HEALEY ALS Platform Trial - Regimen E Trehalose

NCT05136885

Document Date: 10 Oct 2023

RGE 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	RGE: Trehalose
Regimen Partner	Seelos Therapeutics, Inc.
Regulatory Sponsor	Merit E. Cudkowicz, MD
Master Protocol Version	4.0, 31 Aug 2020
RSA Version	2.0, 03 Dec 2021
Master SAP Version	2.0, 01 Aug 2020
R-SAP Version	2.0, 10 Oct 2023

SAP APPROVAL SIGNATURES

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SAP REVISION HISTORY

Version	Date	Description of Changes
1.0	13 Apr 2023	Initial version
2.0	10 Oct 2023	Revision of Section 1.0 Governing Documents to add a reference to analyses specific to the open-label extension period. Revision of Section 2.4 Participant Flow to add a description of participation during the open-label extension period. Revision of Section 2.6 Treatment Allocation to add a description of treatment during the open-label extension period. Addition of Section 2.10 RGE Schedule of Activities (SoA) during the Open-label Extension Period with footnotes. Revision of Section 4.1.3 Exploratory Efficacy Endpoints to switch from ALSAQ-40 symptom index to ALSAQ-40 total score, to specify that time to clinical events is restricted to the placebo-controlled period, to add analyses of survival defined as freedom from death and PAV and freedom from death alone from baseline to the last participant's last visit in the placebo-controlled period, and to add long-term CAFS analyses of ALSFRS-R and SVC as functional measures and freedom from death and PAV or from death alone as survival components. Revision of Section 4.3 Safety Endpoints to add tabulations of adverse events and clinically significant ECG and lab abnormalities that occurred after crossover into the OLE period. Revision of Section 5.4 Biofluid Biomarkers of Neurodegeneration to specify a secondary analysis of serum NfL as an endpoint that excludes measured concentrations less than 4 ng/mL if they are less than 2% of the maximum measured concentration for a given participant. Revision of Section 5.5 ALSAQ-40 to define calculation of the ALSAQ-40 total score and remove reference to an ALSAQ-40 symptom index. Revision of Section 6.1 Analysis Sets to define the Efficacy Relyvrio Free (ERF) analysis set that excludes participants who used sodium phenylbutyrate/taurursodiol at baseline or during their follow-up in the placebo-controlled period. Revision of Section 6.2 Baseline Characteristics to switch from ALSAQ-40 symptom index to ALSAQ-40 total score.

Version	Date	Description of Changes
2.0 (continued)	10 Oct 2023	Revision of Section 6.3 Primary Efficacy Analysis and Supportive Analyses to specify that baseline log-transformed serum NfL will be handled the same as previously specified covariates as described in Section 2.2.5 of the MPRDR and to add the EPP and ERF analysis sets for additional sensitivity analysis. Revision of Section 6.5.2 Combined Assessment of Function and Survival to add the ERF analysis set as an additional sensitivity analysis and to add long-term CAFS analyses of ALSFRS-R and SVC as functional measures and freedom from death and PAV or from death alone as survival components. Revision of Section 6.5.3 Repeated-measures Model to switch from ALSAQ-40 symptom index to ALSAQ-40 total score and to add the ERF analysis set as an additional sensitivity analysis of ALSFRS-R total score and SVC. Revision of Section 6.5.4 Random-slopes Model to switch from ALSAQ-40 symptom index to ALSAQ-40 total score and to fix omission of baseline NfL in the list of covariates in the model specification and template SAS code. Revision of Section 6.7.3 Concomitant Medication Use to add time to event summaries of post-baseline initiation of edaravone, riluzole, or sodium phenylbutyrate/taurursodiol individually and in combination among the subset of participants who were not on a given drug or combination of drugs at baseline. Deletion of Section 6.5.10 Comparison to External Controls.

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
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
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
ECM	Efficacy Common Mode of Administration
ECG	Electrocardiography or Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
ENCALS	European Network for the Cure of ALS
EPP	Efficacy Per-protocol
ERO	Efficacy Regimen-only
FAS	Full Analysis Set
FVC	Forced Vital Capacity

ABBREVIATIONS (continued)

GLI	Global Lung Initiative
HbA1c	Hemoglobin A1c
hCG	Human Chorionic Gonadotropin
HHD	Hand-held Dynamometry
ICF	Informed Consent Form
ITT	Intention-to-treat Principle
IV	Intravenous
LC3-II	Lipid Modified Form of Microtubule-associated Proteins 1A/1B Light Chain 3B
M-SAP	Master Statistical Analysis Plan
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
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
OLE	Open-label Extension
PAV	Permanent Assisted Ventilation
PK	Pharmacokinetics
PRO-ACT	Pooled Resource Open-Access ALS Clinical Trials
RBC	Red Blood Cell
RDW	RBC Distribution Width
RGE	Regimen E (trehalose)
RSA	Regimen-specific Appendix
R-SAP	Regimen-specific Statistical Analysis Plan
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SoA	Schedule of Activities
SOC	MedDRA System Organ Class
SOI	Start of Infusion

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
TRICALS	Treatment Research Initiative to Cure ALS
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
WBC	White Blood Cell
WHODrug	World Health Organization Drug Dictionary Enhanced

Table of Contents

1. Governing Documents

This Regimen-specific Statistical Analysis Plan (R-SAP) for the trehalose regimen (RGE) 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) and adds analyses specific to the open-label extension (OLE) period. 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 RGE Regimen-specific Appendix (RSA). Please refer to the Master Protocol and the RGE 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 RGE RSA and this R-SAP are authoritative in defining the primary and interim analyses. In case of discrepancies between the RGE 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 RGE 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 RGE 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	RGE RSA	MPRDR	M-SAP	RGE 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. RGE evaluates the safety and efficacy of trehalose administered weekly via intravenous (IV) infusion at a dosage of 0.75 g/kg vs. placebo. The RGE 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 trehalose injection, 90.5 mg/mL for IV infusion as compared to placebo on ALS disease progression.

