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PROTOCOL NUMBER: 233AS101/ NCT02623699

PHASE OF DEVELOPMENT: 1/2/3

PROTOCOL 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

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SPONSOR SIGNATURE

Protocol 233AS101 was approved by:

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Biogen MA Inc.

17 June 2021

Date

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1. SPONSOR INFORMATION

Biogen MA Inc. is the Sponsor of the study.

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For urgent medical issues in which the study's Medical Director should be contacted, please refer to the Study Reference Guide's Official Study Contact List for complete contact information.

Biogen may transfer any or all of its study-related responsibilities to a contract research organization and other third parties; however, Biogen retains overall accountability for these activities.

2. LIST OF ABBREVIATIONS

Ab	antibody
AE	adverse event
ALS	amyotrophic lateral sclerosis
[redacted]	
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ANCOVA	analysis of covariance
anti-HBc	total hepatitis B core antibody
anti-HBs	hepatitis B surface antibody
APTT	activated partial thromboplastin time
ASO	antisense oligonucleotide
AUC	area under the concentration-time curve
[redacted]	
CNS	central nervous system
CRF	case report form
CRM	continuous reassessment method
CSF	cerebrospinal fluid
C-SSRS	Columbia Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
DHA	Directions for Handling and Administration
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DPS	diaphragm pacing system
EAC	event adjudication committee
ECG	electrocardiogram
EEG	electroencephalogram
[redacted]	
EOS	end of study
[redacted]	
FIH	first-in-human
FSH	follicle-stimulating hormone
[redacted]	
FU	follow-up
FVC	forced vital capacity
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HED	human equivalent dose
HHD	handheld dynamometry

HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Council for Harmonisation
IDMC	independent data monitoring committee
IgM anti-HBc	immunoglobulin antibody to hepatitis B core antigen
INR	international normalized ratio
IRT	interactive response technology
ITT	intent-to-treat
JRT	joint rank test
LP	lumbar puncture
LTE	long-term extension
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
mITT	modified intent-to-treat
MMSE	Mini-Mental State Examination
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
[redacted]	
NfL	neurofilament light chain
NHP	nonhuman primate
NOAEL	no observed adverse effect level
[redacted]	
PD	pharmacodynamic(s)
[redacted]	
PHI	protected health information
PI	principal investigator
PK	pharmacokinetic(s)
pNfH	phosphorylated axonal neurofilament heavy chain
PT	prothrombin time
RNA	ribonucleic acid
SAD	single ascending dose
SAE	serious adverse event
SD	standard deviation
[redacted]	
SOD1	superoxide dismutase 1
SUSAR	suspected unexpected serious adverse reaction
SVC	slow vital capacity
$t_{½}$	elimination half-life
TEAE	treatment-emergent adverse event
V	visit
[redacted]	

[redacted]

3. SYNOPSIS

Protocol Number:	233AS101
Protocol 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
Version Number	8
Name of Study Treatment:	BIIB067
Study Indication:	Amyotrophic lateral sclerosis and confirmed superoxide dismutase 1 mutation
Study Rationale	ALS is a rare neurodegenerative disease resulting in loss of motor neurons within the cortex, brainstem, and spinal cord. Patients suffer gradual loss of muscle mass, strength, and function in bulbar, respiratory, and voluntary muscle. Decline is inevitable, with death from respiratory failure following 2 to 5 years after diagnosis for most patients. Although the majority of patients suffer from sporadic ALS, a smaller fraction of patients, approximately 2%, have an inherited, or familial, form of ALS caused by a variety of mutations in SOD1 (SOD1-ALS). Since SOD1-ALS was first described in 1993, over 180 SOD1 mutations have been reported to cause this form of ALS. Although the mechanism by which mutations cause SOD1-ALS is not known, data suggest that toxic gain of function, not loss of SOD1 activity, is the likely trigger that initiates the cascade of events resulting in motor neuron death. The only currently approved treatments for ALS are riluzole and edaravone. Riluzole provides a modest increase in survival (2 to 3 months) without noticeable improvement in strength or disability. Edaravone lessens functional decline as measured by ALSFRS-R. The effect of edaravone on survival is unknown. No specific SOD1-ALS treatments are available. Reducing SOD1 mRNA and, subsequently, toxic SOD1 protein may offer therapeutic benefit for SOD1-ALS participants. Delivery of an ASO targeting SOD1 mRNA is a viable method to reduce toxic SOD1 protein.

	BIIB067 is an investigational ASO inhibitor of SOD1 mRNA, under development to reduce levels of SOD1 protein in patients with SOD1-ALS.
Phase of Development:	1/2/3
Study Objectives and Endpoints:	**Parts A (SAD) and B (MAD)** 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 a confirmed SOD1 mutation. The primary endpoints are as follows: • Incidence of AEs and SAEs • Incidence of abnormalities in clinical laboratory assessments, vital signs, physical and neurological examinations, and ECGs • PK measures, including plasma and CSF levels of BIIB067 The secondary objective is to evaluate the effects of BIIB067 on levels of total SOD1 protein in the CSF. • The secondary endpoint is the change from baseline in CSF levels of total SOD1 protein. Exploratory objectives and endpoints are listed in Section 6.1. **Part C (Pivotal)** Primary Objective The primary objective of Part C of this study is to evaluate the clinical efficacy of BIIB067 administered to adult participants with ALS and a confirmed SOD1 mutation. • Primary Efficacy Endpoint The primary efficacy endpoint is the change from baseline to Week 28 in the ALSFRS-R total score.

- Secondary Efficacy Endpoints

  The secondary efficacy endpoints are as follows:

  - Change from baseline to Week 28 in SVC
  - Changes from baseline to Week 28 in HHD megascore to assess muscle strength, as measured by the HHD device
  - Time to death or permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days)
  - Time to death

Secondary Objective

The secondary objective is to evaluate the safety, tolerability, PD, and biomarker effects of BIIB067 administered to adult participants with ALS and a confirmed SOD1 mutation.

- Safety/Tolerability Endpoint

  The safety/tolerability endpoint is the incidence of AEs and SAEs.

- Pharmacodynamic Endpoint

  The PD endpoint is the change from baseline in total SOD1 protein concentration in CSF.

- Biomarker Endpoint

  The biomarker endpoint is the change from baseline in NfL concentration in plasma.

Exploratory objectives and endpoints are listed in Section 6.2.

Study Design:

This is a randomized, double-blind, placebo-controlled, 3-part dose escalation study to examine the efficacy, safety, tolerability, PK, and PD of BIIB067, administered by intrathecal bolus injection to up to approximately 183 adult participants with ALS and a confirmed SOD1 mutation.

Part A (SAD)

Part A will be a randomized, double-blind, placebo-controlled, SAD study of up to 4 dose levels of BIIB067 administered to participants with SOD1-ALS. The dose levels will be evaluated sequentially. Final sample size

	for Part A will be determined by the incidence of DLT. If no DLTs are encountered, a minimum of 20 participants will be randomized. The maximum sample size for Part A is 36 participants. Part B (MAD) 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 to approximately 48 participants with SOD1-ALS. Part C (Pivotal) Part C will be a randomized, double-blind, placebo-controlled evaluation of 100 mg of BIIB067 administered 8 times over approximately 24 weeks to approximately 99 participants with SOD1-ALS. Participants will be randomized to receive BIIB067 or placebo in a 2:1 (active:placebo) ratio. The primary analysis population (N = ~60) will comprise participants who meet prognostic enrichment criteria for rapid disease progression, defined as those with a prerandomization ALSFRS-R slope decline of at least 0.9/month OR a protocol-defined SOD1 mutation and prerandomization ALSFRS-R slope decline of at least 0.2/month. Randomization will be stratified by 3 factors: • whether a participant meets the prognostic enrichment criteria for rapid disease progression • whether a participant uses edaravone at baseline • whether a participant uses riluzole at baseline – Use of both edaravone and riluzole will not be a separate stratum but will be classified as only edaravone use.
Study Location:	Approximately 42 sites are planned in approximately 15 countries globally.
Number of Planned Participants:	Approximately 183 participants are planned to be dosed: 36 participants in Part A, 48 participants in Part B, and 99 participants in Part C.
Study Population:	This study will be conducted in participants who are at least 18 years of age with SOD1-ALS. Detailed criteria are described in Section 8.

Treatment Groups:	Cohorts 1 through 4 (Part A) and Cohorts 5 through 8 (Part B) will be randomized to receive BIIB067 or placebo in a 3:1 (active:placebo) ratio. Cohort 9 (Part C) will be randomized to receive BIIB067 or placebo in a 2:1 (active:placebo) ratio. Part A (SAD) Up to 4 cohorts will be enrolled in Part A (SAD). Cohorts 1 through 3 will enroll at least 4 participants each: 1 participant will be administered placebo, and 3 participants will be administered BIIB067 at 10, 20, or 40 mg. Cohort 4 will enroll approximately 8 participants: 2 participants will be administered placebo, and 6 participants will be administered BIIB067 at 60 mg. Up to 16 additional participants may be enrolled into any of the cohorts (up to 4 placebo and up to 12 BIIB067), based on the incidence of DLT. If no DLTs are observed in the 2 lower dose cohorts, then participants within these 2 lower dose cohorts may re-enroll, after a washout period of at least 5 times the $t_{½}$ [~20 weeks including the 8-week FU period], within the 2 higher dose cohorts. These participants will repeat the screening assessments prior to re-enrolling and will be rerandomized into either the BIIB067 or placebo group. Part B (MAD) Up to 4 cohorts will be enrolled in Part B (MAD). Each cohort will enroll approximately 12 participants: 3 participants per cohort will be administered placebo, and 9 participants per cohort will be administered BIIB067 at 20, 40, 60, or 100 mg. Part C (Pivotal) A single cohort of approximately 99 participants will be enrolled in Part C (Pivotal): approximately 33 participants will be administered placebo, and approximately 66 participants will be administered BIIB067 at 100 mg.

Duration of Treatment and Follow-Up:	Part A (SAD) Duration of the study for each participant participating only in Part A (SAD) will be up to approximately 15 weeks, which will include up to 7-week screening period and an 8-week FU period. For participants 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 × t½ (~12 weeks) after the 8-week postdose FU visit (total washout of 5 × t½, or ~20 weeks). Part B (MAD) For participants participating in only Part B (MAD), the duration will be up to approximately 31 weeks, which will include up to 7-week screening period, 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 FU period. Part A (SAD) and Part B (MAD) For participants participating in both Part A (SAD) and Part B (MAD), duration will be approximately 51 weeks. This will include 15 weeks in Part A, approximately 12 weeks between the end of Part A and dosing in Part B, 12 weeks of dosing in Part B, and the 12-week FU period in Part B. For participants who enroll in 2 cohorts during Part A and participate in Part B, the total duration will be approximately 71 weeks. Part C (Pivotal) For participants in Part C (Pivotal), the study duration will be approximately 32-36 weeks including a 4-week screening period, a 24-week treatment period (3 loading doses 2 weeks apart followed by 5 maintenance doses 4 weeks apart), and a 4- to 8-week FU period as follows: • Part C participants who enroll (uninterrupted) in the LTE study (233AS102): Week 28 Visit will serve as EOS Visit • Part C 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.

- Part C participants who do not enroll in the LTE study: Week 32 Visit will serve as EOS (either in person or by telephone contact) Visit.

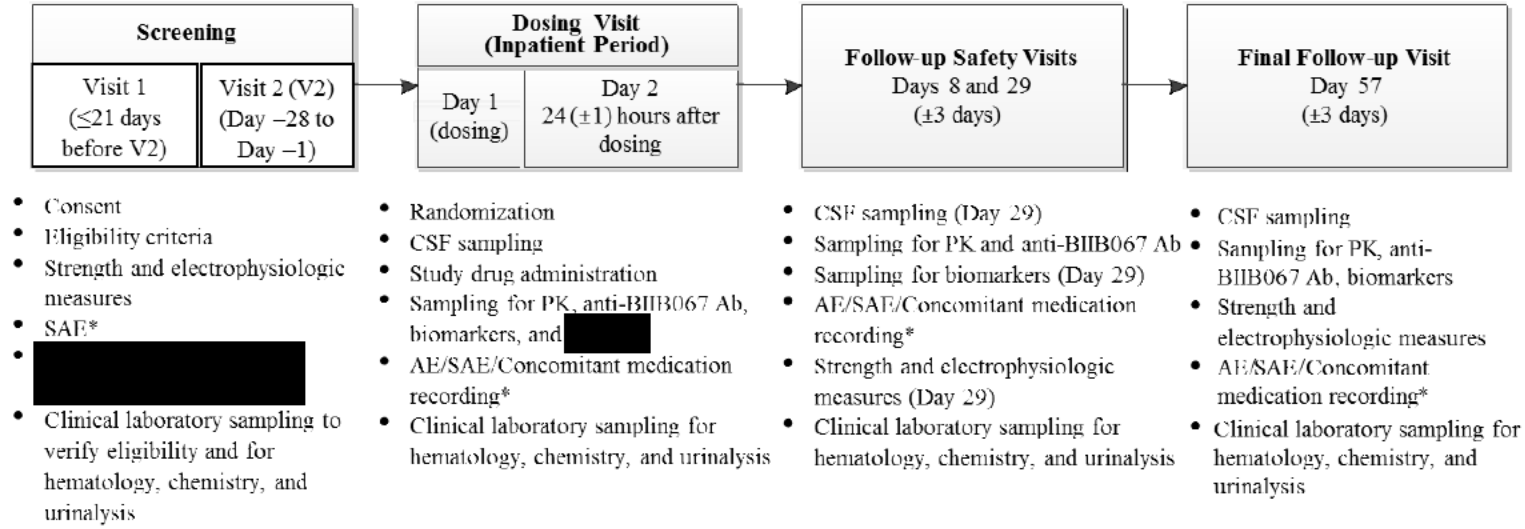
In the event of a decision by the Study Sponsor to terminate the study earlier on the grounds that conducting a placebo-controlled study is no longer deemed ethical based on the assessment of risk-benefit of Study 233AS101, all participants will be invited for an EOS visit, during which all Week 28 assessments will be conducted. For participants not entering the LTE study or in the event the study is stopped early, there will be an additional safety FU visit 8 weeks after the last dose of study treatment.

4. STUDY SCHEMATIC AND SCHEDULE OF ACTIVITIES FOR STUDY 233AS101

Key assessments are included in Figure 1, Figure 2, and Figure 3 for Parts A, B, and C of the study, respectively. For a full list of assessments and their timing, please see Table 1, Table 2, and Table 3 for Parts A, B, and C, respectively.

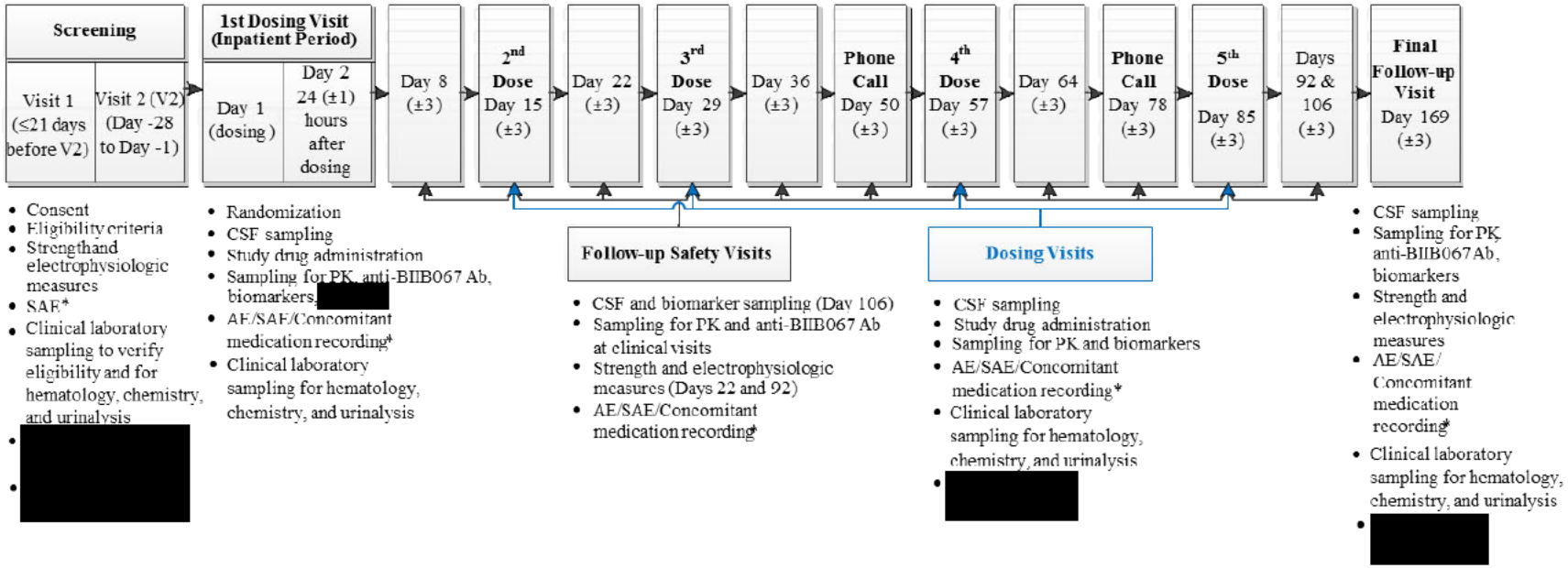
4.1. Study Schematic

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



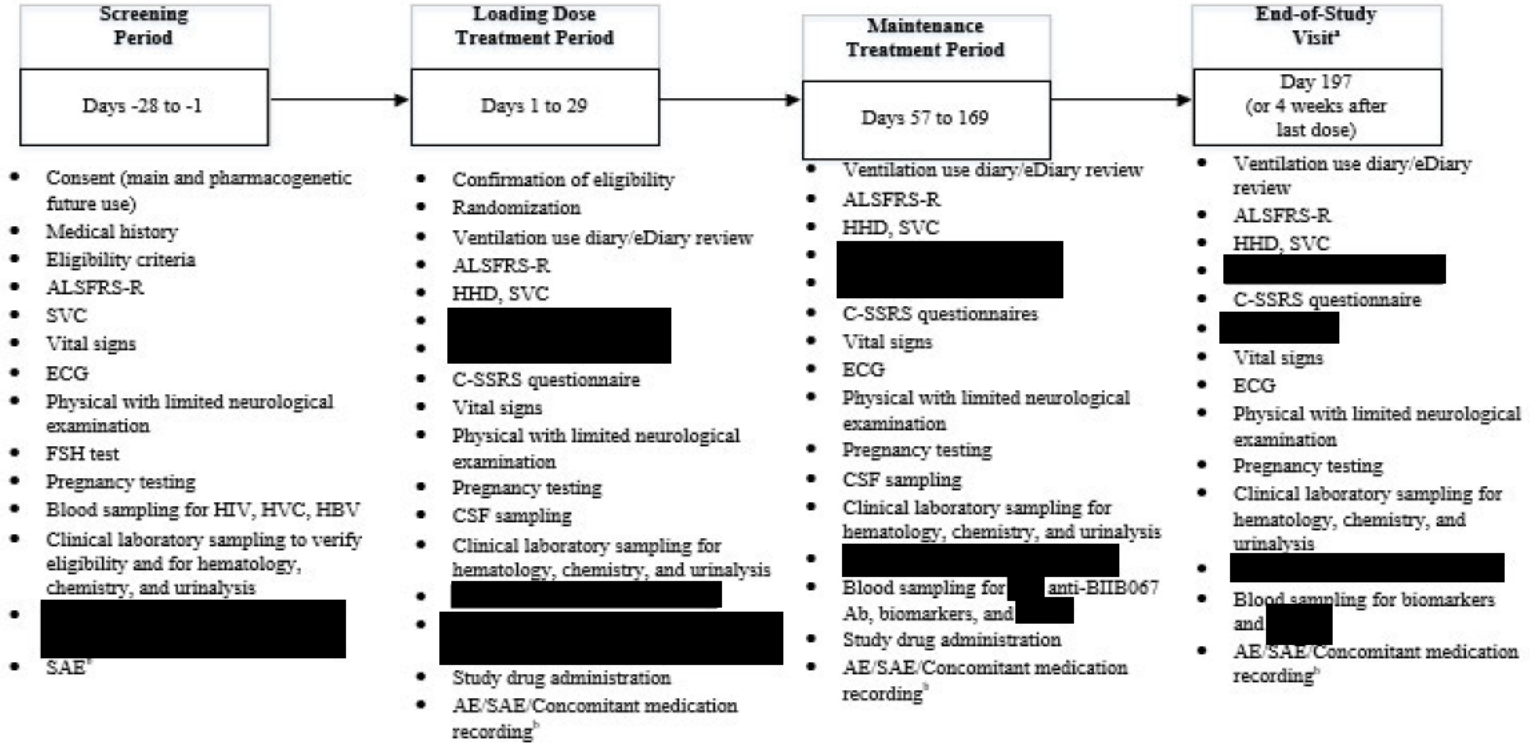
* SAE monitoring will be ongoing starting at the Screening Visit; AE and concomitant medication monitoring will be ongoing starting on Day 1.

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



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

Figure 3: Study Design: Part C (Pivotal)



[a] Participants who do not roll over into the LTE study (233AS102) will have an additional safety FU visit at Week 32 to collect AEs, SAEs, and concomitant medications or procedures. Participants with delays between their Week 28 Visit and enrolment into the LTE study will have a Safety FU (Alternative EOS) Visit to collect AEs, SAEs, and concomitant medications or procedures.
[b] SAE monitoring will be ongoing starting at the Screening Visit; AE and concomitant medication monitoring will be ongoing starting on Day 1.

4.2. Schedule of Activities

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

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

[1] Required only for participants 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 participant burden.
[3] Not required if collected during Screening Visit 1.
[4] Including blood samples for HIV, HCV, and HBV 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. Participants 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 participant 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 participants.
[12] Day 29 only.
[13] LP will be performed to collect CSF samples for PK, PD, 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. Participants will remain under observation in the clinic for approximately 1 hour after the LP procedure and will receive a safety FU telephone contact ~24 hours after the procedure.

[redacted]

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

Table 2: Schedule of Activities: Part B – Multiple Ascending Dose

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 (±3 days) via Telephone Contact	Day 169 (±3 days) /Early Termination Visit
			Predose	Dosing/ Postdose					
Informed Consent (main)	X	X[4]							
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
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]

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 (±3 days) via Telephone Contact	Day 169 (±3 days) /Early Termination Visit
			Predose	Dosing/ Postdose					
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]									
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

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 (±3 days) via Telephone Contact	Day 169 (±3 days) /Early Termination Visit
			Predose	Dosing/ Postdose					
[redacted]									
Blood Samples for Biomarkers			X			X	X[22]		X
[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)----------								

[1] Required only for participants 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 participant burden.
[3] Participants 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 HIV, HCV, and HBV 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. Participants 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 components of the limited neurological examination are 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 participant 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 participants.
[17] Day 22 and Day 92 for [redacted] HHD; Day 92 for ALSFRS-R [redacted]
[18] LP will be performed to collect CSF samples for PK, PD, 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. Participants will remain under observation in the clinic for approximately 6 hours after the LP procedure and will receive a safety FU telephone contact ~24 hours after the procedure.
[20] To be collected on Day 106 only. Participants will remain under observation in the clinic for approximately 1 hour after the LP procedure and will receive a safety FU telephone contact ~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.

Table 3: 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	
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**		
[redacted]															
C-SSRS Questionnaire[8]		X						X[13]			X[13]			X[13]	
Height	X														
Weight[14]	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**		
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)	
[redacted]															
Blood Sampling for HIV, HCV, and HBV Testing	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		
Clinical Laboratory Samples for Hematology, Coagulation, Chemistry, and Urinalysis[8,23]	X	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		
[redacted]															
Study Drug Administration[28]			X			X			X			X			
AE and Concomitant Therapy and Procedures Recording		----------X (ongoing)----------													
SAE Recording	----------X (ongoing)----------														

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

[2] After completing the EOS Visit, participants will be considered study completers and will have the option to participate in the LTE study 233AS102 (see Section 7.5).

[3] Participants 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 (see Section 10.1).

[4] Participants who do not roll over into the LTE study will have an additional safety FU 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 participants are unable to travel to the site; then, a home visit may be possible at the discretion of the Investigator.

[redacted]

[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] Participants 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 participant has rested in a sitting position for at least 5 minutes.

[16] Triplicate 12-lead ECGs will be obtained after the participant 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.

[17] To be assessed just prior to drawing PK blood samples at 2, 4, and 6 hours postdose on Day 1 and Day 85 only.

[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 participants 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.

[redacted]

[28] Participants 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 participants have adequately recovered from the dosing procedure.

5. INTRODUCTION

BIIB067 is an investigational second-generation ASO inhibitor of SOD1 mRNA, under development to reduce levels of total SOD1 protein in patients with ALS and a confirmed SOD1 mutation (SOD1-ALS). ASOs are short synthetic strings of nucleotides designed to prevent the expression of a targeted protein by selectively binding to the mRNA that encodes the protein with high affinity and selectivity through well-characterized Watson-Crick base pairing (hybridization).

5.1. Overview of Amyotrophic Lateral Sclerosis

ALS is a rare neurodegenerative disease resulting in loss of motor neurons within the cortex, brainstem, and spinal cord. Patients suffer gradual loss of muscle mass, strength, and function in bulbar, respiratory, and voluntary muscle. Decline is inevitable, with death from respiratory failure following 2 to 5 years after diagnosis for most patients. Although the majority of patients suffer from sporadic ALS, a smaller fraction of patients, approximately 2%, have an inherited, or familial, form of ALS caused by a variety of mutations in SOD1. Since SOD1-ALS was first described in 1993, over 180 SOD1 mutations have been reported to cause this form of ALS (referred to as SOD1-ALS) [ALSoD 2015; Rosen 1993]. Disease progression for individual mutations is variable, with survival of less than 15 months seen with the most severe mutations [Cudkowicz 1997]. Although the mechanism by which mutations cause SOD1-ALS is not known, compelling data suggest that toxic gain of function, not loss of SOD1 activity, is the likely trigger that initiates the cascade of events resulting in motor neuron death [Bruijn 1998; Rosen 1993].

5.2. Current Therapies for Amyotrophic Lateral Sclerosis

The only currently approved treatments for ALS are riluzole and edaravone. Riluzole provides a modest increase in survival (2 to 3 months) without noticeable improvement in strength or disability [Miller 2012]. Edaravone lessens functional decline as measured by the ALSFRS-R. The effect of edaravone on survival is unknown [Writing Group and Edaravone (MCI-186) ALS 19 Study Group 2017]. No specific SOD1-ALS treatments are available.

5.3. Profile of Previous Experience with BIIB067

5.3.1. Nonclinical Experience

Four in vivo nonclinical toxicology studies were performed to support the development of BIIB067: a repeated-dose, 12-week, subcutaneous bolus injection study in mice (25 and 150 mg/kg); a single-dose, intrathecal bolus injection study in rats (0.1, 0.3, 1.0, and 3.0 mg); a repeated-dose, 13-week, intrathecal bolus injection study in cynomolgus monkeys (4, 12, and 35 mg); and a repeated-dose, 9-month, intrathecal bolus injection study in cynomolgus monkeys (4, 12, and 35 mg).

See the Investigator's Brochure for detailed information on nonclinical studies.

5.3.1.1. Toxicology

In mice, subcutaneous administration of BIIB067 was well tolerated, with the highest dose evaluated (150 mg/kg) being the NOAEL. The NOAELs in the toxicology studies in which the clinical route of administration (intrathecal) was used were 1 mg (rat) and 35 mg (monkey).

In rats, a single intrathecal bolus delivery of BIIB067 resulted in 1 early death in a rat receiving the highest dose evaluated (3 mg). Transient acute tactile hypersensitivity was noted approximately 25 minutes postdose in animals receiving 3 mg. Decreases in arousal, gait, mobility, respiration and sensorimotor observations were also noted 3 hours postdose in the 3-mg group.

In cynomolgus monkeys, repeated intrathecal administration of BIIB067 for 13 weeks was well tolerated up to the highest dose evaluated (35 mg); although, 2 animals in the 35-mg group had transient clinical signs (reduced locomotor activity). Cytoplasmic vacuolation in some neurons of the hippocampus, and to a lesser degree of the cerebral cortex, was observed at all doses (4, 12, and 35 mg). The neuronal vacuolation was not associated with any morphological evidence of cell degeneration or necrosis, and was fully reversible within a 13-week recovery period. Furthermore, intrathecal injection of BIIB067 caused mononuclear inflammatory cell infiltrates in the meninges at the lumbar spinal cord injection site, possibly as a local proinflammatory effect caused by ribonuclease H-based ASOs. Remnants of such inflammatory infiltrates were still detectable after 13 weeks of recovery, although at a lower magnitude. In addition, vacuolated histiocytes were found in lymph nodes as well as in the Virchow-Robin space of the brain; this finding was not fully reversible within 13 weeks. Neuronal vacuolation in the spinal cord was observed in 1 recovery animal, which was not observed in any animal at the end of the 13-week dosing period. The relationship of this finding to BIIB067 is unknown. Based on the lack of any evidence of cell degeneration or necrosis, and absence of clinical or neurological abnormalities associated with the vacuolation, it was concluded that the microscopic findings are not adverse.

From the 9-month cynomolgus monkey toxicology study, the NOAEL for repeated, intrathecally-administered BIIB067 was 12 mg. This was based on adverse clinical observations for 1 female administered 35 mg. This female exhibited neurological signs after the second dose, characterized by transient muscle cramping (seen immediately after dosing on multiple days), prolonged recovery from anesthesia, and intermittent tremors (during the last months of the dosing phase). Treatment with diazepam was required on several dosing occasions. An EEG on this animal revealed altered postdose signals (with effects on high frequency bands) but confirmed that muscle cramping during EEG recording was not a seizure and could have been related to arousal. This finding was considered test-article related; however, there were no correlates in clinical and anatomic pathology.

