STATISTICAL ANALYSIS PLAN (SAP)

Protocol Number:	Trehalose EAP
Protocol Title:	An Expanded Access Protocol of Intravenous Trehalose Injection 90.5 mg/mL Treatment of Patients with Amyotrophic Lateral Sclerosis
Protocol Version and Date:	Version 3.0, 31 July 2023
Compound Name:	SLS-005
Study Phase:	Not Applicable
Regulatory Agency Identifier(s):	IND Number: 151860 EUDRACT Number: Not Applicable
Registry Identifier(s):	

Registry Name	Registry ID
clinicaltrials.gov	NCT05597436

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SAP Version and date:	Version 2.0, 20 Mar 2025

SAP APPROVAL

An Expanded Access Protocol of Intravenous Trehalose Injection 90.5 mg/mL Treatment of Patients with Amyotrophic Lateral Sclerosis

I approve the Statistical Analysis Plan, Version 1.0, dated 23 Aug 2024.

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SAP VERSION HISTORY

SAP Version	Approval Date	Based on Protocol (Version, Approval Date)	Brief Description of Change(s)/Revision(s) and Rationale
1.0	23 August 2024	Version 3.0, 31 July 2023	Original document creation.
1.1	28 November 2024	Version 3.0, 31 July 2023	1.Section 4.3: Add summary on end of treatment and duration of study. 2.Section 4.4.1: add baseline characteristics of Presence of NIV, Gastrointestinal tube insertion. 3.Section 4.9.1: add new exposure summary for participants with TEAE related to study rug. 4.Section 4.9.2: add new AE tables 5.Throughout this SAP document, the term 'historical placebo' is updated to 'historical control'. The chosen external Answer ALS database also contains participants with medications administered for ALS, and no specific field can be used to identify all the participants without medications, so it is agreed to use all available data from Answer ALS. 6.Section 4.1.1: add definition of Baseline and Day 1 for historical control group which has no first dose date collected and uses the date of first study visit as baseline time point. 7.Section 4.4.3: For balance diagnosis between Trehalose group and historical control group after PS matching and stratification: exclude the p-value which should be used with caution. It is sufficient to only present the standard mean difference which is the most commonly used statistic to examine the balance of covariate distribution between treatment groups.
1.1	08 January 2025	Version 3.0, 31 July 2023	1.Section 4.7.1.2: For OS comparison analysis under PSM, the bulbar onset (yes/no) is excluded from the stratification factor since the PS matched ID is constructed based on the PS score which includes the variable of bulbar onset. 2.Section 4.7.3: For % predicted VC comparison between PS matched Trehalose and historical control group, more details about the model are added.
2.0	20 March 2025	Version 3.0, 31 July 2023	Finalized version 2.0.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Description
AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire-40
ALSFRS-R	ALS Functional Rating Scale – Revised
ANCOVA	Analysis of Covariance
ATC	Anatomic Therapeutic Chemical
BMI	body mass index
C-SSRS	Columbia-Suicide Severity Rating Scale
CI	confidence interval
DSMB	Data Safety Monitoring Board
DPP	Data Presentation Plan
EAP	Expanded Access Protocol
eCRF	electronic case report form
FALS	Familial Amyotrophic Lateral Sclerosis
FAS	Full Analysis Set
FDA	Food and Drug Administration (US)
FVC	Forced Vital Capacity
HR	hazard ratio
ID	identifier
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
Nfl	neurofilament light
NIV	ventilation, noninvasive
OPMD	oculopharyngeal muscular dystrophy
OS	overall survival
PAV	permanent assisted ventilation
PMLE	penalized maximum likelihood estimation

LIST OF ABBREVIATIONS

Abbreviation	Description
PPS	Per-Protocol Set
PS	propensity score
PT	preferred term
Q1	lower quartile
Q3	upper quartile
SAE	serious adverse event
SALS	Sporadic Amyotrophic Lateral Sclerosis
SAP	Statistical Analysis Plan
SCA3	spinocerebellar ataxia type 3
SD	standard deviation
SE	standard error
SOC	system organ class
SOD	superoxide dismutase
SVC	Slow Vital Capacity
TEAE	treatment emergent adverse event
TFL	table, figure, listing
VC	Vital Capacity
WHODD	World Health Organization Drug Dictionary

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a serious, life-threatening, rare degenerative disorder of large motor neurons of the cerebral cortex, brain stem and spinal cord that results in progressive wasting and paralysis of voluntary muscles [1]. The incidence of ALS is currently approximately 2/100,000/year [2,3] and may be increasing [4]. The lifetime ALS risk is 1 in 600 to 1 in 1000. Even though the incidence of ALS is similar to that of multiple sclerosis [5], the prevalence is only 4- 6/100,000 (about 25,000 participants in the United States), due to the higher mortality rate. Fifty percent of people with ALS die within three years of onset of symptoms and 90% die within five years [5]. The median age of onset is 55 years. The cause in most cases is unknown. Age and gender are the only risk factors repeatedly documented in epidemiological studies [6]. There is a slight male predominance (3:2 male to female ratio) in sporadic ALS.

No treatment prevents, halts or reverses the disease, although riluzole use is associated with a 10% prolongation of survival [7, 8], and edaravone, an antioxidant, slows illness by approximately 33% in some people [9]. The majority of people with ALS have sporadic (SALS) disease; 10% are familial (FALS). Natural history studies have determined that age at onset, site of onset, delay from first symptom to entering ALS clinic, and rate of change in respiratory function are significant covariates of survival [12,13,14,15].

Trehalose is a disaccharide that is well known for its protein-stabilizing properties [16,17] and its ability to activate autophagy [18]. This was the basis for human trials of intravenous (IV) trehalose in oculopharyngeal muscular dystrophy (OPMD) and spinocerebellar ataxia type 3 (SCA3 or Machado Joseph disease). Trehalose has been shown to penetrate both muscle and brain. In recent years it became clear that trehalose exposure results in the activation of cellular pathways that are relevant to its use as a potential treatment of ALS. Trehalose is not absorbed well in the human gut because of trehalase enzymes in the brush border that cleave the molecule into two glucose molecules. Seelos Therapeutics has developed an IV formulation to circumvent the breakdown of trehalose in the gut.

The clinical safety profile of trehalose and the nonclinical toxicology data support the investigation of SLS-005 as a potential treatment of ALS. Weekly dosing of SLS-005 is the only regimen to be evaluated in clinical trials to date (participants with OPMD and SCA3) and will be used in this trial. Participants with either sporadic or familial ALS accumulate TDP43 and participants with the superoxide dismutase (SOD) mutation accumulate SOD aggregates and thus have the potential to benefit from treatment with SLS-005 through activation of autophagy.

The is an intermediate expanded access protocol (EAP) trial to provide access to the investigational product, SLS-005, to participants with ALS who are not eligible to participate in clinical trials. The results might be included in a regulatory submission.

This statistical analysis plan (SAP) describes the detailed procedures for the planned statistical analyses of the efficacy, safety, and biomarker data that are collected. The endpoints are defined, and the statistical methods used to analyze them are presented. Table shells for the planned tables, figures, and listings (TFL) are included in a separate document, entitled “Data Presentation Plan” (DPP).

This version of the SAP has been developed using protocol version 1.0, dated 31 July 2023. Changes from the statistical methodology planned in the protocol are described in Section **7**.

1.1 Study Objectives and Endpoints

Table 1 Objectives and Endpoints

Objective	Endpoint
PRIMARY	
To provide access to the investigational product, SLS-005, to about seventy (70) participants with ALS who are not eligible to participate in clinical trials.	Not applicable
EXPLORATORY	
To assess the disease progression in a broad population.	Overall survival
	Changes in ALS Functional Rating Scale-Revised (ALSFRS-R) scores
	Changes in Slow Vital Capacity (SVC)
	Changes in Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 (ALSAQ-40) score
BIOMARKER	
To assess the markers of neurodegeneration	Changes in levels of serum neurofilament light (NfL) chain protein
SAFETY	
To assess the long-term safety of SLS-005 in a broad population of participants diagnosed with ALS who are not eligible to participate in clinical trials.	The incidence of treatment-emergent adverse events (TEAEs), and assessments of vital signs, clinical laboratory tests, and Columbia-Suicide Severity Rating Scale (C-SSRS)

1.2 Study Design

The is an intermediate EAP trial to provide access to the investigational product, SLS-005, to participants with ALS who are not eligible to participate in clinical trials. Planned enrolment is approximately 70 people living with ALS at up to 25 sites in the US. Participants will receive weekly 60- or 90-minute IV infusions of trehalose, 90.5 mg/mL, at a dose of 0.75g/kg at the study center or at home. This is the same treatment dosage and dosing interval used in regimen E of the HEALEY ALS Platform Trial.

