HEALEY ALS Platform Trial – Regimen G for DNL343

NCT05842941

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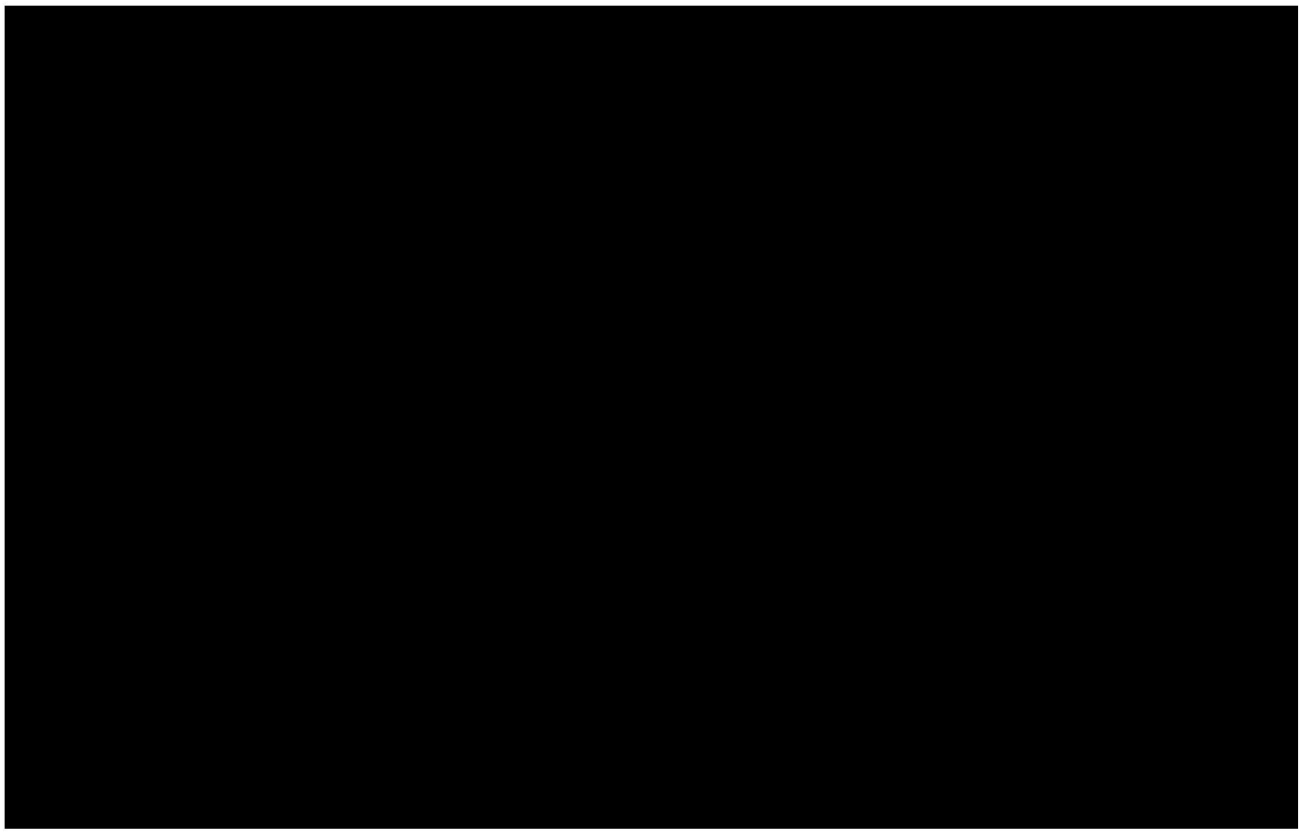
RGG REGIMEN-SPECIFIC STATISTICAL ANALYSIS PLAN (R-SAP)

Title	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	RGG: DNL343
Regimen Partner	Denali Therapeutics
Regulatory Sponsor	Merit E. Cudkowicz, MD
Master Protocol Version	5.0, 15 Dec 2022
RSA Version	2.0, 18 Dec 2023
Master SAP Version	3.0, 06 Feb 2023
R-SAP Version	3.0, 16 Sep 2024

SAP APPROVAL SIGNATURES



SAP REVISION HISTORY

Version	Date	Description of Changes
1.0	11 Jan 2024	Initial version
2.0	03 Jul 2024	Revisions throughout to incorporate analyses of data from the active treatment extension (ATE). Revision of Section 2.6 Treatment Allocation to describe crossover to active study drug at the start of ATE participation. Addition of Section 2.10 RGG Schedule of Activities – Active Treatment Extension to describe assessments completed during ATE participation. Revision of Section 4.2.1 Continuous Endpoints to describe additional time points at which selected endpoints would be analyzed. Revision of Section 4.2.2 Time-to-event Endpoints to include additional time points at which selected endpoints would be analyzed. Revision of Section 4.3 Safety Endpoints to include additional time points at which selected endpoints would be analyzed. Revision of Section 5.4 Biofluid Biomarkers to describe data to use for analysis serum neurofilament light chain protein (NfL) concentrations. Revision of Section 5.6 Survival to describe additional time points at which selected endpoints would be analyzed. Revision of Section 5.8 TRICALS Risk Score to add an exponent on age at symptom onset in the equation for the profile risk score. Revision of Section 6.1 Analysis Sets to describe versions of the efficacy regimen-only (ERO) analysis set that include differing amounts of ATE data. Revision of Section 6.4 Interim Analyses to describe an interim analysis of the RGG ATE using all RGG RCT and ATE data collected up to and including the time point at which the last participant had an opportunity to complete RCT Week 24. Revision of Section 6.5.1 Hierarchical Testing to add a survival endpoint of death or PAV evaluated at the time point at which the last participant had an opportunity to complete the RCT Week 76 (ATE Week 52) visit. Revision of Section 6.5.2 Repeated-measures Model to describe the model for analysis sets with more than 3 contributing regimens and inclusion of regimen-specific random effects.

Version	Date	Description of Changes
2.0 (continued)	03 Jul 2024	Revision of Section 6.5.4 Time-to-event Endpoints to describe additional time points at which selected endpoints would be analyzed. Revision of Section 6.5.5 CAFS to describe additional time points at which selected endpoints would be analyzed. Revision of Section 6.5.7 Subgroup Analyses to specify efficacy endpoints and remove several subgroups from planned analyses. Addition of Section 6.5.11 Partial-linear Spline Mixed Models to describe partial-linear spline models for analysis of long-term change in selected endpoints. Revision of Section 6.6 Safety Analyses to describe additional summaries of safety data collected during ATE participation. Revision of Section 6.7.1 Participant Disposition to describe summaries of initiation, completion, and withdrawal from ATE participation. Revision of Section 6.7.2 Study Drug Compliance and Tolerance to describe additional summaries of study drug exposure during ATE participation. Revision of Section 6.7.3 Concomitant Medication Use to describe summaries of initiation of concomitant medications after first dose of ATE study drug Revision of Appendix 1, Section 3.1 Simulation Scenarios to clarify assumptions related to the effects of standard of care medications.
3.0	16 Sep 2024	Revision of Section 5.8 TRICALS Risk Score to reference the source of coefficients used in the TRICALS risk score calculation. Revision of Section 6.1 Analysis Sets to define the Efficacy Common Mode of Administration (ECM), Efficacy Per-protocol (EPP), and Safety Narrow (STN) analysis sets. Revision of Section 6.3 Primary Efficacy Analysis and Supportive Analyses to specify use of the ECM and EPP analysis sets. Revision of Section 6.5.3 Random-slopes Model to include an equation describing the model and to update the template SAS code to identify change from baseline as the response measure. Revision of Section 6.5.4 Time-to-event Endpoints to specify that log-rank p-values will be calculated using a randomization test if fewer than 10 events are observed in either treatment group.

Version	Date	Description of Changes
3.0 (continued)	16 Sep 2024	Revision of Section 6.6.1 Treatment-emergent Adverse Events to specify additional summaries of treatment-emergent adverse events in the STN analysis set. Revision of Section 6.7.2 Study Drug Compliance and Tolerance to specify summaries of compliance with planned study drug exposure. Revision of Appendix 1 Section 1.0 Introduction to specify that the primary analysis population excludes RGF participants who were also randomized within RGG.

ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ALD	After Last Dose
ALS	Amyotrophic Lateral Sclerosis
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire, 40-item version
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale, Revised
ATE	Active Treatment Extension
BLQ	Below the Limit of Quantitation
CAFS	Combined Assessment of Function and Survival
CBC	Complete Blood Count
CGI-C	Clinical Global Impression of Change
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
DILI	Drug-induced Liver Injury
DNA	Deoxyribonucleic Acid
DRR	Disease Rate Ratio
ECC	Efficacy Concurrent Control
ECG	Electrocardiography or Electrocardiogram
ENCALS	European Network for the Cure of ALS
ERO	Efficacy Regimen-only
ET	Early Termination
FAS	Full Analysis Set
FTD	Frontotemporal Dementia
GLI	Global Lung Initiative
HHD	Hand-held Dynamometry
ICF	Informed Consent Form
INR	International Normalized Ratio
LPLV	Last Participant Last Visit
M-SAP	Master Statistical Analysis Plan
MedDRA	Medical Dictionary for Regulatory Activities

ABBREVIATIONS (continued)

MITOS	ALS Milano-Torino Staging
MMRM	Mixed-model Repeated-measures
MPRDR	ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report
NEALS	Northeast ALS
NfL	Neurofilament Light Chain Protein
PADSR	Primary Analysis, Design & Simulation Report
PAV	Permanent Assisted Ventilation
PGI-C	Patient Global Impression of Change
PGI-S	Patient Global Impression of Severity
PK	Pharmacokinetics
PKA	Pharmacokinetics Analysis Set
PRO-ACT	Pooled Resource Open-Access ALS Clinical Trials
PT	Prothrombin Time
PTT	Activated Partial Thromboplastin Time
PRO	Patient-reported Outcome
QD	Qua Dia (Once Daily)
RCT	Placebo-controlled Period
RGF	Regimen F (ABBV-CLS-7262)
RGG	Regimen G (DNL343)
RMST	Restricted Mean Survival Time
ROADS	Rasch-built Overall ALS Disability Scale
RSA	Regimen-specific Appendix
R-SAP	Regimen-specific Statistical Analysis Plan
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SCC	Safety Concurrent Controls
SoA	Schedule of Activities
SOC	MedDRA System Organ Class
SRO	Safety Regimen-only
STF	Safety and Tolerability Full

ABBREVIATIONS (continued)

SVC	Slow Vital Capacity
T4	Thyroxine
TEAE	Treatment-emergent Adverse Event
TRICALS	Treatment Research Initiative to Cure ALS
TSH	Thyroid Stimulating Hormone