Secondary Efficacy Objectives:

- To evaluate the effect of trehalose injection, 90.5 mg/mL for IV infusion on selected secondary measures of disease progression, including survival

Safety Objectives:

- To evaluate the safety of trehalose injection, 90.5 mg/mL for IV infusion for patients with ALS

Exploratory Efficacy Objectives:

- To evaluate the effect of trehalose injection, 90.5 mg/mL for IV infusion on selected biomarkers and endpoints.

2.3 Study Population

In addition to eligibility criteria specified in the Master Protocol, participants in RGE must not have a current diagnosis of or be treated for diabetes mellitus, have a screening serum glucose level greater than or equal to 140 mg/dL, have previously been treated with IV trehalose or have a known hypersensitivity to trehalose, currently be taking oral trehalose, be unable to return to the clinic for weekly infusions prior to approval for home infusions, or have a screening body weight greater than 144 kg. Detailed criteria are specified in the Master Protocol and the RGE 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 RGE 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 for the placebo-controlled period.

Participants in RGE who complete the placebo-controlled period may extend their participation into the OLE period. Consent to the OLE will be obtained prior to RGE screening and will typically be confirmed at the Week 16 visit. Participants determined eligible to enter the OLE will be dispensed OLE study drug at the Week 24 visit. This visit will serve as Day 0 for the OLE period. Note that references to "baseline" refer to the Baseline visit of the placebo-controlled period. References to the start of participation in the OLE will refer to OLE Day 0.

The duration and visit schedule of the OLE is not specified in the Master Platform protocol, but the guidance is to replicate the visit schedule of the RCT period over the first 24 weeks of the OLE, and the OLE period is typically extended to 52 weeks. Under that schedule, participants who are dispensed OLE study drug would be seen in clinic 4, 8, 16, 28, 40, and 52 weeks after OLE Day 0 and would complete telephone visits at 2, 12, 20, and 24 weeks after OLE Day 0 and

28 days after last dose of study drug or as otherwise specified in the applicable RSA. The OLE visit schedule and duration for a given regimen are specified in the applicable RSA.

Detailed descriptions of study procedures and timing are specified in the Master Protocol and the RGE RSA.

2.5 Regimen Allocation

Participants in RGE are those determined eligible for Master Protocol-level inclusion and exclusion criteria and randomly assigned to RGE, 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 RGE are randomly allocated in a 3:1 ratio to weekly IV trehalose at a dosage of 0.75 g/kg 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. All participants who enter the OLE period receive weekly IV trehalose at a dosage of 0.75 g/kg starting at OLE Day 0.

2.7 Treatment Administration

Trehalose and placebo are supplied as sterile fluids for IV administration. Each infusion bag of active study drug contains 300 mL of 90.5 mg/mL trehalose in aqueous solution. Each infusion bag of placebo contains 300 mL of US Pharmacopeia grade 0.9% sodium chloride in aqueous solution.

The first infusion of study drug should be administered while in the office/clinic on the day of the Baseline Visit after all visit assessments are complete.

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

2.8 Allocation Concealment

Allocation concealment is the same as described in the M-SAP. Both active and placebo infusion bags are identical in appearance.

2.9 RGE Schedule of Activities (SoA) during the Placebo-controlled Period

Activity	MP Scrn[1]	RGE Scrn[1]	Base-line[20]	Week 2[21]	Week 4	Week 8	Week 12[21]	Week 16[13]	Week 20[21]	Week 24/ET	Final Call[10]
	Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
	-42d to -1d[15]	-41d to 0d[15]	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	
Vital Signs at Infusions[5]				At weekly IV infusions Weeks 1 to 24							

Activity	MP Scrn[1] Clinic -42d to -1d[15]	RGE Scrn[1] Clinic -41d to 0d[15]	Base-line[20] Clinic Day 0	Week 2[21] Phone Day 14 ±3	Week 4 Clinic Day 28 ±7	Week 8 Clinic Day 56 ±7	Week 12[21] Phone Day 84 ±3	Week 16[13] Clinic Day 112 ±7	Week 20[21] Phone Day 140 ±3	Week 24/ET Clinic Day 168 ±7	Final Call[10] Phone 28d ±3 ALD
Slow Vital Capacity	X[14]		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	
12-Lead ECG	X									X	
Clinical Safety Labs[6, 17]	X		X		X	X		X		X	
Biomarker Blood Collection			X			X		X		X	
Biomarker Urine Collection[17]			X			X		X		X	
PK Blood Collection[14]			X			X					
DNA Collection[8] (optional)			X								
CSF Collection (optional)			X					X[12]			
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	
Adverse Event Review[7]	X	X	X	X	X	X	X	X	X	X	X
Medical Review at Infusions				At weekly IV infusions Weeks 1 to 24							
Suicidality C-SSRS			X		X	X		X		X	
Assignment to Regimen	X										
Randomization within Regimen			X								
Adjust Dose as Needed[20]					X	X		X		X[19]	
Administer/Dispense Study Drug			X[9]	At weekly IV infusions Weeks 1 to 24						X[10]	
Drug Accountability/Compliance				At weekly IV infusions Weeks 1 to 24[22]						X	
Exit Questionnaire										X	
Vital Status Determination[11]										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, ET = early termination, IV = intravenous, MP = Master Protocol, PK = pharmacokinetic, RGE = the trehalose 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 blood pressure, respiratory rate, heart rate, and temperature. Height measured at Master Protocol Screening Visit only. If an infusion will occur at the completion of the visit, vital signs should be collected within 30 minutes of the start of infusion to serve as the pre-infusion vital signs collection.

