means and geometric mean ratios in addition to arithmetic means.
- A 2-sample student's t-test to compare each dose of BIIB067 and placebo at a 2-sided alpha level of 0.1 for clinical functions and SOD1. Instead, a 2-sample Wilcoxon rank sum test and MMRM are used to analyze the clinical functions and PD parameters, as this

minimizes any undue influence of extreme values. Although 90% confidence intervals were presented for interim analyses, all confidence intervals will be 95% CI. The nature of the study and these endpoints were originally intended to be exploratory and so 90% confidence intervals were deemed as appropriate at the time.

- Pharmacogenomic populations is not defined; the biomarker data is to be analyzed using PD population.
- Biomarker NFL will be analysed as post-hoc due to availability of data.
- Physical examination is collected under adverse events in case of any abnormalities and therefore there is no separate collection of these data and subsequently no separate analysis of these data.

6. References

[redacted]

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Appendix A: Derivation of C-SSRS

The Columbia Suicide Severity Rating Scale (C-SSRS) is an assessment that evaluates suicidal ideation and behavior. C-SSRS measurements are collected with respect to "Lifetime: time he/she felt most suicidal" at baseline, and with respect to "Since last visit" at last visit (Day 57 for Part A and Day 169 for Part B) and ET visit.

There are 11 common "Yes/No" questions at baseline and post-baseline visits. Five questions on *suicidal ideation* and five questions on *suicidal behavior* are re-ordered and follow increasing severity order respectively as shown in Table A1. In particular, only patients who answered "Yes" to question 2 will proceed to question 3, 4 and 5. Thus, for any subjects who answered "No" to question 2, an answer "No" will also be assumed to question 3, 4, and 5. An additional "Yes/No" question is used to record if subject had committed suicide in post-baseline visits.

Table A1: C-SSRS re-ordered questions

Suicidal Ideation	
Question 1	Wish to be dead
Question 2	Non-specific active suicidal thoughts
Question 3	Active suicidal ideation with any methods (not plan) without intent to act
Question 4	Active suicidal ideation with some intent to act, without specific plan
Question 5	Active suicidal ideation with specific plan and intent
Suicidal Behavior	
Question 6	Preparatory acts or behavior
Question 7	Aborted attempt
Question 8	Interrupted attempt
Question 9	Actual attempt

Question 10	Suicidal behavior
Question 11 (post-baseline visit only)	Suicide
Self-Injurious Behaviour without Suicidal Intent	
Question 12	Self-injurious behavior without suicidal intent

A subject is considered to have *suicidal ideation* at the period of interest if a "Yes" is answered to any of the five suicidal ideation questions (Question 1-5). A subject is considered to have *suicidal behavior* at the period of interest if a "Yes" is answered to any of the five suicidal behavior questions (Question 6-10) at baseline or a "Yes" is answered to any of the six suicidal behavior questions (Question 6-11) at post-baseline visit.

A subject's *Suicidal Ideation Score* is defined as the maximal suicidal ideation question number (maximal of 1-5) with an answer "Yes" per visit. The score is defined as 0 if the subject answered "No" to all 5 Suicidal Ideation questions at that visit. A subject is considered to have treatment-emergent suicidal ideation if the subject had either new or worsening suicidal ideation. A subject is considered to have new suicidal ideation if the subject's Suicidal Ideation Score increased at post-baseline visit compared to a score 0 at baseline. A subject is considered to have worsening suicidal ideation if the subject's Suicidal Ideation Score increased at post-baseline visit compared to a positive score at baseline.

A subject is considered to have treatment-emergent suicidal behavior if the subject answered "Yes" to any suicidal behavior questions at any post-baseline visit while answered "No" to all suicidal behavior questions at baseline.

Appendix B: Derivation and Imputation of Clinical Function Endpoint

ALSFRS-R

The ALSFRS-R has been demonstrated to predict survival. The ALSFRS-R measures 4 functional domains, comprising bulbar function (speech, salivation, swallowing), fine motor skills (handwriting, cutting food and handling utensils, dressing and hygiene), gross motor skills (turning in bed, walking, climbing stairs), and respiratory function (dyspnea, orthopnea, respiratory insufficiency). Each domain consists of 3 items, each scored from 0 to 4 with higher scores representing better function. Therefore, the total possible score for ALSFRS-R is 48.

The ALSFRS-R total score will be considered missing if more than 3 individual item scores are missing. If no more than 3 (≤ 3) individual item scores are missing and each functional domain has at least one individual item score available, the functional domain score with any missing item score(s) will be calculated by the following formula:

ALSFRS-R domain score = [(average of all answered item scores) x 3].

The ALSFRS-R total score is the sum of the 4 functional domain scores. If 3 individual item scores are missing from the same functional domain, then the ALSFRS-R domain score is considered as missing and the total score is calculated by the following formula:

ALSFRS-R total score = average of all answered item scores from other 3 domains x 12.

Percent Predicted SVC

Vital capacity will be measured by means of an SVC test, administered in the upright position.

Upright SVC will be determined by performing 3 to 5 measures, in accordance with criteria established by the American Thoracic Society and the European Respiratory Society. The maximum of the percent predicted upright SVC values at each visit will be used for the analysis. The percent predicted SVC will be calculated as [observed SVC divided by predicted SVC] x 100%. The predicted SVC is adjusted by sex, age, height, which is programmed into and performed by the equipment used.

[redacted]

[redacted]

HHD

Sixteen muscle groups (left and right shoulder flexion, left and right elbow flexion, left and right wrist extension, left and right abduction index finger, left and right abduction thumb, left and right abduction 5th digit, left and right knee extension, and left and right ankle dorsiflexion) will

be examined in both upper and lower extremities. At each visit, each muscle will be collected as Best Trial 1 and Best Trial 2 and the maximum value of the measures will be used for the analysis.

Any missing muscle strength with a reason of 'UNABLE TO TEST' will be imputed as 0 for analysis.

Megascore

Mean and standard deviation (SD) for each muscle group will be established from the baseline values of all patients in this trial. For the purpose of calculating megascore, the mean and SD of the baseline value will be used across all patients regardless of dose/treatment group. The muscle strength values will be normalized to Z scores as (post-baseline measurements – mean)/SD and averaged to provide HHD overall megascore. The overall megascore is created by averaging all eight bilateral measurement Z scores, if no more than 10 (≤ 10) measures are missing. The overall megascore will be considered missing if more than 10 measures are missing out of 16 total measures.

HHD zero strength (for Part B)

The zero strength will be derived based on the combined muscle group of 4 selected muscle pairs: first dorsal interosseous left/right, elbow flexion left/right, knee extension left/right, ankle dorsiflexion left/right. The zero strength is defined as: an observed zero strength on HHD on any 1 of the 4 selected muscle pairs that is not zero or missing at baseline or has at least one positive postbaseline strength before that observed zero, for which the observed zero strength is subsequently confirmed at the next available measurement, where the confirmation can be in the form of an observed zero strength or patient death. The time to zero strength may be derived. The time to zero strength is defined as the time since randomization to the time of the first observed zero that is subsequently confirmed (note: time to the first observed zero; not time to confirmation). The unit of time to zero strength will be in months. If a patient does not experience a zero strength, withdraws or is lost to follow-up during the study, the patient's time to zero strength in the study will be marked as right-censored. Time-to-zero strength will be also derived for each of the 16 individual muscles.

[redacted]

[redacted]

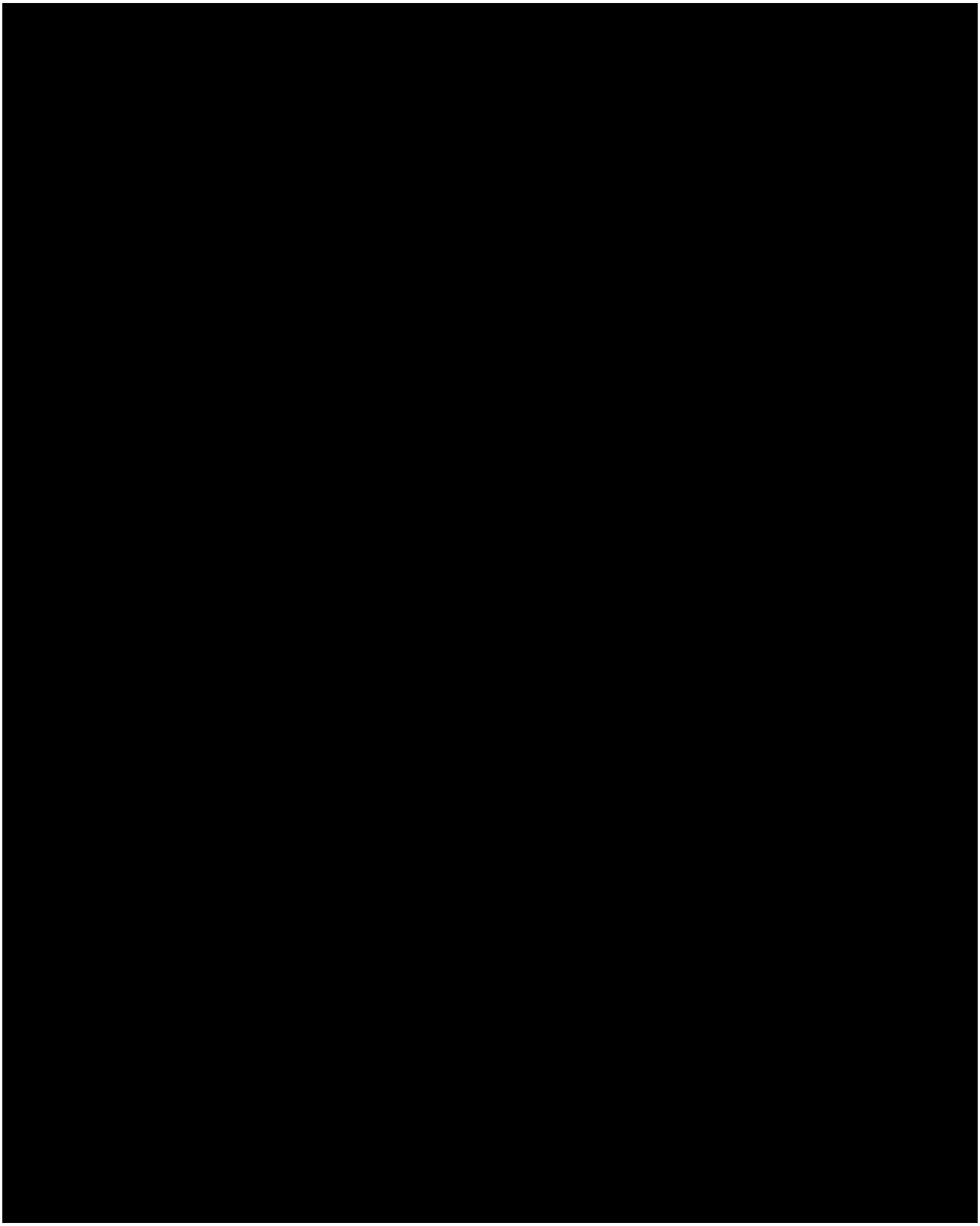
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



Statistical Analysis Plan – Study 233AS101 Part C

Final Version No.: 2.0, 14 August 2021

Study Title: A Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis and Confirmed Superoxide Dismutase 1 Mutation

Name of Study Treatment: Tofersen (BIIB067)

Protocol No.: 233AS101/ NCT02623699

Study Phase: 1/2/3

This document has been reviewed and approved by:		
[redacted]	[redacted] A5C54A0ADD4B45F...	14-Aug-2021
SMT Statistician	Signature	Date
[redacted]	DocuSigned by: [redacted] 58D50B1E41B2400...	14-Aug-2021
Program Statistician	Signature	Date
[redacted]	DocuSigned by: [redacted] 1D2C38148DDE4A2...	14-Aug-2021
SMT Medical Director	Signature	Date

VERSION HISTORY

SAP Version	Date	Primary Reasons for Amendment
1.0	09June2021	Final version
2.0	14Aug2021	Amended primary efficacy analysis for ALSFRS-R and primary analysis for percent predicted SVC and included rationale in changes to planned analyses; additional analyses added for ALSFRS based on FDA feedback; included tipping point analysis as sensitivity analysis for ALSFRS-R; updated AE of note table; added listing of site transfers; listed samples unavailable for analysis; added sensitivity/supplementary analyses for ALFSRS; addition of covariate for SVC; clarifications on sensitivity analyses for ALSFRS; clarification on ventilation data; sensitivity analysis for time to death or permanent ventilation added; clarification on subgroup analyses; indicated CSF NfL may not be available at time of DBL; amended SMQ for immunogenicity analysis; made minor clarifications/corrections

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List of Abbreviations

Ab	antibodies
ADM	abductor digit minimi
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSFRS-R	ALS Functional Rating Scale (revised)
[redacted]	
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
APB	abductor pollicis brevis
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the curve
BB	bicep
BLQ	below limit quantitation
BMI	body mass index
bpm	beats per minute
BUN	blood urea nitrogen
CDISC	Clinical Data Interchange Standards Consortium
[redacted]	
CI	confidence interval
[redacted]	
Cmax	maximum concentration
CO2	bicarbonate
COVID-19	Coronavirus Disease 2019
CRF	case report form
CSF	Cerebral-spinal fluid
C-SSRS	Columbia suicide severity rating scale
CTCAE	common terminology criteria for adverse events
DNA	deoxyribonucleic acid

EAC	Event Adjudication Committee
EAIR	exposure adjusted incidence rates
ECG	electrocardiogram
EDB	extensor digitorum
EOS	end of study (visit)
[redacted]	
ET	early termination (visit)
FDIO	first dorsal interosseous
FSH	follicle-stimulating hormone
[redacted]	
FU	follow-up
FWER	family-wise error rate
GGT	gamma-glutamyl transferase
GGW	generalized Gehan-Wilcoxon
GH	general health
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HHD	hand-held dynamometry
HIV	human immunodeficiency virus
ICC	intraclass correlation
ICH	International Council for Harmonisation
IDMC	independent data monitoring committee
INR	international normalized ratio
IRT	interactive randomization system
ITT	intent-to-treat
LLOQ	lower limit of quantitation
LP	lumbar puncture
LOCF	last observation carried forward
LS	least square
LTE	long term extension
MAD	multiple-ascending dose
MCMC	Markov Chain Monte Carlo
[redacted]	
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
MH	mental health
MI	multiple imputation
MITOS	Milano-Torino Staging
mITT	modified intent to treat
mmHg	millimeters of mercury

MMRM	mixed model for repeated measures
MMSE	mini-mental state exam
[redacted]	
N	number of subjects or observations
NfH	neurofilament heavy chain
NfL	neurofilament light chain
ng/mL	nanogram/milliliter
PAV	permanent assisted ventilation
[redacted]	
PD	pharmacodynamics
PF	physical functioning
[redacted]	
pg/mL	picogram/milliliter
PK	pharmacokinetic(s)
[redacted]	
PRO	patient reported outcome
PT	prothrombin time
QTc	interval between the start of the QRS complex and the end of the T wave, corrected for heart rate
QTcF	QTc interval using Fridericia's formula
RBC	red blood cell (count)
RE	role emotional
RNA	ribonucleic acid
RP	role physical
RR	inter-beat
TA	tibialis anterior
Tmax	time to maximum concentration
SAD	single-ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis system
SD	standard deviation
SDTM	standard data tabulation model
SE	standard error
SF	social functioning
[redacted]	
SOC	system organ class
SOD1	superoxide dismutase 1

SVC	slow vital capacity
ULN	upper limit of normal
UN	unstructured
[redacted]	
VT	vitality
WBC	white blood cell (count)
WHO	World Health Organization
[redacted]	

1. Introduction

Study 233AS101 is a randomized, double-blind, placebo-controlled, 3-part study to examine the efficacy, safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of BIIB067, administered by intrathecal bolus injection in subjects with ALS and a confirmed SOD1 mutation.

BIIB067 will be referred to as tofersen throughout the rest of the statistical analysis plan.

Parts A and B are single-ascending dose (SAD) and multiple-ascending dose (MAD) components, respectively, in which subjects will be randomized to receive tofersen or placebo in a 3:1 (active:placebo) ratio. In Part C, approximately 99 subjects will be randomized to receive tofersen 100 mg or placebo in a 2:1 (active:placebo) ratio. This statistical analysis plan (SAP) is prepared for the clinical study report(s) for Part C; a separate SAP exists for Parts A and B. Subjects have the option of enrolling into a long-term extension study (233AS102) on completion of Study 233AS101. Separate SAPs will be prepared for the analyses of 233AS102 and integrated analyses across the studies. A separate SAP will also detail analyses for defining clinical meaningfulness on Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised (ALSFRS-R).

Blinded safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. Unblinded safety data will be reviewed on an ongoing basis by an Independent Data Monitoring Committee (IDMC).

This SAP also covers how the impact of the Coronavirus Disease 2019 (COVID-19) pandemic will be handled.

1.1 Primary objective and endpoints

The primary objective of Part C of this study is to evaluate the clinical efficacy of tofersen administered to adult subjects with ALS and a confirmed SOD1 mutation.

The primary efficacy endpoint is the change from baseline to Week 28 (Day 197) in the ALSFRS-R total score.

The secondary efficacy endpoints are as follows:

- Change from baseline to Week 28 (Day 197) in slow vital capacity (SVC).
- Change from baseline to Week 28 (Day 197) in handheld dynamometry (HHD) megascore to assess muscle strength, as measured by the HHD device.
- Time to death or permanent assisted ventilation, defined as the time to the earliest occurrence of one of the following events:
  - Death.
  - Permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days).

  This endpoint will be adjudicated by a blinded, independent group of experienced clinicians, the Event Adjudication Committee (EAC), based on review of clinical study data and supporting information.
- Time to death.

[redacted]

1.2 Secondary objectives and endpoints

The secondary objectives of Part C are to evaluate the safety, tolerability, pharmacodynamic (PD) and biomarker effects of tofersen administered to adult subjects with ALS and a confirmed SOD1 mutation.

- The safety/tolerability endpoint is the incidence of AEs and SAEs.
- The PD endpoint is the change (i.e. ratio) from baseline in total SOD1 concentration in cerebral-spinal fluid (CSF).
- The biomarker endpoint is the change (i.e. ratio) from baseline in neurofilament light chain (NfL) concentration in plasma.
- Other safety endpoints are changes and shifts in abnormalities for:
  - clinical laboratory tests (serum chemistry, haematology, urinalysis, CSF laboratory parameters and coagulation)
  - Electrocardiogram (ECG)
  - Vital signs
  - Weight

- Neurological Exam
- Mini-mental state exam (MMSE)
- Columbia suicide severity rating scale (C-SSRS)

[redacted]

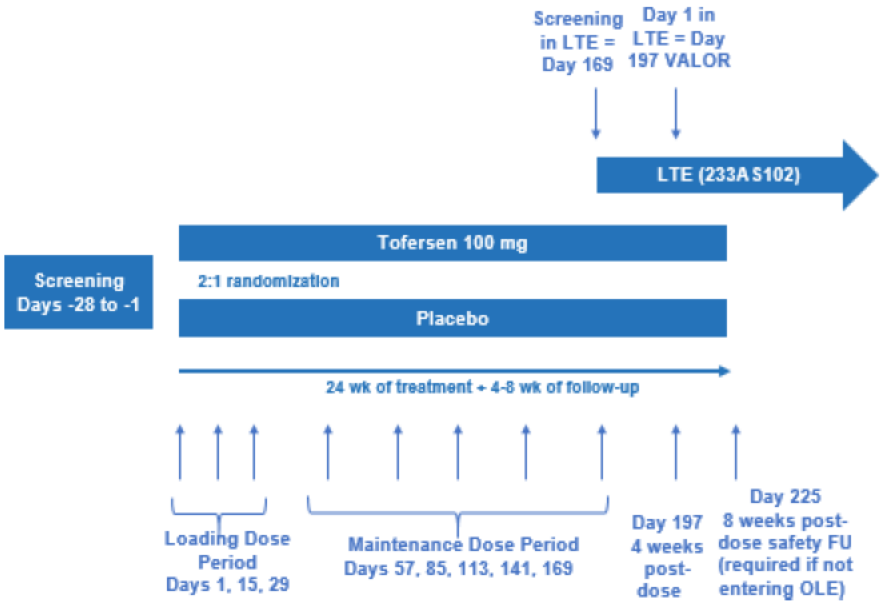
[redacted]

2. Study Design and Conduct

2.1 Study Description

Part C is a randomized, double-blind, placebo-controlled evaluation of 100 mg of tofersen administered 8 times over approximately 24 weeks to approximately 99 subjects with SOD1-ALS (Figure 1).

Figure 1: Study Design



It is planned to randomize approximately 99 subjects total, including approximately 60 subjects who meet the protocol-defined prognostic enrichment criteria for rapid disease progression.

Subjects included in the study must have weakness attributable to ALS and a confirmed SOD1 mutation.

i. SOD1 mutation must be confirmed by the central reader based on the sample obtained during the Screening Visit; subjects with a SOD1 mutation interpreted by the central reader to be pathogenic or likely pathogenic will be eligible.

ii. Additionally:

- Prognostic enrichment criteria for rapid disease progression (subjects may be eligible based on 1 of the following 2 criteria) [Hamidou 2017; Proudfoot 2016]

    a. One of the following SOD1 mutations and a prerandomization ALSFRS-R decline slope ≥ 0.2 per month (calculated as [48-baseline score]/time since symptom onset):

    p.Ala5Val, p.Ala5Thr, p.Leu39Val, p.Gly42Ser, p.His44Arg, p.Leu85Val, p.Gly94Ala, p.Leu107Val, and p.Val149Gly

    OR

    b. SOD1 mutation other than those listed in item ‘a.’ with prerandomization ALSFRS-R decline slope ≥ 0.9 per month (calculated as [48-baseline score]/time since symptom onset)

- Criteria for all other eligible subjects: SOD1 mutation other than those listed in item ‘a.’ (no ALSFRS-R decline slope requirement). These are subjects who have a pathogenic or likely pathogenic SOD1 mutation not listed in ‘a’ with a prerandomization ALSFRS-R decline slope < 0.9 per month.

    Note: subjects with a SOD1 mutation listed in item ‘a’ who have a prerandomization decline slope <0.2 per month are not included in the study.

For participants who meet prognostic enrichment criteria for rapid disease progression, SVC ≥ 65% of predicted value at screening as adjusted for sex, age, and height (from the sitting position). For all other eligible subjects must have SVC ≥ 50% of predicted value as adjusted for sex, age, and height (from the sitting position).

Randomization will be stratified by the following 3 factors:

- whether a subject meets the prognostic enrichment criteria for rapid disease progression;
- whether a subject uses edaravone at baseline;
- whether a subject uses riluzole (but not edaravone) at baseline.
    - Use of both edaravone and riluzole will not be a separate stratum but will be classified as edaravone use.

For subjects participating in Part C (Pivotal), the study duration will be approximately 32 to 36 weeks in duration including a 4-week screening period, a 24-week treatment period (3 loading doses of tofersen or placebo 2 weeks apart, followed by 5 maintenance doses of tofersen or placebo 4 weeks apart), and a 4 to 8 week follow-up period as follows:

Part C subjects who enroll (uninterrupted) in the long term extension study 233AS102 Week 28/Day 197 Visit will serve as EOS.

After completing the EOS visit, subjects will be considered study completers and they will have the option to participate in 233AS102, if eligible. This will be done without unblinding to subject's treatment group.

For subjects with delays between their Week 28/Day 197 Visit and enrollment in 233AS102 study, a Safety FU (Alternative EOS) Visit will be conducted (either in person or by telephone contact) to collect AEs, SAEs, and concomitant medications or procedures. This Safety FU (Alternative EOS) Visit can be performed at Week 32 or prior to (within 2 days) enrollment in 233AS102, whichever comes first.

For Part C subjects who do not enroll in 233AS102, Week 32/Day 225 will serve as the EOS (either in person or by telephone contact) to collect AEs, SAEs, and concomitant medications or procedures.

End of study date is the last visit or contact with the subject.

In the event of a decision by the Study Sponsor to terminate the study earlier on the grounds that conducting a placebo-controlled study is no longer deemed ethical based on the assessment of risk-benefit of Study 233AS101, all subjects will be invited for an EOS visit, during which all Week 28/Day 197 assessments will be conducted. These subjects not entering 233AS102 will have an additional safety follow-up visit 8 weeks after the last dose of study treatment.

Subjects who discontinue study treatment in Part C will be encouraged to remain in the study and complete all appropriate protocol-specified tests and assessments. It is recommended that subjects who terminate early (i.e., discontinue both study treatment and study assessments) perform the assessments of the Week 28/Day 197 End of Study (EOS) visit within 4 weeks after the last dose of study treatment. Home assessments will be allowed at the discretion of the Investigator for subjects who cannot come to the site in person for their Early Termination Visit.

2.2 General Trial Conduct Mitigation Strategy under COVID-19 Pandemic

In order to mitigate risk of missing dosing or assessments during the time of the COVID-19 pandemic, the following has been put into place:

- If a site is not closed due to COVID-19 and the site enables the subject to attend the visit in-clinic, then delayed visits within a reasonable timeframe are allowed. In the analysis, for the loading dose period, a 7-day window will be used and a 14-day window will be used for the maintenance visits.