Table of Contents

1. Governing Documents

This Regimen-specific Statistical Analysis Plan (R-SAP) for the Denali DNL343 regimen (RGG), including the "ALS Master Protocol Regimen G (DNL343) Primary Analysis, Design & Simulation Report" (RGG PADSR) as Appendix 1, specifies any modification from the default outcome measures, analysis samples, and planned analyses for the placebo-controlled period (RCT) of the HEALEY ALS Platform Trial as specified in the Master SAP (M-SAP), including planned analyses and summaries of long-term survival, change in function, and safety using all available data from the RCT and active treatment extension (ATE) periods at the times of the first data freeze after the last participant had an opportunity to complete each of the RCT Week 24, RCT Week 52 (ATE Week 28) and RCT Week 76 (ATE Week 52) visits. The M-SAP and this R-SAP supplement the Master Protocol, the "ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report" (MPRDR, Appendix 1 of the M-SAP), and the RGG Regimen-specific Appendix (RSA). Please refer to the Master Protocol and the RGG 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 MPRDR, any regimen-specific deviations described in the RGG RSA, and this R-SAP, including Appendix 1, are authoritative in defining the primary and interim analyses. In case of discrepancies between the RGG 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 RGG 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. In case of discrepancies between the MPRDR and RGG PADSR (Appendix 1 of this R-SAP) concerning the primary and interim analyses, the RGG PADSR is authoritative. On all matters not related to analysis, the Master Protocol and the RGG 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	RGG RSA	MPRDR	M-SAP	RGG R-SAP
Use of shared placebos	1	4	2	3	5
Primary and interim analysis specifications not related to use of shared placebo	1	4	3	2	5
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. RGG evaluates the safety and efficacy of DNL343 administered orally, once daily (QD) at a dosage of 200 mg vs. placebo. The RGG 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 and Endpoints

Primary Efficacy Objective:

- To evaluate the efficacy of DNL343 as compared to placebo on ALS disease progression.

Secondary Efficacy Objective:

- To evaluate the effect of DNL343 on selected secondary measures of ALS disease progression, including survival.

Safety Objectives:

- To evaluate the safety of DNL343 in participants with ALS.

Exploratory Objectives:

- To evaluate the effect of DNL343 on selected biomarkers and endpoints.
- To evaluate DNL343 concentrations in plasma and cerebrospinal fluid (CSF).

Primary Efficacy Endpoint:

- Change from baseline through Week 24 in disease severity as measured by the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) total score and survival.

Secondary Efficacy Endpoints:

- Change in ALSFRS-R total score from baseline to Week 24
- Combined Assessment of Function and Survival (CAFS) to Week 24
- Change in respiratory function as assessed by slow vital capacity (SVC) from baseline to Week 24
- Change in muscle strength as measured isometrically using hand-held dynamometry and grip strength from baseline to Week 24
- Survival evaluated as time to death or permanent assisted ventilation (PAV)
- Survival evaluated as time to death
- Change in log-transformed serum neurofilament light protein (NfL) concentration from baseline to Week 24

Safety Endpoints:

- Treatment-emergent adverse events and serious adverse events
- Changes in laboratory values and treatment-emergent and clinically significant laboratory abnormalities
- Changes in ECG parameters and treatment-emergent and clinically significant ECG abnormalities
- Treatment-emergent suicidal ideation and suicidal behavior

Exploratory Endpoints:

- Changes in patient reported outcomes (PROs) (Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 [ALSAQ-40], Center for Neurologic Study Bulbar Function Scale [CNS-BFS], Rasch-built Overall Amyotrophic Lateral Sclerosis Disability Scale [ROADS], Patient Global Impression of Change [PGI-C], Patient Global Impression of Severity [PGI-S])
- Change in body weight
- Changes in biofluid biomarkers (urine, plasma, optional CSF)
- DNL343 plasma and optional CSF concentrations and CSF-to-plasma concentration ratios
- Changes in clinician reported outcome (Clinician Global Impression of Change [CGI-C])

2.3 Study Population

In addition to eligibility criteria specified in the Master Protocol, participants in RGG must not have a diagnosis of epilepsy, a recent history of seizures, hypersensitivity to DNL343 or its excipients, or recent use of several classes of drugs with potential adverse drug-drug interactions. Detailed eligibility criteria are specified in the Master Protocol and the RGG RSA.

Participants will be recruited from approximately 80 centers located throughout the US that are part of the Northeast ALS (NEALS) Consortium.

2.4 Participant Flow

Participants in RGG 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, including follow-up during both the RCT and ATE periods. PAV use and vital status are tracked for all participants throughout their follow-up during both the RCT and ATE periods. A final assessment of PAV use and vital status will be completed at the time of the last participant, last visit (LPLV) of each of the RCT and ATE periods. Detailed descriptions of study procedures and timing are specified in the Master Protocol and the RGG RSA.

2.5 Regimen Allocation

Assignment of participants to RGG is the same as described in the M-SAP.

2.6 Treatment Allocation

Approximately 240 participants assigned to RGG who are determined eligible will be randomly allocated to DNL343 or placebo. Approximately 180 will be randomized to DNL343 administered QD at a dosage of 200 mg, approximately 60 participants will be randomized to placebo. Treatment assignments are based on a pre-specified permuted-block randomization schedule stratified for all combinations of use vs. non-use of riluzole, edaravone, and sodium phenylbutyrate/ursodoxicoltaurine (Relyvrio® or Albrioza®) at the time of screening for the Master Protocol. After completion of 24-week RCT follow-up, all participants still on study will enter the ATE period and receive DNL343 200 mg QD for a minimum of an additional 52 weeks to a maximum of an additional 78 weeks.

2.7 Treatment Administration

Details of treatment administration are described in the RGG RSA.

2.8 Allocation Concealment

Allocation concealment is the same as described in the M-SAP.

2.9 RGG Schedule of Activities – Placebo-Controlled Period

Activity	Master Protocol Screening[1]	Regimen Specific Screening[1]	Baseline[1]	Week 2	Week 4[12]	Week 8[12]	Week 12	Week 16[12]	Week 20	Week 24 or ET Visit[19]	Follow-Up Safety Call[10]
	Clinic	Clinic	Clinic	Phone	Clinic	Clinic	Phone	Clinic	Phone	Clinic	Phone
	42 to 1 Days[13]	41 to 0 Day[13]	Day 0	Day 14 ±3	Day 28 ±7	Day 56 ±7	Day 84 ±3	Day 112 ±7	Day 140 ±3	Day 168 ±7	28 days ALD ± 3 days
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[12]		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		X	
CNS-BFS			X			X		X		X	
ROADS			X					X		X	
PGI-C[17]								X		X	
PGI-S[17]			X					X		X	
CGI-C[18]								X		X	
12-Lead ECG	X		X		X	X		X		X	
Clinical Safety Labs[5]	X		X		X	X		X		X	
Coagulation Laboratory Tests (PT, PTT, INR)	X							X			
Endocrinology Safety Laboratory Tests (blood ACTH, cortisol, free T4)[14]			X							X (for all participants prior to ATE)	
Biomarker Blood Collection			X			X		X		X	
Pharmacokinetic Blood Collection[15]					X (pre, post)	X (post)		X (post)		X (pre, post)	
Biomarker Urine Collection			X			X		X		X	
DNA Collection[7] (optional)			X								
CSF Collection[16] (optional)			X							X	
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
Columbia-Suicide Severity Rating Scale			X		X	X		X		X	
Assignment to the Regimen	X										
Randomization within the Regimen			X								
Administer/Dispense Study Drug			X[8]		X	X		X		X[9]	
Study Drug Accountability/Compliance				X[20]	X	X	X[20]	X	X[20]	X	
Review Contraception Method[21]		X	X	X	X	X	X	X	X	X	X
Exit Questionnaire										X	
Vital Status Determination[11]										X	

Abbreviations: ACTH, adrenocorticotropic hormone; ALD, after last dose, ALS, amyotrophic lateral sclerosis; ALSAQ-40, Amyotrophic Lateral Sclerosis Assessment Questionnaire-40; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ATE, active treatment extension; CBC, complete blood cell count; CGI-C, Clinician Global Impression of Change; CNS, central nervous system; CNS-BFS, Center for Neurologic Study Bulbar Function Scale; CSF, cerebrospinal

fluid; ECG, electrocardiogram; ET, early termination; fT4, free thyroxine; ICF, informed consent form; INR, international normalized ratio; PK, pharmacokinetic; ROADS, Rasch-built Overall Amyotrophic Lateral Sclerosis Disability Scale; PAV, permanent assisted ventilation; PGI-C, Patient Global Impression of Change; PGI-S, Patient Global Impression of Severity; PK, pharmacokinetic; PT, prothrombin time; PTT, partial thromboplastin time; TEAE, treatment-emergent adverse event; TSH, thyroid-stimulating hormone.

[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 ICF. After a participant is randomly assigned 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 will be measured at the Master Protocol Screening Visit only.

[5] Clinical safety laboratory tests include hematology (CBC with differential), complete chemistry panel, thyroid function, and urinalysis. Serum pregnancy testing will occur in participants of childbearing potential at the Master Protocol Screening Visit and as necessary during the study. Urine pregnancy testing will occur at every in clinic visit for women of childbearing potential.

[6] Adverse events that occur after signing the consent form will be recorded. If a TEAE is reported that results in dose modification or dose interruption or for severe TEAEs that require an in-clinic evaluation, an unscheduled PK sample should be obtained as needed.

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

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

[9] Study drug will only be dispensed at this visit if the participant continues in the ATE.

[10] Participants will only have a Follow-Up Safety Call at this time if they terminate early from the study. Otherwise, participants will have a Follow-Up Safety Call after their last dose of study drug during the ATE period. Refer to Section 6.9 for additional details regarding the process for early terminations.

[11] Vital status, defined as a determination of date of death or PAV status 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, last visit (LPLV) of the Regimen. We may also ascertain vital status (death or date last known alive only) at later time points by using publicly available data sources as described in Section 8.15 of the Master Protocol.

[12] 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, disability, or a medically necessary reason. Refer to Section 6.9 for additional details regarding the process for early terminations.

[13] Master Protocol Screening and Regimen-Specific Screening Visit windows are relative to Baseline (Day 0).

[14] Endocrinology safety laboratory tests (blood ACTH, cortisol, free T4) may be performed additionally during the study as clinically indicated based on signs and symptoms. Collect blood sample for measurement of ACTH/cortisol at approximately the same time in the morning for each endocrinology laboratory assessment, if possible. It is not considered a protocol deviation if not taken at the same time in the morning as previous endocrinology laboratory assessments.