[5] During each infusion, vital signs associated with the infusion (systolic and diastolic blood pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion, 60 minutes after the start of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug, an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.

[6] Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function, and urinalysis. Hemoglobin A1C (HbA1c) will be included in labs at baseline, week 16, and week 24. 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.

[7] Adverse events that occur after signing the Master Protocol consent form will be recorded.

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

[9] Administer first dose of study drug only after Baseline Visit procedures are completed.

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

[11] 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 patient 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.

[12] 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.

[13] 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 reason.

[14] 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).

[15] Master Protocol Screening and Regimen Specific Screening visit windows are relative to Baseline (Day 0).

[16] Participants completing the PK collection will have blood collected for PK analysis at Baseline (Day 0) and Week 8 (Day 56) at the following time points: pre-dose and 1 hour ± 5 minutes, 2 hours ± 5 minutes, and 4 hours ± 5 minutes post start of infusion. At each time point, 4 mL of blood will be collected.

[17] All urine samples must be collected prior to IP administration.

[19] Dose is adjusted only for those participants continuing into the OLE.

[20] The weight collected during each in-clinic visit will be used to calculate the participant's dose. The participant will remain on a stable dose until the next in-clinic visit (Weeks 4, 8, 16) when weight is collected, at which point the dose may be adjusted if the participant's weight has increased or decreased by 2 kg. Additional information on dose adjustments is included in Section 5.6 Dosing Changes of the RGE RSA.

[21] If the participant receives an in-clinic infusion within the visit window, this visit may be conducted while the participant is in-clinic for their weekly infusion.

[22] Drug accountability will not be done at phone visits. Compliance will be automatically calculated in the EDC.

2.10 RGE SoA during the Open-label Extension Period

Activity	Week 2[8]	Week 4	Week 8[7]	Week 12[8]	Week 16[7]	Week 20[8]	Week 24[8]	Week 28[7]	Week 40[7]	Week 52/ET[6, 7]	Follow-Up Safety Call[4, 6]
	Clinic during Infusion	Clinic	Clinic	Phone	Clinic	Phone	Phone	Clinic	Clinic	Clinic	Phone
		Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day168 ±3	Day 196 ± 14	Day 280 ± 14	Day 364 ± 14	28d ±3 ALD
Vital Signs[1]		X	X		X			X	X	X	
Vital signs associated with infusion[9]	Weekly in conjunction with investigational product administration from OLE week 1 through OLE week 52										
Slow Vital Capacity		X	X		X			X	X	X	
ALSFRS-R		X	X	X	X	X	X	X	X	X	
ALSAQ-40								X		X	
CNS Bulbar Function Scale			X		X			X	X	X	
Clinical Safety Labs[2]		X	X		X			X	X	X	
Biomarker Blood Collection					X			X		X	
Biomarker Urine Collection					X			X		X	
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	
Adverse Event Review[3]	X	X	X	X	X	X	X	X	X	X	X
Document changes in health, medications, and infusion-related AEs	Weekly in conjunction with Investigational Product administration from OLE week 1 through OLE week 52										
Suicidality C-SSRS		X	X		X			X	X	X	
Adjust Dose as Needed[10]		X	X		X			X	X		
Administer/Dispense Study Drug	Weekly IV infusion from OLE week 1 through week 52										
Drug Accountability/Compliance	Weekly throughout study in conjunction with drug administration[11]										

Abbreviations: AE = adverse event, ALD = after last dose, ALSAQ-40 = ALS Assessment Questionnaire, ALSFRS-R = ALS Functional Rating Scale Revised, C-SSRS = Columbia-Suicide Severity Rating Scale, d = day, ET = early termination.

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

[2] Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function and urinalysis. HbA1c will be added to the safety labs at weeks 16, 28 and 52.

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.

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

[4] Participants who continue into OLE will have a Follow-Up Safety Call (as described in the body of the RGE RSA) after their last dose of investigational product during the OLE period.

[5] The duration of the OLE is 52 weeks.

[6] Participants who continue into the OLE and then withdraw consent or early terminate will be asked to complete an Early Termination Visit and Follow-Up Safety Call as described in the RGE RSA.

[7] 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 reason.

[8] If the participant receives an in-clinic infusion within the visit window, this visit may be conducted while the participant is in clinic for their weekly infusion.

[9] During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), 60 minutes after the start of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.