This EAP will enroll two cohorts of participants:

- Cohort 1: participants who do not qualify for any reasonably accessible ongoing clinical trials. This population represents a completely different participant population than that represented in the Platform Trial.
- Cohort 2: participants who have completed the Platform Trial Regimen E RCT and/or OLE and are not eligible for enrollment in another treatment regimen of the platform study. At the completion of their participation in the EAP, these participants may have been exposed to up to 76 weeks of IV trehalose – important long-term safety data.

Specifically, this EAP will capture safety, biomarker, and clinical efficacy data to augment the Phase 2/3 Platform Trial data of IV Trehalose.

Participants may be screened over and up to a 21-day period prior to treatment initiation. Participants who meet the inclusion criteria and none of the exclusionary criteria may be enrolled into the EAP. Participants may initiate treatment on the same day as the combined Screening/Baseline Visit, provided consent was obtained and all inclusion and exclusion criteria have been satisfied and eligibility has been confirmed by the investigator prior to treatment initiation.

Participants may receive up to 24 weeks of weekly infusions per protocol. There are up to 25 infusions over 24 weeks (first infusion occurs at the Screening/Baseline Visit). In addition to infusion visits, participants will have three scheduled in-clinic visits at Screening/Baseline, Week 3, and Week 24, and 1 phone call or telemedicine visits at Week 12 during the treatment period. The follow-up safety call will take place 28 +/- 7 days after the participant's last dose of the investigational medical product.

A Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The DSMB Charter provides additional details.

Figure 1 below displays the study design for the study. Table 7 and Table 8 in Appendix 1 displays the schedule of assessments in the study for Cohort 1 and Cohort 2, respectively.

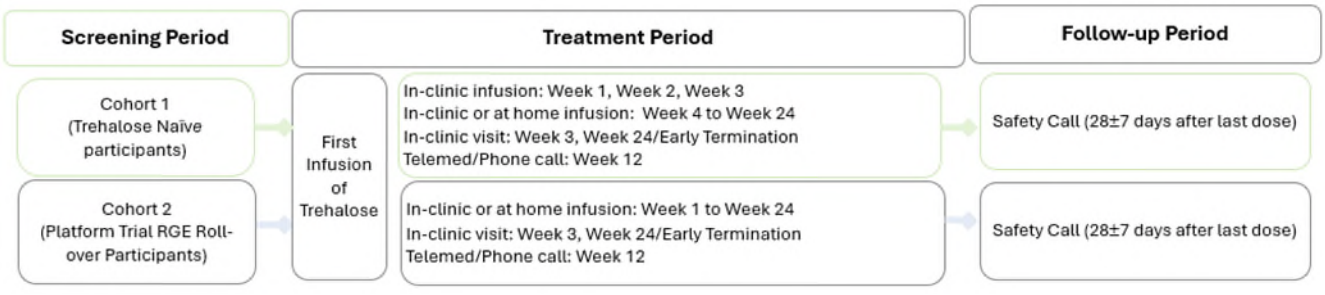

Figure 1 Study Diagram

2. STATISTICAL HYPOTHESES AND MULTIPLICITY ADJUSTMENT

No formal hypothesis testing will be conducted in this study. All analyses will be descriptive in nature. Any modeling that is performed will focus on parameter estimation. The Trehalose treated EAP participants will be compared to predicted placebo progression using historical controls, the p-values will be included to characterize associations and treatment effect but will mainly be used for the exploratory purpose and will not be adjusted for multiplicity.

3. ANALYSIS SETS

3.1 Definition of Analysis Sets

The following analysis sets are defined for this study:

Analysis Set	Definition
Screened Set	All participants who signed the informed consent form.
Full Analysis Set (FAS)	All participants who received at least one dose of Trehalose.
Per-Protocol Set (PPS)	All participants included in FAS and who satisfy all the criteria below: - Did not have a major protocol deviation that affected the scientific integrity of the trial. - Received at least 85% and not more than 125% of the prescribed infusion volume up to the point of drug withdrawal/ last infusion. Data after drug withdrawal/ last infusion will not be included. The PPS will be finalized prior to the database closure.

3.2 Violations and Deviations

Protocol deviations are failures to adhere to the inclusion/exclusion criteria and protocol requirements and will be classified as major or minor protocol deviations.

Major protocol deviations will be identified and categorized by medical review before database lock for analysis.

The following deviations will be collected and reported:

- Informed consent violation
- Study treatment not taken/dispensed per protocol
- Concomitant medication, vitamins, minerals and/or supplements restriction violated
- Study procedure violated
- Visit deviation
- Adverse event (AE) reporting deviation
- Eligibility deviation
- Other deviation

4. STATISTICAL ANALYSIS

4.1 General Considerations

All data collected will be provided in listings, except data collected only for confirmation of study entry criteria and for study administrative purposes. If any participant is found to not have valid documented informed consent, that participant's data will be excluded from all study datasets, summaries, and listings, except as necessary to document the error.

Unless specified otherwise, the tables and listings for participant dispositions will be based on the Screened Set, all other TFLs will be based on FAS. TFLs will present results by treatment group (or cohort), visit and timepoint as applicable. The Trehalose treatment group labels used in analyses are displayed in Table 2. In addition, the historical control groups constructed in Section 4.2 will be included for comparison analysis between combined Trehalose group with historical control groups.

Table 2 Treatment Group Label

Treatment Group Label
Cohort 1 – Trehalose Naive
Cohort 2 – Platform Trial RGE Roll-over
Trehalose Total

All medical history and adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) 23.0; the primary system organ class (SOC) will be used for analyses. The medications will be coded using the World Health Organization (WHO) Drug Dictionary: WHODrug Global B3 September 1, 2020, a few medications not available in this version will be coded using WHODrug Global B3 March 1, 2023.

Except where specified otherwise, all continuous variables will be summarized with descriptive statistics (the number of non-missing values, mean, standard deviation (SD), median, lower quartile (Q1), upper quartile (Q3), minimum and maximum) and all categorical variables will be summarized with frequency counts and percentages in each category, by treatment group.

For conversion of days to years, months, or weeks, the following conversion factors will be used:

- 1 year = 365.25 days
- 1 month = 30.4375 days
- 1 week = 7 days

All analyses will be conducted using SAS version 9.4 or higher.

4.1.1 Definitions, Derivations and Classifications

Definition of Baseline

The baseline value for all endpoints (i.e., laboratory parameters and vital signs) will be the last available value prior to the first dose of study treatment in Trehalose group, and the date of first study visit in historical control group.

Assessments taken on the same day as study treatment will be assumed to be before study treatment unless indicated to be obtained post-study treatment.

Definition of Day 1

Study Day 1 is defined as the first day of study treatment in Trehalose group, and the date of first study visit in historical control group. This will also be considered the reference start date.

Definition of Study Day

Study Day will be computed as follows:

- Study Day = (Date of event – Date of study day 1) + 1 if the date of the event is on or after the date of study day 1.
- Study Day = (Date of event – Date of study day 1) if the date of the event is prior to the date of the study day 1.

Derivations Involving Dates

The number of days between two dates, the date of interest and the reference date, will be calculated as (Date of event – Reference date) + 1 when the event date is on or after the reference date; or, as (Reference date – Date of event) + 1 when the event date is before the reference date. The resulting value is then converted to years, months, or weeks, as appropriate. Partial dates will be imputed before calculating the number of days (See Appendix 2 for partial or unknown date imputation rules).

Common Calculations

Change from baseline (CHG) will be calculated as:

Change from baseline = Actual (observed) value at post baseline visit – baseline value

Percent change from baseline (PCHG) will be calculated as:

Percent change from baseline (%) = 100*(Change from baseline at post baseline visit/Baseline value)

If baseline is not available, the change from baseline and percent change from baseline will not be calculated and will remain missing.

Demographics

Age in years at time of enrollment is not collected in eCRF and will be derived relative to the date of informed consent as: (informed consent date – date of birth + 1) / 365.25 and truncated to 1 decimal point. Age in years at time of first study treatment administration will be derived for participants treated and used for analysis summary. It will be calculated as (date of first study treatment – date of birth + 1) / 365.25 and truncated to 1 decimal point.

Body mass index (BMI) in kg/m^2 will be calculated as by weight in kilograms (kg) divided by height in meters squared (i.e., weight / [height*height]).

4.1.2 Windowing, Unscheduled Visits and Repeat Test Results

All assessments collected at scheduled and unscheduled visits will be included in the by-participant data listings.