5.3.1.2. Pharmacokinetics

To characterize the PK properties of BIIB067, CSF [monkey only], plasma (monkey and mouse only), and tissue (monkey, mouse, and rat) concentrations were assessed following intrathecal (monkey and rat) or subcutaneous (mouse) administration.

Following bolus intrathecal administration in monkeys, BIIB067 concentrations in CSF declined in a multiphasic manner with a rapid distribution phase, followed by a slower and longer

elimination phase. The rapid distribution phase was due to rapid and broad distribution from CSF to CNS tissues and clearance from CSF due to transfer to systemic circulation (including plasma and systemic tissues such as kidney and liver). The estimated CSF $t_{½}$ was 20 to 37 days.

BIIB067 concentrations in plasma peaked 1 to 4 hours after the intrathecal bolus injection in monkeys and then rapidly declined during the first 48 hours postdose, with a much slower elimination phase thereafter. The estimated plasma $t_{½}$ was 22 to 51 days. In mice, BIIB067 concentrations in plasma peaked 0.5 hour after subcutaneous administration, indicating rapid absorption into the systemic circulation and, then similar to monkeys, declined rapidly over the next 48 hours due to extensive distribution to systemic tissues.

Dose-dependent tissue distribution of BIIB067 to CNS and peripheral tissues was seen in all species studied (monkey, rat, and mouse), with no clear or consistent evidence of a sex difference in distribution to tissues.

Following intrathecal administration, distribution to CNS tissues was broad in both rats and monkeys, with the highest CNS concentrations seen in the lumbar spinal cord, consistent with the intrathecal route of administration. In monkeys, relatively higher concentrations were observed in the liver and kidney, indicating a significant proportion of the administered dose was distributed from CSF to the systemic circulation. During the recovery period, BIIB067 appeared to be cleared slowly from CNS and peripheral tissues. The estimated tissue $t_{½}$ was similar to CSF and plasma (31 to 40 days in CNS tissues, 15 to 20 days in the liver, and 18 to 23 days in the kidney). BIIB067 was rapidly and extensively distributed from plasma to liver and kidney following subcutaneous administration in mice.

The long CSF and CNS tissue $t_{½}$ observed in monkeys support an infrequent clinical dosing regimen following intrathecal administration.

5.3.2. Clinical Experience

This is the FIH study of BIIB067. In the single and multiple dose escalation parts (Parts A and B) of this study, BIIB067 at dose levels up to and including 100 mg was generally well tolerated. Most of the AEs were mild or moderate in severity. Plasma concentrations of BIIB067 were dose proportional; CSF concentrations showed a less than dose proportional response. A statistically significant reduction in total CSF SOD1 protein concentration was observed in the BIIB067 100 mg group (37% reduction) compared with the placebo group (no reduction). Interim exploratory analyses showed a slowing of decline in functional (ASLFRS-R), respiratory (SVC), and strength measures (HHD Megascore). These data support the continued development of BIIB067 for the treatment of SOD1-ALS.

5.4. Study Rationale

BIIB067 is an investigational ASO inhibitor of SOD1 mRNA, under development to reduce levels of SOD1 protein in patients with SOD1-ALS.

Overexpression of mutant SOD1 in mice or rats recapitulates important aspects of ALS in humans [Bruijn and Cleveland 1996; Gurney 1994]; however, loss of SOD1, while resulting in eventual motor neuron dysfunction, does not result in motor neuron death [Fischer 2012; Reaume 1996]. Furthermore, neither loss nor increase of SOD1 activity in mouse models of

SOD1-ALS alters survival [Bruijn 1998]. In humans, individual disease mutations are associated with varying levels of SOD1 activity; some patients with disease-causing mutations have apparently normal SOD1 activity [Saccon 2013]. Thus, correlation between disease severity and normal SOD1 activity has not been observed [Andersen 1997; Ratovitski 1999; Saccon 2013]. In contrast, reduction of SOD1 with intracerebroventricular-delivered SOD1 ASO extends survival in a rodent model of SOD1-ALS [Smith 2006]. These observations strongly suggest that SOD1-ALS is caused by toxic properties of the mutant SOD1 protein. Reducing SOD1 mRNA and, subsequently, toxic SOD1 protein may offer therapeutic benefit for patients with SOD1-ALS. Intrathecal delivery of an ASO targeting SOD1 mRNA is a viable method to reduce toxic SOD1 protein.

Parts A (SAD) and B (MAD)

Pharmacology and toxicology data in rats, mice, and monkeys indicate that BIIB067 is well tolerated. Parts A and B of the study will evaluate the safety, tolerability, and PK profile of a range of single and multiple intrathecal BIIB067 doses from a projected minimal pharmacologic dose to either the MTD or the highest planned dose of 100 mg. MTD for this study is defined as the highest tested dose with a less than 33% DLT rate at Day 15. See Section 7.2.3 for the definition of DLT.

Part C (Pivotal)

Based on the interim data from Parts A and B summarized in Section 5.3.2, a randomized, double-blind, placebo-controlled, pivotal phase (Part C) has been designed to assess the efficacy and safety of BIIB067 100 mg versus placebo. Approximately 99 participants with SOD1-ALS will be randomized to receive BIIB067 or placebo and treated for 24 weeks.

5.5. Rationale for Dosing Regimen

BIIB067 will be administered via intrathecal bolus at single ascending doses to cohorts in Part A, and at multiple ascending doses to cohorts in Part B.

The BIIB067 dosing regimens for Parts A and B of this study were selected based on nonclinical toxicology and PK observations in NHP studies using repeated-dosing intrathecal administration for 13 weeks and 9 months, and target tissue concentrations from SOD1 transgenic mouse models.

Based on pharmacology and PK results in SOD1 transgenic mice, the estimated tissue concentrations of BIIB067 needed to produce a 50%, and the upper 95% confidence interval of an 80%, SOD1 mRNA reduction within the human spinal cord are 0.9 µg/g and 4.7 µg/g, respectively. Evaluations of cortex from the same experiment indicate that the estimated tissue concentration of BIIB067 needed to produce a 50% cortical SOD1 mRNA reduction is 8 µg/g.

The lowest dose selected for this study (10 mg in Part A) is predicted to achieve greater than 0.9 µg/g tissue concentration in the spinal cord. The initial highest proposed dose (60 mg, multiple dose in Part B) was predicted to achieve greater than 4.7 µg/g steady-state tissue concentration in the spinal cord, and approximately 1.5 µg/g steady-state tissue concentration in the cortex, which is expected to yield approximately 15% to 20% SOD1 mRNA reduction in that tissue. The addition of a 100-mg cohort is predicted to achieve steady-state exposures in the cortex of approximately 2.5 µg/g, which is predicted to be sufficient for a meaningful reduction

in total CSF SOD1 protein of 25% to 30%. This reduction is expected to be clinically meaningful, based on the expectation that the reduction of SOD1 protein in tissues (spinal cord and cortex) would be greater than the 20% to 30% reduction observed in CSF. This prediction is based on observations in NHP studies, where approximately 50% reduction in CSF corresponded to approximately 50% or greater reduction in the spinal cord and cortex. In rodent efficacy experiments, doses of BIIB067 that reduced tissue total SOD1 protein by approximately 30% or more were found to improve measures of electrophysiology, function, and neurofilament.

Based on nonclinical PK and pharmacology data, and taking into consideration participant safety, the inconvenience of repeated intrathecal injections, and the rapid, fatal nature of ALS, the following dose intervals were selected for Part B of the study (MAD): 3 loading doses, once every 2 weeks, and 2 maintenance doses, administered once every 4 weeks. These intervals were selected based on the estimated $t_{½}$ of BIIB067 (~1 month) in the target CNS tissues (spinal cord and brain cortex), to achieve and maintain the target tissue concentration of BIIB067 at a steady state level and within the estimated pharmacologically active range, and to facilitate effective total SOD1 protein reduction in the CSF within Part B of the study (MAD).

From the 9-month NHP toxicology study, the NOAEL for repeated, intrathecally-administered BIIB067 was 12 mg. This NOAEL was converted to an HED based on the NHP to human CSF volume scaling (approximately 10-fold difference). CSF volume scaling conservatively estimates the needed scaling factor and has predicted HED with reasonable accuracy. The HED for the 9-month, intrathecal NHP toxicology study was calculated to be 120 mg. This provides a 12-fold safety margin for the BIIB067 starting dose (10 mg), and a 2-fold safety margin for the 60-mg dose. The safety margin for a 100-mg clinical dose (highest planned dose) would be 1.2-fold. Preliminary PK data (AUC from time 0 to 24 hours) from the 20-mg MAD cohort indicate that the safety margin based on exposure is 2.2-fold relative to a 100-mg clinical dose. The 1.2-fold safety margin based on CSF volume scaling from the NHP toxicology study was chosen over the 2.2-fold safety margin calculated from the plasma steady-state exposures since the former provides a more conservative estimate.

Therefore, all BIIB067 doses planned for 233AS101 are predicted to be pharmacologically active, while maintaining sufficient safety margins calculated from the 9-month intrathecal NHP toxicology study.

For Part C (Pivotal), the dose of 100 mg of BIIB067 was determined based on the interim analyses of data from participants in Part B (MAD) Cohorts 5 to 8 treated for 85 days. Safety analyses of these data suggested that all doses through 100 mg had been well tolerated with a safety profile supportive of continued development of BIIB067 in ALS participants. The selection of the 100 mg BIIB067 dose for Part C is supported by PK/PD and exploratory efficacy analyses of data from participants in Part A (SAD) and Part B (MAD) [see Section 5.3.2].

6. STUDY OBJECTIVES AND ENDPOINTS

6.1. Parts A (SAD) and B (MAD)

6.1.1. Primary Objective

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

6.1.1.1. Primary Endpoints

The primary endpoints are as follows:

- Incidence of AEs and SAEs.
- Incidence of abnormalities in clinical laboratory assessments, vital signs, physical and neurological examinations, and ECGs.
- PK measures, including plasma and CSF levels of BIIB067.

6.1.2. Secondary Objective

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

6.1.2.1. Secondary Endpoint

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

6.1.3. Exploratory Objectives and Endpoints

To evaluate the effect of BIIB067 on [redacted] 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:
  - ALSFRS-R scores
  - SVC
  - [redacted]

To explore possible relationships between [redacted] phosphorylated axonal pNfH, and NfL.

[redacted]

6.2. Part C (Pivotal)

6.2.1. Primary Objective

The primary objective of Part C of this study is to evaluate the clinical efficacy of BIIB067 administered to adult participants with ALS and a confirmed SOD1 mutation.

6.2.1.1. Primary Efficacy Endpoint

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

6.2.1.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

- Change from baseline to Week 28 in SVC.
- Changes from baseline to Week 28 in HHD megascore to assess muscle strength, as measured by the HHD device.
- Time to death or permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days).
- Time to death.

[redacted]

6.2.2. Secondary Objective

The secondary objective is to evaluate the safety, tolerability, PD, and biomarker effects of BIIB067 administered to adult participants with ALS and a confirmed SOD1 mutation.

6.2.2.1. Safety/Tolerability Endpoint

The secondary safety/tolerability endpoint is the incidence of AEs and SAEs.

6.2.2.2. Pharmacodynamic Endpoint

The secondary PD endpoint is the change from baseline in total SOD1 protein concentration in CSF.

6.2.2.3. Biomarker Endpoint

The secondary biomarker endpoint is the change from baseline in NfL concentration in plasma.

[redacted]

7. STUDY DESIGN

7.1. Study Overview

This is a randomized, double-blind, placebo-controlled, 3-part study to examine the efficacy, safety, tolerability, PK, and PD of BIIB067, administered by intrathecal bolus injection to up to approximately 183 participants with ALS and a confirmed SOD1 mutation. Parts A and B are single and multiple dose escalating parts, respectively, in which participants will be randomized to receive BIIB067 or placebo in a 3:1 (active:placebo) ratio. In Part C, participants will be randomized to receive BIIB067 100 mg or placebo in a 2:1 (active:placebo) ratio. The study will be conducted at approximately 42 sites in approximately 15 countries globally. See Figure 1, Figure 2, and Figure 3 for Parts A, B, and C of the study, respectively.

7.1.1. Part A: SAD Evaluation

Part A will be a randomized, double-blind, placebo-controlled, SAD study of up to 4 dose levels of BIIB067 administered to participants 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 DLT. If no DLTs are encountered, a minimum of 20 participants will be randomized. The maximum sample size for Part A is 36 participants. The first 3 dose levels will be assessed in cohorts of 4 participants each: 1 participant will be administered placebo, and 3 participants will be administered BIIB067 at 10, 20, or 40 mg. The last dose level will be assessed in a cohort of 8 participants: 2 participants will be administered placebo, and 6 participants will be administered BIIB067 at 60 mg. Up to 16 additional participants may be enrolled into the selected dose levels (4 administered placebo and 12 administered BIIB067), based on the incidence of DLT. See Section 7.2.1 for a detailed description of dose escalation.

If no DLTs are observed in the 2 lower dose cohorts, then participants within these 2 lower dose cohorts may re-enroll, after a washout period of at least 5 times the $t_{½}$ (~20-week; including the 8-week FU period), within the 2 higher dose cohorts. These participants 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 participant enrolled in Part A will be up to approximately 15 weeks, which will include up to 7-week screening period and an 8-week FU period. For participants 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 $t_{½}$ (~12 weeks) after the 8-week FU visit (total washout of 5 times the $t_{½}$, or ~20 weeks). Participants 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 participants.
- A maximum of 36 participants have been enrolled.

7.1.2. Part B: MAD Evaluation

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, approximately 48 participants with SOD1-ALS. Each dose level will be assessed in cohorts of 12 participants: 3 participants will be administered placebo, and 9 participants will be administered BIIB067 at 20, 40, 60, or 100 mg.

The duration of study participation for each participant enrolled in Part B will be up to approximately 31 weeks, which will include up to 7-week screening period, 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 FU period.

7.1.2.1. Parts A and B

Participants with SOD1-ALS who participate in Part A (SAD) of the study will be able to re-enroll in Part B (MAD), after a washout period of at least 3 times the $t_{½}$ (~12-week), at a dose level that is either the same or at the next level up from the dose received in Part A (for participants who choose to re-enroll, this will be the last dose level received in Part A). For participants participating in both the SAD and the MAD portion of the study, duration will be approximately 51 weeks (71 weeks for participants who enroll in 2 cohorts during Part A). This will include all Part A visits, a period of at least 3 times the $t_{½}$ (~12 weeks) between the 8-week FU visit of Part A and the first dosing visit of Part B (~20-week total washout), and all Part B visits, excluding the Part B screening period, which can occur during the washout period, within 28 days before Day 1.

7.1.3. Part C: Pivotal

Part C will be a randomized, double-blind, placebo-controlled evaluation of 100 mg of BIIB067 administered 8 times over approximately 24 weeks to approximately 99 participants with SOD1-ALS.

Part C: Screening-Only Sites

Some sites may be designated as screening-only sites and will only perform screening visit assessments. Participants who complete screening at screening-only sites and are deemed eligible for participation in Part C will be transferred to a full participation site for the remainder of the study. Participants may also be screened at full treatment sites and transferred to other full treatment sites for randomization.

Part C: Full Participation Sites

Randomization will occur only at full participation sites. It is planned to randomize approximately 99 participants total, of whom approximately 60 participants will meet the protocol-defined prognostic enrichment criteria for rapid disease progression (see Section 8.2.1).

Randomization will be stratified by the following 3 factors:

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

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

- Part C participants who enroll (uninterrupted) in the LTE study (233AS102): Week 28 Visit will serve as EOS Visit
- Part C 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.
- Part C participants who do not enroll in the LTE study: Week 32 Visit will serve as EOS Visit (either in person or by telephone contact)

In the event of a decision by the 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 participants will be invited for an EOS Visit, during which all Week 28 assessments will be conducted. For participants not entering the LTE study or in the event the study is stopped early, there will be an additional safety FU visit 8 weeks after the last dose of study treatment.

7.2. Study Specifics

7.2.1. Dose Escalation

7.2.1.1. Part A: Single Ascending Dose

The dose levels will be evaluated sequentially. After the 14-day safety review for the last participant within a cohort is completed (Day 15), and prior to dosing of the next cohort (the next planned dose level), a Biogen Medical Director and a Global Safety and Regulatory Sciences: Global Safety Physician will review participants' available blinded safety data with the PI. These data include AEs and SAEs, vital signs, physical and neurological examinations, ECGs, and clinical laboratory safety tests. Review of blinded safety data may be followed by review of unblinded safety data of the current cohort and all available safety data from preceding cohorts by the Biogen Medical Director, Global Safety Physicians, Biostatistician, Clinical Pharmacologist, and, if required, any ad hoc members. Before escalating to the next higher dose level, there must be agreement that the current emerging safety and tolerability data support dose

escalation. Progression to each subsequent dose level will be based on the number of DLTs, as defined in Section 7.2.3, observed in participants treated with BIIB067. For each dose level, if any DLT is observed, then the Biogen Medical Director, Global Safety Physician, Biostatistician, Clinical Pharmacologist, and, if required, any ad hoc members will review the safety profile, integrating results from the CRM [see Appendix A], to decide dose expansion, escalation, or de-escalation. A maximum of 12 participants can be dosed at each dose level. In general, the occurrence of DLTs in at least 3 out of 9 participants treated with BIIB067 in a cohort (i.e., ≥ 33.3% DLT rate) will result in the cohort being considered dose limiting. In this situation, the prior dose may be considered the MTD. The prior cohort may be expanded, if necessary, to confirm there is an acceptable toxicity profile at the lower dose prior to its designation as the MTD.

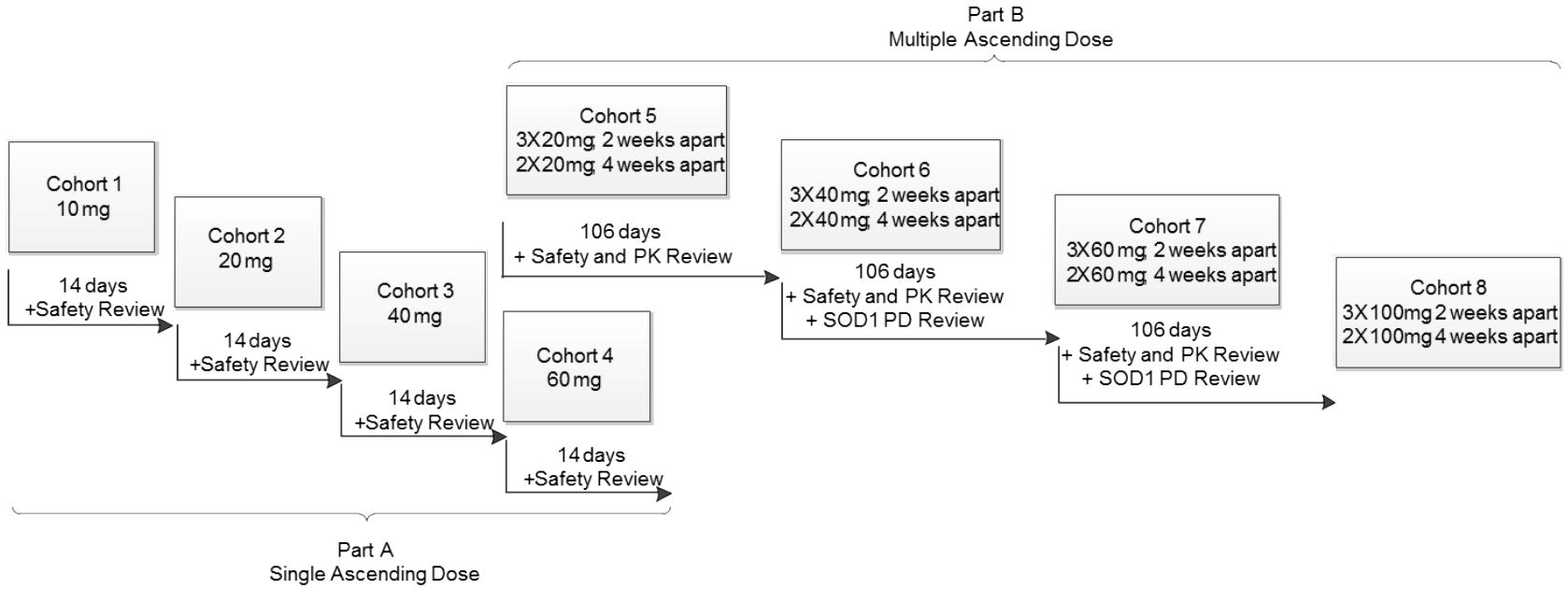
See Figure 4 for a dose escalation schematic.

7.2.1.2. Part B: Multiple Ascending Dose

After a minimum of 10 participants within a cohort complete the Day 106 FU Visit (~3 weeks after last dose), and prior to dosing of the next, higher dose cohort, a Biogen Medical Director and a Global Safety Physician will review all available blinded safety data with the PI. Review of blinded safety data may be followed by review of unblinded safety data of the current cohort and all available safety data from preceding cohorts by the Biogen Medical Director, Global Safety Physician, Biostatistician, Clinical Pharmacologist, and, if required, any ad hoc members. In addition to review of the safety data, a Biogen Medical Director, Clinical Pharmacologist, and, if required, any ad hoc members will review PK data through the trough levels after the fourth dose (samples collected predose on Day 85) for a minimum of 6 dosed participants within a cohort, prior to dosing of the next, higher dose cohort. Before escalating to the next, higher dose level, there must be agreement that the current emerging safety, tolerability, and PK data support dose escalation. The progression to each subsequent dose level will be based on the number of DLTs observed in participants treated with BIIB067. Prior to continuing to the 60-mg and 100-mg dose cohorts, available CSF of the previous cohorts will be assessed to determine the level of total SOD1 protein reduction. Based on this total SOD1 protein level data, the decision will be made to proceed with the planned dosing regimen, to alter the dosing regimen, to conduct an interim analysis after the 60-mg cohort or 100-mg cohort, or to stop the study. Section 7.2.2.2 provides specifics related to dosing Cohort 8.

Dosing for the first cohort of Part B (Cohort 5) may begin simultaneously with the last cohort of Part A (Cohort 4). See Figure 4 for a dose escalation schematic.

Figure 4: Dose Escalation Schematic (Parts A and B)



7.2.2. Within Cohort Staggered Dosing

7.2.2.1. Part A: Single Ascending Dose

For the first 3 dose levels in Part A (10, 20, and 40 mg), participants will be randomized in blocks of 4 to receive BIIB067 or placebo in a 3:1 (active treatment:placebo) ratio. The first 2 participants in each cohort will be dosed sequentially, each followed by a 72-hour safety data review period, during which the Biogen Medical Director and Global Safety Physician will review blinded safety data with the PI. The remaining participants within the cohort will then be dosed at least 24 hours apart, to allow for review of safety data. Depending on the accumulated DLT data from the first 3 cohorts, the 8 participants in Cohort 4 (60 mg) can either be randomized in a single block of 8, or be divided into 2 sequential blocks of 4, with the second block of 4 participants starting dosing only after the 14-day safety review for the last participant within the first block is completed, and the accumulated DLT data have been fully reviewed. Dosing within each randomized block of either 8 or 4 participants will be staggered similarly as for Cohorts 1-3.

7.2.2.2. Part B: Multiple Ascending Dose

Within Cohorts 5 to 7 in Part B, participants will be randomized in blocks of 4 to receive BIIB067 or placebo in a 3:1 (active treatment:placebo) ratio. The 3 blocks of each cohort will be dosed sequentially, each followed by a 72-hour safety data review period, during which the Biogen Medical Director and Global Safety Physician will review blinded safety data with the PI.

Given that safety and tolerability of the 100-mg dose of BIIB067 were not explored in Part A (SAD), both a sentinel dosing strategy and a review of safety data for every participant after the first dose will be incorporated into Cohort 8.

In Cohort 8, the first 2 participants (1 participant receiving placebo and 1 participant receiving BIIB067) will be dosed as a sentinel group, followed by a 72-hour safety data review period, during which the Biogen Medical Director and Global Safety Physician will review blinded safety data with the PI. For the remaining participants in the cohort, no more than 2 participants will be given the first dose of study treatment on the same day throughout the study.

Cohort 8 follows the same loading dose/maintenance dose as the other MAD cohorts. While a 100-mg dose has not been administered in the SAD portion of the study, the safety data collected between Week 2 and Week 4 postdose (or even Week 1 and Week 4 postdose) did not yield any additional information when compared with safety data collected for the first 2 weeks postdose. This justifies the absence of a 4-week safety observation period between doses 1 and 2.

Additional to the 72-hour safety review period, for every participant in Cohort 8, a review of all available safety and tolerability data will be performed approximately 1 week after the first dose is administered. This review will be performed by the Biogen Medical Director and Global Safety Physician with the PI. The second dose cannot be given until this review is complete.

7.2.2.3. Part C

As the initial safety and tolerability of 100 mg BIIB067 have previously been explored in Cohort 8, staggered dosing is not required for Part C of the study.

7.2.3. Dose-Limiting Toxicity

For Parts A and B only, a DLT will be defined as an AE that, in the judgment of an Investigator and/or the Sponsor, is of sufficient significance to be dose limiting, is related to study treatment (i.e., the AE is substantially less likely to occur in participants not treated with study treatment), and is not a known 1) sign or symptom of ALS, or 2) effect of the LP procedure.

For Part C, a single dose level of BIIB067 (100 mg) is to be used, and DLT evaluation will not be done. The dose level of 100 mg BIIB067 has been determined from interim analyses of data from Parts A and B of this study, in which BIIB067 at dose levels up to and including 100 mg was generally well tolerated.

7.3. Overall Study Duration and Follow-Up

The study period will consist of screening, dosing, and FU.

7.3.1. Screening

7.3.1.1. Part A: Single Ascending Dose

For participants who do not have documentation of an SOD1 mutation, the screening period will involve an additional visit (Screening Visit 1), where a blood sample will be collected for DNA analysis. The rationale for the extra screening visit is to ensure DNA analysis results prior to assessing the rest of the eligibility criteria. Participant eligibility for the study will be determined during Screening Visit 2, up to approximately 21 days after Screening Visit 1 and within 28 days prior to admission to the inpatient facility for dosing (Day 1). Participants in the 2 lowest dose cohorts, who choose to re-enroll into 1 of the 2 highest dose cohorts, will have to repeat screening assessments before receiving the second dose. This additional Screening Visit can occur during the washout period, within 28 days prior to their second admission to the inpatient facility for dosing. [redacted]

The Screening Visit 2 assessments may be performed over 2 days, which do not need to be consecutive, to minimize participant burden. All assessments must be completed before Day 1.

7.3.1.2. Part B: Multiple Ascending Dose

For participants who do not have documentation of an SOD1 mutation, the screening period will involve an additional visit (Screening Visit 1), [redacted]. Participant eligibility for the study will be determined during Screening Visit 2, up to approximately 21 days after Screening Visit 1, and within 28 days before admission to the inpatient facility for dosing (Day 1).

The Screening Visit 2 assessments may be performed over 2 days to minimize participant burden. All assessments must be completed before Day 1.

7.3.1.3. Part C

Participant eligibility for Part C of the study will be determined during the Screening Visit, up to approximately 4 weeks prior to the first dose (Day 1). All assessments must be completed before Day 1.

7.3.2. Dosing

7.3.2.1. Part A: Single Ascending Dose

Eligible participants will report to the study site for admission to the inpatient unit on Day 1 to complete baseline assessments and reaffirm eligibility. Participants will stay in the clinic until completion of all 24-hour postdose assessments.

7.3.2.2. Part B: Multiple Ascending Dose

Eligible participants will report to the study site for admission to the inpatient unit on Day 1 to complete baseline assessments and reaffirm eligibility. Participants will stay in the clinic until completion of all 24-hour postdose assessments. Following the inpatient stay on Day 1, participants will return to the clinic on an outpatient basis to receive 4 more doses on Days 15, 29, 57, and 85. CSF sampling on Days 15, 29, 57, and 85 will be performed predose. On dosing days, participants will remain under observation for approximately 6 hours after the LP procedure and will receive a safety FU telephone contact approximately 24 hours after the procedure.

7.3.2.3. Part C: Pivotal

Following the initial dosing on Day 1, participants will return to the clinic on an outpatient basis to receive up to 7 additional doses at Weeks 2 (Day 15), 4 (Day 29), 8 (Day 57), 12 (Day 85), 16 (Day 113), 20 (Day 141), and 24 (Day 169). CSF sampling will be performed predose at all dosing visits. On dosing days/CSF sampling days, participants will remain under observation at the study site for at least 1 hour after the LP procedure and can be discharged at the discretion of the Investigator and in compliance with the institutional requirements once the participants have adequately recovered from the dosing procedure. Participants will receive a safety FU telephone contact approximately 24 hours after the procedure to provide information on AEs, SAEs, and concomitant medications or procedures.

7.3.3. Follow-Up Procedures

7.3.3.1. Part A: Single Ascending Dose

Following the inpatient stay, participants participating in Part A will return to the clinic for FU visits on Days 8, 29, and 57. On the day of CSF sampling during the FU period (Days 29 and 57), participants will remain under observation for approximately 1 hour after the LP procedure and will receive a safety FU telephone contact approximately 24 hours after the procedure.