- Site transfers are encouraged where possible, so that subjects can be transferred to another site for assessment. Instructions are in place to enable sites to do this including transfer of the database and source documents for the patient from the transferring site to the receiving site. This will be the optimal option in order to perform dosing and all assessments for the subject, since dosing can only be performed in the clinic setting due to intrathecal administration. In particular, if screening cannot be performed in the clinic, subjects will be screened at a screening site and subsequently transferred to another site. Site transfer instructions and travel plans were already in place prior to COVID-19 pandemic to allow for subjects to travel to sites if a site was not open in their country of residence.
- If site transfers cannot be performed, subjects will be allowed to have home visits if agreed to by the participating site. Instructions are in place on how this will work. If a home visit is performed, ALSFRS-R will still be performed by the blinded rater at the site via telephone. Due to the nature of the ALSFRS-R, as a status assessment, the informed consent allows for telephone visits to collect safety data. It has been shown that there is a strong correlation (r^2=0.941, P < 0.05) between ALSFRS-R administered to patients in the clinic and by telephone (Mannino, 2006). Ventilation diaries recorded on paper will be collected by the staff performing home visits and provided directly to the sites.
- Currently if home visits are not possible or not yet set up for the participating site, a phone visit can be used to collect safety data (AEs, SAEs, C-SSRS, concomitant medications/procedures) and ALSFRS-R.
- Patient reported outcomes were not allowed to be collected by telemedicine based on the original informed consent. An addendum to the global informed consent will allow these assessments to be collected over the telephone after the date it is effective.
- Due to regulations at sites, some sites may allow a clinic visit but not allow SVC to be performed in which case it is preferable to perform this assessment as a home visit or transfer the patient to another site.
- Transferring sites are encouraged to enter and clean all data before transferring the subject to the receiving site. The database for a subject will remain with the transferring site until data are entered and cleaned as much as possible, but the transferring site must have access to the subject in IRT and copies of all source documents. Blinding of staff will be maintained as appropriate. Any queries raised later on for data belonging to visits at the transferring site will be cleaned by the receiving site through the source documents, and interaction between monitors at the receiving and transferring sites. The transferring site will no longer have access to the database for a subject they have transferred but the monitors will. The Subject ID will remain the same when a subject is transferred to another site, but the transfer can be tracked via the Site ID.

2.3 Detail of Assessments Not Collected According to Protocol

In order to handle data appropriately for analysis, the method of collection for assessments is also being collected in the database. Table 1 shows the schedule of assessments. There are some points to note around differences in data collection between protocol amendments for 233AS101 Part C.

- The protocol specified that the same qualified and trained ALSFRS-R rater should consistently perform the ALSFRS-R for a subject. Protocol Version 6 allowed for a trained and qualified back-up rater to perform the ALSFRS-R in case the primary rater was not available. The raters should remain blinded to subjects' treatment assignments and to the results of other assessments. Having a back-up rater is also important in light of the COVID-19 pandemic where it may not be possible to keep the same rater throughout. In an inter-rater reliability assessment (Kaufman, 2007), the primary raters performed 54 ratings on 9 patients showing ICC = 0.93 (95% CI 0.84–0.98). For back-up raters, 32 ratings on nine patients resulted in ICC = 0.93 (95% CI 0.82–0.98). The intra- rater reliability for in- person interviews was ICC = 0.95 (95% CI 0.92–0.98). The reliability of telephone administration compared to in- person interviews was ICC = 0.97 (95% CI 0.93–0.98).
- [redacted]
- In addition MMSE was initially collected at the same time as Neurological Examination but in Protocol Version 6 the frequency of the MMSE was reduced to every 3 months during the treatment period of Part C, as there had been no significant safety findings on this assessment seen in Part B of the study. Therefore, some subjects will have MMSE collected pre- and postdose at all postbaseline visits while some subjects will only have MMSE collected at Days 85, 169 and 197 during the treatment period.
- [redacted].
- [redacted]
- At Day 197, blood samples of [redacted], and anti-BIIB067 Ab as well as CSF samples were intended to be collected as part of baseline for 233AS102. However, they were inadvertently omitted from the schedule of activities at Day 197 of 233AS101 Part C. Also, for subjects who do not enter 233AS102, blood samples would need to be collected

at Day 197. There may be a few patients who do not have the plasma or CSF samples available at Day 197. In particular, for subjects who cannot attend a clinic visit due to COVID-19 or subjects who do not enter 233AS102 they may not have these samples collected (blood samples can be collected at home visits, if a home visit is possible). CSF sampling is associated with a lumbar puncture and is generally only performed as part of study drug administration therefore some subjects are unlikely to have CSF samples available at Day 197.

- ECG was intended to be collected postdose as well as predose but this was inadvertently omitted from the schedule of activities until Protocol Version 6. In addition, there was a misinterpretation of which dosing days ECG should be collected at and was being collected at all dosing visits, although it should only have been collected at dosing Days 85 and 169. This was clarified under Protocol Version 7. Some subjects therefore also have additional ECG data at Days 15, 29, 57, 113 and 141.

As a general note, Day 197 should serve as the same visit as Baseline in 233AS102 for the majority of subjects. The data should be entered under the 233AS101 Part C database but there may be a few exceptions where the incorrect lab kit was used from 233AS102 and so the data are entered under Baseline in 233AS102. In that situation where Day 197 for 233AS101 Part C and Baseline for 233AS102 are the same visit, the data will be taken from the 233AS102 database and incorporated into the datasets at the CDISC SDTM level. There is also the scenario where Baseline for 233AS102 did not occur at Day 197 of 233AS101 Part C but a Day 197 lab kit was used for baseline. Therefore, in that scenario, the baseline data will be taken from Day 197 of 233AS101; it will be clear from date of sample collection as to which visit these data are associated with. This will be handled at the SDTM level.

Table 1: Schedule of Activities: Part C (Pivotal)

	Screening[1]	**Loading Dose Treatment Period**									**Maintenance Treatment Period**			**EOS Visit**[2,3]	**Safety FU (Alternative EOS) Visit** [4] **(In Person or Telephone Contact)**
		Baseline (Dose 1)			**Week 2 (Dose 2)**			**Week 4 (Dose 3)**			**Week 8, 12, 16, 20, and 24**			**Week 28 OR (4 Weeks After Last Dose)**	**Week 32 OR (8 Weeks After Last Dose)**
Tests and Assessments[5]	**Day -28 to Day -1**	**Day 1 (±3 days)**			**Day 15 (±3 days)**			**Day 29 (±3 days)**			**Days 57, 85, 113, 141, and 169 (±3 days)**			**Day 197 OR 4 Weeks After Last Dose (±3 days)**	**Day 225 OR 8 Weeks After Last Dose (±3 days)**
		Pre dose	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**		
Informed Consent (main)[6]	X														
[redacted]															
Medical History	X														
Confirmation of Eligibility Criteria	X	X													
Randomization		X													
Telephone Contact[7]				X			X			X			X		
Ventilation Use[8, 9]		X			X			X			X			X	
ALSFRS-R[8]	X	X			X			X			X			X	
SVC[8,10]	X	X			X			X			X			X	

	Screening[1]	**Loading Dose Treatment Period**									**Maintenance Treatment Period**			**EOS Visit**[2,3]	**Safety FU (Alternative EOS) Visit** [4] **(In Person or Telephone Contact)**
		Baseline (Dose 1)			**Week 2 (Dose 2)**			**Week 4 (Dose 3)**			**Week 8, 12, 16, 20, and 24**			**Week 28 OR (4 Weeks After Last Dose)**	**Week 32 OR (8 Weeks After Last Dose)**
Tests and Assessments[5]	**Day -28 to Day -1**	**Day 1 (±3 days)**			**Day 15 (±3 days)**			**Day 29 (±3 days)**			**Days 57, 85, 113, 141, and 169 (±3 days)**			**Day 197 OR 4 Weeks After Last Dose (±3 days)**	**Day 225 OR 8 Weeks After Last Dose (±3 days)**
		Pre dose	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**		
HHD[8]		X			X			X			X			X	
[redacted]															

	Screening[1]	**Loading Dose Treatment Period**									**Maintenance Treatment Period**			**EOS Visit**[2,3]	**Safety FU (Alternative EOS) Visit** [4] **(In Person or Telephone Contact)**
		Baseline (Dose 1)			**Week 2 (Dose 2)**			**Week 4 (Dose 3)**			**Week 8, 12, 16, 20, and 24**			**Week 28 OR (4 Weeks After Last Dose)**	**Week 32 OR (8 Weeks After Last Dose)**
Tests and Assessments[5]	**Day -28 to Day -1**	**Day 1 (±3 days)**			**Day 15 (±3 days)**			**Day 29 (±3 days)**			**Days 57, 85, 113, 141, and 169 (±3 days)**			**Day 197 OR 4 Weeks After Last Dose (±3 days)**	**Day 225 OR 8 Weeks After Last Dose (±3 days)**
		Pre dose	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**		
C-SSRS Questionnaire[8]		X						X[13]			X[13]			X[13]	
Height	X														
Weight[14]	X	X									X			X	
Vital Signs (temperature, blood pressure, pulse rate, respiratory rate)[14,15]	X	X		X	X		X	X		X	X		X	X	
12-Lead ECG[14,16]	X	X		X[17]							X[11]		X[17]	X	
Physical Examination[14]	X	X			X[18]			X[18]			X[18]			X[18]	
Limited Neurological Examination[14,19]	X	X		X	X		X	X		X	X		X	X	
MMSE	X	X		X							X[11]		X[11]	X	
FSH Test[20]	X														
Pregnancy Test[14,21,22]	X (serum)	X			X			X			X			X (serum)	

	Screening[1]	**Loading Dose Treatment Period**									**Maintenance Treatment Period**			**EOS Visit**[2,3]	**Safety FU (Alternative EOS) Visit** [4] **(In Person or Telephone Contact)**
		Baseline (Dose 1)			**Week 2 (Dose 2)**			**Week 4 (Dose 3)**			**Week 8, 12, 16, 20, and 24**			**Week 28 OR (4 Weeks After Last Dose)**	**Week 32 OR (8 Weeks After Last Dose)**
Tests and Assessments[5]	**Day -28 to Day -1**	**Day 1 (±3 days)**			**Day 15 (±3 days)**			**Day 29 (±3 days)**			**Days 57, 85, 113, 141, and 169 (±3 days)**			**Day 197 OR 4 Weeks After Last Dose (±3 days)**	**Day 225 OR 8 Weeks After Last Dose (±3 days)**
		Pre dose	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**		
CSF Sampling for [redacted], PD, Safety, and Biomarkers[14,23,24]		X			X			X			X			X	
Blood Sampling for HIV, HCV, and HBV Testing	X														
Clinical Laboratory Samples for Hematology, Coagulation, Chemistry, and Urinalysis[8,23]	X	X			X			X			X			X	
[redacted]															
[redacted]															

	Screening[1]	**Loading Dose Treatment Period**									**Maintenance Treatment Period**			**EOS Visit**[2,3]	**Safety FU (Alternative EOS) Visit** [4] **(In Person or Telephone Contact)**
		Baseline (Dose 1)			**Week 2 (Dose 2)**			**Week 4 (Dose 3)**			**Week 8, 12, 16, 20, and 24**			**Week 28 OR (4 Weeks After Last Dose)**	**Week 32 OR (8 Weeks After Last Dose)**
Tests and Assessments[5]	**Day -28 to Day -1**	**Day 1 (±3 days)**			**Day 15 (±3 days)**			**Day 29 (±3 days)**			**Days 57, 85, 113, 141, and 169 (±3 days)**			**Day 197 OR 4 Weeks After Last Dose (±3 days)**	**Day 225 OR 8 Weeks After Last Dose (±3 days)**
		Pre dose	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**	**Pre dose**	**LP**	**Post dose**		
Blood Samples for anti-BIIB067 Ab[8]		X			X			X			X			X	
[redacted]															
Blood Sampling for Biomarkers[8]		X			X			X			X			X	
[redacted]															
Study Drug Administration[28]			X			X			X			X			
AE and Concomitant Therapy and Procedures Recording		X (ongoing)													
SAE Recording	X (ongoing)														

Ab = antibody; AE = adverse event; ALS = amyotrophic lateral sclerosis; [redacted] ALSFRS-R = ALS Functional Rating Scale- Revised; EOS = end of study; [redacted]; CSF = cerebrospinal fluid; C-SSRS = Columbia Suicide Severity Rating Scale; DNA = deoxyribonucleic acid; ECG = electrocardiogram; [redacted]; FSH = follicle-stimulating hormone; [redacted]; FU = follow-up; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HHD = handheld dynamometry; LP = lumbar puncture; LTE = long-term extension; MMSE = Mini-Mental State Examination; [redacted]; PD = pharmacodynamics; [redacted] SAE = serious adverse event; [redacted] SVC = slow vital capacity; [redacted]

[1] Some sites may only participate in screening procedures.

[2] After completing the EOS visit, subjects will be considered study completers and will have the option to participate in the LTE study 233AS102.

[3] Subjects who terminate early (i.e., discontinue both study treatment and assessments) will be asked to complete the assessments of the EOS Visit within 4 weeks after the last dose of study treatment.

[4] Subjects who do not roll over into the LTE study will have an additional safety follow-up visit at Week 32 or 8 weeks after the last dose of study treatment. For participants with delays between their Week 28 Visit and enrollment in the LTE study, a Safety FU (Alternative EOS) Visit will be conducted (either in person or by telephone contact) to collect AEs, SAEs, and concomitant medications or procedures. This Safety FU (Alternative EOS) Visit can be performed at Week 32 or prior to (within 2 days) enrollment in the LTE, whichever comes first.

[5] All visits are expected to take place on site unless subjects are unable to travel to the site; then, a home visit may be possible at the discretion of the Investigator.

[6] The main informed consent form will include a genetic consent for collection of DNA samples to confirm presence of SOD1 mutation and to be used for analyses of specific genes related to ALS or the response to BIIB067. DNA and RNA collection for possible future research will be optional in all regions where not prohibited by regulatory authorities or ethics committees. Consent for optional sample collection will be collected in a separate document.

[7] Telephone contact to be performed approximately 24 hours after LP.

[8] Predose assessments can be performed in a single visit up to 2 days prior to dosing.

[9] Subjects will use a diary/eDiary to record ventilation use. The diary/eDiary should be completed only for days when the participant uses mechanical ventilation. This diary/eDiary will be reviewed with study site staff at each visit.

[10] If a facemask is used at screening and/or baseline it should be used for all SVC assessments for the duration of the study. If a facemask is not used at screening and / or baseline it should not be used for the duration of the study. Upright SVC will be determined by performing 3 to 5 measures. The results will be overread by a central reader to confirm that these criteria (at least 3 acceptable tests with the 2 highest acceptable (largest and next largest) efforts within 150 mL of vital capacity) have been achieved.

[11] To be performed on Week 12 (Day 85) and Week 24 (Day 169) only.

[redacted]

[13] Use "Since Last Visit" version of C-SSRS.

[14] Assessment must occur on the day of dosing.

[15]Temperature, systolic and diastolic blood pressure, pulse rate and respiratory rate will be measured after the subject has rested in a sitting position for at least 5 minutes.

[16]Triplicate 12-lead ECGs will be obtained after the subject has rested in a supine position for at least 10 minutes. All ECGs will be centrally read; ECGs will be read by the Investigator at collection and then sent to a central ECG reader for further evaluation and to confirm eligibility at Screening. The first ECG will be interpreted, and the last 2 will be checked for consistency and quality.

[redacted]

[18]Limited physical examination will be conducted at the Investigator's discretion.

[19]The components of the limited neurological examination include motor, reflexes, and coordination/cerebellar function.

[20]To confirm postmenopausal status (in postmenopausal female subjects only).

[21]Results from urine or serum pregnancy tests must be reviewed predose in order to determine if dose should be administered or held.

[22]For women of childbearing potential only. Serum test to be performed at Screening and EOS visit; urine or serum test to be performed at all other timepoints.

[23]The results of most recent (i.e., obtained at previous visit) centrally read coagulation tests and platelet count must be reviewed before LP can be performed. Coagulation tests may be repeated at local laboratory once if, in the opinion of the Investigator, values of the initial tests are out of range but deemed not clinically significant)

[24]CSF samples collected during the LP procedure will be analyzed to evaluate for blood contamination. CSF samples for safety will be tested at local laboratories.

[25]Required for all subjects in order to document SOD1 mutation, regardless of presence or absence of prior documentation. For subjects who do not have previously documented SOD1 mutation genotype, it is strongly recommended to review the centrally read results before continuing any other screening assessments. Centrally read results for all subjects must be available before randomization

[redacted]

[28]Subjects will remain at the study site for at least 1 hour postdose for safety monitoring and can be discharged at the discretion of the Investigator and in compliance with the institutional requirements once the subjects have adequately recovered from the dosing procedure.

3. Sample Size Justification

For Part C, approximately 99 subjects will be randomized, with approximately 66 subjects administered tofersen 100 mg and approximately 33 subjects administered placebo in a 2:1 ratio.

This sample size for Part C is selected primarily based on the joint rank test combining the Week 28 change from baseline in ALSFRS-R and mortality, in the mITT population (N = 60). The observed results from an interim analysis of Study 233AS101 in the fast progressor group as well as the observed ALSFRS-R decline in subjects who received placebo in a similar fast progressor ALS population with SOD1 mutation [Benatar 2018] are the basis of assumed treatment effect and the variability. Subjects from both datasets were matched with the prognostic enrichment criteria for rapid disease progression specified in this protocol for Part C (i.e., one of the protocol-defined SOD1 mutations and a pre-randomization ALSFRS-R decline slope ≥ 0.2 per month or a pre-randomization ALSFRS-R decline slope ≥ 0.9 with SOD1 mutation NOT on the protocol-defined list). This resulted in a total of 12 placebo control matched subjects (8 from the historical placebo data [Benatar 2018] and 4 from Study 233AS101) and 4 matched subjects treated with tofersen 100 mg from interim data of a very small cohort in Study 233AS101. The observed mean slope of decline was -3.83 for the matched placebo subjects and -0.74 for the matched tofersen 100 mg subjects, with a pooled SD of 3.166. In the 12 matched subjects on placebo, there was 1 death and 2 subjects with permanent assisted ventilation less than 6 months but none in the tofersen 100 mg matched subjects. Based on this and overall survival data in fast progressors from the literature on subjects with an A4V SOD1 mutation, the survival at Week 28 was assumed to be 82% in the placebo control and 90% in the tofersen 100 mg group.

Under the above assumptions, with N = 60 subjects in the mITT population and a two-sided significance level of 0.05, the joint rank test gives 84% power. Because events of death have been factored into the analysis, no sample size overage is planned for missing values.

For the population outside of the mITT population, the primary focus will be on the PD endpoint, total CSF SOD1 protein concentration, as this population is anticipated to have a slower decline in clinical function compared to the mITT population. A sample size of 26 subjects in the treated group and 13 subjects in the placebo group for the population outside of the mITT would provide 97% power to detect a 25% reduction in total CSF SOD1 from baseline in the treated group, with an assumed SD of 0.216 (natural log scale), compared to the placebo group.

The COVID-19 mitigation plan outlined earlier lowers the risk of missed doses and assessments and is in place to avoid the need for a sample size increase.

4. General Considerations

4.1 Statistical Methods: General Considerations

Descriptive summary statistics will be presented for all primary, secondary and exploratory endpoints collected. Unless otherwise specified, for continuous endpoints, the summary statistics will generally include number of subjects with data, mean, standard deviation, median, 25th

percentile, 75th percentile, minimum and maximum. For categorical endpoints, the summary statistics will generally include number of subjects with data, and the percentage of those with data in each category.

All summaries and listings will be presented by treatment group unless otherwise specified. For the purpose of the efficacy and pharmacodynamic outputs, the mITT and non mITT populations will be used to describe the subset of subjects who meet the prognostic enrichment criteria for rapid disease progression, and all other eligible subjects, respectively. For the purpose of safety and pharmacokinetic outputs, any summaries for disease progression subgroup will present the subgroup categories as 'enriched' for subjects who meet the prognostic enrichment criteria for rapid disease progression, and "other" for all other eligible subjects. Listings will also indicate which subgroup the subject belongs to ("enriched" "other"). Visits in listings will be displayed as per CRF data collection rather than analysis visits.

Treatment groups will be labelled as "Placebo" and "tofersen 100 mg".

Unless otherwise specified, the baseline value is defined as the last non-missing value prior to first dose. Change from baseline is defined as postbaseline value minus baseline value.

Unless otherwise stated, all statistical tests for primary and secondary efficacy endpoints will be 2-sided with overall Type I error control at the 5% statistical significance level.

The statistical software, SAS®(Version 9.4) will be used for all summaries and statistical analyses.

4.2 Analysis Populations

- Subjects in Part C Randomized subjects

These are subjects enrolled who received a randomization treatment assignment from the Interactive Response Technology (i.e. tofersen 100 mg or placebo).

- ITT Population

The overall ITT population is defined as all subjects in Part C who are randomized and received at least 1 dose of study treatment.

- Modified ITT (mITT) Population

The modified intent-to-treat (mITT) population is defined as all subjects who meet the prognostic enrichment criteria for rapid disease progression in Part C who are randomized and received at least 1 dose of study treatment. The criteria are defined in Section 2.

- Non mITT Population

This is the subset of subjects in the overall ITT population who are not included in the mITT population i.e. all other eligible subjects (criteria defined in Section 2).

- Per-protocol population

This is the subset of subjects in the mITT population who did not have a protocol deviation that violated the following criteria:

- Missed at least one dose of study drug during the loading dose period (i.e. Day 1 to Day 29)
- ALSFRS-R not performed by a blinded rater (i.e. not blinded to other assessments for the subject) as recorded on the protocol deviation log (or if for some reason the rater was not certified at the time of assessment).

- [redacted]

- Safety Population

The safety population is defined as all subjects in Part C who are randomized and received at least one dose of study treatment (i.e. the overall ITT population of subjects in Part C).

- Immunogenicity Population

The analysis population for immunogenicity is defined as all subjects who received at least one dose of study treatment and have at least 1 postdose sample evaluated for immunogenicity in Part C.

Populations used for analysis

The primary analyses of clinical function will be evaluated in the mITT population, comprising the subset of subjects who meet prognostic enrichment criteria for rapid disease progression who are randomized and receive at least 1 dose of study treatment. The primary analysis population comprised participants who met prognostic enrichment criteria for rapid disease progression to enable detection of a statistically significant and clinically meaningful treatment effect on clinical function over the 6 month treatment duration. The treatment effect of clinical function endpoints outside of the mITT population (i.e. non mITT) will be analyzed where relevant, as described in Section 6. The clinical function data in the overall ITT population will be summarized and analyzed where appropriate.

[redacted]

All safety evaluations will be summarized using the safety population.

[redacted]

There will not be a separate pharmacodynamic population for the PD endpoints [redacted] These endpoints will all be analyzed/summarized as appropriate for each of the mITT population, the non mITT population and the overall ITT population, as described in the relevant sections.

Dealing with errors in randomization, study drug dispensation and stratification

Analyses using the mITT population, non mITT population and overall ITT population will be performed according to the treatment assignment at the time of randomization.

In case of any subjects who are incorrectly stratified when they are randomized the analyses will be based on the treatment they were randomized to. If the subject was stratified to disease progression subgroup incorrectly according to prognostic enrichment criteria ("enriched" "other") at randomization, the mITT population will be based on the correct population according to the clinical database. For analysis purposes e.g. when summarizing any of the stratification factors (population ("enriched" "other"), riluzole use, edaravone use) or including in statistical models or subgroup analyses, subjects will be included under the correct categorization according to the clinical database.

For the safety [redacted] populations, subjects will be analyzed under the actual treatment they received. If a subject received one or more doses of tofersen then the subject will be included under the tofersen treatment group for safety [redacted]. If a subject only received placebo then the subject will be included under the placebo group for safety.

If a subject is misrandomized, e.g. does not meet eligibility criteria, then the subject will be included in the overall ITT and safety populations. Depending on whether the subject is classed as part of the "enriched" population or not the subject would also be included under the relevant population for the mITT or the non mITT.

A listing of subjects whose actual treatment assignment is different from their randomized treatment group will be provided.

4.3 Visit Windows for Early Withdrawal, Unscheduled Visits and Early Termination Visits in the Event of Early Study Closure

For subjects who are early terminated (ET), EOS visit is defined as the scheduled follow-up visit 4 weeks after last dose (i.e. Week 28/Day 197 EOS visit). Week 32/Day 225 is only intended to collect adverse events and concomitant medications for participants who do not proceed into the 233AS102 extension study and thus will not need to be windowed.

If, due to the COVID-19 pandemic, subjects could not attend clinic visits during protocol defined windows, they were given some allowance to have a delayed visit. There may also have been subjects who attended visits outside the protocol defined windows for other reasons. Regardless of whether the delayed visit was due to the COVID-19 pandemic or not, these are recorded under scheduled visits and are also considered to be protocol deviations. Visit windows will not be applied to scheduled visits for analysis purposes. If a subject had a phone visit followed by a delayed clinic visit, the clinic visit is still recorded as the scheduled visit. The scheduled visit will always take precedence over unscheduled visits.

Data from early withdrawal visits, postbaseline unscheduled assessments and early termination visits in the event of early study closure will be assigned to an appropriate scheduled postbaseline visit using a windowing scheme for assessments that are tabulated or summarized by visit. Scheduled visits will not be windowed.