[15] For visits with a predose PK sample (Week 4 and Week 24), these visits should be scheduled for the morning and participants should take their dose in the clinic at approximately the same time in the morning as usual. The predose sample should be collected prior to dosing (ie, any time 0 to 2 hours before the dose) and approximately 24 hours after the most recent dose. The postdose sample can be collected anytime between 1 to 8 hours postdose. For the Week 4 Visit, if it is not possible to collect a predose PK sample, 2 postdose samples should be collected (at least 1 hour apart). The Week 24 Visit is the first day of the ATE and, therefore, the ATE dose would be provided in the clinic with a predose and postdose PK sample obtained. Additional unscheduled PK samples should be collected as needed as part of assessment for TEAEs leading to dose reduction or dose interruption or for severe TEAEs requiring an unscheduled visit for assessment.

[16] CSF collection is scheduled to occur for this Regimen during the placebo-controlled period. CSF collection may be performed on a separate date from the other assessments scheduled for the visits. For the Baseline visit, CSF may be collected at any time within one week prior to administration of the first dose of study medication after Regimen G eligibility is confirmed. Post treatment CSF collection will only be collected for participants with baseline CSF collected. For the Week 24 Visit, CSF may be collected at any time within the allowed visit window of the Week 24 Visit as defined in the protocol. For participants continuing into ATE, CSF collection must occur prior to taking the first dose of study medication from the ATE period. For the early termination (ET) visit, CSF collection is to be completed in participants with a Baseline CSF collection and only if the ET visit occurs at or after the Week 12 visit.

[17] PGI-C and PGI-S should be completed by the participant prior to other clinical assessments scheduled during the visit whenever possible.

[18] CGI-C should be completed after all clinical assessments are completed by the investigator whenever possible.

[19] The assessments listed must be completed for Early Termination Visits that occur prior to Week 24 in the placebo-controlled treatment period.

[20] 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.

[21] For female participants of childbearing potential and for men with a female partner of childbearing potential, review and document current contraceptive method(s) and ensure they meet study requirements.

2.10 RGG Schedule of Activities – Active Treatment Extension

Study Week in ATE [5]	Week 2	Week 4 [7]	Week 8 [7]	Week 12	Week 16 [7]	Week 20	Week 24	Week 28 [7]	Week 40 [7]	Week 52 or ET Visit [6,8]	All visits occurring after week 52 (every 13weeks) [7]	Follow-Up Safety Call [4,6]
Study Week from Baseline Visit	Week 26	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52	Week 64	Week 76	Week 89 and Week 102	
	Phone	Clinic	Clinic	Phone	Clinic	Phone	Phone	Clinic	Clinic	Clinic	Clinic	Phone
Activity	Day 14 ± 3	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	84 days (± 14 days) from last visit after 52 weeks	28 days after last dose ± 3 days
Vital Signs [1]		X	X		X			X	X	X	X	
12-lead ECG		X	X		X			X	X	X	X	
Slow Vital Capacity		X	X		X			X	X	X	X	
ALSFRS-R		X	X	X	X	X	X	X	X	X	X	
ALSAQ-40								X		X[11]		
CNS-BFS			X		X			X	X	X	X	
ROADS								X		X[11]		
PGI-C								X		X[11]		
PGI-S								X		X[11]		
CGI-C								X		X[11]		
Clinical Safety Laboratory Tests [2]		X	X		X			X	X	X	X	
Biomarker Blood Collection					X			X		X	X	
Biomarker Urine Collection					X			X		X	X	
Concomitant Medication Review	X	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	X
Columbia-Suicide Severity Rating Scale		X	X		X			X	X	X	X	
Administer/ Dispense Study Drug			X		X			X	X	X[9]	X[9]	
Study Drug Accountability/ Compliance	X[12]	X	X	X[12]	X	X[12]	X[12]	X	X	X	X	
Review contraception method[13]	X	X	X	X	X	X	X	X	X	X	X	X
Vital Status Determination[10]										X	X	

Abbreviations: ALSAQ-40, Amyotrophic Lateral Sclerosis Assessment Questionnaire-40; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ATE, active treatment extension; CBC, complete blood cell count; CGI-C, Clinician Global Impression of Change; CNS, central nervous system; CNS-BFS, Center for Neurologic Study Bulbar Function Scale; ECG, electrocardiogram; ET, early termination; PAV, permanent assisted ventilation; PGI-C, Patient Global Impression of Change; PGI-S, Patient Global Impression of Severity; PK, pharmacokinetic; ROADS, Rasch-built Overall Amyotrophic Lateral Sclerosis Disability Scale; RSA, Regimen-Specific Appendix; TEAE, treatment-emergent adverse event.

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

[2] Clinical safety laboratory tests include hematology (CBC with differential), complete chemistry panel, thyroid function, and urinalysis. Serum pregnancy testing will occur in participants of childbearing potential at the Master Protocol Screening Visit

and as necessary during the study. Urine pregnancy testing will occur at every in clinic visit for women of childbearing potential.

[3] Adverse events that occur after signing the consent form will be recorded. If a TEAE is reported that results in dose modification or dose interruption or for a severe TEAE that requires an in-clinic evaluation, an unscheduled PK sample should be obtained.

[4] All participants will have a Follow-Up Safety Call (as described in the body of this RSA) after their last dose of study drug during the ATE period. Refer to Section 6.9 for additional details regarding the process for early terminations.

[5] The duration of the ATE is a minimum of 52 weeks with a maximum of 78 weeks per participant and will be completed when all active participants have completed the 52-week Visit in the ATE.

[6] Participants who withdraw consent or early terminate during the ATE will be asked to complete an Early Termination Visit and Follow-Up Safety Call as described in the body of this RSA. Refer to Section 6.9 for additional details regarding the process for early terminations.

[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, disability, or a medically necessary reason.

[8] All visits after the 52-week Visit in the ATE will be in-clinic visits that occur at 13-week (96 days ±14 days) intervals. Participants will continue in the ATE until all active participants have completed a minimum of 52 weeks of the ATE; no participant will exceed 78 weeks in the ATE.

[9] For participants returning for another visit only.

[10] Vital status, defined as a determination of date of death or PAV status or date last known alive, will be determined for each participant at the end of the ATE portion of their follow-up. If at that time the participant is alive, his or her vital status should be determined again at the time of the last participant, last visit (LPLV) of the Regimen ATE. We may also ascertain vital status (death or date last known alive only) at later time points by using publicly available data sources as described in Section 8.15 of the Master Protocol.

[11] Not required for early terminations that occur after Week 52 in the ATE.

[12] 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.

[13]For female participants of childbearing potential and for men with a female partner of childbearing potential, review and document current contraceptive method(s) and ensure they meet study requirements.

3. General Considerations for Data Analysis

3.1 Statistical Software

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

3.2 Summary Statistics

Data will be summarized with respect to disposition, demographics, pre-treatment characteristics, efficacy endpoints, safety endpoints, and tolerability. Summary statistics for continuous variables will include the number of observations, the mean, median, standard deviation, inter-quartile range, and range. Summaries of categorical data will include counts, denominators, and percentages.

3.3 Precision

Results will generally be reported to 3 significant figures. Percentages will generally be reported to 0.1 percentage points. P-values will generally be reported to two decimal places when greater than or equal to 0.09995, to four decimal places when greater than or equal to 0.0001 and less than 0.09995, and as <0.0001 for smaller values.

3.4 Transformations

Data that are strictly positive, continuous, and strongly right skewed will typically be log-transformed prior to any inferential testing. Skewness greater than 3 will be used as a guide in determining which variables to transform. This R-SAP will be updated as needed prior to regimen data lock to indicate which variables warrant transformation and how they will be transformed. Original, untransformed values will be used for all summaries. Any data transformations used in the primary and interim analyses are described in the MPRDR (Appendix 1 of the M-SAP).

3.5 Multiplicity Adjustments

A single primary analysis is planned. The default criterion for significance at the final analysis is adjusted to ensure an overall one-sided type 1 error rate less than 0.025 in the absence of early stopping for futility. If the primary efficacy endpoint meets the success criteria, secondary efficacy endpoints will be tested comparing randomization to DNL343 versus placebo in a pre-specified order using a fixed sequence procedure with a two-sided alpha level of 0.05 for each endpoint. After the first failure to declare significance, no endpoints lower in the sequence can be significant. Nominal comparison-wise p-values will also be reported for all analyses unless otherwise specified.

3.6 Missing Data

If baseline values of a given measure are missing, the last observed pre-treatment value will be used. Missing baseline covariates of specified analyses will be imputed using the mean of the respective covariate. For any baseline covariate that is transformed for analysis, means will be imputed after transformation. Instances of missing baseline covariates are assumed to be rare. This R-SAP will be updated as needed prior to regimen data lock if the prevalence of missing baseline covariates warrants more specific handling.

For analyses that depend on visit-specific data, off-schedule post-baseline observations, e.g., those collected as part of an Early Termination Visit or an unscheduled visit, will be used in place of the closest missing scheduled visit that preserves the true visit sequence if the observation time is as close or closer to the missing scheduled visit than to any other post-baseline scheduled visit. Other observations will not be carried forward. For analyses that do not depend on visit-specific data, observations will be analyzed according to the actual time observed.

The planned mixed model analyses, where applied, yield estimates that are unbiased conditional on the observed values under a missing at random assumption. Secondary analyses of efficacy endpoints that accommodate missing values are described below (see Sections 6.5.5).

4. Study Endpoints and Comparisons

4.1 Efficacy Endpoints

The primary efficacy endpoint is change from baseline through Week 24 in disease severity as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) total score and survival.

Secondary efficacy endpoints include change from baseline through Week 24 in ALSFRS-R total score, CAFS analysis of ALSFRS-R and death or death-equivalent to Week 24, change from baseline through Week 24 in respiratory function as assessed by slow vital capacity (SVC),

change from baseline through Week 24 in serum neurofilament light chain protein (NfL), change from baseline through Week 24 in muscle strength of upper extremities as measured isometrically using hand-held dynamometry (HHD) and grip strength as a percentage of baseline, time to death or death-equivalent to RCT Week 24 and to end of follow-up at the time points at which the last participant had an opportunity to complete each of the RCT Week 52 (ATE Week 28) and the RCT Week 76 (ATE Week 52) visits, and time to death alone.

4.2 Exploratory Endpoints

4.2.1 Continuous Endpoints

The following continuous exploratory endpoints will be evaluated for treatment-dependent differences in change from baseline to Week 24 by inferential testing:

- Muscle strength:
  - HHD and grip strength upper extremity z-score,
  - HHD lower extremity percentage and z-score,
  - HHD global percentage and z-score,
- Patient-reported outcomes:
  - ALSAQ-40 total score,
  - ROADS total score.