7.3.3.2. Part B: Multiple Ascending Dose

Participants will return to the study site for FU visits after each dose on Day 8 (after first dose), Day 22 (after second dose), Day 36 (after third dose), Day 64 (after fourth dose), Days 92

and 106 (after fifth dose), and on the final study visit on Day 169. On the days of CSF sampling (Day 106 and Day 169), participants will remain under observation for approximately 1 hour after the procedure and will receive a safety FU telephone contact approximately 24 hours after the procedure. Participants will also receive FU telephone contacts on Day 50 (after third dose) and Day 78 (after fourth dose) to provide information on AEs, SAEs, and concomitant medications or procedures. Participants 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.

7.3.3.3. Part C: Pivotal

Participants participating in Part C (Pivotal) will return to the site for an EOS Visit 4 weeks after their last dose (at Week 28), as described in the Schedule of Activities (Table 3) they will have the option to participate in the LTE study (233AS102), if eligible. This will be done without unblinding the treatment group.

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.

Participants who do not roll over into the LTE study will have an additional safety FU visit 8 weeks after their last dose (at Week 32) to collect information on AEs, SAEs, and concomitant medications or procedures (in person or by telephone contact).

7.4. Study Stopping Rules

7.4.1. Dose Suspension (Parts A and B only)

If 1 participant experiences either an SAE or a clinically significant AE (as defined by the Investigator or the Sponsor), the Biogen Medical Director and Global Safety Physician will discuss the event with the Investigator and review the SAE form (if applicable). If the AE is assessed as unrelated to the study treatment by both the Investigator and the Sponsor (e.g., it is a known sign or symptom of ALS, or it is an effect of the LP procedure), dose suspension is not necessary.

If the SAE or clinically significant AE (as determined by the Investigator or the Sponsor) is assessed as related to the study treatment by the Investigator or the Sponsor, all dosing will be suspended until the event has been fully evaluated by the Biogen Medical Director, Global Safety Physician, and, if required, any ad hoc members. Depending on the nature, severity, suspected on- or off-target toxicity, outcome, and frequency of the event, a decision (documented with supporting rationale) will be made to proceed with one of the following:

- Request additional safety data
- Continue dosing remaining participants within the cohort
- Enroll additional participants to the current cohort

- Proceed with the planned dose escalation in the next cohort
- Enroll additional participants to previous (lower-dose) cohort
- Stop the study

The review will also include available preliminary PK data from the preceding cohort(s).

Dosing of the cohort cannot resume until Biogen completes the safety evaluation, and the Investigator has received written approval from Biogen to resume dosing.

Dose suspension rules are not applicable for Part C of the study.

7.4.2. Dose Termination

7.4.2.1. Parts A and B

After evaluation of safety, tolerability, and PK data by the Biogen Medical Director, Global Safety Physician, and, if required, any ad hoc members, further dosing at the current level and dose escalation will be terminated if any of the following is observed:

- Two similar SAEs (unless clearly unrelated to BIIB067 upon medical review) are reported for 2 participants on active study treatment within the same cohort.
- Three or more similar AEs (unless clearly unrelated to BIIB067 upon medical review) that are either not tolerable (as reported by the participants) or deemed a medically unacceptable risk by the Biogen Medical Director, Global Safety Physician, and, if required, any ad hoc members.
- Sponsor requests that dosing be terminated.

The study will be terminated if no dose is deemed to be safe. Biogen may terminate this study at any time, after informing the Investigator. The Investigator will be notified by Biogen if the study is placed on hold, completed, or closed.

7.4.2.2. Part C

An IDMC will be formed to review ongoing safety and tolerability data. Members of the IDMC will not be allowed to participate as Investigators in this study. The IDMC will review safety data on an ongoing basis to ensure safe and proper treatment of participants. Regular IDMC meetings will occur approximately every 3 months after the first meeting. An IDMC charter will provide full guidance on the function and practices to be followed by the IDMC.

Biogen may terminate this study at any time, after informing the Investigator. The Investigator will be notified by Biogen if the study is placed on hold, completed, or closed.

7.5. End of Study

The EOS is last participant, last visit for Part C (either in person or by telephone contact). In the event of a decision by the 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 participants will be invited for the EOS Visit, during which all Week 28 assessments will be conducted. The EOS Visit should occur 4 weeks after the last dose of study

treatment. After completing the EOS Visit, participants will be considered study completers and they will have an option to participate in the LTE study, if eligible. 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. For participants not entering the LTE study or in the event the study is stopped early, there will be an additional safety FU visit 8 weeks after the last dose of study treatment.

8. SELECTION OF PARTICIPANTS

8.1. Parts A (SAD) and B (MAD)

8.1.1. Inclusion Criteria

To be eligible to participate in this study, candidates must meet the following eligibility criteria at Screening, or at the timepoint specified in the individual eligibility criterion listed:

1. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use PHI in accordance with national and local participant privacy regulations. In the case that a participant is legally incapable of providing informed consent, the participant's legally authorized representative will be able to provide the informed consent.
2. Aged ≥ 18 years at the time of informed consent.
3. Weakness attributable to ALS and documented SOD1 mutation at Screening Visit 2.
4. An FVC ≥ 50% of predicted value as adjusted for sex, age, and height (from the sitting position). Participants with stable FVC < 50% but ≥ 45%, whose FVC has not declined by more than 5% in the last 6 months may be considered for inclusion, at the discretion of the Investigator.
5. If taking riluzole, participant must be on a stable dose for ≥ 30 days prior to Day 1 and expected to remain at that dose until the final study visit.
6. Medically able to undergo the study procedures, and to adhere to the visit schedule at the time of study entry, as determined by the Investigator.
7. Screening values of coagulation parameters including platelet count, INR, PT, and APTT should be within normal ranges. Coagulation tests may be repeated once if, in the opinion of the Investigator, values of the initial tests are only slightly out of range. Participants with nonclinically significant and stable out-of-range values may be eligible to enroll in the study at the discretion of the Investigator and after a consultation with the Sponsor. (For normal ranges, please refer to the study reference guide).
8. Participants of childbearing potential must agree to practice effective contraception during the study and be willing and able to continue contraception for 5 months after

their last dose of study treatment. For further details of contraceptive requirements for this study, please refer to Section 15.5.

8.1.2. Exclusion Criteria

Candidates will be excluded from study entry if any of the following exclusion criteria exist at Screening, or at the timepoint specified in the individual criterion listed:

1. History of or positive test result for HIV.
2. History of, or positive test result at Screening, for HCV Ab.
3. Current hepatitis B infection (defined as positive for HBsAg and/or anti-HBc). Participants with immunity to hepatitis B from previous natural infection (defined as negative HBsAg, positive IgM anti-HBc, and positive anti-HBc) or vaccination (defined as positive anti-HBs) are eligible to participate in the study.
4. Treatment with another investigational drug (including investigational drugs for ALS through compassionate use programs), biological agent, or device within 1 month or 5 half-lives of study agent, whichever is longer. Specifically, no prior treatment with small interfering RNA, stem cell therapy, or gene therapy is allowed.
5. Current enrollment in any other interventional study.
6. Current or anticipated need, in the opinion of the Investigator, of a DPS during the study period.
7. Current or recent (within 1 month) use, or anticipated need, in the opinion of the Investigator, of copper (II) (diacetyl-bis(N4-methylthiosemicarbazone)) or pyrimethamine.
8. Current or recent use (within 30 hours prior to screening), or anticipated need of edaravone (Radicava®).
9. Presence of any implanted vascular devices.
10. History of drug abuse or alcoholism within ≤ 6 months of study enrollment that would limit participation in the study, as determined by the Investigator.
11. Tracheostomy.
12. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period.
13. Ongoing medical condition (e.g., wasting or cachexia, severe anemia) that according to the Investigator would interfere with the conduct or assessments of the study.
14. Female participants who are pregnant or currently breastfeeding.
15. Significant cognitive impairment, clinical dementia, or unstable psychiatric illness, including psychosis, suicidal ideation, suicide attempt, or untreated major depression ≤ 90 days, as determined by the Investigator.
16. History of allergies to a broad range of anesthetics.

17. Presence of risk for increased or uncontrolled bleeding and/or risk of bleeding that is not managed optimally and could place a participant at an increased risk for intraoperative or postoperative bleeding. These could include, but are not limited to, anatomical factors at or near the LP site (e.g., vascular abnormalities, neoplasms, or other abnormalities) and underlying disorders of the coagulation cascade, platelet function, or platelet count (e.g., hemophilia, Von Willebrand's disease, liver disease).
18. Anticipated need, in the opinion of the Investigator, for administration of any antiplatelet or anticoagulant medication (e.g., clopidogrel) for 7 days before or 48 hours after an LP.
19. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter.
20. Clinically significant abnormalities in hematology or clinical chemistry parameters, as determined by the Investigator, which would render the participant unsuitable for enrollment. For a list of normal, acceptable, and exclusionary ranges, please refer to the study reference guide.
21. Clinically significant, as determined by the Investigator, 12-lead ECG abnormalities, including corrected QT interval using Fridericia's correction method of > 450 ms for males and > 470 ms for females.
22. Inability to comply with study requirements.
23. Other unspecified reasons that, in the opinion of the Investigator or Sponsor, make the participant unsuitable for enrollment.

8.2. Part C (Pivotal)

8.2.1. Inclusion Criteria

To be eligible to participate in Part C (Pivotal) of this study, candidates must meet the following eligibility criteria at Screening, or at the timepoint specified in the individual eligibility criterion listed:

1. Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use PHI in accordance with national and local participant privacy regulations. In the case that a participant is legally incapable of providing informed consent, the participant's legally authorized representative will be able to provide the informed consent.
2. Aged ≥ 18 years at the time of informed consent.
3. Weakness attributable to ALS and a confirmed SOD1 mutation.
    a. SOD1 mutation must be confirmed by the central reader based on the sample obtained during the Screening Visit; participants with an SOD1 mutation interpreted by the central reader to be pathogenic or likely pathogenic will be eligible.
    b. Additionally:

- Prognostic enrichment criteria for rapid disease progression (participants 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 slope decline of ≥ 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 slope decline of ≥ 0.9 per month (calculated as [48-baseline score]/time since symptom onset)

- Criteria for all other eligible participants: SOD1 mutation other than those listed in item 'a.' (no ALSFRS-R slope decline requirement).

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

   Note: For SVC testing, at least 3 acceptable tests with the 2 highest acceptable (largest and next largest) efforts within 150 mL of vital capacity should be achieved.

5. If taking riluzole, participant must be on a stable dose for ≥ 30 days prior to Day 1 and expected to remain at that dose until the final study visit.

6. If taking edaravone, participant must have initiated edaravone ≥ 60 days (2 treatment cycles) prior to Day 1 and expected to remain at that dose until the final study visit, unless the Investigator determines that edaravone should be discontinued for medical reasons, in which case it may not be restarted during the study. Edaravone may not be administered on dosing days of this study.

7. Medically able to undergo the study procedures and to adhere to the visit schedule at the time of study entry, as determined by the Investigator.

8. All women of childbearing potential must agree to practice effective contraception during the study and be willing and able to continue contraception for 5 months after their last dose of study treatment. For further details of contraceptive requirements for this study, please refer to Section 15.5.

8.2.2. Exclusion Criteria

Candidates will be excluded from study entry if any of the following exclusion criteria exist at Screening, or at the timepoint specified in the individual criterion listed:

1. History of or positive test result for HIV.

2. Current hepatitis C infection (defined as positive HCV Ab and detectable HCV RNA). Participants with positive HCV Ab and undetectable HCV RNA are eligible to participate in the study (United States Centers for Disease Control and Prevention).

3. Current hepatitis B infection (defined as positive for HBsAg and/or anti-HBc). Participants with immunity to hepatitis B from previous natural infection (defined as negative HBsAg, positive anti-HBc, and positive anti-HBs) or vaccination (defined as negative HBsAg, negative anti-HBc, and positive anti-HBs) are eligible to participate in the study.
4. Treatment with another investigational drug (including investigational drugs for ALS through compassionate use programs), biological agent, or device within 1 month or 5 half-lives of study agent, whichever is longer. Specifically, no prior treatment with small interfering RNA, stem cell therapy, or gene therapy is allowed.
5. Current enrollment in any other interventional study.
6. Current or anticipated need, in the opinion of the Investigator, of a DPS during the study period.
7. Current or recent (within 1 month) use, or anticipated need, in the opinion of the Investigator, of copper (II) (diacetyl-bis(N4-methylthiosemicarbazone)) or pyrimethamine.
8. History of drug abuse or alcoholism within ≤ 6 months of study enrollment that would limit participation in the study, as determined by the Investigator.
9. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period.
10. Ongoing medical condition (e.g., wasting or cachexia, severe anemia) that according to the Investigator would interfere with the conduct or assessments of the study.
11. Female participants who are pregnant or currently breastfeeding.
12. Significant cognitive impairment, clinical dementia, or unstable psychiatric illness, including psychosis, suicidal ideation, suicide attempt, or untreated major depression ≤ 90 days, as determined by the Investigator.
13. History of allergies to a broad range of anesthetics.
14. Presence of risk for increased or uncontrolled bleeding and/or risk of bleeding that is not managed optimally could place a participant at an increased risk for intraoperative or postoperative bleeding. These could include, but are not limited to, anatomical factors at or near the LP site (e.g., vascular abnormalities, neoplasms, or other abnormalities) and underlying disorders of the coagulation cascade, platelet function, or platelet count (e.g., hemophilia, Von Willebrand's disease, liver disease).
15. Anticipated need, in the opinion of the Investigator, for administration of any antiplatelet or anticoagulant medication that cannot be safely held before and/or after an LP procedure according to local or institutional guidelines and/or Investigator determination.
16. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter.
17. Clinically significant abnormalities in hematology or clinical chemistry parameters, as determined by the Investigator, which would render the participant unsuitable for

enrollment. For a list of normal, acceptable, and exclusionary ranges, please refer to the study reference guide.

18. Clinically significant, as determined by the Investigator, 12-lead ECG abnormalities, including corrected QT interval using Fridericia's correction method of > 450 ms for males and > 470 ms for females.
19. Inability to comply with study requirements.
20. Other unspecified reasons that, in the opinion of the Investigator or Sponsor, make the participant unsuitable for enrollment.

9. ENROLLMENT, REGISTRATION, AND RANDOMIZATION

9.1. Screening and Enrollment

Participants (or their legally authorized representative [e.g., spouse], where applicable) must provide informed consent before any screening tests are performed (see Section 17.3). When a participant signs the ICF, that participant is considered to be enrolled in the study. Participating study sites are required to document all screened candidates initially considered for inclusion in this study. If a participant is excluded from the study, the reasons for exclusion will be documented in the participant's source documents and on the screening log.

For Part C (Pivotal), participants who fail screening can be rescreened up to one time at the discretion of the Investigator.

All screening assessments would need to be repeated during rescreening except for the following:

- FSH level in female participants with postmenopausal state confirmed by level obtained in previous screening for Part C of this study
- Confirmation of SOD1 mutation by the central reader if available from previous screening for Part C (Pivotal) of this study

9.2. Randomization and Registration of Participants

Parts A and B

Participants will be registered and randomized on Day 1, after all predose assessments have been completed, and after the Investigator has verified that the participants are eligible per criteria in Sections 8.1 and 8.2. No participant may begin treatment prior to assignment of a unique identification number (registration) and randomization. Any participant identification numbers that are assigned will not be reused, even if the participant does not receive treatment.

Participants who withdraw from the study prior to completing the final FU visit may be replaced at the discretion of the Sponsor. Up to 8 replacement participants may be enrolled in Part B of the study (2 per cohort). These additional participants will be assigned to receive the same study treatment as the withdrawn participants they will replace.

Part C

For Part C, participants will be registered at Screening when a centralized IRT system will assign a participant identification number to each participant. Once a participant has been allocated an identification number, he or she is considered registered for the study. The participant's identification number will be used on all of that participant's CRFs and on all laboratory and other requisition forms. Any participant identification numbers that are assigned will not be reused even if the participant does not receive treatment.

If a participant is excluded from the study, the reasons for exclusion will be documented in the participant's source documents and on the screening log. Participants in Part C will be randomized on Day 1 after all predose assessments have been completed and after the Investigator has verified that the participants are eligible per criteria in Sections 8.2.1 and 8.2.2

and that all criteria to determine which stratum the participant should be randomized to have been correctly entered in the IRT system. These criteria include whether the participant meets the prognostic enrichment criteria for rapid disease progression, SVC, riluzole use, and edaravone use. No participant may begin treatment prior to randomization.

Eligible participants will be randomized in a 2:1 ratio to receive BIIB067 100 mg or placebo, respectively. Randomization will be stratified by 3 factors: 1) whether a participant meets the prognostic enrichment criteria for rapid disease progression; 2) whether a participant uses edaravone at baseline; and 3) whether a participant uses riluzole at baseline. Use of edaravone and riluzole will not be a separate stratum but will be classified as edaravone use.

Refer to the study reference guide for details on registration and randomization.

9.3. Blinding Procedures

This is a randomized, double-blind, placebo-controlled study.

Investigators, study staff (except for a designated pharmacist/technician), and study participants will be blinded to the randomized study treatment assignments. The designated unblinded Study Pharmacy Staff will dispense the study treatment according to the DHA and randomization schedule and will not have responsibilities for any other study assessments.

To maintain the study blind, it is imperative that participant treatment assignments are not shared with the participants, their families, or any member of the study team, either at the study site or at Biogen, except the unblinded designated Study Pharmacy Staff and designated Biogen personnel.

For Parts A and B only, the Biogen Medical Director, Global Safety Physician, Biostatistician, Clinical Pharmacologist, and, if required, any ad hoc members, may be unblinded in order to review all safety and available PK data, to assess safety and tolerability, and to make informed decisions regarding dose escalation (Section 7.2.1).

At each study site, a qualified and trained study site staff member will consistently perform the ALSFRS-R for a participant and will remain blinded both to the participants' treatment assignments and to the results of other assessments.

At the end of the study (i.e., once the clinical study report is finalized), if unblinding will not jeopardize the results of ongoing related studies, Biogen will provide the randomization codes to Investigators, who then can inform their participants about the treatment received.

For information pertaining to unblinding for medical emergencies, see Section 15.4.3.1.

10. DISCONTINUATION OF STUDY TREATMENT OR WITHDRAWAL OF PARTICIPANTS FROM THE STUDY

10.1. Withdrawal from the Study (Part A) or Discontinuation of Study Treatment (Parts B and C)

A participant *must* be withdrawn from Part A of the study (SAD), or permanently discontinue study treatment in Part B (MAD) and Part C (Pivotal) of the study, for any of the following reasons:

- The participant becomes pregnant. Study treatment must be discontinued immediately. Report the pregnancy according to the instructions in Section 15.4.1.
- The participant withdraws consent to continue study treatment.
- The participant experiences a medical emergency that necessitates permanent discontinuation of study treatment.
- The participant experiences a medical emergency that necessitates unblinding of the participant's treatment assignment.
- The participant is unwilling or unable to comply with the protocol.
- At the discretion of the Investigator or Sponsor.

The reason for discontinuation of study treatment must be recorded in the participant's CRF.

Participants who discontinue treatment in Part B may remain in the study and continue protocol-required tests and assessments.

Participants 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 participants who terminate early (i.e., discontinue both study treatment and study assessments) perform the assessments of the EOS Visit (Week 28 Visit) within 4 weeks after the last dose of study treatment. Home assessments will be allowed at the discretion of the Investigator for participants who cannot come to the site in person for their Early Termination Visit.

11. STUDY TREATMENT USE

11.1. Regimen

Each participant will receive a 15-mL intrathecal bolus (over 1-3 minutes) of study treatment (BIIB067 or placebo). Prior to injection, approximately 10 mL of CSF will be collected for analyses. Depending on institutional guidelines, anesthesia or sedation may be used for the LP procedure. Detailed instructions are provided in the DHA.

11.1.1. BIIB067

In Part A (SAD), a single dose of BIIB067 will be administered by intrathecal bolus over 1-3 minutes at the following planned dose levels:

Cohort 1: BIIB067 10 mg (FIH dose)

Cohort 2: BIIB067 20 mg

Cohort 3: BIIB067 40 mg

Cohort 4: BIIB067 60 mg

In Part B (MAD), 5 doses of BIIB067 will be administered by intrathecal bolus over 1-3 minutes as follows: a loading regimen of 3 doses on Days 1, 15, and 29, followed by maintenance dosing on Days 57 and 85 for each cohort. The following dose levels are planned:

Cohort 5: BIIB067 20 mg

Cohort 6: BIIB067 40 mg

Cohort 7: BIIB067 60 mg

Cohort 8: BIIB067 100 mg

In Part C (Pivotal), 8 doses of BIIB067 100 mg will be administered by intrathecal bolus over 1-3 minutes as follows: a loading regimen of 3 doses on Days 1, 15, and 29, followed by 5 maintenance doses every 28 days (or 4 weeks).

11.1.2. Placebo

Matching placebo will be administered by intrathecal bolus over 1-3 minutes, following the same dosing regimen as BIIB067, for each cohort.

11.2. Modification of Dose and/or Treatment Schedule

The dosage should not be modified, other than as stated in Section 7.2.1. However, for Parts A and B only, if necessary (e.g., dose is not tolerated), after review of all available data by the Biogen Medical Director, a Global Safety Physician, the PI, and, if required, any ad hoc members, an alternative dose or dosing regimen may replace the planned dose or dosing regimen for the subsequent cohort.

No dosage modification will be done in Part C.

11.3. Concomitant Therapy and Procedures

11.3.1. Concomitant Therapy

A concomitant therapy is any drug or substance administered between Day 1 and the final study visit (Day 57 for participants participating in Part A [SAD] only; Day 169 for participants participating in both Parts A [SAD] and B [MAD] or in Part B [MAD]; and Day 197 or Day 225 for participants participating in Part C [Pivotal] only).

11.3.1.1. Allowed Concomitant Therapy

Participants taking concomitant riluzole at study entry must be on a stable dose for 30 days prior to the first dose of study treatment (Day 1) and must continue with the same dose regimen throughout the study, unless the Investigator determines that riluzole should be discontinued for medical reasons, in which case it may not be restarted.

For Part C, participants taking concomitant edaravone at study entry must have initiated edaravone≥ 60 days (2 treatment cycles) prior to the first dose of study treatment (Day 1) and must continue with the same dose regimen throughout the study, unless the Investigator determines that edaravone should be discontinued for medical reasons, in which case it may not be restarted during the study. Edaravone may not be administered on dosing days of this study.

Concomitant medication for symptom management during the study is at the discretion of the Investigator. Participants with questions about allowed concomitant therapy should seek medical guidance from the Investigator.

Daily intake of all concomitant vitamins and supplements should be stabilized at least 14 days prior to Day 1. Doses of vitamins, minerals, and supplements greater than the recommended daily dose should be discouraged during the study.

Supplements that are subject to dose limits from at least 14 days prior to Day 1 and that should remain stable throughout the study are as follows: creatine 5 g per day and vitamin E 1000 IU per day. These daily limits include the total doses obtained through the combination use of daily multivitamins and supplements.

11.3.1.2. Disallowed Concomitant Therapy

Any antiplatelet or anticoagulant medication that cannot be safely held before and/or after an LP procedure according to local or institutional guidelines and/or Investigator determination is prohibited for the duration of the study.

Use of DPS, copper (II) (diacetyl-bis(N4-methylthiosemicarbazone)), or pyrimethamine is prohibited for the duration of the study.

Off-label use of any disease-modifying treatment for ALS is prohibited for the duration of the study.

Participants should be instructed to contact their Investigator before taking any new medications, including nonprescription drugs and herbal preparations.

11.3.2. Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the time the participant is enrolled in the study and the final FU visit.

12. STUDY TREATMENT MANAGEMENT

Study site staff should follow the DHA for specific instructions on the handling, preparation, administration, and disposal of the study treatment. The DHA aligns with all other references, including the protocol.

Study treatment must be dispensed only by a Pharmacist or appropriately qualified staff. Study treatment is to be dispensed only to participants enrolled in this study. Once study treatment is prepared for a participant, it can be administered only to that participant. Study treatments are for one-time use only; do not use any study treatment remaining in the vial for another participant.

12.1. BIIB067

For Parts A and B, Biogen will supply BIIB067 as a liquid in vials containing approximately 5.5 mL per vial, to ensure 5.0 mL deliverable volume. The drug product is a sterile, parenteral solution formulation that contains BIIB067 drug product 20 mg/mL, sodium dihydrogen phosphate dihydrate 0.050 mg/mL, sodium phosphate dibasic anhydrous 0.097 mg/mL, sodium chloride 7.597 mg/mL, potassium chloride 0.224 mg/mL, calcium chloride dihydrate 0.206 mg/mL, and magnesium chloride hexahydrate in water for injection 0.163 mg/mL. If necessary, pH is adjusted to a target pH of 7.2 with hydrogen chloride or sodium hydroxide during compounding.

For Part C, Biogen will supply BIIB067 as a liquid in vials containing approximately 16.4 mL per vial, to ensure 15.0 mL deliverable volume after withdrawal from vial and dose preparation. The drug product is a sterile, parenteral solution formulation that contains BIIB067 drug product 6.7 mg/mL, sodium dihydrogen phosphate dihydrate 0.03 mg/mL, sodium phosphate dibasic anhydrous 0.11 mg/mL, sodium chloride 8.77 mg/mL, potassium chloride 0.22 mg/mL, calcium chloride dihydrate 0.21 mg/mL, and magnesium chloride hexahydrate in water for injection 0.16 mg/mL, pH 7.2.

The contents of the BIIB067 label will be in accordance with all applicable regulatory requirements. At a minimum, the label will include a study reference code, study treatment identifier, quantity of dosage units, lot number, and other pertinent information in accordance with local law. The expiry or use-by date will be stored in the IRT system, and printable assignment reports will be available to site personnel. BIIB067 should not be used after the expiration date.

12.1.1. BIIB067 Preparation

The individual preparing BIIB067 should carefully review the instructions provided in the DHA.

If the packaging is damaged, or if there is anything unusual about the appearance or attributes of the vials or study treatment, then the study treatment should not be used. The vial in question should be saved at the study site, and the problem should be reported to Biogen immediately.

12.1.2. BIIB067 Storage

Study treatment must be stored in a secure location.

BIIB067 is to be protected from light and stored at 2°C to 8°C (36°F to 46°F), in a monitored, locked refrigerator, with limited access. For the most up-to-date storage requirements, follow the instructions provided in the DHA.

12.1.3. BIIB067 Handling and Disposal

The Investigator must return all used and unused vials of BIIB067 as instructed by Biogen, unless approved for onsite destruction.

If any BIIB067 supplies are to be destroyed at the study site, the institution or appropriate site personnel must obtain prior approval from Biogen, by providing, in writing, the destruction policy or details of the method of destruction. After such destruction, Biogen must be notified, in writing, of the details of the study treatment destroyed (e.g., lot or kit numbers, quantities), the date of destruction, and proof of destruction.

12.1.4. BIIB067 Accountability

Accountability for study treatment is the responsibility of the Investigator. The study site must maintain accurate records demonstrating dates and amount of study treatment received, to whom dispensed (participant-by-participant accounting), and accounts of any study treatment accidentally or deliberately destroyed or lost.

Unless otherwise notified, all vials both used and unused, must be saved for study treatment accountability. At the end of the study, reconciliation must be made between the amount of BIIB067 supplied, dispensed, and subsequently destroyed, lost, or returned to Biogen. A written explanation must be provided for any discrepancies.

12.2. Placebo Product

Biogen manufactures the matching placebo (artificial CSF), supplied as a liquid in vials containing approximately 20 mL per vial for use in Parts A and B and approximately 21 mL per vial for use in Part C. The drug supply label will include conditions for storage, lot number, and other pertinent information.

Placebo is to be stored at 2°C to 8°C (36°F to 46°F), in a monitored, locked cabinet with limited access.

13. CLINICAL FUNCTION, PHARMACOKINETIC, AND PHARMACODYNAMIC ASSESSMENTS

13.1. Assessments of the Effect of BIIB067 on Clinical Function

The following clinical assessments will be performed to evaluate the effect of BIIB067 on clinical function. See Section 4 for the timing of assessments. All study visits are expected to occur at the site unless the participant is unable to travel to the site then a home visit may be possible at the discretion of the Investigator.

13.1.1. ALS Functional Rating Scale-Revised

The ALSFRS-R has been demonstrated to predict survival. The ALSFRS-R measures 4 functional domains, including respiratory, bulbar function, gross motor skills, and fine motor skills. There are 12 questions, each scored from 0 to 4, for a total possible score of 48 [Cedarbaum 1999], with higher scores representing better function. At each study site, the same qualified and trained study site staff member will consistently perform the ALSFRS-R for a participant. 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 participants' treatment assignments and to the results of other assessments.

13.1.2. Slow Vital Capacity

Vital capacity will be measured by means of an SVC test, administered in the upright position. The procedure will be performed according to the study Pulmonary Procedure Manual. 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 [Miller 2005; Pellegrino 2005; Wanger 2005]. 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.

13.1.3. Participant Diary/eDiary (Part C only)

At Baseline, participants will be given diaries/eDiaries to record the date and time of ventilation use. Diaries will be reviewed by study site staff at each visit.

[redacted]

[redacted]

13.1.6. Handheld Dynamometry

Muscle strength is an important determinant of both function and ultimate survival in ALS. Quantitative muscle strength will be evaluated using HHD, which tests isometric strength of multiple muscles using standard participant positioning. Approximately 8 muscle groups will be examined (per each side) in both upper and lower extremities.

[redacted]

[redacted]

13.2. Pharmacokinetic Assessments

BIIB067 concentrations in the plasma and CSF will be determined using validated assays.

Samples for analysis of BIIB067 concentrations in plasma and CSF will be collected from each participant at the timepoints specified in Section 4.