The visit windowing for efficacy [redacted] is given in the tables below:

Table 2: Visit Windows for Primary and Secondary Efficacy Endpoints

	ALSFRS-R/SVC/HHD		
Visit	Lower Bound	Upper Bound	Target Day
Day 15	8	21	15
Day 29	22	42	29
Day 57	43	70	57
Day 85	71	98	85
Day 113	99	126	113
Day 141	127	154	141
Day 169	155	182	169
Day 197	183	211	197

[redacted]

The visit windowing for safety assessments is given in the tables below:

Table 4: Visit Windows for Safety Endpoints Part 1 and [redacted] PD/Biomarker Endpoints

	Safety Endpoints*		
Visit	Lower Bound	Upper Bound	Target Day
Day 15	8	21	15
Day 29	22	42	29
Day 57	43	70	57
Day 85	71	98	85
Day 113	99	126	113
Day 141	127	154	141
Day 169	155	182	169
Day 197	183	211	197

* This windowing covers the following safety endpoints: vital signs, neurological exam (including MMSE), all lab tests (including pregnancy; CSF sampling; lab tests for haematology, chemistry, coagulation and urinalysis; urine sample for biomarkers, blood samples for anti-BIIB067 antibodies, [redacted] and blood samples for biomarkers) Note: Weight is not collected at Days 15 and 29 so no assessments will be windowed to those visits. All other windows are as above. Based on Protocol Version 6, some subjects will have MMSE collected less frequently during postbaseline i.e., Days 85, 169 and 197 so these subjects will not be summarized at other postbaseline visits. The outputs will include a clarification footnote.

Table 5: Visit Windows for Safety Endpoints Part 2

	C-SSRS			ECG*		
Visit	Lower Bound	Upper Bound	Target Day	Lower Bound	Upper Bound	Target Day
Day 15	n/a	n/a	n/a	n/a	n/a	n/a
Day 29	8	42	29	n/a	n/a	n/a
Day 57	43	70	57	43	70	57
Day 85	71	98	85	71	98	85
Day 113	99	126	113	99	126	113
Day 141	127	154	141	127	154	141
Day 169	155	182	169	155	182	169
Day 197	183	211	197	183	211	197
* ECG is not intended to be collected at Days 57, 113 and 141 but due to a misinterpretation a substantial number of subjects have ECG collected at these visits and so these data will also be summarized. Not all subjects will have data at these additional visits and so the outputs will include a clarification footnote.						

Considering the high frequency of postbaseline visits and the relatively small gap between adjacent visits, the lower bound and the upper bound for the visit windows are based on the midpoints of the scheduled visits. The date of first dose will be the reference point (Day 1).

- If more than one observation is within the same window, data from the regular scheduled visit will be used for that visit.
- If neither of the observations are from a regular scheduled visit the observation closest to the planned target date will be used. However, if both the observations are equidistant from the target date, the latest one will be used; if repeated measurements are on the same day, then the last measurement will be used.
- If there is more than one observation in a window for a dosing visit (Day 15 to Day 169 visits), the observation on the day of dosing will be chosen over the observation where dosing did not occur on the same day.
- Some of the safety assessments are collected postdose in addition to the predose assessments (e.g. vital signs and neurological exam at Day 15 to Day 169 visits, ECG and MMSE at Day 169, [redacted] When windowing any predose assessments these should be the last available assessment prior to the dose. When windowing any postdose assessments they should correspond to the same dose as the predose assessment used for that visit. These summaries will present predose and

postdose separately on by-visit summaries. If one of these assessments is collected at an unscheduled visit where there is no dosing, the assessment will be assigned as a predose assessment.

- As a general note, Day 197 should serve as the same visit as Baseline in 233AS102 for the majority of subjects. The data should be entered under the 233AS101 Part C database but there may be a few exceptions where the incorrect lab kit was used from 233AS102 and so the data are entered under Baseline in 233AS102. In that situation where Day 197 for 233AS101 Part C and Baseline for 233AS102 are the same visit, the data will be taken from the 233AS102 database and incorporated into the datasets at the CDISC SDTM level. If samples are accidentally collected under both 233AS101 Part C and the 233AS102, the data under 233AS101 Part C will be used for Day 197. Similarly, for Screening Visit of 233AS102 and Week 24 of 233AS101 Part C, data will be taken from 233AS101 Part C Week 24 in case lab kits from both studies were used

[redacted]

5. Study Subjects

5.1 Subject Accountability

The number (and percentage) of subjects randomized; dosed; completed the treatment; completed the study; completed the study but missed one or more doses; discontinued treatment and the reasons for discontinuation; and withdrew from study early and the reasons for withdrawal will be summarized in a table. These summaries will be presented by disease progression subgroup (“enriched” “other”) and treatment.

If there are any subjects who discontinued from treatment or withdrew from study due to COVID-19 pandemic related reasons, a separate summary will be presented to summarize reasons for discontinuation and withdrawal from study for COVID-19 related reasons. Adverse events, deaths and other reasons may fall into COVID-19 related categories. The Other category will be broken down into the following categories:

Other – COVID-19 (Movement Restrictions Related to COVID-19 Pandemic)

Other – COVID-19 (Subject Fear Related to COVID-19 Pandemic)

Other – COVID-19 (Site Closed Due to COVID-19 Pandemic)

Other – COVID-19

A listing of subjects who discontinued treatment/withdrew from study and the associated reasons for discontinuation/withdrawal will be presented. A separate listing will also be presented for subjects who discontinued treatment/withdrew from study due to the COVID-19 pandemic.

The number (and percentage) of subjects randomized by country and site, the number (and percentage) of subjects who completed the study by country and site, and number (and percentage) of subjects by analysis population will be summarized by disease progression subgroup (”enriched” “other”) and treatment group, and for all subjects.

Listings will be provided to show subjects excluded from each population.

A summary of the stratification factors used for randomization (i.e. disease progression subgroup (“enriched” “other”), riluzole use and edaravone use) will also be presented by treatment group for all randomized subjects, based on the data in the clinical database. A randomization listing will be provided and will include stratification factors (disease progression subgroup (“enriched” “other”), riluzole use and edaravone use).

5.2 Demography and Baseline Disease Characteristics

All demographics and ALS disease history data will be summarized for the mITT population, the non mITT population and the overall ITT population by treatment group and overall.

Demographic data, including age (years), age category (18-<35,35-<50, 50-<65, ≥65), gender, ethnicity, race, height, weight and body mass index (BMI). The derivation for BMI is detailed in Appendix A.

ALS disease history will also be summarized descriptively and will include summaries of:

- confirmed SOD1 mutation (per analysis report of genetic sample collected at screening) i.e. Yes/No
- protocol defined mutation (i.e. p.Ala5Val, p.Ala5Thr, p.Leu39Val, p.Gly42Ser, p.His44Arg, p.Leu85Val, p.Gly94Ala, p.Leu107Val, and p.Val149Gly) (Not applicable for non mITT population)
- mutation type;
- time since ALS symptom onset (months);
- time since ALS diagnosis (months);

- site of onset (bulbar, lower limbs, upper limbs, respiratory, thoracic; subjects may be summarized under more than one site if subject has multiple sites of onset);
- pre-randomization ALSFRS-R slope;
- disease progression subgroup ("enriched" vs "other");
- riluzole usage/duration and edaravone usage/duration including number and percentage with both riluzole and edaravone use;
- ALS Milano-Torino Staging (MITOS) at baseline [Chio 2015] described in Appendix B;
- King's Stage for ALS at baseline [Balendra 2014] described in Appendix B.

A separate table will also be presented for the mITT population that lists the Subject ID, Treatment, SOD1 mutation, pre-randomization ALSFRS-R slope, Classification of SOD1 mutation (pathogenic/likely pathogenic), time since ALS diagnosis, screening and baseline total ALSFRS-R score, screening and baseline percent predicted SVC, time since symptom onset, site of onset, riluzole and edaravone usage/duration.

A listing will be presented for all screened subjects with details of the genetic testing at screening, including information on those who were reclassified for pathogenicity on their genetic test.

Details for any derivations are given in Appendix A.

Descriptive statistics for baseline clinical function for each of ALSFRS-R total score, percent predicted SVC and HHD megascore at screening and baseline will also be presented in a separate summary table. The table will also include a summary of total CSF SOD1 protein and NfL in plasma at baseline, a summary of whether a subject used a facemask for SVC at screening and baseline assessments, and whether a subject had any ventilation use at screening or baseline (determined also eDiary records if recorded, from Concomitant Non-Drug Treatment eCRF page in addition to the question asked on ALS Disease History eCRF page; the ALSFRS-R question on respiratory insufficiency will also be used as a check to ensure consistency). The summary will also present number and percentage of subjects with percent predicted SVC <65% at baseline in the mITT population, and with percent predicted SVC <50% at baseline in the non mITT population. For percent predicted SVC, a summary will also be presented of number and percentage of subjects with a baseline assessment that did not meet acceptability and repeatability ATS criteria A per Appendix B.

Medical history will be classified using MedDRA version 24.0. A summary of medical history by system organ class and preferred term will also be provided for the safety population. All demographics, baseline characteristics, disease history and medical history will also be listed.

A listing of subjects who transferred sites will also be included with the site ID for transferring and receiving site as well as the timing of the transfers.

5.3 Extent of Exposure and Study Drug Compliance

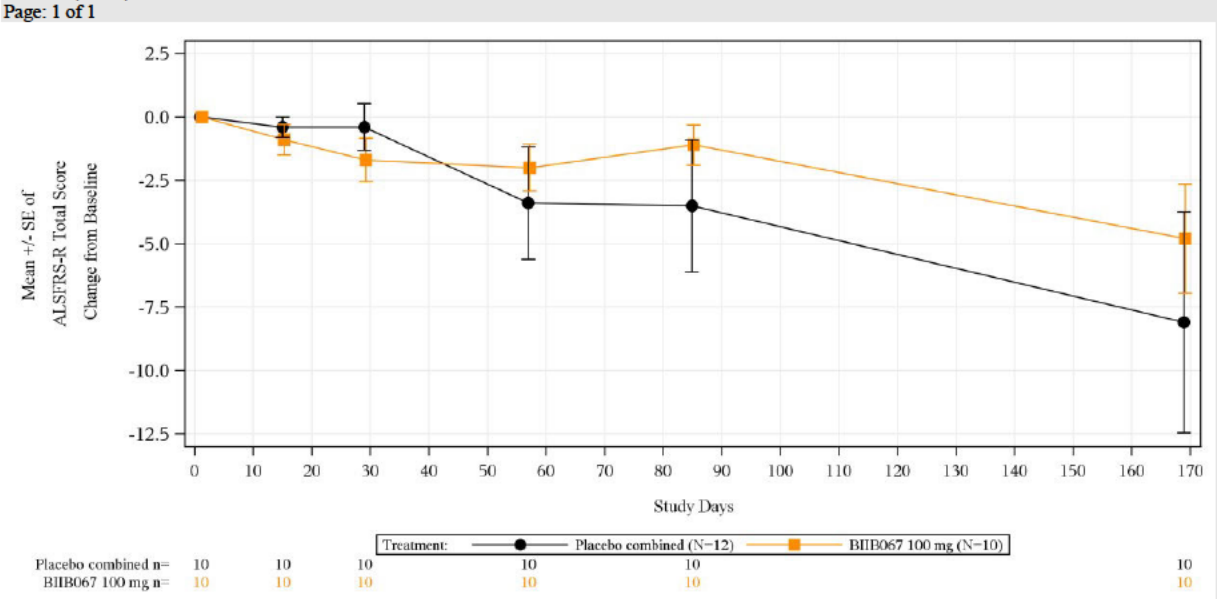
Summaries for extent of exposure to study treatment and study drug compliance will be summarized for the mITT population, the non mITT population and the overall ITT population.

A frequency distribution for number of doses received per subject will be summarized by treatment group, and descriptive statistics will be presented for total cumulative dose in mg. Total cumulative dose will be based on actual dose administered (calculated as volume administered multiplied by dose, and divided by 15mL).

Based on Phase 1 data from Part B following withdrawal of study drug and the half-life of tofersen (~1 month), missing more than 2 consecutive doses within the 24 week study period is considered likely to impact pharmacodynamic and efficacy results. Figure 2 shows the Phase 1 MAD observed data for ALSFRS-R total score in the tofersen 100 mg and placebo subjects who completed the study with data at all visits. The last dose is at Day 85, and ALSFRS-R shows a rebound at Day 169 after study drug withdrawal (i.e. approximately 3 months after the last dose).

Figure 2: Phase 1 MAD Data for ALSFRS-R Total Score in Completers (tofersen 100 mg mg vs Placebo)

ALSFRS-R total score mean change from baseline values +/- SE by visit (observed data) for completers to Day 169 (BIIB067 100 mg and placebo) - Part B (MAD) clinical function set
Page: 1 of 1



NOTE 1: Baseline is defined as day 1 value prior to the study drug and presented as Day 1 If day 1 value is missing, the non-missing value(including screening visit) closest to and prior to the first dose will be used as the baseline value
NOTE 2: A positive change indicates improvement
NOTE 3: To aid interpretation of completers, Day 92 is not presented as this assessment was not required for subjects prior to the protocol amendment
NOTE 4: Data points where n = 1 have not been presented
Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised; MMRM = mixed model for repeated measures
Source:biib067/233as101/fda/f-cf-byvis-p100-meanchg-od-c169n-alsfrs-t sas **Run Date:**20OCT2020

windows in Table 6:

Table 6: Protocol-Defined Visit/Dose Windows

Visit	Lower Bound	Upper Bound	Target Day
Day 15	12	18	15
Day 29	26	32	29
Day 57	54	60	57
Day 85	82	88	85
Day 113	110	116	113
Day 141	138	144	141
Day 169	166	172	169
Day 197	194	200	197

The following summaries will be presented by treatment group within each of the mITT, non mITT and overall ITT populations:

- Total number of expected doses across subjects based on actual subject duration in study; total number of missed doses and total number of delayed doses (percentages calculated out of total number of expected doses);
- A frequency distribution for number of consecutive missed doses per subject;
- A frequency distribution for overall number of missed doses per subject (i.e. regardless of whether they were consecutive). The mean (SD) will be presented for the mean number of missed doses per subject. Subjects with no missed doses will be included as having 0 missed doses.
- The mean (SD) number of delayed doses per subject will also be summarized. Subjects with no delayed doses will be included as having 0 delayed doses. A delayed dose is defined in Table 6 based on regular scheduled visits, as dosing cannot be recorded under unscheduled visits.
- A by-visit summary will also show the number of subjects who missed a dose at that visit i.e. no dose recorded at the regular scheduled visit.

The summaries described above for missed and delayed doses will be repeated based on doses that are missed or delayed due to COVID-19 impact.

A listing will also be presented to show dosing history for all subjects who missed at least one dose, indicating whether any of the missed doses are related to COVID-19. If any of the doses are due to COVID-19, then the number of impacted doses will be presented.

Study drug compliance percentage up to the last dose of study drug received will be defined as the number of doses actually received divided by the number of doses that the subject is expected to receive during the study period and will be summarized by descriptive statistics. For subjects who withdrew from study early, the number of expected doses is the planned number of doses before the time of study withdrawal.

Separate listings will be provided showing what subjects were randomized to as well as study drug administration data which will include lot numbers, actual treatment received, cumulative number of doses and cumulative dose. A listing of dosing errors will also be provided i.e. where either the incorrect dose was administered as specified in protocol deviation log and kit description from IRT system, or where less than 15mL of study drug was administered. Actual dose will be calculated as volume administered multiplied by dose, and divided by 15mL. The nominal dose will also be presented.

A listing of study drug administration records for placebo subjects who received any active treatment will be provided.

Time on study will be summarized descriptively by treatment. Overall time on study will be calculated as last date on study – first dose date + 1 in days. Last date on study is defined as the date of the latest visit or evaluation or telephone contact, or time of death from all available data for a given subject. The durations will also be categorized and summarized using the following categories: ≤29 days, >29 to 57 days, >57 days to 85 days, >85 to 113 days, >113 to 141 days, >141 to 169 days, >169 to 197 days, >197 days.

Subjects are considered to be exposed to study drug for approximately 5 half-lives. Therefore, given the half-life of tofersen (approximately 1 month), subjects would be considered to be exposed to study drug for 5 months after their last dose. Based on the 6-month duration of the study, a separate summary for exposure will not be provided. However, a summary of time to treatment discontinuation will be provided for those subjects who discontinued study drug, calculated as last dose date – first dose date +1.

5.4 Concomitant Medications and Non-Drug Treatments/Procedures

Concomitant medications will be coded using the World Health Organization (WHO) dictionary WHODRUG (March 2021) and concomitant non-drug treatments/procedures using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0.

Medications and non-drug treatments are considered concomitant if they are taken during the study. This includes medications/treatment/procedures that were started prior to the date and time of first dose of study drug if their use continued on or after the date and time of first dosing. If the start date is available but start time is missing and the medications/treatment/procedure start date is the same as the first dose date, the medications/treatment/procedure will be considered concomitant. Similarly, if the stop date is available but stop time is missing and the medications/treatment/procedure stop date is the same as the first dose date, the medications/treatment/procedure will also be considered concomitant. Medications/treatment/procedures with missing start or stop dates and times, will also be considered as concomitant in the following situations:

- If both the start and stop dates and times of a medication/treatment/procedure are missing;
- If the start date and time of a medication/treatment/procedure is missing and the stop date and time of the medication/therapy occurred on or after the date and time of first dose of study drug;

- If the start date and time of a medication/treatment/procedure therapy occurred prior to the date and time of first dose of study drug and the stop date and time of the medication/treatment/procedure is missing and the medication/treatment/procedure is listed as ongoing;

In a rare situation where the start date and time of a medication/treatment/procedure occurred prior to the date and time of first dose of study drug and both the stop date and time of the medication/treatment/procedure is missing and the medications/treatment/procedure is not listed as ongoing, then the medication/treatment/procedure will also be considered as concomitant.

For medications/treatment/procedures with a partial start date, the year/month will be compared to that of the first dosing date to determine whether the medications/treatment/procedure is concomitant.

The number and percentage of subjects taking any concomitant medications will be summarized by treatment group for the safety population. The number and percentage of subjects taking any concomitant non-drug treatments will also be summarized by treatment group for the safety population.

A review of concomitant medications will be performed by a medical reviewer to identify disallowed medications as defined in the protocol. This review will be on an ongoing basis prior to the database lock and unblinding for this study. A summary showing the number and percentage of subjects taking any disallowed concomitant medication will be summarized by treatment group for the safety population.

These summaries will also be presented by disease progression subgroup (“enriched” “other”). All medications/treatment/procedures will also be listed.

A listing of any changes to riluzole or edaravone use during the study will be presented.

If there are a sufficient number of tests or treatments reported with indication of COVID-19, a separate summary of these will be provided. This will include a summary of the number and percentage of subjects with COVID-19 diagnostic tests with the following recorded verbatim terms (these will be coded using MedDRA dictionary):

“COVID-19 Diagnostic Test Result Positive”

“COVID-19 Diagnostic Test Result Negative”

“COVID-19 Diagnostic Test Result Pending”

"COVID-19 Diagnostic Test Result Inconclusive"

and the number and percentage of subjects with COVID-19 antibody tests with the following recorded verbatim terms (these will be coded using MedDRA dictionary):

"COVID-19 Antibody Test Result Positive"

"COVID-19 Antibody Test Result Negative"

"COVID-19 Antibody Test Result Pending"

"COVID-19 Antibody Test Result Inconclusive"

If there are very few COVID-19 tests or treatments the summary will not be presented but a listing of these will be provided.

5.5 Protocol Deviations and Compliance

Protocol deviations identified during site monitoring will be captured in a protocol deviation log. All protocol deviations will be listed and a summary of major protocol deviations by treatment group will also be provided for each of the mITT population, non mITT population and overall ITT population. A separate listing will present site level deviations for deviations that do not impact any subjects (e.g. temperature excursions where no subjects were administered impacted doses),

Subjects with incorrect stratification i.e. any mismatch between the stratification used for randomization on the IRT system and the actual stratification according to the eCRF will also be listed. A separate summary of major protocol deviations related to the COVID-19 pandemic will be presented. A listing will also present all minor and major PDs related to the COVID-19 pandemic.

A summary will be presented by visit and overall showing the number and percentage of subjects with the following type of visit:

Clinic visit (with dosing)

Clinic visit (with no dosing)

Home visit only

Home visit and telephone visit

Telephone visit only

Missed visit

The overall summary will be based on number of expected visits. This summary will also present the number of delayed visits (i.e. outside protocol defined window as per Table 6) per subject across the study and the number of missed visits per subject.

A summary showing the number and percentage of subjects by visit will be presented with the breakdown of the reason for the missed visit i.e.

COVID-19 – Subject diagnosed with Covid-19 disease

COVID-19 – Subject fear related to Covid-19 pandemic

COVID-19 – Movement restrictions related to Covid-19 pandemic

COVID-19 – Site closed due to COVID-19 pandemic

Other

This summary will also show the total number of visits missed per subject for each reason.

Although only a protocol deviation before Protocol Version 6, a summary will be provided to show number of ALSFRS-R raters per site (i.e. mean and SD, as well as frequency distribution). The summary will also indicate number and percentage of subjects assessed by a rater that was not blinded to other assessments, as well as number of visits affected per subject. The proportion of assessments not rated by a blinded rater will also be presented (i.e. total number of assessments across all sites and subjects not performed by a blinded rater over total number of expected assessments).

The acceptability criteria for SVC is defined in Appendix B of this SAP. The number and percentage of subjects with a least one invalid reading graded F according to the ATS criteria will be presented, as well as number of visits per subject with an invalid reading.

Subjects who use a facemask for the SVC assessment should ideally use throughout the subsequent assessments. The number of subjects with an SVC reading at both baseline and Day 197 will be presented. Of these, the number and percentage of subjects with facemask use will be presented as follows:

- Those who used a facemask at both baseline and Day 197
- Those who did not use a facemask at baseline and Day 197

- Those with inconsistent or missing facemask use at baseline and Day 197

At Site [redacted], a HHD rater performed this assessment for 3 subjects after [redacted] certification expired at multiple visits. These subjects are all in the non mITT population. In addition, due to the ALSFRS-R rater at this site not being available during the COVID-19 pandemic, the ALSFRS-R for one of these subjects was also performed by a non-certified rater. This impacted only 1 subject ([redacted]) at 2 unscheduled visits that are not mapped to any regular scheduled visits and therefore impacts no analysis. These are all recorded as protocol deviations. For HHD these deviations will be included in the main analyses but will be assessed via sensitivity analysis. Although action has been taken to mitigate risk of non-certified raters performing assessments, any additional cases will be documented as protocol deviations and handled in the same way for analysis. If ALSFRS-R is impacted at any regular visits that are required for analysis, a sensitivity analysis will be conducted for non-mITT. For mITT this would be handled via the Per-protocol population.

At the same site ([redacted]), there was a subject who had C-SSRS performed by non-certified raters. These are also recorded as protocol deviations. As this is a safety assessment all data will be used for analysis of C-SSRS.

5.6 Protocol Alternations

Protocol alternations are alternative methods of assessments due to the COVID-19 pandemic. For subjects who could not attend clinic visits due to the pandemic, protocol alternations in place for this study are home visits or telephone assessments. A listing of subjects with protocol alternations will be provided.

6. Efficacy Data

6.1 General Considerations

The primary efficacy population (mITT) will be used for all analyses of efficacy data. In addition, descriptive statistics and/or analyses may also be performed on the non mITT population as specified. Although it is unlikely that any testing of clinical function endpoints will show statistical significance in the non mITT population given the study duration, nominal p-values will be presented. For the overall ITT population, there will be no formal testing.

Unless stated otherwise, baseline data are defined as the data collected prior to the time and/or on the date of first dose, which is usually the same day as the Day 1/Baseline visit. If there is more than one value on/before the date of the first dose, the non-missing value closest to and prior to the first dose will be used as the baseline value.

For each of the primary efficacy and secondary efficacy endpoints the key analysis which is in the mITT population and is used for formal testing is classed as the 'primary analysis' for that efficacy endpoint.

The analyses in the non mITT population are classed as secondary analyses of each of the primary and secondary efficacy endpoints, as are other definitions for the endpoint and/or time point analyzed. An alternative test such as a non-parametric test, or any other analysis to check the robustness of the assumptions for the primary analysis is classed as a sensitivity analysis i.e. any analysis that confirms that the estimate derived is reliable for interpretation. All other analyses are classed as supplementary analyses i.e. any analyses to more fully investigate and understand the data.

The primary, secondary, sensitivity and supplementary analyses for the primary and key secondary efficacy endpoints are listed in Tables 7 and 8.