[10] The weight collected during each in clinic visit will be used to calculate the participant's dose. The participant will remain on a stable dose until the next in clinic visit (Weeks 4, 8, 16, 28, 40) when weight be collected, at which point the dose may be adjusted if the participant's weight has increased or decreased by 2 kg. Additional information on dose adjustments is included in Section 5.6 Dosing Changes of the RGE RSA.

[11] Drug accountability will not be done at phone visits. Compliance will be automatically calculated in the EDC.

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.

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. Exploratory endpoints and safety evaluations will report unadjusted p-values.

3.6 Missing Data

Handling of missing data is the same as described in the M-SAP except as indicated below. Missing baseline covariates will be mean imputed. Any transformed covariates will be transformed prior to mean imputation. Clinic-based assessments that are missing due to COVID-19 restrictions or disruptions are considered missing completely 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), 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 analysis of continuous 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 or reach a death equivalent before reaching the pre-specified time-to-event will be considered as having the unfavorable event being analyzed at the time of death or death equivalent.

4. Study Endpoints

4.1 Efficacy Endpoints

4.1.1 Primary Efficacy Endpoint

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

4.1.2 Key Secondary Efficacy Endpoints

The following key secondary efficacy endpoints will be evaluated hierarchically (see Section 6.5.1):

- Combined analysis of function and survival (CAFS) using ALSFRS-R total score as the functional measure, freedom from death and permanent assisted ventilation (PAV) as the survival component, evaluated to the Week 24 time point, and adjusting the rank-sums for baseline covariates of pre-baseline slope of ALSFRS-R, time since symptom onset, use of edaravone, use of riluzole, and log-transformed serum NfL level,

- Change from baseline to Week 24 in ALS functioning as assessed using ALSFRS-R total score change from baseline,
- Change from baseline to Week 24 in respiratory function as assessed by slow vital capacity (SVC) and measured as percent predicted using Global Lung Initiative (GLI) normal values,
- Change from baseline to Week 24 in upper limb muscle strength as assessed isometrically using hand-held dynamometry (HHD) and grip strength and measured as average percent change from baseline among maneuvers with non-zero strength at baseline,
- Change from baseline to Week 24 in lower limb muscle strength as assessed isometrically using HHD and measured as average percent change from baseline among maneuvers with non-zero strength at baseline, and
- Survival defined as freedom from death and PAV from baseline to Week 24.

4.1.3 Exploratory Efficacy Endpoints

The following exploratory efficacy endpoints will be evaluated:

- Change from baseline to Week 24 in ALSFRS-R domain scores: bulbar, fine motor, gross motor, respiratory, and fine and gross motor combined,
- Change from baseline to Week 24 in combined upper and lower limb muscle strength as assessed isometrically using HHD and grip strength and measured as average percent change from baseline among muscles or muscle groups with non-zero strength at baseline,
- Time to loss of measurable strength of the first (HHD0) and second ($HHD0^2$) muscle or muscle group with non-zero strength at baseline,
- Change from baseline to Week 24 in biofluid biomarkers of neurodegeneration, neuromuscular degeneration, and autophagy: serum creatinine, serum and cerebrospinal fluid (CSF) neurofilament light chain (NfL), and the lipid modified form of microtubule-associated proteins 1A/1B light chain 3B (LC3-II) in CSF,
- 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 total score, and CNS-BFS total score,
- Time to clinical events during the placebo-controlled period: 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 PAV, or death,
- Survival defined as freedom from death and PAV and freedom from death alone from baseline to the last participant's last visit in the placebo-controlled period (RCT LPLV) analyzed in the ERO analysis set,
- CAFS analyses of ALSFRS-R total score and SVC as functional measures, freedom from death and PAV or from death alone as survival components, evaluated to the RCT LPLV time point, and adjusting the rank-sums for baseline covariates of pre-baseline slope of ALSFRS-R, time since symptom onset, use of edaravone, use of riluzole, and log-transformed serum NfL level analyzed in the ERO analysis set.

4.2 Pharmacokinetic Endpoints

The following pharmacokinetic endpoints will be evaluated:

- Serum trehalose concentration at the following time points: pre-dose, 60 (±5) min after start of infusion (SOI), 120 (±5) min after SOI, and 240 (±5) min after SOI.

4.3 Safety Endpoints

The safety endpoints are the same as those described in the M-SAP with the addition of tabulations of adverse events and clinically significant ECG and lab abnormalities that occurred after crossover into the OLE period.

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 the 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 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, whichever occurs first.

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 last date that an HHD assessment was performed up to the end of the Week 24 Visit window.

5.4 Biofluid Biomarkers of Neurodegeneration

Blood biomarkers of neurodegeneration and neuromuscular degeneration 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. All serum NfL results that pass Quanterix quality control criteria will be analyzed for primary analyses using serum NfL, both as a baseline covariate and as an endpoint. A secondary analysis of serum NfL as an endpoint will exclude measured concentrations less than 4 ng/mL if they are less than 2% of the maximum measured concentration for a given participant. Levels of serum and CSF NfL will be log-transformed in all analyses.

5.5 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 total score will be calculated as the mean of all 40 items multiplied by 25 (range 0 to 100; Jenkinson et al. 2003). Domain scores and the total score will be missing if more than 20% of the applicable items are missing; otherwise, item non-response will be mean-imputed from other completed items from the same assessment. Higher scores indicate worse quality of life.

5.6 CNS-BFS

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

5.7 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 RGE participant. The primary analysis of survival will evaluate PAV-free survival to the Week 24 Visit time point.