The following PK parameters will be assessed in plasma, when feasible:

- Maximum observed concentration
- Time to reach the maximum observed concentration
- AUC from time 0 to infinity
- AUC from time 0 to time of the last measurable concentration
- Apparent terminal $t_{½}$

The $t_{½}$ will be assessed in CSF, when feasible.

Additional PK parameters may be calculated at the discretion of the Pharmacokineticist.

13.3. Pharmacodynamic Assessments

The following tests will be performed to assess the PD properties of BIIB067. Refer to Section 4 for the timing of assessments.

- [redacted]
- [redacted]
- The biomarkers to be analyzed may include, but are not limited to, the following:
  - [redacted]
  - pNfH
  - NfL
  - [redacted]

[redacted]

[redacted]

14. SAFETY ASSESSMENTS

Refer to Section 4 for the timing of all safety assessments.

14.1. Clinical Safety Assessments

The following clinical assessments will be performed to evaluate the safety profile of BIIB067:

- Medical history
- Physical examinations
- Limited neurological examinations (to be assessed by a trained specialist) of:
  - cranial nerves, coordination/cerebellar function, reflexes, motor, and MMSE (a 30-point questionnaire that is used to measure cognitive impairment) [in Part B]
  - MMSE, motor, reflexes, and coordination/cerebellar function (in Part C)
- Vital sign measurements: temperature, pulse rate, systolic and diastolic blood pressure, and respiratory rate will be performed after the participant has rested in a sitting position for at least 5 minutes
- Weight measurements
- 12-Lead ECGs in triplicate (paper, as applicable)
- C-SSRS
- Concomitant therapy and procedure recording
- AE and SAE recording

14.2. Laboratory Safety Assessments

The following laboratory assessments will be performed to evaluate the safety profile of BIIB067:

- Hematology: complete blood count with differential and platelet count.
- Coagulation: INR, PT, and APTT
- Blood chemistry: total protein, albumin, creatinine, blood urea nitrogen, uric acid, bilirubin (total and direct), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, glucose, calcium, phosphorus, bicarbonate, chloride, sodium, and potassium
- Urinalysis: dipstick for blood, protein, and glucose (microscopic examination, if abnormal)
- Urine and/or serum pregnancy tests
- CSF analysis: red blood cell count, white blood cell count, protein, and glucose

14.3. Product-Specific Safety Assessments

Anti-BIIB067 Ab assessments will be performed according to the schedule provided in Section 4.

15. SAFETY DEFINITIONS, RECORDING, REPORTING, AND RESPONSIBILITIES

Throughout the course of the study, every effort must be made to remain alert to possible AEs. If an AE occurs, the first concern should be for the safety of the participant. If necessary, appropriate medical intervention should be provided.

At the signing of the ICF, each participant or his/her legally authorized representative and/or main caregiver must be given the names and telephone numbers of study site staff for reporting AEs and medical emergencies.

15.1. Definitions

15.1.1. Adverse Event

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

Determination of whether an abnormal laboratory value meets the definition of an AE will be made by the Investigator. Although abnormal laboratory values are typically not considered AEs, the following considerations may result in an abnormal laboratory value being considered an AE:

- A laboratory test result that meets the criteria for an SAE
- A laboratory test result that requires the participant to receive specific corrective therapy
- A laboratory abnormality that the Investigator considers to be clinically significant

15.1.2. Serious Adverse Event

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

- Results in death
- In the view of the Investigator, places the participant at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Is a medically important event

An SAE may also be any other medically important event that, in the opinion of the Investigator, may jeopardize the participant or may require intervention to prevent one of the other outcomes

listed in the definition above. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization.)

15.1.3. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment, including hospitalization that is part of the study design (e.g., admission to the clinic for the purposes of dosing or post-LP observation) will not be considered an SAE, even if the participant is hospitalized. The study site must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the participant's consent to participate in the study.
- The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the participant's consent to participate in the study and the time of the procedure or treatment.
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission.
  - If a participant is hospitalized due to local requirements for administration of the study treatment, the hospitalization should not be considered an SAE unless one of the requirements in Section 15.1.2 is met.

15.2. Safety Classifications

15.2.1. Investigator Assessment of Events

All events must be assessed to determine the following:

- If the event meets the criteria for an SAE as defined in Section 15.1.2.
- The relationship of the event to study treatment as defined in Section 15.2.2.
- The severity of the event as defined in Section 15.2.3.

15.2.2. Relationship of Events to Study Treatment

The following definitions should be considered when evaluating the relationship of AEs and SAEs to the study treatment.

Relationship of Event to Study Treatment	
Not related	An AE will be considered "not related" to the use of the investigational drug if there is not a reasonable possibility that the event has been caused by the product under investigation. Factors pointing toward this assessment include, but are not limited to, the lack of reasonable temporal relationship between administration of the drug and the event, the presence of a biologically implausible relationship between the product and the AE, or the presence of a more likely alternative explanation for the AE.
Related	An AE will be considered "related" to the use of the investigational drug if there is a reasonable possibility that the event may have been caused by the product under investigation. Factors that point toward this assessment include, but are not limited to, a positive rechallenge, a reasonable temporal sequence between administration of the drug and the event, a known response pattern of the suspected drug, improvement following discontinuation or dose reduction, a biologically plausible relationship between the drug and the AE, or a lack of an alternative explanation for the AE.

Relationship of Event to Lumbar Puncture	
Not related	An AE will be considered "not related" to the LP procedure if there is not a reasonable possibility that the event has been caused by the LP procedure. Factors pointing toward this assessment include, but are not limited to, the lack of reasonable temporal relationship between the LP procedure and the event, the presence of a biologically implausible relationship between the LP procedure and the AE, or the presence of a more likely alternative explanation for the AE.
Related	An AE will be considered "related" to the LP procedure if there is a reasonable possibility that the event may have been caused by the LP procedure. Factors that point toward this assessment include, but are not limited to, a reasonable temporal sequence between the LP procedure and the event, a known response pattern of the LP procedure (e.g., bleeding from the puncture site), a biologically plausible relationship between the LP procedure and the AE, or a lack of an alternative explanation for the AE.

15.2.3. Severity of Events

The severity of AEs and SAEs will be graded using the National Cancer Institute CTCAE, version 4. Any AE not listed in the CTCAE will be graded as follows:

Severity of Event	
Grade	Definition
1	Mild AE
2	Moderate AE
3	Severe or medically significant AE
4	Life-threatening AE
5	Death related to AE

15.2.4. Expectedness of Events

Expectedness of all AEs will be determined by Biogen according to the Investigator's Brochure.

15.3. Monitoring and Recording Events

15.3.1. Adverse Events

Any AE experienced by the participant between the time of first dose of study treatment and the last FU visit is to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. At each study visit, each post-LP telephone contact, the EOS Visit (Part C Week 28), or the Safety FU (Alternative EOS) Visit (Week 32) for participants who do not roll over or have delays in rollover into the LTE Study 233AS102), the Investigator will assess the participant for AEs and will record any new AEs or updates to previously reported AEs on the CRF.

15.3.2. Serious Adverse Events

Any SAE experienced by the participant between the time of the signing of the first (main) ICF and the last FU visit is to be recorded on an SAE form, regardless of the severity of the event or its relationship to study treatment. SAEs must be reported to Biogen within 24 hours as described in Section 15.3.3. FU information regarding an SAE also must be reported within 24 hours.

Participants will be followed for all SAEs until the final study visit. Thereafter, the event should be reported to Biogen only if the Investigator considers the SAE to be related to study treatment.

Any SAE that is ongoing when the participant completes or discontinues the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status.

15.3.3. Immediate Reporting of Serious Adverse Events

In order to adhere to all applicable laws and regulations for reporting an SAE, the study site must formally notify Biogen within 24 hours of the study site staff becoming aware of the SAE. It is the Investigator's responsibility to ensure that the SAE reporting information and procedures are used and followed appropriately.

Reporting Information for SAEs
A report ***must be submitted*** to Biogen regardless of the following: • Whether or not the participant has undergone study-related procedures • Whether or not the participant has received study treatment • The severity of the event • The relationship of the event to study treatment To report initial or FU information on an SAE, fax or email a completed SAE form; refer to the Study Reference Guide for complete contact information.

15.3.3.1. Deaths

Death is an outcome of an event. The event that resulted in death should be recorded on the appropriate CRF. All causes of death must be reported as SAEs within 24 hours of the site becoming aware of the event. The Investigator should make every effort to obtain and send death certificates and autopsy reports to Biogen. The term death should be reported as an SAE only if the cause of death is not known and cannot be determined.

15.3.4. Suspected Unexpected Serious Adverse Reactions

SUSARs are SAEs that are unexpected and judged by the Investigator or Biogen to be related to the study treatment administered.

Appropriate personnel at Biogen will unblind SUSARs for the purpose of regulatory reporting. Biogen will submit SUSARs (in blinded or unblinded fashion) to regulatory agencies according to local law. Biogen will submit SUSARs to Investigators in a blinded fashion.

15.4. Procedures for Handling Special Situations

15.4.1. Pregnancy

Participants should not become pregnant during the study and for 5 months after their last dose of study treatment. If a female participant becomes pregnant, study treatment must be discontinued *immediately*.

The Investigator must report a pregnancy occurring in a female participant by faxing or emailing the appropriate form to Biogen within 24 hours of the study site staff becoming aware of the pregnancy; refer to the Study Reference Guide for complete contact information. The Investigator or study site staff must report the outcome of the pregnancy to Biogen. A pregnancy is not considered an AE and should not be recorded on the AE CRF.

Congenital abnormalities and birth defects in the offspring of male or female participants should be reported as an SAE if conception occurred during the study treatment period.

15.4.2. Overdose

An overdose is any dose of study treatment administered to a participant or taken by a participant that exceeds the dose assigned to the participant according to the protocol. Overdoses are not considered AEs and should not be recorded as an AE on the CRF; however, all overdoses must be recorded on an Overdose form and faxed or emailed to Biogen within 24 hours of the site becoming aware of the overdose. An overdose must be reported to Biogen even if the overdose does not result in an AE. If an overdose results in an AE, the AE must be recorded. If an overdose results in an SAE, both the SAE and Overdose forms must be completed and faxed or emailed to Biogen; refer to the Study Reference Guide for complete contact information. All study treatment-related dosing information must be recorded on the dosing CRF.

15.4.3. Medical Emergency

In a medical emergency requiring immediate attention, study site staff will apply appropriate medical intervention, according to current standards of care. The Investigator (or designee) should contact the study's Medical Director. Refer to the Study Reference Guide's Official Study Contact List for complete contact information.

15.4.3.1. Unblinding for Medical Emergency

In a medical emergency when knowledge of the participant's treatment assignment may influence the participant's clinical care, the Investigator and, if applicable, designated personnel at Biogen may access the participant's treatment assignment by IRT. The Investigator must document the reasons for unblinding in the participant's source documents. The Investigator is strongly advised not to divulge the participant's treatment assignment to any individual not directly involved in managing the medical emergency, nor to personnel involved with the analysis and conduct of the study. The Investigator can contact Biogen or designee to discuss such situations.

15.5. Contraception Requirements

All women of childbearing potential must ensure that effective contraception is used during the study and for 5 months after their last dose of study treatment. In addition, female participants should not donate eggs for the duration of the study and for at least 5 months after their last dose of study treatment.

For the purposes of this study, women who do not meet one of the following criteria are considered to be physiologically capable of becoming pregnant and are, therefore, defined as women of childbearing potential:

- Postmenopausal
  - 12 continuous months of natural (spontaneous) amenorrhea without an alternative medical cause and a serum FSH level > 40 mIU/mL
  - 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Posthysterectomy
- Female surgical sterilization (e.g., bilateral tubal ligation)

For the purposes of the study, effective contraception is defined as use of at least 1 of the following:

For females:

- Established use of oral, injected, implanted, intravaginal, or transdermal hormonal methods of contraception.
- Placement of an intrauterine device or intrauterine hormone-releasing system.
- Barrier methods of contraception with use of a spermicide: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide.
- Sex with a male who has undergone surgical sterilization (with the appropriate postvasectomy documentation of the absence of sperm in the ejaculate).

True abstinence, when this is consistent with the preferred and usual lifestyle of the participant, can be considered an acceptable method of contraception based on the evaluation of the Investigator who should also take into consideration the duration of the clinical trial. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not considered acceptable methods of contraception.

Pregnancy reporting is described in Section 15.4.1.

15.6. Safety Responsibilities

15.6.1. The Investigator

The Investigator's responsibilities include the following:

- Monitor and record all AEs, including SAEs, regardless of the severity or relationship to study treatment.
- Determine the seriousness, relationship, and severity of each event.
- Determine the onset and resolution dates of each event.
- Monitor and record all pregnancies, and follow up on the outcome of the pregnancy in female participants.
- Complete an SAE form for each SAE and fax or email it to Biogen within 24 hours of the study site staff becoming aware of the event.
- Pursue SAE FU information actively and persistently. FU information must be reported to Biogen within 24 hours of the study site staff becoming aware of new information.
- Ensure all AE and SAE reports are supported by documentation in the participants' medical records.
- Pursue AE FU information, if possible, until the event has resolved or become stable.

- Report SAEs to local ethics committees, as required by local law.

15.6.2. Biogen

Biogen's responsibilities include the following:

- Before study site activation and participant enrollment, the Clinical Monitor is responsible for reviewing with study site staff the definitions of AE and SAE, as well as the instructions for monitoring, recording, and reporting AEs and SAEs.
- Biogen is to notify all appropriate regulatory authorities, central ethics committees, and Investigators of SAEs, as required by local law, within required time frames.

16. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The objectives of the study and the endpoints to be analyzed are listed in Section 6.

16.1. Part A (SAD) and Part B (MAD)

The ITT population is defined as all randomized participants who receive at least 1 or a part of 1 dose of study treatment.

16.1.1. Clinical Function

16.1.1.1. Analysis Population

The clinical function set is defined as the subset of the ITT population who have at least 1 postdose measurement.

All participants receiving placebo will be combined to form placebo control groups for Part A (SAD) and for Part B (MAD). A separate statistical analysis will be performed for Part A (SAD) and for Part B (MAD).

16.1.1.2. Methods of Analysis

The change and percent change from baseline in [redacted] HHD, ALSFRS-R, SVC, [redacted] will be summarized by visit and dose level using descriptive statistics and, where warranted, presented graphically. A 2-sample Student's t-test will be used to compare these data between each dose of BIIB067 and placebo at a 2-sided alpha level of 0.10.

16.1.2. Pharmacokinetics

16.1.2.1. Analysis Population

The PK population is defined as the subset of the ITT population of participants with at least 1 postdose PK measurement.

16.1.2.2. Methods of Analysis

Plasma and CSF PK parameters and BIIB067 concentrations in plasma and CSF for the PK population will be summarized using descriptive statistics and, where warranted, presented graphically.

16.1.3. Pharmacodynamics

16.1.3.1. Analysis Population

The PD population is defined as the ITT population of participants with at least 1 PD measurement after baseline.

16.1.3.2. Methods of Analysis

Changes and percent changes from baseline for total SOD1 protein levels will be summarized and presented by visit and by dose level. A 2-sample Student's t-test will be used to compare each dose of BIIB067 and placebo at a 2-sided alpha level of 0.10.

16.1.4. Biomarker and Pharmacogenomic Analyses

16.1.4.1. Analysis Population

The biomarker population is defined as the ITT population of participants with at least 1 postbaseline biomarker measurement.

16.1.4.2. Methods of Analysis

CSF and blood samples may be assayed for biomarkers that may include, but will not be limited to, [redacted] pNfH, and NfL.

Exploratory potential biomarker candidates related to BIIB067 biological activity will be summarized using descriptive statistics and will be presented by dose level.

Summary statistics (raw and logarithmic scale) for the above potential biomarkers will be calculated and presented by dose level and by visit, and their mean and median levels will be plotted over time, as appropriate.

See Section 4 for information on when samples will be collected.

Sampling for this analysis will be approved at the discretion of each site's ethics committee. If a site's ethics committee does not approve the sampling for the analysis, this section will not be applicable to that site.

16.1.5. Safety

16.1.5.1. Analysis Population

The safety population is defined as the ITT population of participants.

16.1.5.2. Methods of Analysis

16.1.5.2.1. Adverse Events

AEs will be coded using MedDRA.

The incidence of all AEs and SAEs will be summarized by system organ class and preferred term for each dose level. AEs will also be summarized by severity and relationship to study treatment. Narratives of deaths, SAEs, and AEs that led to study withdrawal, at a minimum, will also be provided.

16.1.5.2.2. Clinical Laboratory Results

Clinical laboratory evaluations include hematology, coagulation, blood chemistry, and urinalysis. Laboratory data will be summarized using shift tables. The number and percentage of participants with shifts from baseline to high or low status for hematology, coagulation, and

blood chemistry, and shifts from baseline to high or positive status for urinalysis will be presented by dose level. In addition, summaries of the shift from baseline to the maximum postbaseline value and the shift from baseline to the minimum postbaseline value will presented by cohort.

16.1.5.2.3. Vital Signs

Changes from baseline will be summarized for all vital signs by dose levels. A separate summary will be presented for incidence of clinically significant changes from baseline.

16.1.5.2.4. Columbia Suicide Severity Rating Scale

C-SSRS data will be summarized by dose level using descriptive statistics (number of participants, mean, SD, median, minimum, and maximum) for continuous variables, and using frequency and percentage for discrete variables.

16.1.5.2.5. Physical Examinations

The changes from baseline will be summarized by dose level.

16.1.5.2.6. Limited Neurological Examinations

The changes from baseline in the limited neurological examination (MMSE, cranial nerves, coordination/cerebellar function, motor and reflexes) will be summarized by dose level.

16.1.5.2.7. Electrocardiogram

The changes from baseline will be summarized using shift tables. The number and percentage of participants with shifts to the categorical values (abnormal not AE, or abnormal and AE) will be summarized by dose level.

16.1.6. Antigenicity/Immunogenicity Data

16.1.6.1. Analysis Population

The analysis population for immunogenicity is defined as all participants who receive at least 1 dose of study treatment and have at least 1 postdose immunogenicity sample collected.

16.1.6.2. Methods of Analysis

Results will be summarized by dose level.

16.1.7. Interim Analyses

In Part A (SAD) of the study, CRM will be used as supporting evidence for continuous assessment of MTD for dose escalation decisions. In Part B (MAD) of the study, in addition to the safety review (blinded), a designated team of staff will review the available unblinded but de-identified PD and/or biomarker data (e.g., levels of total SOD1 protein and pNfH) from all previous cohorts at the completion of the last Day 106 visit of each cohort to determine whether to proceed with the subsequent planned dose cohorts. An interim analysis of key safety, secondary, and exploratory endpoints may be conducted based on data up to the 60-mg and/or

100-mg dose cohort to allow for the planning of the pivotal efficacy and safety study. The participants, investigative staff, and sponsor's staff responsible for site monitoring and data management will remain blinded to treatment assignment during the ongoing data review, as well as during interim analyses, to minimize the potential for bias in the data cleaning process. Details of the interim analysis will be provided in a separate statistical analysis plan.

16.1.8. Sample Size Considerations

The maximum sample size of 36 participants for Part A of the study (SAD) is based on clinical rather than statistical considerations. Participants will be randomized to receive BIIB067 or placebo in a 3:1 ratio in each cohort. Each cohort will have a minimum of 4 participants (3 active and 1 placebo), and a maximum of 12 participants (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 participants (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 participant exposure at subtherapeutic single doses.

For Part B (MAD), up to 48 participants will be enrolled in up to 4 cohorts, with up to 9 participants randomized to receive BIIB067 and 3 participants randomized to receive placebo for each cohort. This design will provide approximately 80% power to detect a difference in total SOD1 protein 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.

16.2. Part C

The ITT population is defined as all participants who are randomized and receive at least 1 dose of study treatment.

16.2.1. Clinical Function

16.2.1.1. Analysis Population

The primary analyses of clinical function will be evaluated in the mITT population (see Section 8.2.1 for definition) comprising the subset of the population 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 will comprise participants who meet 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 in the non-mITT population will be analyzed, where relevant, with nominal p-values presented. The clinical function data in the overall ITT population will be summarized and analyzed where relevant.

16.2.1.2. Methods of Analysis

16.2.1.2.1. Analysis of the Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline to Week 28 in the ALSFRS-R total score, which will be analyzed as follows, depending on 2 scenarios:

1. 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 JRT methodology to account for mortality [Berry 2013] will be used for the primary inference. When implementing the JRT methodology, 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 MI. The corresponding nominal p-value from the ANCOVA will be presented as a sensitivity analysis.
2. 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 as a sensitivity analysis.

The primary estimand is described as follows:

The primary analysis for the primary endpoint is the composite estimand [ICH 2017]. The estimand of the primary analysis is defined as follows:

- Population: all participants 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 participants who have these intercurrent events are ranked against each other and against participants without any intercurrent event using the joint rank methodology based on MI datasets. The relevant intercurrent events are deaths and withdrawals. These participants will be handled using the composite strategy and ranked as described in this section. 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 for change from baseline to Day 197, based on the MI datasets. The ANCOVA model will include treatment group as a fixed effect and covariates for ALSFRS-R total score, baseline disease duration since symptom onset, and use of riluzole or edaravone.

  For the JRT analysis, the ranked scores will be analyzed for each of the 100 MI-complete datasets using an ANCOVA 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. The p-value will be based on combining the estimates for the treatment differences using PROC MIANALYZE. The difference between treatment groups in median changes from baseline to each visit will be presented with 95% confidence intervals based on the MI datasets, as well as the p-value from the JRT for Day 197. The joint rank analysis will only be performed for the mITT population.

The joint rank procedure allows for a statistical test of the treatment effect on the ALSFRS-R total score while accounting for truncation of data due to deaths. In this analysis, a participant's joint rank score will be calculated by comparing each participant to every other participant in the

study, resulting in a score of +1 if the outcome was better than the participant being compared, -1 if worse, and 0 if the same. The participant's score will then be calculated by summing their comparison to all the other participants in the study.

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

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

MI will be used to impute all missing data before determining the rank score, including intermittent data and data after withdrawal from the study except after death.

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

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

Further details will be described in the statistical analysis plan.

The primary inference will be based on the mITT population. The treatment comparison will be based on a 2-sided α level of 0.05.

If the second scenario occurs, i.e., in both the active and placebo treatment groups combined the number of participants who died is < 3 (e.g., < 5% deaths out of a total of 60 participants in the mITT population), the primary efficacy analysis will be the change from baseline in ALSFRS-R total score, analyzed using an ANCOVA based on data imputed using MI. The ANCOVA model will include treatment group as a fixed effect and covariates for each of the corresponding baseline value for ALSFRS-R total score, baseline disease duration since symptom onset and use of riluzole or edaravone. The primary inference will be based on the treatment comparison at Week 28. A 2-sided α level of 0.05 will be used. Treatment differences at other visits will also be presented with nominal p-values.

Sensitivity, supplementary and subgroup analyses of the primary efficacy endpoint will be specified in the statistical analysis plan.

For the non-mITT population, the analysis will be the change from baseline in ALSFRS-R total score, analyzed using an ANCOVA based on data imputed using MI. The ANCOVA model will include treatment group as a fixed effect and covariates for each of the corresponding baseline value for ALSFRS-R total score, baseline disease duration since symptom onset, and use of riluzole or edaravone. Least square means with standard errors will be presented for each treatment group. The difference between treatment groups in least square means for change from baseline at each visit, with corresponding standard errors and 95% confidence intervals, will be presented with nominal p-values.

16.2.1.2.2. Analysis of the Secondary Efficacy Endpoints

Formal testing of secondary efficacy endpoints will be in the mITT population alone. For the non-mITT and ITT populations, there will be no formal testing of efficacy endpoints. Testing of PD endpoints is discussed in Section 16.2.3. The analyses of secondary endpoints for the mITT population will be based on a sequential testing procedure in the order of the rank of secondary endpoints as listed below if the primary endpoint is statistically significant at a 2-sided α level of 0.05:

- Change from baseline (i.e., ratio) to Week 28 (Day 197) in CSF total 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 SVC
- Change from baseline to Week 28 (Day 197) in 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

The treatment effect will be assessed at each step at a 2-sided α level of 0.05. The primary inference at each step will be based on the mITT population.

This procedure 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.

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.

The first and second endpoints in the ranking are PD endpoints, for which the analysis is described in Section 16.2.3.

The third secondary endpoint will be change from baseline to Day 197 in percent predicted SVC. 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 primary inference will be based on the JRT methodology with MI to impute missing data; ANCOVA with MI for change from baseline will be used to obtain the estimates, and nominal p-values will be presented. 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 of this endpoint. The ANCOVA model will include treatment group as a fixed effect and covariates for baseline percent predicted SVC, baseline disease duration since symptom onset, and use of riluzole or edaravone. Treatment differences at other visits will also be derived from the model for exploratory purpose.

The secondary efficacy endpoints, time to death or permanent ventilation and time to death, will each be analyzed as time to event endpoints. Kaplan-Meier estimates of the cumulative probability of event occurrence over time will be determined. Treatment comparison will be based on a stratified log rank test. A Cox regression model will be used to obtain the hazard ratio and 95% confidence intervals for each of these endpoints. The analysis for each endpoint will also be repeated for the non-mITT population with nominal p-values presented.

[redacted]

16.2.3. Pharmacodynamics

16.2.3.1. Analysis Population

The analysis of the PD and biomarker endpoints will be performed primarily for the mITT population but also for the non-mITT population.

16.2.3.2. Methods of Analysis

The PD endpoint, CSF total SOD1 protein at Day 197, will be the primary endpoint in the non-mITT population due to their likely slow clinical function decline. This is also the highest-ranked secondary endpoint in the sequential testing procedure for the mITT population, and NfL in plasma at Day 197 is the second highest ranked secondary endpoint. For other biomarkers, analyses will be performed for each of the mITT and non-mITT populations with nominal p-values presented. For each of the PD/biomarker endpoints, MI will be used to impute missing values. An ANCOVA model for log ratio to baseline will be performed on change from baseline values on the log scale for each of the 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. Least square means (i.e., geometric mean ratios) for each treatment group with standard errors as well as treatment differences will be presented with 95% confidence intervals and p-values. The other exploratory PD/biomarker endpoints that will be analyzed in this way are NfL in CSF, and pNfH in CSF and plasma. [redacted]. Treatment differences at other visits will also be presented with nominal p-values.

16.2.4. Safety

16.2.4.1. Analysis Population

The safety population will be defined as all participants who received at least 1 dose of study treatment (i.e., the overall ITT population).

16.2.4.2. Methods of Analysis

All AEs, laboratory data, ECG, neurological and physical examinations, and vital signs will be evaluated for safety.

16.2.4.2.1. Adverse Events

Only TEAEs will be presented in the summary tables. Treatment-emergent is defined as having an onset date that is on or after start of study treatment, or that is worsened after the start of study treatment.

Incidence of TEAEs will be summarized by treatment groups, overall, by severity, by relationship to LP, and by relationship to study treatment. SAEs, discontinuations and withdrawals due to AEs, AEs leading to hospitalization, and deaths will also be summarized or listed, depending on number of events. The summary tables will include incidence estimates for overall system organ class as well as for preferred terms within each system organ class. AEs will be coded using MedDRA. A subset of AE outputs will also be presented by disease progression subgroup (“participants who meet prognostic enrichment criteria for rapid disease progression”, “other eligible participants”).

16.2.4.2.2. Clinical Laboratory Results

Laboratory data will be summarized using shift tables. Shifts from baseline to high/low status for hematology and blood chemistry parameters, and shifts from baseline to high/positive status for urinalysis will be presented. In addition, the shift from baseline to the maximum postbaseline value and the shift from baseline to the minimum postbaseline status will be presented for each laboratory test by treatment group. Summary statistics for actual values and change from baseline will also be presented for quantitative laboratory data.

16.2.4.2.3. Vital Signs

The analysis of vital signs will focus on clinically relevant abnormalities.

16.2.4.2.4. Electrocardiogram

The number and percentage of participants with shifts to categorical values (abnormal not AE, or abnormal AE) will be summarized by treatment group.

16.2.4.2.5. Columbia Suicide Severity Rating Scale

The C-SSRS data will be summarized by treatment group.

16.2.5. Participant-Reported Outcomes on Quality of Life

16.2.5.1. Analysis Population

The analysis of the participant-reported outcome endpoints will be performed primarily for the mITT population but also for the non-mITT population.

16.2.5.2. Methods of Analysis

The quality of life measures will be analyzed using an ANCOVA with MI with fixed effect for treatment and covariates for corresponding baseline value, baseline disease duration since symptom onset and use of riluzole or edaravone. The treatment difference at Day 197 will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose. Nominal p-values will be presented.

16.2.6. Interim Analyses

There will be no interim analysis performed.

16.2.7. Sample Size Considerations

For Part C, approximately 99 participants will be randomized, with approximately 66 participants administered BIIB067 100 mg and approximately 33 participants administered placebo in a 2:1 ratio.