For Safety endpoints, the data will be analyzed according to the nominal visits except Baseline which is defined as last available value prior to the first dose of study treatment. The results collected at scheduled visits per

protocol will be included in the by-visit summary tables. For laboratory data analysis at the scheduled visits, central-laboratory values will (preferentially) be selected for a given visit; however, if there is no non-missing central-laboratory value available and there is a non-missing local-laboratory value collected at the visit, then a local-laboratory value will be used.

In addition, for Safety endpoints of laboratory data, vital signs and C-SSRS assessments, the last assessment during the post-baseline period will also be summarized using the analysis visit name "End of Treatment Period", which is defined as the last measurement from all post-baseline visits including the unscheduled visits and early termination visit.

For Efficacy and Biomarker endpoints, the protocol defined visit windowing in Table 3 will be used to window the unscheduled visits or early termination visit to scheduled visit when it doesn't exist. If unscheduled visits or early termination assessments fall in the same window, then select the assessment closest to the target assessment day. If there are two assessments that fall into a visit window and are equidistant from the target assessment day, then the later assessment will be selected.

Table 3 Analysis Visit Windows for Efficacy (ALSFRS-R, SVC, ALSAQ-40) and Biomarker

Analysis Visit	Nominal Day	Lower Limit	Upper Limit
Baseline	1	None	Time of first dose of study treatment
Week 3	22	19	25
Week 12	85	78	92
Week 24	169	155	183

4.1.3 Dropouts, Missing/Incomplete Data, and Outliers

Missing data occurs when any requested data is not provided, leading to blank fields on the collection instrument. These data will be indicated using a "blank" in participant listing displays. Answers such as "Not applicable" and "Not evaluable" are not considered to be missing data and should be displayed as such. In general, missing data will not be imputed unless specifically stated in this SAP.

The missing/incomplete dates for adverse events, and prior/concomitant medications, procedures (for the purpose of computing durations and treatment emergent status of adverse events, medications and procedures status (prior and/or concomitant)), ALS symptom onset date and diagnosis date will be imputed according to the rules specified in Appendix 2. Partial dates will still be displayed in listings.

Uncoded events at the time of analysis will be assigned the string "UNCODED" as the system organ class, and the reported term will be used as the preferred term so they can be included in the summary tables. In the final dataset, all the adverse events should be coded.

Uncoded medications at the time of analysis will be assigned the string "UNCODED" as the Anatomical-Therapeutic-Chemical (ATC) summary level(s) and the verbatim term will be used as the preferred name, so they can be included in the summary tables. In the final dataset, all the medications will be coded.

4.2 Historical Controls

Due to the nature of this EAP study, there is no treatment-naive randomized control group. To estimate the treatment effect, historical controls are introduced to compare with Trehalose treated participants. The historical controls will be constructed through the propensity score (PS) matching and PS based stratification approaches, respectively.

4.2.1 Eligible Historical Control Analysis Set

The eligible historical control analysis set will be constructed from the available databases relating to clinical outcomes in ALS consisting of previous ALS clinical research study participants who:

- met major eligibility criteria from this EAP study if related information was collected in historical database, including:
  - Inclusion criteria 2: Age 18 years or older.
  - Exclusion criteria 2: Screening glucose >=140 mg/dl
  - Exclusion criteria 6: Screening body weight >144 kilograms
- and had known mortality information
- and had baseline and at least one post-baseline ALSFRS-R total scores recorded
- and/or had baseline and at least one post-baseline measurements of Vital Capacity (VC) including the collected SVC or Forced Vital Capability (FVC)

4.2.2 PS Estimation

PS method will be used to balance the population-level baseline characteristics between the Trehalose treated group and the eligible historical control analysis set. In order to calculate PS, the following selected common baseline demographics and characteristics variables of known prognostic significance will be used as covariates in PS estimation if applicable, other covariates may also be included.

- Age (years) [19]
- BMI (kg/m^2)
- Bulbar onset (Yes/No)
- Riluzole use (Yes/No)
- Time since ALS symptom onset (months) [19]
- Time since ALS diagnosis (months)
- Baseline ALSFRS-R total score
- Pre-baseline ALSFRS-R slope (points/month) [20]
- Baseline % predicted VC [21]

PS is the probability of a participant being assigned to a treatment instead of the other one conditional on a given set of baseline characteristics. It is determined by a logistic regression with all above common baseline covariates. To reduce any potential bias due to rare events, PS will be estimated using penalized maximum

likelihood estimation (PMLE) (Firth, 1993). The PS will be estimated for participants from Trehalose group and the eligible historical control analysis set. Overlap of PS between both groups will be assessed using standardized mean differences table and/or plot.

4.2.3 PS Matching

PS matching method (Rosenbaum and Rubin, 1985) matches Trehalose participants with eligible historical control participants based on PS such that matched Trehalose participants and placebo participants are comparable in terms of covariates used in PS determination. This process mimics randomization to create two comparable groups with a caution of limitation that the matching is conditional on included covariates.

PS matching will be conducted in a 1:1 ratio using a more optimal caliper width based on the standard deviation of the logit of the propensity score [22]. The constructed historical control group will be used for the main analysis on the exploratory efficacy endpoints defined in Section 4.7. In addition, the propensity score matching will be performed with other different caliper widths if available to construct additional matched historical control groups for sensitivity analysis of the related exploratory efficacy endpoints.

Participants who are not selected in the matching process will be excluded from further analysis. Following matching, baseline covariates will be summarized for participants in PS matched Trehalose group and PS matched historical control group to check if the balance is generally achieved.

The PS matched identifier (ID) will be included as stratification factor to estimate the overall treatment effect and difference between the PS matched Trehalose group with historical control group in the analysis of exploratory efficacy endpoints defined in Section 4.7.

4.2.4 PS Stratification

The participants from Trehalose group and the eligible historical control analysis set will be sequentially stratified into appropriate strata according to the PS quintiles of the Trehalose treated participants. This allows each stratum to contain roughly an equal number of Trehalose treated participants. The strata that do not contain both Trehalose and historical control participants will be dropped from the matched samples. As participants in each stratum have similar propensity scores, each stratum can be conceptualized as a quasi-RCT, and the observed covariates will be better balanced between the two groups under the assumption that there are no unobserved confounders and the PS model has been correctly specified.

The overlap of PS distributions between the two groups within each stratum will be assessed. The covariate balance between the two groups after the PS stratification will be checked.

The PS stratification-built strata will be included as stratification factor to estimate the overall treatment effect and difference between the PS stratification-based Trehalose group and historical control group in the analysis of exploratory efficacy endpoints defined in Section 4.7.

4.3 Participant Disposition

A summary of participant disposition will be provided for Screened Set by treatment cohort and overall for the following categories. The number and percentage of participants will be presented.

- Participants screened (signed the informed consent form)
- Screen failures with reasons for failure

- Participants treated
- Participants with major protocol deviation
- Participants completed study or discontinued from study with discontinuation reasons
- Participants completed treatment or discontinued from treatment with discontinuation reasons

The study duration (weeks), defined as (date of study completion/discontinuation – date of informed consent signed + 1) / 7, will be summarized descriptively by treatment cohorts and overall.

The participant disposition for FAS and the participants who failed screening with reasons (if any) will be listed.

Protocol deviations for FAS will be provided in a listing including the deviation type and deviation description for major and minor deviations, respectively.

The number and percentage of participants included and excluded from the analysis sets FAS and PPS will be tabulated overall and for each treatment cohort. Reason(s) for exclusion from each set will be summarized overall and by reason for exclusion. The ALSFRS-R and SVC evaluable participants who have baseline and at least one post-baseline assessment of ALSFRS-R or SVC will be summarized under both FAS and PPS. The participants excluded from analysis sets and exclusion reasons will be listed for all Screened Set.

4.4 Participant Characteristics

The participant characteristics described in this section will generally be performed for FAS by treatment cohort and overall, unless otherwise specified. All related data will be provided in listings unless specified otherwise.

4.4.1 Demographic and Baseline Characteristics

Demographic data will be summarized descriptively including age (years) at time of first study treatment administration, age group (< 65 years, ≥ 65 years), sex at birth, reproductive capacity, race, ethnicity and baseline physical measurements of height (cm), weight (kg), BMI (kg/m^2).