Table 7 Analysis for Primary Efficacy Endpoint

Endpoint	Analysis	Analysis Populations	SAP Section
Change from baseline in ALSFRS-R total score at Week 28/Day 197	Primary: Joint Rank methodology using multiple imputation (MI); analysis of ranked scores using analysis of covariance	mITT	6.2.1
	Sensitivity: Joint Rank methodology; analysis using generalized Gehan-Wilcoxon test	mITT	6.2.2
	Sensitivity: multiple imputation (MI) to impute missing values; analysis of covariance (ANCOVA) with covariates for Day 197. LS Mean Treatment differences, 95% CI and p-values at visits other than Day 197 will also be presented as secondary analyses by fitting separate ANCOVA for each visit	mITT	6.2.2
	Sensitivity: subjects who died will be excluded from the MI model; joint rank p-value only	mITT	6.2.2
	Sensitivity: Trimmed Mean	mITT	6.2.2

	Sensitivity: Tipping point analysis for primary efficacy (Joint Rank + MI): p-values from each shift parameter	mITT	6.2.2
	Sensitivity: Tipping point analysis for primary efficacy (ANCOVA + MI): LS Mean Treatment differences, 95% CI and p-values based on ANCOVA for Day 197 presented for each shift parameter	mITT	6.2.2
	Sensitivity: Include baseline plasma NfL as an additional covariate in ANCOVA model for both ranked scores and for change from baseline: MI to impute missing values: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented. As secondary analysis all visits other than Day 197 will also be presented.	mITT	6.2.2
	Secondary: MI to impute missing values; ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits	non mITT	6.2.2
	Secondary: joint rank test to obtain p-value where permanent ventilation is incorporated as an event; analysis of ranked scores using analysis of covariance	mITT	6.2.3.1
	Secondary: joint rank test to obtain p-value where permanent ventilation and withdrawals due to disease progression are incorporated as an event; analysis of	mITT	6.2.3.2

	ranked scores using analysis of covariance		
	Secondary: Include baseline plasma NfL as an additional covariate in ANCOVA model: MI to impute missing values at each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits.	non mITT	6.2.2
	Supplementary: Q5a and Q11 from Japanese Ohashi version of ALSFRS-R for Japanese subjects used; Joint Rank methodology	mITT	6.2.4.1
	Supplementary: Exclusion of Japanese subjects; Joint Rank methodology	mITT	6.2.4.1
	Supplementary: Q5a and Q11 from Japanese Ohashi version of ALSFRS-R for Japanese subjects used; MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits	mITT non mITT	6.2.4.1
	Supplementary: Exclusion of Japanese subjects; MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits	mITT non mITT	6.2.4.1
	Supplementary: If such protocol deviations occur, exclusion of assessments by non-certified rater across sites; MI to impute missing values – the values for assessments by a non-certified rater at affected visits will be set to missing and instead imputed values used; ANCOVA including	non mITT	6.2.4.2

	covariates – performed for Day 197; LS Mean Treatment difference, 95% CI and p-value.		
	Supplementary: Exclusion of mITT subjects with percent predicted SVC <65% at baseline: MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented	mITT	6.2.4.3
	Supplementary: exclusion of prespecified protocol deviations from the mITT; MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; P-value from joint rank will also be presented	Per-protocol	6.2.4.4
	Supplementary: Exclusion of carriers of SOD1 mutations characterized as “indeterminate”) (i.e., heterozygous D91A carriers); MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented	mITT	6.2.4.6
	Subgroup analysis: Subjects with pre-randomization slope decline ≥0.9; missing data imputed using multiple imputation model for this subset of subjects for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented	mITT	6.5

	Subgroup analysis for gender, baseline disease duration since symptom onset, baseline NfL plasma level, site of onset, geographic region and riluzole/edaravone use: MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; (no p-values for overall ITT) Observed data descriptive statistics.	mITT non mITT Overall ITT	6.5
	Additional analysis : missing data imputed using multiple imputation separately for mITT and non mITT; ANCOVA includes both populations i.e disease progression subgroup ('enriched' 'other') in the model and interaction for disease progression subgroup by treatment as well as covariates – performed for Day 197; LS Mean treatment difference for each subgroup and across the subgroups and corresponding 95% CI	Overall ITT	6.2.5
	Additional analysis: missing data imputed using multiple imputation model for this subset of subjects; ANCOVA using MI includes only subjects with slope decline ≥0.9 from Screening to Day 15 inclusive. The ANCOVA will be performed for Day 197 and will include covariates; LS mean treatment difference, 95% CI and p-value; p-value from joint rank will also be presented	Subset of Overall ITT	6.2.5
	Additional analysis : includes only subjects from mITT with slope decline ≥0.9 from Screening to Day 15 inclusive. MI model will include this subset of subjects. The ANCOVA using MI will be performed for Day 197 and	Subset of mITT	6.2.5

	will include covariates; LS mean treatment difference, 95% CI and p-value; p-value from joint rank will also be presented		
ALSFRS-R slope of decline	Supplementary: slope of decline analyzed using analysis of covariance; supportive analysis for ranked data using Mann-Whitney test	mITT non mITT	6.2.4.5
ALSFRS-R changes from baseline at each visit	Secondary: missing data imputed using multiple imputation separately for mITT and non mITT; ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI	Overall ITT	6.2.3
ALSFRS-R actual values and changes from baseline at each visit	Secondary: observed data; descriptive statistics only	mITT non mITT overall ITT	6.2.3

Table 8 Analysis for Secondary Efficacy Endpoints

Endpoint	Analysis	Analysis Populations	SAP Section
Change from baseline in percent predicted SVC at Week 28/Day 197	Primary: Joint Rank methodology using multiple imputation (MI); analysis of ranked scores using analysis of covariance	mITT	6.3.1.1
	Sensitivity: Joint Rank methodology; analysis using generalized Gehan-Wilcoxon test	mITT	6.3.1.2
	Sensitivity: MI to impute missing values for each visit: ANCOVA including covariates for Day 197. LS Mean Treatment differences, 95% CI and p-values at visits other than Day 197 will also be presented; separate	mITT	6.3.1.2

	ANCOVA for each visit as secondary analysis		
	Secondary: MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at visits other than Day 197 will also be presented	non mITT	6.3.1.2
	Supplementary: exclusion of subjects who wore a facemask inconsistently at baseline and Day 197 visits : MI to impute missing values: ANCOVA including covariates for Day 197; LS Mean Treatment differences, 95% CI and p-value; p-value from joint rank will also be presented	mITT	6.3.1.3
	Supplementary: exclusion of prespecified protocol deviations from the mITT; MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented	Per-protocol	6.3.1.3
	Subgroup analysis: Subjects with pre-randomization slope decline ≥0.9; missing data imputed using multiple imputation model for this subset of subjects for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits; p-value from joint rank will also be presented	mITT	6.5
	Subgroup analysis for gender, baseline disease duration since symptom onset, baseline NfL plasma level, site of onset, geographic region and	mITT non mITT overall ITT	6.5

	riluzole/edaravone use: MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit and/or descriptive statistics based on observed data; LS Mean Treatment differences, 95% CI and p-values at all visits if using ANCOVA (no p-values for overall ITT)		
Percent predicted SVC actual values and changes from baseline at each visit	Sensitivity: observed data; descriptive statistics only	mITT non mITT overall ITT	6.3.1.2
Percent predicted SVC changes from baseline at each visit	Secondary: missing data imputed using multiple imputation separately for mITT and non mITT; ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI	Overall ITT	6.3.1.2
Change from baseline in HHD megascore at Week 28/Day 197	Primary: MI to impute missing values for each visit: ANCOVA including covariates for Day 197 – also performed as secondary analysis for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at visits other than Day 197 will also be presented	mITT	6.3.2.1
	Secondary: MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit LS Mean Treatment differences, 95% CI and p-values at visits other than Day 197 will also be presented	non mITT	6.3.2.2
	Supplementary: Exclusion of subjects assessed by non-certified rater across sites; MI to impute missing values – the	mITT non mITT	6.3.2.3

	values for assessments by a non-certified rater at affected visits will be set to missing and instead use imputed values: ANCOVA including covariates – performed for Day 197 LS Mean Treatment difference, 95% CI and p-value		
	Supplementary: exclusion of prespecified protocol deviations from the mITT; MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits	Per-protocol	6.3.2.3
	Supplementary: Upper and lower extremities HHD megascore; MI to impute missing values: ANCOVA including covariates – performed at each visit LS Mean Treatment difference, 95% CI and p-value	mITT non mITT	6.3.2.3
	Subgroup analysis: Subjects with pre-randomization slope decline ≥0.9; MI model will include only this subset of patients to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI and p-values at all visits	mITT	6.5
	Subgroup analysis for gender, baseline disease duration since symptom onset, baseline NfL plasma level, site of onset, geographic region and riluzole/edaravone use : MI to impute missing values for each visit: ANCOVA including covariates – performed for each postbaseline visit and/or descriptive statistics; LS Mean Treatment differences, 95% CI and p-	mITT non mITT overall ITT	6.5

	values at all visits if using ANCOVA (no p-values for overall ITT)		
HHD megascore actual values and changes from baseline at each visit	Sensitivity: observed data; descriptive statistics only	mITT non mITT overall ITT	6.3.2.2
HHD megascore changes from baseline at each visit	Secondary: missing data imputed using multiple imputation separately for mITT and non mITT; ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI	Overall ITT	6.3.2.2
HHD upper and lower extremities megascore actual values and changes from baseline at each visit	Supplementary: observed data; descriptive statistics only	mITT non mITT overall ITT	6.3.2.3
HHD upper and lower extremities megascore changes from baseline at each visit	Supplementary: missing data imputed using multiple imputation separately for mITT and non mITT; ANCOVA including covariates – performed for each postbaseline visit; LS Mean Treatment differences, 95% CI	Overall ITT	6.3.2.3
Time to death or permanent ventilation	Primary: stratified log-rank test and Cox proportional hazards model	mITT	6.3.3.1
	Secondary: stratified log-rank test and Cox proportional hazards model	non mITT Overall ITT	6.3.3.2
	Supplementary: time to death or mechanical ventilation for ≥22 hours per day for ≥7 consecutive days	mITT non mITT Overall ITT	6.3.3.3
	Supplementary: time to death or mechanical ventilation for ≥22 hours per day for ≥21 consecutive days using imputed data	mITT non mITT Overall ITT	6.3.3.3

Time to death	Primary: stratified log-rank test and Cox proportional hazards model	mITT	6.3.4.1
	Secondary: stratified log-rank test and Cox proportional hazards model	non mITT Overall ITT	6.3.4.2

Derivation of efficacy endpoints

The derivations for each clinical function are provided in Appendix B.

Covariates

The covariates for the statistical models will be as follows:

- Corresponding baseline score for the endpoint (continuous)
- Baseline disease duration since symptom onset as derived in Appendix A i.e. time since symptom onset in months (continuous)
- Riluzole or Edaravone use which will take 3 levels as follows:
    - Edaravone Use (Edaravone = Yes; Riluzole = Yes OR No)
    - Riluzole Only (Riluzole = Yes and Edaravone = No)
    - Neither (Edaravone = No and Riluzole = No)

Edaravone use may only occur for a very small number of subjects in which case this could cause problems for statistical modelling. If Edaravone use is limited to <5 subjects within the relevant population (i.e. mITT or non mITT) then riluzole and edaravone use will be combined so that there are only 2 levels:

- Edaravone or Riluzole Use (Edaravone = Yes and Riluzole = Yes; OR Edaravone = Yes and Riluzole = No; OR Edaravone = No and Riluzole=Yes)
- Neither (Edaravone = No and Riluzole = No)

For ANCOVA models for percent predicted SVC the model will also adjust for baseline ALSFRS-R total score. Given that there is a restriction on SVC for enrolment into the study the rate of progression may not be adequately adjusted for based on duration since symptom onset and baseline SVC alone; baseline ALSFRS-R score is more likely to reflect stage of disease and rate of progression in combination with these other covariates.

Missing data summaries

A summary of missing data by visit and overall for each of the following assessments will be presented. The summary will also be broken down by whether the visit was missed due to COVID-19 or other reason. These will include missing data after withdrawal to show the extent of data imputed for analysis.

- ALSFRS-R
- SVC
- HHD
- Plasma collection for biomarkers
- CSF collection for biomarkers
- [redacted]

A summary of each of the above assessments will also be presented to show the number and percentage of subjects at each visit broken down by the type of visit conducted for that assessment and if a clinic visit was not conducted whether the alternative method implemented was due to COVID-19. [redacted]

All summaries will be presented for each of the mITT, non mITT and ITT populations.

Missing data for individual items in scales

The imputation of individual components of each endpoint are described in Appendix B. Imputation will be performed within a scale for each endpoint by prorating the observed scores at the same visit, for example, if there are missing responses to some of the individual questions on a scale at a given visit the endpoint may only be derived for that visit if there are a certain number of individual items/components with available data. Specific details are provided for each assessment/scale in Appendix B, as this differs across assessments/scales. These imputations within a visit will be performed prior to applying imputations on the endpoint at the visit level (e.g. multiple imputation) and will be applied consistently across visits where required. Multiple imputation will not be applied on an individual item/question within an assessment/scale.

Last observation carried forward (LOCF)

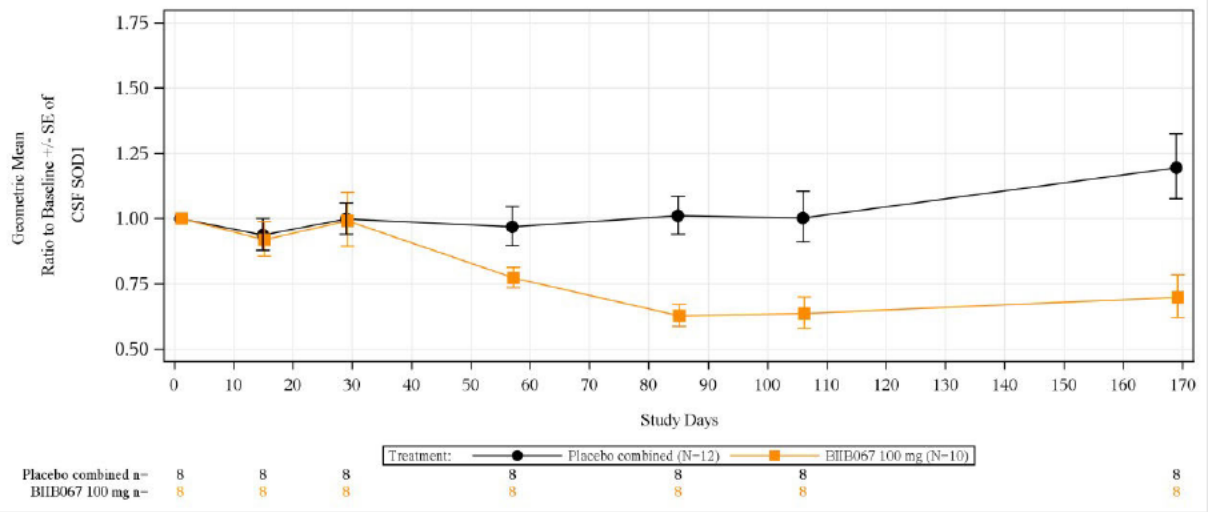
As there is not likely to be meaningful change between Day 169 and Day 197 in CSF and plasma biomarkers (i.e. total CSF SOD1 protein and neurofilament), last observation carried forward (LOCF) approach will be used as a sensitivity analysis to impute Day 197, when missing, as long as there is a result available at Day 169. The primary analysis of these endpoints will use MI to impute all missing values.

CSF samples are linked to dosing visits as this requires a lumbar puncture. If a subject cannot attend the clinic for a visit, the CSF sample cannot be collected. For subjects who do not continue into 233AS102 or those subjects who cannot attend the clinic for their Day 197 visit and have a delayed enrolment into 233AS102, these subjects are likely to have a missing CSF sample at Day 197. LOCF will be used to impute missing values at Day 197 for CSF and plasma biomarkers listed above, where the patient has Day 169 data available. Otherwise MI will be applied. Based on Phase 1 data, this approach is conservative as the value 1 month after dosing is either flat or further reducing so the levels remain relatively stable over time. The imputed value at Day 197 will be used for the analysis of these endpoints. Data at other timepoints will be imputed using multiple imputation. If neither Day 169 nor Day 169 values are available, multiple imputation will be used to impute these values.

Figure 3 shows the Phase 1 MAD observed data for total CSF SOD1 in the tofersen 100 mg and placebo subjects who completed the study with data at all visits. The last dose is at Day 85, and total CSF SOD1 is still stable at Day 106 but there is a slight rebound at Day 169 in subjects who received tofersen 100mg.

Figure 3: Phase 1 MAD Data for Total CSF SOD1 in Completers (tofersen 100 mg vs Placebo)

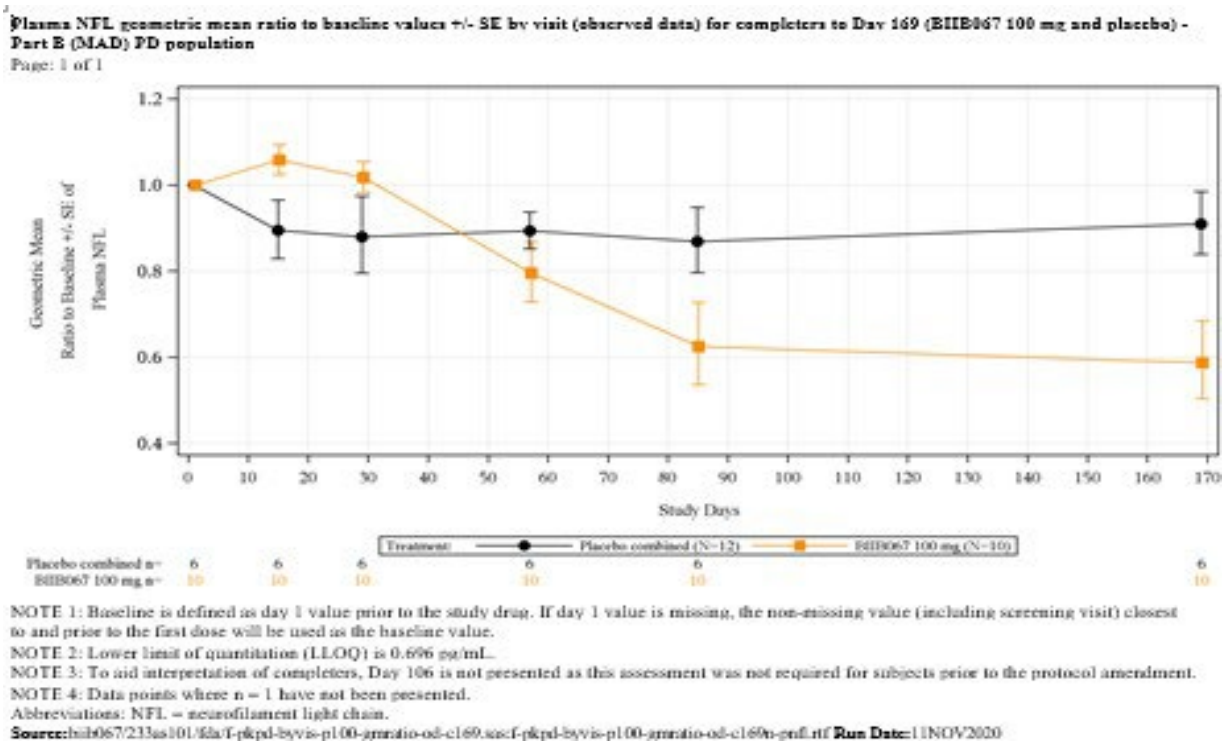
CSF SOD1 geometric mean ratio to baseline values +/- SE by visit (observed data) for completers to Day 169 (BIIB067 100 mg and placebo) - Part B (MAD) PD population
Page: 1 of 1



NOTE 1: Baseline is defined as day 1 value prior to the study drug If day 1 value is missing, the non-missing value (including screening visit) closest to and prior to the first dose will be used as the baseline value
NOTE 2: Lower limit of quantitation (LLOQ) is 15 6 ng/mL
NOTE 3: Due to some anomaly for the CSF sample at Day 15 for Subject 101-301, the result was BLQ and looks like it is an extreme outlier Therefore the value for this subject at Day 15 is set to missing
NOTE 4: Data points where n = 1 have not been presented
Abbreviations: CSF = cerebrospinal fluid; SOD1 = superoxide dismutase 1; BLQ = below limit of quantitation
Source:biib067/233as101/fda/f-pkpd-byvis-p100-gmratio-od-c169 sas f-pkpd-byvis-p100-gmratio-od-c169-sod1 rtf Run Date:16OCT2020

Figure 4 shows the Phase 1 MAD observed data for NfL in plasma in the tofersen 100 mg and placebo subjects who completed the study with data at all visits. The last dose is at Day 85, and pNF-H continues to show a reduction at Day 106 followed by stabilization to Day 169 in subjects who received tofersen 100 mg.

Figure 4: Phase 1 MAD Data for NfL in Plasma in Completers (tofersen 100 mg vs Placebo)



Multiple imputation (MI)

Missing data for postbaseline assessments (Day 15 through to Day 197 visits) will be imputed using the multiple imputation method [Schafer 1997, Schafer 1999]. Any missing data for baseline will also be imputed if there is no assessment available on or prior to Day 1. Visit windowing as described in Section 4.3 will be applied prior to any imputations. Any imputation on an item level within a scale will be performed prior to performing MI (see Appendix B). LOCF for CSF and plasma biomarkers will also be applied to Day 197 prior to performing MI, as described earlier in this section for the purpose of sensitivity analysis.

MI will be performed on actual values, domains, total scores or summary scores depending on the endpoint and not performed at the item level; the complete datasets can then be used to calculate change from baseline.

All available data will be used where subjects have missed doses but continued to perform assessments; otherwise any missing values will be imputed using MI. A subject may discontinue treatment but may still continue in the study, for example, if a subject has disease progression

home visits may be conducted at the discretion of the investigator. In this situation, all available data will be used. Only data after withdrawal from study will be imputed in this scenario.

The Markov Chain Monte Carlo (MCMC) will be used under the multivariate normality assumption to impute the missing postbaseline scores in the endpoint by treatment group. As some subjects will have intermittent missing data and other subjects will only have data missing in a monotone manner, the MI model will be run iteratively so that data will be imputed up to and including the first intermittent missing record.

Prior to performing multiple imputation, the dataset will be sorted by ascending unique subject identifier (USUBJID). The treatment variable and riluzole/edaravone use will be coded for inclusion in the MI model. The variable list in the model for imputations will include treatment group, riluzole/edaravone use, the corresponding baseline value for the endpoint, and all available postbaseline values. The MI model will be performed by population (mITT and non mITT). A set of 100 complete imputed datasets will be generated. This number of imputed datasets needs to be large enough for applying the multiple imputation method in this study as the sample size is small. The relative efficiency parameter will also be checked to determine the acceptability of the imputed results i.e. it should be close to 98% or higher. The seed used for the multiple imputation is specified in Table 9.

Table 9: Seed used for each endpoint in MI

Endpoint	Seed
ALSFRS-R Bulbar function Respiratory function Fine motor skills Gross motor skills (Total score will not be imputed separately; instead the imputed datasets from the functional domains will be used to calculate the total score. A separate model will be used for each functional domain.)	5846892
Percent predicted SVC	8746890
HHD megascore (overall, upper extremities, lower extremities)	4279059
[redacted]	[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Imputed values that are outside of the expected range of a scale will be reset: for example, for ALSFRS-R bulbar function should only be in the range 0 to 12. Values outside of this range will be reset so that values below 0 are set to 0 and values above 12 are set to 12. Values will be used as imputed, and will not be rounded prior to analysis.

For each of the 100 imputed datasets, the endpoint will be compared between treatment groups using an ANCOVA model for continuous endpoints (primary, secondary and exploratory efficacy and pharmacodynamic/biomarker endpoints). The ANCOVA model will include covariates for each of the corresponding baseline value for the endpoint, baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*). For ANCOVA models for percent predicted SVC the model will also adjust for baseline ALSFRS-R total score (as specified in Section 6.1 under *Covariates*). The model will be used to present overall LS means and standard errors for each treatment group, and LS mean differences for treatment with corresponding 95% CI and p-values. A combined estimate of the LS means, LS mean differences and standard errors of the 100 imputed datasets will be obtained, where standard errors will be used to obtain 95% CI from PROC MIANALYZE [Little et al, 2002]. The primary inference will be based on Day 197 but separate ANCOVA models will be used to obtain estimates, 95% confidence intervals and p-values for other visits. For subjects who died, the imputed values after death will be used in the ANCOVA analysis for change from baseline endpoints (e.g., change from baseline in ALSFRS-R total score).

Multiple imputation will be performed separately on each population mITT and non mITT. For the overall ITT population, the MI datasets for the mITT and non mITT populations will be combined. For each of the 100 imputed datasets, the endpoint will be compared between treatment groups using an ANCOVA model for continuous endpoints (primary, secondary and pharmacodynamic/biomarker endpoints). The ANCOVA model will include covariates for each of the corresponding baseline value for the endpoint (also baseline ALSFRS-R total score for

percent predicted SVC), baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*). The model will be used to present overall LS means and standard errors for each treatment group, and LS mean differences for treatment with corresponding 95% CI.

Joint rank methodology

The joint rank methodology [Berry 2013] allows for a statistical test of the treatment effect on the endpoint while accounting for truncation of data due to deaths. In this analysis, a subject's joint rank score will be calculated by comparing each subject to every other subject in the study, resulting in a score of +1 if the outcome was better than the subject being compared, -1 if worse, and 0 if the same. The subject's score will then be calculated by summing their comparison to all the other subjects in the study.

For the purpose of this calculation, subjects will be grouped into the following 2 categories:

- Group 1: Subjects who complete the study and have data available at the Day 197 assessment AND subjects who withdraw from the study due to reasons other than death;
- Group 2: Subjects who die

The criteria for withdrawals are based on withdrawal from study. A subject may discontinue treatment but still continue in the study. Data from assessments conducted after treatment will be included for analysis.

Joint rank methodology + MI

MI will be used to impute all missing data before determining the rank score, including data after withdrawal from the study except after death. MI will be performed as described earlier in this section. Subjects who died will be included in the MI model but any values imputed after death will not be used for determining the rank score. Therefore, all subjects except withdrawals due to death will have a value at Day 197 (either observed or imputed).

In each of the 100 imputed datasets, subjects will be ranked as follows:

- Subjects in Group 2 will be given lower ranks than subjects in Group 1, with the lowest ranks being given to the subjects who die in the shortest time after first dose. Progressively higher ranks will be given to subjects who die at longer times after first dose.
- Subjects in Group 1 will rank higher than subjects in Group 2. Progressively higher ranks will be given to subjects with a higher change from baseline at Day 197 i.e. smaller decline at Day 197.

For example, for 2 subjects in Group 1 their comparison score will be based on the endpoint of Day 197 change from baseline in ALSFRS-R (or SVC). The subject being ranked will be given

a comparison score of -1 if their ALSFRS-R (or SVC) change from baseline is worse than that of the comparison subject. If the change is the same in both subjects their comparison will be considered a tie and so a comparison score of 0 will be assigned.

For subjects who do not survive, a subject who dies earlier than the comparator subject will be given a comparison score of -1.

If two subjects die the same number of days from their first dose of study medication their comparison will be considered a tie i.e. comparison score of 0 will be assigned.