Change from baseline to other time points during the RCT period and average rates of change from baseline to Week 24 of secondary and the exploratory endpoints listed above will also be estimated.

Change from baseline to RCT Week 52 (ATE Week 28) will be estimated for the following endpoints:

- ALSFRS-R total score,
- SVC percent-predicted,
- Serum NfL concentration.

Combined analysis of function and survival (CAFS) will be performed using ALSFRS-R total score as the functional component and using death/death-equivalent and death alone as the survival component in separate analyses to the following two time points:

- the time point at which the last participant had an opportunity to complete RCT Week 52 (ATE Week 28),
- the time point at which the last participant had an opportunity to complete RCT Week 76 (ATE Week 52).

The following exploratory endpoints will be summarized descriptively by treatment group and visit during both the RCT and ATE periods, as applicable:

- Patient-reported outcomes:
  - ALSAQ-40 total score, physical mobility, independence in activities of daily living, eating and drinking, communications, and emotional reactions domain scores,

  - ROADS total score,
  - CNS-BFS total score,
  - Patient Global Impression of Change (PGI-C),
  - Patient Global Impression of Severity (PGI-S),
- ALSFRS-R total and domain scores (bulbar, fine motor, gross motor, fine and gross motor combined, and respiratory),
- Clinician Global Impression of Change (CGI-C),
- Clinical staging systems: King's stage and ALS Milano-Torino Staging (MITOS) stage,
- Biofluid biomarkers: cerebrospinal fluid (CSF) NfL (assessed only during the RCT).

4.2.2 Time-to-event Endpoints

The following time-to-event exploratory endpoints will be evaluated for treatment-dependent differences in time to progression by 1 or more stages to Week 24 by inferential testing:

- King's stage,
- MITOS stage.

The following time-to-event exploratory endpoints will be evaluated for treatment-dependent differences in event-free survival to end of follow-up at the time point at which the last participant had an opportunity to complete the RCT Week 76 (ATE Week 52) by inferential testing:

- Death or death-equivalent,
- Death alone.

4.2.3 Pharmacokinetic Endpoints

The following pharmacokinetic endpoints will be summarized descriptively by treatment group and visit:

- DNL343 plasma and CSF concentrations and CSF-to-plasma concentration ratio.

4.3 Safety Endpoints

The following safety endpoints will be summarized descriptively by treatment group during both the RCT and ATE periods:

- Treatment-emergent adverse and serious adverse events, including laboratory and ECG abnormalities,

The following safety endpoints will be summarized descriptively by treatment group and visit during both the RCT and ATE periods:

- Changes in laboratory values and treatment-emergent and laboratory abnormalities,
- Changes in ECG parameters and treatment-emergent and ECG abnormalities,
- Changes in vital signs, and
- Treatment-emergent suicidal ideation and suicidal behavior.

Reported proportions will use as their denominator all participants in the applicable Safety sample (see Section 6.1 below).

5. Measurement Definitions

5.1 ALSFRS-R

The definition and scoring of the ALSFRS-R are the same as described in the M-SAP.

5.2 SVC

The definition and quantitation of SVC are the same as described in the M-SAP.

5.3 HHD and Grip Strength

The definition of HHD and grip strength are the same as described in the M-SAP. In addition to quantitation of HHD upper extremity, lower extremity, and global percentages, muscle strength will be quantified by calculating z-scores for each muscle group or maneuver based on the mean and standard deviation of participants in regimens A, B, C, D, and E at baseline, and then averaging applicable z-scores for a given body region.

The data used to calculate z-scores will parallel the data used for calculating muscle strength percentages. The muscle group specific means and standard deviations used to calculate z-scores will be calculated using only non-zero baseline measurements. The baseline z-score averages for a given participant will be calculated using only non-zero measurements. The follow-up z-score averages for a given participant will only include muscle groups and maneuvers that were non-zero at baseline but will include measurements of zero strength that are measured during follow-up.

5.4 Biofluid Biomarkers

The definition and quantitation of serum and CSF NfL are the same as described in the M-SAP with the following modification. For analyses of longitudinal change in serum NfL concentration, data obtained from the same plate will be used.

5.5 Patient-reported Outcomes

5.5.1 ALSAQ-40

The Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40, Jenkinson et al. 1999; Jenkinson et al. 2003) is a 40-item ALS-specific health-related quality-of-life instrument completed by participants for assessing quality of life in five domains: physical mobility (10 items), independence in activities of daily living (10 items), eating and drinking (3 items), communication (7 items), and emotional reactions (10 items). For each item, participants are asked to rate the degree to which a specific statement describing difficulties of living with ALS applies to the participant's personal experience over the past two weeks on a 5-point scale from 0 ("Never") to 4 ("Always").

The total score will be calculated as the mean of all 40 items multiplied by 25 (range 0 to 100). Each of the five domains will be scored as the mean of all domain-specific items multiplied by 25 (range 0 to 100). The total score and each domain 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.

5.5.2 CNS-BFS

The Center for Neurologic Study Bulbar Function Scale (CNS-BFS, Smith et al. 2018) is a 21-item instrument completed by participants for assessing bulbar function in three domains: speech, swallowing, and sialorrhea. For each domain, participants are asked to rate the degree to which each of seven statements describing an aspect of bulbar dysfunction apply to the participant's personal experience over the past week on a scale from 1 ("Does not apply") to 5 ("Applies most of the time"). Subjects unable to speak are assigned a value of 6 for each item comprising the speech domain. The total score is the sum of all items (range 21 to 112). Higher scores indicate worse bulbar dysfunction.

5.5.3 ROADS

The Rasch-built Overall ALS Disability Scale (ROADS, Fournier et al. 2020) is a 28-item instrument completed by participants for assessing overall functional disability associated with ALS. Each item assesses the participant's ability to perform a stated task on a scale from 0 ("unable to perform") to 2 ("normal"). Participants are instructed to rate their ability based on how a given task is usually performed; items should be scored as 2 (normal) if the task is performed as quickly and easily as it was before experiencing symptoms of ALS; items should be scored as 1 (abnormal) if it is harder to perform the task, the task takes more time or effort, or the task is performed with the assistance of a device or another person. The sum of the item scores is normed to a linearly-weighted total score (range 0 to 146). Higher scores indicate better function.

5.6 Survival

The definition of the survival endpoints of death or death-equivalent and death-only for the placebo-controlled period are the same as described in the M-SAP. The survival endpoints will also be assessed over an extended time interval which includes all available data from the RCT and ATE periods at the time of the first data freeze after the last participant had an opportunity to complete the RCT Week 52 (ATE Week 28) visit. The same summaries and analyses will be repeated at the time of the first data freeze after the last participant had an opportunity to complete the RCT Week 76 (ATE Week 52) visit.

5.7 Clinical Staging Systems

5.7.1 King's ALS Clinical Staging System

The definition and scoring of the King's ALS Clinical Staging System are the same as described in the M-SAP with the addition of a fifth stage indicating death. Participants who die or reach a death equivalent will be assigned to stage 5.

5.7.2 MITOS Clinical Staging System

The ALS Milano-Torino Staging (MITOS) clinical staging system (Chiò et al. 2015) is a 6-level ordinal scale focused on loss of independence in four key domains assessed by the ALSFRS-R: movement, swallowing, communicating, and breathing. Stage 0 indicates no loss of independence in any domain; stages 1–4 indicate loss of independence in the associated count of the four key domains; and stage 5 indicates death.

Loss of function in the movement domain is defined as a score 0 or 1 on one or both of ALSFSR-R question 6, Dressing and hygiene, and ALSFRS-R question 8, Walking. Loss of function in the swallowing domain is defined as a score 0 or 1 on ALSFSR-R question 3,

Swallowing. Loss of function in the communicating domain is defined as a score 0 or 1 on both of ALSFSR-R question 1, Speech, and ALSFRS-R question 4, Handwriting. Loss of function in the breathing domain is defined as a score 0 or 1 on ALSFSR-R question 10, Dyspnea, or a score of 0, 1, or 2 on ALSFRS-R question 12, Respiratory insufficiency.

5.8 TRICALS Risk Score

The Treatment Research Initiative to Cure ALS (TRICALS) Risk Profile is based on the European Network for the Cure of ALS (ENCALS) survival prediction model (Westeneng et al. 2018). The TRICALS risk score differs from the full ENCALS risk score in omitting *C9ORF72* expansion status, yielding slightly different coefficients (van Eijk 2022). The TRICALS risk score will be calculated at baseline as follows:

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

where *VC* is vital capacity in units of percent-predicted using Global Lung Initiative (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 risk scores will be calculated assuming that participants do not have frontotemporal dementia.

5.9 Pharmacokinetic Concentrations

Methods for measuring DNL343 concentration in the plasma and CSF will be described at a later date.

5.10 Study Drug Exposure

Study drug exposure will be summarized by the following metrics:

- Time from first exposure to last exposure,
- Cumulative days dosed,
- Total stick packs dispensed and not returned, lost, or destroyed, and
- Proportion of participants with dose reductions, suspensions, and discontinuations (including deaths, early termination, loss to follow-up, and any discontinuation more than 2 days prior to a participant's Week 24 visit).

5.11 Clinical Safety Laboratory Tests

The list of clinical safety labs and the definitions of measures of potential drug-induced liver injury (DILI) are the same as described in the M-SAP.