Baseline characteristics including ALS history, ALS diagnosis and ALS therapy as listed in items below will be summarized:

- Family history of ALS
- Site of onset (Limb, Bulbar, Axial, Respiratory, Generalized)
- Bulbar onset (Yes/No)
- Time since ALS symptom onset (months)

  This equals to (date of first study treatment - date of ALS symptom onset + 1) / 30.4375
- Time since ALS diagnosis (months)

  This equals to (date of first study treatment - date of ALS diagnosis + 1) / 30.4375
- Time since ALS symptom onset to diagnosis (months)

  This equals to (date of ALS diagnosis – date of ASL symptom onset + 1) / 30.4375
- Has participant undergone genetic testing

- Has a genetic mutation been identified
- Type of identified mutation
- ALS Diagnosis made according to Revised El Escorial criteria
- Revised El Escorial Criteria for ALS
- Have genetically definite ALS
- Presence of tracheostomy (Yes/No)
- Presence of ventilation, noninvasive (NIV) (Yes/No)
- Gastrointestinal tube insertion (Yes/No)
- ALS medication use
    - None of Riluzole, Edaravone and Relyvrio
    - Any of Riluzole, Edaravone and Relyvrio (including subcategories: Riluzole, Edaravone, Relyvrio, Riluzole only, Edaravone only, Relyvrio only, Riluzole and Edaravone only, Edaravone and Relyvrio only, All of Riluzole, Edaravone and Relyvrio)
    - Neither Riluzole nor Edaravone, Both Riluzole and Edaravone

  Participants' usage of these medications will be collected in concomitant medication form. These medications can be identified by the medication preferred term. Relyvrio is also called sodium phenylbutyrate/ursodoxicoltaurine, sodium phenylbutyrate/taurursodiol.

4.4.2 Baseline Assessments

Descriptive statistics will be used to summarize, by treatment cohort and overall, the following baseline assessments in the FAS.

- ALSFRS-R Total Score
- ALSFRS-R Severity Group: All ALSFRS-R Items ≥ 2, One or More ALSFRS-R Items < 2
- Pre-baseline ALSFRS-R Slope (points/month)

  This equals to (48 – baseline ALSFRS-R total score) / Time since ALS symptom onset (months)
- % Predicted SVC
- ALSAQ – Symptom Index
- ALSAQ - Physical Mobility
- ALSAQ - Activities of Daily Living/Independence
- ALSAQ - Eating and Drinking
- ALSAQ – Communication
- ALSAQ - Emotional Reactions

4.4.3 Baseline Characteristics Comparison with Historical Control

The common demographics and baseline characteristics outlined in Section 4.2.2 will be tabulated for PS matched (based on the pre-specified caliper width for main analysis) Trehalose group and historical control group under FAS and PPS, as well as PS stratification-based Trehalose group and historical control group under FAS and PPS.

The descriptive statistics for each baseline characteristics will be summarized; the standardized mean differences table and/or plot will be provided to assess the balance between two groups.

4.4.4 Medical History

Medical history will be classified using MedDRA terminology. Medical history will be summarized using numbers and percentages by primary system organ class and preferred term for each treatment cohort and overall for FAS.

4.4.5 Medications, Therapies and Procedures

4.4.5.1 Prior and Concomitant Medications

Prior medications are medications and therapies with a start date before the first dose of study treatment. Concomitant medications are medications with a start date after the first dose of study treatment. Medications with a start date before the first dose of study treatment and with either an end date after the first dose of study treatment or are ongoing will be classified as both prior and concomitant medications. If a medication or therapy cannot be classified due to completely missing dates, it will be classified as both prior and concomitant.

Prior and concomitant therapies/medications will be summarized by ATC drug class (i.e., 4th level, or most specific level available if 4th level is unavailable) and preferred name for each treatment cohort and overall based on the FAS.

4.4.5.2 Prior and Concomitant Devices/Procedures

The pre-specified key devices and procedures will be summarized by phase (Prior or Concomitant) and devices/procedure names for each treatment cohort and overall based on the FAS.

Prior devices/procedures are those with a start date before the first dose of study treatment. Concomitant devices/procedures are those with a start date after the first dose of study treatment. Devices/procedures with a start date before the first dose of study treatment and with either an end date after the first dose of study treatment or are ongoing will be classified as both prior and concomitant procedures. If a device/procedure cannot be classified due to completely missing dates, it will be classified as both prior and concomitant.

4.5 Primary Endpoint Analyses

There is no statistical primary endpoint analysis based on the nature of this EAP study’s primary objective is to provide access to the investigational product, SLS-005, to about seventy participants with ALS who are not eligible to participate in clinical trials.

4.6 Secondary Endpoint Analyses

Not applicable.

4.7 Exploratory Endpoints Analyses

This section includes the clinical efficacy analyses on overall survival, decline of ALSFRS-R, % predicted SVC and ALSAQ-40 scores. All endpoints will be summarized descriptively for EAP study treatment cohorts and overall. The first three efficacy endpoints (overall survival, ALSFRS-R, % predicted SVC) will also compare the treatment effect between combined Trehalose group with the historical control groups under both FAS and PPS as appropriate.

4.7.1 Overall Survival

4.7.1.1 Definition of Endpoint

The overall survival (OS) will be calculated in months, defined as the time from the date of first study treatment administration to the date of death due to any cause. Participants not known to have died at the time of the analysis will be censored on the date they were last known to be alive.

OS time (months) = (date of event/censored - date of first study treatment administration + 1) / 30.4375.

4.7.1.2 Main Analytical Approach

OS will be summarized for both EAP study treatment groups and historical control groups based on the FAS.

The number and percentage of participants with event or censored will be presented by treatment group. The Kaplan-Meier estimates of the quartiles of the survival distribution along with corresponding 95% confidence intervals will be calculated. The survival rate (%) and 95% CI will be displayed for 2 months, 4 months, 6 months, 7 months, and 8 months.

To assess the differences in OS between PS matched (based on the pre-specified caliper width for main analysis) Trehalose group and historical control group: the hazard ratio (HR) and corresponding 95% confidence interval will be estimated using the Cox proportional-hazards model, stratified by PS matched ID. The two-sided log-rank test stratified by PS matched ID will be performed for generation of the two-sided p-value, which will be considered statistically significant if $p<0.05$. The Kaplan-Meier plot for Trehalose and historical control groups will be presented.

4.7.1.3 Sensitivity Analyses

To assess the treatment effect in OS, the following sensitivity analyses will be performed using similar approaches as described in the main analysis:

- OS comparison between PS matched (based on other caliper widths if available) Trehalose group and historical control group, including PS matched ID as stratification factor
- OS comparison between PS stratification-based Trehalose group and historical control group, including PS stratification-built strata and bulbar onset as stratification factors

Besides, for EAP study Trehalose group, the OS will also be evaluated by including the usage of permanent assisted ventilation (PAV) as event outcome together with death. A participant will be classified as having an event or being censored based on the earliest occurrence of outcomes as outlined in Table 4. The analytical approach will be the same as the approach detailed in Section 4.7.1.2.

Table 4 Censoring Rules for OS (Including PAV)

Scenario	Date of Event or Censored	Event or Censored
Usage of PAV	Date PAV started	Event
Death	Date of death	Event
Others	Date of last known to be alive	Censored

4.7.1.4 Supplementary Analyses

For OS (Including PAV), one additional comparison between PS stratification-based Trehalose group and historical control group under FAS will be provided. Besides, the following analyses will be performed for the participants in PPS, using the same approaches as described above.

- OS and OS (Including PAV) summary for EAP study by treatment cohort and overall
- OS and OS (Including PAV) comparison between PS matched (based on the pre-specified caliper width for main analysis) Trehalose group and historical control group. If the stratified analysis is invalid due to lack of event in some strata, the unstratified HR and 95% CI, as well as unstratified log-rank p-value will be provided
- OS and OS (Including PAV) comparison between PS stratification-based Trehalose group and historical control group

4.7.2 ALSFRS-R

4.7.2.1 Definition of Endpoints

The endpoint is the mean change from baseline in ALSFRS-R total score measured at Week 24.

The ALSFRS-R is a validated instrument for evaluating the levels of the functional status of participants with ALS in 4 areas, including bulbar, gross motor activity, fine motor activity, and respiratory functions. The scale includes 12 functional items, and each item is rated on a 0 to 4 scale, with a maximum total score of 48. A higher score indicates greater retention of function. The total score collected in eCRF will be used for the analysis.

4.7.2.2 Main Analytical Approach

For participants in FAS, the ALSFRS-R total score as well as the change from baseline will be summarized descriptively at scheduled visits (Baseline, Week 3, Week 12 and Week 24) by treatment group. The linear mixed-effects model for repeated measures (MMRM) will be used to calculate the least square mean for each treatment group, and the treatment difference between PS matched (based on the pre-specified caliper width for main analysis) Trehalose group vs. historical control group.