The subjects will be ranked according to their subject rank scores in each of the 100 MI datasets. In the instance of ties, the mean of the corresponding ranks will be assigned. Hence, in general, these comparisons will result in subjects who die being assigned the worst scores and ranked according to time of death. Subjects who survive and complete the study and subjects who withdraw from the study will be ranked more favourably than subjects who die.

For each of the 100 imputed datasets, the ranked scores will be compared between treatment groups using an ANCOVA model. The ANCOVA model will include covariates for each of the corresponding baseline value for the endpoint (also baseline ALSFRS-R total score for percent predicted SVC), baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*). The model will be used to obtain the p-value from PROC MIANALYZE for the joint rank. The median change from baseline to Day 197 will be calculated for each of the 100 imputed datasets and these medians will be averaged across the 100 imputed datasets. The estimates for the treatment effect will be based on the ANCOVA model for the change from baseline in ALSFRS-R (or SVC) as described earlier in the section describing MI. Estimates from the ANCOVA for ranked scores will also be presented, as supportive only.

The joint rank methodology will only be performed for the mITT population.

Adjustment for multiple testing

The analyses of secondary endpoints will be based on a sequential testing procedure in the order of the rank of secondary endpoints as listed below:

- Change from baseline (i.e. ratio) to Week 28 (Day 197) in total CSF SOD1 protein
- Change from baseline (i.e. ratio) to Week 28 (Day 197) in NfL in plasma
- Change from baseline to Week 28 (Day 197) in slow vital capacity (SVC).
- Change from baseline to Week 28 (Day 197) in handheld dynamometry (HHD) megascore to assess muscle strength, as measured by the HHD device.

- Time to death or permanent ventilation, which is defined as the time to the earliest occurrence of one of the following events:
  - Death.
  - Permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days).
- Time to death.

This will control the Type I error for the secondary endpoints. If statistical significance is not achieved for a secondary endpoint, all secondary endpoints of a lower rank will not be considered statistically significant. Further details are also described in Section 6.6.

There will be no adjustment for multiple testing for the sensitivity or supplementary analyses for the primary and secondary endpoints, the exploratory endpoints or subgroup analyses.

6.2 Primary Efficacy Endpoint

6.2.1 Primary analysis

The primary efficacy endpoint is the change from baseline to Week 28 in the ALSFRS-R total score, which will be analyzed using the joint rank test (JRT) methodology to account for mortality [Berry 2013] for the primary inference. When implementing the JRT methodology, multiple imputation (MI) will be used to handle withdrawals. The estimates will be obtained from an ANCOVA for change from baseline in ALSFRS-R at Week 28 with missing data imputed using multiple imputation. The corresponding nominal p-value from the ANCOVA will be presented as sensitivity analysis.

The primary analysis of the primary endpoint of Week 28/Day 197 change from baseline in ALSFRS-R is the composite estimand [ICH E9 (R1) Addendum 2017]. The estimand of the primary analysis is defined as:

- Population: all subjects in the mITT population
- Variable: change from baseline to Day 197 in the ALSFRS-R total score
- Handling of intercurrent events: Relevant intercurrent events are handled using a composite strategy in which subjects who have these intercurrent events are ranked against each other and against subjects without any intercurrent event using the joint rank methodology based on MI imputed datasets as detailed in Section 6.1. The relevant intercurrent events are deaths and withdrawals. These subjects will be handled using the

composite strategy and ranked as above using the assessment at Day 197. For withdrawals this will be based on the imputed value for Day 197 from the MI datasets.

- Summary statistics: difference between treatment groups in least square means of Day 197 change from baseline with corresponding standard errors and 95% confidence intervals taken from the ANCOVA described in 6.1 based on the multiple imputation dataset. The ranked scores will be analyzed for each of the 100 MI complete datasets using an analysis of covariance model with treatment included as a fixed effect and adjusted for the following covariates: baseline disease duration since symptom onset , baseline ALSFRS-R total score, and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*). The p-value for the joint rank test will be based on combining the estimates for the treatment differences using PROC MIANALYZE.

  In addition, median changes from baseline to Day 197 for each treatment group with corresponding 95% confidence intervals will be provided by treating death as worse than the median (i.e. assign a low value) and will be obtained for each of the 100 imputed datasets. The standard error will be back calculated from the 95% confidence intervals and used in PROC MIANALYZE to obtain a single estimate for the median and 95% confidence intervals. The difference between treatment groups in median changes from baseline to each visit will also be presented with 95% confidence intervals based on the multiple imputation datasets. The treatment difference for the median change from baseline will be obtained by determining the Hodges-Lehmann estimate for each of the 100 imputed datasets and using PROC MIANALYZE to obtain a single estimate of the median treatment difference and corresponding 95% confidence intervals.

  As supportive only, least square means from the ANCOVA for rank scores will also be presented along with standard errors, as well as the treatment difference and 95% confidence intervals. The analysis will only be performed for the mITT population.

  The observed actual values and changes from baseline to each visit in ALSFRS-R total score will be presented. The frequency and percentage of subjects in each of Groups 1 and 2 will also be presented.

6.2.2 Sensitivity analysis of primary endpoint

Gehan-Wilcoxon

In addition, the treatment difference in the joint ranked scores will be compared using the generalized Gehan-Wilcoxon (GGW) test [Finkelstein 1999] as a sensitivity analysis of the primary endpoint. The T-statistic will first be calculated for the ranked scores obtained from the 100 MI complete datasets and the corresponding p-value will be obtained from PROC MIANALYZE.

ANCOVA+MI

MI will be applied to each of the 4 functional domain scores for ALSFRS-R separately and then the 100 imputed datasets for each of the domains will be used to calculate firstly the ALSFRS-R total score and then the change from baseline in total score. This will be calculated for each of the 100 imputed datasets. The change from baseline in ALSFRS-R total score for the 100 imputed datasets will be analyzed using an ANCOVA. The MI and ANCOVA are as described in Section 6.1. The primary inference will be based on the treatment comparison at Day 197. For subjects who died or withdrew, the imputed Day 197 value will be used. Treatment differences at other visits will be derived from separate ANCOVA models for exploratory purposes. LS means and treatment differences will be presented with 95% confidence intervals and p-values at each visit.

The joint rank methodology will not be used in the non mITT population as it is unlikely that there would be sufficient deaths in this population over the duration of Study 233AS101.

The ANCOVA analysis based on MI datasets will also be performed for the non mITT population as a secondary analysis, as described in Section 6.1 and will be the main analysis for ALSFRS-R in this population. LS means and treatment differences will be presented with 95% confidence intervals and p-values at each visit. The ANCOVA analysis will also be provided for the Overall ITT but no p-values will be presented; the MI datasets from the non mITT and mITT populations will be used. LS means and LS mean changes over time will also presented in line plots.

A waterfall plot will be presented for the mITT population showing the changes from baseline in ALSFRS-R total score to Day 197 for completers. Deaths and withdrawals will be presented based on observed data i.e. their last available ALSFRS-R assessment; the day of this assessment will be indicated on the plot. A separate waterfall plot will be presented for the non mITT population.

Exclusion of deaths from MI model

The MI model will be run for ALSFRS-R for the mITT population excluding data from any subjects who died, so that missing data for survivors are not influenced by any systematic difference there may be in data from subjects who died. The joint rank analysis will then be conducted using these imputed datasets for survivors and deaths will be ranked the lowest based on time of death. The p-value from the joint rank analysis will be presented.

Trimmed mean

As a sensitivity analysis, the change from baseline in ALSFRS-R total score between treatment groups will be compared using the trimmed mean method to account for informative censoring due to death or withdrawal [Permutt and Li 2015]. This analysis will only be performed in the mITT population.

The trimmed mean is an averaging method which eliminates a partial percentage of the smallest values before evaluating the standard mean of the given data. The trimmed mean difference can be interpreted as the mean difference of the top fraction of responses between the two groups. To do this, responses are ranked and subjects who have died or withdrew are considered as having worse outcomes than those who survived and completed the trial. To ensure that subjects who died or withdrew receive the lowest rank in response an arbitrary value of -999 will be assigned. The choice of this value does not impact the analysis results because these subjects are eventually excluded from the calculation of the trimmed mean statistics. Subject response scores in the two groups will then be ranked from the largest to the smallest separately. The top 100*p% response scores from the two groups will be retained and the mean response difference is calculated as the trimmed mean statistics. Here, p is the minimum of the proportions of subjects who survived and completed the assessment in the two groups.

As a hypothetical example, assume at the end of the study in which 60 subjects were randomized and dosed, the number of subjects who died or withdrew is 3 out of 40 (7.5%) in the tofersen 100 mg group and 5 subjects out of 20 (25%) in the placebo group. The 8 subjects who died or withdrew in the two groups are assigned a response score of -999. The ALSFRS-R scores at Day 197 in the two groups are then ranked from largest to smallest separately. The top 75% of scores from each group will be retained and the mean difference is then calculated as the trimmed mean statistic.

The p-value associated with the observed test statistic is then calculated using a reference distribution generated by the re-randomization procedure described below:

1. For all 60 subjects randomized in the mITT population, randomly assign subjects in a 2:1 ratio to treatment (tofersen 100 mg or placebo) such that there are 40 subjects in the tofersen 100 mg group and 20 in the placebo group. This can be done using simple random sampling without replacement. The seed used will be 5846894.
2. Calculate the trimmed-mean test statistic as described above using the random treatment assignment in Step 1

3. Repeat Steps 1 and 2 N times (N=10,000). As a result, N trimmed-mean statistics will be generated. The empirical distribution of the N trimmed-mean statistics will then serve as the reference distribution for p-value calculations

Denote the observed trimmed mean statistic as T_0. Once the reference distribution has been established, the p-value associated with the observed test statistic is calculated as proportion of simulated test statistics that are either greater than $|T_0|$ or less than - $|T_0|$.

Tipping point analysis

The tipping point analysis evaluates the impact of missing data on the reliability of treatment effect by varying the assumptions about the outcome of patients due to dropouts. The varying assumptions will include the scenarios where dropouts on tofersen 100 mg had worse future outcome than dropouts on placebo. The tipping region is defined as the combinations of δ_c and δ_t such that the treatment effect is no longer statistically significant (p-value greater than the significance level). The clinical plausibility of the underlying assumptions for the tipping point region will be discussed to evaluate the robustness of the primary findings.

This tipping point analysis will follow the steps below:

Step1: Missing data for placebo group and tofersen 100 mg group will be imputed using the MI approach described in the primary analysis

Step 2: Adjust the Day 197 imputed value by adding a specified shift parameters δ_c and δ_t to placebo group and tofersen 100 mg group respectively

Step 3: Perform the analysis and calculate the p-value for each imputed scenario, reflecting various degree of treatment effect about the dropouts in both groups.

This analysis will be performed as 2 sensitivity analyses for: i) joint rank + MI based on primary efficacy analysis and ii) ANCOVA + MI. In the first sensitivity analysis, subjects will be ranked as per the primary efficacy analysis for each of the 100 adjusted MI datasets. The p-value for each shift parameter will be presented. The ANCOVA for ranked scores will be performed on the adjusted MI datasets, and for the second sensitivity analysis the ANCOVA will be performed based on the Day 197 value from the adjusted MI datasets. For each sensitivity analysis the results from the 100 ANCOVA analyses will then be combined using PROC MIANALYZE to obtain the p-value for inference. LS means, LS mean differences and corresponding 95% CI and p-values will be presented for each shift parameter.

Inclusion of baseline plasma NfL as a covariate in ANCOVA

Given the potential utility of neurofilament as a biomarker of ALS disease activity, baseline plasma NfL will be added as a covariate in the ANCOVA model for the primary efficacy endpoint as a sensitivity analysis for the mITT population. This analysis will be performed for the primary efficacy endpoint, change from baseline to Day 197 in ALSFRS-R total score, based on both joint rank methodology as used in the primary efficacy analysis and ANCOVA based on MI imputed datasets. This analysis will also be performed for the non mITT population as a secondary analysis.

A forest plot will present the estimate of the treatment difference and corresponding 95% CI for change from baseline in ALSFRS-R to Day 197 based on all the primary, secondary, supplementary and sensitivity analyses.

6.2.3 Secondary analysis of primary endpoint

The following secondary analyses will be performed to assess the robustness of the primary efficacy analysis. All the secondary analyses will be performed using the mITT population. Some secondary analyses may also be specified for the non mITT and overall ITT population.

Descriptive statistics will be presented for actual values and changes from baseline in ALSFRS-R total score based on observed data over time for each of the mITT, non mITT and overall ITT populations.

6.2.3.1 Joint rank test using permanent ventilation as an event

As a secondary analysis, permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days) will also be incorporated into the joint rank analysis as an event. Subjects with an event of death will be ranked the lowest, where subjects with shortest time to death will be ranked lowest. Subjects with an event of permanent ventilation will be ranked higher than subjects who died but lower than all other subjects who completed the study or withdrew early. Within this group, subjects will be compared using their time to reaching permanent ventilation (as defined above) i.e. the first day of the ventilation period. Subjects with shortest time to permanent ventilation will be ranked lowest within this group. Analyses will be repeated as for the primary analysis to present the p-value from the joint

rank test. An event of permanent ventilation will be based on an adjudicated event (i.e. adjudicated by an independent committee as having met permanent ventilation based on the protocol-defined criteria). If a subject is adjudicated as having an event, the day of the event will be date of death or first date of ventilation use during the period in which the subject met the permanent ventilation criteria. If a subject died and reached permanent ventilation prior to this, the subject will be included under deaths and not permanent ventilation.

6.2.3.2 Joint rank test using permanent ventilation and withdrawal from study due to disease progression as an event

As a secondary analysis, both withdrawal from study due to disease progression and permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days) will be incorporated into the joint rank analysis as separate events. Withdrawal from study due to disease progression will be determined from the end of study eCRF page where reason for withdrawal is recorded as 'DISEASE PROGRESSION'. The analysis will be performed as follows:

1. Deaths will all be ranked lowest based on time of death i.e. where subjects with shortest time from baseline (Day 1) to death will be ranked lowest
2. Patients with an event of permanent ventilation will be ranked higher than deaths but lower than those who withdrew from the study for any reason and completers. Within this group, subjects will be compared using their time to reaching the event i.e. the first day of at least 21 consecutive days with at least 22 hours of ventilatory support. Subjects with shortest time to permanent ventilation will be ranked lowest within this group
3. Withdrawals due to disease progression will be ranked above those subjects in 1 and 2 above but below all other withdrawals and completers. Within this group, subjects will be compared using their time to reaching the event i.e. day of withdrawal (end of study day). Subjects with shortest time to study withdrawal due to disease progression will be ranked lowest within this group
4. Completers and all other study withdrawals will be ranked highest based on Day 197 value using MI for patients without a Day 197 value

Analyses will be repeated as for the primary analysis to present the p-value from the joint rank test. An event of permanent ventilation will be based on an adjudicated event. If a subject is

adjudicated as having an event, the day of the event will be date of death or first date of ventilation use during the period in which the subject met the permanent ventilation criteria. If a subject reached permanent ventilation and subsequently died, this subject will be ranked with deaths based on their date of death rather than initiation of permanent ventilation. If a subject withdrew from the study for any reason and reached permanent ventilation prior to this, the subject will be included under the permanent ventilation group.

6.2.4 Supplementary analysis of primary endpoint

6.2.4.1 Japanese version of ALSFRS-R

For eligibility assessment and primary efficacy analysis, ALSFRS-R scores for Japanese subjects will be assessed with a Japanese translation of the English ALSFRS-R. The English ALSFRS-R used in this study and the Japanese translation of the English ALSFRS-R used in the study will be referred to as the "Global ALSFRS-R" and "Japanese ALSFRS-R," respectively.

There is a version of the ALSFRS-R that has questions specifically developed for Japanese culture [Ohashi et al. 2001] (henceforth referred to as "Ohashi version" of the ALSFRS-R). The Ohashi version of the ALSFRS-R has translated Questions 1-4, 5b-10, and 12 from the original English-language ALSFRS-R publication [Cedarbaum 1999] into Japanese. However, Question 5a (cutting foods and using utensils) and Question 11 (orthopnea) were modified to reflect Japanese culture.

Japanese subjects will be asked to respond to Questions 5a and 11 from the Ohashi version of the ALSFRS-R, in addition to completing the "Japanese ALSFRS-R." These additional questions will be termed the "Ohashi Version additional Questions 5a and 11" [Ohashi 2001].

The intention was that the Japanese ALSFRS-R would be translated directly from the Global ALSFRS-R. However, it was later found that the Global ALSFRS-R and Japanese ALSFRS-R differed. The Global ALSFRS-R is a version of the ALSFRS-R adapted from the original publication [Cedarbaum 1999] by Barrow Neurological Institute. This adaptation included updates to questions 4 (handwriting) and 11 (orthopnea) from the original wording to clarify answers and provide guidance on how to score these items.

The Japanese ALSFRS-R was translated directly from the original Cedarbaum 1999 ALSFRS-R publication with the exception of

- Question 11 which was translated with an error in answer 3 ("more than two" translated to "two or more") (Table 10)

The wording of responses for each question of the ALSFRS-R is provided for each of the ALSFRS-R versions in Table 10, with meaningful differences highlighted.

Table 10 Response Choices for ALSFRS-R questionnaire versions used in 233AS101

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
1. Speech	**4 Normal speech process** Speech is as it was prior to onset of illness **3 Detectable speech disturbance** Some detectable alteration in speech **2 Intelligible with repeating** Some repetition is required to understand the speech **1 Speech combined with non-vocal communication** Gestures or communication aids are required to understand the speech **0 Loss of useful speech** Impossible for the person to communicate verbally	4-Normal speech processes 3-Detectable speech disturbance 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 night time 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	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	

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
3. Swallow-ing	**4 Normal eating habits** No difficulty swallowing, can eat any foods/liquids of choice **3 Early eating problems – occasional choking** Ask whether patient avoids any foods because they get caught in his/her throat; can still eat all foods of choice but with occasional choking **2 Dietary consistency changes** Avoids certain foods or requires that consistency of foods be changed **1 Needs supplemental tube feeding** **0 NPO** Exclusively parental or enteral feeding	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. Hand-writing	**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 pen** **0 Unable to grip pen**	4-Normal 3-Slow or sloppy; all words are legible 2-Not all words are legible 1-Able to grip pen but unable to write 0-Unable to grip pen	

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
5a. Cutting food and handling utensils without gastrostomy	**4 Normal** No difficulty cutting or handling utensils by methods used prior to disease onset **3 Somewhat slow and clumsy, but no help needed** Some difficulty cutting or handling utensils by methods used prior to disease onset but patient continues to do so independently **2 Can cut most foods (> 50%), although slow and clumsy; some help needed** Some difficulty cutting or handling utensils by methods used prior to disease onset; patient requires assistance, but still tries to cut some foods, and still does >50% of the task successfully **1 Food must be cut by someone, but can still feed slowly** Patient cannot cut foods by methods used prior to disease onset, but still tries to feed him/herself and succeeds at least occasionally **0 Needs to be fed**	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	4 – Normal 3 – Somewhat slow and clumsy, but no help needed 2 – Can use a fork: cannot use chopsticks 1 – Food must be cut by someone, but can still feed using a fork or a spoon 0 – Needs to be fed
5b. Cutting food and handling utensils 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 caregiver** **0 Unable to perform any aspect of task**	4-Normal 3-Clumsy but able to perform all manipulations independently 2-Some help needed with closures and fasteners 1-Provides minimal assistance to caregiver 0-Unable to perform any aspect of task	

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
6. Dressing and Hygiene	**4 Normal function** Patient has NO difficulty, and is still completely independent in dressing and hygiene by methods used prior to disease onset **3 Independent; Can complete self-care with effort or decreased efficiency** Patient still completely independent in dressing but requires more effort to dress; no substitute methods are used to dress **2 Intermittent assistance or substitute methods** Patient requires occasional assistance or the use of assistive devices or substitute methods (e.g., pull-on clothes, velcro closures or shoes, pre buttoned shirt, lying down to don pants) in dressing and hygiene. Methods used are now different than those used prior to disease onset **1 Needs attendant for self-care** Means patient needs **daily** caregiver assistance with dressing but patient has some level of function **0 Total dependence**	4-Normal function 3-Independent and 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** Patient can turn alone or adjust sheets, but completes task with great difficulty; no help needed **1 Can initiate, but not turn or adjust sheets alone** **0 Helpless**	4-Normal 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	

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
8. Walking	**4 Normal** **3 Early ambulation difficulties** Notes some difficulty, but walks without assistance **2 Walks with assistance** Includes AFO, cane, walker, or a caregiver **1 Non-ambulatory functional movement only** Patient is able to move lower extremities partially for functional movement; able to stand for transfers, but unable to walk **0 No purposeful leg movement**	4-Normal 3-Early ambulation difficulties 2-walks with assistance 1-Non-ambulatory functional movement only 0-No purposeful leg movement	
9. Climbing stairs	**4 Normal** **3 Slow** **2 Mild unsteadiness or fatigue** Patient needs to rest between steps, or feels unsteady, but does not need rail **1 Needs assistance** Patient needs assistance including handrail or caregiver **0 Cannot do**	4-Normal 3-Slow 2-Mild unsteadiness or fatigue 1-Needs assistance 0-Cannot do	
10. 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**	4-None 3-Occurs when walking 2-Occurs with one or more of the following: eating, bathing, dressing (ADL) 1-Occurs at rest, difficulty breathing when either sitting or lying 0-Significant difficulty, considering using mechanical Respiratory support	

Question	233AS101 Global ALSFRS-R Non-Japanese subjects	233AS101 Japanese ALSFRS-R Japanese subjects	233AS101 Ohashi Version Additional Questions (5a and 11) Japanese subjects
11. 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 pillows in order to sleep (more than two)** **1 Can only sleep sitting up** **0 Unable to sleep without mechanical assistance**	4-None 3-Some difficulty sleeping at night due to shortness of breath. Does not routinely use two or more pillows 2-Needs extra pillow in order to sleep (two or more) 1-Can only sleep sitting up 0-Unable to sleep	4 – None 3 – Some difficulty sleeping at night due to shortness of breath 2 – Needs a supporting pillow in order to sleep 1 – Can only sleep sitting up 0 – Unable to sleep
12. Respiratory insufficiency	**4 None** **3 Intermittent use of BiPAP** **2 Continuous use of BiPAP during the night** **1 Continuous use of BiPAP during day & night** **0 Invasive mechanical ventilation by intubation or tracheostomy**	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	

In summary, the ALSFRS-R completed by Japanese subjects differ from the ALSFRS-R completed by non-Japanese subjects in two ways:

1. The Japanese ALSFRS-R wording differs from the Global ALSFRS-R in Questions 4 and 11.
2. Japanese subjects also completed the Ohashi Version additional Questions 5a and 11, in addition to the Japanese ALSFRS-R.

The Global and Japanese ALSFRS-Rs will be used in the primary efficacy analysis. Due to the differences between the Global and Japanese ALSFRS-R versions, an additional analysis will be performed based on the dataset used for the primary efficacy analysis, where subjects from Japan will be excluded from the analysis for each of the mITT and non mITT populations. This analysis will be performed on the primary efficacy endpoint using both the joint rank analysis

and ANCOVA analysis at each visit based on MI imputed datasets that were used for the primary efficacy analysis.

To consider the effect of the Japanese culturally-specific Qusetions 5a and 11, a supplementary analysis will be performed by substituting the Ohashi Version additional Questions 5a and 11 for the Japanese ALSFRS-R Questions 5a and 11. Both the joint rank analysis and ANCOVA analysis based on MI imputed datasets will be performed. For all other questions, besides Questions 5a and 11, the responses from the Japanese ALSFRS-R questions will be used; these are the same as that used for the primary efficacy analysis. For subjects outside of Japan, the responses from the Global ALSFRS-R will be used as per the primary analysis. This analysis will also be performed for the non mITT population, using a ANCOVA for the MI imputed datasets at each visit.

For Japanese subjects, a listing will also be presented to show the responses from Questions 5a and 11 from both the Global Japanese ALSFRS-R and the Ohashi Version additional questions at each visit. Any differences in responses will be flagged. The total ALSFRS-R score for observed data at each visit will also be included in the listing, based on each of the sets of responses.

6.2.4.2 Exclusion of assessments by non-certified rater for ALSFRS-R

As detailed in Section 5.5 for protocol deviations, this supplementary analysis will be performed if there are cases where ALSFRS-R is assessed by a non-certified rater. A supplementary analysis will be performed for the non mITT population by setting all values performed by the non-certified rater to missing and using MI to impute these values. These imputed datasets will be used to perform a sensitivity analysis using the ANCOVA on change from baseline in ALSFRS-R at Day 197.

6.2.4.3 Exclusion of mITT subjects with percent predicted SVC <65% at baseline

The “enriched” population is defined based on mutation and pre-randomization ALSFRS-R slope, but should also have percent predicted SVC ≥65% based on their screening value. It may be possible that some subjects have a decline in SVC between their screening assessment and baseline assessment, by which time their percent predicted SVC may fall below 65%. If ≥10% subjects in the mITT population across treatment groups have a baseline value <65% or an invalid assessment (i.e. “F”) at baseline for percent predicted SVC a supplementary analysis will be performed for the primary efficacy endpoint, change from baseline to Day 197 in ALSFRS-R total score, based on both joint rank methodology as used in the primary efficacy analysis and ANCOVA based on MI imputed datasets. The sensitivity analysis will exclude any subjects in

the mITT population who have percent predicted SVC <65% at baseline or all readings are invalid at baseline i.e. "F".