This sample size for Part C is selected primarily based on the JRT 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 participants who met the prognostic criteria for rapid disease progression as well as the observed ALSFRS-R slope decline in participants who received placebo in a similar ALS population with SOD1 mutation [Benatar 2018] are the basis of assumed treatment effect and the variability. Participants from both datasets were matched with the prognostic criteria for rapid disease progression specified in this protocol for Part C (i.e., one of the protocol-defined SOD1 mutations and a prerandomization ALSFRS-R slope decline of ≥ 0.2 per month or a prerandomization ALSFRS-R slope decline of ≥ 0.9 with SOD1 mutation NOT on the protocol-defined list). This resulted in a total of 12 placebo control participants who met the prognostic criteria for rapid disease progression (8 from the historical placebo data [Benatar 2018] and 4 from Study 233AS101) and 4 matched participants treated with BIIB067 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 participants and -0.74 for the matched BIIB067 100 mg participants, with a pooled SD of 3.166. In the 12 matched participants on placebo, there was 1 death and 2 participants with permanent assisted ventilation less than 6 months but none in the BIIB067 100 mg matched participants. Based on this and overall survival data in patients who met the prognostic criteria for rapid disease progression from the literature on patientss with an A4V SOD1 mutation, the survival at Week 28 was assumed to be 82% in the placebo control and 90% in the BIIB067 100 mg group.

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

For the non-mITT population, the primary focus will be on the PD endpoint, CSF total 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 participants in the treated group and 13 participants in the placebo group for the non-mITT population would provide 97% power to detect a 25% reduction in CSF total SOD1 protein from baseline in the treated group, with an assumed SD of 0.216 (natural log scale), compared to the placebo group.

17. ETHICAL REQUIREMENTS

Biogen and the Investigator must comply with all instructions, regulations, and agreements in this protocol and applicable ICH and GCP guidelines and conduct the study according to local regulations.

The Investigator may delegate responsibilities for study-related tasks where appropriate to individuals sufficiently qualified by education, training, and experience, in accordance with applicable ICH and GCP guidelines. The Investigator should maintain a list of the appropriately qualified persons to whom significant study-related duties have been delegated.

17.1. Declaration of Helsinki

This study will be performed in alignment with the ethical principles outlined in the Declaration of Helsinki.

17.2. Ethics Committee

The Investigator must obtain ethics committee approval of the protocol, ICF, and other required study documents prior to starting the study. Biogen will submit documents on behalf of the investigational sites.

If the Investigator makes any changes to the ICF, Biogen must approve the changes before the ICF is submitted to the ethics committee. A copy of the approved ICF must be provided to Biogen. After approval, the ICF must not be altered without the agreement of the relevant ethics committee and Biogen.

It is the responsibility of the Investigators to ensure that all aspects of institutional review are conducted in accordance with current applicable regulations.

Biogen must receive a letter documenting ethics committee approval, which specifically identifies the protocol, protocol number, and ICF, prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the ethics committee at required intervals and not less than annually.

At the completion or termination of the study, the investigational site must submit a close-out letter to the ethics committee and Biogen.

17.3. Participant Information and Consent

Prior to performing any study-related activities under this protocol, including screening tests and assessments, written informed consent with the approved ICF must be obtained from the participant or participant's legally authorized representative (e.g., spouse), as applicable, in accordance with local practice and regulations.

The background of the proposed study, the procedures, the benefits and risks of the study, and that study participation is voluntary for the participant must be explained to the participant (or the participant's legally authorized representative). The participant must be given sufficient time to consider whether to participate in the study.

Participants will be informed, where applicable, that their race and ethnicity will be collected during the study (unless the collection is not permitted by applicable law or not approved by the governing ethics committee) and the data will be used during analysis of study results.

A copy of the signed and dated ICF must be given to the participant or the participant's legally authorized representative. The signed and dated ICF will be retained with the study records. Local regulations must be complied with in respect to the final disposition of the original (wet signature) and copies of the signed and dated ICFs.

Confirmation of informed consent must also be documented in the participant's medical record.

17.4. Participant Data Protection

Prior to any testing under this protocol, including screening tests and assessments, candidates must also provide all authorizations required by local law (e.g., PHI authorization in North America).

During the study, participants' race and ethnicity will be collected (unless the collection is not permitted by applicable law or not approved by the governing ethics committee). These data will be used in the analysis of the safety and/or PK profile of the study treatment. It is unknown whether the effects of the study treatment are influenced by race or ethnicity.

The participant will not be identified by name in the CRF or in any study reports, and these reports will be used for research purposes only. Biogen, its partners and designees, ethics committees, and various government health agencies may inspect the records of this study. Every effort will be made to keep the participant's personal medical data confidential.

17.5. Compensation for Injury

Biogen maintains appropriate insurance coverage for clinical studies and will follow applicable local compensation laws.

17.6. Conflict of Interest

The Investigators should address any potential conflicts of interest (e.g., financial interest in Biogen or partnering companies) with the participant before the participant makes a decision to participate in the study.

17.7. Registration of Study and Disclosure of Study Results

Biogen will register the study and post study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

18. ADMINISTRATIVE PROCEDURES

18.1. Study Site Initiation

The Investigator must not screen any participants prior to completion of a study initiation visit, conducted by Biogen or designee. This initiation visit will include a detailed review of the protocol and study procedures.

18.2. Quality Assurance

During and/or after completion of the study, quality assurance officers named by Biogen or the regulatory authorities may wish to perform onsite audits or inspections. The Investigator will be expected to cooperate with any audit or inspection and to provide assistance and documentation (including source data) as requested.

18.3. Monitoring of the Study

The Investigator must permit study-related monitoring by providing direct access to source data and to the participants' medical histories. Source data must be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data must be traceable, not obscure the original entry, and be explained if necessary (e.g., with an audit trail). The Investigator should maintain a record of the location(s) of essential documents.

The Clinical Monitor will visit the Investigator at regular intervals during the study and after the study has completed, as appropriate. During these visits, CRFs and supporting documentation related to the study will be reviewed and any discrepancies or omissions will be resolved.

Remote evaluation of data (centralized monitoring) and remote verification of source documentation may also be conducted and reported as defined in the monitoring plan.

Monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

18.4. Study Funding

Biogen is the Sponsor of the study and is funding the study. All financial details are provided in the separate contracts between the institution, Investigator, and Biogen.

18.5. Publications

Details are included in the clinical trial agreement for this study.

19. FURTHER REQUIREMENTS AND GENERAL INFORMATION

19.1. Public Health Emergencies

In the event of a public health emergency that results in site closure, travel restrictions, and/or the study being deprioritized at the site such that clinic visit(s) cannot occur, a protocol deviation would be incurred for any deviation from the protocol-specified visits and assessments, with additional notation that this protocol deviation is due to the public health emergency. If a protocol-specified clinical visit cannot occur due to a public health emergency, the following mitigating options should be pursued, in order of preference (in which the highest preference option that is feasible should be done): 1) transfer to another active study site that is open, 2) home visit, 3) telemedicine visit (e.g., by telephone or web conference), and 4) local laboratory visit. These mitigating options only apply in the setting of a public health emergency in which a protocol-specified clinic visit cannot occur and should not be pursued solely due to participant's preference.

A third-party vendor has been engaged to perform the following assessments at the study participant's home:

1. Limited neurological examination, including the MMSE
2. Physical examination
3. Vital signs
4. Height and body weight collection
5. SVC

[redacted]

7. Pregnancy test (if applicable)
8. HHD

[redacted]

10. Collection/shipment of ventilation diary records

The following assessments can be performed via the phone (telemedicine) by the site staff:

1. ALSFRS-R

[redacted]

4. Assessing changes in signs and symptoms as well as review of concomitant medications and AEs

The following assessments can be performed at the study participant's home OR via the phone (telemedicine) by the site staff:

1. Health outcome measures
   a. [redacted]

[redacted]

g. C-SSRS

[redacted]

19.2. External Contract Organizations

19.2.1. Contract Research Organization

Biogen will be responsible for administrative aspects of the study including, but not limited to, study initiation, monitoring, management of SAE reports, and data management. Before participants are screened at each study site, Biogen will review study responsibilities with the Investigators and other study site staff, as appropriate.

19.2.2. Interactive Response Technology

IRT will be used in this study. Before participants are screened or enrolled, the IRT vendor will provide each study site with the necessary training, a user manual, and access rights to the system.

19.2.3. Electronic Data Capture

Participant information will be captured and managed by study sites on electronic CRFs by a Web-based electronic data capture tool developed and supported by Medidata and configured by Biogen. Ventilation use information will be recorded on paper initially and then via eDiaries developed and supported by Signant Health.

19.2.4. Laboratory Assessments

A central laboratory has been selected by Biogen to analyze hematology, blood chemistry, and urine samples collected for the assessment of safety in this study.

Urine pregnancy tests will be collected and analyzed at the clinic. Serum pregnancy tests will be collected and analyzed at a central laboratory; where applicable, serum pregnancy tests performed on dosing days may be analyzed at the local laboratory. CSF samples will be collected in the clinic and analyzed at the local laboratory. At each CSF collection, 2 sample tubes will be sent to the local laboratories for routine cell count, differential count, and for protein and glucose analysis. In addition, should repeat coagulation or hematology tests be required, these may be collected in the clinic and sent to the local laboratory for analysis.

19.3. Study Committees

19.3.1. Safety Surveillance Team

A safety surveillance team, consisting, at a minimum, of the Biogen Medical Director and a Global Safety Physician will monitor dose escalation, as described in Section 7.2.1.

19.3.2. Independent Data Monitoring Committee

An IDMC will be formed to review ongoing safety and tolerability data, as described in Section 7.4.2.2.

19.3.3. Endpoint Adjudication Committee

In Part C, 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.

19.4. Changes to Final Study Protocol

All protocol amendments must be submitted to the ethics committee and regulatory authorities if required by local law. Protocol modifications that affect participant safety, the scope of the investigation, or the scientific quality of the study must be approved by the ethics committee before implementation of such modifications to the conduct of the study. If required by local law, such modifications must also be approved by the appropriate regulatory agency prior to implementation.

However, Biogen may, at any time, amend this protocol to eliminate an apparent immediate hazard to a participant. In this case, the appropriate regulatory authorities will be notified subsequent to the modification.

In the event of a protocol modification, the ICF may require similar modifications (see Section 17).

19.5. Ethics Committee Notification of Study Completion or Termination

Where required, the regulatory authorities and ethics committees must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

19.6. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations. Prior to proceeding with destruction of records, the Investigator must notify Biogen in writing and receive written authorization from Biogen to destroy study records. In addition, the Investigator must notify Biogen of any changes in the archival arrangements, including but not limited to, archival at an offsite facility or transfer of ownership if the Investigator leaves the site.

19.7. Study Report Signatory

Biogen will designate one of the participating Study Investigators as a signatory for the study report. This determination will be made by several factors, including but not limited to, the Investigator's experience and reputation in the studied indication; the Investigator's contribution to the study in terms of design, management, and/or participant enrollment; or by other factors determined to be relevant by Biogen.

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21. SIGNED AGREEMENT OF THE STUDY PROTOCOL

I have read the foregoing protocol, "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," and agree to conduct the study according to the protocol and the applicable ICH guidelines and GCP regulations, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

_____

Investigator's Signature Date

_____

Investigator's Name (Print)

_____

Study Site (Print)

APPENDIX A. CONTINUOUS REASSESSMENT METHOD

A CRM is a working model that operates by continuously refining the assumptions about the dose toxicity relationship after each participant or group of participants are dosed and observed [O'Quigley 1990]. By utilizing the information from nonclinical and clinical studies, and continuously updating the information during the study, the CRM is able to minimize the number of participants exposed to lower doses, which are not expected to be effective, and obtain a more accurate estimate of the MTD. The MTD for this therapy is defined as the highest tested dose with a less than 33% DLT rate at Day 15.

With this CRM, the DLT rate will be continuously assessed as data from groups of 4 participants (3 randomized to active treatment and 1 to placebo) becomes available. The first group will receive the lowest dose level (10 mg). For dose escalation or de-escalation decisions, no more than 1 dose level will be allowed at a time. The CRM algorithm requires specifying prior distributions on the model parameter. A vague prior distribution is normally utilized based on nonclinical animal toxicology data and relevant clinical data. Whenever DLT data from each group of participants are available, the parametric model will be updated with all the accumulated DLT data. The posterior probability of DLT of each dose will be calculated and used to find the highest dose that has at most 50% posterior probability of being intolerable (i.e., DLT rate > 33%).

Specifically, the probability of observing a DLT, $p(x)$ at each dose level (d) will be estimated through a 2-parameter logistic model:

$$P(Y = 1|\text{dose} = x=\log(d/10)) = p(x) = \exp(\beta_0+ \beta_1 x)/[1+ \exp(\beta_0+ \beta_1 x)],$$

where Y is the binary indicator of a DLT for a given dose d, and x is the standardized dose for d, in reference to the 10-mg dose. At the beginning of the study, we set slightly informative prior distributions for the 2 logistic regression parameters, the intercept (β_0) and the slope (β_1) as follows: $\beta_0 \sim N(-3.89, 1.0^2)$ and $\beta_1 \sim N(1.82, 0.3^2)I(0, \infty)$. The truncated normal distribution for β_1 ensures that the probability of DLT is a monotonic increasing function of dose. After DLT data from 15 participants receiving active treatment are available, the prior distribution for β_0 will be replaced by $N(-3.89, 2.0^2)$. Similarly, after DLT data from the 60 mg cohort are available, the prior distribution for β_1 will be replaced by $N(1.82, 0.6^2)I(0,\infty)$. Such change will ensure that the dose toxicity curve is more driven by the safety data collected toward the end of the study.

These initial prior distributions are selected so that the induced prior distributions on DLT rate will yield a median DLT rate of approximately 2% (95% credible interval 0.003, 0.127) at the 10-mg dose, and approximately 35% (95% credible interval 0.054, 0.832) at the 60-mg dose. The expected DLT rates were determined based on the safety data on BIIB067 from the toxicology study in monkeys, where no clinical findings were observed, and from prior clinical trials, where up to 12 mg of other ASO treatments were shown to be well tolerated in repeated-dose studies in patients with ALS or spinal muscular atrophy. Toward the end of the study, the prior distributions $\beta_0 \sim N(-3.89,2.0^2)$ and $\beta_1 \sim N(1.82, 0.6^2)I(0,\infty)$ will induce prior distributions with the same median DLT rate but much wider 95% credible intervals (0, 0.513 for 10-mg and 0.006, 0.979 for 60-mg dose, respectively).

The details of the CRM algorithm are as follows:

Step 1: The first group of participants will be assigned to the lowest dose level, i.e., 10 mg. Set d_1*=log(d_1/10)=0.

Step 2: For any group k, we calculate the posterior probability of DLT rate ≥ 33% for all d_1*,… d_r*, where d_i* =log(d_i/10),

$$\pi_k(d_i^*) = P(p(d_i^*) \geq 0.33 \mid y_k),\ i=1,\ldots, r,$$

where y_k denotes the DLT data collected from the first (k-1) groups.

The kth group will be assigned to the highest planned dose level whose posterior probability of DLT rate larger than 33% is less than 50%, with a restriction to a change of no more than one dose level at a time.

Step 2 will be repeated until one of the following conditions is encountered:

1. a total of 27 active and 9 placebo participants have been enrolled, or
2. the highest planned dose has tested 6 active and 2 placebo participants and the posterior probability of DLT rate of the highest dose larger than 33% is less than 20%, or
3. MTD dose has tested 9 active and 3 placebo participants.

At the completion of the trial, the highest tested dose with less than 50% posterior probability of being intolerable will be labeled as the MTD, or highest tested safe dose, which will be based on all the trial data, including the last group of participants.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

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

Version 8

Date: 15 June 2021

EUDRA CT Number: 2015-004098-33

Version 8 of the protocol has been prepared for this amendment, which supersedes Version 7.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 233AS101 is to update to reflect the final statistical analysis plans.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 16.2.1.2.1, Analysis of the Primary Efficacy Endpoint

Change: The statistical methodology was updated consistent with the study's Statistical Analysis Plan.

Now reads:

~~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 methodology to account for mortality [Berry 2013]. This joint ranking procedure allows for a statistical test of the treatment effect on the ALSFRS-R total score while accounting for loss of data due to deaths. In this analysis, a subject's Combined Assessment of Function and Survival (CAFS) 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 example, if 2 subjects complete the study up to Week 28, their comparison score will be based on the change from baseline in ALSFRS-R total score at Week 28. A subject who dies will rank lower than any subject who completes the study up to Week 28. Two subjects who both die will be ranked based on the time of death, with a longer time to death corresponding to a higher rank. 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 will be ranked more favorably than subjects who die.~~

~~The ranked scores will be analyzed 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. The primary inference will be based on the mITT population. The treatment comparison will be based on a 2-sided α level of 0.05.~~

~~If there are insufficient number of deaths, the change from baseline in ALSFRS-R total score will be analyzed using a mixed model for repeated measures (MMRM) with fixed effect for treatment, time (as a categorical variable), treatment by time interaction and adjustment for baseline ALSFRS-R score, baseline ALSFRS-R score by time interaction, baseline disease duration since symptom onset, and use of riluzole or edaravone. An unstructured covariance (UN) matrix will be used to model the within subject variance-covariance errors. The primary~~

~~inference will be based on the treatment comparison at Week 28. A 2-sided α level of 0.05 will be used.~~

~~Sensitivity analyses of the primary efficacy endpoint will be specified in the statistical analysis plan.~~

The primary efficacy endpoint is the change from baseline to Week 28 in the ALSFRS-R total score, which will be analyzed as follows, depending on 2 scenarios:

1. **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 JRT methodology to account for mortality [Berry 2013] will be used for the primary inference. When implementing the JRT methodology, 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 MI. The corresponding nominal p-value from the ANCOVA will be presented as a sensitivity analysis.**

2. **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 as a sensitivity analysis.**

The primary estimand is described as follows:

The primary analysis for the primary endpoint is the composite estimand [ICH E9 (R1) Addendum 2017]. The estimand of the primary analysis is defined as follows:

- **Population: all participants 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 participants who have these intercurrent events are ranked against each other and against participants without any intercurrent event using the joint rank methodology based on MI datasets. The relevant intercurrent events are deaths and withdrawals. These participants will be handled using the composite strategy and ranked as described in this section. 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 for change from baseline to Day 197, based on the MI datasets. The ANCOVA model will include treatment group as a fixed effect and covariates for ALSFRS-R total score, baseline disease duration since symptom onset, and use of riluzole or edaravone.**

For the JRT analysis, the ranked scores will be analyzed for each of the 100 MI-complete datasets using an ANCOVA 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. The p-value will be based on combining the estimates for the treatment differences using PROC MIANALYZE. The difference between treatment groups in median changes from baseline to each visit will be presented with 95% confidence intervals based on the MI datasets, as well as the p-value from the JRT for Day 197. The joint rank analysis will only be performed for the mITT population.

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

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

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

MI will be used to impute all missing data before determining the rank score, including intermittent data and data after withdrawal from the study except after death.

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

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

Further details will be described in the statistical analysis plan.

The primary inference will be based on the mITT population. The treatment comparison will be based on a 2-sided α level of 0.05.

If the second scenario occurs, i.e., in both active and placebo treatment groups combined the number of participants who died is < 3 (e.g., < 5% deaths out of a total of 60 participants in the mITT population), the primary efficacy analysis will be the change from baseline in ALSFRS-R total score, analyzed using an ANCOVA based on data imputed using MI. The ANCOVA model will include treatment group as a fixed effect and covariates for each of the corresponding baseline value for ALSFRS-R total score, baseline disease duration since symptom onset and use of riluzole or edaravone. The primary inference will be based on the treatment comparison at Week 28. A 2-sided α level of 0.05 will be used. Treatment differences at other visits will also be presented with nominal p-values.

Sensitivity, supplementary and subgroup analyses of the primary efficacy endpoint will be specified in the statistical analysis plan.

For the non-mITT population, the analysis will be the change from baseline in ALSFRS-R total score, analyzed using an ANCOVA based on data imputed using MI. The ANCOVA model will include treatment group as a fixed effect and covariates for each of the corresponding baseline value for ALSFRS-R total score, baseline disease duration since symptom onset, and use of riluzole or edaravone. Least square means with standard errors will be presented for each treatment group. The difference between treatment groups in least square means for change from baseline at each visit, with corresponding standard errors and 95% confidence intervals, will be presented with nominal p-values.

Rationale:

The primary efficacy endpoint and analysis have been clarified to distinguish between the methodology used for statistical testing and the analysis for obtaining the estimates of the treatment difference. The joint rank methodology is a statistical test of the treatment effect on the ALSFRS-R total score while accounting for truncation of data due to deaths. A rank score is calculated to obtain the p-value. As the range of this rank score depends on the sample size, it is not possible to provide a clinically meaningful interpretation using this score. Therefore, an ANCOVA on the change from baseline is used to obtain the estimates of the treatment difference.

The traditional joint rank method for the CAFS has also been replaced by the joint rank methodology with multiple imputation for handling missing data due to withdrawals. This provides a more robust method for the joint rank test.

For the alternative analysis when there are an insufficient number of deaths, ANCOVA with multiple imputation will be used to account for missing data as a more robust method, instead of MMRM.

There is clarification on what is deemed as an insufficient number of deaths to determine whether the joint rank test will be used for the primary efficacy analysis. This threshold is based on the duration of the study and the requirement of SVC ≥ 65% at screening for participants in the mITT population.

Section 16.2.1.2.2, Analysis of the Secondary Efficacy Endpoints

Change: The ordering of secondary endpoints in the sequential testing procedure for the mITT population and the statistical methodology for secondary endpoints was amended for consistency with the study's Statistical Analysis Plan.

Now reads:

~~The analyses of secondary efficacy endpoints will be based on a sequential closed testing procedure in the order of the rank of secondary endpoints as listed in Section 6.2. The treatment effect will be assessed at each step at a 2-sided α level of 0.05. The primary inference at each step will be based on the mITT population.~~

~~The first secondary efficacy endpoint will be change from baseline to Week 28 in percent predicted SVC, which will be analyzed using a MMRM with fixed effect for treatment, time (as a categorical variable), treatment by time interaction and adjustment for baseline SVC, baseline SVC by time interaction, baseline disease duration since symptom onset, use of riluzole or edaravone. Overall treatment difference across visits and treatment difference at Week 28 will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose.~~

~~The second and third secondary efficacy endpoints, VAFS and overall survival, respectively, will be analyzed as time to event. Kaplan-Meier estimates of the cumulative probability of event occurrence over time will be determined. Treatment comparison will be based on a stratified log rank test. A Cox regression model will be used to obtain the hazard ration and 95% confidence intervals for each of these endpoints.~~

~~The muscle strength as measured by HHD will be summarized by individual muscles and megascore. The megascore will be analyzed using a MMRM with fixed effect for treatment, time (as a categorical variable), treatment by time interaction and adjustment for baseline HHD megascore, baseline HHD megascore by time interaction, baseline disease duration since symptom onset, and use of riluzole or edaravone. Overall treatment difference across visits and treatment difference at Week 28 will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose.~~

Formal testing of secondary efficacy endpoints will be in the mITT population alone. For the non-mITT and ITT populations, there will be no formal testing of efficacy endpoints. Testing of PD endpoints is discussed in Section 16.2.3. The analyses of secondary endpoints for the mITT population will be based on a sequential testing procedure in the order of the rank of secondary endpoints as listed below if the primary endpoint is statistically significant at a 2-sided α level of 0.05:

- **Change from baseline (i.e., ratio) to Week 28 (Day 197) in CSF total 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 SVC**
- **Change from baseline to Week 28 (Day 197) in 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**

The treatment effect will be assessed at each step at a 2-sided α level of 0.05. The primary inference at each step will be based on the mITT population.

This procedure 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.

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.

The first and second endpoints in the ranking are PD endpoints for which the analysis is described in Section 16.2.3.

The third secondary endpoint will be change from baseline to Day 197 in percent predicted SVC. 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 primary inference will be based on the JRT methodology with MI to impute missing data; ANCOVA with MI for change from baseline will be used to obtain the estimates, and nominal p-values will be presented. 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 of this endpoint. The ANCOVA model will include treatment group as a fixed effect and covariates for baseline percent predicted SVC, baseline disease duration since symptom onset, and use of riluzole or edaravone. Treatment differences at other visits will also be derived from the model for exploratory purpose.

The secondary efficacy endpoints, time to death or permanent ventilation and time to death, will each be analyzed as time to event endpoints. Kaplan-Meier estimates of the cumulative probability of event occurrence over time will be determined. Treatment comparison will be based on a stratified log rank test. A Cox regression model will be used to obtain the hazard ratio and 95% confidence intervals for each of these endpoints. The

analysis for each endpoint will also be repeated for the non-mITT population with nominal p-values presented.

Rationale:

The hierarchy for the sequential closed testing procedure has been modified to account for the likelihood of statistical success for each endpoint in the 28-week duration of this study. CSF SOD1 and plasma NfL are also now included in the closed testing procedure and placed above SVC. Due to the COVID-19 pandemic, the level of missing data for SVC is likely to be higher due to individual site restrictions for handling the pandemic. The time-to-event endpoints are ranked lowest as the duration of the study and the inclusion criteria for SVC ≥ 65% at Screening in the mITT population reduces the chance to observe a large number of events in these endpoints over 28 weeks. It is expected that a greater treatment effect may be observed on SVC and muscle strength measured by HHD than on the time-to-event endpoints.

Change from baseline in SVC will be analyzed similarly to change from baseline in ALSFRS-R to account for mortality, depending on the number of deaths. The analysis specified provides a more robust method for estimating the treatment effect on this endpoint.

ANCOVA with multiple imputation is also specified as the analysis of HHD megascore instead of MMRM to provide a more robust method for handling missing data.

Section 6.2.1.2 Secondary Efficacy Endpoints

Change: The time to event endpoints were renamed so that VAFS was relabeled as time to death or permanent ventilation; and overall survival was renamed to time to death.

Now reads:

The secondary efficacy endpoints are as follows:

- ~~Ventilation assistance-free survival (VAFS), which 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).~~
- ~~Overall survival.~~
- Time to death or permanent ventilation (≥ 22 hours of mechanical ventilation [invasive or noninvasive] per day for ≥ 21 consecutive days)
- Time to death

Rationale: The endpoints were updated to reflect the intended analyses that are being performed for each of these endpoints and the statistics that will be estimated from the Kaplan-Meier analysis.

[redacted]

Section 16.2.3.2, Methods of Analysis

Change: The statistical analysis methods for pharmacodynamic endpoints/biomarkers were updated to be consistent with the study's Statistical Analysis Plan.

Now reads:

The PD endpoint, CSF total SOD1 ~~concentrations~~ **protein at Day 197**, will be the primary endpoint in the ~~subject~~ **non-mITT** population ~~outside of the mITT~~ due to their likely slow clinical function decline. **This is also the highest ranked secondary endpoint in the sequential testing procedure for the mITT population, and NfL in plasma at Day 197 is the second highest ranked secondary endpoint. For other biomarkers, analyses will be performed for each of the mITT and non-mITT populations with nominal p-values presented. For each of the PD/biomarker endpoints, MI will be used to impute missing values. An ANCOVA model for log ratio to baseline will be performed on change from baseline values on the log scale for each of the 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. Least square means (i.e., geometric mean ratios) for each treatment group with standard errors as well as treatment differences will be presented with 95% confidence intervals and p-values. The other exploratory PD/biomarker endpoints that will be analyzed in this way are NfL in CSF, and pNfH in CSF and plasma.** [redacted] ~~The data will be analyzed using a MMRM on a logarithmic scale, with baseline disease duration since symptom onset and baseline total SOD1 concentration as covariates. Overall treatment difference across visits and treatment difference at Week 28 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 purpose~~

presented with nominal p-values. ~~CSF, NFL, p-NFH [redacted] will be analyzed similarly.~~

Rationale: ANCOVA with multiple imputation is also specified as the analysis of PD/biomarker endpoints instead of MMRM to provide a more robust method for handling missing data.

This change also affects Section 6.2.3.1, Analysis Population.

Section 16.2.4.2.1, Adverse Events

Change: Included additional key AE summaries and listings to be consistent with the study's Statistical Analysis Plan.

Now reads:

Incidence of TEAEs will be summarized by treatment groups, overall, by severity, **by relationship to LP,** and by relationship to study treatment. **SAEs, discontinuations and withdrawals due to AEs, AEs leading to hospitalization, and deaths will also be summarized or listed, depending on number of events.** The summary tables will include incidence estimates for overall system organ class as well as for preferred terms within each system organ class. AEs will be coded using ~~the~~ MedDRA. **A subset of AE outputs will also be presented by disease progression subgroup ("participants who met prognostic enrichment criteria for rapid disease progression", "other eligible participants").**

Rationale: The protocol was updated to clarify some of the additional AE summaries presented. The subgroup analysis was added in line with the analysis populations used for clinical function and pharmacodynamic endpoints.

Section 16.2.5.2, Methods of Analysis

Change: Amended statistical methodology to be consistent with study's Statistical Analysis Plan

Now reads:

The quality of life measures will be analyzed using ~~a MMRM with baseline disease duration since symptom onset, baseline value, and use of riluzole or edaravone as covariates~~ **an ANCOVA with MI with fixed effect for treatment and covariates for corresponding baseline value, baseline disease duration since symptom onset and use of riluzole or edaravone.** ~~Overall treatment differences across visits and~~ **The** treatment difference at ~~Week 28~~ **Day 197** will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose. **Nominal p-values will be presented.**

Rationale: ANCOVA with multiple imputation is specified as the analysis of quality of life endpoints instead of MMRM to provide a more robust method for handling missing data.

Section 16.2.6, Interim Analyses

Change: Specified that no interim analysis will be performed.

Now reads:

~~An interim efficacy analysis may be performed. The specifics around the optional interim analysis will be detailed in the statistical analysis plan. In order to maintain the treatment blind, an independent unblinded team not to be involved in the conduct of the study after unblinding will perform the interim analysis. The unblinded team will present the unblinded interim analysis to the IDMC.~~ **There will be no interim analysis performed.**

Rationale: No interim analysis will be conducted due to potential impact to study power with minimal opportunity to accelerate study timelines.