The MMRM model will include the ALSFRS-R total score change from baseline at scheduled post-baseline visits as the dependent variable, with fixed effects for treatment, bulbar onset (Yes/No) and baseline value, visit (as categorical variable), treatment-by-visit interaction, and baseline value-by-visit interaction, with visit as the repeating factor, participant as a random effect. PS matched ID will also be included as a random effect for comparison between Trehalose group vs. historical control group.

The MMRM model will use restricted maximum likelihood (REML) to estimate least squares means and treatment differences. An unstructured covariance matrix will be used to model within-participant errors. Data

from all scheduled visits over the treatment period will be used to fit the model. Missing data will not be imputed.

If the computational algorithm fails to converge, the following structures will be executed in the order specified until convergence is achieved: Toeplitz with heterogeneity (TOEPH), autoregressive with heterogeneity (ARH(1)), compound symmetry with heterogeneous variances (CSH), autoregressive (AR(1)), Toeplitz (TOEP), and compound symmetry without heterogeneous variances (CS).

At the post-baseline visits of Week 3, Week 12 and Week 24, the least square mean and standard error (SE), as well as 2-sided 95% CI for each treatment group will be provided. The difference (SE) and 95% CI, p-value between PS matched Trehalose group and historical control group will be summarized.

4.7.2.3 Sensitivity Analyses

To assess the treatment effect in ALSFRS-R total score, the following sensitivity analyses will be performed using similar approaches as described in main analysis:

- ALSFRS-R total score comparison between PS matched (based on other caliper widths if available) Trehalose group and historical control group, including PS matched ID as random effect
- ALSFRS-R total score comparison between PS stratification-based Trehalose group and historical control group, including PS stratification-built strata as fixed effect

4.7.2.4 Supplementary Analyses

The following analyses will also be performed for the participants in PPS, using the same approaches as described above.

- ALSFRS-R total score summary for EAP study by treatment cohort and overall
- ALSFRS-R total score comparison between PS matched (based on the pre-specified caliper width for main analysis) Trehalose group and historical control group
- ALSFRS-R total score comparison between PS stratification-based Trehalose group and historical control group

In addition, an analysis of the total ALSFRS-R score will be performed using a general linear mixed effects model to compare the rate of decrease (ie, slope) of total ALSFRS-R scores over the 24-week treatment period between the PS matched (based on the pre-specified caliper width for main analysis) Trehalose and historical control, and between the PS stratification based Trehalose and historical control), respectively, for both FAS and PPS.

The model will fit a random intercept and slope for each participant and will include fixed effects for treatment group, bulbar onset (Yes/No), visit, and treatment by visit interaction. For comparison between the PS matched Trehalose and historical control, PS matched ID will be included as random effect; for comparison between PS stratification-based Trehalose and historical control, the PS stratification-built strata will be included as fixed effect.

An unstructured covariance structure will be used to model the within-participant errors. Visit will be expressed in weeks as a continuous variable and will include all scheduled visits including baseline as Week 0 (i.e., Week 0, Week 3, Week 12, Week 24). Missing values will not be imputed.

Estimates of the slope will be presented by the treatment group along with corresponding 95% CIs. The estimate of the difference between slopes (Trehalose vs. historical control) and the 95% CI of the difference between slopes will be presented. The null hypothesis of no difference in slopes between the treatment groups will be determined by testing the significance of the treatment by visit interaction term.

4.7.3 SVC

4.7.3.1 Definition of Endpoints

The endpoint is the mean change from baseline in % predicted SVC (or VC) at Week 24.

For this EAP study, the SVC is measured at Screening/Baseline and Week 24 visit with the 3 best trials recorded on the eCRF. The % predicted SVC value will be calculated at each visit for analysis following the calculation algorithm in Appendix 3. For historical control group, the % predicted VC (based on collected SVC or FVC) will be used.

4.7.3.2 Main Analytical Approach

For participants in FAS, the % predicted SVC (or VC) as well as the change from baseline will be summarized descriptively at Baseline and Week 24 by treatment group.

For EAP study treatment groups, an analysis of covariance (ANCOVA) model with change from baseline at Week 24 as the dependent variable, including main effects for treatment group, bulbar onset (Yes/No) and baseline value will be performed. The % predicted SVC change from baseline's least square mean (SE) and 2-sided 95% CI for each treatment group will be provided at Week 24.

For comparison between PS matched (based on the pre-specified caliper width for main analysis) Trehalose group vs. historical control group, a linear mixed effects model will be used, including the % predicted VC change from baseline at Week 24 as the dependent variable, with fixed effects for treatment group, bulbar onset (yes/no), and baseline value, with propensity score matched ID as the random effect. At Week 24, the % predicted vital capacity change from baseline's least square mean (SE) and 2-sided 95% CI for PS matched Trehalose and historical control groups will be presented. The difference (SE) and 95% CI, p-value for PS matched Trehalose vs. historical control will be summarized.

4.7.3.3 Sensitivity Analyses

To assess the treatment effect in %predicted VC, the following sensitivity analyses will be performed using similar approaches as described in the main analysis:

- % Predicted VC comparison between PS matched (based on other caliper widths) Trehalose group and historical control group, including PS matched ID as random effect using the linear mixed effect model
- % Predicted VC comparison between PS stratification-based Trehalose group and historical control group, including PS stratification-built strata as fixed effect using ANCOVA model

4.7.3.4 Supplementary Analyses

The following analyses will also be performed for the participants in PPS, using the same approaches as described above.

- % Predicted SVC summary for EAP study by treatment cohort and overall
- % Predicted VC comparison between PS matched (based on the pre-specified caliper width for main analysis) Trehalose and historical control groups
- Predicted VC comparison between PS stratification-based Trehalose group and historical control group

4.7.4 ALSAQ-40

The ALSAQ-40 consists of forty questions that are specifically used to measure the subjective well-being of participants with ALS and motor neuron disease. It evaluates 5 dimensions of health status that are affected by the disease: physical mobility (10 items), activities of daily living and independence (10 items), eating and drinking (3 items), communication (7 items), and emotional reactions (10 items). Refer to Table 5 for dimensions and corresponding items from ALSAQ-40 questionnaire.

For each question, participants are asked to select one of the five options according to the frequency of each event experienced during the last two weeks, and a score is assigned for each option: 0=never, 1=rarely, 2=sometimes, 3=often, 4=always or cannot do at all.

The score of items for each dimension will be summed up, then transformed on a scale of 0 to 100 using the equation: (score of each dimension/4) x 100, with 0 indicating perfect health and 100 indicating worst possible health status. The ALSAQ-40 total score represented as symptom index will be calculated as (sum of scores of the 40 individual items)/4 x 100. The specific dimensions and symptom index scoring algorithms are shown in Table 5.

Table 5 ALSAQ-40 Dimensions and Symptom Index Scoring Algorithms

Dimension Scale	Number of Items	Item (Question)	Scoring Algorithm
Physical Mobility	10	Q1 - Q10	If two or fewer of the 10 items are missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.
Activities of Daily Living/Independence	10	Q11 - Q20	If two or fewer of the 10 items are missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.
Eating and Drinking	3	Q21 - Q23	If none of the 3 items is missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.
Communication	7	Q24 - Q30	If one or fewer of the 7 items is missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.
Emotional Reactions	10	Q31 - Q40	If two or fewer of the 10 items are missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.
Symptom Index (for total score)	40	Q1- Q40	If eight or fewer of the 40 items are missing, then scale score = 25 x mean of (all available items raw score); otherwise, set the scale score as missing.

For ALSAQ symptom index and each subscale, the score and change from baseline will be summarized descriptively at Baseline and Week 24 for EAP study treatment groups for FAS. At week 24, the least square mean (SE) and 2-sided 95% CI for each treatment group will be provided using the analysis of covariance (ANCOVA) model with change from baseline at Week 24 as the dependent variable, including main effects for treatment group, bulbar onset (Yes/No) and baseline value as covariate.

4.8 Other Analyses

4.8.1 Biomarker Analyses

A descriptive analysis of change in NfL levels will be performed at Baseline and Week 24 for FAS by EAP study treatment groups.

In addition, the NfL levels will be performed using a general linear mixed effects model to compare the rate of decrease (ie, slope) of NfL levels over the 24-week treatment period for FAS. The model will fit a random intercept for each participant and will include fixed effects for treatment group, bulbar onset, visit, and treatment by visit interaction. An unstructured covariance structure will be used to model the within-participant errors. Visit will be expressed in months (using actual study days / 30.4375) as a continuous variable including the baseline visit and all post-baseline visits. Missing values will not be imputed.

Estimates of the slope will be presented by treatment group along with corresponding 95% CIs.