5.8 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.

5.9 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.

5.10 TRICALS Risk Profile

The Treatment Research Initiative to Cure ALS (TRICALS) Risk Profile is based on the the European Network for the Cure of ALS (ENCALS) survival prediction model (Westeneng et al. 2018). The TRICALS score will be calculated at baseline as follows:

$$\begin{aligned}\text{Profile} = {} & 0.474\left[(VC/100)^{-1} + (VC/100)^{-1/2}\right] - 2.376\left[(DD/10)^{-1/2} + ln(DD/10)\right] \\ & - 1.839(dFRS + 0.1)^{-1/2} - 0.264(AAO/100) + 0.271\ \text{Bulbar} \\ & + 0.238\ \text{Definite} + 0.415\ \text{FTD}\end{aligned}$$

where *VC* is vital capacity in units of percent-predicted using GLI norms as defined in Section 5.2, *DD* is diagnostic delay calculated as (date of diagnosis – date of symptom onset) / 365.25 x 12, *dFRS* is pre-baseline slope of ALSFRS-R total score as defined in Section 5.1, *AAO* is age at symptom onset calculated as (date of symptom onset – date of birth) / 365.25, Bulbar is an indicator of initial site of onset in the bulbar region, Definite is an indicator for classification as definite ALS by revised El Escorial criteria, and FTD is an indicator for frontotemporal dementia. While participants are not evaluated for frontotemporal dementia at baseline, given that presence of dementia defined broadly is an exclusion criterion at screening, TRICALS scores will be calculated assuming that participants do not have frontotemporal dementia.

5.11 Pharmacodynamic Biomarkers

CSF LC3-II levels in samples collected at Baseline and Weeks 16 or 24 will be assayed. The method of analysis of CSF LC3-II will be specified once known. Levels of CSF LC3-II that are reported to be below the limit of quantitation will be imputed at the limit of quantitation. Levels of CSF LC3-II will be log-transformed in all analyses.

5.12 Clinical Safety Laboratory Tests

Clinical safety labs include hematology, blood chemistry panel, liver function tests, thyroid function, urinalysis, and pregnancy testing in women of childbearing potential as specified in Section 9.1.2 Clinical Safety Laboratory Tests of the Master Protocol:

- Hematology: hematocrit, hemoglobin, platelet count, red blood cell (RBC) count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, RBC distribution width (RDW), RBC morphology, white blood cell (WBC) count, and counts and percentages of basophils, eosinophils, lymphocytes, monocytes, and neutrophils;
- Blood chemistry panel: bicarbonate, chloride, potassium, sodium, calcium, magnesium, phosphate, blood urea nitrogen, creatinine, estimated glomerular filtration rate (eGFR) calculated using the Modification of Diet in Renal Disease (MDRD) four-variable equation, creatinine clearance calculated using the Cockcroft-Gault equation, glucose, and hemoglobin A1c;
- Liver function tests: alanine aminotransferase (ALT [SGPT]), aspartate aminotransferase (AST [SGOT]), alkaline phosphatase (ALP), albumin, total protein, total bilirubin (TBL);
- Thyroid function tests: thyroid-stimulating hormone (TSH);
- Urinalysis: clarity, color, specific gravity, pH, microalbumin, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, and blood; and

- Pregnancy: qualitative and quantitative serum human chorionic gonadotropin (hCG).

Clinical safety labs will also include derived measures of potential drug-induced liver injury (DILI), including those that potentially meet the Hy's law criteria, as distinct safety lab outcomes.

Three potential DILI criteria will be defined:

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

where ULN is upper level of normal and all levels are measured on the same day.

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 RGE plus placebo participants from regimens A-D who had an opportunity to complete placebo-controlled follow-up, 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 RGE plus placebo participants from regimens A-D who are not known to be ineligible for RGE 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 RGE is defined as follows:

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

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.

- 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, assessment, or use of other disease-modifying treatments for ALS, 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 are truncated for inclusion in the EPP analysis set due to a time-dependent event (e.g., non-adherence to protocol-specified dosing, initiation of a prohibited medication, initiation of edaravone, riluzole, or sodium phenylbutyrate/taurursodiol), 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.

An analysis set that excludes participants who used sodium phenylbutyrate/taurursodiol during their follow-up for the placebo-controlled period is defined as follows:

- Efficacy Relyvrio Free (ERF) Set: The subset of participants in the ERO who were not using sodium phenylbutyrate/taurursodiol at baseline and did not start using sodium phenylbutyrate/taurursodiol after baseline and prior to completion of their Week 24 visit or death, earlier termination, withdrawal, or loss to follow-up prior to the end of the Week 24 visit window if they did not complete a Week 24 visit.

Applicable analysis sets (FAS, ECC, EPP, and STF) will include shared placebo participants from regimens A, B, C, and D. As RGE is the only regimen among the contributing regimens that is administered as an IV infusion, the ERO and ECM analysis sets are synonymous and only the ERO analysis set will be referenced. Similarly, the SRO and STN analysis sets are synonymous and only the SRO analysis set will be referenced.

6.2 Baseline Characteristics

The baseline characteristics summarized for participants randomized within RGE are the same as those specified in the M-SAP with the addition of ALSAQ-40 total and domain scores, CNS-BFS total score, King's stage, TRICALS score, weight, body mass index (BMI), serum creatinine concentration, serum NfL concentration, and baseline use of sodium phenylbutyrate/taurursodiol.