6.2.4.4 Per-protocol population

The per-protocol population will account for treatment gaps during the loading dose period and subjects whose ALSFRS-R is not performed by a blinded rater, by excluding subjects who violated these criteria under protocol deviations. These deviations for blinded rater will be determined based on the protocol deviation log where standard text is entered to identify these cases i.e. 'ALSFRS-R rater not blinded at Week X'. Treatment gaps during the loading dose period (i.e. Day 1, Day 15 or Day 29 missed doses) will be determined programmatically. The primary efficacy endpoint, change from baseline in ALSFRS-R total score, will be analyzed using this population using both joint rank methodology as in the primary efficacy analysis and using an ANCOVA at each visit based on MI imputed datasets.

6.2.4.5 Analysis of ALSFRS-R slope of decline

As a supplementary analysis, the slope of ALSFRS-R total scores measured from baseline to Day 197 will be analyzed for each of the mITT and non mITT populations. A mixed effects random intercept and random slope model will be used separately for each population where the response will be the absolute score for ALSFRS-R. The model will include baseline ALSFRS-R total score, baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*) as covariates. The model will also include fixed effects for treatment group, time (i.e. actual time as a continuous variable), and interaction for treatment group by time, and a random intercept for each subject and random slope for each subject over time. There will be a fixed intercept for ALSFRS across all subjects. The model will use an unstructured covariance matrix and will be used to present LS means, standard errors and 95% CI for each treatment group at each visit, and LS mean differences for treatment with corresponding 95% CI and p-values at each visit. All postbaseline assessments (scheduled and unscheduled) included under 233AS101 Part C will be included in the model right through to end of Study 233AS101.

In addition, as a supportive analysis, the slope of each individual subject will be derived using simple least square estimates (i.e. using all scores collected from baseline onwards), and treatment comparison using the Mann Whitney test, based on ranked data, will be made. A scatterplot (or boxplot) of pre-randomization slope versus post-randomization slope for each subject will be presented.

6.2.4.6 Exclusion of carriers of mutations classified as "indeterminate"

As a supplementary analysis, subjects who carry mutations adjudicated by the central lab as "indeterminate" (i.e. heterozygous p.Asp91Ala (D91A; D90A) mutation carriers)will be excluded from the analysis for the mITT population. The primary efficacy endpoint, change from baseline in ALSFRS-R total score, will be analyzed using this population using both joint rank methodology as in the primary efficacy analysis and using an ANCOVA at each visit based on MI imputed datasets.

6.2.5 Additional analysis of primary endpoint

6.2.5.1 Assessment of treatment difference for 'enriched' vs 'other'

The primary efficacy endpoint, change from baseline in ALSFRS-R total score at Day 197, will be analyzed using the overall ITT population using an ANCOVA based on MI imputed datasets. The MI datasets from the mITT and non mITT populations will be stacked to use for this analysis. The ANCOVA model for each of the 100 imputed datasets will include covariates for baseline ALSFRS-R total score, disease progression subgroup ('enriched' 'other'), baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*) as well as treatment group and the disease progression by treatment group interaction. The model will be used to present the LS mean treatment difference for each of the enriched and other disease progression subgroups with corresponding 95% CI, as well as the LS mean treatment difference and 95% CI across the subgroups.

6.2.5.2 Subset of subjects with a slope decline ≥0.9 from screening to Day 15

The following analyses will also be conducted:

1) All subjects with an observed slope decline ≥0.9 from screening to Day 15 inclusive (regardless of whether they meet protocol defined 'enriched' or 'other' criteria) i.e. subset of Overall ITT population. The MI model for ALSFRS-R will include only data from this subset of subjects and will not distinguish between subjects from the 'enriched' or 'other' population based on the protocol definition. The primary efficacy endpoint, change from baseline in ALSFRS-R total score at Day 197, will be analyzed for this subset of the ITT population based on both joint rank methodology as used in the primary efficacy analysis and ANCOVA based on MI . The ANCOVA model for each of the 100 imputed datasets will include covariates for baseline ALSFRS-R total score, baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*) as well as treatment group. The model will be used to present LS means and standard errors for each treatment group and LS mean differences for treatment with corresponding 95% CI.

2) All subjects in the predefined mITT population with an observed slope decline ≥0.9 from screening to Day 15 inclusive. The MI model for ALSFRS-R will include only data from this subset of subjects. The primary efficacy endpoint, change from baseline in ALSFRS-R total score at Day 197, will be analyzed for this subset of the mITT population based on both joint rank methodology as used in the primary efficacy analysis and ANCOVA based on MI . The ANCOVA model for each of the 100 imputed datasets will include covariates for baseline ALSFRS-R total score, baseline disease duration since symptom onset and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*) as well as treatment group. The model will be used to present LS means and standard errors for each treatment group and LS mean differences for treatment with corresponding 95% CI.

6.3 Secondary Efficacy Endpoints

6.3.1. Percent predicted SVC

6.3.1.1. Primary analysis of percent predicted SVC

The primary analysis for percent predicted SVC will be the joint rank method using joint rank + MI for the mITT population as described for the primary efficacy analysis of ALSFRS-R in Section 6.2.1.

6.3.1.2. Sensitivity and secondary analysis of percent predicted SVC

Sensitivity analysis for percent predicted SVC will be the treatment difference in the joint ranked scores will be compared using the generalized Gehan-Wilcoxon (GGW) test [Finkelstein 1999] as described for ALSFRS-R in Section 6.2.2.

The change from baseline in percent predicted SVC will also be analyzed using an ANCOVA based on data imputed using MI, as described in Section 6.1. The primary inference will be based on the treatment comparison at Day 197. The treatment differences at each visit will also be presented as secondary analysis with nominal p-values. A line plot of LS means and LS mean changes from baseline over time will also be provided.

The joint rank methodology will not be used in the non mITT population as it is unlikely that there would be sufficient deaths in this population over the duration of Study 233AS101.

The ANCOVA based on MI imputed datasets will also be performed for the non mITT population as described in Section 6.1. LS means and treatment differences will be presented with 95% confidence intervals and nominal p-values at each visit. The analysis will also be performed for the overall ITT population but no p-values will be performed.

The observed actual values and changes from baseline in percent predicted SVC will be presented at each visit for the mITT, non mITT and overall ITT populations.

Supplementary analysis for change from baseline to Day 197 in percent predicted SVC will be performed using the Per-protocol population. The analysis will be based on both joint rank methodology as used in the primary analysis and ANCOVA using MI imputed datasets at each visit.

A separate supplementary analysis will exclude subjects who used a facemask inconsistently at baseline and Day 197 (i.e. exclude if a subject used a facemask at baseline but not at Day 197 or if a subject did not use a facemask at baseline but used one on Day 197; also exclude if facemask use is unknown or missing at baseline or Day 197) . The analysis will be performed as for the primary analysis of this endpoint and using ANCOVA on MI imputed datasets at Day 197.

6.3.2. HHD

6.3.2.1. Primary analysis of HHD megascore

The muscle strength as measured by HHD will be summarized by individual muscles and megascore. See Appendix B for calculation of the megascore. The normalization of individual muscles is performed separately for mITT and non mITT populations using the relevant baseline mean and standard deviation for the population. The change from baseline in HHD megascore will be analyzed using the ANCOVA based on MI imputed datasets as described in Section 6.1 for the mITT population. The primary inference will be based on the treatment comparison at Day 197.

6.3.2.2. Sensitivity and secondary analysis of HHD megascore

Treatment differences at each visit will also be presented with nominal p-values. A line plot of LS means and LS mean changes from baseline over time will also be provided.

The ANCOVA based on MI imputed datasets will also be performed for the non mITT population as described in Section 6.1. LS means and treatment differences will be presented with 95% confidence intervals and p-values at each visit. The ANCOVA analysis will also be provided for the Overall ITT but no p-values will be presented; the MI datasets from the non mITT and mITT populations will be used.

The observed actual values and changes from baseline in individual muscles and HHD megascore will be presented at each visit for the mITT, non mITT and overall ITT populations.

6.3.2.3. Supplementary analysis of HHD

Supplementary analysis for change from baseline to Day 197 in HHD megascore will be performed using the Per-protocol population. The analysis will be as for the primary analysis of HHD megascore i.e. the p-value and estimates at each visit will be taken from ANCOVA using MI imputed datasets.

For the change from baseline to Day 197 in HHD megascore a supplementary analysis will also be performed on both the mITT and non mITT populations by excluding the assessments performed by a non-certified rater, and imputing these using MI. The ANCOVA using MI imputed datasets will be presented for Day 197.

A supplementary analysis will also be performed for each of HHD upper extremities megascore (left and right shoulder flexion, left and right elbow flexion, left and right wrist extension, left and right abduction index finger, left and right abduction thumb, left and right abduction 5th digit) and for HHD lower extremities megascore (left and right knee extension, and left and right ankle dorsiflexion). The change from baseline in each of the upper and lower extremities HHD megascores will be analyzed using the ANCOVA based on MI imputed datasets as for the overall HHD megascore. These analyses will be performed at each visit for both the mITT and non mITT populations. Nominal p-values will be presented at each visit with LS means, treatment differences and 95% confidence intervals. The analysis will also be performed for the overall ITT population but no -p-values will be presented.

The observed actual values and changes from baseline in upper and lower extremities HHD megascores will be presented at each visit for the mITT, non mITT and overall ITT populations.

6.3.3. Time to death or permanent ventilation

6.3.3.1. Primary analysis of time to death or permanent ventilation

Time to death or permanent ventilation is defined as the time to the earliest occurrence of 1 of the following events:

- Death.
- Permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days).

Endpoint Adjudication Committee (EAC)

Time to death or permanent ventilation will be determined in a blinded fashion by a central, independent EAC. Procedures for reviewing and adjudicating events are described in the Charter that governs the operation of the EAC. As per the charter, to reach permanent ventilation, this

may be any combination of invasive and non-invasive ventilation as long as it occurs for the number of hours and days specified.

Non-invasive ventilation (NIV) refers to the administration of ventilatory support without using an invasive artificial airway (endotracheal tube or tracheostomy tube). Modes of NIV may include, but not restricted to: bilateral positive airway pressure (biPAP), continuous positive airway pressure (CPAP), intermittent positive pressure ventilation (AVAPS), proportional-assist ventilation (PAV). Noninvasive ventilation can be applied including nasal and orofacial masks, mouthpieces, nasal pillows, total face masks, and even a helmet device.

Invasive ventilation refers to the administration of ventilatory support via an endotracheal tube or tracheostomy tube.

Ventilatory support

Ventilation use during the study will be collected via the daily ventilation diary (paper or electronic). Other source documents in addition to the ventilation diary may be used; it is to the discretion of the Principal Investigator to determine the number of hours of ventilation use per day and the type of ventilation support used. The ventilation data from the different sources are entered into the ventilation log in the case report form, with the exception of the electronic diary data which will serve as its own source. The site should try to resolve any discrepancies between sources.

- For example if a subject does not enter ventilation use in the diary for a period of time but other source documents such as hospital records indicate there was ventilation use during this period, the Principal Investigator is responsible for assessing and determining whether there was ventilation use. If there are such discrepancies the Principal Investigator should enter the correct data in the ventilation log which will take precedence over other data.
- There were also some issues in the ediary where multiple entries for the same date were entered. These instances will be resolved by the Principal Investigator in review with the subject, and the correct entry provided in the ventilation log.
- If a subject's ALSFRS-R Q12 for respiratory insufficiency indicates any use of ventilatory support, this could be used to identify any missing gaps or discrepancies in the diary and completed under the ventilation log. If the subject responded 2 (Continuous use of BiPAP during the night) or 1 (Continuous use of BiPAP during day and night) this is an indicator of continuous use of ventilatory support for a period of time. The site may not know the number of exact hours but can enter in the 'If Unknown, reason' field or in the 'If Yes, Please Explain' field for ventilatory support that the source used was

ALSFRS-R (based on the response and interaction with the subject on completion of ALSFRS-R) and additionally whether it was continuous use during day per day and night. If the record does not specify exact number of hours but the site comment indicates that they used ALSFRS-R as a source the entry will be mapped to the corresponding response on ALSFRS-R. If the subject had continuous use of BiPAP during the night it will be assumed that this is 8 hours for the purpose of supplementary analysis; if it indicates that subject responded as continuous use of BiPAP during the day and night for analysis purpose it will be assumed that this is 22 hours. This determination will be made only for the supplementary analysis of time to death or permanent ventilation and for the profile plots and summaries; not for the primary analysis using adjudicated events. A programmatic search in the comments fields of the ventilation log will be applied to look for ALSFRS-R.

Analysis

The analysis is to compare the time to death or permanent ventilation between the treatment groups in the mITT population using the log-rank test stratified by riluzole or edaravone use (as specified in Section 6.1 under Covariates). Only events that were adjudicated by the EAC as meeting the protocol defined criteria for permanent ventilation or death will be included in the primary analysis. Subjects who do not meet the endpoint definition will be censored on the subject's last contact in Study 233AS101. Follow-up will end on the last occasion that the subject was seen in the 233AS101 study (either as an in-person visit or by home visit or by telephone contact). This is regardless of whether the subject completed their full course of treatment, whether the subject completed the study or withdrew prematurely.

Kaplan-Meier estimates of the cumulative probability of the time to death or permanent ventilation over time will be determined for the mITT population. Treatment comparison of time to death or permanent ventilation will be based on a stratified log rank test. Stratification factors will be riluzole or edaravone use (as specified in Section 6.1 under Covariates). Kaplan-Meier plots will be presented. The median time to death or permanent ventilation, percentiles (5^{th}, 10^{th}, 25^{th}, 75^{th}) and associated 95% confidence limits, and proportion of subjects who meet an event by Day 197 will be estimated using the Kaplan-Meier. A Cox proportional hazards model will be used to obtain the hazard ratio and 95% confidence intervals. The Cox proportional hazards model will adjust for baseline disease duration since symptom onset, baseline ALSFRS-R total score, and riluzole or edaravone use (as specified in Section 6.1 under Covariates).

The start date for calculating time to death or permanent ventilation in days i.e. death, permanent ventilation or censoring will be date of first dose. If a subject is adjudicated as having an event,

the day of the event will be date of death or first date of ventilation use during the period in which the subject met the permanent ventilation criteria.

The proportion of subjects meeting the endpoint, time to death or permanent ventilation, will be presented by treatment group. For subjects who met the endpoint, time to death or permanent ventilation, the number of days per subject at which ventilation use was at least 22 hours per day will be summarized.

6.3.3.2. Sensitivity and secondary analysis of time to death or permanent ventilation

As a secondary analysis the analysis specified in Section 6.3.3.1 for the mITT population will be repeated for the non mITT population and the Overall ITT population. No p-values will be presented for the Overall ITT population.

6.3.3.3. Supplementary analysis of time to death or mechanical ventilation

Two sets of supplementary analyses for time to death or permanent ventilation will also be analyzed using the following definitions based on ventilation data in the log and ediary records from the clinical database and not the adjudicated events:

1) Time to the earliest occurrence of 1 of the following events:

- Death.
- Mechanical ventilation for ≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 7 consecutive days.

2) Time to the earliest occurrence of 1 of the following events:

- Death.
- Mechanical ventilation for ≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days.

Events for mechanical ventilation will be determined using the number of hours specified in the ediary or ventilation log. As detailed in 6.3.3.1, if exact number of hours are not specified and the comment in the ventilation log indicates that ALSFRS-R was used as a source then depending on the response 8 hours will be imputed only for those specific records if the subject used BiPAP continuously at night (a score of "2" on the respiratory insufficiency domain of the ALSFRS-R at the visit corresponding to the diary/log entries) and 22 hours will be imputed if the subject used BiPAP continuously during day and night (a score of "1" on the respiratory insufficiency domain of the ALSFRS-R at the visit corresponding to the diary/log entries). The number of hours will not be imputed if the ALSFRS-R score was anything other than 1 or 2; if number of hours is specified and in addition the source indicates ALSFRS-R there will be no

imputation as number of hours recorded in the ediary or vent log will take precedence. If there are ventilation records indicating vent use and there are no hours entered and ALSFRS-R was not specified as a source the number of hours will not be imputed.

The analysis will be performed for both the mITT, non mITT and Overall ITT population. Subjects who do not meet the endpoint definition will be censored on the subject's last contact in Study 233AS101. Follow-up will end on the last occasion that the subject was seen in the 233AS101 study (either as an in-person visit or by home visit or by telephone contact). This is regardless of whether the subject completed their full course of treatment, whether the subject completed the study or withdrew prematurely.

Kaplan-Meier estimates of the cumulative probability of the time to death or permanent ventilation over time and Cox proportional hazards model will be performed as specified for the primary analysis in Section 6.3.3.1.

The start date for calculating time to death or permanent ventilation in days i.e. death, permanent ventilation or censoring will be date of first dose. The day of the event will be date of death or first date of ventilation use during the period in which the subject met the permanent ventilation criteria. The proportion of subjects meeting the endpoint, time to death or permanent ventilation, will be presented by treatment group. For subjects who met the endpoint, time to death or permanent ventilation, the number of days per subject at which ventilation use was at least 22 hours per day will be summarized. For each of the mITT and non mITT populations, the following will also be presented:

- Proportion of subjects using any vent support at Week 28 (or last available assessment for withdrawals and deaths) as recorded on the vent log
- Proportion of subjects with invasive mechanical ventilation recorded at Week 28 (or last available assessment for withdrawals and deaths) as per the ALSFRS-R Question 12 (i.e. score of 0) on respiratory insufficiency.

For subjects in the mITT and non mITT populations with any ventilation data recorded (i.e. >0 hours of vent use on at least 1 day), patient profile plots (lollipop plots) will be presented showing the number of hours of ventilation use by study day. Imputed hours will also be represented if exact number of hours were not specified and ALSFRS-R was used as a source based on the response i.e. 8 hours will be imputed if the subject used BiPAP continuously at night and 22 hours will be imputed if the subject used BiPAP continuously during days and night. If there are ventilation records indicating vent use and there are no hours entered and ALSFRS-R was not used as a source the number of hours will not be imputed. Imputed flags will be denoted on the plot and explained in a footnote.

6.3.4. Time to death

6.3.4.1. Primary analysis of time to death

The overall survival will be analyzed as a time to event endpoint i.e. time to death. Kaplan-Meier estimates of the cumulative probability of death over time will be determined for the mITT population. Treatment comparison for survival time will be based on a stratified log rank test. Stratification factors will be riluzole or edaravone use (as specified in Section 6.1 under Covariates). Kaplan-Meier plots will be presented. The median survival time, percentiles (5^{th}, 10^{th}, 25^{th}, 75^{th}) and associated 95% confidence limits, and proportion of subjects who meet an event by Day 197 will be estimated using the Kaplan-Meier. A Cox proportional hazards model will be used to obtain the hazard ratio and 95% confidence intervals. The Cox proportional hazards model will adjust for baseline disease duration since symptom onset, baseline ALSFRS-R total score, and riluzole or edaravone use (as specified in Section 6.1 under Covariates).

Follow-up will end on the last occasion that the subject was seen in the 233AS101 study (either as an in-person visit or by home visit or by telephone contact). This is regardless of whether the subject completed their full course of treatment, whether the subject completed the study or withdrew prematurely. Censoring will occur on the latest of the date of last contact in 233AS101 for subjects who are alive.

The start date for calculating time to death in days i.e. death or censoring will be date of first dose.

6.3.4.2. Sensitivity and secondary analysis of time to death

As a secondary analysis the analysis specified in Section 6.3.4.1 for the mITT population will be repeated for the non mITT population and the Overall ITT population. No p-values will be presented for the Overall ITT population.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

6.5 Subgroup Analysis

Subgroup analysis will be performed for the primary efficacy endpoint and for the following secondary endpoints: change from baseline in percent predicted SVC, change from baseline in HHD megascore, and ratio to baseline for each of total CSF SOD1 protein and plasma NfL for each of the mITT, non mITT and ITT populations. Descriptive statistics for observed data will be presented by treatment group and each of the subgroup categories.

- Gender (female, male)

- Baseline disease duration since symptom onset by tertiles (tertiles to be defined separately for each of mITT, non mITT and overall ITT) (median values may be used instead of tertiles if insufficient data)
- Baseline NfL plasma level (tertiles to be defined separately for each of mITT, non mITT and overall ITT) (median values may be used instead of tertiles if insufficient data)
- Site of onset (bulbar, other site of onset). If a subject had onset at multiple sites and one of the sites is bulbar the subject will be included under the bulbar category for subgroup analysis.
- Geographic region (North America, Europe, Asia Pacific) – Canada will be included under North America
- Riluzole or edaravone use (Note: If Edaravone use is limited to <5 subjects within the relevant population (e.g. mITT) then riluzole and edaravone use will be combined so that there are only 2 levels)

For the mITT, non mITT populations and the ITT population, each subgroup category will also be analyzed separately using the ANCOVA based on MI. For the overall ITT population, no p-values will be presented.

The 100 imputed datasets will be analysed using an ANCOVA for each subgroup category including baseline value, riluzole/edaravone use, treatment and baseline disease duration. The ANCOVA will not include baseline disease duration if that is the subgroup being analysed. Similarly, use of riluzole or edaravone will not be included as a covariate if that is the subgroup being analysed. If the ANCOVA analysis uses riluzole/edaravone use as a 3 level covariate and the model does not converge, then rilzuole/edaravone may be included as a 2 level covariate for that specific subgroup analysis. Any subgroup category that has an insufficient number of subjects for the ANCOVA analysis to be generated for that category will not be presented, but other categories for the subgroup will still be presented

An analysis will also be performed for the mITT population on the subgroup of subjects with a pre-randomization ALSFRS-R slope decline of at least 0.9 per month. This analysis will be performed for each of the 5 endpoints mentioned above. The MI model for each endpoint will be performed in this subset of subjects prior to running the analysis. Both the ANCOVA using MI and joint rank methodology will be performed for ALSFRS-R and percent predicted SVC. For the other endpoints, ANCOVA using MI will be presented.

6.6 Statistical Testing Procedure

The primary endpoint and secondary endpoints will all be tested sequentially at the two-sided alpha of 0.05 for the final analysis after all subjects have completed the study and final database has locked. There will be no interim analysis.

Below is a description of the sequential testing procedure for secondary endpoints if the primary endpoint is statistically significant at an alpha of 0.05.

- The change from baseline (i.e. ratio) to Week 28/Day 197 in total CSF SOD1 protein will be tested at an alpha of 0.05. If it is statistically significant then proceed to the next step; otherwise all subsequent tests are considered exploratory.
- The change from baseline (i.e. ratio) to Week 28/Day 197 in plasma NfL will be tested at an alpha of 0.05. If it is statistically significant then proceed to the next step; otherwise all subsequent tests are considered exploratory.
- The change from baseline to Week 28/Day 197 in SVC will be tested at an alpha of 0.05. If it is statistically significant then proceed to the next step; otherwise all subsequent tests are considered exploratory.
- Test change from baseline to Week 28/Day 197 in HHD megascore at an alpha of 0.05. If it is statistically significant then proceed to the next step; otherwise all subsequent tests are considered exploratory.
- Test time to death or permanent ventilation at an alpha of 0.05. If it is statistically significant then proceed to the next step; otherwise all subsequent tests are considered exploratory.
- Test time to death at an alpha of 0.05.

If the primary efficacy endpoint is not statistically significant at the final analysis, then the testing of the secondary endpoints will be considered exploratory.

Table 11 summarizes the analysis sets, populations and testing that would be performed.

Table 11 Analysis sets and populations for final analysis

	mITT	non mITT	Overall ITT
Demographics/baseline disease history; drug exposure/compliance	X	X	X
Primary efficacy endpoint	Formal testing	Descriptive and estimates with confidence intervals and nominal p-values	Descriptive and estimates with confidence intervals
Total CSF SOD1 protein	Formal testing only if primary efficacy endpoint is statistically significant (otherwise exploratory)	Formal testing	Descriptive and estimates with confidence intervals and nominal p-values
Plasma NfL	Formal testing only if primary efficacy endpoint is statistically significant (otherwise exploratory)	Descriptive and estimates with confidence intervals and nominal p-values	Descriptive and estimates with confidence intervals
Secondary efficacy endpoints	Formal testing only if primary efficacy endpoint is statistically significant	Descriptive and estimates with confidence intervals and nominal p-values	Descriptive and estimates with confidence intervals

	(otherwise exploratory)		
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

8. Pharmacodynamic Data

All summaries and analyses of PD/biomarker data will be presented for each of the mITT and non mITT populations. In addition, analyses of total CSF SOD1 protein will be presented for the overall ITT population as exploratory analysis.

The following PD/biomarker endpoints will be summarized:

- [redacted] neurofilament light chain (NfL) will be analyzed in plasma.
- Total SOD1 protein will be analyzed in the CSF. In the Phase 1 (233AS101 Part B) study, total CSF SOD1 protein was reduced in tofersen-treated participants, irrespective of their rapidity of disease progression. As such, total CSF SOD1 protein serves as a bridging marker of treatment effect for the non-mITT population in which differentiation from placebo on clinical outcome measures may not be apparent over the 6 month treatment period.

CSF NfL, misfolded or mutant SOD1 and Urinary $p75^{ECD}$ may also be summarized and analyzed depending on the timing of the assay availability. These are unlikely to be available at the time of the readout following database lock, and therefore depending on timing may be included as part of an addendum to the CSR.

Due to technical platform variability observed in other datasets, select neurofilament samples may be re-tested post-hoc (after database lock) in case of any extreme outliers.

Values that are BLQ will be set to half of LLOQ (15.6 ng/mL for total CSF SOD1 protein, [redacted] CSF and plasma, 17.4 pg/mL for NfL in CSF and 0.696 pg/mL for NfL in plasma).

Testing of total CSF SOD1 protein and plasma NfL endpoints is discussed in Section 6.6 as part of the secondary endpoint testing.

MI for imputation

A summary of missing data for each PD/biomarker endpoint will be presented.

