HEALEY ALS Platform Trial - Regimen D Pridopidine

NCT04615923

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RGD REGIMEN-SPECIFIC STATISTICAL ANALYSIS PLAN (R-SAP)

Master Protocol	Platform Trial for the Treatment of Amyotrophic Lateral Sclerosis (ALS): A perpetual multi-center, multi-regimen, clinical trial evaluating the safety and efficacy of investigational products for the treatment of ALS
Regimen	RGD: Pridopidine
Regimen Partner	Prilenia Neurotherapeutics Ltd.
Regulatory Sponsor	Merit E. Cudkowicz, MD
Master Protocol Version	4.0, 31 Aug 2020
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R-SAP Version	3.0, 06 Oct 2022

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SAP REVISION HISTORY

Version	Date	Description of Changes
1.0	19 May 2022	Initial version
2.0	08 Aug 2022	Revision of Section 3.6 Missing Data to clarify that mean imputation of any missing baseline covariates will impute after transformation for any transformed covariates. Revision of Section 4.1 Efficacy Endpoints to clarify reference to CNS-BFS total score and serum NfL level. Revision of Section 4.2 Exploratory Endpoints to identify serum creatinine and serum and CSF neurofilament light chain (NfL) as exploratory biomarkers of neurodegeneration and neuromuscular degeneration and to include ALSAQ-40 domain scores and symptom index (SI) as exploratory endpoints. Revision of Section 5.1 ALSFRS-R to specify details of the calculation of pre-baseline slope. Revision of Section 5.2 SVC to specify details of the calculation of age. Revision of Section 5.5 Quantitative Voice Characteristics to include predicted vital capacity as an additional metric at the Baseline Visit. Revision of Section 5.6 Biofluid Biomarkers of Neurodegeneration to specify the assay techniques used to quantify serum creatinine and serum and CSF NfL and to specify that levels of serum and CSF NfL will be log-transformed in all analyses. Revision of Section 5.7 ALSAQ-40 to specify calculation of domain scores and to revise calculation of overall ALSAQ-40 SI. Revision of Section 5.8 CNS-BFS to specify that the total score is referenced. Revision of Section 5.10 Survival to specify that time at risk begins at each participant's Baseline Visit and to specify that the date of PAV initiation, where applicable, will be imputed as the fifteenth day of a month if not specified more precisely. Revision of Section 6.1 Analysis Sets to add the Efficacy Common Mode of Administration (ECM) analysis set, to remove the restriction on protocol deviations that could be considered for exclusion from the Efficacy Per-protocol (EPP) analysis set must be classified as major protocol deviations, to specify the time point at which data is excluded from the EPP analysis set in the

Version	Date	Description of Changes
2.0 (continued)	08 Aug 2022 (continued)	case of time-dependent exclusions, and to specify that data from placebo participants from other regimens would not be excluded from the EPP analysis set due to non-adherence to protocol-specified dosing. Revision of Section 6.2 Baseline Characterization to include ALSAQ-40 domain scores and SI and clarify reference to CNS-BFS total score and serum NfL level. Revision of Section 6.3 Primary Efficacy Analysis and Supportive Analyses to include the ECM analysis set. Revision of Section 6.5.1 Hierarchical Testing to correct reference to Section 6.5.6 for survival analyses. Revision of Section 6.5.2 Repeated-measures Model to add a fixed term for treatment group (removing the shared-baseline assumption at the recommendation of the FDA) and to specify a separate supportive analysis that includes fixed terms for centered baseline serum NfL level and centered baseline serum NfL level × visit interaction. Revision of Section 6.5.3 Random-slopes Model to add a fixed term for treatment group (removing the shared-baseline assumption) and to specify a separate supportive analysis that includes fixed terms for centered baseline serum NfL level and centered baseline serum NfL level × study month interaction. Revision of Section 6.5.4 Time to First Evidence of Bulbar Dysfunction to reference the FAS and mITT analysis sets and to specify a separate supportive analysis that includes baseline serum NfL level as an additional covariate. Revision of Section 6.5.5 Responder Analysis to specify a separate supportive analysis that includes baseline serum NfL level as an additional covariate. Revision of Section 6.5.6 Survival and Time to Clinical Events to include the mITT and ECM analysis sets, to clarify that survival analyses that include follow-up beyond the placebo-controlled period will be analyzed in the ERO analysis set, to include baseline age as an additional covariate in adjusted models, and to specify an additional adjusted analysis that includes baseline serum NfL level as a covariate. Revision of Section 6.5.7 CAFS to clarify that the primary CAFS analysis is specified in the ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report and to add two additional sets of CAFS analyses that adjust rank scores in

2.0 (continued)	08 Aug 2022 (continued)	linear models, one set that adjusts for time from ALS symptom onset, delta-FRS, baseline use of riluzole, and baseline use of edaravone, and one set that adjusts for the same set of covariates plus baseline serum NfL level. Revision of Section 6.5.8 HHD0 and $HHD0^2$ to remove reference to the shared-baseline assumption of the repeated-measures mixed model of Section 6.5.2 and to specify a separate analysis that adds baseline serum NfL level as an additional covariate. Revision of Section 6.5.9 Quantitative Voice Measures to remove reference to the shared-baseline assumption of the random-slopes mixed model of Section 6.5.3, to add a fixed term for treatment group (removing the shared-baseline assumption), and to specify a separate analysis that includes fixed terms for centered baseline serum NfL level and centered baseline serum NfL level × B-spline interaction. Revision of Section 6.5.10 Placebo Multiple Imputation to specify regression over sequential visits by the fully conditional specification method, to remove reference to the shared-baseline assumption of the repeated-measures mixed model of Section 6.5.2, and to specify a separate analysis that adds baseline serum NfL level as an additional covariate. Revision of Section 6.5.13 Comparison of Controls across Regimens to specify separate analyses that add baseline serum NfL level and relevant interaction terms as additional covariates.
3.0	06 Oct 2022	Revision of Section 5.10 Survival to clarify calculation of censoring time for the composite endpoint of death or death-equivalent. Revision of Section 7.2 [Validation of] Secondary, Exploratory, and Safety Analyses to identify three levels of validation and their application and to specify that analyses that fail due to small sample size or lack of convergence will be omitted.

ABBREVIATIONS

ALD	After Last Dose
ALP	Alkaline Phosphatase
ALS	Amyotrophic Lateral Sclerosis
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire, 40-item version
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale, Revised
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	WHODrug Anatomical, Therapeutic, and Chemical class
ATS	American Thoracic Society
BLQ	Below the Limit of Quantitation
BMI	Body Mass Index
C-SSRS	Columbia Suicide Severity Rating Scale
CAFS	Combined Assessment of Function and Survival
CBC	Complete Blood Count
CKD	Chronic Kidney Disease
COVID-19	Coronavirus Disease 2019
CNS-BFS	Center for Neurologic Study Bulbar Function Scale
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
delta-FRS	Pre-baseline Slope in ALSFRS-R
DAP	Data Analysis Plan
DNA	Deoxyribonucleic Acid
DRR	Disease Rate Ratio
ECC	Efficacy Concurrent Control
ECG	Electrocardiography or Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
EPP	Efficacy Per-protocol
ERO	Efficacy Regimen-only
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set

ABBREVIATIONS (continued)

FVC	Forced Vital Capacity
GLI	Global Lung Initiative
HHD	Hand-held Dynamometry
HLT	MedDRA High Level Term
ICF	Informed Consent Form
ITT	Intention-to-treat Principle
M-SAP	Master Statistical Analysis Plan
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-to-treat
MP	Master Protocol
MPRDR	ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report
NCI	National Cancer Institute
NEALS	Northeast ALS
NfL	Neurofilament Light Chain
NIV	Noninvasive Ventilation
OLE	Open-label Extension
PAV	Permanent Assisted Ventilation
PEG	Polyethylene Glycol
PD	Pharmacodynamics
PK	Pharmacokinetics
PT	MedDRA Preferred Term
RBC	Red Blood Cell
RGD	Regimen D (pridopidine)
RSA	Regimen-specific Appendix
R-SAP	Regimen-specific Statistical Analysis Plan
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	Symptom Index
SoA	Schedule of Activities
SOC	MedDRA System Organ Class

ABBREVIATIONS (continued)

SRO	Safety Regimen-only
STF	Safety and Tolerability Full
STN	Safety and Tolerability Narrow
SVC	Slow Vital Capacity
TBL	Total Bilirubin
TEAE	Treatment-emergent Adverse Event
ULN	Upper Limit of Normal
WHODrug	World Health Organization Drug Dictionary Enhanced

Table of Contents

1. Governing Documents

This Regimen-specific Statistical Analysis Plan (R-SAP) for the pridopidine regimen (RGD) specifies any modification from the default outcome measures, analysis samples, and planned analyses for the placebo-controlled period of the HEALEY ALS Platform Trial as specified in the Master SAP (M-SAP). The M-SAP and this R-SAP supplement the Master Protocol, the "ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report" (Appendix 1 to the Master Protocol), and the RGD Regimen-specific Appendix (RSA). Please refer to the Master Protocol and the RGD RSA for details on the rationale for the study design, eligibility criteria, conduct of the trial, clinical assessments and schedule of assessments, definitions and reporting of adverse events, data management conventions, and regulatory oversight and compliance procedures. The "ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report" (MPRDR) and any regimen-specific deviations described in the RGD RSA and this R-SAP are authoritative in defining the primary and interim analyses. In case of discrepancies between the RGD RSA and this R-SAP concerning use of shared placebos, this R-SAP is authoritative. In case of discrepancies between either SAP and the Master Protocol and the RGD RSA concerning matters of analysis other than the primary and interim analyses and use of shared placebos, the M-SAP and this R-SAP are authoritative. In case of discrepancies between the M-SAP and this R-SAP, this R-SAP is authoritative. On all matters not related to analysis, the Master Protocol and the RGD RSA are authoritative. The following table describes relationships among the relevant documents in adjudicating possible discrepancies with higher numbers indicating greater authority.