4.9 Safety Analyses

Safety data that will be summarized includes drug exposure, adverse events, clinical laboratory assessments, vital signs, physical and neurologic examinations, C-SSRS assessments. All safety analyses will be based on the FAS and will be summarized by EAP study treatment cohort and overall unless otherwise stated. All data will be provided in the participant data listings.

4.9.1 Extent of Exposure

A summary on the participants' study drug dose modification types (dose initiation, drug interrupted, drug withdrawn) along with primary reasons for dosage change during the study will be provided with number and percentage of participants, and number of events by treatment cohort and overall for FAS.

The following study drug dose exposure variables will be derived and summarized descriptively by treatment cohorts and overall, for FAS and PPS, respectively.

- Number of infusions administered as continuous variable and by categories (1-5, 6-10, 11-15, 16-20, 21-25).
- Number of participants with any treatment infusion interruption, and number of infusion interruptions
- Treatment duration (weeks): defined as (date of last administration – date of first administration + 7) / 7.
- Total volume prescribed (mL): defined as sum of all volume prescribed for the treatment duration, including the missed scheduled infusion visits if any before participants' last infusion. For the missed infusion visits without volume prescribed collected in eCRF, the volume prescribed will be imputed using the last available volume prescribed carried forward from the prior visit.

- Total volume infused (mL): defined as sum of all volume infused for the treatment duration.
- Prescribed dose intensity (mL/week): defined as Total volume prescribed (mL) / Treatment duration (weeks).
- Actual dose intensity (mL/week): defined as Total volume infused (mL) / Treatment duration (weeks).
- Relative dose intensity (%): defined as 100 x (Actual dose intensity / Prescribed dose intensity)

  The relative dose intensity (%) will be summarized as continuous variable and presented categorically (< 85%, 85% to 125%, > 125%).

In addition, the same study drug exposure analyses above will be performed for participants with TEAEs related to study drug. Only the infusion data prior to the onset of study drug related TEAEs will be included. Treatment duration (weeks) is defined as (date of last administration prior to the onset of TEAE related to study drug - date of first administration + 7)/7, other exposure variables will be calculated within this treatment duration period.

4.9.2 Adverse Events

Participants will be monitored for adverse events from the time they sign consent for the EAP until completion of their participation as defined in the schedule of assessments.

Severity of AEs

Severity of AEs will be graded as Mild, Moderate, Severe. Missing grades will not be imputed, the AEs with missing grade will be summarized into the category of 'Not Reported' in the tabulation summary.

Relationship of AEs to Study Drug

The relationship of AE to study drug will be determined by Investigator. The relatedness categories include Related and Not Related.

TEAEs

AEs with the date of onset or worsening on or after the date of the first dose of study drug administration will be considered treatment emergent.

TEAEs Summary

AEs with onset after informed consent obtained but before the first dose of study drug will be collected but excluded from adverse event tabulations and presented in listing, only TEAEs are included in the summary by treatment cohort and overall for FAS. The number and percentage of participants with TEAEs will be presented for the summaries as described in **Table 6**. For table of overall summary, the number of events will also be provided.

Table 6 TEAE Summaries

Endpoint	Description
Overall summary	• TEAE • TEAE related to study drug • Serious TEAE

Endpoint	Description
	• Serious TEAE related study drug • Severe TEAE • Severe TEAE related study drug • TEAE leading to study drug dose reduced • TEAE leading to study drug interrupted • TEAE leading to study drug discontinuation • TEAE leading to early study discontinuation • TEAE leading to death (i.e., outcome is fatal)
TEAE	• Overall summary by SOC and PT • Overall summary by SOC, PT, and maximum severity grade • Overall summary by SOC, PT, and time to onset • Overall summary by PT
Most frequently-occurring (≥ 5%) TEAE related to study drug	• Overall summary by PT
TEAE related to study drug	• Overall summary by SOC and PT • Overall summary by SOC, PT, and maximum severity grade • Overall summary by SOC, PT, and time to onset
Most frequently-occurring (≥ 5%) TEAE related to study drug	• Overall summary by PT
Serious TEAE	• Overall summary by SOC and PT • Overall summary by SOC, PT, and time to onset
Serious TEAE related to study drug	• Overall summary by SOC and PT
Severe TEAE	• Overall summary by SOC and PT
Severe TEAE related to study drug	• Overall summary by SOC and PT
TEAE leading to study drug discontinuation	• Overall summary by SOC and PT
TEAE leading to early study discontinuation	• Overall summary by SOC and PT
TEAE leading to death	• Overall summary by SOC and PT

- Time to onset is defined as the number of days from the date of the first dose of study drug administration to the date of AE start. The time to onset will be categorized as the following intervals: 0 to 12 weeks, 12 to 24 weeks, and > 24 weeks.
- The summary of most frequently-occurring (⩾ 5%) TEAE includes the TEAEs experienced by at least 5% participants in the FAS.
- For AE counting at participant level: A participant having the same event (SOC or PT) more than once will be counted only once. When summarizing events by causality and severity by participants, each participant will be counted only once within a SOC or a PT by using the event with the greatest relationship and maximum severity within each classification.

- For TEAE tables by SOC and PT, the AEs will be presented in descending order of total frequency of both SOC and PT within each SOC. If multiple SOCs or PTs have the same frequency, then they will be sorted in alphabetical order. For TEAE tables by PT only, the AEs will be listed in the descending order of total frequency.

AE listings

Individual listings for the following adverse events will be provided:

- Adverse events
- Serious adverse events
- Adverse events leading to early study discontinuation
- Adverse events leading to death

4.9.3 Laboratory Data

Clinical laboratory data includes the measurements of lab parameters from hematology (including HgbA1C), chemistry, urinalysis and serum pregnancy collected from both central laboratory and local laboratory.

- Laboratory data in summary tables and participant data listings will be presented in the International System of Units (SI units; Système International d'Unités).
- If the same lab test has measurements from both central and local labs at same time point, the one from central lab will be used for analysis in tabulation summary.
- Given the lab measurements' baseline and post-baseline results are collected from different labs (most baseline lab measurements are completed at local labs while most Week 24 lab measurements are collected at central lab), the lab results will not be compared with baseline to investigate the change from baseline trend over time except HgbA1C (%). The HgbA1C is a significant parameter as a measure of diabetes, the change from baseline will be calculated and presented in table.
- All scheduled hematology and chemistry parameters' normality assessments will be summarized by visit for each treatment cohort and overall. The assessments will be classified "Normal", "High" and "Low" with respect to the parameter-specific reference ranges established in central lab; and classified as "Normal" and "Abnormal" (with "clinically significant" information) compared to reference ranges by investigator in local lab. The local labs don't collect reference ranges in eCRF, then the status of "High" or "Low" can't be derived programmatically for measurements from local lab, so the summary will be a mixture of assessment values from both central lab and local labs. The number and percentage of participants in each assessment category will be provided.
- All laboratory data will be presented in participant data listings with normality assessments included. The laboratory measurements identified as abnormal (ie, outside the normal range) will also be listed separately by participant, laboratory category, lab parameters at relevant time points.

4.9.4 Vital Signs

Vital sign parameters to be summarized include Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Heart Rate (beats/min), Respiratory rate (breaths/min), Temperature (C) and Weight (kg).

Vital signs will be summarized with descriptive statistics by visit and treatment cohort and overall. Descriptive statistics will be presented for results and change from baseline at each visit and time point where parameters were scheduled to be collected. For parameters Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg), Heart Rate (beats/min), Respiratory rate (breaths/min) at infusion visits, the changes associated with the infusion from pre-dose to 30 mins post start of infusion (SOI), 60 minutes post the SOI, 90 minutes post the SOI, and 30 minutes post end of infusion (EOI) if collected will also be summarized.

4.9.5 Other Safety Assessments

4.9.5.1 Physical Examination

The physical examinations are scheduled at Screening/Baseline visit and will be presented in participant data listing including the body system, result (Normal, Abnormal) and description of abnormal findings.

4.9.5.2 Neurological Examination

The neurological examinations are scheduled at Screening/Baseline visit to assess the normality of body systems under the following categories. The results will be presented in a participant data listing.

- General Category
- Cranial Nerves
- Reflexes
- Plantar Reflex
- Motor System – General Category
- Motor System - Muscle Strength
- Motor System - Muscle Tone
- Coordination/Cerebellar Function
- Sensation - Upper Extremities
- Sensation - Lower Extremities

4.9.5.3 C-SSRS Assessment

The most severe suicidal ideation and most severe suicidal behavior will be summarized descriptively by visit for each treatment cohort and overall under FAS. In addition, the most severe assessments during the treatment period for suicidal ideation and suicidal behavior will be summarized as well, according to the table below which list the ideation and behavior from most severe to least severe.