6.3 Primary Efficacy Analysis and Supportive Analyses

The primary analysis for RGE 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 MPRDR with the addition of baseline log-transformed serum NfL as a covariate. Baseline log-transformed serum NfL will be handled the same as previously specified covariates with respect to standardization, imputation of missing values, model for covariate effect, and prior distributions as described in Section 2.2.5 of the MPRDR. The Bayesian shared-parameter, repeated-measures model will be applied to the FAS analysis set as the primary analysis and to the ECC, ERO, EPP, and ERF analysis sets as sensitivity analyses.

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: weekly IV infusion of trehalose at a dosage of 0.75 g/kg 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

RGE will be considered for early stopping for futility according to the interim analysis schedule and definition specified in the MPRDR. RGE 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. The primary CAFS analysis is described in Section 6.5.2. Primary estimates and inference for 24-week change of continuous endpoints will be by a mixed model repeated-measures analysis (see Section 6.5.3 below). Primary estimates and inference for time-to-event endpoints will be by Kaplan-Meier product-limit estimates and log-rank test (see Section 6.5.5 below). The sequence for testing secondary efficacy endpoints is the following:

1. CAFS using ALSFRS-R total score as the functional measure, freedom from death and PAV as the survival component, evaluated to the Week 24 time point, and adjusting the rank-sums for baseline covariates of pre-baseline slope of ALSFRS-R, time since symptom onset, use of edaravone, use of riluzole, and log-transformed serum NfL level,
2. Change from baseline to Week 24 in ALS functioning as assessed using ALSFRS-R total score change from baseline in the FAS,
3. Change from baseline to Week 24 in respiratory function as assessed by slow vital capacity (SVC) and measured as percent predicted using Global Lung Initiative normal values in the FAS,
4. Change from baseline to Week 24 in upper limb muscle strength as assessed isometrically using HHD and grip strength and measured as average percent change from baseline among maneuvers with non-zero strength at baseline in the FAS,
5. Change from baseline to Week 24 in lower limb muscle strength as assessed isometrically using HHD and measured as average percent change from baseline among maneuvers with non-zero strength at baseline in the FAS, and

6. Survival defined as freedom from death and permanent assisted ventilation (PAV) from baseline to Week 24 in the FAS.

If the primary analysis indicates a significant slowing in disease progression, 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.

6.5.2 Combined Assessment of Function and Survival

The first key secondary analysis for RGE is a Combined Assessment of Function and Survival (CAFS). CAFS is a joint-rank test of a composite endpoint combining function as measured by change in ALSFRS-R total scores and survival as measured by time to death or death equivalent. CAFS will be applied to the FAS as the principal CAFS analysis with the addition of baseline log-transformed serum NfL level as a covariate (see MPRDR for more details) and to the ECC, ERO, and ERF analysis sets as sensitivity analyses.

The estimand is the stochastic probability that a randomly selected active participant will rank higher than a randomly selected placebo participant based on time to death or death equivalent or change from baseline in ALSFRS-R in the FAS. The estimand is defined by the following attributes:

- Treatment: weekly IV infusion of trehalose at a dosage of 0.75 g/kg vs. placebo.
- Population: FAS as defined in Section 6.1.
- Variables: the rank-sum score for each participant derived from comparing each study participant's outcome to all others in the analysis set in a series of pairwise comparisons, based on first on survival up to Week 24 and second on absolute change from baseline in ALSFRS-R total score.
- 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 a 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. Pairs of participants will be ranked at the maximum follow-up time at which both participants have an observation during the placebo-controlled period. Missing data post-treatment will not be imputed and will be handled via a missing at random assumption.
- Population-level summary: the ANCOVA derived least-square mean rank-sum score for each treatment group, the LS mean estimated difference in rank-sum scores (adjusted for covariates: time since symptom onset, delta-FRS, baseline riluzole use, baseline edaravone use, and baseline log-transformed serum NfL) as well as the corresponding 2-sided p-value will be presented. A Gehan-Wilcoxon rank sum test will be performed for the FAS as a sensitivity analysis to the primary analysis.

Inference from this analysis is supportive of inference from the Bayesian shared-parameter, repeated-measures model described in MPRDR.

Including the analysis above, all combinations of the following elements will be tested in separate joint-rank analyses:

1. Survival endpoint: (a) death or death-equivalent, and (b) death alone.

2. Function endpoint: (a) ALSFRS-R total score, and (b) SVC percent-predicted.

3. Analysis: (a) Wilcoxon rank-sum test by t-distribution approximation, and (b) adjusted mixed model or linear regression controlling for time since ALS symptom onset, delta-FRS, baseline log-transformed serum NfL level, use of riluzole at baseline, and use of edaravone at baseline, and including a random effect of regimen in all analyses that include at least three regimens or a fixed effect of regimen otherwise.

Additional CAFS analyses of long-term change in function and survival will be performed using ALSFRS-R total score and SVC percent-predicted as functional measures, freedom from death and PAV and freedom from death alone as survival components, both evaluated to the RCT LPLV time point and adjusting the rank-sums for baseline covariates of pre-baseline slope of ALSFRS-R, time since symptom onset, use of edaravone, use of riluzole, and log-transformed serum NfL level analyzed in the ERO analysis set.