Issues potentially requiring adjudication	Master Protocol	RGD RSA	MPRDR	M-SAP	RGD R-SAP
Use of shared placebos	1	4	2	3	5
Primary and interim analysis specifications not related to use of shared placebo	1	5	4	2	3
Statistical analysis specifications not related to use of shared placebo or primary and interim analyses	1	3	2	4	5
All matters not related to statistical analysis	4	5	1	2	3

2. Study Design

2.1 Overview

The HEALEY ALS Platform Trial is a perpetual multi-center, multi-regimen clinical trial evaluating the safety and efficacy of investigational products for the treatment of ALS. RGD evaluates the safety and efficacy of pridopidine administered orally at a dosage of 45 mg twice daily (BID) vs. placebo. The RGD RSA describes the nature of the intervention and its mechanism of action, the mode and frequency of administration, additional eligibility criteria beyond those specified in the Master Protocol, additional enrollment procedures, and additions and modifications of safety and efficacy assessments relative to those outlined in the Master Protocol.

2.2 Study Objectives

Primary Efficacy Objective:

- To evaluate the efficacy of pridopidine as compared to placebo on ALS disease progression.

Secondary Efficacy Objectives:

- To evaluate the effect of pridopidine on selected secondary measures of disease progression, including survival

Safety Objectives:

- To evaluate the safety of pridopidine in ALS patients.

Exploratory Efficacy Objectives:

- To evaluate the effect of pridopidine on selected biomarkers and endpoints.
- To explore plasma pharmacokinetics (PK) of pridopidine and its metabolites.
- To explore relationship between PK of pridopidine and pharmacodynamic endpoints.

2.3 Study Population

In addition to eligibility criteria specified in the Master Protocol, participants in RGD must not have a confirmed prolonged Fridericia-corrected QT (QTcF) interval or clinically significant heart disease or selected cardiac abnormalities, must not have used selected prohibited medications within 4 weeks prior to the Regimen-specific Screening Visit, must not currently use selected medications above specified dosage thresholds at the time of the Regimen-specific Screening Visit, and must not have a history of allergy to any ingredient of study drug. Detailed criteria are specified in the Master Protocol and the RGD RSA.

Participants will be recruited from approximately 60 centers located throughout the US that are part of the Northeast ALS (NEALS) Consortium.

2.4 Participant Flow

Participants in RGD follow the consenting, Master screening, regimen assignment, regimen-specific screening, randomization to active or placebo treatment, and follow-up procedures and timing described in the M-SAP. Detailed descriptions of study procedures and timing are specified in the Master Protocol and the RGD RSA.

2.5 Regimen Allocation

Participants in RGD are those determined eligible for Master Protocol-level inclusion and exclusion criteria and randomly assigned to RGD, stratified by use of riluzole, edaravone, both, or neither at the time of screening for the Master Protocol. Details of regimen assignment are described in the Platform Trial Regimen Assignment Plan.

2.6 Treatment Allocation

Participants in RGD are randomly allocated in a 3:1 ratio to pridopidine 45 mg BID or placebo treatment based on a pre-specified permuted-block randomization schedule, stratified by use of riluzole, edaravone, both, or neither at the time of screening for the Master Protocol.

2.7 Treatment Administration

Pridopidine and placebo are supplied as capsules for oral administration. Each capsule of active study drug contains 45 mg of pridopidine plus silicified microcrystalline cellulose and magnesium stearate as inactive excipients.

The first dose of study drug should be administered while in the office/clinic on the day of the Baseline Visit after all visit assessments are complete except for post-dose ECG recordings.

From the Baseline Visit to the end of Week 2, participants should take one capsule of study drug orally once daily in the morning (the QD dose schedule). Starting at the beginning of Week 3 and throughout the remainder of the study, participants should take one capsule of study drug twice daily, once in the morning and once in the afternoon (approximately 7 to 10 hours after the morning dose; the BID dose schedule).

After the 2-week up-titration period, participants who are unable to tolerate the BID dose schedule should reduce their dosage to the QD dose schedule for one week. If a participant is tolerant of the QD dose schedule, then they should resume the BID dose schedule. If the participant cannot tolerate the second increase to the BID dose schedule, then they should continue on the QD dose schedule for the remainder of the study.

Additional details of treatment administration are described in the RGD RSA.

2.8 Allocation Concealment

Allocation concealment is the same as described in the M-SAP. Both active and placebo formations of study drug are identical in appearance.

2.9 RGD Schedule of Activities (SoA)

Activity	MP Scrn[1]	RGD Scrn[1]	Base-line	Week 2	Week 4[14]	Week 8[19]	Week 12	Week 16[19]	Week 20	Week 24/ET[14, 22]	Final Call[11]
	Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
	-42d to -1d	-41d to 0d	Day 0	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day 168 ±7	28d ±3 ALD
Written Informed Consent[2]	X	X									
Inclusion/Exclusion Review	X	X[3]									
ALS & Medical History	X										
Demographics	X										
Physical Examination	X										
Neurological Exam	X										
Vital Signs[4]	X		X		X	X		X		X	
Slow Vital Capacity	X[20]		X			X		X		X	
Home Spirometry	X[20]		X			X		X		X	
Muscle Strength Assessment			X			X		X		X	
ALSFRS-R	X		X		X	X	X	X	X	X	
ALSAQ-40			X							X	
CNS Bulbar Function Scale			X			X		X		X	
CNS Lability Scale			X			X		X		X	
12-Lead ECG	X	X[15]	X[16]		X[16]					X[15, 16, 17]	

Activity	MP Scrn[1]	RGD Scrn[1]	Base-line	Week 2	Week 4[14]	Week 8[19]	Week 12	Week 16[19]	Week 20	Week 24/ET[14, 22]	Final Call[11]
	Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
	-42d to -1d	-41d to 0d	Day 0	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day 168 ±7	28d ±3 ALD
Clinical Safety Labs[5, 21]	X		X		X	X		X		X	
Biomarker Blood Collection[21]			X			X		X		X	
Plasma PK collection[21]					X					X	
Biomarker Urine Collection			X			X		X		X	
DNA Collection[7, 21] (optional)			X								
CSF Collection (optional)			X					X[13]			
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	
Adverse Event Review[6]	X	X	X	X	X	X	X	X	X	X	X
Suicidality C-SSRS			X		X	X		X		X	
Install Smartphone App[23]			X								
Voice Recording[9]			X		X	X		X		X	
Uninstall Smartphone App										X	
Randomization to Regimen	X										
Randomization within Regimen			X								
Administer/Dispense Study Drug			X[8]		X[18]	X		X		X[10,18]	
Drug Accountability/Compliance				X[24]	X	X	X[24]	X	X[24]	X	
Exit Questionnaire										X	
Vital Status Determination[12]										X	

Abbreviations: ALD = after last dose, ALS = amyotrophic lateral sclerosis, ALSAQ-40 = ALS Assessment Questionnaire, ALSFRS-R = ALS Functional Rating Scale Revised, CSF = cerebrospinal fluid, C-SSRS = Columbia-Suicide Severity Rating Scale, d = day, DNA = deoxyribonucleic acid, ECG = electrocardiogram, MP = Master Protocol, RGD = the pridopidine regimen, Scrn = Screening Visit.

[1] Master Protocol Screening procedures must be completed within 42 days to 1 day prior to the Baseline Visit. The Regimen-Specific Screening Visit and Baseline Visit should be combined, if possible.

[2] During the Master Protocol Screening Visit, participants will be consented via the Platform Trial informed consent form (ICF). After a participant is randomized to a regimen, participants will be consented a second time via the regimen-specific ICF.

[3] At the Regimen Specific Screening Visit, participants will have regimen-specific eligibility criteria assessed.

[4] Vital signs include weight, systolic and diastolic pressure, respiratory rate, heart rate and temperature. Height measured at Master Protocol Screening Visit only.

[5] Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function and urinalysis. Serum pregnancy testing will occur in women of child-bearing potential at the Master Protocol Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.

[6] Adverse events that occur after signing the master protocol consent form will be recorded.

[7] The DNA sample can be collected after Baseline Visit if a baseline sample is not obtained or the sample is not usable.

[8] Administer first dose of IP only after Baseline Visit procedures are completed, except for the post-dose ECG.

[9] In addition to study visits outlined in the SOA, participants will be asked to complete twice weekly voice recordings at home.

[10] Drug will only be dispensed at this visit if the participant continues in the OLE.

[11] Participants will only have a Follow-Up Safety Call at this time if they *do not* continue on in the OLE. Participants who continue into OLE will have a Follow-Up Safety Call after their last dose of IP during the OLE period.

[12] Vital status, defined as a determination of date of death or death equivalent or date last known alive, will be determined for each randomized participant at the end of the placebo-controlled portion of their follow-up (generally the Week 24 Visit, as indicated). If at that time the participant is alive, his or her vital status should be determined again at the time of the last participant's last visit (LPLV) of the placebo-controlled portion of a given regimen. We may also ascertain vital status at later time points by using publicly available data sources as described in section 8.15 of the Master Protocol.

[13] If the CSF collection is unable to be performed for logistical reasons, such as scheduling, at the Week 16 Visit, it may be performed at the Week 24 Visit.

[14] If a participant's visit occurs in the morning, the participant should be instructed that the morning dose of study medication should not be taken, and the morning dose should be administered in-clinic 1-2 hours prior to the POST-DOSE ECG. If a participant's visit occurs in the afternoon, the participant should be instructed to take the morning dose immediately upon waking in the morning, and the second dose should be administered in-clinic 1-2 hours prior to the POST-DOSE ECG.

[15] At the regimen-specific Screening visit (and Week 24 for participants enrolling in OLE), QTcF will be determined by the mean of three, 10-second recordings of a 12-Lead ECG (performed at least 1 minute a part) BEFORE IP administration. For participants on Nuedexta, the Nuedexta dose should be taken within 6 hours BEFORE the ECG, refer to the MOP for additional guidelines.