This change also affects Section 9.3, Blinding Procedures.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 1.1, Synopsis

The synopsis was revised to reflect changes made throughout the protocol.

Section 4.2, Schedule of Activities

Change: Footnote 9 in Table 3 was clarified to specify that the diary/eDiary will be completed only for days when the participant uses mechanical ventilation.

Now reads:

[9] Participants 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.

Rationale: Clarification

Section 8.2.1, Inclusion Criteria

Change: The nomenclature for the disease progression subgroups (previously "fast progressors" and "non-fast progressors") was modified to more accurately represent the population enrolled.

Now reads:

3. Weakness attributable to ALS and a confirmed SOD1 mutation.
    a. SOD1 mutation must be confirmed by the central reader based on the sample obtained during the Screening Visit; ~~subjects~~**participants** with an SOD1 mutation interpreted by the central reader to be pathogenic or likely pathogenic will be eligible.
    b. Additionally:

- ~~Fast progressor criteria~~ **Prognostic enrichment criteria for rapid disease progression** (~~subjects~~**participants** 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 slope decline of ≥ 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 slope decline of ≥ 0.9 per month (calculated as [48-baseline score]/time since symptom onset)

- ~~Non-fast progressor criterion~~ **Criteria for all other eligible participants**: SOD1 mutation other than those listed in item ‘a.’ (no ALSFRS-R slope decline requirement).

4. For ~~fast progressors~~ **participants who meet prognostic enrichment criteria for rapid disease progression**, SVC ≥ 65% of predicted value as adjusted for sex, age, and height (from the sitting position). For ~~non-fast progressors~~ **all other eligible participants**, SVC ≥ 50% of predicted value as adjusted for sex, age, and height (from the sitting position).

Rationale:

The primary analysis population was enriched, based on SOD1 mutation type and prerandomization ALSFRS-R slope decline, for participants more likely to have rapid disease progression during the study. The terminology used to describe this population was changed from "fast progressors" to "participants who met prognostic enrichment criteria for rapid disease progression" to more accurately reflect the population enrolled. Consistently, the terminology for the broader population enrolled (previously "non-fast progressors") was updated to "other eligible participants." No changes have been made to the criteria to define either subgroup.

This change also affects Sections 7.1.3, Part C: Pivotal; 9.2, Randomization and Registration of Participants; 16.2.1.1, Analysis Population, and 16.2.7, Sample Size Considerations.

Section 6.2.2, Secondary Objective

Change: Added biomarker effects to the secondary objective.

Now reads: The secondary objective is to evaluate the safety, tolerability, ~~and~~ PD, **and biomarker** effects of BIIB067 administered to adult participants with ALS and **a** confirmed SOD1 mutation.

Rationale: Updated to clarify that biomarker effects are also a secondary objective.

Section 6.2.2.1, Safety/Tolerability Endpoint

Change: Added “secondary” to the endpoint.

Now reads: The **secondary** safety/tolerability endpoint is the incidence of AEs and SAEs.

Rationale: Clarification.

Section 6.2.2.2, Pharmacodynamic Endpoint

Change: Specified that the change from baseline in total SOD1 protein concentration in CSF is a secondary PD endpoint.

Now reads: The **secondary** PD endpoint is the change from baseline in total SOD1 **protein** concentration in CSF

Rationale: Updated to reflect that total SOD1 protein concentration in CSF will be tested as a secondary endpoint.

Section 6.2.2.3, Biomarker Endpoint

Change: The key secondary biomarker and matrix were changed.

Now reads: The **secondary** biomarker endpoint is the change from baseline in ~~p-NFH~~ **NfL** concentration in ~~CSF~~ **plasma**.

Rationale: Updated to reflect that plasma NfL will be tested as a key secondary (biomarker) endpoint. Plasma NfL was selected given the potential utility of a blood-based biomarker in the future, along with assay characteristics. Plasma NfL will be evaluated on the Siemens Healthineers NfL assay which has good analytical performance and utilizes a fully automated instrument.

Section 6.2.3.2, Additional Biomarkers

Change: Clarified additional biomarkers to be measured in serum, plasma, and/or CSF.

Now reads:

- Blood **(serum)** and CSF concentrations of ~~NFL~~**NfL**
- Blood ~~concentration~~ **(plasma, serum) and/or CSF concentrations** of ~~pNF-H~~pNfH

Rationale: Clarification.

Section 7.1.3, Part C: Pivotal

Change: Updated to clarify that a Safety Follow-up (Alternative EOS) Visit should occur when enrollment in the LTE study is delayed.

Now reads:

~~For subjects participating in Part C (Pivotal) and continuing (uninterrupted) in the LTE study (233AS102), the study duration will be approximately 32 weeks. This will include up to a 4-week screening period, a 24-week treatment period (consisting of 3 loading doses of BIIB067 administered approximately once every 2 weeks, followed by 5 maintenance doses of BIIB067~~

~~administered approximately once every 4 weeks), and an EOS Visit at Week 28 (4 weeks after the last dose). After completing the EOS visit, subjects will be considered study completers and they will have the option to participate in the LTE study (233AS102). This will be done without unblinding to subject's treatment group.~~

~~For subjects who do not roll over into the LTE study, the duration will be approximately 36 weeks, as the subjects will have an additional safety follow-up visit at Week 32 (8 weeks after the last dose) [either in person or by telephone contact]. End of study date is the last visit or contact with the subject.~~

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

- **Part C participants who enroll (uninterrupted) in the LTE study (233AS102): Week 28 Visit will serve as EOS Visit**
- **Part C 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.**
- **Part C participants who do not enroll in the LTE study: Week 32 Visit will serve as EOS Visit (either in person or by telephone contact)**

Rationale: Updated to clarify that a Safety FU (Alternative EOS) Visit should occur at Week 32 or prior to (within 2 days) enrollment in the LTE study, whichever is first, when there is a delay between the Week 28 visit and enrollment in the LTE study. This follow-up visit will ensure continuity of monitoring (adverse events, serious adverse events, concomitant medications, concomitant procedures) through entry in the LTE or for at least 8 weeks after the last dose of study treatment.

This change also affects Section 4.1, Study Schematic; Section 4.2, Schedule of Activities; Section 7.3.3.3, Part C: Pivotal; Section 7.5, End of Study; and 15.3.1 Adverse Events.

Section 12, Study Treatment Management

Change: Replaced "supersedes" with "aligns with"

Now reads:

The DHA ~~supersedes~~**aligns with** all other references, ~~(e.g., protocol)~~**including the protocol**.

Rationale: Wording was modified to align with updates to the protocol template.

Section 18.3, Monitoring of the Study

Change: Implemented remote evaluation of data and source documents.

Now reads:

The Investigator must permit study-related monitoring by providing direct access to source data and to the ~~subjects'~~**participants'** medical histories. **Source data must be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data must be traceable, not obscure the original entry, and be explained if necessary (e.g., with an audit trail). The Investigator should maintain a record of the location(s) of essential documents.**

The Clinical Monitor will visit the Investigator at regular intervals during the study and after the study has completed, as appropriate. During these visits, CRFs and supporting documentation related to the study will be reviewed and any discrepancies or omissions will be resolved.

Remote evaluation of data (centralized monitoring) and remote verification of source documentation may also be conducted and reported as defined in the monitoring plan.

Monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

Rationale: Remote evaluation of data and source documents is being implemented to allow the sponsor and CRO to continue to provide oversight to site performance and ensure overall patient safety and data integrity. This process is being implemented because several sites/institutions have restricted on -site access for the CRO monitors during this ongoing COVID-19 pandemic.

Section 19.1, Public Health Emergencies

Change: Added contingency plan in the event of site closure, travel restrictions, or other events resulting from a public health emergency.

Now reads:

19.1, Public Health Emergencies

In the event of a public health emergency that results in site closure, travel restrictions, and/or the study being deprioritized at the site such that clinic visit(s) cannot occur, a protocol deviation would be incurred for any deviation from the protocol-specified visits and assessments, with additional notation that this protocol deviation is due to the public health emergency. If a protocol-specified clinical visit cannot occur due to a public health emergency, the following mitigating options should be pursued, in order of preference (in which the highest preference option that is feasible should be done): 1) transfer to another active study site that is open, 2) home visit, 3) telemedicine visit (e.g., by telephone or web conference), and 4) local laboratory visit. These mitigating options only apply in the setting

of a public health emergency in which a protocol-specified clinic visit cannot occur and should not be pursued solely due to participant's preference.

A third-party vendor has been engaged to perform the following assessments at the study participant's home:

1. **Limited neurological examination, including the MMSE**
2. **Physical examination**
3. **Vital signs**
4. **Height and body weight collection**
5. **SVC**
6. [redacted]
7. **Pregnancy test (if applicable)**
8. **HHD**
9. [redacted]
10. **Collection/shipment of ventilation diary records**

The following assessments can be performed via the phone (telemedicine) by the site staff:

1. **ALSFRS-R**
2. [redacted]
3. [redacted]
4. **Assessing changes in signs and symptoms as well as review of concomitant medications and AEs**

The following assessments can be performed at the study participant's home OR via the phone (telemedicine) by the site staff:

1. **Health outcome measures**
2. [redacted]

g. C-SSRS

[redacted]

Rationale: In the event of a public health emergency, mitigating options provide flexibility with respect to the collection of data in the interest of participant safety and to protect the integrity of the data when participants are unable to attend a clinic visit.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- A new signatory's name and title were added. The previous signatory's name and title were removed.
- Section 2, List of Abbreviations, was updated.
- Replaced "subject" with "participant" throughout the protocol (with exception of the title).
- Deleted "tertiary/" preceding exploratory objectives.
- The abbreviation for neurofilament light was changed from "NFL" to NfL" and the abbreviation for phosphorylated axonal neurofilament heavy chain was changed from "pNF-H" to "pNfH."
- Differentiated between SOD1 gene/mutation and total SOD1 protein.
- Abbreviations were deleted from the footnotes in tables and figures and added to the List of Abbreviations to align with the updated protocol template
- The expanded forms of abbreviations were deleted at first use to align with the updated style guide
- Replaced "population outside the mITT" with "non-mITT population."
- Typographical errors and formatting were corrected.

LIST OF ABBREVIATIONS

AE	adverse event
ALS	amyotrophic lateral sclerosis
[redacted]	
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ANCOVA	analysis of covariance
CAFS	Combined Assessment of Function and Survival
[redacted]	
CRF	case report form
CRO	contract research organization
CSF	cerebrospinal fluid
CSR	clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
DHA	Directions for Handling and Administration
EOS	end of study
[redacted]	
FU	follow-up
GCP	Good Clinical Practice
HHD	handheld dynamometry
ICH	International Council for Harmonisation
IDMC	independent data monitoring committee
ITT	intent-to-treat
JRT	joint rank test
LTE	long-term extension
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
mITT	modified intent-to-treat
MMRM	mixed model for repeated measures
MMSE	Mini-Mental State Examination
NfL	neurofilament light chain
[redacted]	
PD	pharmacodynamic(s)
[redacted]	
PK	pharmacokinetic(s)
pNfH	phosphorylated axonal neurofilament heavy chain
SAE	serious adverse event
[redacted]	
SOD1	superoxide dismutase 1
SVC	slow vital capacity

TEAE	treatment-emergent adverse event
UN	unstructured covariance
VAFS	ventilation assistance-free survival
[redacted]	

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

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

Version 7

Date: 30 September 2019

EUDRA CT Number: 2015-004098-33

Version 7 of the protocol has been prepared for this amendment, which supersedes Version 6, dated 19 September 2019.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 233AS101 is to add a provision to collect [redacted] in all regions where not prohibited by regulatory authorities or ethics committee.

New text is shown in **bold** type.

[redacted]

Now reads:

[redacted]

[redacted]

Rationale: [redacted]

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

No major changes are being made in this amendment.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Section 4.2, Schedule of Activities (Table 3 – Schedule of Activities: Part C [Pivotal]), [redacted]
- Section 6.2.3.2, Additional Biomarkers, sub-bullet blood and CSF concentrations of pNF-H was updated to blood concentration of pNF-H, as change in CSF concentration of p-NFH is already covered as a biomarker endpoint under Section 6.2.2.3 (Biomarker Endpoint).
- Typographical errors and formatting were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

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

Version 6

Date: 19 September 2019

EUDRA CT Number: 2015-004098-33

Version 6 of the protocol has been prepared for this amendment, which supersedes Version 5 dated 20 December 2018.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 233AS101 is to describe the changes to the primary endpoint and statistical methodology, sample size considerations, and inclusion of an optional interim efficacy analysis for Part C (Pivotal) of the study.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 6.2.1., Primary Objective

Now reads:

6.2.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint ~~of Part C~~ is the ~~postrandomization slope of the ALSFRS-R score measured~~ **change** from baseline to Week 28 **in the ALSFRS-R total score.**

Section 16.2.1.2.1., Analysis of the Primary Efficacy Endpoint

Now reads:

The primary efficacy endpoint is the ~~postrandomization slope of ALSFRS-R scores measured from baseline to Week 28. The postrandomization ALSFRS-R score over all time points~~ **change from baseline to Week 28 in the ALSFRS-R total score, which will be analyzed using the joint rank methodology to account for mortality [Berry 2013]. This joint ranking procedure allows for a statistical test of the treatment effect on the ALSFRS-R** ~~score over all time points~~ **total score while accounting for loss of data due to deaths. In this analysis, a subject's Combined Assessment of Function and Survival (CAFS) 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 example, if 2 subjects complete the study up to Week 28, their comparison score will be based on the change from baseline in ALSFRS-R total score at Week 28. A subject who dies will rank lower than any subject who completes the study up to Week 28. Two subjects who both die will be ranked based on the time of death, with a longer time to death corresponding to a higher rank. 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 will be ranked more favorably than subjects who die.

The ranked scores will be analyzed 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 (yes or no), and use of edaravone (yes or no with yes including the use of both edaravone and riluzole). The primary inference will be based on the mITT population. The treatment comparison will be based on a 2-sided α level of 0.05.

If there are insufficient number of deaths, the change from baseline in ALSFRS-R total score will be analyzed using a mixed model for repeated measures (MMRM) with fixed effect for treatment, time (as a ~~continuous~~ **categorical** variable), treatment by time interaction~~,~~ **and adjustment for** baseline ALSFRS-R score, baseline **ALSFRS-R score by time interaction, baseline** disease duration since symptom onset, ~~use of riluzole (yes or no),~~ and use of **riluzole or** edaravone ~~(yes or no with yes including the use of both edaravone and riluzole)~~. An unstructured covariance (**UN)** matrix will be used to model the within-subject variance-covariance errors ~~If the unstructured covariance structure matrix results in a lack of convergence, the first-order autoregressive covariance structure will be used. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. The primary inference will be based on the estimated treatment by time interaction in the mITT population. The estimated difference in slope of decline between BIIB067 and placebo, and the associated 95% confidence interval, will be derived from the model. The treatment effect will be assessed at a 1-sided α level of 0.05 (or 2-sided α level of 0.10). In addition, as a supportive analysis, the slope of each individual subject will be derived using simple least square estimate, and treatment comparison using the Mann-Whitney test, based on ranked data, will be made. As a further sensitivity analysis, the slope of decline in 92 days observed in subjects from Part B who received placebo or BIIB067 100 mg will be pooled with Part C slopes over 92 days, and the Mann-Whitney test will be performed on the pooled data. The treatment effect in the overall ITT population will also be assessed similarly. The ALSFRS-R score will be set to 0 upon occurrence of death~~**. The primary inference will be based on the treatment comparison at Week 28. A 2-sided α level of 0.05 will be used.**

Sensitivity analyses of the primary efficacy endpoint will be specified in the statistical analysis plan.

Rationale for Section 6.2.1 and Section 16.2.1.2.1: The primary efficacy endpoint was revised from ALSFRS-R slope to total score (change from baseline to Week 28) and will be analyzed via a joint-rank analysis that combines the ALSFRS-R score and survival time. This approach minimizes the dependency on linearity assumptions (of the ALSFRS-R slope decline) and enables a robust mechanism for accounting for missing data due to death.

Section 16.2.7., Sample Size Considerations

Now reads:

For Part C, ~~up to 60 subjects~~ **approximately 99 subjects** will be~~ enrolled in a single cohort~~ **randomized**, with ~~40~~ **approximately 66** subjects administered BIIB067 100 mg and ~~20~~ **approximately 33** subjects administered placebo in a 2:1 ratio.

This sample size for Part C is selected primarily based on the ~~primary endpoint,~~ **joint rank test combining** the ~~slope of decline~~ **Week 28 change from baseline** in ALSFRS-R **and mortality**, in the mITT population (fast progressor group**, N = 60**). The observed results from an interim analysis of ~~BIIB067 100 mg from~~ Study 233AS101 in the fast progressor ~~subject~~ group as well as the observed ALSFRS-R decline in subjects who receive**d** placebo in a similar fast progressor ALS population with SOD1 mutation [Benatar 2018] are the basis of assumed treatment effect and the variability. ~~The observed mean ± SD of the slope of decline in the historical placebo data [Benatar 2018] was 3.2 ± 2.5 per month (markedly more rapid than that in the overall ALS population). The interim data of a very small cohort in Study 233AS101 suggested a treatment difference between BIIB067 100 mg and placebo of 5.8 per month in favor of BIIB067 100 mg with an estimated pooled SD of 1.4 and an estimated mean decline in the active group of 0.8 per month. Given the small sample size for the treatment comparison in Study 233AS101, a conservative assumption of a treatment difference of 2.4 per month (the difference between historical placebo mean and observed mean of BIIB067 100 mg in Study 233AS101) and a SD of 2.5 (from historical data) was made. With a 2:1 randomization ratio, a sample size of 36 subjects in the fast progressor population (mITT population) would provide the study with 85% power at 1-sided α of 0.05 to detect such treatment differences. Because events of death or permanent ventilation assistance have been factored in the analysis, no sample size overage is planned for missing values.~~ **Subjects from both datasets were matched with the criteria for a fast progressor 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 fast progressors (8 from** the historical placebo data [Benatar 2018] **and 4 from Study 233AS101) and 4 fast progressors treated with BIIB067 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 BIIB067 100 mg subjects, with a pooled SD of 3.166. In the 12 matched fast progressors on placebo, there was 1 death and 2 subjects with PAV less than 6 months but none in the BIIB067 100 mg potential fast progressors. 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 BIIB067 100 mg group.**

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

For the subject population outside of the mITT population, the primary focus will be on the PD endpoint, CSF SOD1 concentration, ~~due to their likely slow clinical function decline~~ **as this population is anticipated to have a slower decline in clinical function compared to the mITT population**. A sample size of ~~16~~ **26 subjects in** ~~this group~~ **the treated group and 13 subjects in the placebo group for the population outside of the mITT** would provide ~~80%~~ 97% power to detect a ~~19%~~ 25% reduction **in CSF SOD1** from baseline ~~within group~~ **in the treated group,** with an assumed SD of ~~0.28~~ **0.216** (~~in~~ natural log scale), ~~as observed in this population in Study 233AS101~~ compared to the placebo group.

Rationale: The sample size for Part C of the study was increased to approximately 99 subjects (increased from up to 60 subjects) based on the following changes:

- Revised primary efficacy endpoint of change in ALSFRS-R total score from baseline to Week 28 analyzed via the joint-rank methodology
- Two-sided alpha of 0.05 for the primary analysis
- Revised survival assumptions based on further review of natural history data and data from an interim analysis of Part B of this study (82% survival in the placebo control group and 90% survival in the BIIB067-treated group)

Under these assumptions, approximately 60 subjects (increased from 36) are needed in the mITT population (fast progressors) to achieve approximately 84% power. The target sample size for the non-fast progressor population was increased to approximately 39 subjects (increased from 24) to enable adequate power to detect a statistically significant reduction in CSF SOD1 concentrations.

Also, the assumptions for the sample size needed for the population outside the mITT were updated based on current results from the Part B of this study (233AS101).

This change also affects Section 5.4, Study Rationale and Section 7.1, Study Overview.

Section 16.2.6, Interim Analyses

Now reads:

~~In Part C of the study, a blinded sample size re-estimation may be conducted when approximately 18 subjects in the fast progressor population complete Day 169 assessments of ALSFRS-R or have an outcome of death or permanent ventilation assistance. At this interim timepoint, the SD for the primary endpoint will be estimated based on the blinded data. The sample size of the fast progressor group may be increased to up to 60 (potentially increasing the total sample size to approximately 80 subjects) if the SD is estimated to be ≥3. As this is based on blinded data, the type I error rate is maintained.~~

An interim analysis may be performed. The specifics around the optional interim analysis will be detailed in the statistical analysis plan. In order to maintain the treatment blind, an independent unblinded team not to be involved in the conduct of the study after unblinding will perform the interim analysis. The unblinded team will present the unblinded interim analysis to the IDMC.

~~A futility analysis may be performed at the interim. The futility criteria will be prespecified in the analysis plan, and if conducted, all necessary procedures to safeguard the integrity of the blind will be set in place. No early stopping for efficacy will be made at this interim analysis.~~

Section 7.1.3, Part C: Pivotal

Now reads:

…

…

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 assessments will be conducted. For subjects not entering the LTE study or in the event the study is stopped early, there will be an additional safety follow-up visit 8 weeks after the last dose of study treatment.

Rationale for Section 16.2.6 and Section 7.1.3: An optional interim efficacy analysis has been included to enable the earliest opportunity to detect a clinically meaningful effect and potential to minimize placebo exposure should the study be terminated early due to a positive interim analysis. Correspondingly, the text around futility analysis was removed.

This change also affects Section 4.2 (Table 3), Schedule of Activities: Part C (Pivotal); and Section 9.3, Blinding Procedures.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 6.2.1.2, Secondary Efficacy Endpoints; Section 6.2.3, Tertiary/Exploratory Objectives; and Section 6.2.3.3, Quality of Life Endpoints

Change: The Combined Assessment of Function and Survival (CAFS) and change from baseline in ALSFRS-R total score were removed as secondary endpoints. The assessments related to quality of life assessments were moved from the secondary efficacy endpoints section to the exploratory endpoints section.

Now reads:

6.2.1.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

- ~~Combined assessment of function and survival (CAFS) up to Week 28.~~
- ~~Change from baseline to Week 28 in ALSFRS-R score.~~
- Change from baseline to Week 28 in SVC.
- Ventilation assistance-free survival (VAFS), which is defined as the time to the earliest occurrence of 1 of the following events:
  - Death.
  - ~~First use of non-invasive ventilation (NIV) for ≥22 hours per day for ≥10 consecutive days.~~ **Permanent ventilation (≥ 22 hours of mechanical ventilation (invasive or noninvasive) per day for ≥ 21 consecutive days).**
  - ~~First use of permanent assisted ventilation (PAV) for ≥22 hours per day for ≥7 consecutive days.~~
- Overall survival.

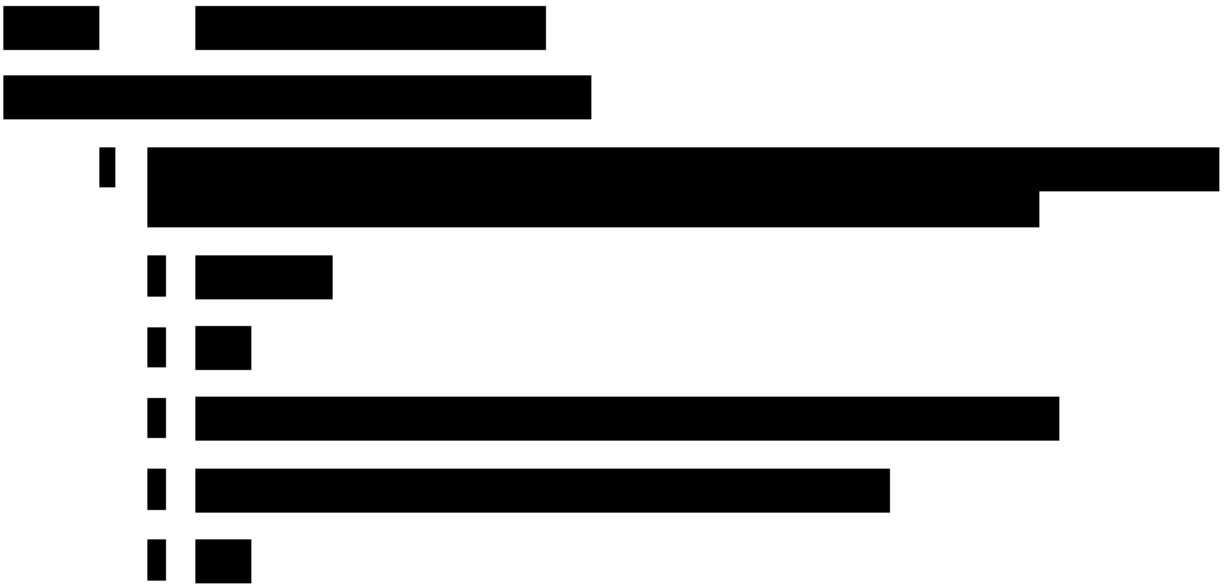
- Changes from baseline to Week 28 in **HHD megascore to assess** muscle strength**, as** measured by **the** HHD device.

…

6.2.3. Tertiary/Exploratory Objective

The tertiary/exploratory objectives ~~is~~ **are** to evaluate the PK of BIIB067 and other potential biomarkers in adult subjects with ALS and confirmed SOD1 mutation~~.~~ **and to assess the effect of BIIB067 on changes in subject quality of life and caregiver burden.**

…



– [redacted]

[redacted]

Rationale: In light of the changes to the primary endpoint, the CAFS and change from baseline in ALSFRS-R total score have been removed as secondary endpoints to avoid redundancy.

The criteria for VAFS were updated to incorporate a conservative threshold best representative of permanent ventilation that accounts for acute reversible illness and variability in patient/family preference and resources, geographic differences in standard of care, etc.

[redacted]

These changes also affect Section 4.2, Schedule of Activities (Table 3 – Schedule of Activities: Part C [Pivotal]); Section 13.1, Assessments of the Effect of BIIB067 on Clinical Function; Section 16.2.1.2.1, Analysis of Primary Efficacy Endpoint; Section 16.2.1.2.2, Analysis of Secondary Efficacy Endpoint; and Section 19.2.3. Endpoint Adjudication Committee.

Section 16.2.1.2.2., Analysis of the Secondary Efficacy Endpoints

Change: Details for the analysis of the secondary endpoints were updated.

Now reads:

The analyses of secondary efficacy endpoints will be based on a sequential closed testing procedure in the order of the rank of secondary endpoints as listed in procedure in the order of the rank of secondary endpoints as listed in Section 6.2. The treatment effect will be assessed at each step at a ~~1-sided α level of 0.05 (or~~ 2-sided α level ~~of 0.10)~~**05.** The primary inference at each step will be based on the mITT population. ~~However, the treatment effect in the population in the overall ITT population will also be assessed~~.

The first secondary efficacy endpoint will be ~~will be CAFS [Berry 2013],~~ **change from baseline to Week 28 in percent predicted SVC,** which ~~incorporates both ranked score of death~~ **will be analyzed using a MMRM with fixed effect for treatment, time (as a categorical variable), treatment by time interaction** and ~~ALSFRS-R score up to Week 28, and will be analyzed using an analysis of covariance with baseline~~ **adjustment for baseline SVC, baseline SVC by time**

interaction, baseline disease duration since symptom onset, ~~baseline ALSFRS-R score, and~~ use of riluzole ~~or edaravone as covariates.~~

~~The second and third secondary efficacy endpoints, ALSFRS-R and SVC, respectively, will be analyzed using a MMRM with baseline disease duration since symptom onset, baseline value (ALSFRS-R and SVC, respectively), and use of riluzole or edaravone as covariates. The ALSFRS-R score and SVC will be set to 0 upon occurrence of death or permanent ventilation assistance.~~ Overall treatment difference across visits and treatment difference at Week 28 will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose.

The ~~fourth~~ **second** and ~~fifth~~ **third** secondary efficacy endpoints, VAFS and overall survival, respectively, will be analyzed as time to event. Kaplan-Meier estimates of the cumulative probability of event occurrence over time will be determined. Treatment comparison will be based on a ~~Cox proportional hazards model with baseline disease duration since symptom onset, baseline ALSFRS-R score, and use of riluzole or edaravone as covariates~~ **stratified log rank test. A Cox regression model will be used to obtain the hazard ration and 95% confidence intervals for each of these endpoints**.

The muscle strength as measured by HHD will be summarized by individual muscles and megascore. The megascore will be analyzed using a MMRM with **fixed effect for treatment, time (as a categorical variable), treatment by time interaction and adjustment for baseline HHD megascore, baseline HHD megascore by time interaction**, baseline disease duration since symptom onset~~, baseline value,~~ and use of riluzole or edaravone**.** ~~as covariates. The HHD megascore will be set to 0 upon occurrence of death or permanent ventilation assistance.~~ Overall treatment difference across visits and treatment difference at Week 28 will be derived from the model. Treatment differences at other visits will also be derived from the model for exploratory purpose.

Rationale: The analysis for the 2 secondary endpoints of CAFS up to Week 28 and change from baseline to Week 28 in ALSFRS-R total score was removed because these endpoints are now incorporated into the primary endpoint.

Section 8.2.1, Inclusion Criteria

Change: Criteria for screening values of coagulation parameters to be within normal range was removed.

Now reads:

…

~~8. Screening values of coagulation parameters including platelet count, INR, PT, and APTT should be within normal ranges. Coagulation tests may be repeated 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 may be eligible to enroll in the study at the discretion of the Investigator. (For normal ranges, please refer to the study reference guide).~~

…

Rationale: Considering the stage of development of BIIB067 in this study and in the overall program, this criterion of coagulation parameters values to be in normal range is not required and was removed. The original criterion was more appropriate for early phase clinical studies.