Suicidal Ideation Severity	**Suicidal Behavior Severity**
• Active suicidal ideation with specific plan and intent • Active suicidal ideation with some intent to act, without specific plan • Active suicidal ideation with any methods (not plan) without intent to act • Non-specific active suicidal thoughts	• Completed suicide • Actual attempt • Interrupted attempt • Aborted attempt

Suicidal Ideation Severity	Suicidal Behavior Severity
• Wish to be dead	• Preparatory acts or behavior

All the C-SSRS assessment information collected will be provided in the data listing.

4.9.5.4 Pregnancy Information

If the female participant/female partner of a male participant become pregnant during the study, the pregnancy history and current pregnancy information will be presented in participant data listing.

4.10 Subgroup Analyses of Interest

Not applicable.

5. INTERIM ANALYSIS AND ANALYSIS SEQUENCE

No formal interim analyses are planned. The DSMB will be convened for safety monitoring of this research study.

6. SAMPLE SIZE DETERMINATION

This is a multi-center intermediate-size EAP to provide access to the investigational product, SLS-005. The planned enrollment is about 70 participants at up to 25 sites.

There are no prior human studies directly measuring effect of IV trehalose on motor axonal integrity in ALS individuals. The estimated SD of log-transformed NfL levels in an longitudinal observational study [23] is 0.30 log-pg/mL. Preliminary data [23] on change in NfL levels over 6 months (range 4 to 8 months) among 46 ALS participants provided an estimate of the person-to-person standard deviation on the natural-log scale of 0.30 log-pg/mL.

With 70 participants anticipated in the EAP, the study would have greater than 99% power to detect a 30% decline in NfL levels over 6 months. The study would have greater than 90% power to detect a true decline as small as 12% over 6 months. This power calculation is aligned with the NfL data from a separate published dataset suggesting that using a mean longitudinal ΔNfL in the untreated state of 0.011 log units/month, a sample cohort size of 26 participants (1:1) would provide 90% power at 5% significance level to detect a treatment difference of 0.065 log units/month (estimated SD of 0.048 log units/month), using a 2-sample t-test [24].

7. CHANGES FROM AND ADDITIONS TO METHODS PLANNED IN THE PROTOCOL

The protocol Section 9.0 mentioned: for cohort 2, the TEAEs and biomarker NfL levels data collected during the parent HEALEY ALS Platform Trial Regimen E study will be combined with cohort 2 data in this EAP study for final analyses.

Changes in SAP: this is re-evaluated by sponsor and decided that the Regimen E study data will not be included in the analysis plan provided by Everest. The integration analysis of EAP study and Regimen E study data may be scheduled in-house by sponsor.

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9. SUPPORTING DOCUMENTATION

Appendix 1 Schedule of Procedures/Assessments

The schedule of assessments for Cohort 1 is given in Table 7, while the schedule of assessments for Cohort 2 is given in Table 8.

- Participants receive a weekly infusion of SLS-005 for up to 24 weeks. Participants who join from the HEALEY ALS Platform Trial Regimen E OLE may do all infusions at home. Participants who join from the HEALEY ALS Platform Trial Regimen E RCT and/or have not previously received SLS-005 in the HEALEY ALS Platform Trial will complete the first four infusion visits in-clinic, then these participants may be permitted, based on safety and tolerability assessments, to have infusions administered at home. There are 25 infusions over 24 weeks (first infusion occurs at the Screening/Baseline Visit).
- In addition to infusion visits, participants will have three scheduled in-clinic visits at Screening/Baseline, Week 3, and Week 24, and 2 phone call or telemedicine visits at Week 12 and approximately 28 days following the last dose of the investigational medical product.

Table 7 Schedule of Activities – Cohort 1 (Trehalose Naïve Participants)

Activity	**Screening/ Baseline Combined[7]**	**Week 1, Week 2**	**Week 3**	**Week 12**	**Week 24 or Early Term.**	**Follow-up Safety Call**
	Clinic	In-clinic infusion-only[5]	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 7 ±3, Day 14 ±3	Day 21 ±3	Day 84 ±7	Day 168 ±14	28±7 days after last dose
Written Informed Consent	X					
Inclusion/Exclusion Review	X					
ALS & Medical History	X					
Demographics	X					
Physical Examination	X					
Neurological Exam	X					

Activity	**Screening/ Baseline Combined**[7]	**Week 1, Week 2**	**Week 3**	**Week 12**	**Week 24 or Early Term.**	**Follow-up Safety Call**
	Clinic	In-clinic infusion-only[5]	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 7 ±3, Day 14 ±3	Day 21 ±3	Day 84 ±7	Day 168 ±14	28±7 days after last dose
Vital Signs[1]	X Weekly documentation of infusion-associated vital signs					
Slow Vital Capacity (SVC)	X				X	
ALSFRS-R	X		X	X	X	
ALSAQ-40	X				X	
Clinical Safety Labs[2]	X		X		X	
Biomarker Collection (Serum)	X				X	
Concomitant Medication Review	X		X	X	X	
Adverse Event Review	X		X	X	X	X
Document infusion-related adverse events[3]	X Weekly documentation of infusion-related adverse events.					
Columbia-Suicide Severity Rating Scale	X		X		X	
Adjust Dose as Needed[4]			X			
Administer/Dispense Study Drug[5]	X Weekly infusions will continue through Week 24. Based on safety and tolerability assessments, infusions may be administered at home after the first 4 in-clinic infusions at Screening/Baseline, Week 1, Week 2, and Week 3.					
Drug Accountability/Compliance[6]	X Weekly infusions will continue through Week 24. Refer to the Pharmacy Manual, site policy, and GCP guidelines on drug accountability, compliance, and source documentation.					
Vital Status Determination[8]					X	X

1 Vital signs are collected at the Screening/Baseline, Week 3, and Week 24 visits and weekly in conjunction with study drug administration from Baseline through Week 24. Vital signs collected at in-clinic visits (Screening/Baseline, Week 3, Week 24) include systolic and diastolic pressure, respiratory rate, heart rate, temperature, and weight. Height is measured at Screening/Baseline Visit only. If significant weight change is reported or suspected at any point during the study, the SI may choose to collect an additional in-clinic weight at their discretion. During each weekly infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), 60 minutes after the SOI (± 10 mins), and 90 minutes (± 10 mins) after the SOI. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug, an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.

Activity	Screening/ Baseline Combined[7]	Week 1, Week 2	Week 3	Week 12	Week 24 or Early Term.	Follow-up Safety Call
	Clinic	In-clinic infusion-only[5]	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 7 ±3, Day 14 ±3	Day 21 ±3	Day 84 ±7	Day 168 ±14	28±7 days after last dose

2 Clinical safety labs include hematology (CBC with differential), complete chemistry panel and urinalysis at Screening/Baseline, Week 3, and Week 24. Hb A1C will be included in labs at Screening/Baseline and Week 24. If labs are abnormal at Week 3, they may need to be repeated per investigator judgement. Serum pregnancy testing will occur in women of child-bearing potential at the Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy. All urine samples must be collected prior to IP administration. Clinical safety labs may be drawn at the study site or obtained at a local site convenient for the participant. Clinical safety lab results obtained locally may be used to assess participant eligibility.

3 Infusion-related AEs are documented weekly in conjunction with study drug administration from Screening/Baseline through week 24.

4 The weight collected at the Screening/ Baseline clinic visit will be used to calculate the participant's initial dose. The participant will remain on a stable dose until the next in-person visit when weight be collected, at which point the dose may be adjusted if the participant's weight has increased or decreased by 2kg. Additional information on dose adjustments is included in Protocol section 6.7.4 Dosing Changes.

5 Administer first dose of study drug only after Screening/Baseline Visit procedures are completed. For participants in cohort 1, infusions occur in-person at clinic at the Screening/Baseline study visit, Week 1, Week 2, and the Week 3 study visits. Weekly infusions will continue through Week 24. An in-clinic infusion and clinic visit may occur on the same day, provided both visits are conducted in-person and within window. There should be no fewer than 4 days (96 hours) between study drug infusions.

6 Refer to the Pharmacy Manual, site policy, and GCP guidelines on site investigator responsibilities, drug accountability, compliance, and source documentation. Any and all accountability records may be requested by sponsor for review at any time.

7 The combined Screening/ Baseline visit may be split into a Screening and a Baseline visit as needed. If baseline infusion occurs on the same day as screening, it must occur after obtaining written informed consent, completing all screening procedures, and confirming participant eligibility.

8 Vital status, defined as a determination of date of death or death equivalent or date last known alive, will be determined for each enrolled participant at the end of their EAP participation (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 EAP. We may also ascertain vital status at later time points by using publicly available data sources as described in section 6.6.10 of the Protocol.