[16] At Baseline and Week 4 (and Week 24 for participants enrolling in OLE) QTcF will be determined by a single 10-second 12-lead ECG, 1-2 hours AFTER IP administration. If stopping or monitoring rules are met, refer to Section 4.4. For participants on Nuedexta, the Nuedexta dose should be taken within 6 hours BEFORE the ECG, refer to the MOP for additional guidelines.

[17] For participants that will NOT be enrolling into the OLE, QTcF will be determined by a single 10-second 12 lead ECG BEFORE administration of the IP. If stopping or monitoring rules are met, refer to Section 4.4. For participants on Nuedexta, the Nuedexta dose should be taken within 6 hours BEFORE the ECG, refer to the MOP for additional guidelines.

[18] IP must be administered at Week 4 (and Week 24 for participants enrolling into the OLE) 1-2 hours prior to the POST-DOSE ECG.

[19] Visit may be conducted via phone or telemedicine with remote services instead of in-person if this is needed to protect the safety of the participant due to a pandemic, or other reasons.

[20] If required due to pandemic-related restrictions, Forced Vital Capacity (FVC) performed by a Pulmonary Function Laboratory evaluator or with a study-approved home spirometer, or sustained phonation using a study approved method may be used for eligibility (Master Protocol Screening ONLY).

[21] All blood draws should occur after the ECG is collected, or at least 1 hour prior to the ECG.

[22] If participant's visit occurs in the morning, the participant should be instructed not to take the morning dose at home but rather in-clinic after the PRE-DOSE ECG.

If visit occurs in the afternoon, morning dose should be taken immediately upon waking in the morning and the second dose after the PRE-DOSE ECG.

[23] Two smartphone apps should be installed on the participant's phone, one to collect the voice recordings and one to collect home spirometry.

[24] Drug accountability will not be done at phone visits. A drug compliance check-in should be held during phone visits to ensure participant is taking drug per dose regimen and to note any report of missed doses.

3. General Considerations for Data Analysis

3.1 Statistical Software

Statistical software use for analyses is the same as described in the M-SAP.

3.2 Summary Statistics

Data summaries are the same as described in the M-SAP.

Summary statistics for time-to-event endpoints will include the percentage of subjects having the event and relevant percentiles of the survival curve depending on frequency of a given event. Summary statistics for responder endpoints will include the percentage of responders.

3.3 Precision

Precision of reported results is the same as described in the M-SAP.

3.4 Transformations

Data transformations are the same as described in the M-SAP.

3.5 Multiplicity Adjustments

The primary endpoint will be tested first, and key secondary endpoints will be tested next in a fixed sequence with overall family-wise error rate controlled by a closed-testing procedure as indicated in Section 6.5.1 Hierarchical Testing below.

3.6 Missing Data

Handling of missing data is the same as described in the M-SAP except as indicated below. Mean imputation of any missing baseline covariates will impute after transformation for any transformed covariates. Clinic-based assessments that are missing due to COVID-19 restrictions or disruptions are considered missing at random.

Handling of missing data depends upon the type and pattern of missing data (monotone, non-monotone, deaths), the endpoint analyzed (ALSFRS-R, SVC, HHD, etc.), the method of analysis (continuous, time to event, responder), and the reason for missing data (related to lack of efficacy, related to tolerability, administrative reasons unrelated to lack of efficacy or tolerability, and unknown reasons).

Deaths are considered a special type of missing data.

For continuous analysis of all endpoints, the method described in the M-SAP for handling missing data will be followed.

For time-to-event analyses that depend on scheduled assessments, participants lacking any post-baseline assessment not due to death or disease progression will be censored at baseline and functionally omitted from the analysis. If data are missing not due to death, no imputation will be made for the primary analysis. As a sensitivity analysis, multiple imputations as described in Section 6.5.10 Placebo Multiple Imputation below will be used. All subjects who die, reach a death equivalent, or early terminate due to disease progression before reaching the pre-specified time-to-event will be considered as having the unfavorable event being analyzed (e.g., worsening by 1 or more points in bulbar subscale score) at the time of death, death equivalent or early termination, respectively.

For responder analysis, participants experiencing the unfavorable event (e.g., worsening in bulbar subscale score) are considered non-responders. All participants who die, reach a death equivalent, or early terminate due to disease progression will be considered non-responders. Data missing not due to death, death equivalent, or early terminate due to disease progression will be imputed under an assumption that data are conditionally missing at random (MAR). Under MAR, monotone missing data for participants will be imputed using available data from participants within the treatment group to which the participant was randomized. Sensitivity analyses based on multiple imputation under an assumption that data are missing not at random (MNAR) will be performed as described in the M-SAP.

4. Study Endpoints

4.1 Efficacy Endpoints

The primary efficacy endpoint is the same as described in the M-SAP.

The following secondary efficacy endpoints are adjusted for multiplicity and will be evaluated hierarchically:

- Time to first decline of 2 points or greater post baseline in the ALSFRS-R total score among participants in the FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,

- Proportion of participants experiencing no worsening in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS,
- Rate of change in respiratory function as measured by slow vital capacity (SVC) maximum percent predicted from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Time to first decline of 1-point or greater post baseline in the ALSFRS-R bulbar domain (Q1-Q3) score among all participants in FAS,
- Time to death or death equivalent through Week 24 among all participants in FAS.

Other Secondary Efficacy Endpoints (pre-specified non multiplicity adjusted non-hierarchical)

- Time to first decline of 2 points or greater post baseline in the ALSFRS-R total score among participants in FAS,
- Proportion of participants experiencing 5 points or less decline in the ALSFRS-R total score from baseline through Week 24 among all participants in FAS,
- Proportion of participants experiencing 5 points or less decline in the ALSFRS-R total score from baseline through Week 24 among all participants in FAS with baseline ALSFRS-R greater than or equal to 36 (Early in disease),
- Proportion of participants experiencing 5 points or less decline in the ALSFRS-R total score from baseline through Week 24 among all participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Rate of change in ALSFRS-R Total Score from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Proportion of participants experiencing no or only a 1-point decline in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Time to first decline of 1 or more points post baseline in the ALSFRS-R bulbar domain (Q1-Q3) score among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Proportion of participants experiencing no worsening in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS with delta-FRS slower than -0.75 points/month (slow progressors),
- Proportion of participants with ALSFRS-R bulbar domain (Q1-Q3) score greater than or equal to 9 (out of 12) at Week 24 among all participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,

- Proportion of participants experiencing no worsening in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS with baseline ALSFRS-R greater than or equal to 36 (Early in disease),
- Rate of change in CNS-BFS total score from baseline through Week 24 among all participants in FAS,
- Rate of change in CNS-BFS total score from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Change in HHD upper extremity percentage from baseline through Week 24,
- Change in HHD lower extremity percentage from baseline through Week 24,
- Change in log transformed serum NfL from baseline through Week 24 among all participants in FAS,
- Change in log transformed serum NfL from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Change in log transformed serum NfL from baseline through Week 24 among participants in FAS by NfL median baseline split (slow vs fast progressors),
- Rate of change in ALSFRS-R total score from baseline through Week 24 among participants in FAS who were not on Nuedexta at baseline,
- Rate of change in ALSFRS-R total score from baseline through Week 24 among participants in FAS who were not on Nuedexta at baseline and with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Rate of change in CNS-BFS total score from baseline through Week 24 among participants in FAS who were not on Nuedexta at baseline,
- Rate of change in CNS-BFS total score from baseline through Week 24 among participants in FAS who were not on Nuedexta at baseline and with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Proportion of participants experiencing no worsening in the CNS-BFS total score from baseline through Week 24 among all participants in FAS who were not on Nuedexta at baseline,
- Time to first increase of 5 points or more post baseline in the CNS-BFS total score among all participants in FAS who were not on Nuedexta at baseline, and
- Rate of change in ALSFRS-R total score from baseline through Week 24 among participants in FAS who were on Nuedexta at baseline.

4.2 Exploratory Endpoints

The following categories of exploratory endpoints will be evaluated:

- Rate of change in ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among participants in FAS,

- Rate of change in ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
- Proportion of participants experiencing no worsening in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS with delta-FRS less than -0.75 points/month (fast progressors),
- Rate of change in ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS with delta-FRS greater than -0.75 points/month (slow progressors),
- Time to first decline of 1 or more points post baseline in the ALSFRS-R bulbar domain (Q1-Q3) score among participants in FAS with no bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of 12,
- Change in other ALSFRS-R domain scores (fine motor, gross motor, respiratory, and fine and gross motor combined),
- Change in strength: HHD global percentage, HHD0, and $HHD0^2$,
- Change in quantitative voice characteristics as measured by Aural Analytics: maximum phonation time, pause rate, breathy vocal quality, pitch instability, regulation of voicing, articulatory precision, speaking rate, articulation rate, and monotonicity,
- Change in biofluid biomarkers of neurodegeneration and neuromuscular degeneration: serum creatinine, and serum and cerebrospinal fluid (CSF) neurofilament light chain (NfL),
- Change in patient-reported outcomes: ALSAQ-40 physical mobility, independence in activities of daily living, eating and drinking, communications, and emotional reactions domain scores and ALSAQ-40 symptom index, CNS-BFS total score, and CNS Lability Scale (CNS-LS),
- Change in plasma concentration of pridopidine and its metabolites,
- Change in respiratory function as assessed by home spirometry, and
- Time to clinical events: first hospitalization due to a serious adverse event (SAE), first hospitalization due to an ALS-related SAE, first use of assisted ventilation, first placement of a feeding tube, first time reaching King's stage 4a or 4b, and first instance of any of the following events: hospitalization for an SAE, feeding tube placement, tracheostomy, initiation of permanent assisted ventilation (PAV), or death.

Safety Endpoints

In addition to the safety endpoints described in the M-SAP, the following RGD regimen-specific safety endpoint will be evaluated:

- QT prolongation: Proportion of participants meeting ECG stopping rules or meeting ECG monitoring rules 1, 2, or 3 times.