6. Statistical Methodology

6.1 Analysis Sets

The following analysis sets will be used for testing efficacy, pharmacokinetics, and safety endpoints:

- Efficacy Full Analysis Set (FAS): Participants who were randomized within RGG plus placebo participants from Regimens A, B, C, D, E, F who had an opportunity to complete 24-week placebo-controlled follow-up, classified according to their randomized treatment assignment. Observations completed after regimen data lock will be excluded. Participants determined to not meet ALS diagnostic criteria are excluded.
- Efficacy Concurrent-controls (ECC) Set: The subset of participants in the FAS analysis set who were randomized within RGG or randomized to placebo within RGF, exclusive of RGF participants who were also randomized within RGG.
- Efficacy Regimen-only (ERO) Set: The subset of participants in the FAS analysis set who were randomized within RGG. Four distinct versions of the ERO analysis set will be analyzed:
    - ERO to RCT Week 24 (ERO): RCT data up to and including RCT Week 24,
    - ERO to LP RCT Week 24 (ERO24): RCT and ATE data up to and including the time point at which the last participant had an opportunity to complete RCT Week 24 or the Final Safety Visit if the participant did not enter the ATE,
    - ERO to LP RCT Week 52 (ATE Week 28) (ERO52): RCT and ATE data up to and including the time point at which the last participant had an opportunity to complete RCT Week 52 (ATE Week 28),
    - ERO to LP RCT Week 76 (ATE Week 52) (ERO76): RCT and ATE data up to and including the time point at which the last participant had an opportunity to complete RCT Week 76 (ATE Week 52).
- Efficacy Common Mode of Administration (ECM) Set: The subset of participants in the FAS analysis set who were in regimens in which study drug was administered orally, specifically, Regimens B, C, D, F, and G.
- Efficacy Per-protocol (EPP) Set: The subset of participants in the ECC analysis set who initiated their assigned study treatment, took at least 80% of planned 24-week study drug exposure, and never took study drug to which they were not assigned.
- Pharmacokinetic Analysis (PKA) Set: Participants who received at least one dose of active DNL343 within RGG and from whom at least one PK sample was collected.
- Safety Full (STF) Set: Participants who received at least one dose of study drug within RGG plus placebo participants from Regimens A, B, C, D, E, F who had an opportunity to complete placebo-controlled follow-up and who received at least one dose of study drug in their respective regimen, classified according to the treatment they actually received. Observations completed after regimen data lock are excluded.

- Safety Concurrent Control set (SCC): The subset of participants in the STF analysis set who were randomized within RGG or randomized to placebo within RGF, exclusive of RGF participants who were also randomized within RGG.
- Safety Narrow (STN) Set: The subset of participants in the STF analysis set who were exposed to orally administered study drug, specifically, Regimens B, C, D, F, and G.
- Safety Regimen-only (SRO) Set: The subset of participants in the STF analysis set who were randomized within RGG.

The meaning of regimen data lock and the criteria for inclusion of data for placebo participants assigned to other regimens at the time of regimen data lock will be defined in the HEALEY ALS Platform Trial Data Management Plan and the HEALEY ALS Platform Trial Data Sharing Plan.

6.2 Baseline Characterization

The specification of baseline characteristics is the same as described in the M-SAP with the addition of ALSFRS-R domain scores, ALSAQ-40 total and domain scores, CNS-BFS total score, ROADS total score, TRICALS risk score (continuous and by category: < -4.5, ≥ -4.5 to < -3.5, ≥ -3.5), MITOS stage, and CSF NfL concentration.

6.3 Primary Efficacy Analysis and Supportive Analyses

The primary analysis for RGG is a Bayesian shared-parameter model of function and survival. The model will include DNL343 and placebo treatment groups with primary inference based on the ECC analysis set, with sensitivity analyses conducted also in the FAS, ERO, ECM, and EPP analysis sets.

Details of the model, including documentation of operating characteristics under a range of scenarios, are provided in the MPRDR (Appendix 1 of the M-SAP) with the modification that the threshold to declare success is 0.975 (vs. 0.979 in the MPRDR), TRICALS risk score is added as a covariate and time from symptom onset and delta-FRS are removed as covariates. Additional details of operating characteristics specific to RGG are provided in Appendix 1 of this R-SAP.

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 ECC 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: DNL343 200 mg treatment vs. placebo.
- Population: ECC 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 and will be handled via missing at random assumption.

- Population-level summary: mean ratio of hazard or progression rate of active treatment relative to placebo estimated by DRR.

6.4 Interim Analyses

The RGG RCT will be considered for early stopping for futility at 12-week intervals. The RGG RCT will not be considered for early stopping for success. Details of the schedule for futility analyses, the futility stopping rule, and documentation of operating characteristics under a range of scenarios, are provided in the PADSR (Appendix 1 of this R-SAP). In particular, the baseline covariates will be time from symptom onset, delta-FRS, and baseline use of edaravone, riluzole, and sodium phenylbutyrate/ursodoxicoltaurine. Baseline serum NfL and TRICALS risk scores are not used as covariates in interim analyses.

A single interim analysis of the RGG ATE will be performed using all RGG RCT and ATE data collected up to and including the time point at which the last participant had an opportunity to complete RCT Week 24 or the Final Safety Visit if the participant did not enter the ATE. The interim analysis will include the following subset of disposition summaries, efficacy analyses, and safety summaries using the ERO24 analysis set:

- Summaries of participant disposition as described in Section 6.7.1,
- Summaries of ALSFRS-R total score, SVC, and serum NfL by treatment group and visit with analyses as described in Sections 6.5.5 and 6.5.11,
- Summaries of adverse events as described in Section 6.6.1,
- Summaries of clinical safety labs as described in Section 6.6.2.

6.5 Secondary Efficacy Analyses

6.5.1 Hierarchical Testing

Principal inference for secondary efficacy endpoints will be based on comparison of DNL343 vs. placebo in the ECC analysis set using a repeated-measures linear mixed model for functional endpoints (see Section 6.5.2 below) and the FAS for 24-week time-to-event endpoints and the ERO analysis set for RCT Week 52 (ATE Week 28) LPLV time-to-event endpoints. The default sequence for testing is the following:

1. Change in ALSFRS-R total score from baseline to Week 24
2. Change in respiratory function as assessed by slow vital capacity (SVC) from baseline to Week 24
3. Change in serum NfL from baseline to Week 24
4. Change in muscle strength of upper extremities as measured isometrically using hand-held dynamometry and grip strength from baseline to Week 24
5. Survival evaluated as time to death or PAV to Week 24

6. Survival evaluated as time from RCT baseline to death or PAV at the time point at which the last participant had an opportunity to complete the RCT Week 52 (ATE Week 28) visit
7. Survival evaluated as time from RCT baseline to death or PAV at the time point at which the last participant had an opportunity to complete the RCT Week 76 (ATE Week 52) visit.

If the primary analysis indicates a significant slowing in disease progression from the Bayesian shared-parameter model of function and survival, then each secondary efficacy endpoint would be declared significant in the specified sequence using a criterion of two-sided alpha of 0.05. After the first failure to declare significance, no endpoints lower in the hierarchy can be significant. Nominal comparison-wise p-values for secondary efficacy endpoints will also be reported.

Note that the analysis of the secondary endpoint of CAFS at Week 24 will be considered supportive of the primary endpoint analysis, and for this reason it is not included in the hierarchical testing sequence. Likewise, the endpoints of survival evaluated as time to death to Week 24, and survival evaluated as time to death to the time point at which the last participant had an opportunity to complete the RCT Week 52 (Week 28 ATE) visit or the RCT Week 76 (Week 52 ATE) visit will be considered as supportive of the corresponding survival evaluated as time to death or PAV secondary endpoints, and also not included in the hierarchical testing sequence.

6.5.2 Repeated-measures Model

The specification of the repeated-measures model for analysis of data from the RCT period is modified from that described in the M-SAP by changing the response measure to be a calculated change from baseline, removing baseline observations from the response, adding the baseline observation and TRICALS risk score and their interactions with visit as additional covariates, and removing time from symptom onset and delta-FRS and their interactions with visit as covariates.

The following equations describe the model with fixed effects for regimen for the principal analysis of ALSFRS-R and other continuous endpoints in the ECC analysis set:

$$\begin{gathered}\left(Y_{ij} - Y_{i0}\right) = \boldsymbol{\gamma_0'} a_{k(i)} + \gamma_1 t_i + \gamma_{2,j} v_j + \boldsymbol{\gamma_3'} \boldsymbol{z_i} + \gamma_{4,j}\, t_i v_j + \boldsymbol{\gamma_{5,j}'} \boldsymbol{z}_i\, v_j + \epsilon_{ij} \\ \boldsymbol{\epsilon_{i\cdot}} \sim N(\boldsymbol{0}, \boldsymbol{R})\end{gathered} \qquad \text{(eqn. 1)}$$

where Y_{ij} is a given efficacy endpoint measured for participant i at visit j, Y_{i0} is a given efficacy endpoint measured for participant i at baseline, $a_{k(i)}$ is an indicator variable 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 baseline value of the given efficacy endpoint, centered TRICALS risk score, centered baseline log-transformed serum NfL level, centered baseline riluzole use, centered baseline edaravone use, and centered baseline sodium phenylbutyrate/ursodoxicoltaurine use) for participant i, t_i is an indicator variable for treatment t to which participant i was assigned, γ_0, 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 vector of residuals for a given participant are normally distributed with mean $\boldsymbol{0}$ and an unstructured covariance matrix $\boldsymbol{R}$.

In models with random effects for regimens, the regimen fixed effects will be removed, regimen-specific random effects are normally distributed with mean 0 and variance σ^2_r, and regimen-specific random effect for a given participant and residuals for that participant are uncorrelated (eqn. 2):

$$\begin{gathered}\left(Y_{ij} - Y_{i0}\right) = b_{k(i)} + \gamma_1 t_i + \gamma_{2,j} v_j + \boldsymbol{\gamma}_3' \boldsymbol{z_i} + \gamma_{4,j}\, t_i v_j + \boldsymbol{\gamma}_{5,j}' \boldsymbol{z}_i\, v_j + \epsilon_{ij} \\ b_{k(i)} \sim N(\mathbf{0}, \sigma_r^2), \boldsymbol{\epsilon}_{i\cdot} \sim N(\mathbf{0}, \boldsymbol{R}), \mathrm{Cov}\left(b_{k(i)}, \boldsymbol{\epsilon}_{i\cdot}\right) = 0\end{gathered} \quad \text{(eqn. 2)}$$

where $b_{k(i)}$ is the random effect for regimen k to which participant i was assigned and other terms are the same as those described in equation 1 above.