Table 8 Schedule of Activities – Cohort 2 (Platform Trial RGE Roll-over Participants)[10]

Activity	Screening/ Baseline Combined[1,8]	Week 3	Week 12	Week 24 or Early Term.	Follow-up Safety Call
	Clinic	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 21 ±3	Day 84 ±7	Day 168 ±14	28 ±7 days after last doses
Written Informed Consent	X				
Inclusion/Exclusion Review	X				
ALS & Medical History	X				
Demographics	X				
Physical Examination[10]	X				
Neurological Exam[10]	X				
Vital Signs[2]	X[1,10]	X Weekly documentation of infusion-associated vital signs			
Slow Vital Capacity (SVC)[10]	X[1]			X	
ALSFRS-R	X[1]	X	X	X	
ALSAQ-40[10]	X[1]			X	
Clinical Safety Labs[3]	X[1]	X		X	
Biomarker Collection (Serum)[10]	X			X	
Concomitant Medication Review	X[1]	X	X	X	
Adverse Event Review	X[1]	X	X	X	X
Document infusion-related adverse events[4]	X[1]	X Weekly documentation of infusion-related adverse events.			
Columbia-Suicide Severity Rating Scale	X[1]	X		X	
Adjust Dose as Needed[5]		X			
Administer/Dispense Study Drug[6]	X Weekly infusions will continue through Week 24. Participants who join from OLE may do all infusions at home.				
Drug Accountability/Compliance[7]	X Weekly infusions will continue through Week 24. Refer to the Pharmacy Manual, site policy, and GCP guidelines on drug accountability, compliance, and source documentation.				

Activity	Screening/ Baseline Combined[1,8]	Week 3	Week 12	Week 24 or Early Term.	Follow-up Safety Call
	Clinic	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 21 ±3	Day 84 ±7	Day 168 ±14	28 ±7 days after last doses
Vital Status Determination[9]				X	X

1 Participants who join from HEALEY ALS Platform Trial Regimen E RCT and/or OLE may leverage assessments completed during the last Platform Trial visit if the last Platform Trial visit is completed on the same day as EAP Screening visit. Only those assessments collected after obtaining written informed consent for the EAP may be used in such cases.

2 Vital signs are collected at Screening/Baseline, Week 3, and Week 24 visits and weekly in conjunction with study drug administration from Baseline through Week 24. Vital signs collected at in-clinic visits (Screening/ Baseline, Week 3, Week 24) include weight, systolic and diastolic pressure, respiratory rate, heart rate, and temperature. Height is measured at Screening/Baseline Visit only. If significant weight change is reported or suspected at any point during the study, the SI may choose to collect an additional in-clinic weight at their discretion. During each weekly infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), 60 minutes after the SOI (± 10 mins), and 90 minutes (± 10 mins) after the SOI. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.

3 Clinical safety labs include hematology (CBC with differential), complete chemistry panel and urinalysis at Screening/Baseline, Week 3, and Week 24. Hb A1C will be included in labs at Screening/Baseline and Week 24. If labs are abnormal at Week 3, they may need to be repeated per investigator judgement. Serum pregnancy testing will occur in women of child-bearing potential at the Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy. All urine samples must be collected prior to IP administration. Clinical safety labs may be drawn at the study site or obtained at a local site convenient for the participant. Clinical safety lab results obtained locally may be used to assess participant eligibility.

4 Infusion-related AEs are documented weekly in conjunction with study drug administration from Screening/Baseline through week 24.

5 The weight collected at the Screening/ Baseline clinic visit will be used to calculate the participant's initial dose. The participant will remain on a stable dose until the next in-person visit when weight be collected, at which point the dose may be adjusted if the participant's weight has increased or decreased by 2kg. Additional information on dose adjustments is included in Protocol section 6.7.4 Dosing Changes.

6 Administer first dose of study drug only after Screening/Baseline Visit procedures are completed. For participants in cohort 2 who join from HEALEY ALS Platform Trial Regimen E OLE, all infusions may occur at-home. For participants in cohort 2 who join from HEALEY ALS Platform Trial Regimen E RCT, infusions occur in-person at clinic at the Screening/Baseline study visit, Week 1, Week 2, and the Week 3 study visits. Weekly infusions will continue through Week 24. An in-clinic infusion and clinic visit may occur on the same day, provided both visits are conducted in-person and within window. There should be no fewer than 4 days (96 hours) between study drug infusions.

7 Refer to the Pharmacy Manual, site policy, and GCP guidelines on site investigator responsibilities, drug accountability, compliance, and source documentation. Any and all accountability records may be requested by sponsor for review at any time.

8 The combined Screening/ Baseline visit may be split into a Screening and a Baseline visit as needed. If baseline infusion occurs on the same day as screening, it must occur after obtaining written informed consent, completing all screening procedures, and confirming participant eligibility.

9 Vital status, defined as a determination of date of death or death equivalent or date last known alive, will be determined for each enrolled participant at the end of their EAP participation (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 EAP. We may also ascertain vital status at later time points by using publicly available data sources as described in section 6.6.10 of the Protocol.

Activity	Screening/ Baseline Combined[1,8]	Week 3	Week 12	Week 24 or Early Term.	Follow-up Safety Call
	Clinic	Clinic	Telemed/ Phone	Clinic	Telemed/ Phone
	Day -21 to 0	Day 21 ±3	Day 84 ±7	Day 168 ±14	28 ±7 days after last doses

10 Complete remote participation may be considered for select participants who join from HEALEY ALS Platform Trial Regimen E OLE on a case-by-case basis with permission from the Healey Coordination Center and Sponsor. Refer to Protocol section 6.2.1.

Appendix 2 Date Imputation Rules

The following rule will be used for missing/partial date imputation.

Item	Missing or Partial Date Variable	Imputation Rule
Adverse events, prior/concomitant medications and procedures	Start date	**The day is missing but month and year are available** • If the month and year of occurrence are the same as month and year of the first study treatment, the start date will be set to the date of the first study treatment. • Otherwise, if the month and year of occurrence are not equal to the month and year of first study treatment, the start date will be set to the first day of the month of occurrence. **The day and month are missing but year is available** • If the year of occurrence is the same as the year of the first study treatment, the start date will be set to the date of the first study treatment. • Otherwise, if the year of occurrence is not the same as the year of the first study treatment, the start date will be set to January 1 of the year of occurrence. **The start date is completely missing** • The start date will not be imputed. • Adverse events will be considered treatment emergent unless the end date suggests otherwise. • Medications/Procedures will be considered both prior and concomitant unless the end date suggests otherwise. EXCEPTION: If complete end date is available and the imputed start date is greater than the end date, then the assumed start date should be set to the end date.
	End date	**The day is missing but month and year are available** • If the participant died and the partial stop date is in the same month and year as the date of death, the end date will be set to the date of death. • Otherwise, the end date will be set to the last day of the month of occurrence. **The day and month are missing but year is available** • If the participant died and the partial end date is in the same year as the date of death, the end date will be set to the date of death. • Otherwise, the end date will be set to December 31 of the year of occurrence. **The end date is completely missing** • The end date will not be imputed.

		EXCEPTION: If the imputed end date is less than the start date, then the end date should be set to the start date.
ALS	Symptom onset date, diagnosis date	**The day is missing but month and year are available** • Impute the missing day to be 15. **The day and month are missing but year is available** • Impute the missing month and day to be July 1. **The date is completely missing** • The date will not be imputed.

Appendix 3 Calculation of % Predicted SVC

The % predicted SVC at each SVC measurement visit could be derived using the algorithm below:

1. Calculate the Predicted SVC.

Sex	**Height (cm)**	**Age at SVC measurement (age_SVC)**	**Predicted SVC**
Female	>=142.24	[18, 20)	(0.0699 * age_SVC) + (0.0416 * height) - 4.4470
		[20, 70)	(-0.0169 * age_SVC) + (0.0444 * height) - 3.1947
		[70, 89)	(-0.0296 * age_SVC) + (0.0313 * height) - 0.1889
		Other	Missing
	Other	Any	Missing
Male	>=147.32 and <= 203.2	[18, 25)	(0.0739 * age_SVC) + (0.0590 * height) - 6.8865
		[25, 86)	(-0.0298 * age_SVC) + (0.0844 * height) - 8.7818
		Other	Missing
	Other	Any	Missing

Where age_SVC = floor((intck("month", date of birth date, date of measurement) - (day(date of measurement) < day(date of birth))) / 12).

2. For each visit, set max_SVC = the maximal SVC result from multiple measurements
3. For each visit, % predicted SVC = 100 * max_SVC / Predicted SVC, round to 1 decimal.