5. Measurement Definitions

5.1 ALSFRS-R

The definitions of ALSFRS-R scores are the same as described in the M-SAP with the clarification that scores will be considered missing in cases of item nonresponse. Pre-baseline slope in ALSFRS-R (delta-FRS) is defined as 48 minus the baseline ALSFRS-R total score then divided by the number of months from onset of symptomatic weakness to the Baseline Visit. The number of months will be calculated as the difference in days from onset of symptomatic weakness to the Baseline Visit multiplied by 12 / 365.25. The date of onset of symptomatic weakness will be imputed as the fifteenth day of a month if not specified more precisely.

5.2 SVC

The derivation of SVC percent-predicted of normal is the same as described in the M-SAP with age calculated as number of days from date of birth to the date of a given SVC assessment divided by 365.25 and with the following correspondence between self-identified race and race defined by Global Lung Initiative (GLI) classification:

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

5.3 Home Spirometry

Home spirometry assesses FVC remotely using a smartphone app (ZEPHYRx, Albany, NY) and a handheld spirometer (Spirobank Smart, Medical International Research, Rome, Italy). Coordinators guide participants through 3 to 8 maneuvers with live-video coaching using the ZEPHYRx platform. Flow loops are classified for acceptability and repeatability using American Thoracic Society (ATS) criteria and are manually reviewed by the NEALS Outcomes Center (Barrow Neurological Institute, Phoenix, AZ). The maximum acceptable FVC is converted to percent of predicted normal using GLI norms based on sex, age at time of assessment, height at time of screening, and race. Higher values indicate greater respiratory function.

5.4 HHD and Grip Strength

The derivation of HHD upper and lower extremity scores and HHD0 are the same as described in the M-SAP with the revision that HHD0 is a composite endpoint with death or death equivalent.

A second HHD time-to-event endpoint is defined as the time from the Baseline Visit to the second post-baseline occurrence of a muscle with a strength recording of 0 among those muscles that were non-zero at baseline, or time to death or death equivalent, whichever occurs first ($HHD0^2$).

Time at risk for HHD0 and $HHD0^2$ will be censored at the Week 24 Visit, if completed, the date of consent withdrawal, if withdrawn, or the last date at which vital status is known prior to the end of the Week 24 Visit window for participants lost to follow-up.

HHD global average percentage, HHD0, and $HHD0^2$ are measures of the secondary endpoint HHD and grip strength.

5.5 Quantitative Voice Characteristics

Voice samples will be collected using the Aural Analytics app installed on either an Android or iOS-based smartphone. At each assessment, participants perform a set of speaking tasks: reading 5 prespecified sentences, reading 5 sentences chosen at random from a large sentence bank, repeating a consonant-vowel sequence, producing a sustained phonation, and counting on a single breath. Speech analysis will be performed by Aural Analytics to derive the following quantitative voice characteristics: maximum phonation time, pause rate, breathy vocal quality, pitch instability, regulation of voicing, articulatory precision, speaking rate, articulation rate, and monotonicity. Aural Analytics will use data on quantitative voice characteristics and participant age, sex, race, height, and weight to derive a prediction of vital capacity at the Baseline Visit.

5.6 Biofluid Biomarkers of Neurodegeneration

Blood biomarkers of neurodegeneration, including biomarkers of neuromuscular dysfunction, will be assayed. These will include serum creatinine and serum and CSF neurofilament light chain (NfL). Serum creatinine will be assayed by the kinetic Jaffe method (test 001370, Labcorp, Burlington, NC). NfL will be assayed by single-molecule array (Simoa; Quanterix, Billerica, MA). Levels of serum and CSF NfL that are reported to be below the limit of quantitation will be imputed at the limit of quantitation. Levels of serum and CSF NfL will be log-transformed in all analyses.

5.7 ALSAQ-40

The description of the ALSAQ-40 instrument and item-level scores are the same as described in the M-SAP. Each of the five domains will be scored as the mean of all domain-specific items multiplied by 25 (range 0 to 100). An overall symptom index (SI) will be scored as the mean of the five domain scores. A domain score will be missing if more than 20% of the items are missing; otherwise, item non-response will be mean-imputed from other completed items from the same assessment. The ALSAQ-40 SI will be missing if any domain scores are missing. Higher scores indicate worse quality of life.

5.8 CNS-BFS

The definition of CNS-BFS total score is the same as described in the M-SAP.

5.9 CNS-LS

The Center for Neurologic Study Lability Scale (CNS-LS, Moore et al. 1997) is a 7-item instrument completed by participants for assessing emotional lability. For each item, participants are asked to rate the perceived frequency at which they experienced a manifestation of pseudobulbar affect (PBA) over the past week on a scale from 1 ("Applies never") to 5 ("Applies most of the time"). The total score is the sum of all items (range 7 to 35). Higher scores indicate greater emotional lability. A CNS-LS score of 13 or higher may suggest PBA.

5.10 Survival

The primary definition of survival time is the same as described in the M-SAP with the clarifications that PAV is defined as more than 22 hours per day of noninvasive or invasive mechanical ventilation for more than seven consecutive days, that time at risk should not be censored at date of consent withdrawal, and that time at risk should be censored at date last known alive. The date of PAV initiation, where applicable, will be imputed as the fifteenth day of a month if not specified more precisely. Any participant on PAV at baseline will be censored at baseline. A secondary survival endpoint of death alone, independent of any death equivalent (including a death equivalent that occurs prior to baseline), is also defined and will be censored at the Week 24 Visit if completed or at the earlier of the end of the Week 24 Visit window or date last known alive if a Week 24 visit is not completed. An exploratory survival endpoint of death or death-equivalent will censor at the earlier of last known PAV-free or last known alive.

Time at risk for the composite endpoint of death or death equivalent and time at risk for the endpoint of death alone will be measured from each participant's Baseline Visit. Time at risk will be censored at two time points: (1) at the Week 24 Visit as defined in the M-SAP, and (2) at a subsequent assessment of death or death equivalent scheduled approximately at the end of placebo-controlled follow-up of the last RGD participant. The primary analysis of survival will evaluate PAV-free survival to the Week 24 Visit time point.

5.11 King's ALS Clinical Staging System

The King's ALS Clinical Staging System (Roche et al. 2012) is a 4-level ordinal scale with the first three levels indicating the number (1, 2, or 3) of distinct central nervous system regions (bulbar, upper limb, and lower limb) with neuromuscular dysfunction and levels 4a and 4b indicating nutritional or respiratory failure secondary to ALS, respectively.

Participants will be classified to King's stage 1, 2, 3, 4a, or 4b based on scores from ALSFRS-R assessments according to a published derivation (Balendra et al. 2014). Bulbar involvement is defined as a score less than 4 on any of the ALSFRS-R questions in the bulbar domain (questions 1, 2, and 3). Upper limb involvement is defined as a score less than 4 on either of the ALSFRS-R questions related to hand function (questions 4 and 5A). Lower limb involvement is defined as a score less than 4 on the ALSFRS-R question about walking (question 8). Nutritional failure is defined as responding that the participant uses gastrostomy for greater than 50% of their nutrition. Respiratory failure is defined as a score of 0 on the ALSFRS-R question addressing dyspnea (question 10 or R-1) or a score less than 4 on the ALSFRS-R question about use of mechanical ventilation (question 12 or R-3). Participants without evidence by ALSFRS-R scores of involvement of any of the three central nervous system regions will be scored as King's stage 1 due to their confirmed diagnosis with ALS. Participant may meet criteria for both King's stage 4a and 4b.

5.12 Hospitalization and Other Clinical Events

Times to the following clinically relevant events are defined:

- Time to first hospitalization due to a serious adverse event (SAE),
- Time to first hospitalization due to an ALS-related SAE,
- Time to first use of assisted ventilation,

- Time to first placement of a feeding tube,
- Time to King's stage 4a or 4b, and
- Time to first instance of hospitalization for an SAE, feeding tube placement, tracheostomy, initiation of PAV, or death.

Time at risk for each event will be measured from each participant's Baseline Visit. Time to first hospitalization excludes hospitalizations for elective procedures. ALS-related SAEs are those indicated as related to ALS disease progression by the site investigator. Participants who are already using assisted ventilation or have a feeding tube at the time of the Baseline Visit will be excluded from analysis of those endpoints. Death or death equivalent will be considered an outcome for each of the events listed, forming a composite endpoint.

Time at risk for these events will be censored at the Week 24 Visit, if completed, the date of consent withdrawal, if withdrawn, or the last date at which the status of each endpoint is known prior to the end of the Week 24 Visit window for participants lost to follow-up. Time to King's stage 4a or 4b is interval censored between ALSFRS-R assessments.

5.13 Pharmacokinetics of Pridopidine

Plasma samples collected at the Week 4 and Week 24 visits will be analyzed to quantify the concentration of pridopidine. Details of the assay technique will be added once the procedures are confirmed.

6. Statistical Methodology

6.1 Analysis Sets

The ITT analysis set is henceforth referred to as the Full Analysis Set (FAS) and defined as follows:

- Full Analysis Set (FAS): Participants who were randomized within RGD plus placebo participants from specified regimens, classified according to their randomized treatment assignment. Participants who discontinue study drug but remain in the study will be included in the FAS. Observations completed after regimen data lock are excluded. Participants determined to not meet ALS diagnostic criteria are excluded.

The definition of the STF analysis set is revised as follows:

- Safety Full (STF) Set: Participants who initiated treatment within RGD plus placebo participants from specified regimens who are not known to be ineligible for RGD and who initiated treatment in their respective regimen, classified according to the treatment they actually received. Observations made after premature permanent discontinuation of study drug are included in this sample, should such participants remain on study. Observations completed after regimen data lock are excluded.

An analysis set restricting shared placebo participants to those regimens in which study drug is administered by the same route as RGD is defined as follows:

- Efficacy Common Mode of Administration (ECM) Set: The subset of participants in the FAS analysis set who are in regimens in which study drug is administered by the same route as RGD.