Satterthwaite's approximation will be used to estimate denominator degrees of freedom.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd visit;
  model Delta = trtrnd|visit regimen
                baseval|visit tricals|visit nfl|visit
                rlz|visit edv|visit amx|visit / solution cl ddfm=sat;
  repeated visit / subject=id(regimen) type=un;
```

where `regimen` is a regimen identifier, `id` is a participant study identifier, `trtrnd` is the randomly assigned treatment group, `visit` is the visit identifier, `Delta` is change from baseline of the efficacy endpoint being tested for a given participant at a given visit, `baseval` is the baseline value of the efficacy endpoint, `tricals` is TRICALS risk score, `nfl` is baseline log-transformed serum NfL level, `rlz` is an indicator of riluzole use at baseline, `edv` is an indicator of edaravone use at baseline, and `amx` is an indicator of sodium phenylbutyrate/ursodoxicoltaurine use at baseline.

The following SAS code specifies the primary linear contrast for an endpoint measured every 8 weeks assuming that the sort order for treatment group is placebo and DNL343 200 mg, and that visits are sorted chronologically:

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

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 DNL343 treatment group relative to the placebo group in the ECC population. The estimand is defined by the following attributes:

- Treatment: DNL343 200 mg treatment vs. placebo.
- Population: ECC set 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 and will be handled via missing at random assumption implemented through MMRM framework.

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

A nominally significant difference in 24-week change from baseline in the direction of greater improvement or less worsening among participants randomized to DNL343 would support inference of benefit from DNL343 for the efficacy endpoint being tested. Inference from this analysis is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint.

Primary inference will be based on analysis of the ECC set. Where applicable, results from the FAS, and ERO, analysis sets will be interpreted as sensitivity analyses.

Secondary endpoints will be analyzed by this model in the ECC, FAS, and ERO analysis sets.

Continuous exploratory endpoints for which treatment-dependent differences will be estimated will be analyzed by this model in the ECC analysis set, and generally either in the FAS, or the ERO analysis set, if FAS is not applicable (e.g., for change in ROADS), unless otherwise specified.

The muscle strength exploratory endpoints calculated as average percentage change will be analyzed in the ECC, FAS and ERO analysis sets, similar to the corresponding muscle strength secondary endpoint.

All muscle strength endpoints calculated as average of z-scores will be analyzed in the ECC analysis set.

6.5.3 Random-slopes Model

The specification of the random-slopes model for analysis of data from the RCT period is the same as described in the M-SAP with the following modifications. The model terms are revised to include the baseline value of a given efficacy endpoint and TRICALS risk score and their interactions with month since baseline as additional covariates and to remove time from symptom onset and delta-FRS and their interactions with month since baseline as covariates. Satterthwaite's approximation will be used to estimate denominator degrees of freedom.

The following equations describe the model with regimen fixed effects:

$$\begin{aligned}\left(Y_{ij} - Y_{i0}\right) &= \gamma_1 + \boldsymbol{\gamma_2'} a^0_{k(i)} + b^0_i + \gamma_3 t_i + \boldsymbol{\gamma_4'} \boldsymbol{z_i} \\ &+ \left(\gamma_5 + \boldsymbol{\gamma_6'} a^1_{k(i)} + b^1_i + \gamma_7 t_i + \boldsymbol{\gamma_8'} \boldsymbol{z_i}\right) m_{ij} + \epsilon_{ij} \qquad \text{(eqn. 3)} \\ \{b^0_k, b^1_k\} &\sim N\left(\mathbf{0}, \boldsymbol{\Sigma_p}\right), \epsilon_{ij} \sim N(0, \sigma^2_\epsilon), \text{and } \mathrm{Cov}(\boldsymbol{b}_k, \boldsymbol{\epsilon}_{i\cdot}) = \mathbf{0}\end{aligned}$$

where b^0_i and b^1_i are random intercept and slope 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, γ_1, $\boldsymbol{\gamma_2}$, γ_3, $\boldsymbol{\gamma_4}$, γ_5, $\boldsymbol{\gamma_6}$, γ_7, and $\boldsymbol{\gamma_8}$ are estimated parameters and vectors of parameters for the fixed effects, and other terms are the same as those described in equation 1 above. 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 participant-specific random effects and residuals are uncorrelated.

The following SAS code specifies the model with fixed effects for regimen:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd;
  model Delta = trtrnd|month regimen|month
                baseval|month tricals|month nfl|month
                rlz|month edv|month amx|month / solution cl ddfm=sat;
  random intercept month / subject=id(regimen) 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 following SAS code specifies the primary linear contrast assuming that the sort order for treatment group is placebo and DNL343 200 mg:

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

The estimand estimated by the primary linear contrast of the random-slopes model is the difference in mean rate of change from baseline to the Week 24 visit of a given continuous efficacy endpoint in the DNL343 treatment group relative to the placebo group in the ECC population. The estimand is defined by the following attributes:

- Treatment: DNL343 200 mg treatment vs. placebo.
- Population: ECC population as defined in Section 6.1.
- Variables: 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, including data missing due to death, will not be imputed and will be handled via missing at random assumption implemented through the random-slopes model.
- Population-level summary: difference in rate of change of active treatment relative to placebo.

A nominally significant difference in slopes in the direction of greater improvement or less worsening among participants randomized to DNL343 would support inference of benefit from DNL343 for the efficacy endpoint being tested. Inference from this analysis is supportive of inference from the Bayesian shared-parameter, repeated-measures model for the primary endpoint.

Secondary endpoints, as well as the exploratory muscle strength endpoints calculated as average percentage change will be analyzed by this model in the ECC analysis set and in the FAS.

Other continuous exploratory endpoints (including muscle strength endpoints calculated as average of z-scores, change in ALSAQ-40, change in ROADS) will be analyzed in the ECC analysis set.

6.5.4 Time-to-event Endpoints

Time to death or death-equivalent and time to death alone evaluated at the Week 24 time point will be summarized in the FAS, ECC, and ERO analysis sets. Time to progression by 1 or more stages of the King's staging system and progression by 1 or more stages of the MITOS staging system will be summarized in the FAS and ECC analysis sets. Time to death or death-equivalent and time to death alone evaluated at the ATE Week 28 LPLV time point will be summarized in the ERO analysis set. Death or death-equivalent and death alone will be analyzed as right censored data. Progression by 1 or more stages of the King's staging system and by 1 or more stages of the MITOS staging system will be analyzed as interval censored data.

For right-censored endpoints, summaries will include number at risk, cumulative number of events, and estimated survival probabilities at 8-week intervals and over the full placebo-controlled period. For interval censored endpoints, summaries will include the number of participants at risk at baseline and the number of participants left censored, interval censored, and right censored at the end of RCT follow-up. Summaries will include treatment-specific times to 10%, 25%, and 50% percentiles of the estimated survival curves.

Survival probabilities and times to specific survival percentiles will be provided as point estimates and 95% log-log confidence intervals. Summaries will include restricted mean survival time (RMST) as point estimates and 95% confidence intervals calculated from bias-corrected standard error estimates. Hazard ratios will be provided as point estimates and likelihood-profile 95% confidence intervals. Differences in RMST will be provided by point estimates and 95% confidence intervals.

The primary test of treatment effect on survival at the Week 24, LP RCT Week 52 (ATE Week 28), and LP RCT Week 76 (ATE Week 52) time points will be the unstratified log-rank test of the death or death-equivalent endpoint. If there are fewer than 10 events in either treatment group, p-values will be calculated by randomization test using 10,000 random relabelings of participant treatment assignments at the observed frequency. Otherwise, p-values will be calculated assuming that the log-rank test statistic follows a one degree of freedom Chi-squared distribution. Analysis of the death alone endpoint will be supportive. If at least one occurrence of a given survival endpoint is observed in each treatment group, then the treatment-dependent hazard ratio with profile likelihood confidence bounds will be estimated by unadjusted Cox proportional hazards regression.

For other time-to-event endpoints evaluated at the Week 24, LP RCT Week 52 (ATE Week 28), or LP RCT Week 76 (ATE Week 52) time points, treatments will be compared by stratified log-rank test, adjusted Cox regression, and difference in stratified RMST.

For the time to progression of 1 or more clinical stages endpoints, the log-rank test and RMST analyses will be stratified by baseline riluzole use, TRICALS risk score (< -4.5, ≥ -4.5 to < -3.5, ≥ -3.5) and baseline clinical stage (1, 2, or 3 or more for King's staging and 0 vs. 1 or more MITOS staging). Cox regression models will adjust for the baseline clinical state, baseline TRICALS risk score, baseline log-transformed serum NfL level, baseline riluzole use, baseline edaravone use, and baseline sodium phenylbutyrate/ursodoxicoltaurine use.

For the time to death or death-equivalent and time to death alone evaluated at the LP RCT Week 52 (ATE Week 28) and LP RCT Week 76 (ATE Week 52) time points, the log-rank test and RMST analyses will be stratified by baseline riluzole use, and TRICALS risk score (< -4.5,

≥ -4.5 to < -3.5, ≥ -3.5). Cox regression models will adjust for the baseline TRICALS risk score, baseline log-transformed serum NfL level, baseline riluzole use, baseline edaravone use, and baseline sodium phenylbutyrate/ursodoxicoltaurine use.

6.5.5 CAFS

The specification of CAFS analyses of data from the RCT period is the same as described in the M-SAP with the following modification. Only ALSFRS-R total score will be used for comparing functional component, only death/death-equivalent to the RCT Week 24 time point will be used for the survival component, and analysis will adjust for TRICALS risk score, baseline use of edaravone, baseline use of riluzole, baseline use of sodium phenylbutyrate/ursodoxicoltaurine, and baseline log-transformed serum NfL.

This is a key sensitivity analysis of the primary Bayesian shared-parameter model. Primary inference will be based on analysis of the ECC set. The analysis will also be performed with the FAS and ERO analysis sets.

CAFS analyses will also be performed using ALSFRS-R total score as the functional component, using death/death-equivalent and death alone as the survival component in separate analyses, using all data up to the LP RCT Week 52 (ATE Week 28) and LP RCT Week 76 (ATE Week 52) in separate analyses, and adjusting for TRICALS risk score, baseline use of edaravone, baseline use of riluzole, baseline use of sodium phenylbutyrate/ursodoxicoltaurine, and baseline log-transformed serum NfL.

6.5.6 Global Impressions

Participant Global Impression of Change (PGI-C), Patient Global Impression of Severity (PGI-S), and Clinician Global Impression of Change (CGI-C) will be summarized by treatment group and visit in the ERO analysis set.

6.5.7 Subgroup Analyses

Differences in treatment efficacy across subgroups will be estimated both in the repeated-measures model (Section 6.5.2) to estimate 24-week change from baseline and in the random-slopes model (Section 6.5.3) to estimate average rate of change over the 24-week placebo-controlled period for ALSFRS-R total score, SVC, serum NfL, and HHD upper extremity percentage. Terms for subgroup, subgroup × visit, and subgroup × treatment × visit will be added to the repeated-measures model. Terms for subgroup, subgroup × time, and subgroup × treatment × time will be added to the random-slope model. All analyses will use the ECC and FAS analysis sets.

- Baseline use of standard of care medications (riluzole alone, edaravone or sodium phenylbutyrate/ursodoxicoltaurine, or none of riluzole, edaravone, or phenylbutyrate/ursodoxicoltaurine),
- El Escorial definite and time from symptom onset less than 18 months (yes vs. no),
- El Escorial definite or probable and time from symptom onset less than 24 months (yes vs. no),
- Time from symptom onset (less than 18 months, 18 to less than 24 months, 24 months or more),

- Baseline serum NfL (by ECC analysis set tertiles),
- TRICALS risk score (< -4.5, ≥ -4.5 to < -3.5, ≥ -3.5),
- King's stage (stage 1, stage 2, stage 3 or higher),
- MITOS stage (stage 0, stage 1 or higher),
- Bulbar onset or current bulbar dysfunction (bulbar site of onset or baseline ALSFRS-R bulbar domain score less than 12) (yes vs. no),
- Delta-FRS (by ECC analysis set tertiles),
- SVC % predicted less than 80% (yes vs. no).

A given level of a specified subgroup will be included only if the sample size in each treatment group is at least 20 participants and at least 15% of the total sample size within that treatment group. If the subgroup includes more than two levels, subgroup levels that do not meet these criteria will be pooled with the smaller of either adjacent category. This same rule applies to the sample size available with Week 24 data for subgroup analyses by repeated-measures models.

For each subgroup specified, the following estimates will be obtained:

- the treatment-group specific estimate within each subgroup level,
- the treatment difference within each subgroup level,
- the difference of differences between each pair of subgroup levels, and
- for subgroups with more than two levels, the F-test for the subgroup × treatment × 24-week change from baseline contrast from repeated-measures models and the subgroup × treatment × time term from random-slopes models.

Forest plots of subgroup-specific treatment-dependent differences will be provided.

6.5.8 Comparison of Controls across Regimens

The specification of comparisons of controls across regimens is the same as described in the M-SAP with the modification that comparisons will be restricted to the FAS and ECC analysis sets and the following endpoints only: ALSFRS-R total score, SVC percent-predicted and muscle strength upper extremity.

6.5.9 Pharmacodynamic Biomarker Analyses

Change in pharmacodynamic biomarkers will be summarized by treatment group and visit in the ERO analysis set, and the ECC analysis set (if applicable). Summary statistics 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. Summaries will include observed values, absolute change from baseline, and percent change from baseline at each scheduled assessment time point.

6.5.10 Pharmacokinetic Analyses

Serum and CSF concentrations of pharmacokinetic biomarkers will be summarized by treatment group and visit in the ERO analysis set. Summary statistics 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.

6.5.11 Partial-linear Spline Mixed Models

Change from RCT baseline to RCT Week 52 (ATE Week 28) in ALSFRS-R, SVC percent-predicted, and log-transformed serum NfL concentration will be estimated using partial-linear spline mixed models that assume random period-specific slopes in the ERO analysis set.

The model to RCT Week 52 (ATE Week 28) will include fixed terms for month since RCT Baseline, non-negative months since RCT Week 24 (ATE Day 0), and the interactions of month since RCT Baseline and months since RCT Week 24 (ATE Day 0) with treatment group, TRICALS risk score, baseline log-transformed serum NfL, baseline use of edaravone, baseline use of riluzole, and baseline use of sodium phenylbutyrate/ursodoxicoltaurine. The model will include three participant-specific random effects (an intercept and two period-specific slopes) with unstructured covariance.

The following equations describe the model:

$$Y_{ij} = \gamma_1 + b_i^0 + \gamma_2 t_i + \boldsymbol{\gamma_3'} \boldsymbol{z_i} + (b_i^1 + \gamma_4 + \gamma_5 t_i + \boldsymbol{\gamma_6'} \boldsymbol{z_i})\, m_{ij}^{RCT_0} + (b_i^2 + \gamma_7 + \gamma_8 t_i + \boldsymbol{\gamma_9'} \boldsymbol{z_i})\, m_{ij}^{RCT_{24}} + \epsilon_{ij} \quad \text{(eqn. 3)}$$

$$\{b_i^0, b_i^1, b_i^2\} \sim N(\mathbf{0}, \boldsymbol{\Sigma_p}),\ \epsilon_{ij} \sim N(0, \sigma_\epsilon^2),\ \mathrm{Cov}(\boldsymbol{b_i}, \boldsymbol{\epsilon_{i\cdot}}) = \mathbf{0}$$

where Y_{ij} is a given efficacy endpoint measured in participant i at visit j, $b^0{}_i$ is a random intercept for participant i, $b^1{}_i$ and $b^2{}_i$ are random slopes for participant i during the RCT and ATE periods, respectively, t_i is an indicator variable for active treatment t to which participant i was assigned, $\boldsymbol{z_i}$ is the vector of covariates (centered TRICALS risk score, centered baseline log-transformed serum NfL level, centered baseline riluzole use, centered baseline edaravone use, and centered baseline sodium phenylbutyrate/ursodoxicoltaurine use) for participant i, $m^{RCT[0]}{}_{ij}$ is the time from RCT baseline to observation j for participant i in months calculated as days x 12 / 365.25, $m^{RCT[24]}{}_{ij}$ is the time from RCT Week 24 (ATE Day 0) to observation j for participant i in months calculated as the maximum of zero or the difference from RCT Week 24 (ATE Day 0), $\boldsymbol{\gamma_1}$ through $\boldsymbol{\gamma_9}$ are estimated parameters and vectors of parameters for the fixed effects, and ϵ_{ij} is the residual for observation j for participant i. 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 participant-specific random effects and residuals are uncorrelated. Satterthwaite's approximation will be used to estimate denominator degrees of freedom.

The following SAS code specifies the model:

```
proc mixed data=xxx method=reml;
  class regimen id trtrnd;
  model Value = rctmon trtrnd*rctmon r24mon trtrnd*r24mon
                tricals|rctmon nfl|rctmon rlz|rctmon edv|rctmon amx|rctmon
                tricals|r24mon nfl|r24mon rlz|r24mon edv|r24mon amx|r24mon
```

```
                / solution cl ddfm=sat;
  random intercept rctmon r24mon / subject=id type=un;
```

where `id` is a participant study identifier, `trtrnd` is the randomly assigned treatment group, `Value` is value of the efficacy endpoint being tested for a given participant at a given visit, `rctmon` is time in months from the RCT Baseline Visit (assuming 12 months in an average of 365.25 days per year), `r24mon` is non-negative time in months from RCT Week 24 (ATE Day 0), `tricals` is TRICALS risk score, `nfl` is baseline log-transformed serum NfL level, `rlz` is an indicator of riluzole use at baseline, `edv` is an indicator of edaravone use at baseline, and `amx` is an indicator of sodium phenylbutyrate/ursodoxicoltaurine use at baseline.

The treatment-dependent difference in function at RCT Week 52 (ATE Week 28) and its 95% Wald confidence bound will be used to test whether early initiation of study drug is beneficial (H_o: E[$Y_{i,52}$ | t_i = active] – E[$Y_{i,52}$ | t_i = placebo] = 0). The following SAS code specifies the linear contrast assuming that the sort order for treatment group has the active group last:

```
estimate "AA 52wk vs PA 52wk (abs)" trtrnd*rctmon -52 52 trtrnd*r24mon -28 28 / cl;
```

A significant positive difference in estimated mean ALSFRS-R at RCT Week 52 (ATE Week 28) would support inference that DNL343 treatment reduced the rate of functional decline.

6.6 Safety Analyses

Safety outcomes will be summarized in the SCC, STF, and SRO analysis sets except as described below. Observations made after premature permanent discontinuation of study drug are included should such participants remain on study. Summaries of change in the SRO analysis set will tabulate both change from RCT baseline and change from ATE Day 0 (for ATE period data).

6.6.1 Treatment-emergent Adverse Events

Summaries of treatment-emergent adverse events (TEAEs) during the RCT period are the same as described in the M-SAP with the following modifications: addition of tabulations for the SCC analysis set, addition of summaries without regard to MedDRA term for any TEAE, any serious TEAE, any severe TEAE, any fatal TEAE, any related TEAE, and any TEAE leading to discontinuation of study drug and summaries of TEAEs and serious TEAEs classified by MedDRA preferred term in the STN analysis set, removal of summaries by demographic and weight subgroups, removal of estimates of incidence rates and incidence rate differences, and addition of a table summarizing the number and proportion of participants experiencing TEAEs and the number of TEAEs in the SCC by MedDRA system organ class, high level term, and preferred term for all TEAEs, excluding any with date of onset more than 30 days after the date of last dose of RCT study drug. TEAEs and serious TEAEs inclusive of both RCT and ATE period follow-up will be tabulated separately by RCT treatment group.

6.6.2 Safety Labs

Summaries of safety labs are the same as described in the M-SAP with the addition of the SCC analysis set and extension to include data collected during the ATE period.

6.6.3 ECG Results

Summaries of ECG results are the same as described in the M-SAP with the addition of the SCC analysis set and shift tables for abnormal findings and extension to include data collected during the ATE period.

6.6.4 Vital Signs and Weight

Summaries of vital signs are the same as described in the M-SAP with the addition of the SCC analysis set and extension to include data collected during the ATE period.

6.6.5 Suicidality

Summaries of suicidality are the same as described in the M-SAP with the addition of the SCC analysis set and extension to include data collected during the ATE period.

6.7 Other Analyses

6.7.1 Participant Disposition

Summaries of participant disposition will be made in the ECC, FAS, and ERO analysis sets and are otherwise the same as described in the M-SAP with the addition of summaries of the number of participants who initiated ATE study drug, completed RCT Week 52 (ATE Week 28) follow up, completed RCT Week 76 (ATE Week 52) follow up, and prematurely terminated ATE participation due to death, withdrawal of consent, or administrative termination summarized overall and by RCT treatment group for the ERO and SRO analysis sets. Reasons for withdrawal from the ATE period of the regimen will be presented.

6.7.2 Study Drug Compliance and Tolerance

Summaries of study drug exposure and tolerance are the same as described in the M-SAP with the modification that summaries will be restricted to the SCC and SRO analysis sets and will include exposure during the RCT and ATE periods separately and combined. The percentage of planned study drug exposure taken by each participant relative to full, 24-week follow-up and relative to achieved follow-up and the proportion of participants who took at least 80% of planned study drug exposure relative to each denominator will be summarized.

6.7.3 Concomitant Medication Use

Summaries of concomitant medication use are the same as described in the M-SAP with the modification that summaries will be made in the ECC, FAS, and ERO analysis sets, that use at baseline refers to use of a concomitant medication at the time of first dose of RCT study drug, that initiation after first dose of RCT study drug is defined as first use of a concomitant medication after first dose of RCT study drug that was not being taken at the time of first dose of RCT study drug, and that initiation of concomitant medications after first dose of ATE study drug will be summarized.

6.7.4 Medical History

Summaries of medical histories are the same as described in the M-SAP with the modification that summaries will be made in the ECC, FAS, and ERO analysis sets.

6.7.5 Blindedness

Summaries of blindedness are the same as described in the M-SAP with the modification that summaries will be made in the ECC, FAS, and ERO analysis sets.

6.7.6 Protocol Deviations

Summaries of protocol deviations are the same as described in the M-SAP with the modification that major protocol deviations will be summarized in the ECC, FAS, and ERO analysis sets and minor protocol deviations will only be provided as a listing and only in the ERO analysis set.

7. Validation

7.1 Primary Efficacy Analysis

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

7.2 Secondary, Exploratory, and Safety Analyses

Validation of the secondary, exploratory, and safety analyses is the same as described in the M-SAP.

8. References

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

Chiò A, Hammond ER, Mora G, Bonito V, Filippini G. Development and evaluation of a clinical staging system for amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2015 Jan;86(1):38-44.

Fournier CN, Bedlack R, Quinn C, Russell J, Beckwith D, Kaminski KH, Tyor W, Hertzberg V, James V, Polak M, Glass JD. Development and Validation of the Rasch-Built Overall Amyotrophic Lateral Sclerosis Disability Scale (ROADS). JAMA Neurol. 2020 Apr 1;77(4):480-488.

Jenkinson C, Fitzpatrick R, Brennan C, Swash M. Evidence for the validity and reliability of the ALS assessment questionnaire: the ALSAQ-40. Amyotroph Lateral Scler Other Motor Neuron Disord. 1999 Dec;1(1):33-40.

Jenkinson C, Norquist JM, Fitzpatrick R. Deriving summary indices of health status from the Amyotrophic Lateral Sclerosis Assessment Questionnaires (ALSAQ-40 and ALSAQ-5). J Neurol Neurosurg Psychiatry. 2003 Feb;74(2):242-5.

Smith RA, Macklin EA, Myers KJ, Pattee GL, Goslin KL, Meekins GD, Green JR, Shefner JM, Pioro EP. Assessment of bulbar function in amyotrophic lateral sclerosis: validation of a self-report scale (Center for Neurologic Study Bulbar Function Scale). Eur J Neurol. 2018 Jul;25(7):907-e66.

van Eijk, RPA. Risk Profile Predictor v2.01 (2022-02-20). https://tricals.shinyapps.io/risk-profile/ [accessed 2023-12-13].

Westeneng HJ, Debray TPA, Visser AE, van Eijk RPA, Rooney JPK, Calvo A, Martin S, McDermott CJ, Thompson AG, Pinto S, Kobeleva X, Rosenbohm A, Stubendorff B, Sommer H, Middelkoop BM, Dekker AM, van Vugt JJFA, van Rheenen W, Vajda A, Heverin M, Kazoka M, Hollinger H, Gromicho M, Körner S, Ringer TM, Rödiger A, Gunkel A, Shaw CE, Bredenoord AL, van Es MA, Corcia P, Couratier P, Weber M, Grosskreutz J, Ludolph AC, Petri S, de Carvalho M, Van Damme P, Talbot K, Turner MR, Shaw PJ, Al-Chalabi A, Chiò A, Hardiman O, Moons KGM, Veldink JH, van den Berg LH. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol. 2018 May;17(5):423-433.

Appendix 1.

ALS Master Protocol Regimen G (DNL343)

Primary Analysis, Design & Simulation Report

September 16, 2024

Table of Contents

1.0 Introduction

This document describes the primary endpoint, primary analysis, and interim analyses for Regimen G (DNL343).

Regimen G follows the recommended design, primary endpoint and primary analysis from the ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report Dated February 6, 2023 (Appendix I of the HEALEY ALS Platform Trial Master Statistical Analysis Plan v3.0) with 3 minor differences:

1) The primary analysis population includes all participants in the Full Analysis Set who were randomized within Regimen G or randomized to placebo within Regimen F, exclusive of Regimen F participants who were also randomized within Regimen G (Section 2.1).
2) The two covariates "time since onset" and "pre-baseline slope of ALSFRS-R" are replaced with the baseline TRICALS risk score.
3) Success will be declared at the final analysis if the posterior probability that the treated group is superior to placebo group is greater than .975.

The design for Regimen G enrolls approximately 240 participants. Approximately 180 participants will be randomized to DNL343 administered QD at a dosage of 200 mg and approximately 60 participants will be randomized to placebo.

This document serves to provide:

- A list of sensitivity analyses that will be performed.
- Simulations custom to Regimen G that include 240 participants enrolled to Regimen G (180 active and 60 control) and concurrent shared controls from Regimen F.

2.0 Overview of Primary Analysis and Design

2.1 Primary Analysis

The primary analysis population includes all participants in the Full Analysis Set who were randomized within Regimen G or randomized to placebo within Regimen F, exclusive of Regimen F participants who were also randomized within Regimen G (Efficacy Concurrent Controls analysis set).

The primary analysis is a Bayesian shared parameter analysis of mortality and function (ALSFRS-R) and is described in the ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report Section 2.0 dated February 6, 2023 (Appendix I of the HEALEY ALS Platform Trial Master Statistical Analysis Plan v3.0), except with a slight variation on the included covariates. More specifically, the two covariates "time since onset" and "pre-baseline slope of ALSFRS-R" are replaced with the baseline TRICALS risk score. Hence the primary analysis for Regimen G will include 5 covariates: baseline TRICALS risk score, baseline log-transformed serum NfL level, baseline riluzole use, baseline edaravone use, and baseline sodium phenylbutyrate/ursodoxicoltaurine use as indicated in participants' concomitant medication log.

2.2 Regimen Success

The primary analysis in Regimen G will test those randomized to active treatment compared to placebo. Success will be declared at the final analysis if the posterior probability that the treated group is superior to placebo group is greater than .975:

$$Pr\left(\exp(\theta_1) < 1\right) > .975.$$

The threshold for the final analysis of .975 was chosen to control type I error at 2.5% in the null scenario without futility stopping. This threshold differs from the recommended design due to updated simulations reflecting changes in standard of care (approvals of oral edaravone and sodium phenylbutyrate/ursodoxicoltaurine [Relyvrio]) and different sample sizes compared to the initial regimens in the platform trial.

2.3 Interim Analyses and Regimen Futility

Interim analyses will occur simultaneously for all regimens within the platform with participants active in their placebo-controlled period. Interim analyses occur approximately every 12 weeks. At an interim analysis, Regimen G can stop early for futility. Regimen G is eligible to stop early for futility once there are 40 randomized participants within Regimen G who have had the opportunity to complete at least 24 weeks of follow-up. If a regimen does not stop early for futility at an interim analysis, the Regimen G final analysis will take place after all participants

randomized within Regimen G have had the opportunity to complete 24 weeks of follow-up and final data for the placebo-controlled period has been locked.

The primary analysis population for early interims on futility will include all participants in the Efficacy Concurrent Controls analysis set (defined above).

For interim analyses, due to constraints on when serum NfL data is received, the covariate of baseline log-transformed serum NfL level will not be included in the analysis model.

Futility will be declared for Regimen G at an interim analysis if the posterior probability that the treatment slows disease progression by at least 10% is less than 5%:

$$Pr\left(\exp(\theta_1) < .9\right) < .05$$

2.4 Goodness of Fit Diagnostics and Sensitivity Analyses of Primary Efficacy Analysis

The goodness-of-fit diagnostics, supportive analyses, and sensitivity analyses will be performed for the primary efficacy analysis and will be conducted on the primary analysis population unless otherwise specified. Analyses will follow those specified in Section 4.0 of the ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report Dated February 6, 2023 (Appendix I of the HEALEY ALS Platform Trial Master Statistical Analysis Plan v3.0) with the following modifications:

- Concurrent shared control population will serve as the primary analysis population and a sensitivity analysis with all shared controls from Regimens A-G will be conducted.

3.0 Clinical Trial Simulations

Clinical trial simulation is used to provide simulated example trials and to quantify operating characteristics for each regimen. Virtual patient outcomes are created under different assumptions for key design parameters (see ALS Master Protocol Recommended Statistical Analysis, Design and Simulation Report Section 5.1 dated February 6, 2023 [Appendix I of the HEALEY ALS Platform Trial Master Statistical Analysis Plan v3.0] for description of simulation method). For each set of simulation assumptions (i.e. a scenario), many trials are simulated and virtually executed without futility. Trial operating characteristics are summarized across all simulated trials for each scenario.

3.1 Simulation Scenarios

Trial simulations for Regimen G assume that Regimen G is the 7th regimen with a 6th regimen (Regimen F) also enrolling concurrently with a total of N=300 and 75 controls in Regimen F. Under the primary Efficacy Concurrent Controls analysis set there is expected to be a total of N=180 treated Regimen G and N=135 shared controls in Regimen F and G. Treatment effect assumptions will vary across the active treatment group for Regimen G and operating characteristics will be reported for this regimen.

Additional simulation assumptions are:

- Accrual of 40 participants per month
- Non-mortality dropout rate of 2% per month
- Mortality rate of 5% over 24 weeks

- ALSFRS-R slope distribution and measurement error for Regimen G is the same as PRO-ACT
- Rates of usage and effects of standard of care (SOC). Effects of SOC will be modeled as multiplicative effects to the individual rates of progression and will be applied first, before any treatment effect (see below).
    - Edaravone usage at baseline is 25% with an assumed 30% slowing over natural progression for those participants who are on edaravone
    - Relyvrio usage at baseline is 60% with an assumed 25% slowing over natural progression for those participants who are on Relyvrio
    - The treatment effect for those participants on both edaravone and Relyvrio is capped at 30% slowing.

Treatment effects for Regimen G will be simulated under the Null with 0% slowing in disease progression and under an alternative with 30% slowing in disease progression. The 30% slowing treatment effect for Regimen G is assumed to be an additional additive effect on top of any effect of standard of care (see SOC effect above). This additive effect to the slope will be specific to each SOC group (those not on edaravone or Relyvrio, those only on edaravone, those only on Relyvrio, and those on both) based on the expected rate of progression among control participants within that subgroup to achieve a 30% slowing.

Simulations are performed without futility stopping.

3.2 Operating Characteristics Primary Analysis

For each scenario and treatment effect, we simulate 10,000 clinical trials. We present overall average operating characteristics across all simulated trials. All simulations do not include early futility stopping. For each simulation scenario and treatment effect, we report the following regimen-specific operating characteristics for Regimen G:

- Probability of regimen success
- Average estimate of the treatment effect.

Table 3.2.1 shows the overall operating characteristics for Regimen G under the proposed design for the base simulation scenarios without futility stopping. The regimen has greater than 80% power to detect a 30% slowing of mortality and function. Under the null there is no bias and an estimated 2.5% type I error.

Scenario	Power / Type I Error	Effect Estimate
Null (0% slowing)	0.025	1.00
30% slowing Mortality and Function	0.836	0.70
30% slowing Function only 0% slowing Mortality	0.792	0.71

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