Section 8.2.2, Exclusion Criteria

Change: Details for hepatitis C infection screening for Part C were updated based on the current protocol template guidelines.

Now reads:

Candidates will be excluded from study entry if any of the following exclusion criteria exist at Screening, or at the timepoint specified in the individual criterion listed:

1 History of or positive test result for human immunodeficiency virus.

~~2 History of, or positive test result at Screening, for hepatitis C virus antibody.~~

2 Current hepatitis C infection (defined as positive hepatitis C virus [HCV] antibody and detectable HCV ribonucleic acid [RNA]). Subjects with positive HCV antibody and undetectable HCV RNA are eligible to participate in the study (United States Centers for Disease Control and Prevention).

3 Current hepatitis B infection (defined as positive for HBsAg and/or anti-HBc). Subjects with immunity to hepatitis B from previous natural infection (defined as negative HBsAg, positive ~~IgM~~ anti-HBc, and positive anti-~~HBc~~**HBs**) or vaccination (defined as **negative HBsAg, negative anti-HBc, and** positive anti-HBs) are eligible to participate in the study.

…

15 Anticipated need, in the opinion of the Investigator, for administration of any antiplatelet or anticoagulant medication ~~(e.g., clopidogrel) for 7 days~~ **that cannot be safely held** before a**nd**/or ~~48 hours~~ after an LP **procedure according to local or institutional guidelines and/or Investigator determination.**

Rationale: Additional guidance text was provided to clarify the exclusion criterion of current hepatitis C infection. The hepatitis B criterion was reworded for accuracy and clarity as the text

in the current protocol (version 5) was not clear or completely accurate. These changes in hepatitis B and hepatitis C criteria are in alignment with the current protocol template guidance.

Because study drug administration will occur via lumbar puncture, care must be exercised in the use of antiplatelet or anticoagulant medication. However, the criterion has been updated to allow for the local standard of care to be used, accounting for the many different indications, medications, and individual patient situations that may be relevant.

The change in exclusion criterion 15 also affects Section 11.3.1.2, Disallowed Concomitant Therapy.

Section 9.1, Screening and Enrollment

Change: Rescreening criteria for subjects who fail to complete Day 1 Visit within 28 days of their screening assessments during Part C were added.

Now reads: Subjects (or their legally authorized representative [e.g., spouse], where applicable) must provide informed consent before any screening tests are performed (see Section 17.3). When a subject signs the informed consent form (ICF), that subject is considered to be enrolled in the study. Participating study sites are required to document all screened candidates initially considered for inclusion in this study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and on the screening log.

~~Eligible~~ **For Part C (Pivotal)** subjects who ~~are not able~~ **fail screening can be rescreened up** to ~~complete~~ **one time at** the ~~Day 1 Visit within 28 days~~ **discretion** of ~~their~~ **the Investigator**.

All screening assessments ~~(Screening Visit 2~~**would need to be repeated during rescreening except** for ~~Parts A and B)~~ ~~may be rescreened.~~**the following:**

- **Follicle-stimulating hormone (FSH) level in female subjects with post-menopausal state confirmed by level obtained in previous screening for Part C of this study**
- **Confirmation of SOD1 mutation by the central reader if available from previous screening for Part C (Pivotal) of this study**

Rationale: It was clarified that rescreening during Part C of the study is allowed at the discretion of the Investigator, and the assessments that need not be performed during rescreening are listed.

Section 4.2, Schedule of Activities

Change: Table 3, Schedule of Activities: Part C (Pivotal) was updated to revise the frequency of Mini-Mental State Examination (MMSE) testing and to reflect changes in the assessment timepoints for 12-lead electrocardiogram (ECG) and blood sample for plasma pharmacokinetic (PK) evaluation.

Now reads:

	Screening[1]	Loading Dose Treatment Period									Maintenance Treatment Period			~~Follow Up Visit~~[2] **EOS Visit[2,3] (All Subjects)**	~~End of Study or Telephone Call~~ **Safety FU Visit for Subjects not Continuing in the LTE Study[4] (In Person or Telephone Contact)**
		Baseline[5] (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[4 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		
12-Lead ECG ~~12,14~~14,16	X	X		X[17]							X[11]		**X[17]**	X	
Limited Neurological Examination (~~including MMSE~~)~~12,14~~**14, 19**	X	X		X	X		X	X		X	X		X	X	
MMSE	**X**	**X**		**X**							**X[11]**		**X[11]**	**X**	
[redacted]															

[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.

...

[redacted]

...

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

...

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

...

[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]

[redacted]

Rationale: In Part B of the study no significant safety findings were noted on the MMSE, so the frequency of the MMSE assessment was reduced to every 3 months during the treatment period in Part C of the study

Considering the median plasma T_{max} of 3 hours (range 1 to 24 hours) for the 100 mg cohort of Part B and to support a potential waiver for Thorough QT (TQT) analysis, it was clarified that at the Baseline Visit, the ECGs should be assessed just prior to drawing PK blood samples at 2, 4, and 6 hours postdose. It was also clarified that blood samples for PK will be collected predose at all timepoints and postdose only at Day 1 and Day 85.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Section 1, Sponsor Information, was updated.
- Section 2, List of Abbreviations was updated.
- The following minor changes were made in Section 4.2 Schedule of Activities (Table 3 – Schedule of Activities: Part C [Pivotal]):
  - Footnotes were added to clarify details of the EOS visit (Week 28) and additional safety follow-up visit (Week 32). This change also affects Section 7.1.3, Part C: Pivotal.
  - [redacted]
  - [redacted].
  - An assessment timepoint at Week 28 was added for CSF sample collection, blood sample collection for anti-BIIB067 antibodies, [redacted].
  - A footnote added to clarify the use of a facemask during SVC testing.
  - There is no requirement for a review of SVC and C-SSRS results at predose to determine if the dose should be administered or held, and these criteria were updated.
  - It was clarified that the pregnancy test will be performed using serum during the screening period and at the EOS visit and that it may be done using urine or serum at other visits at Investigator's discretion. This change also affects Section 14.2, Laboratory Safety Assessments and Section 19.1.4, Laboratory Assessments.
  - A provision to use eDiaries in the future to record ventilation use information in this study was added. This was clarified in footnotes of Table 3. This change also affects Section 19.1.3, Electronic Data Capture.
  - [redacted]

[redacted]

- Section 4.1, Study Schematic (Figure 3: Study Design: Part C [Pivotal]) was aligned with the changes made to Table 3.

- In Section 5.2, Current Therapies for Amyotrophic Lateral Sclerosis, information was generalized to include only the generic names riluzole and edaravone, and details on approval status in different countries were removed.

- Section 5.3.2, Clinical Experience, was updated to include interim analysis results for Part A (single ascending dose) and Part B (multiple ascending dose) of this study. This change also affects Section 5.4, Study Rationale, and Section 5.5, Rationale for Dosing Regimen.

- Section 6.2.3.2, Additional Biomarkers, was updated to clarify that the blood and cerebrospinal fluid (CSF) concentrations of phosphorylated axonal neurofilament heavy chain (pNF-H) will be used for additional biomarker evaluation.

- Section 7.1, Study Overview, was updated with the total number of countries and number of planned study sites globally.

- Section 7.2.3, Dose-Limiting Toxicity, was updated to state that dose-limiting toxicity (DLT) evaluation is not applicable for Part C of the study.

- Section 7.3.3.3, Part C: Pivotal, was edited to remove repetitive information.

- In Section 7.4.1, Dose Suspension (Parts A and B only), a statement was added to clarify that the dose suspension rules are not applicable for Part C of the study.

- Section 7.5, End of Study, was updated with details for criteria to conduct an EOS visit.

- Section 8.2.1, Inclusion Criteria (#3), was updated to clarify that the SOD1 mutation in subjects who are enrolled in Part C of the study must be adjudicated by the central reader.

- In Section 11.2, Modification of Dose and/or Treatment Schedule, clarification was added that no dosage modification will be done in Part C of the study.

- Section 13.1.1, ALS Functional Rating Scale-Revised, was updated with information on the backup rater.

- Section 13.1.2, Slow Vital Capacity, was updated to note that at least 3 acceptable tests with the 2 highest acceptable (largest and next largest) efforts within 150 mL of vital capacity are required for SVC testing. This change also affects Section 4.2

(Table 3; footnote 10), Schedule of Activities: Part C (Pivotal) and Section 8.2.1, Inclusion Criteria (#4).

[redacted]

- Section 14.1, Clinical Safety Assessments, was updated to include information on the limited neurological examinations (including MMSE) to be assessed during Part C of the study. Clarification was also added regarding use of paper for 12-lead ECGs.

- Section 8.2.1, Inclusion Criteria, and Section 15.4.1, Pregnancy, were updated to clarify that all women of child bearing potential must not become pregnant during the study or 5 months after the last dose of study treatment.

- Section 16.2, Part C, was updated to include a definition for the overall intent-to-treat (ITT) population. In Section 16.2.1.1, Analysis Population, the definition of the modified-intent-to-treat (mITT) population was updated due to the change in the primary endpoint from ALSFRS-R slope and corresponding slope analysis to the change from baseline in ALSFRS-R using joint rank methodology for analysis. It was also clarified in Sections 16.2.1.1, 16.2.3.1, and 16.2.5.1 that the mITT population will be the primary analysis set.

- A minor update was made in Section 16.2.4.2.2, Clinical Laboratory Results, for presentation of results.

- A minor update made in Section 16.2.5.2, to update the method of analysis for subject-reported outcomes on quality of life.

- Clarification was added for Week 28 visit (EOS visit) and Week 32 visit (safety follow-up visit) throughout the protocol.

- Telephone call was updated to telephone contact throughout the protocol.

- The role of the Safety and Benefit Risk Management (SABR) physician was updated to Global Safety Physician throughout the protocol.

- Clarification was added throughout the protocol for randomization stratification factors for the use of edaravone and riluzole, stating that baseline values will be used for stratification.

- Clarification was added throughout the protocol that home visit/assessments may be allowed at the discretion of the Investigator.

- Typographical errors and formatting were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

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

Version 5

Date: 20 December 2018

EUDRA CT: 2015-004098-33

Version 5 of the protocol has been prepared for this amendment, which supersedes Version 4.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 233AS101 is to include a third part (Part C [Pivotal]) of approximately 60 subjects (2:1 randomization ratio of BIIB067:placebo) to assess the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of BIIB067 at 100 mg. Part C will be conducted over a 4-week screening period, 24-week treatment period and 4-week follow-up period. This inclusion of Part C also warrants a change in the phase of development of the study, the title of the protocol to accurately reflect study design and the objectives and endpoints.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 5.4, Study Rationale

Now reads:

Parts A (SAD) and B (MAD)

Pharmacology and toxicology data in rats, mice, and monkeys indicate that BIIB067 is well tolerated. ~~This Phase 1~~**Parts A and B of the** study will evaluate the safety, tolerability, and PK profile of a range of single and multiple intrathecal BIIB067 doses from a projected minimal pharmacologic dose to either the maximum tolerated dose (MTD) or the highest planned dose of 100 mg. MTD for this study is defined as the highest tested dose with a less than 33% dose-limiting toxicity (DLT) rate at Day 15. See Section 7.2.3 for the definition of DLT.

Part C (Pivotal)

Interim analyses of data from subjects in Part B (MAD) Cohorts 5 to 8 treated for 85 days were conducted. Safety analyses of these data suggested that all doses through 100 mg had been well tolerated with a safety profile supportive of continued development of BIIB067 in ALS subjects. Preliminary and exploratory analyses of clinical function data (ALSFRS-R, vital capacity, and muscle strength) further suggested possible emergence of early clinical efficacy signals. Based on these emerging data from Parts A and B, a randomized, double-blind, placebo-controlled, pivotal phase (Part C) is designed to assess the efficacy and safety of BIIB067 100 mg versus placebo. Approximately 60 subjects with SOD1-ALS will be enrolled and randomized to receive BIIB067 or placebo and treated for 24 weeks.

Rationale: The interim analyses of Study 233AS101 were based on treated subjects in multiple ascending dose Cohorts 5 to 8 treated for 85 days (pending review of 100 mg data for safety and efficacy). Safety analyses of these data suggested that all doses through 100 mg had been well tolerated with a safety profile supportive of continued development of BIIB067 in amyotrophic lateral sclerosis (ALS) subjects. Preliminary and exploratory analyses of clinical function data (Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised [ALSFRS-R], vital capacity, muscle strength) further suggest possible emergence of clinical effect. In order to further assess the treatment, 60 subjects with superoxide dismutase 1 (SOD1)-ALS will be enrolled and randomized to BIIB067 or placebo and treated for 24 weeks duration.

This change also affects the phase of development; the title of the protocol; Section 3, Synopsis; Section 4, Study Schematic and Schedule of Activities for Study 233AS101; Section 4.1, Study

Schematic (Figure 3); Section 4.2, Schedule of Activities (Table 3); Section 5.5, Rationale for Dosing Regimen; Section 6.2, Part C (Pivotal); Section 7.1, Study Overview; Section 7.1.3, Part C: Pivotal; Section 7.2.2.3, Part C; Section 7.3.1.3, Part C; Section 7.3.2.3, Part C: Pivotal; Section 7.3.3.3, Part C: Pivotal; Section 7.4.2.2, Part C; Section 7.5, End of Study; Section 8.2, Part C (Pivotal); Section 9.1, Screening and Enrollment; Section 9.2, Randomization and Registration of Subjects; Section 10.1, Withdrawal from the Study (Part A) or Discontinuation of Study Treatment (Parts B and C); Section 11.1.1, BIIB067; Section 11.3.1, Concomitant Therapy; Section 11.3.1.1, Allowed Concomitant Therapy; Section 12.1, BIIB067; Section 12.2, Placebo Product; Section 13.1.3, Subject Diary (Part C only); Section 13.1.5, [redacted] Section 13.3, Pharmacodynamic Assessments; Section 16.2, Part C; Section 19.2.2, Independent Data Monitoring Committee; and Section 19.2.3, Endpoint Adjudication Committee, Section 21, Signed Agreement of the Study Protocol.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Title Page

Change: The phase of development and protocol title were updated to accurately reflect the addition of Part C (Pivotal).

Now reads:

PHASE OF DEVELOPMENT: 1**/2/3**

PROTOCOL TITLE: A ~~Phase 1, Placebo-Controlled, Single and Multiple Ascending Dose~~ Study to Evaluate the **Efficacy**, Safety, Tolerability, ~~and~~ Pharmacokinetics, **and Pharmacodynamics** of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis **and Confirmed Superoxide Dismutase 1 Mutation**

Rationale: The primary objective of Part C (Pivotal) is efficacy; therefore, the overall phase of development of the study and the title of the study were changed to reflect this new objective and related endpoints.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 5.3.2, Clinical Experience

Change: Added that additional information on the safety, pharmacokinetics, and pharmacodynamics for BIIB067 can be found in the Investigator's Brochure.

Now reads:

This is the first-in-human (FIH) study of BIIB067; ~~therefore, no clinical experience is available to date.~~ **preliminary safety, PK, and pharmacodynamic (PD) information for BIIB067 can be found in the Investigator's Brochure.**

…

Rationale:

Based on data analysis from Parts A and B of the study, the Investigator's Brochure has been updated to reflect the current safety, pharmacokinetic, and pharmacodynamic information for BIIB067.

Section 6, Study Objectives and Endpoints

Change: Clarified that the primary objective of Parts A and B was to evaluate the safety, tolerability, and pharmacokinetics of BIIB067 in adult subjects with ALS and confirmed SOD1 mutation.

Now reads:

6.1. Parts A (SAD) and B (MAD)

~~6.1.~~ **6.1.1.** Primary Objective ~~and Endpoints~~

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

6.1.1.1. Primary Endpoints

…

6.1.21. Secondary Endpoints

…

Rationale: Subjects must have both ALS and confirmed SOD1 mutation in order to be enrolled into any part of the study.

Section 7.1, Study Overview

Change: Addition of Japan as potential location for sites.

Now reads:

…

The study will be conducted at approximately ~~17~~**18** sites in the United States, Canada, **Japan,** and Western Europe.

…

Rationale: Adding Japan as an additional country for potential sites in order to enable eventual filing for Japan.

Section 8.2.1, Inclusion Criteria

Note: all the inclusion exclusion criteria of Part A and Part B of the study are applicable to Part C other than the changes mentioned below.

Change: Inclusion criterion 3 was updated to clarify inclusion of subjects with both non-fast and fast progression of disease.

Now reads:

3. Weakness attributable to ALS and confirmed SOD1 mutation at Screening ~~Visit 2~~ **Visit**. **Additionally:**

- **Fast progressor criteria:**
    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)**
- **Non-fast progressor criterion: SOD1 mutation other than those listed in item 'a.' (no ALSFRS-R decline slope requirement).**

Rationale: The rate of ALS progression can vary widely across both non-genetic and SOD1 ALS. In order to account for non-fast and fast progressor subjects, specific SOD1 mutations and pre-slope criteria have been included.

Change: Inclusion criterion 4, a slow vital capacity (SVC) of ≥65% of predicted value for fast progressors adjusted for sex, age, and height (from sitting position), was added as an inclusion criterion. SVC≥50% for non-fast progressors was also added.

Now reads:

4. **For <u>fast progressors</u>, SVC ≥65% of predicted value** as adjusted for sex, age, and height (from the sitting position). Subjects with stable FVC <50% but ≥45%, whose FVC has not declined by more than 5% in the last 6 months may be considered for inclusion, at the discretion of the Investigator **For <u>non-fast progressors</u>, SVC ≥50% of predicted value as adjusted for sex, age, and height (from the sitting position).**

Rationale: In Parts A and B of this study, criterion for inclusion was forced vital capacity (FVC) ≥50% and study duration was 3 months. In Part C of the study, the duration of treatment is 6 months and thus in order to improve the probability of survival through 6 months the use of SVC ≥65% instead of FVC ≥50% was added to Part C. SVC is commonly used in ALS studies and is easier for patients and sites to perform consistently.

Change: Inclusion criterion 6 permitting the use of edaravone was added as inclusion criterion as long as the subject initiated edaravone ≥60 days (2 treatment cycles) prior to Day 1 and that edaravone may not be administered on dosing days. The use of edaravone or any implanted vascular devices was removed as exclusionary criteria.

Now reads:

6. **If taking edaravone, subject must have initiated edaravone ≥60 days (2 treatment cycles) prior to Day 1 and expected to remain at that dose until the final study visit,**

unless the Investigator determines that edaravone should be discontinued for medical reasons, in which case it may not be restarted during the study. Edaravone may not be administered on dosing days of this study.

Rationale: Edaravone is now an approved medication for treatment of ALS in some regions of the world in which this study is being conducted. In order to not deprive subjects from an approved treatment, use of edaravone is allowed.

This change also affects Section 8.2.2, Exclusion Criteria.

Section 8.2.2, Exclusion Criteria

Change: Exclusion criterion 9 was removed to allow the use of vascular devices.

Now reads:

~~9. Presence of any implanted vascular devices.~~

Rationale: Edaravone is administered via implanted vascular devices, and therefore, the inclusion of edaravone as an acceptable concomitant medication warrants the use of implanted vascular devices for Part C (Pivotal).

Change: Exclusion criterion 11 was removed to allow subjects with a tracheostomy to enroll.

Now reads:

~~11. Tracheostomy.~~

Rationale: Most subjects with ALS will rely on a tracheostomy at some point, and the use of a tracheostomy will not interfere with the study drug administration and data analysis of BIIB067.

Section 13.1, ~~Exploratory~~ Assessments of the Effect of BIBB067 on Clinical Function

Change: Text was included to allow subjects to have a home visit if they are unable to travel to the site.

Now reads:

…

All study visits are expected to occur at the site unless the subject is unable to travel to the site then a home visit may be possible with Investigator approval.

Rationale: In order to relieve the burden on subjects and continue to collect data, subjects who are unable to travel to the site may be able to have a home visit.

Section 13.1.1, ALS Functional Rating Scale-Revised

Change: Text was clarified to state that a dedicated, blinded staff member will perform the ALSFRS-R for a subject.

Now reads:

…

At each study site, the same qualified and trained study site staff member will consistently perform the ALSFRS-R for a subject. This study site staff member will remain blinded to subjects' treatment assignments and to the results of other assessments.

Rationale: The use of a dedicated staff member will help to ensure that the blind is maintained and reduce the risk of breaking the blind.

[redacted]

Change: [redacted]

Now reads:

[redacted]

Rationale: [redacted]

Section 15.5, Contraception Requirements

Change: Contraception requirements were updated.

Now reads:

All women of childbearing potential ~~and all men~~ must ~~practice~~ **ensure that** effective contraception **is used** during the study and for 5 months after their last dose of study treatment. In addition, ~~male~~ **female** subjects should not donate ~~sperm~~ **eggs** for the duration of the study and for at least 5 months after their last dose of study treatment.

For the purposes of this study, women who do not meet one of the following criteria ~~listed below~~ are considered to be physiologically capable of becoming pregnant and are, therefore, defined as women of childbearing potential:

- Postmenopausal

  - 12 **continuous** months of natural (spontaneous) amenorrhea without an alternative medical cause and a serum follicle-stimulating hormone level >40 mIU/mL
  - 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Posthysterectomy
  - Female surgical sterilization (e.g., bilateral tubal ligation)

For the purposes of the study, ~~highly~~ effective contraception is defined as use of **more than 1** of the following:

For females:

- Established use of oral, injected, ~~or~~ implanted**, intravaginal, or transdermal** hormonal methods of contraception.
- Placement of an intrauterine device or intrauterine **hormone-releasing** system.
- ~~Male~~ Barrier methods of contraception with use of a spermicide: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide.
- **Sex with a male who has undergone** surgical sterilization (with the appropriate postvasectomy documentation of the absence of sperm in the ejaculate). ~~(For female subjects participating in the study, male sexual partners must have undergone surgical sterilization.)~~

~~For males:~~

~~A vasectomy with negative semen and analysis at follow up.~~

- True abstinence, when this is consistent with the preferred and usual lifestyle of the subject, can be considered an acceptable method of contraception based on the evaluation of the Investigator who should also take into consideration the duration of the clinical trial. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not considered acceptable methods of contraception.

Pregnancy reporting is described in Section 15.4.1.

Rationale: Contraception requirements were updated to reflect current accepted contraception practices in the parts of the world that the study is being conducted.

Section 17.4, Subject Data Protection

Change: Added text to state that the race and ethnicity of the subjects will be collected unless prohibited by local law.

Now reads:

…

During the study, subjects' race and ethnicity will be collected (unless the collection is not permitted by applicable law or not approved by the governing ethics committee). These data will be used in the analysis of the safety and/or PK profile of the study treatment. It is unknown whether the effects of the study treatment are influenced by race or ethnicity.

…

Rationale: The race and ethnicity of the subjects are important to capture in order to assess any trends in pharmacokinetic data that may be related to race and/or ethnicity.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Clarifications were made throughout the protocol in reference to whether information pertained to Parts A and/or B.
- The Sponsor signatory was updated.
- Section 2, List of Abbreviations, was updated.
- Section 5, Introduction, a description of the mechanism of action information for BIIB067 was added from the Investigator's Brochure.
- Section 8.1.2, Exclusion Criteria; Section 8.2.2, Exclusion Criteria, and Section 11.3.1.2, Disallowed Concomitant Therapy, aspirin was removed as an excluded concomitant medication.
- Section 8.1.2, Exclusion Criteria and Section 8.2.2, Exclusion Criteria, hepatitis B serololigical test abbreviations updated.
- Section 9.3, Blinding Procedures, text was added describing end of study unblinding procedures.
- Section 14.2, Laboratory Safety Assessments, laboratory assessments were updated for clarity.
- Section 15.1.2, Serious Adverse Event, text was updated to classify a medically important event as a serious adverse event (SAE).
- Section 15.3.1, Adverse Events, text was added to clarify that at each study visit, the Investigator will assess the subject's adverse events (AEs) and record any new AEs or updates to previously reported AEs.
- Section 15.3.3, Immediate Reporting of Serious Adverse Events, "Reporting Information for SAEs" text was updated to remove text stating that any SAE between consent and last follow-up visit must be reported within 24 hours, and that thereafter, events should only be reported if related to study treatment.
- Section 15.4.1, Pregnancy, text was added to clarify that a pregnancy is not considered an AE and should not be recorded as such and that pregnancy can also be reported via email.
- Sections 15.4.2, Overdose, and Section 15.6.1, The Investigator, text was added indicating that overdose and SAEs may be reported by email.
- Section 16.1.4.2, Methods of Analysis, p75 was added as a potential biomarker for analysis.
- Section 19.1.4, Laboratory Assessments, text added to clarify that serum pregnancy tests will be analyzed at a central laboratory.

- The vendor information was updated to Sponsor information throughout (changed from [redacted] to “Biogen”).
- New references were added to the reference list.
- Typographical errors and formatting were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

A Phase 1, Placebo-Controlled, Single and Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis

Version 4

Date: 29 November 2017

EUDRA CT: 2015-004098-33

Version 4 of the protocol has been prepared for this amendment, which supersedes Version 3.

PRIMARY REASONS FOR AMENDMENT

The primary reasons for this amendment to Protocol 233AS101 are to 1) add an 8th treatment cohort to assess the safety and tolerability of up to 5 doses of BIB067 100 mg, and 2) include the option of an interim analysis during Part B (multiple-ascending-dose portion) of the study.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 7.1.2, Part B: Multiple-Ascending-Dose Evaluation

Now reads:

Part B will be a randomized, double-blind, placebo-controlled, MAD evaluation of up to ~~3~~**4** dose levels of BIIB067 administered up to 5 times, over approximately 3 months, to up to ~~36~~ **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, ~~or~~ 60**, or 100** mg.

Rationale:

Achieving maximum efficacy in the treatment of superoxide dismutase 1 (SOD1) amyotrophic lateral sclerosis (ALS) is likely dependent on reducing the levels of the toxic, mutant version of SOD1 throughout the central nervous system (CNS) – including the brain (motor cortex) and spinal cord. Emerging preliminary pharmacokinetic (PK) data from the ongoing trial indicated that the exposure to BIIB067 was less than that predicted preclinically. The purpose of adding a higher dose cohort (100 mg) to the trial is to enable a higher exposure to BIIB067, which would more effectively reduce SOD1 in the CNS, and is supported by the safety margins from the 9-month nonhuman primate (NHP) study.

This change also affects Sections 5.4, Study Rationale; 7.1, Study Overview; 7.2.1.2, Part B: Multiple Ascending Dose; Figure 3, Dose Escalation Schematic; 9.2, Randomization and Registration of Subjects; 11.1.1, BIIB067; and 16.8, Sample Size Considerations.

Section 16.7, Interim Analysis

Now reads: In Part A (SAD) of the study, CRM will be used as supporting evidence for continuous assessment of MTD for dose escalation decisions. ~~No formal interim analysis will be performed in Part B (MAD) of the study.~~ **In Part B (MAD) of the study, in addition to the safety review (blinded), a designated team of staff will review the available unblinded but de-identified PD and/or biomarker data (eg., levels of SOD-1 and p-NFH) from all previous cohorts at the completion of the last Day 106 visit of each cohort to determine whether to proceed with the subsequent planned dose cohorts. An interim analysis of key safety, secondary and exploratory endpoints may be conducted based on data up to the 60-mg and/or 100-mg dose cohort to allow for the planning of the pivotal efficacy and safety study. The subjects, investigative staff, and sponsor's staff responsible for site monitoring and data management will remain blinded to treatment assignment during the ongoing data review, as well as during interim analyses, to minimize the potential for bias in the data cleaning process. Details of the interim analysis will be provided in a separate statistical analysis plan.**

Rationale: The implementation of an interim analysis during Part B of the study would allow for the timely determination of a possible effect on key biomarkers, thereby facilitating an earlier transition to pivotal studies without compromising overall study design.

This change also affects Section 7.2.1.2, Multiple Ascending Dose.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

The major changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 5.2, Current Therapies for Amyotrophic Lateral Sclerosis

Change: Included information on edaravone.

Now reads:

The only currently approved treatment**s** for ALS **are** ~~is Rilutek® (~~riluzole **(Rilutek®), which is marketed globally**, **and edaravone (Radicava™),** which **is approved in the United States, Japan, and South Korea. Riluzole** provides a modest increase in survival (2 to 3 months) without noticeable improvement in strength or disability [Miller 2012]. **Edaravone lessens functional decline as measured by the Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised (ALSFRS-R). The effect of edaravone on survival is unknown [Writing Group and Edaravone (MCI-186) ALS 19 Study Group 2017].** No specific SOD1 ALS treatments are available.

Rationale: Edaravone was recently approved as a treatment for ALS; this change updates the information on current therapies for ALS.

Section 5.3.1.1, Toxicology

Change: Added description of a 9-month nonclinical toxicology study in cynomolgus monkeys (NHPs).

Now reads:

From the 9-month cynomolgus monkey toxicology study, the NOAEL for repeated, intrathecally-administered BIIB067 was 12 mg. This was based on adverse clinical observations for 1 female administered 35 mg. This female exhibited neurological signs after the second dose, characterized by transient muscle cramping (seen immediately after dosing on multiple days), prolonged recovery from anesthesia, and intermittent tremors (during the last months of the dosing phase). Treatment with diazepam was required on several dosing occasions. An electroencephalogram (EEG) on this animal revealed altered postdose signals (with effects on high frequency bands) but confirmed that muscle cramping during EEG recording was not a seizure and could have been related to arousal. This finding was considered test-article related; however, there were no correlates in clinical and anatomic pathology.