The definitions of the ECC, ERO, STN, and SRO analysis sets are the same as described in the M-SAP with reference to the ITT analysis set now referencing the FAS analysis set. The following analysis sets are specific to RGD:

- Modified Intention-to-treat (mITT) Set: Participants who were randomized within RGD plus placebo participants from specified regimens, who received at least one dose of study drug, and who completed at least one post-baseline ALSFRS-R assessment, classified according to their randomized treatment assignment. Participants who discontinue study drug but remain in the study will be included in the mITT. Observations completed after regimen data lock are excluded.
- Efficacy Per-protocol (EPP) Set: The subset of participants in the FAS analysis set who initiated study treatment and who were not involved in protocol deviations related to eligibility, treatment, follow-up, or assessment, classified according to the treatment they actually received. Final specification of participants included vs. excluded from the EPP analysis set will be defined prior to data lock. Inclusion or exclusion from the EPP analysis set of any participant for whom treatment assignment was unblinded prior to data lock will be governed by the prespecified criteria above. If a participant's data is truncated for inclusion in the EPP analysis set due to a time-dependent event, e.g., initiation of a prohibited medication or non-adherence to protocol-specified dosing, clinical events observed up to 28 days after the censoring event will be included in the EPP analysis set. Data from placebo participants shared from other regimens will not be truncated due to non-adherence to protocol-specified dosing.

Applicable analysis sets (FAS, mITT, ECM, EPP, STF, and STN) will include shared placebo participants from regimens A, B, and C. As only concurrently enrolling regimens are contributing to efficacy analyses, the FAS and ECC analysis sets are synonymous in RGD and only the FAS analysis set will be referenced. As regimen A is administered by subcutaneous injection and regimens B and C are administered orally, only regimens B and C will contribute shared placebo participants to the ECM and STN analysis sets.

6.2 Baseline Characterization

The baseline characteristics summarized for participants randomized within RGD are the same as specified in the M-SAP with the addition of ALSAQ-40 domain scores and SI, CNS-BFS total score, CNS-LS score, King's stage, weight, body mass index (BMI), serum urate concentration, serum creatinine concentration, serum NfL concentration, and baseline use of Nuedexta.

6.3 Primary Efficacy Analysis and Supportive Analyses

The primary analysis for RGD is a Bayesian shared-parameter, repeated-measures model of ALSFRS-R that accounts for loss of follow-up due to mortality. Details of the model, including documentation of operating characteristics under a range of scenarios, are provided in the "ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report" (Appendix 1 to the Master Protocol). The Bayesian shared-parameter, repeated-measures model will be

applied to the FAS analysis set as the primary analysis, to the ECM and ERO analysis sets as sensitivity analyses, and to the EPP analysis set as a supportive analysis.

The estimand of the primary analysis is the relative rate of disease progression (the "disease rate ratio" or DRR) of active treatment relative to placebo in the FAS population under an assumption that active treatment slows mean time to death or death equivalent by the same proportion as treatment slows the mean rate of functional progression as measured by change in ALSFRS-R total score over time. The estimand is defined by the following attributes:

- Treatment: pridopidine administered orally at a dosage of 45 mg BID vs. placebo.
- Population: FAS population as defined in Section 6.1.
- Variables: time to death or death equivalent and rate of change in ALSFRS-R total score from baseline to the Week 24 Visit.
- Intercurrent event 1: treatment discontinuation due to death or death equivalent: no ALSFRS-R data from participants who reach the death or death equivalent endpoint are included in the analysis, handled via mortality component in model, composite variable strategy approach.
- Intercurrent event 2: treatment discontinuation not due to death or death equivalent: handled via treatment policy approach, all data will be used including data collected during the placebo-controlled period after treatment discontinuation regardless of concomitant medication, for those participants who have not been censored due to mortality. Missing data post-treatment will not be imputed, handled via missing at random assumption.
- Population-level summary: mean ratio of hazard or progression rate of active treatment relative to placebo.

6.4 Interim Analysis

RGD will be considered for early stopping for futility according to the interim analysis schedule and definition specified in the "ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report" (Appendix 1 to the Master Protocol). RGD will not be stopped early for success.

6.5 Secondary Efficacy Analyses

6.5.1 Hierarchical Testing

Primary inference for secondary efficacy endpoints will be based on analysis of the FAS analysis set or subsets of the FAS analysis set as defined below. Primary estimates and inference for time-to-event endpoints will be by Kaplan-Meier product-limit estimates and log-rank test (see Section 6.5.6 below). Primary estimates and inference for responder endpoints will be by logistic regression as described in Section 6.5.5 with missing data handled as indicated in Section 3.6. Primary estimates and inference for rate of change of continuous endpoints will be by a random-slopes linear mixed model (see Section 6.5.3 below). The sequence for testing secondary efficacy endpoints is the following:

1. Time to first decline of 2 points or greater post-baseline in the ALSFRS-R total score among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,

2. Proportion of participants experiencing no worsening in the ALSFRS-R bulbar domain (Q1-Q3) score from baseline through Week 24 among all participants in FAS,
3. Rate of change in respiratory function as measured by slow vital capacity (SVC) maximum percent predicted from baseline through Week 24 among participants in FAS with bulbar dysfunction at baseline defined as an ALSFRS-R bulbar domain (Q1-Q3) score of less than 12,
4. Time to first decline post baseline of 1 point or greater in the ALSFRS-R bulbar domain (Q1-Q3) score among all participants in FAS, and
5. Time to death or death equivalent through Week 24 among all participants in FAS.

If the primary analysis indicates a significant slowing in disease progression from the Bayesian shared-parameter, repeated-measures model of ALSFRS-R and mortality, then each secondary efficacy endpoint in succession would be declared significant in the specified sequence using a comparison-wise criterion of two-tailed $p < 0.05$. After the first failure to declare significance, no endpoints lower in the hierarchy can be significant. This sequential closed-testing procedure controls the overall type 1 error rate at 5%. Nominal comparison-wise p-values for secondary efficacy endpoints will also be reported.

All secondary efficacy analyses specified here for hierarchical testing will also be analyzed in the mITT analysis set as sensitivity analyses.

6.5.2 Repeated-measures Model

The specification of the repeated-measures linear mixed model and the primary linear contrast for estimating differences in 24-week change from baseline in a given continuous efficacy endpoint (ALSFRS-R total and domain scores, SVC, HHD upper extremity, lower extremity, and global average percentages, FVC by home spirometry, serum creatinine, serum NfL, ALSAQ-40 domain scores and SI, CNS-BFS total score, and CNS-LS score) are revised from those specified in the M-SAP to include a main effect of treatment and main effects of centered baseline Nuedexta use, centered site of symptom onset (bulbar vs. non-bulbar), and their interactions with visit as additional covariates.

The model will include fixed terms for discrete visit, treatment group, treatment group × visit interaction, centered time since symptom onset and centered time since symptom onset × visit interaction, centered delta-FRS and centered delta-FRS × visit interaction, centered baseline riluzole use and centered baseline riluzole × visit interaction, centered baseline edaravone use and centered baseline edaravone × visit interaction, centered baseline Nuedexta use and centered baseline Nuedexta use × visit interaction, and centered site of symptom onset (bulbar vs. non-bulbar) and centered site of symptom onset × visit interaction. The following equations describe the model with regimen random effects:

$$Y_{ij} = a_{k(i)} + \gamma_1 t_i + \gamma_{2,j} v_j + \boldsymbol{\gamma_3'} \boldsymbol{z_i} + \gamma_{4,j}\, t_i v_j + \boldsymbol{\gamma_{5,j}'} \boldsymbol{z}_i\, v_j + \epsilon_{ij}$$
$$a_k \sim N(0, \sigma_r^2),\ \boldsymbol{\epsilon_{i\cdot}} \sim N(\boldsymbol{0}, \boldsymbol{R}),\ \mathrm{Cov}\left(b_{k(i)}, \epsilon_{ij}\right) = 0 \qquad \text{(eqn. 1)}$$

where Y_{ij} is a given efficacy endpoint measured in participant i at visit j, $a_{k(i)}$ is a random intercept for regimen k to which participant i was assigned, v_j is an indicator variable for visit j, $\boldsymbol{z}_i$ is the vector of covariates (centered time since onset, centered delta-FRS, centered baseline riluzole use, centered baseline edaravone use, centered baseline Nuedexta use, and centered site

of symptom onset) for participant i, t_i is an indicator variable for treatment t to which participant i was assigned, γ_1, $\gamma_{2,j}$, $\boldsymbol{\gamma_3}$, $\gamma_{4,j}$, and $\boldsymbol{\gamma_{5,j}}$ are estimated parameters and vectors of parameters for the fixed effects, and ϵ_{ij} is the residual for participant i at visit j. The regimen-specific random effects are normally distributed with mean 0 and variance σ^2_r. The vector of residuals for a given participant are normally distributed with mean **0** and an unstructured covariance matrix **R**. The regimen-specific random effect for a given participant and residuals for that participant are uncorrelated.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd visit;
  model Value = trtrnd|visit
                sx2bl|visit dFRS|visit rlz|visit edv|visit
                NDX|visit bulbar|visit / solution cl;
  random intercept / subject=regimen type=vc;
  repeated visit / subject=id type=un;
```

where `id` is a participant study identifier, `trtrnd` is the randomly assigned treatment group, `visit` is the visit identifier, `Value` is value of the efficacy endpoint being tested for a given participant at a given visit, `sx2bl` is years since ALS symptom onset centered at the sample median, `dFRS` is pre-baseline slope centered at the sample median, `rlz` is an indicator of riluzole use at baseline, `edv` is an indicator of edaravone use at baseline, `NDX` is an indicator of baseline use of Nuedexta centered at the sample median, and `bulbar` is an indicator of bulbar site of onset centered at the sample median. The primary estimate will be the treatment-dependent difference in change from baseline to the Week 24 Visit. The estimate and its 95% Wald confidence bounds will be obtained by a linear contrast of adjusted means. The following SAS code specifies the linear contrast for a regimen with one active treatment assuming an endpoint measured every 8 weeks and that the sort order for treatment group has the active group last and visits are sorted chronologically:

```
  estimate "3|Act vs Plb|dWk 24" trtrnd*visit 1 0 0 -1  -1 0 0 1 / cl;
```

A significant difference in 24-week change from baseline in the direction of greater improvement or less worsening among participants randomized to active treatment would support inference of benefit from active treatment for the efficacy endpoint being tested.

The estimand estimated by the primary linear contrast of the repeated-measures linear mixed model is the mean difference in 24-week change from baseline of a given continuous efficacy endpoint in the active treatment group relative to the placebo group in the FAS population. The estimand is defined by the following attributes:

- Treatment: pridopidine administered orally at a dosage of 45 mg BID vs. placebo.
- Population: FAS population as defined in Section 6.1.
- Variables: absolute change in endpoint from baseline to the Week 24 Visit.
- Intercurrent event: treatment discontinuation: handled via treatment policy approach, all data will be used including data collected during the placebo-controlled period after treatment discontinuation. Missing data post-treatment, including data missing due to death, will not be imputed, handled via missing at random assumption.

- Population-level summary: difference in conditional means of active treatment relative to placebo.

Inference from this analysis is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint. A separate supportive analysis will include centered baseline serum NfL level and the interaction of centered baseline serum NfL level and visit as additional covariates.

6.5.3 Random-slopes Model

The specification of the random-slopes linear mixed model and the primary linear contrast for estimating differences in mean rate of progression in a given continuous efficacy endpoint (ALSFRS-R total and domain scores, SVC, HHD upper extremity, lower extremity, and global average percentages, FVC by home spirometry, quantitative voice characteristics, serum creatinine, serum NfL, ALSAQ-40 domain scores and SI, CNS-BFS total score, and CNS-LS score) are revised from those specified in the M-SAP to include a main effect of treatment and main effects of centered baseline Nuedexta use, centered site of symptom onset (bulbar vs. non-bulbar), and their interactions with visit as additional covariates and to specify that study months are calculated as the difference in days from the Baseline Visit to the date of assessment of a given endpoint multiplied by 12 / 365.25.

The model will include fixed terms for month since the Baseline Visit, treatment group, treatment group × month interaction, centered years since ALS symptom onset and centered years since ALS symptom onset × month interaction, centered delta-FRS and centered delta-FRS × month interaction, centered baseline riluzole use and centered baseline riluzole use × month interaction, centered baseline edaravone use and centered baseline edaravone use × month interaction, centered baseline Nuedexta use and centered baseline Nuedexta use × month interaction, and centered site of symptom onset (bulbar vs. non-bulbar) and centered site of symptom onset × month interaction. The following equations describe the model with regimen random effects:

$$\begin{gathered} Y_{ij} = \gamma_1 + a^0_{k(i)} + b^0_i + \gamma_2 t_i + \boldsymbol{\gamma_3' z_i} \\ + \left(\gamma_4 + a^1_{k(i)} + b^1_i + \gamma_5 t_i + \boldsymbol{\gamma_6' z_i}\right) m_{ij} + \epsilon_{ij} \\ \{a^0_k, a^1_k\} \sim N(\mathbf{0}, \boldsymbol{\Sigma_r}), \{b^0_k, b^1_k\} \sim N\left(\mathbf{0}, \boldsymbol{\Sigma_p}\right), \epsilon_{ij} \sim N(0, \sigma^2_\epsilon) \\ \text{Cov}(\boldsymbol{a}_k, \boldsymbol{b}_k) = \mathbf{0}, \text{Cov}(\boldsymbol{a}_k, \boldsymbol{\epsilon}_{i\cdot}) = \mathbf{0}, \text{and Cov}(\boldsymbol{b}_k, \boldsymbol{\epsilon}_{i\cdot}) = \mathbf{0} \end{gathered} \quad \text{(eqn. 2)}$$

where Y_{ij} is a given efficacy endpoint measured in participant i at visit j, $a^0_{k(i)}$ and $a^1_{k(i)}$ are random intercept and slope for regimen k to which participant i was assigned, b^0_i and b^1_i are random intercept and slope for participant i, $\boldsymbol{z}_i$ is the vector of covariates (centered time since onset, centered delta-FRS, centered baseline riluzole use, centered baseline edaravone use, centered baseline Nuedexta use, and centered site of symptom onset) for participant i, m_{ij} is the time from baseline to observation j for participant i in months calculated as days x 12 / 365.25, t_i is an indicator variable for treatment t to which participant i was assigned, γ_1, γ_2, $\boldsymbol{\gamma_3}$, γ_4, γ_5, and $\boldsymbol{\gamma_6}$ are estimated parameters and vectors of parameters for the fixed effects, and ϵ_{ij} is the residual for observation j for participant i. The regimen-specific random effects are normally distributed with mean $\mathbf{0}$ and unstructured covariance matrix $\boldsymbol{\Sigma_r}$. The participant-specific random effects are normally distributed with mean $\mathbf{0}$ and unstructured covariance matrix $\boldsymbol{\Sigma_p}$. The residuals for a

given participant are normally distributed with mean 0 and variance σ^2_ϵ. The regimen-specific random effects, participant-specific random effects, and residuals are uncorrelated.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd;
  model Value = trtrnd|month
                sx2bl|month dFRS|month rlz|month edv|month
                NDX|month bulbar|month / solution cl;
  random intercept month / subject=regimen type=un;
  random intercept month / subject=id type=un;
```

where `month` is time in months from the Baseline Visit (assuming 12 months in an average of 365.25 days per year) and other fields are the same as identified above in Section 6.5.2. The primary estimand will be the treatment-dependent difference in slopes. The estimate and its 95% Wald confidence bounds will be obtained by a linear contrast of adjusted means. The following SAS code specifies the linear contrast for a regimen with one active treatment assuming that the sort order for treatment group has the active group last:

```
  estimate "3|Act vs Plb|Slope (/mn)" month 0 trtrnd*month -1 1 / cl;
```

A significant difference in slopes in the direction of greater improvement or less worsening among participants randomized to active treatment would support inference of benefit from active treatment for the efficacy endpoint being tested.

The estimand estimated by the primary linear contrast of the random-slopes linear mixed model is the difference in mean rate of progression of a given continuous efficacy endpoint in the active treatment group relative to the placebo group in the FAS population. The estimand is defined by the following attributes:

Treatment: pridopidine administered orally at a dosage of 45 mg BID vs. placebo.

Population: FAS population as defined in Section 6.1.

Variables: mean rate of change in endpoint from baseline to the Week 24 Visit.

Intercurrent event: treatment discontinuation: handled via treatment policy approach, all data will be used including data collected during the placebo-controlled period after treatment discontinuation. Missing data post-treatment will not be imputed, handled via missing at random assumption.

Population-level summary: difference in conditional mean slopes of active treatment relative to placebo.

Inference from this analysis is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint and is primary for the secondary endpoint of rate of change in SVC. A separate supportive analysis will include centered baseline serum NfL level and the interaction of centered baseline serum NfL level and study month as additional covariates.

6.5.4 Time to First Evidence of Bulbar Dysfunction

The time to first evidence of bulbar dysfunction will be compared between treatments by Kaplan-Meier product-limit estimates and log-rank test in the subset of participants with an ALSFRS-R bulbar domain (Q1-Q3) score of 12 at the Baseline Visit in the FAS, mITT, ERO, and EPP samples. If greater than 10% of participants develop bulbar dysfunction after baseline, treatments will be compared by Cox proportional hazards regression adjusting for time since ALS symptom onset, delta-FRS, use of riluzole at baseline, use of edaravone at baseline, and use of Nuedexta at baseline with a random gamma-distributed regimen-specific frailty term. If convergence is not obtained, then the frailty term will be dropped. A separate supportive analysis will include centered baseline serum NfL level as an additional covariate.

6.5.5 Responder Analysis

Analysis of responder endpoints will use logistic regression. The dependent variable will be whether the participant is a responder or not, while the independent variables will be treatment group, baseline ALSFRS-R score, site of onset (any bulbar vs. non-bulbar), riluzole use at baseline, edaravone use at baseline, and delta-FRS. A separate supportive analysis will include centered baseline serum NfL level as an additional covariate.

6.5.6 Survival and Time to Clinical Events

Survival and time to hospitalizations and clinical events will be analyzed in the FAS, mITT, ECM, ERO, EPP, STF, and SRO analysis sets. Survival analyses that include follow-up beyond the placebo-controlled period will be analyzed in the ERO analysis set. The summaries and analyses of time to death or death equivalent are the same as specified in the M-SAP with the addition of baseline age as a covariate in adjusted models, with the addition that the endpoints of time to death independent of occurrence of death equivalents and time to each of the hospitalization and clinical events will be separately analyzed using the same models, and with an additional adjusted analysis that includes baseline serum NfL level as a covariate. Analysis of time to King's stage 4a or 4b will accommodate interval censoring between ALSFRS-R assessments and will be stratified by baseline King's stage.

6.5.7 CAFS

The primary CAFS analysis is as specified in the MPRDR. Additional, unadjusted CAFS analyses are the same as specified in the M-SAP, including specification that pair-wise comparison of change in ALSFRS-R total score for participants who cannot be ranked by time to death or death equivalent is to the maximum follow-up time at which both participants have an observation, and with the following additions:

1. HHD upper and lower extremity percentage and SVC will be analyzed by CAFS by substituting change from baseline for those secondary efficacy endpoints in place of ALSFRS-R total score,
2. An additional set of CAFS analyses will use multiple imputation to extend follow-up of ALSFRS-R total score, HHD upper and lower extremity percentage and SVC for participants who early terminate, withdraw consent, or are lost to follow-up,
3. An additional set of CAFS analyses will use time to death alone independent of any death equivalent,
4. An additional set of CAFS analyses for ALSFRS-R total score, HHD upper and lower extremity percentage, and SVC will adjust rank scores in a linear model with the

following covariates: time from ALS symptom onset, delta-FRS, baseline use of riluzole, and baseline use of edaravone, and

5. An additional set of CAFS analyses for ALSFRS-R total score, HHD upper and lower extremity percentage, and SVC will adjust rank scores in a linear model with the following covariates: time from ALS symptom onset, delta-FRS, baseline use of riluzole, baseline use of edaravone, and baseline serum NfL level.