6.5.3 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, serum creatinine, serum NfL, ALSAQ-40 total and domain scores, and CNS-BFS total score) are revised from those specified in the M-SAP to include a main effect of treatment and its interaction with visit as additional covariates and to add the ERF analysis set for analysis of ALSFRS-R total score and SVC.

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, and centered baseline log-transformed serum NfL and centered baseline log-transformed serum NfL × visit interaction. The following equations describe the model with regimen random effects:

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

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, and centered baseline log-transformed serum NfL) 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 NfL|visit
                / solution cl;
  random intercept / subject=regimen type=un;
  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, `dFRS` is pre-baseline slope , `rlz` is an indicator of riluzole use at baseline, `edv` is an indicator of edaravone use at baseline, and `NfL` is baseline log-transformed serum NfL level. Covariates will be centered at their sample means. 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 an endpoint measured every 8 weeks and assuming 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. The estimand is defined by the following attributes:

- Treatment: weekly IV infusion of trehalose at a dosage of 0.75 g/kg vs. placebo.
- Population: FAS as defined in Section 6.1.
- Variables: absolute change in endpoint from baseline to the Week 24 Visit.
- Intercurrent event: treatment discontinuation: handled via a 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, and will be handled via a missing at random assumption.
- Population-level summary: difference in least-square means of active treatment relative to placebo.

Inference from these analyses is supportive of inference from the Bayesian shared-parameter, repeated-measures model.

6.5.4 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, serum creatinine, serum NfL, ALSAQ-40 total and domain scores, and CNS-BFS total score) are revised from those specified in the M-SAP to include a main effect of treatment and its interactions with study month 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, and centered baseline log-transformed serum NfL and centered baseline log-transformed serum NfL × month interaction. The following equations describe the model with regimen random effects:

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

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, and centered baseline log-transformed serum NfL) 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 $\sigma^2{}_\epsilon$. 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 NfL|visit
                / 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 6.5.3. Between-within denominator degrees of freedom will be used for endpoints with only two observations per participant; otherwise, denominator degrees of freedom will be calculated using containment rules. 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 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. The estimand is defined by the following attributes:

Treatment: weekly IV infusion of trehalose at a dosage of 0.75 g/kg vs. placebo.

Population: FAS 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 a 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 and will be handled via a 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.

6.5.5 Survival and Time to Clinical Events

Survival and time to hospitalizations and clinical events will be analyzed in the FAS, ECC, ERO, 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, baseline log-transformed serum NfL level, and baseline TRICALS score as covariates in adjusted models, and 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. 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.

6.5.6 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 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.

6.5.7 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 analyses of ALSFRS-R total score and SVC in the FAS.

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 NfL Wk00);
  fcs reg(Wk16 = sx2bl dFRS rlz edv NfL Wk00 Wk08);
  fcs reg(Wk24 = sx2bl dFRS rlz edv NfL Wk00 Wk08 Wk16);
  mnar model( Wk08 Wk16 Wk24 / modelobs=(trtrnd="0"));
  var sx2bl dFRS rlz edv NfL 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 . [missing value indicator] for SVC).

Inference from these analyses is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint and SVC in the FAS.

6.5.8 Inverse Probability Weights

To reduce bias from possible treatment-dependent loss to follow-up due to death, intolerance, or other causes and possible treatment-dependent initiation of other disease-modifying treatments for ALS, the effect of treatment on ALSFRS-R and SVC will be estimated in the mixed model repeated-measures analysis using visit-specific stable inverse probability weights (Avagyan et al. 2021) in the FAS and ERO analysis sets with censoring of observations collected after any post-baseline initiation of edaravone, riluzole, or sodium phenylbutyrate/taurursodiol. Weights will be calculated as the inverse of the predicted probability of loss to follow-up or initiation of other disease-modifying treatments, winsorized to the 5th and 95th percentiles. Probability of loss to follow-up or initiation other disease-modifying treatments for ALS will be predicted from a conditional random forests model (Hothorn et al. 2006) using all baseline characteristics specified in Section 6.2 Baseline Characteristics.

6.5.9 Principal Stratification

To reduce bias from possible treatment-dependent loss to follow-up due to death, intolerance, or other causes and possible treatment-dependent initiation of other disease-modifying treatments for ALS, the effect of treatment on ALSFRS-R and SVC will be estimated using the potential

outcome framework and principal stratification (Rubin 2006). We will estimate the visit-specific profile of average causal effects in the treatment- and visit-specific principal strata always survivors (i.e., the participants who would have lived to a given visit under both the active and placebo treatment exposures) and never initiators (i.e., the participants who would have not initiated another disease-modifying treatment for ALS prior to a given visit under both the active and placebo treatment exposure) in the FAS and ERO analysis sets. Time to loss to follow-up or initiation other disease-modifying treatments for ALS will be predicted from a conditional survival random forests model (Hothorn et al. 2006) using all baseline characteristics specified in Section 6.2 Baseline Characteristics.

6.5.10 Additional Sensitivity Analyses of Primary and Key Secondary Outcomes

Sensitivity analyses of primary and key secondary efficacy outcomes are as described in this R-SAP.