For each of the PD/biomarker endpoints, any missing postbaseline will be imputed. Baseline will not be imputed.

The primary analysis will use MI to impute all missing values. The MI model will be performed based on log transformed data for each PD/biomarker endpoint using the methods described in Section 6.1. There will be no resetting of imputed values. The seed used for each PD/biomarker endpoint is given in Table 12. The model will be performed by population (i.e. for mITT and non mITT). Before running the model the dataset will be sorted by population (mITT first), and by USUBJID.

Table 12: Seed used for each PD/biomarker endpoint in MI

Endpoint	Seed
Total SOD1 protein in CSF	1327538
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
NfL in plasma	4311930

A sensitivity analysis will use LOCF to impute missing Day 197 values, using Day 169 value if available (see Section 6.1). Otherwise MI will be used to impute missing values at all other visits.

Analysis

An ANCOVA model for log ratio to baseline for each of the mITT and non mITT populations will be performed on change from baseline values on log scale ((i.e. log(postbaseline) – log(baseline)) for each of the 100 imputed datasets for each PD/biomarker endpoint. The model will include covariates for the corresponding baseline value i.e. log value, baseline disease duration since symptom onset, and use of riluzole or edaravone (as specified in Section 6.1 under *Covariates*). A separate ANCOVA model will be run on total CSF SOD1 protein and plasma NfL for the overall ITT population based on the MI models run on each of the mITT and non mITT populations. The ANCOVA model will be as described.

The least square (LS) i.e. adjusted mean of each treatment group, as well as the treatment group differences will be presented with 95% confidence intervals (CI) and the p-value.

Presentations of data

Descriptive statistics of actual values and absolute changes from baseline for each PD/biomarker endpoint at each visit and ratio to baseline will be summarized by treatment group. The summary statistics will include arithmetic mean and SD for the ratio to baseline as well as the geometric mean ratio and corresponding standard error. The geometric mean ratio will be obtained by back-transforming change from baseline on log transformed data. Arithmetic means and geometric means will be presented for the actual values and absolute changes from baseline at each visit. The descriptive summaries will be presented for each of the mITT, non mITT and overall ITT populations. Descriptive statistics of actual values, absolute changes from baseline and ratio to baseline will be presented using observed data.

A plot of the geometric mean and standard deviation for the actual values over time will be presented based on observed data.

Listings of individual data will also be provided.

The treatment difference at Day 197 will be derived from the model and back-transformed to the original scale. Treatment differences at other visits will also be derived from the model for exploratory purposes. For each PD/biomarker endpoint least-squares (LS) means for each treatment group each visit as well as the treatment differences at each visit will be presented, along with 95% CI and p-values. The back-transformed LS means here represent geometric mean ratios compared to baseline, and the back-transformed LS means differences represent geometric mean ratios of the ratios compared to baseline between tofersen 100 mg and placebo. These geometric mean ratios will also be plotted.

The ANCOVA will be performed for both the mITT population and non mITT population as described in Section 6.1. For total CSF SOD1 protein and plasma NfL the ANCOVA will also be performed for the overall ITT population. LS means and treatment differences will be presented with 95% confidence intervals and p-values at each visit (no p-values will be presented in the Overall ITT for plasma NfL). Subgroup analyses as specified in Section 6.5 will also be presented for CSF SOD1 and plasma NfL; these also include an analysis for the mITT population on the subgroup of subjects with a pre-randomization ALSFRS-R slope decline of at least 0.9 per month.

9. Safety Data

The safety population will be used for the analyses of the safety data. All summaries of AEs and CSF laboratory parameters will also be presented by population ("enriched" "other"). Safety data will be summarized using frequency counts and percentages and descriptive statistics by treatment group.

A summary of missing safety data by visit and overall (i.e. C-SSRS, laboratory data broken down by panel, vital signs, ECG, neurological exam, MMSE) will be presented. The summary will also be broken down by whether the visit was missed due to COVID-19 or other reason.

9.1 Adverse Events

All AEs will be classified using MedDRA version 24.0. All AEs will be listed but only treatment emergent AEs will be summarized, where treatment emergence will be relative to the first dose of study drug. A treatment emergent AE/SAE is defined as any AE/SAE with an onset date and time that is on or after the first dose of study drug or any pre-existing condition that has worsened in severity after the first dose of study drug. In case of missing dates,

- any AE/SAE with both a missing onset date and resolution date, or any AE/SAE with a missing onset date and a resolution date which is after the first dose of study drug, will be considered treatment emergent.
- If the onset date is available but onset time is missing and the AE onset date is the same as the first dose date the AE will be considered treatment emergent.
- For AEs with a partial start date, the year/month of the event date will be compared to that of the first dose date to determine whether the event is treatment emergent.

The incidence of treatment emergent AEs will be summarized by treatment group as follows (these summaries will be presented overall and by population ("enriched" "other")):

- by primary system organ class (SOC) and MedDRA preferred term sorted by decreasing frequency
- by SOC and MedDRA preferred term sorted by alphabetical order
- by MedDRA preferred term
- by SOC
- The most common treatment emergent AEs i.e. occurring in 3 or more subjects in any treatment group will be presented by MedDRA preferred term.

- The most common treatment emergent AEs i.e. occurring in 2 or more subjects in any active treatment group compared to placebo will be presented by MedDRA preferred term.
- by maximum CTCAE grade, primary SOC and MedDRA preferred term
- by maximum CTCAE grade and MedDRA preferred term
- AEs with a CTCAE grade>=3 by primary SOC and MedDRA preferred term
- related AEs by primary SOC and MedDRA preferred term
- SAEs by primary SOC and MedDRA preferred term
- SAEs by MedDRA preferred term
- Related SAEs by primary SOC and MedDRA preferred term
- AEs related to lumbar puncture (as assessed by the investigator) by primary SOC and MedDRA preferred term
- AEs that occurred within 24 hours of dosing by primary SOC and MedDRA preferred term (in case of partial AE start date, if the month and year are available and day is missing, the AE will be included for any dose that occurs in the same month and year as the AE)
- AEs led to discontinuation of study drug by primary SOC and MedDRA preferred term
- AEs led to withdrawal from study by primary SOC and MedDRA preferred term
- AEs led to drug interrupted by primary SOC and MedDRA preferred term
- AEs led to hospitalization by primary SOC and MedDRA preferred term (showing number and percentage of subjects with at least one occurrence in that SOC or preferred term, as well as total number of events). Further summaries of hospitalization data are also included later in this section.
- AEs that led to death
- AEs of note (serious neurologic events, falls, CSF laboratory abnormalities) for which preferred terms will be based on a medical review before final analysis and unblinding. This summary will include number and percentage of subjects with at least one event and total number of events. The follow-up adjusted incidence rates based on number of subjects as well as number of events will also be presented in the same table. See calculation later in this section on incidence rates. If any of these are defined based on more than one preferred term, the preferred terms will also be broken down under the overall category.

The sorting order for AE incidence tables, unless otherwise specified, will be by decreasing frequency order of "tofersen 100 mg" column. For the AE summary by primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term. For the summary of AEs by maximum CTCAE grade, primary system organ class and preferred term, subjects will be counted only once within each primary SOC/MedDRA preferred term and will only be counted under the maximum CTCAE grade. For the summary of related AEs, if the relationship is missing then this will be summarized as unknown.

For AEs that occurred within 24 hours of dosing, if time of AE is missing and the event occurs on the same day as the day for dosing or the day following dosing, the AE will be counted as occurring within 24 hours of dosing.

An overall summary of AEs will also be presented. A separate table will also be presented to show an overall summary of COVID-19 pandemic related AEs.

Listings of the following events will be produced.

- AEs led to discontinuation of study drug
- AEs led to withdrawal from study
- AEs led to drug interrupted
- AEs related to lumbar puncture
- AEs led to hospitalization
- SAEs
- AEs

A listing of deaths will be provided if applicable.

An additional table for hospitalizations will present the number and percentage of subjects in each treatment group with at least one hospitalization, the number of hospitalizations per subject, total number of hospitalizations, and a descriptive summary for the total cumulative duration of hospitalizations per subject.

Pre-treatment SAEs, i.e. those that occurred between screening and first dose are not considered treatment emergent, and will be summarized separately if there are a sufficient number. Pre-treatment AEs should not be collected unless classed as serious. It is likely that there may be a few non-serious AEs related to study procedures (e.g. lumbar puncture) that occurred on the same date as first dose but prior to the start time of dosing. These AEs will be summarized

separately. All SAEs will be listed, with an indicator for pretreatment SAEs. Only treatment emergent AEs will be summarized, unless otherwise specified.

A separate summary will be presented for each of confirmed COVID-19 AEs and suspected COVID-19 AEs. These will be identified as AEs that are recorded with an entry of '(Confirmed)' and '(Suspected)'. As per the guidance, confirmed cases are those confirmed via a diagnostic test and suspected cases are those diagnosed based on typical symptoms, travel/contact history. A listing will also be provided.

Incidence rate tables

Incidence and incidence rate will be provided in incidence rate tables and will be summarized by primary SOC and MedDRA preferred term by treatment group. Two different kinds of incidence rate tables will be provided. Definitions are provided below:

(1) Follow-up adjusted incidence rate – defined as the number of subjects who experienced an event divided by the total of entire follow-up time among the subjects in the analysis population. The entire follow-up time for subject is defined as the time from the first dose until the last day on study. Each subject will be counted only once within each category. A similar table will also be based on total number of events divided by the total of entire follow-up time among the subjects in the analysis population. This may count a subject more than once within each category of they experienced an event more than once.

(2) Exposure-adjusted incidence rate (EAIR) – defined as the number of subjects who experience an event divided by the total exposure adjusted follow-up time among the subjects in the analysis population. The exposure adjusted follow-up time is defined as the time from the first dose until the initial occurrence of the event for those who experienced an event, or from the first dose until the end of follow-up (the last day on study) for those who did not. Each subject will be counted only once within each category. If the initial occurrence of the event has a partial start date, the following rules will be applied. If only month and year of the AE are available, and the month and year are same as that of the first dose then it will be assumed that the event started on date of first dose. Otherwise it will be assumed that the event started on the first of the month. If only year is available, and the year is same as that of the first dose then it will be assumed that the event started on the date of first dose. Otherwise it will be assumed that it started on the 1st January.

9.2 Laboratory Data

Laboratory data will be evaluated to determine the incidence of abnormalities that emerge during the course of the study. Changes in laboratory evaluations will be presented relative to baseline, which is defined as the closest visit prior to the subject starting treatment.

The following clinical laboratory parameters are assessed in the protocol:

- Hematology panel: complete blood count with differential and platelet count (hematocrit, hemoglobin, platelets, red blood cell count [RBC], white blood cell count [WBC], basophils, eosinophils, lymphocytes, monocytes, neutrophils)
- Blood chemistry panel: albumin, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase (ALT) (SGPT), aspartate aminotransferase (AST) (SGOT), gamma-glutamyl transferase (GGT), sodium, potassium, calcium, chloride, phosphate, blood urea nitrogen (BUN), creatinine, uric acid, bicarbonate (CO2), glucose, total protein.
- Urinalysis: blood, glucose, protein, and microscopic examination if abnormal
- CSF analysis: RBC, WBC, protein, glucose.
- Coagulation: PT (prothrombin time), aPTT（activated partial thromboplastin time), and INR (international normalized ratio).

If multiple samples are collected at the same visit, the samples collected at the earliest time will be analyzed after applying visit window rules as described in Section 4.3. For CSF laboratory parameters, tube 2 results will be analyzed if available.

Baseline value is defined as data collected which are prior to and/or on the date of the first dose, usually also the same day as the Day 1 visit. If there is more than one value on or before Day 1, then the last non-missing value prior to (including on) the date of first dose will be used as the baseline value.

Each hematology, blood chemistry, coagulation and CSF laboratory parameter will be flagged as “low” or “high” relative to the parameter’s normal range or as “unknown” if no result is available. For each urinalysis laboratory parameter, the number and percentage of subjects experiencing post-dosing shifts to abnormal will be summarized. For each hematology, blood chemistry, coagulation and CSF parameter, the number and percentage of subjects experiencing post-dosing shifts to ‘low’ or ‘high’ will be summarized. In each summary, the denominator for the percentage is the number of subjects at risk for the shift. The number at risk for the shift to low is the number of subjects whose baseline value was not low and who had at least one postbaseline value. The number at risk for the shift to high is the number of subjects whose

baseline value was not high and who had at least one-post baseline value. Subjects will be counted only once for each parameter and each shift regardless of how many post-dosing assessments had that type of shift. Subjects with shift will be listed by laboratory parameter and shift type. All postbaseline data will be used in the shift tables, regardless of whether a scheduled or unscheduled visit. For CSF laboratory parameters, both tubes will be used in this assessment for the shift tables.

Summary statistics for actual values and change from baseline in laboratory values will be summarized by treatment group and visit. Line plots for chemistry, hematology and CSF showing mean value for each treatment group at each visit will also be presented.

To evaluate potential serious hepatotoxicity subjects with a postbaseline AST and/or ALT value ≥3 times the upper limit of normal (ULN) and a postbaseline bilirubin value >2 times ULN at any time, not necessarily concurrent, will be listed with their values. In addition, a plot will be presented with each subject's maximum postbaseline AST or ALT value relative to the ULN against the subject's maximum postbaseline bilirubin value relative to the ULN; values do not have to be concurrent. All postbaseline data will be used to identify abnormalities, regardless of whether a scheduled or unscheduled visit.

Listings of all chemistry, hematology, coagulation, CSF, urinalysis values and pregnancy test (serum and urine) will be provided. Abnormal values and potentially clinically significant values will be flagged.

Subjects with at least one postbaseline CSF WBC values >5 will have their CSF WBC plotted over time in a spaghetti plot. A separate plot will also be presented for any subjects who have a postbaseline CSF WBC value >10. Corresponding listings will also be presented with AEs that occurred at any time on or after the subject had a postbaseline CSF WBC value >5 and any time on or after the subject had a postbaseline CSF WBC value >10. A summary table will also present number and percentage of subjects with at least one postbaseline CSF leukocyte >5 and number and percentage with at least one postbaseline CSF leukocyte >10. The summary will also present total number and percentage of individual values >5 and total number and percentage of individual values >10. All postbaseline data will be used in this summary, regardless of whether a scheduled or unscheduled visit.

A summary table and listing of lumbar puncture and CSF sample collection data other than CSF laboratory results (i.e. lumbar puncture position, number of attempts, interspaces, additional guidance used, CSF volume collected, needle and gauge information) will also be provided.

9.3 Vital Signs

Summary statistics for actual values and change from baseline will be presented for each vital sign parameter (temperature, weight, pulse, respiration, systolic and diastolic blood pressure) by treatment group and visit. A listing of vital sign data will also be provided.

Vital signs (temperature, pulse, systolic and diastolic blood pressure) will also be examined to determine the incidence of potentially clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with potentially clinically relevant abnormalities will be presented. All postbaseline data will be used to identify abnormalities, regardless of whether a scheduled or unscheduled visit. The criteria for potentially clinically relevant post-dosing abnormalities are shown in Table 13 below:

Table 13: Criteria to determine potentially clinically relevant abnormalities in vital signs

Vital Sign	Criteria for Abnormalities
Temperature	<36°C >38°C
Pulse	>100 beats per minute (bpm) <60 bpm
Systolic Blood Pressure	<90 mmHg >140 mmHg >160 mmHg
Diastolic Blood Pressure	<50 mmHg >90 mmHg >100 mmHg
Weight	7% or more increase from baseline 7% or more decrease from baseline
Respiratory Rate	<12 >20

9.4 ECG

The ECG test includes heart rate, PR interval, QRS interval, QT interval, QTc interval using Bazett's formula, QTc interval using Fridericia's formula, and RR interval. Summary statistics for actual values and change from baseline in each ECG parameter will be presented by treatment group and visit.

The number and percentage of subjects with shifts from normal to each of the categorical values denoting an abnormal scan (abnormal not AE, abnormal AE) will be summarized by treatment group. All postbaseline data will be used to identify abnormalities, regardless of whether a scheduled or unscheduled visit. A listing of subjects with abnormal status in ECG will be presented.

QTc (interval using Fridericia's formula) will also be examined to determine the incidence of clinically relevant abnormalities. The number of subjects evaluated and the number of subjects with clinically relevant abnormalities will be presented. The criteria for clinically relevant post-dosing abnormalities are:

- Maximum increase from baseline QTcF > 30 to 60 ms
- Maximum increase from baseline QTcF > 60 ms
- Maximum postbaseline QTcF > 480 to 500 ms
- Maximum postbaseline QTcF > 500 ms

9.5 Mini-Mental State Exam (MMSE)

Summary statistics for actual values and change from baseline of the total score of MMSE will be presented for each visit by treatment group. When there are multiple assessments during a single visit, the minimal value will be taken as the value for that visit. A listing of MMSE data for individual patients will also be provided.

9.6 Limited neurological examinations

The number and percentages of the status in each assessment of coordination/cerebellar function and each assessment of reflexes will be summarized by treatment group and visit. The reflexes neurological examination of upper and lower extremities will be analyzed as continuous variables (0 = absent, 1 = trace, 2 = normal, 3 = brisk, 4 = clonus). The descriptive statistics will be summarized by treatment group and visit. Stacked bar charts showing the proportion of subjects in each response category in each treatment group over time will be generated to show shifts over time.

The motor neurological examination will be analyzed as continuous variable (0 = no contraction or can't position limb; 1 = flicker or trace contraction, no movement; 2 = movement only with

gravity eliminated; 3 = movement against gravity; 4 = movement against gravity and resistance; 5 = normal strength). Summary statistics will be presented by treatment group and visit. Stacked bar charts showing the proportion of subjects in each response category in each treatment group over time will be generated to show shifts over time.

Baseline values will be taken from Day 1 predose unless an entire section of the neurological exam (i.e. coordination/cerebellar function, reflexes, motor) is missing or not done, in which case for that specific section baseline will be taken from the Screening visit for all items within that section of the exam.

Listings will also be provided with complete details for the neurological examinations.

9.7 Columbia Suicide Severity Rating Scale (C-SSRS)

The details of derivation and imputation for C-SSRS is described in Appendix D. The following analyses on C-SSRS measurements will be conducted:

- Descriptive summary of subjects who answered “Yes” to any question 1-12 as well as subjects who had suicidal ideation or suicidal behavior at baseline and at any postbaseline visit. The denominator for baseline summary is the number of subjects who were dosed and had baseline assessment; the denominator for postbaseline summary is the number of subjects who were dosed and had at least one postbaseline assessment for each question.

- Descriptive summary of subjects who had treatment-emergent suicidal ideation, subjects who had new suicidal ideation as well as subjects who had worsening suicidal ideation. The denominator is the number of dosed subjects with both baseline and at least one postbaseline suicidal ideation assessment.

- Descriptive summary of subjects who had treatment-emergent suicidal behavior. The denominator is the number of subjects who answered “No” to all suicidal behavior questions at baseline and had at least one postbaseline suicidal behavior assessment.

Listing of subjects having treatment-emergent suicidal ideation will be provided. Subjects who had new suicidal ideation and subjects who had worsening suicidal ideation will be flagged. The listing will display both baseline and postbaseline Suicidal Ideation Scores for each subject. Listing of subjects having treatment-emergent suicidal behavior will also be provided.

9.8 Interim Safety Analyses

Blinded safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor, while unblinded safety data are reviewed on a quarterly basis by the IDMC. The IDMC has been assembled to perform ongoing review of safety and tolerability data collected on

tofersen during this study. Based on its ongoing assessment of the safety and tolerability of tofersen, the IDMC will provide recommendations to the Sponsor for modifying or continuing the study as planned.

Details on the safety assessments, frequency of review, meeting schedules and controlled access to unblinded data at the interim safety analyses are outlined in the IDMC Charter.

10. Immunogenicity Data

The immunogenicity population, described in Section 4.2, will be used for all analyses of immunogenicity data.

- For immunogenicity, the baseline value is defined as the latest immunogenicity data collected at any time prior to the first dose. If no immunogenicity data are collected prior to the first dose, the baseline value is missing and will be imputed as anti-drug antibody negative for immunogenicity analyses.
- Subjects with at least one confirmed post-treatment positive result will be considered treatment-emergent positive for anti-drug antibodies if their baseline result is negative.
- Subjects where none of the post-treatment samples were positive for anti-drug antibodies will be considered negative regardless of their baseline result
- For subjects that are confirmed positive at baseline and have at least one post-treatment sample with a ≥ 2-fold increase in titer will be considered positive for anti-drug antibodies. Subjects that are positive at baseline, with subsequent post-treatment samples titers that are within 2-fold will be considered negative for anti-drug antibodies. If there are any samples which the lab was able to confirm the screen was ADA positive but could not determine the magnitude of the positive response in time for database lock these will be considered as being ≥ 2-fold for the purpose of this analysis.
- Numbers and percentage of subjects who develop antibodies will be determined and summarized by treatment groups.

The same summary will be provided for subjects with positive baseline results and for subjects with negative or missing baseline results, separately.

In addition, for subjects that are considered anti-drug antibody positive (using definition above, based on whether they have negative or positive baseline) with final immunogenicity data, the following may be evaluated:

  - Persistent anti-drug antibody response:

- More than one postbaseline positive time point that are ≥112 days apart

or

- One or more postbaseline positive time point, but < 112 days of evaluable data post first positive time point.

o Transient anti-drug antibody response:

- A single positive postbaseline time point, followed by ≥112 days results which are all negative.

or

- Two or more positive postbaseline data points with <112 days apart, with later negative samples that are ≥112 days apart from the first positive result.

The categorization of subjects may change when integrated with further data in the integrated analyses.

The incidence of AEs selected by specified SMQ (SMQ for each of hypersensitivity, anaphylactic reaction and angioedema) will be presented for subjects with at least 1 positive ADA result at post-baseline. Percentages will be calculated out of the immunogenicity population.

The incidence of AEs selected by specified SMQ (SMQ for each of hypersensitivity, anaphylactic reaction and angioedema) will also be presented for subjects with negative ADA result at post-baseline. Percentages will be calculated out of the immunogenicity population.

Descriptive statistics for AUC and Cmax calculated for tofersen PK concentrations will be presented separately for subjects with at least 1 positive ADA result at post-baseline, and for subjects with negative ADA result at post-baseline.

A summary of missing data will be presented for immunogenicity overall and broken out by relationship to COVID-19.

11. Changes to Planned Analyses

The protocol is being amended to reflect the changes in statistical analyses and will be issued as Global Protocol Amendment 8.0.

The protocol specified that the primary efficacy endpoint, change from baseline to Week 28 in the ALSFRS-R total score, will be analyzed as follows depending on 2 scenarios:

i) if the number of deaths across both treatment arms is ≥ 3 (i.e., ≥ 5% deaths out of a total of 60 patients in the mITT population) the joint rank test (JRT) methodology to account for mortality [Berry 2013] will be used for the primary inference. When implementing the JRT methodology, multiple imputation (MI) will be used to handle withdrawals. The estimates will be obtained from an ANCOVA for change from baseline in ALSFRS-R at Week 28 with missing data imputed using multiple imputation. The corresponding nominal p-value from the ANCOVA will be presented as sensitivity analysis.

ii) If the number of deaths is < 3 (i.e., < 5% deaths out of a total of 60 patients in the mITT population), ANCOVA with MI will be used as the primary analysis method for both inference and estimates. The p-value from the JRT will be presented for sensitivity analysis.

This analysis was also proposed for one of the key secondary endpoints, change from baseline in percent predicted SVC.

This has been amended so that JRT+ MI will be used as the primary efficacy analysis for change from baseline to Week 28 in ALFSRS-R total score and also as the primary analysis for change from baseline to Week 28 in percent predicted SVC. ANCOVA+MI will be performed as a sensitivity analysis for both endpoints. There is the potential that in a small primary population even a single patient could have undue influence on the analysis; with this uncertainty the JRT may be more appropriate to account for mortality.

The protocol states that for the ANCOVA analysis for percent predicted SVC (i.e. third ranking secondary endpoint) the covariates included will be baseline percent predicted SVC, baseline disease duration since symptom onset, and use of riluzole or edaravone. As stated earlier in Section 6.1, ANCOVA models for percent predicted SVC will also adjust for baseline ALSFRS-R total score. Given that there is a restriction on SVC for enrolment into the study the rate of progression may not be adequately adjusted for based on disease duration since symptom onset and baseline SVC alone; baseline ALSFRS-R score is more likely to reflect stage of disease and rate of progression in combination with these other covariates.