The multiple imputation model used to extend follow-up of functional scores for participants who early terminate, withdraw consent, or are lost to follow-up will use linear regression with covariates of: time since symptom onset, delta-FRS, baseline riluzole use, baseline edaravone use, and each observed functional score prior to a missing assessment.

Inference from CAFS analyses is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary outcome and supportive of inference from the random-slopes model for the secondary outcome of SVC. Primary inference from CAFS analyses will compare survival by time to death or death equivalent, will compare change in function to the last jointly observed time point, and will adjust for the specified covariates.

6.5.8 HHD0 and HHD0^2

Analyses of HHD0 are the same as specified in the M-SAP with the addition of parallel analyses of HHD0^2, with a separate analysis that includes baseline serum NfL level as an additional covariate, and with the clarification that time to zero strength for both analyses is interval censored between HHD assessments.

6.5.9 Quantitative Voice Measures

Given the high frequency of voice recordings, a repeated-measures analysis with unstructured covariance is overly flexible but the assumption of linear change required by the random-slopes model may be overly rigid. To complement estimates from the random-slopes linear mixed model, quantitative voice characteristics will be analyzed in a linear mixed model in which the temporal profile for both fixed and random terms is modeled using cubic B-splines with knots at 8 and 16 weeks. The model will include fixed terms for B-splines (4 terms), treatment group (2 levels), treatment group × B-spline interaction, centered time since symptom onset and centered time since symptom onset × B-spline interaction, centered delta-FRS and centered delta-FRS × B-spline interaction, centered baseline riluzole use and centered baseline riluzole use × B-spline interaction, centered baseline edaravone use and centered baseline edaravone use × B-spline interaction, centered baseline Nuedexta use and centered baseline Nuedexta use × B-spline interaction, and centered site of symptom onset (bulbar vs. non-bulbar) and centered site of symptom onset × B-spline interaction. The model will include random regimen-specific intercepts and slopes with unstructured covariance, random participant-specific B-splines (5 terms) with unstructured covariance, and a first-order autoregressive structure for residuals. A simplified covariance structure assuming no regimen-level covariance, heterogeneous compound symmetric covariance among the random B-splines, conditional independence of residuals, or a combination of the three simplifying assumptions will be used if the full model fails to converge. The primary estimand will be the treatment-dependent difference in 24-week change from baseline. The estimate and its 95% Wald confidence bounds will be obtained by a linear contrast of adjusted means. A separate analysis will include centered baseline serum NfL level and the interaction of centered baseline serum NfL level and B-splines as additional covariates.

6.5.10 Placebo Multiple Imputation

Placebo multiple imputation analyses are the same as specified in the M-SAP with the addition of baseline serum NfL level as a covariate and will be applied to the mixed model repeated-measures analysis of ALSFRS-R total score and the random-slopes analysis of SVC in the FAS analysis set.

The following SAS code specifies the imputation for an endpoint measured every 8 weeks:

```
proc mi data=work.outcomes seed=xxx nimpute=50 out=work.out_mi01
        minimum=. . . . . x x x x maximum=. . . . . y y y y minmaxiter=1000;
  class rlz edv trtrnd;
  fcs reg(Wk08 = sx2bl dFRS rlz edv Wk00);
  fcs reg(Wk16 = sx2bl dFRS rlz edv Wk00 Wk08);
  fcs reg(Wk24 = sx2bl dFRS rlz edv Wk00 Wk08 Wk16);
  mnar model( Wk08 Wk16 Wk24 / modelobs=(trtrnd="0"));
  var sx2bl dFRS rlz edv Wk00 Wk08 Wk16 Wk24;
run;
```

where `Wk00`, `Wk08`, `Wk16`, and `Wk24` are the values of a given efficacy endpoint at the Baseline, Week 8, Week 16, and Week 24 Visits, respectively, `trtrnd` has a value of zero (0) for participants randomized to placebo, and `x` and `y` take appropriate values to specify the range of a given outcome measure (i.e., 0 and 48 for ALSFRS-R total score; 0 and . for HHD upper and lower extremity percentages and SVC).

Inference from these analyses is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint and assess sensitivity to the missing data assumption of the random-slopes linear mixed model for SVC in the FAS analysis set. A separate analysis will include centered baseline serum NfL level as an additional covariate in both imputation stages.

6.5.11 Additional Sensitivity Analyses of Primary and Key Secondary Outcomes

Sensitivity analyses of primary and key secondary efficacy outcomes are the same as specified in the M-SAP.

6.5.12 Subgroup Analyses

In addition to the subgroups specified in the M-SAP, the following additional subgroups will be analyzed in the random-slope model (see Section 6.5.3) for ALSFRS-R total score and SVC in the FAS analysis set:

- Baseline use of riluzole and edaravone (neither, riluzole only, edaravone only, both),
- Age (less than 65 years vs. 65 years or older),
- Sex (female vs. male),
- Race (white vs. any minority race with greater than 5% prevalence in the sample),
- Ethnicity (Hispanic or Latino vs. non-Hispanic or Latino),
- Weight (less than 43 kg, 43 to less than 56 kg, 56 to less than 77 kg, 77 to less than 150 kg, 150 kg or more),

- BMI (less than 18.5 kg/m^2, 18.5 to less than 25 kg/m^2, 25 to less than 30 kg/m^2, 30 to less than 40 kg/m^2, 40 kg/m^2 or more),
- Chronic kidney disease (CKD) stage (stage 1 or better [eGFR 90 mL/min/1.73m^2 or more], stage 2 [eGFR 60 to 89 mL/min/1.73m^2], stage 3 [eGFR 30 to 59 mL/min/1.73m^2], stage 4 [eGFR 15 to 29 mL/min/1.73m^2], stage 5 [eGFR less than 15 mL/min/1.73m^2]),
- Time since onset of weakness (less than 18 months vs. 18 months or longer),
- El Escorial and time since onset of weakness (El Escorial definite and less than 18 months vs. not both),
- Baseline symptom severity (all ALSFRS-R questions scored 2 or greater vs. any question scored 0 or 1),
- Early disease state (all ALSFRS-R questions scored 2 or greater, SVC 80%-predicted or greater, and time since symptom onset less than 24 months vs. not meeting all three criteria),
- Baseline serum urate concentration (less than 5.5 mg/dL vs. 5.5 mg/dL or greater),
- Baseline plasma NfL concentration (by median split), and
- Site (individual sites with at least 5 participants per treatment group and all participants from sites with fewer than 5 participants per treatment group pooled).

For each classification, unknown, not reported, and missing will be considered one group. All individuals not included in a specified subgroup will be combined into a mixed, "other" group. The "other" group will be included in analyses if its prevalence is greater than 5%; otherwise, the "other" group will be excluded.

6.5.13 Comparison of Controls across Regimens

Comparisons of placebo participants across regimens are the same as specified in the M-SAP with separate analyses that include baseline serum NfL level as an additional covariate in adjusted analyses plus applicable interaction terms as relevant to a given model.

6.5.14 Pharmacokinetic Analyses of Pridopidine

Plasma concentrations of pridopidine will be summarized by treatment group and visit in the ERO sample. Concentrations below the limit of quantitation (BLQ) will be replaced with one half of the lower limit of quantitation. Summaries will include number of observations, number and percentage with concentrations BLQ, arithmetic mean, median, standard deviation, minimum, maximum, geometric mean, geometric coefficient of variation (calculated as sqrt(exp(variance of log-transformed concentrations) – 1)), and 95% confidence bounds for the geometric mean assuming log-normally distributed data.

Plasma concentration data of pridopidine may be subjected to population pharmacokinetic analysis to derive population estimates of pharmacokinetic parameters and test the effect of various covariates such as age, weight, and sex. Details of the analysis will be described in a separate data analysis plan (DAP). This analysis may be performed by combining data from the current study with data from other studies of pridopidine, if deemed appropriate. The population pharmacokinetic analysis will be performed by Prilenia and reported in a separate modelling report.

6.6 Safety Analyses

6.6.1 Treatment-emergent Adverse Events

Summaries and analyses of treatment-emergent adverse events (TEAE) are the same as specified in the M-SAP with the following revisions.

TEAEs are defined as those adverse events with onset dates in the interval from treatment initiation to either the Final Safety Visit if completed, the date of death if the participant dies, 40 days after last study contact if the participant early terminates or is lost to follow-up, or the date of first dose of study drug during participation in the OLE, if so exposed. The interval is inclusive except for adverse events that occur on the day of treatment initiation and are known to precede first exposure to study drug.

In addition to summaries specified in the M-SAP, TEAEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term for fatal TEAEs, TEAEs that occurred during a participant's COVID-19 infection (defined as 5 days prior to symptom onset to end of COVID-19 symptoms or end of double-blind follow-up, if ongoing), and TEAEs and serious TEAEs stratified by the following subgroups: baseline riluzole use, baseline edaravone use, age, sex, race, ethnicity, weight, BMI, and CKD stage. Subgroup classifications will be the same as described in Section 6.5.12 Subgroup Analyses except that the "other" group will be retained regardless of prevalence.

Treatment-dependent differences in the proportion of participants experiencing a given type of TEAE will not be tested. Treatment-dependent differences in TEAE incidence rates will be estimated as differences rather than ratios and will include comparison-wise 95% confidence intervals with variance estimates obtained by the delta method.

6.6.2 Safety Labs

Summaries and analyses of clinical safety labs are the same as specified in the M-SAP with the revision that abnormal levels will be classified to a toxicity grade based on quantitative grading using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 and with the addition that maximum toxicity over all post-baseline visits will be included in shift tables along with visit-specific shifts.