6.5.11 Subgroup Analyses

The following subgroups will be analyzed in the random-slopes model (see Section 6.5.4) for ALSFRS-R total score and SVC in the FAS by including subgroup, subgroup × month, and subgroup × treatment × month terms:

- El Escorial definite or probable and less than 18 months since onset of weakness (both criteria vs. not both),
- Pre-baseline ALSFRS-R slope (high vs. low by median split),
- Baseline plasma NfL concentration (high vs. low by median split),
- Baseline TRICALS score (more negative vs. less negative by median split),
- Baseline use of riluzole by concomitant medication log (yes vs. no),
- Baseline use of edaravone by concomitant medication log (yes vs. no), and
- Baseline use of riluzole and edaravone (neither, riluzole only, edaravone only, both).

Conditional on findings from initial analyses, the following subgroups will also be analyzed:

- 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),
- BMI (less than 25 kg/m^2, 25 to less than 30 kg/m^2, 30 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 or worse [eGFR less than 60 $mL/min/1.73m^2$]),
- 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.

6.5.12 Comparison of Controls across Regimens

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

6.5.13 Pharmacokinetic Analyses

Serum trehalose concentrations will be summarized by treatment group, time point (pre-dose and 1, 2, and 4 hours after SOI), 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.

Serum trehalose concentration data 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 trehalose, if deemed appropriate. The population pharmacokinetic analysis will be performed by Seelos and reported in a separate modelling report.

6.5.14 Pharmacodynamic Biomarker Analyses

Change in LC3-II levels in CSF will be summarized by treatment group and visit in the ERO. Summary statistics will be provided for the values, change from baseline, and percent change from baseline at each scheduled assessment time point. The effect of treatment on pharmacodynamic response will be estimated in the repeated-measure model (see Section 6.5.3). Pearson and Spearman correlations between change in LC3-II levels in the CSF and concurrent change in ALSFRS-R total scores and SVC percent-predicted will be estimated among participants with complete data.

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, 28 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.

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 lab results collected more than 28 days after last dose of study drug will not be tabulated, 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 that occur within 28 days or fewer after last dose of study drug will be included in shift tables along with visit-specific shifts.

6.6.3 ECG Results

Summaries of ECG parameters and findings are the same as specified in the M-SAP.

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 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 RGE vs. final screening for a non-RGE contributing 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 RGE screen failure will be summarized separately for all participants screened for RGE whether that was their final screening experience or not.

All participants in the FAS 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 that were not taken at baseline will be separately summarized and will be classified by ATC Therapeutic class and WHODrug Preferred base name.

Time to post-baseline initiation of edaravone, riluzole, or sodium phenylbutyrate/taurursodiol individually and in combination will be summarized by Kaplan-Meier product-limit estimates among the subset of participants who were not on a given drug or combination of drugs at baseline.

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. 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 . 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 and listed.

7. Validation

7.1 Primary Efficacy Analysis

Validation of the primary efficacy analysis is as specified in the M-SAP. In addition, Seelos will perform a replication of the primary efficacy results (including any sensitivity analyses) using an independent programmer.

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:

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Eric A. Macklin, PhD emacklin@mgh.harvard.edu Instructor in Medicine Eric Macklin Security Level: Email, Account Authentication (Required)	Eric A. Macklin, PhD Signature Adoption: Pre-selected Style Signature ID: 8E847DD4-0E85-42A1-B334-DFC089673751 Using IP Address: 12.191.26.4 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/10/2023 4:02:37 PM Viewed: 10/10/2023 4:07:44 PM Signed: 10/10/2023 4:08:09 PM
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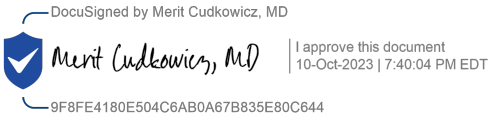
Signer Events	Signature	Timestamp
Merit Cudkowicz, MD cudkowicz.merit@mgh.harvard.edu Chief of Neurology Security Level: Email, Account Authentication (Required)	DocuSigned by Merit Cudkowicz, MD  I approve this document 10-Oct-2023 | 7:40:04 PM EDT 9F8FE4180E504C6AB0A67B835E80C644 Signature Adoption: Pre-selected Style Signature ID: 9F8FE418-0E50-4C6A-B0A6-7B835E80C644 Using IP Address: 174.242.65.152 Signed using mobile With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/10/2023 4:02:36 PM Viewed: 10/10/2023 7:39:08 PM Signed: 10/10/2023 7:40:25 PM
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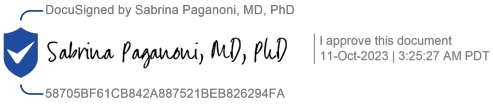
Sabrina Paganoni, MD, PhD spaganoni@mgh.harvard.edu MD PhD MGH Security Level: Email, Account Authentication (Required)	DocuSigned by Sabrina Paganoni, MD, PhD  I approve this document 11-Oct-2023 | 3:25:27 AM PDT 58705BF61CB842A887521BEB826294FA Signature Adoption: Pre-selected Style Signature ID: 58705BF6-1CB8-42A8-8752-1BEB826294FA Using IP Address: 73.182.245.163 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 10/10/2023 4:02:36 PM Viewed: 10/11/2023 6:25:09 AM Signed: 10/11/2023 6:25:29 AM
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Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
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Certified Delivered	Security Checked	10/11/2023 9:38:32 AM
Signing Complete	Security Checked	10/11/2023 9:39:09 AM
Completed	Security Checked	10/13/2023 3:23:41 PM

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