12. Summary of Changes from the Previous Version of the SAP

- Specified samples will be unavailable for analysis at time of database lock
- Included a listing showing transfer of subjects between sites
- Clarification on cumulative dose calculation
- Amended primary efficacy analysis and specified details and rationale in changes to planned analyses. Similarly amended primary analysis for percent predicted SVC.
- Included tipping point analysis as a sensitivity analysis to address any assumptions about missing at random
- Included sensitivity analysis excluding subjects who died from the MI model
- Included sensitivity analysis for ALSFRS-R total score where baseline NfL is included as a covariate
- Included supplementary analysis for ALSFRS-R total score where subjects with a heterozygous D91A mutation are excluded
- Included additional analyses based on feedback from FDA to compare the treatment difference in the mITT population with the non mITT population, and to look at the treatment difference in a subset of the population who meet a prespecified slope decline between Screening and Day 15.
- Included baseline ALSFRS-R as a covariate for all ANCOVA analyses of percent predicted SVC, and specified this under changes to planned analyses
- Clarified that HHD megascore supplementary analysis is for each of upper and lower extremities.
- Removed joint rank p-value from subgroup analysis of HHD megsacore as it is not applicable
- Clarified MI model where treatment is being included in the model, and the model will be run separately for each population
- Clarified sensitivity analyses of joint rank for ALSFRS-R
- Clarified for the ALSFRS-R slope of decline analysis that all postbaseline assessments will be included
- [redacted]
- Clarified exploratory analyses for Kings and Mitos disease staging
- Clarified ventilation diary reconciliation and handling of discrepancies, presentation of ventilation profile plots and supplementary analysis for time to death or permanent ventilation

- Added supplementary analysis for time to death or permanent ventilation using also ALSFRS-R as a source where hours of vent use are unknown
- For subgroup analysis indicated that median values may be used instead of tertile to determine subgroup categories for baseline disease duration since symptom onset and baseline NfL plasma level. Clarification of inclusion of riluzole/edaravone in the model
- Removed descriptive statistics from MI imputed datasets for pharmacodynamic/biomarker endpoints as this is not appropriate
- Indicated that CSF plasma may not be available at time of database lock and may be analyzed at a later stage
- Added clarification that subgroup analysis will be performed in key pharmacodynamic/biomarker endpoints
- Changed radiculitis/myelitis in AEs of note to a broader category for serious neurologic events. Also amended so that incidence rates are also presented.
- Included SMQ for anaphylactic reaction and angioedema as part of the hypersensitivity SMQ selection when summarizing relationship with immunogenicity

13. References

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Appendix A: Derivation of Demographic and Baseline Characteristics

BMI will be calculated as weight (kg) / height^2 (m^2).

Time since ALS symptom onset will be calculated in months as (date of baseline ALSFRS-R score – date of ALS symptom onset)/30.4375. Time since ALS diagnosis will be calculated in months as (date of baseline ALSFRS-R score – date of ALS diagnosis)/30.4375. For the purpose of these calculations partial dates for ALS symptom onset or ALS diagnosis will be imputed as follows: missing day will be imputed with 15th and missing month/day will be imputed with January 15th.

The prerandomization ALSFRS-R slope will be calculated using the ALSFRS-R score at baseline (Day 1) i.e. Baseline ALSFRS-R score – maximum possible score of 48/duration of symptom onset, where duration of symptom onset will be calculated as (date of baseline ALSFRS-R score – date of symptom onset)/30.4375. Partial dates for symptom onset will be handled as defined above.

Appendix B: Derivation and Imputation of Clinical Function Endpoint

ALSFRS-R

The ALSFRS-R has been demonstrated to predict survival. The ALSFRS-R measures 4 functional domains, comprising bulbar function (speech, salivation, swallowing), fine motor skills (handwriting, cutting food and handling utensils, dressing and hygiene), gross motor skills (turning in bed, walking, climbing stairs), and respiratory function (dyspnea, orthopnea, respiratory insufficiency). Each domain consists of 3 items, each scored from 0 to 4 with higher scores representing better function. Therefore, the total possible score for ALSFRS-R is 48.

At each study site, the same qualified and trained study site staff member will consistently perform the ALSFRS-R for a subject. A qualified and trained backup ALSFRS-R rater will be identified in case the primary rater is unavailable. This study site staff member will remain blinded to subjects' treatment assignments and to the results of other assessments.

The ALSFRS-R total score will be considered missing if more than 3 individual item scores are missing. If no more than 3 (≤ 3) individual item scores are missing and each functional domain has at least one individual item score available, the functional domain score with any missing item score(s) will be calculated by the following formula:

ALSFRS-R domain score = [(average of all answered item scores) x 3].

The ALSFRS-R total score is the sum of the 4 functional domain scores. If 3 individual item scores are missing from the same functional domain, then the ALSFRS-R domain score is considered as missing and the total score is calculated by the following formula:

ALSFRS-R total score = average of all answered item scores from other 3 domains x 12.

<u>ALS Milano-Torinos Staging (MITOS)</u>

This staging system captures the observed progressive loss of independence and function in ALS. This defines clinical milestones in ALS progression by los of independence in 4 key domains on the ALSFRS: swallowing, walking/self-care, communicating and breathing.

Stages are defined as follows:

Stage 0: functional involvement but no loss of independence on any domain;

Stages 1-4: number of domains in which independence was lost;

Stage 5: death.

Figure 5 below demonstrates how the ALSFRS-R domains are mapped to the stages [Chio 2015]:

Figure 5: Functional domains and stages

ALSFRS domain	Item	Score	Functional score*
Movement (walking/self-care)†	8	4 Normal	0
	Walking	3 Early ambulation difficulties	
		2 Walks with assistance	
		1 Non-ambulatory functional movement only	1
	OR	0 No purposeful leg movement	
	6	4 Normal function	0
	Dressing and hygiene	3 Independent and complete self-care with effort or decreased efficiency	
		2 Intermittent assistance or substitute methods	
		1 Needs attendant for self-care	1
		0 Total dependence	
Swallowing	3	4 Normal eating habits	0
	Swallowing	3 Early eating problems; occasional choking	
		2 Dietary consistency changes	
		1 Needs supplemental tube feeding	1
		0 NPO (exclusively parenteral or enteral feeding)	
Communicating†	1	4 Normal speech processes	0
	Speech	3 Detectable speech with disturbances	
		2 Intelligible with repeating	
		1 Speech combined with non-vocal communication	1
	AND	0 Loss of useful speech	
	4	4 Normal	0
	Handwriting	3 Slow or sloppy; all words are legible	
		2 Not all words are legible	
		1 Able to grip pen but unable to write	1
		0 Unable to grip pen	
Breathing†	10	4 None	0
	Dyspnea	3 Occurs when walking	
		2 Occurs with one or more of: eating, bathing, dressing	
		1 Occurs at rest, difficulty breathing when either sitting or lying	1
	OR	0 Significant difficulty, considering using mechanical respiratory support	
	12	4 None	0
	Respiratory insufficiency	3 Intermittent use of NIPPV	
		2 Continuous use of NIPPV during the night	1
		1 Continuous use of NIPPV during the night and day	
		0 Invasive mechanical ventilation by intubation or tracheostomy	
ALS-MITOS	Stage	Functional domains lost	
	0	None	
	1	1 domain	
	2	2 domains	
	3	3 domains	
	4	4 domains	
	5	Death	

*Staging determined by the sum of functional score of 1 for each domain.
†Where two items were used, scoring was based on either or both item scores as indicated.
ALSFRS, Amyotrophic Lateral Sclerosis Functional Rating Scale; ALS-MITOS, Amyotrophic Lateral Sclerosis Milano-Torino Staging; NIPPV, nasal intermittent positive pressure ventilation; NPO, nothing by mouth.

A score is assigned for each of the 4 functional domains: movement, swallowing, communicating, breathing

For movement domain,

If a patient scores 0 or 1 on Q8 OR if they score 0 or 1 on Q6 they get assigned a functional score of 1 for this domain. Any other scores on these 2 questions the patient is assigned 0 functional score for this domain.

For swallowing domain,

If a patient scores 0 or 1 on Q3 they get assigned a functional score of 1 for this domain. Any other scores on this question the patient is assigned 0 functional score for this domain.

For communicating domain,

If a patient scores 0 or 1 on Q1 AND if they score 0 or 1 on Q4 they get assigned a functional score of 1 for this domain. Any other combination of scores on these 2 questions the patient is assigned 0 functional score for this domain.

For breathing domain,

If a patient scores 0 or 1 on Q10 OR if they score 0, 1 or 2 on Q12 they get assigned a functional score of 1 for this domain. Any other scores on these 2 questions the patient is assigned 0 functional score for this domain.

Their overall ALS-MITOS Staging is then assigned by summing the scores across the domains:

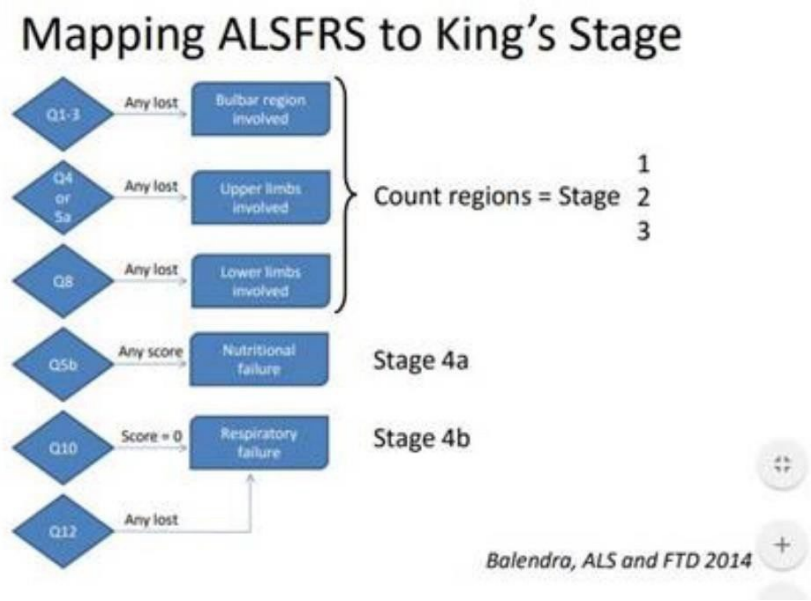
Stage	Functional domain lost
0	None
1	1 domain
2	2 domain
3	3 domains
4	4 domains
5	death

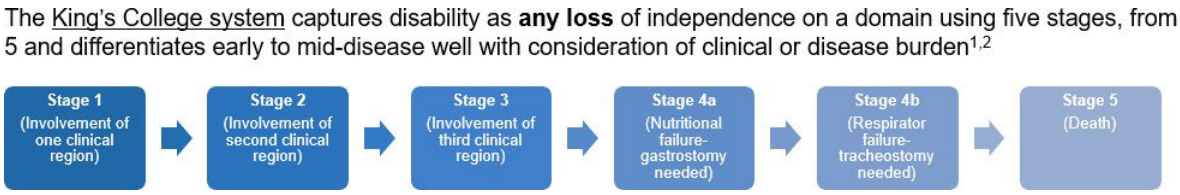
The staging will be applied to each subject using the ALSFRS-R scores at each visit. Observed data will be used.

Kings' Staging

King's staging is based on clinical milestones that consider involvement of 1-3 anatomical regions (Stages 1, 2, 3) and the need for gastrostomy (Stage 4a) and non-invasive ventilations (Stage 4b). Figure 6 demonstrates how King's staging is determined based on ALSFRS.

Figure 6: Kings' Staging





Stages 1, 2 and 3 are based on the number of regions with loss in function. Stage 1 is loss of function in 1 region, stage 2 is loss of function in 2 regions and stage 3 is loss of function in all 3 regions. The regions are bulbar, lower limb and upper limb, defined as follows:

If Q1 (speech), Q2 (salivation) or Q3 (swallowing) are scored 0 to 3 (i.e. anything except normal) this indicates loss of function in the bulbar region

If Q4 (handwriting) or Q5a (cutting food and handling utensils (subjects without gastrostomy)) are scored 0 to 3 this indicates loss of function in the upper limbs

If Q8 (walking) is scored 0 to 3 i.e. anything except normal, this indicates loss of function in the lower limbs

Stages 4a and 4b are defined as follows:

If a patient has completed Q5b (cutting food and handling utensils (subjects with gastrostomy)) then King's stage =4a

If Q10 (dyspnea) is scored 0 i.e. significant difficulty considering mechanical respiratory support or Q12 (respiratory insufficiency) is scored 0 to 3 i.e. any sort of respiratory insufficiency, then King's stage =4b

Stage 5 is defined as death.

The higher stage takes precedence and is assigned to the subject. If a subject does not fall into any of these stages, the subject will be assigned Stage 0 i.e. not yet reached Stage 1. This staging will be applied to each subject using the ALSFRS-R scores at each visit. Observed data will be used.

Percent Predicted SVC

Vital capacity will be measured by means of an SVC test, administered in the upright position.

Upright SVC will be determined by performing 3 to 5 measures, in accordance with criteria established by the American Thoracic Society and the European Respiratory Society. The results will be overread by a central reader to confirm that these criteria (at least 3 acceptable tests with the 2 highest acceptable (largest and next largest) efforts within 150 mL of vital capacity) have been achieved. Study eligibility will be based on acceptable tests. Table 13 provides a summary of the definition for acceptability and abnormalities. All tests defined as A, B, D (1), or D (2) will be used for analysis purposes. Codes defined as F are failures and will not be used for analysis. Given the uncertainty around the reason for the failed attempt (e.g. disease progression versus quality issue), these values will be set to missing and imputed using multiple imputation. If a subject has a code F at baseline, the screening value will be used.

TABLE 13 – SVC Abnormality Code Mapping

Abnormality Code	Criteria
A	At least 3 acceptable tests AND the difference between the best VC values is equal to or less than 150 ml.
B	At least 2 acceptable tests AND the difference between the best VC values is equal to or less than 150 ml.
D (1)	At least 2 acceptable trials but the results are not reproducible according to 'B'.
D (2)	Only one acceptable trial
F	No acceptable test available

The maximum i.e. best effort of the percent predicted upright SVC values at each visit will be used for the analysis. Only acceptable tests will be used for analysis. The percent predicted SVC

will be calculated as [observed SVC divided by predicted SVC] x 100%. The predicted SVC is adjusted by sex, age, height, which is programmed into and performed by the equipment used.

[redacted]

HHD

Sixteen muscle groups (left and right shoulder flexion, left and right elbow flexion, left and right wrist extension, left and right abduction index finger, left and right abduction thumb, left and right abduction 5th digit, left and right knee extension, and left and right ankle dorsiflexion) will be examined. These are split into upper extremities (left and right shoulder flexion, left and right elbow flexion, left and right wrist extension, left and right abduction index finger, left and right abduction thumb, left and right abduction 5th digit) and lower extremities (left and right knee extension, and left and right ankle dorsiflexion). At each visit, each muscle will be collected as Best Trial 1 and Best Trial 2 and the maximum value of the measures will be used for the analysis. In some cases a third trial may be required and will also be collected.

Any missing muscle strength with a reason of 'UNABLE TO TEST' will be imputed as 0 for analysis.

Megascore

Mean and standard deviation (SD) for each muscle will be established separately for the mITT and non mITT populations from the baseline values of subjects in the relevant population in this trial. For the purpose of calculating megascore the mean and SD of the baseline value will be used across all subjects in the corresponding population regardless of treatment group. The

muscle strength values will be normalized to Z scores as (measurement at visit – baseline mean)/baseline SD and averaged to provide HHD overall megascore. The overall megascore is created by averaging all eight bilateral measurement Z scores, if no more than 10 (≤ 10) measures are missing. The overall megascore will be considered missing if more than 10 measures are missing out of 16 total measures.

A megascore is also calculated separately for each of upper and lower extremities using the relevant muscles only. The upper extremities megascore is created by averaging all six bilateral measurement Z scores, if no more than 8 (i.e. ≤ 8) measures are missing. The upper extremities megascore will be considered missing if more than 8 measures are missing out of 12 total measures. The lower extremities megascore is created by averaging both bilateral measurement Z scores, if no more than 2 (i.e. ≤ 2) measures are missing. The lower extremities megascore will be considered missing if more than 2 measures are missing out of 4 total measures.

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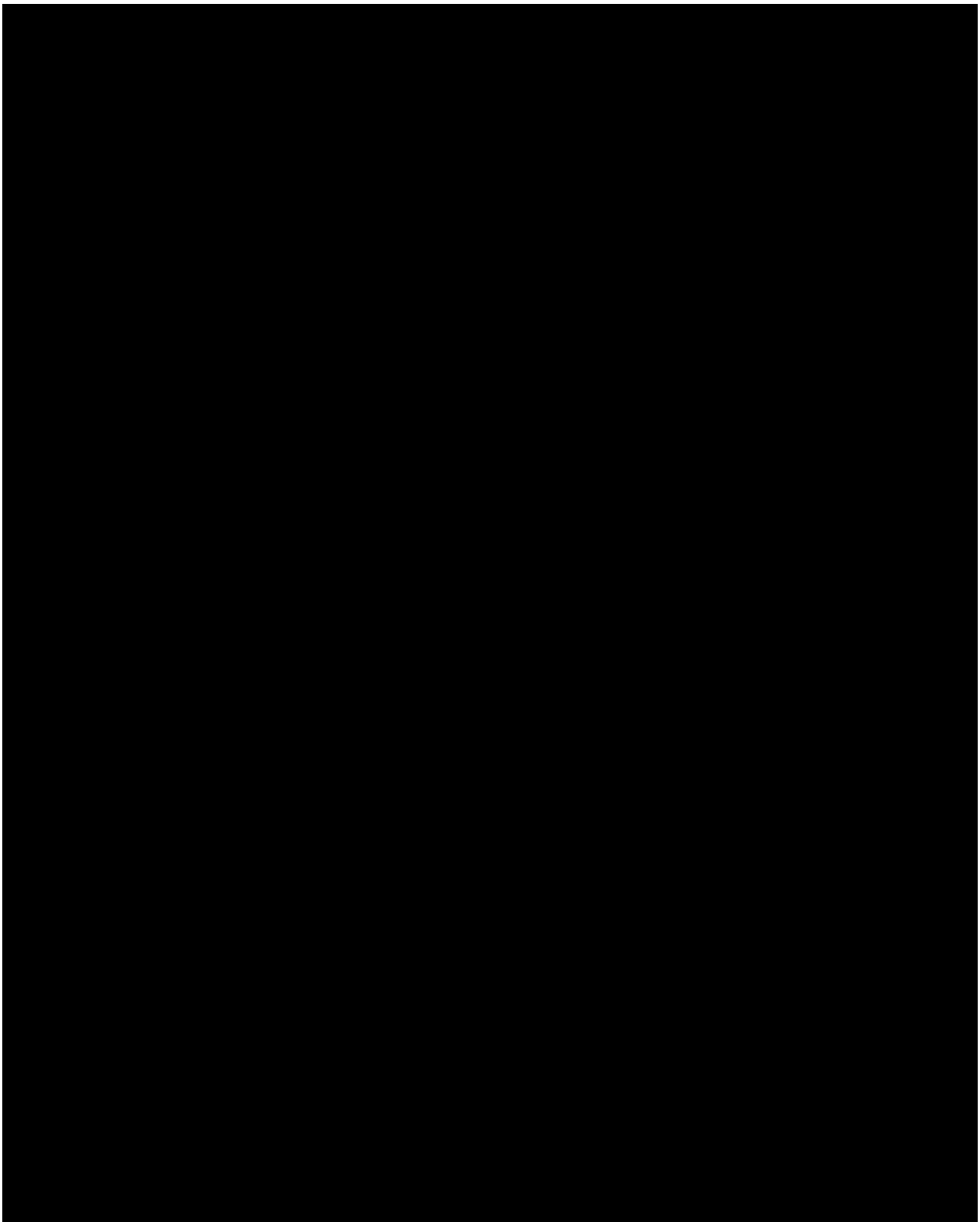

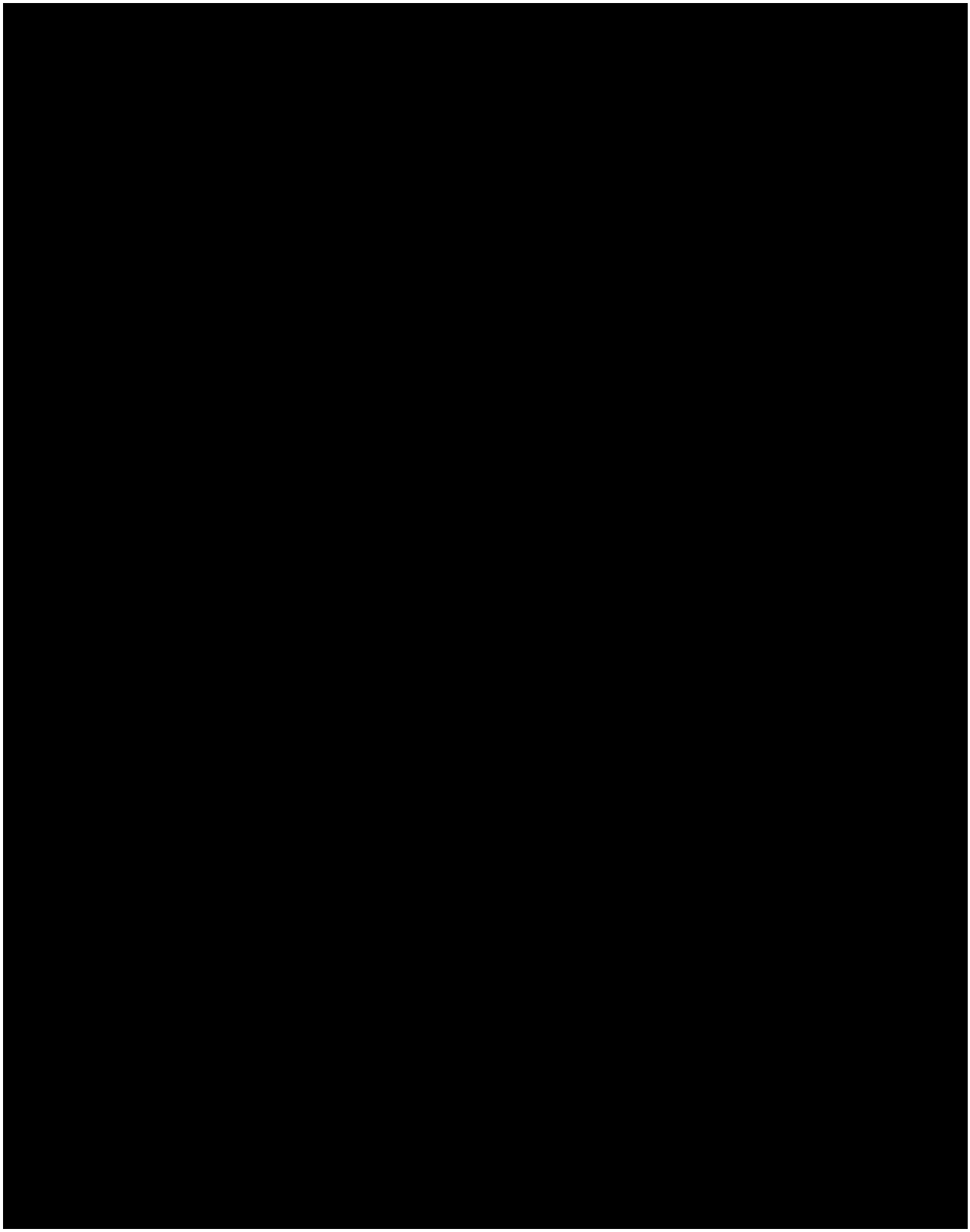

Appendix D: Derivation of C-SSRS

The Columbia Suicide Severity Rating Scale (C-SSRS) is an assessment that evaluates suicidal ideation and behavior. C-SSRS measurements are collected with respect to "Lifetime: time he/she felt most suicidal" at baseline, and with respect to "Since last visit" at last visit (Day 57 for Part A and Day 169 for Part B) and ET visit.

There are 11 common "Yes/No" questions at baseline and postbaseline visits. Five questions on *suicidal ideation* and five questions on *suicidal behavior* are re-ordered and follow increasing severity order respectively as shown in Table D1. In particular, only patients who answered "Yes" to question 2 will proceed to question 3, 4 and 5. Thus, for any subjects who answered "No" to question 2, an answer "No" will also be assumed to question 3, 4, and 5. An additional "Yes/No" question is used to record if subject had committed suicide in postbaseline visits.

Table D1: C-SSRS re-ordered questions

Suicidal Ideation	
Question 1	Wish to be dead
Question 2	Non-specific active suicidal thoughts
Question 3	Active suicidal ideation with any methods (not plan) without intent to act
Question 4	Active suicidal ideation with some intent to act, without specific plan
Question 5	Active suicidal ideation with specific plan and intent
Suicidal Behavior	
Question 6	Preparatory acts or behavior
Question 7	Aborted attempt
Question 8	Interrupted attempt
Question 9	Actual attempt

Question 10	Suicidal behavior
Question 11 (postbaseline visit only)	Suicide
Self-Injurious Behaviour without Suicidal Intent	
Question 12	Self-injurious behavior without suicidal intent

A subject is considered to have *suicidal ideation* at the period of interest if a "Yes" is answered to any of the five suicidal ideation questions (Question 1-5). A subject is considered to have *suicidal behavior* at the period of interest if a "Yes" is answered to any of the five suicidal behavior questions (Question 6-10) at baseline or a "Yes" is answered to any of the six suicidal behavior questions (Question 6-11) at postbaseline visit.

A subject's *Suicidal Ideation Score* is defined as the maximal suicidal ideation question number (maximal of 1-5) with an answer "Yes" per visit. The score is defined as 0 if the subject answered "No" to all 5 Suicidal Ideation questions at that visit. A subject is considered to have treatment-emergent suicidal ideation if the subject had either new or worsening suicidal ideation. A subject is considered to have new suicidal ideation if the subject's Suicidal Ideation Score increased at postbaseline visit compared to a score 0 at baseline. A subject is considered to have worsening suicidal ideation if the subject's Suicidal Ideation Score increased at postbaseline visit compared to a positive score at baseline.

A subject is considered to have treatment-emergent suicidal behavior if the subject answered "Yes" to any suicidal behavior questions at any postbaseline visit while answered "No" to all suicidal behavior questions at baseline.

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Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
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Operating Systems:	Windows2000? or WindowsXP?
Browsers (for SENDERS):	Internet Explorer 6.0? or above
Browsers (for SIGNERS):	Internet Explorer 6.0?, Mozilla FireFox 1.0, NetScape 7.2 (or above)
Email:	Access to a valid email account
Screen Resolution:	800 x 600 minimum
Enabled Security Settings:	•Allow per session cookies •Users accessing the internet behind a Proxy Server must enable HTTP 1.1 settings via proxy connection

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