Potential drug-induced liver injury (DILI), including cases that potentially meet the Hy's law criteria, will be reported as distinct safety lab outcomes. Three potential DILI criteria will be defined:

- ALT or AST >3xULN with TBL >1.5xULN
- AST or ALT >3xULN with TBL >2xULN
- AST or ALT >3xULN with TBL >2xULN and ALP <2xULN (potential Hy's Law cases)

where ALT is alanine transaminase level, AST is aspartate transaminase level, TBL is total bilirubin level, ALP is alkaline phosphatase level, ULN is upper level of normal, and all levels are measured on the same day.

6.6.3 ECG Results

Summaries of ECG parameters and findings are the same as specified in the M-SAP with the additional of summaries of the proportion of participants meeting ECG stopping rules or meeting ECG monitoring rules 1, 2, or 3 times.

6.6.4 Vital Signs and Weight

Summaries and analyses of vital signs and weight are the same as specified in the M-SAP.

6.6.5 Suicidality

Summaries of suicidality are the same as specified in the M-SAP.

6.7 Other Analyses

6.7.1 Participant Disposition

All participants consented to the Master Protocol between the time of the first and last consent of a participant assigned to a regimen included in the FAS analysis set will be summarized as a single set for the following events: consented to the Master Protocol, failed screening for the Master Protocol, other reasons not assigned to a regimen (including timing out of the screening window, death, withdrawal of consent, early termination, and administrative termination), and assigned to a regimen. Reasons for Master Protocol screen failure will be summarized.

All participants in the above sample assigned to a regimen will be summarized as two sets (final screening for RGD vs. final screening for a non-RGD regimen) for the following events: consented to a regimen, failed screening for a regimen, other reasons not randomized within a regimen (including timing out of the screening window, death, withdrawal of consent, early termination, and administrative termination), and randomized within a regimen. If a given individual is screened multiple times prior to randomization within a regimen, then the final screening experience of that individual will be summarized. Reasons for RGD screen failure will be summarized separately for all participants screened for RGD whether that was their final screening experience or not.

All participants in the FAS analysis set will be summarized as two sets (randomization to active study drug vs. randomization to placebo) for the following events: initiated regimen-specific study drug, prematurely terminated study participation due to death, withdrawal of consent, early termination, loss to follow-up, or administrative termination, completed 24-week follow up, and completed a safety follow-up visit vs. continued into the OLE. Reasons for withdrawal of consent or early termination after randomization will be summarized.

6.7.2 Study Drug Compliance and Tolerance

Summaries of study drug compliance and tolerance are the same as specified in the M-SAP with the clarification that summaries will be reported for the ERO and SRO analysis sets and that date of permanent discontinuation of study drug is the date of last use of double-blind study drug among all participants in a given analysis set. The number of days of exposure to study drug will be calculated as the number of days from dose initiation to drug withdrawal, inclusive, less any day(s) during which use of study drug was interrupted. The proportion of participants who interrupted study drug or reduced study drug dosage and the time to first study drug interruption or dosage reduction will be summarized. The number of days of exposure to a reduced dosage of study drug will be summarized.

6.7.3 Concomitant Medication Use

Summaries of concomitant medication use are the same as specified in the M-SAP with the clarification that medications taken at baseline and those initiated after first dose of study drug will be separately summarized and will be classified by ATC Therapeutic class and WHODrug Preferred base name.

6.7.4 Medical History

Medical histories will be summarized by MedDRA system organ class, high level term, and preferred term in the STF and SRO analysis sets.

6.7.5 Blindedness

The proportions of participants and site investigators who report on the Exit Questionnaire a guess of active vs. placebo treatment assignment, each level of surety of that guess, and each of five pre-specified reasons for making a treatment assignment will be summarized by treatment group in the FAS and ERO analysis sets. Treatment-dependent differences in the proportion guessing active treatment assignment will be tested among all respondents and among those stating they are at least somewhat sure of their guess by Fisher's exact test and the difference in proportion guessing active treatment assignment will be estimated with confidence bounds.

6.7.6 Impact of COVID-19 Pandemic

The proportions of planned assessments missed due to COVID-19 restrictions or disruptions will be summarized by treatment group, visit, and type of assessment in the FAS and ERO analysis sets. Protocol deviations that resulted from COVID-19 restrictions or disruptions will be summarized by treatment group and type of deviation in the FAS and ERO analysis sets and listed.

7. Validation

7.1 Primary Efficacy Analysis

Validation of the primary efficacy analysis is the same as specified in the M-SAP.

7.2 Secondary, Exploratory, and Safety Analyses

Validation of secondary, exploratory, and safety analyses are the same as specified in the M-SAP with the following clarifications.

Three levels of validation will be used:

Level 1: Replication of results by an independent programmer.

Level 2: When a macro is used to generate multiple results, one result will be replicated by an independent programmer.

Level 3: Log files will be inspected for error messages and / or relevant warnings.

A separate validation document will be created in which the validation level applied for each analysis is specified. The level of validation will be based on whether the result is considered core or supplementary. Core results will be based on validation level 1 or level 2. Supplementary results will be based on level 3 validation. Examples of core results include primary and key

secondary efficacy and safety endpoints. Examples of supplementary results include exploratory efficacy endpoints, selected sensitivity analyses, and selected sensitivity analysis data sets for secondary efficacy endpoints. Any analyses that fail due to small sample size or lack of convergence will be omitted with comment rather than programmed separately to find a post-hoc, work-around solution.

8. References

The following references are cited in addition to those specified in the M-SAP:

Balendra R, Jones A, Jivraj N, Knights C, Ellis CM, Burman R, Turner MR, Leigh PN, Shaw CE, Al-Chalabi A. Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale. Amyotroph Lateral Scler Frontotemporal Degener. 2014 Jun;15(3-4):279-84.

Roche JC, Rojas-Garcia R, Scott KM, Scotton W, Ellis CE, Burman R, Wijesekera L, Turner MR, Leigh PN, Shaw CE, Al-Chalabi A. A proposed staging system for amyotrophic lateral sclerosis. Brain. 2012 Mar;135(Pt 3):847-52.

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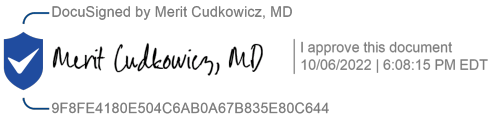
Merit Cudkowicz, MD cudkowicz.merit@mgh.harvard.edu Chief of Neurology Security Level: Email, Account Authentication (Required)	DocuSigned by Merit Cudkowicz, MD  I approve this document 10/06/2022 | 6:08:15 PM EDT 9F8FE4180E504C6AB0A67B835E80C644 Signature Adoption: Pre-selected Style Signature ID: 9F8FE418-0E50-4C6A-B0A6-7B835E80C644 Using IP Address: 132.183.13.4 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/6/2022 4:56:52 PM Viewed: 10/6/2022 6:08:02 PM Signed: 10/6/2022 6:08:18 PM
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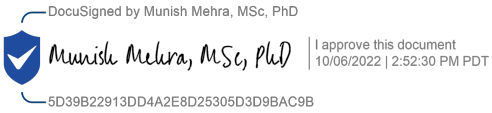
Munish Mehra, MSc, PhD munish.mehra@tigermedgrp.com Project Statistician Security Level: Email, Account Authentication (Required)	DocuSigned by Munish Mehra, MSc, PhD  I approve this document 10/06/2022 | 2:52:30 PM PDT 5D39B22913DD4A2E8D25305D3D9BAC9B Signature Adoption: Pre-selected Style Signature ID: 5D39B229-13DD-4A2E-8D25-305D3D9BAC9B Using IP Address: 96.255.202.248 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/6/2022 4:56:53 PM Viewed: 10/6/2022 5:51:52 PM Signed: 10/6/2022 5:52:40 PM
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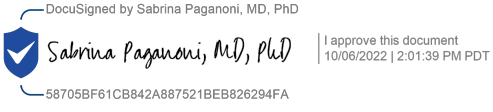
Sabrina Paganoni, MD, PhD spaganoni@mgh.harvard.edu MD PhD MGH Security Level: Email, Account Authentication (Required)	DocuSigned by Sabrina Paganoni, MD, PhD  I approve this document 10/06/2022 | 2:01:39 PM PDT 58705BF61CB842A887521BEB826294FA Signature Adoption: Pre-selected Style Signature ID: 58705BF6-1CB8-42A8-8752-1BEB826294FA Using IP Address: 73.182.245.163 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/6/2022 4:56:54 PM Viewed: 10/6/2022 5:00:43 PM Signed: 10/6/2022 5:01:41 PM
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Suzanne Hendrix, PhD shendrix@pentara.com CEO Suzanne Hendrix Security Level: Email, Account Authentication (Required)	 Signature Adoption: Drawn on Device Signature ID: 4702CD2D-FA58-4E06-88AE-96D6F0925B7F Using IP Address: 97.117.97.193 Signed using mobile With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/6/2022 4:56:55 PM Viewed: 10/6/2022 10:21:51 PM Signed: 10/6/2022 10:23:08 PM
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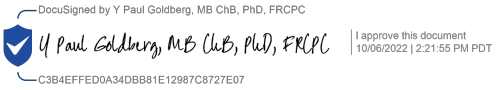
Y Paul Goldberg, MB ChB, PhD, FRCPC paul.goldberg@prilenia.com CSO, Head of Early Development Security Level: Email, Account Authentication (Required)	 DocuSigned by Y Paul Goldberg, MB ChB, PhD, FRCPC I approve this document 10/06/2022 | 2:21:55 PM PDT C3B4EFFED0A34DBB81E12987C8727E07 Signature Adoption: Pre-selected Style Signature ID: C3B4EFFE-D0A3-4DBB-81E1-2987C8727E07 Using IP Address: 70.79.152.36 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/6/2022 4:56:55 PM Viewed: 10/6/2022 5:21:25 PM Signed: 10/6/2022 5:22:09 PM
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In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

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Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	10/6/2022 4:56:55 PM
Certified Delivered	Security Checked	10/6/2022 5:21:25 PM
Signing Complete	Security Checked	10/6/2022 5:22:09 PM
Completed	Security Checked	10/7/2022 9:50:18 AM

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