Rationale: A long-term nonclinical toxicology study was recently completed to support clinical development of BIIB067, and the data generated in NHPs were used to update the dose rationale in this first-in-human study of BIIB067.

This change also affects Section 5.3.1, Nonclinical Experience.

Section 5.5, Rationale for Dosing Regimen

Change: Updated the rationale for dosing regimen to include the 100-mg dose of BIIB067.

Now reads:

BIIB067 will be administered via intrathecal bolus at single ascending doses (SAD) to cohorts in Part A, and at multiple ascending doses (MAD) to cohorts in Part B.

The BIIB067 dosing regimens for this study were selected based on nonclinical toxicology and PK observations in nonhuman primate (NHP) studies using repeated-dosing intrathecal administration for 13 weeks **and 9 months**, and target tissue concentrations from SOD1 transgenic mouse models.

Based on pharmacology and PK results in SOD1 transgenic mice, the estimated tissue concentrations of BIIB067 needed to produce a 50%, and the upper 95% confidence interval of an 80%, SOD1 mRNA reduction within the human spinal cord are 0.9 µg/g and 4.7 µg/g, respectively. **Evaluations of cortex from the same experiment indicate that the estimated tissue concentration of BIIB067 needed to produce a 50% cortical SOD1 mRNA reduction is 8 µg/g.**

The lowest dose selected for this study (10 mg) is predicted to achieve greater than 0.9 µg/g tissue concentration in the spinal cord**.** ~~and cortex, while t~~**T**he **initial** highest proposed dose (60 mg, **multiple dose**) ~~is~~**was** predicted to achieve greater than 4.7 µg/g **steady-state** tissue concentration in the spinal cord**,** and **approximately 1.5 µg/g steady-state tissue concentration in the** cortex, **which is expected to yield approximately 15% to 20% SOD1 mRNA reduction in that tissue**. ~~These tissue concentrations are predicted to span the pharmacologically active range.~~ **The addition of a 100-mg cohort is predicted to achieve steady-state exposures in the cortex of approximately 2.5 µg/g, which is predicted to be sufficient for a meaningful CSF SOD1 reduction of 25% to 30%. This reduction is expected to be clinically meaningful, based on the expectation that the reduction of SOD1 in tissues (spinal cord and cortex) would be greater than the 20% to 30% reduction observed in CSF. This prediction is based on observations in NHP studies, where approximately 50% reduction in CSF corresponded to approximately 50% or greater reduction in the spinal cord and cortex. In rodent efficacy experiments, doses of BIIB067 that reduced tissue SOD1 by approximately 30% or more were found to improve measures of electrophysiology, function, and neurofilament.**

Based on nonclinical PK and pharmacology data, and taking into consideration subject safety, the inconvenience of repeated intrathecal injections, and the rapid, fatal nature of ALS, the following dose intervals were selected for Part B of the study (MAD): 3 loading doses, once every 2 weeks, and 2 maintenance doses, administered once every 4 weeks. These intervals were selected based on the estimated t½ of BIIB067 (~1 month) in the target CNS tissues (spinal cord and brain cortex), to achieve and maintain the target tissue concentration of BIIB067 at a steady state level and within the estimated pharmacologically active range, and to facilitate effective SOD1 protein reduction in the CSF within Part B of the study (MAD).

From the ~~13~~**9-** ~~week~~**month** NHP toxicology study, the NOAEL for repeated, intrathecal**ly**-administered BIIB067 was ~~35~~ **12** mg. ~~No clinical or histologic observations were deemed~~

~~adverse in this study.~~ This NOAEL was converted to a human equivalent dose (HED) based on the ~~nonclinical species~~ **NHP** to human CSF volume scaling **(approximately 10-fold difference)**. CSF volume scaling conservatively estimates the needed scaling factor and has predicted HED with reasonable accuracy. The HED for the ~~13~~**9-**~~week~~**month**, intrathecal NHP toxicology study was calculated to be ~~350~~ **120** mg. This provides a ~~35~~**12**-fold safety margin for the BIIB067 starting dose (10 mg), and a ~~5.8~~**2**-fold safety margin for the ~~highest planned dose (60~~ **60-**mg **dose**~~)~~. **The safety margin for a 100-mg clinical dose (highest planned dose) would be 1.2-fold. Preliminary PK data (plasma area under the concentration-time curve [AUC] from time 0 to 24 hours) from the 20-mg MAD cohort indicate that the safety margin based on exposure is 2.2-fold relative to a 100-mg clinical dose. The 1.2-fold safety margin based on CSF volume scaling from the NHP toxicology study was chosen over the 2.2-fold safety margin calculated from the plasma steady-state exposures since the former provides a more conservative estimate.**

Therefore, all BIIB067 doses planned for 233AS101 are predicted to be pharmacologically active, while maintaining sufficient safety margins calculated from the ~~13~~**9-**~~week~~**month** intrathecal NHP toxicology study.

Rationale: This change was made based on BIIB067 exposure predictions related to addition of the 100-mg dose and updated information on the 9-month NHP toxicology study. The human equivalent dose and the safety margin relative to the new highest planned dose of 100 mg (Cohort 8) were also included.

Section 7.2.2.2, Multiple Ascending Dose

Change: Added details of within cohort staggering for Cohort 8.

Now reads:

Within ~~each~~cohort**s 5-7** in Part B, subjects will be randomized in blocks of 4 to receive BIIB067 or placebo in a 3:1 (active treatment:placebo) ratio. The 3 blocks of each cohort will be dosed sequentially, each followed by a 72-hour safety data review period, during which the Biogen Medical Director and SABR Physician will review blinded safety data with the PI.

Given that safety and tolerability of the 100-mg dose of BIIB067 were not explored in Part A (SAD), both a sentinel dosing strategy and a review of safety data for every subject after the first dose will be incorporated into Cohort 8.

In Cohort 8, the first 2 subjects (1 subject receiving placebo and 1 subject receiving BIIB067) will be dosed as a sentinel group, followed by a 72-hour safety data review period, during which the Biogen Medical Director and SABR Physician will review blinded safety data with the PI. For the remaining subjects in the cohort, no more than 2 subjects will be given the first dose of study treatment on the same day throughout the study.

Cohort 8 follows the same loading dose/maintenance dose as the other MAD cohorts. While a 100-mg dose has not been administered in the SAD portion of the study, the safety data collected between week 2 and week 4 postdose (or even week 1 and week 4 postdose) did not yield any additional information when compared with safety data collected for the first 2 weeks postdose. This justifies the absence of a 4-week safety observation period between doses 1 and 2.

Additional to the 72-hour safety review period, for every subject in Cohort 8, a review of all available safety and tolerability data will be performed approximately 1 week after the first dose is administered. This review will be performed by the Biogen Medical Director and SABR Physician with the PI. The second dose cannot be given until this review is complete.

Rationale: These changes were made to ensure safety evaluation of a single dose of BIIB067 100 mg before proceeding with multiple dosing, as this dose level was not evaluated in the single ascending dose portion of this study.

Section 7.3.3.2, Part B: Multiple Ascending Dose

Change: To include the option of home visits.

Now reads:

Subjects will return to the study site for follow-up visits after each dose on Day 8 (after first dose), Day 22 (after second dose), Day 36 (after third dose), Day 64 (after fourth dose), Days 92 and 106 (after fifth dose), and on the final study visit on Day 169. On the days of CSF sampling (Day 106 and Day 169), subjects will remain under observation for approximately 1 hour after the procedure and will receive a safety follow-up telephone call approximately 24 hours after the procedure. Subjects will also receive follow-up telephone calls on Day 50 (after third dose) and Day 78 (after fourth dose) to provide information on AEs, SAEs, and concomitant medications or procedures. **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.**

Rationale: The Sponsor has a contract with Global Care to provide home visits; however, this option has not been used as the language was missing from the protocol.

This change also affects Section 4.1, Study Schematic: Figure 2; and Section 4.2, Schedule of Activities: Table 2, footnote 3.

Section 7.4.1, Dose Suspension

Change: Updated language related to managing a serious adverse event (SAE) or a clinically significant adverse event (AE).

Now reads:

If 1 subject experiences either an SAE or a clinically significant AE (as defined by the Investigator or the Sponsor), **the Biogen Medical Director and SABR Physician will discuss the event with the Investigator and review the SAE form (if applicable). If the AE is assessed as unrelated to the study treatment by both the Investigator and the Sponsor (e.g., it is a known sign or symptom of ALS, or it is an effect of the LP procedure), dose suspension is not necessary.**

If the SAE or clinically significant AE (as determined by the Investigator or the Sponsor) is assessed as related to the study treatment by the Investigator or the Sponsor, all dosing will be suspended until the event has been fully evaluated~~, including for potential relatedness to study drug,~~ by the Biogen Medical Director, SABR Physician, ~~Biostatistician, Clinical Pharmacologist,~~ and, if required, any ad hoc members. Depending on the nature, severity, suspected on- or off-

target toxicity, outcome, and frequency of the event, a decision (documented with supporting rationale) will be made to proceed with one of the following:

- Request additional safety data
- Continue dosing remaining subjects within the cohort
- Enroll additional subjects to the current cohort
- Proceed with the planned dose escalation in the next cohort
- Enroll additional subjects to previous (lower-dose) cohort
- Stop the study

Rationale: This update was included to prevent any unnecessary dose suspensions for events clearly not related to the study treatment.

Section 7.4.2, Dose Termination

Change: Updated dose termination criteria in case of recurrent SAEs or AEs. Also updated the individuals evaluating safety, tolerability, and PK data.

Now reads:

After evaluation of safety, tolerability, and PK data by the Biogen Medical Director, SABR Physician, ~~Biostatistician, Clinical Pharmacologist,~~ and, if required, any ad hoc members, further dosing at the current level and dose escalation will be terminated if any of the following is observed:

- **Two similar SAEs (unless clearly unrelated to BIIB067 upon medical review) are reported for 2 subjects on active study treatment within the same cohort**~~Current dose is not deemed to be safe~~**.**

- **Three or more similar AEs (unless clearly unrelated to BIIB067 upon medical review) that are either not tolerable (as reported by the subjects) or deemed a medically unacceptable risk by the Biogen Medical Director, SABR Physician, and, if required, any ad hoc members.**

- Sponsor requests that dosing be terminated.

Rationale: This change was made to explain the situations that could trigger dose termination if the dose administered is not deemed safe for the subjects.

Section 8.2, Exclusion Criteria

Change: Updated hepatitis C- and B-related exclusion criteria.

Now reads:

2. History of, or positive test result **at Screening**, for hepatitis C virus antibody**.**

~~2.~~3. **Current hepatitis B infection (defined as positive for hepatitis B surface antigen [HBsAg] and/or hepatitis B core antibody [HBcAb]). Subjects with immunity to hepatitis B from previous natural infection (defined as negative HBsAg, positive hepatitis B surface antibody immunoglobulin G, and positive HBcAb) or vaccination (defined as positive anti-**

HBs) are eligible to participate in the study~~or hepatitis B virus (defined as positive for both hepatitis B surface antigen and hepatitis B core antibody)~~.

Rationale: This update is to clarify the timing for the hepatitis C antibody test and to provide a more detailed definition for hepatitis B exclusion criteria.

Change: Included the use of edaravone as a criterion for exclusion from the study.

Now reads:

8. Current or recent use (within 30 hours prior to screening), or anticipated need of edaravone (Radicava).

Rationale: Edaravone was recently approved as a treatment for ALS in the United States, and although its possible effect on various exploratory ALS biomarkers such as neurofilament or SOD1 clinically has not been reported, nonclinical models showed that it reduced SOD1 levels in rodent spinal cord. The use of edaravone was included as a criterion for exclusion from the study to avoid the interference from any of its potential clinical effects.

Change: Included the presence of implanted vascular devices as a criterion for exclusion from the study.

Now reads:

9. Presence of any implanted vascular devices.

Rationale: This change was made to avoid associated risks, including infection.

Section 9.1, Screening and Enrollment

Change: To permit rescreening of subjects who met eligibility but were not dosed within the 28-day screening window.

Now reads:

Subjects (or their legally authorized representative [e.g., spouse], where applicable) must provide informed consent before any screening tests are performed (see Section 17.3). When a subject signs the informed consent form (ICF), that subject is considered to be enrolled in the study. Participating study sites are required to document all screened candidates initially considered for inclusion in this study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and on the screening log.

Eligible subjects who are not able to complete the Day 1 Visit within 28 days of their screening assessments (Screening Visit 2) may be rescreened.

Rationale: This change may help reduce the impact of an expired screening window on recruitment time and allow the team to include subjects who would otherwise be eligible for the study but for the screening window expiration.

14.1, Clinical Safety Assessments

Change: Included a list of the type of neurological examinations to be conducted at the sites.

Now reads:

- **Limited** ~~N~~**n**eurological examinations (to be assessed by a trained specialist) **of cranial nerves, coordination/cerebellar function, reflexes, motor, and MMSE (Mini-Mental State Examination – a 30-point questionnaire that is used to measure cognitive impairment; in Part B only)**~~. In Part B of the study, the neurological examination will include the Mini-Mental State Examination (MMSE) – a 30-point questionnaire that is used to measure cognitive impairment~~

Rationale: This change will eliminate confusion and enable consistency in the type of neurological examinations performed across all sites conducting the study.

This change also affects Sections 4.2, Schedule of Activities: Table 2; and 16.5.2.6, Limited Neurological Examinations.

14.2, Laboratory Safety Assessments

Change: To separate coagulation from other hematology assessments and to exclude total cell count from CSF analysis.

Now reads:

- Hematology: complete blood count with differential, platelet count, and absolute neutrophil count.~~ INR, PT, and APTT will also be measured~~
- **Coagulation: INR, PT, and APTT**
- Blood chemistry: electrolytes, total protein, albumin, creatinine, blood urea nitrogen, uric acid, bilirubin (total and direct), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, glucose, calcium, phosphorus, bicarbonate, chloride, sodium, and potassium
- Urinalysis: dipstick for blood, protein, and glucose (microscopic examination, if abnormal)
- Urine pregnancy tests
- CSF analysis: ~~total cell count,~~ red blood cell count, white blood cell count, protein, and glucose

Rationale: These changes were made for clarity, as coagulation is not part of the standard hematology assessments and the total cell count is not required for standard CSF analysis.

This change also affects Sections 4.2, Schedule of Activities, Table 2; and 16.5.2.2, Clinical Laboratory Results.

17.3, Subject Information and Consent

Change: Included update that subjects will be informed about the collection of sensitive data (race and ethnicity).

Now reads:

Subjects will be informed, where applicable, that their race and ethnicity will be collected during the study (unless the collection is not permitted by applicable law or not approved

by the governing ethics committee) and the data will be used during analysis of study results.

Rationale: This update was made to keep subjects informed about the collection of sensitive data

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Sponsor address in the United States was updated.
- The List of Abbreviations was updated.
- Section 4.1, Study Schematic, Figures 1 and 2 were replaced to correct minor errors.
- Section 9.3, Blinding Procedures, was updated for consistency in the description of safety review blinding.
- Use of abbreviation only (vs. spelled-out term) was fixed in Section 13.2, Pharmacokinetic Assessments.
- [redacted]
- A new reference was added to the list.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

A Phase 1, Placebo-Controlled, Single and Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis

Version 3

Date: 17 November 2016

EUDRA CT 2015-004098-33

Version 3 of the protocol has been prepared for this amendment, which supersedes Version 2.

PRIMARY REASON FOR AMENDMENT

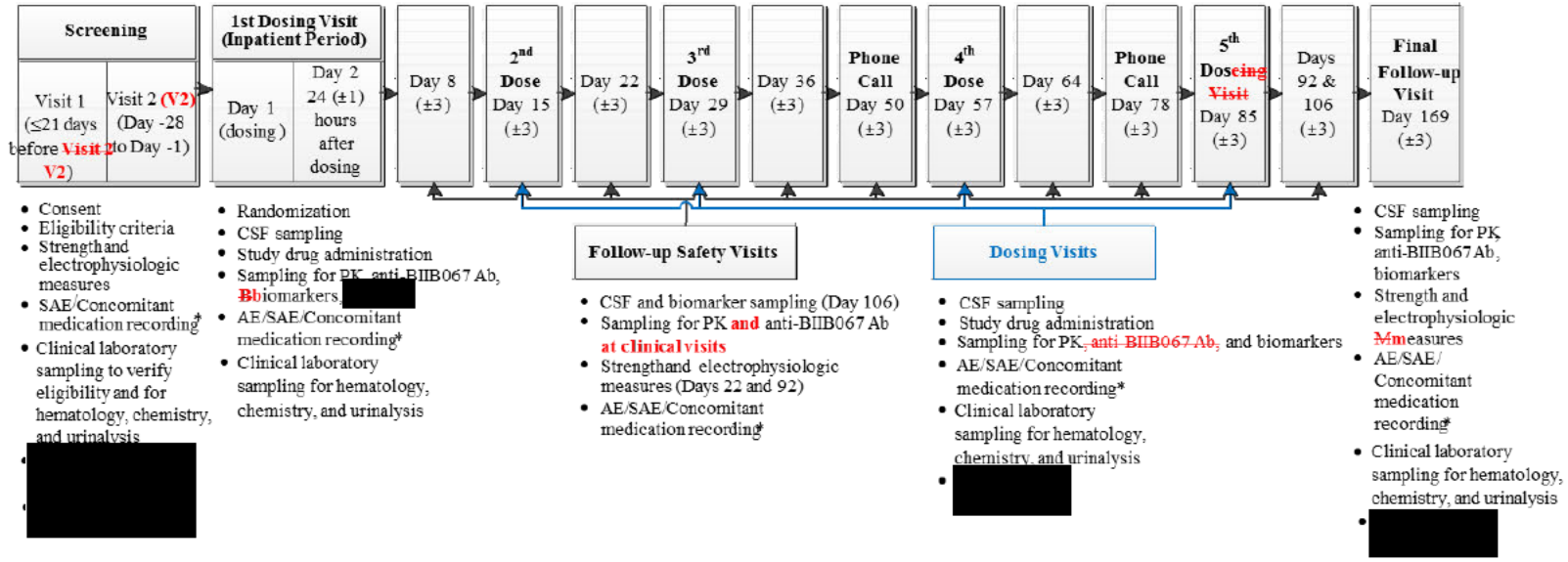
The primary reason for this amendment to Protocol 233AS101 is to correctly reflect in Figure 2 of the protocol that sampling for anti-BIIB067 antibody (Ab) will occur during nondosing clinical visits (except for predose on Day 1).

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4.1, Study Schematic (Figure 2: Study Design: Part B – Multiple Ascending Dose)

Now reads:

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



Ab = antibody; AE = adverse event; CSF = cerebrospinal fluid; ; ;
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.

Rationale:

The schematic for Part B incorrectly lists collection of anti-BIIB067 Ab samples under dosing visits for doses 2 through 5. The sampling for anti-BIIB067 Ab should occur at all clinical follow-up visits, but not on the day of dosing per the Schedule of Events table. Currently, Figure 2 indicates that sampling for anti-BIIB067 Ab occurs at the Follow-up Safety Visits and Dosing Visits.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4.2, Schedule of Activities (Table 2: Schedule of Activities: Part B – Multiple Ascending Dose)

Change: Table 2 Schedule of Activities: Part B – Multiple Ascending Dose was updated to add the collection of height at Visit 2.

Now reads:

Table 2: Schedule of Activities: Part B – Multiple Ascending Dose

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, 22, 36, 64, 92, and 106 (±3 days)	Days 50 & 78 (±3 days) via Telephone	Day 169 (±3 days) /Early Termination Visit
			Predose	Dosing/ Postdose					
Physical Examination		X	X		X[5]	X[5]	X[5]		X[5]
Weight		X	X						X
Height		**X**							

Rationale:

Height was being collected in Part A – Single Ascending Dose phase of the study and was not being collected in Part B – Multiple Ascending Dose phase. Collection of height was added to Part B to align both components of the study.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Minor editorial changes were made as appropriate (e.g., addition of abbreviations to figure and table footnotes).
- The sponsor signature page was updated.
- Section 4.1 Study Schematic, Figure 1 Study Design: Part A – Single Ascending Dose was updated to reflect the sampling of peripheral blood mononuclear cell at the dosing visit and the sampling of biomarkers only at Day 29 to align with Table 1.

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AMENDMENT SUMMARY

Biogen Protocol 233AS101

A Phase 1, Placebo-Controlled, Single and Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of BIIB067 Administered to Adult Subjects with Amyotrophic Lateral Sclerosis

Global Amendment Version 2

Date: 10 October 2016

EUDRA CT Number: 2015-004098-33

Version 2 of the protocol has been prepared for this amendment, which supersedes Version 1.

PRIMARY REASON FOR AMENDMENT

The primary reasons for this amendment to Protocol 233AS101 are 1) to add the review of pharmacokinetic (PK) data and clarify the review of safety data prior to each dose escalation in Part B (multiple-ascending-dose [MAD]) of the study and 2) to add the Mini-Mental State Examination (MMSE) as part of the neurological examination.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 7.2.1.2, Multiple Ascending Dose

Now reads:

After ~~the last subject~~ **a minimum of 10 subjects** within a cohort complete~~s~~ the Day 106 Follow-Up Visit (~3 weeks after last dose), and prior to dosing of the next, higher dose cohort, a Biogen Medical Director and a SABR Physician will review ~~the~~ **all available** blinded safety data with the PI. Review of blinded safety data may be followed by review of unblinded safety data of the current cohort and all available safety data from preceding cohorts by the Biogen Medical Director, SABR Physician, Biostatistician, Clinical Pharmacologist, and, if required, any ad hoc members. **In addition to review of the safety data, a Biogen Medical Director, Clinical Pharmacologist, and, if required, any ad hoc members will review PK data through the trough levels after the fourth dose (samples collected predose on Day 85) for a minimum of 6 subjects within a cohort, prior to dosing of the next, higher dose cohort.** Before escalating to the next**,** higher dose level, there must be agreement that the current emerging safety, ~~and~~ tolerability**, and PK** data support dose escalation.

Rationale: To confirm that PK data will be obtained and analyzed before transitioning to the subsequent cohort for safety reasons and to ensure the assessment of PK at a steady state.

This change also affects Figure 3: Dose Escalation Schematic.

Section 14.1, Clinical Safety Assessments

Now reads:

The following clinical assessments will be performed to evaluate the safety profile of BIIB067:

- Medical history
- Physical examinations
- Neurological examinations (to be assessed by a trained specialist)**. In Part B of the study, the neurological examination will include the Mini-Mental State Examination (MMSE) – a 30-point questionnaire that is used to measure cognitive impairment**

Rationale: The MMSE was added to assess possible changes in the subject's cognitive performance.

This change also affects Section 4.2, Schedule of Activities (Table 2: Part B – Multiple Ascending Dose).

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 6.3, Exploratory Objectives and Endpoints

Change: Questionnaires were added to the list of exploratory endpoints.

Now reads:

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)
  - ~~s~~**S**low vital capacity (SVC)~~.~~

[redacted]

Rationale: The questionnaires will enable assessment of any changes in subjects' quality of life, which is an important component of clinical function.

This change also affects Section 4.1, Study Schematic (Figure 2: Study Design: Part B – Multiple Ascending Dose); Section 4.2, Schedule of Activities (Table 2: Part B – Multiple Ascending Dose); Section 13.1, Exploratory Assessments of the Effect of BIIB067 on Clinical Function; and Section 16.1.2, Methods of Analysis.

Section 7.1.1, Part A: Single-Ascending-Dose Evaluation:

Change: Eligibility criterion for Part A (single-ascending dose [SAD]) of the study was changed to allow only those patients with amyotrophic lateral sclerosis (ALS) caused by superoxide dismutase 1 (SOD1) mutation to enter the study.

Now reads:

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 dose levels will be evaluated sequentially. Final sample size for Part A of the study will be determined by the incidence of DLT. If no DLTs are encountered, a minimum of 20 subjects will be enrolled. The

maximum sample size for Part A is 36 subjects. ~~Every attempt will be made to enroll subjects with SOD1 ALS into each dose cohort.~~ 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.

Rationale: This change was made to exclude subjects with sporadic ALS as these subjects were not expected to benefit from the treatment with the study drug.

This change also affects Section 8.1, Inclusion Criteria 3.

Section 7.3.1.1, Part A: Single-Ascending Dose

Change: The duration of Part A screening period was increased [redacted]

Now reads:

For subjects who do not have documentation of a SOD1 mutation, the screening period will involve an additional visit (Screening Visit 1), where a blood sample will be collected for deoxyribonucleic acid (DNA) analysis. The rationale for the extra screening visit is to ensure DNA analysis results prior to assessing the rest of the eligibility criteria. Subject eligibility for the study will be determined **during Screening Visit 2, up to approximately 21 days after Screening Visit 1 and** within 28 days prior to admission to the inpatient facility for dosing (Day 1). Subjects in the 2 lowest dose cohorts, who choose to re-enroll into 1 of the 2 highest dose cohorts, will have to repeat screening assessments before receiving the second dose. ~~The~~ **This** ~~second~~ **additional** Screening Visit can occur during the washout period, within 28 days prior to their second admission to the inpatient facility for dosing. [redacted].

The Screening Visit **2** assessments may be performed over 2 days, which do not need to be consecutive, to minimize subject burden. All assessments must be completed before Day 1.

Rationale: The duration of the study for Part A was increased [redacted].

[redacted]

Section 9.2, Randomization and Registration of Subjects

Change: The number of replacement subjects that could be enrolled per cohort in Part B of the study was specified.

Now reads:

Up to 6 replacement subjects may be enrolled in Part B of the study (2 per cohort). These additional subjects will be assigned to receive the same study treatment as the withdrawn subjects they will replace.

Rationale: This update was made to suggest that if subject(s) withdrew in Part B (multiple-ascending dose), and if the Sponsor decided to replace the subjects, then 2 subjects per

cohort (i.e., up to 14 subjects in each cohort) could be enrolled and would each receive the same treatment as the subject being replaced.

[redacted]

Change: [redacted]

Now reads:

[redacted]

[redacted]

Rationale: [redacted]

Section 15.5, Contraception Requirements

Change: Barrier method was removed from the list of highly effective contraception.

Now reads:

For the purposes of the study, highly effective contraception is defined as use of the following:

For females:

- Established use of oral, injected, or implanted hormonal methods of contraception.
- Placement of an intrauterine device or intrauterine system.
- ~~Barrier methods of contraception with use of a spermicide: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/cream suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide.~~
- Male surgical sterilization (with the appropriate postvasectomy documentation of the absence of sperm in the ejaculate). (For female subjects participating in the study, male sexual partners must have undergone surgical sterilization.)

For males:

- ~~Effective male contraception includes a~~**A** vasectomy with negative semen analysis at follow up~~, or the use of condoms with spermicide~~.

Rationale: It was decided that barrier method will not be considered as highly effective contraception to be compliant with requirements of European Regulatory Agencies.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- A sponsor signature page was added to the protocol.
- Section 2, List of Abbreviations, was updated to reflect changes in the protocol. This change also affects Section 17, Ethical Requirements, for the definition of International Council for Harmonisation.Section 4.2, Schedule of Activities (Table 2: Part B – Multiple Ascending Dose), footnotes were updated to indicate that the ALSFRS-R assessment [redacted] HHD will be performed on Day 22 and Day 92. A footnote was added to indicate that blood sampling for biomarkers will be collected on Day 106. The table was also updated to move the FSH assessment from Screening V1 to Screening V2.
- Section 5.3.2, Clinical Experience, the name of the company was updated from ISIS Pharmaceuticals to Ionis Pharmaceuticals to reflect the company's recent name change.
- Section 8.1, Inclusion Criterion 7, was updated to indicate that screening coagulation values should be within normal ranges. It was clarified that subjects with nonclinically significant and stable out-of-range values may be eligible to enroll in the study at the discretion of the Investigator and after a consultation with the Sponsor. This change also affects Section 4.2, Schedule of Activities, Table 1 and Table 2, Footnote 4.
- Section 8.2, Exclusion Criterion 3, was updated to clarify that subjects receiving treatment with another investigational drug, including investigational drugs for ALS through compassionate use programs, will not be eligible to participate in the study.
- Section 11.3.1.2, Disallowed Concomitant Therapy, was updated to remove memantine and coenzyme Q that were included in the protocol as examples of off-label medications for ALS treatment to avoid any confusion resulting from citing an incomplete list of medications.
- Section 13, Efficacy, Pharmacokinetic, and Pharmacodynamic Assessments, was updated to change all instances of the use of the term "efficacy" in the protocol to "clinical function" and to emphasize that the clinical function measurements will only be exploratory in nature. This change also affects Section 16.1.1, Analysis Population, wherein the "efficacy set" is termed "clinical function set."
- Section 14.2, Laboratory Safety Assessments, was updated to clarify that cerebrospinal fluid (CSF) analysis will also include red blood cell count and white blood cell count. Updates were made to clarify that platelet and coagulation tests will be collected at every visit (including screening) and analyzed centrally as safety laboratory tests for the study.

- Section 15.3.2, Serious Adverse Events, was updated to clarify that the ICF mentioned in this section refers to the first (main) ICF.
- Section 19.1.4, Laboratory Assessments, was updated to clarify that urine pregnancy tests will be collected and analyzed in the clinic; however, CSF samples will be collected in the clinic and analyzed at the local laboratory. In addition, it was clarified that repeat coagulation or hematology tests, if required, will be collected in the clinic and sent to the local laboratory for analysis. The section header was updated to reflect this change.
- Section 19.2, Publications, was moved to Section 18.5 to adhere to template guidance.
- Section 20, References, was updated to reflect changes in the protocol. Reference to the article by Brooks et al was removed because the reference to sporadic ALS has been removed from Inclusion Criteria 3.