**HEALEY ALS Platform Trial - Regimen E Trehalose**

**NCT05136885**

**Document Date: 03 Dec 2021**

# REGIMEN-SPECIFIC APPENDIX E

# FOR SLS-005 – TREHALOSE

**Regimen-Specific Appendix Date:** 12/3/2021

**Version Number:** 2.0

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**SIGNATURE PAGE**

I have read the attached Regimen-Specific Appendix (RSA) entitled, "REGIMEN E: Trehalose" dated December 3, 2021 (Version 2.0) and agree to abide by all described RSA procedures. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice, applicable FDA regulations and guidelines identified in 21 CFR Parts 11, 50, 54, and 312, central Institutional Review Board (IRB) guidelines and policies, and the Health Insurance Portability and Accountability Act (HIPAA).

By signing the RSA, I agree to keep all information provided in strict confidence and to request the same from my staff. Study documents will be stored appropriately to ensure their confidentiality. I will not disclose such information to others without authorization, except to the extent necessary to conduct the study.

Site Name: _____

Site Investigator: _____

Signed: _____ Date: _____

# LIST OF ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| AE | Adverse Event |
| ALSAQ-40 | Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 |
| ASLFRS-R | ALS Functional Rating Scale-Revised |
| $AUC_{inf}$ | Area Under the Curve to Time Infinity |
| CNS-BFS | Center for Neurologic Study Bulbar Function Scale |
| FVC | Forced Vital Capacity |
| g | Grams |
| GLP | Good Laboratory Practice |
| HHD | Hand-held Dynamometry |
| LC-3 II | Microtubule-associated Protein 1A/1B-light chain 3 |
| LPLV | Last Patient Last Visit |
| MTD | Maximum Tolerated Dose |
| OLE | Open Label Extension |
| OPMD | Oculopharyngeal Muscular Dystrophy |
| PK | Pharmacokinetics |
| RSA | Regimen Specific Appendix |
| SAE | Serious Adverse Event |
| SLS-005 | Trehalose |
| SOA | Schedule of Activities |
| SOI | Start of Infusion |
| SVC | Slow Vital Capacity |
| TFEB | Transcription Factor EB |

## REGIMEN-SPECIFIC APPENDIX SUMMARY

### Regimen-Specific Appendix E

For SLS-005, also known as Trehalose injection, 90.5 mg/mL for intravenous infusion.

### Rationale and RSA Design

Trehalose is a disaccharide that is well known for its protein-stabilizing properties (Elbein 2003, Emanuele 2014) and its ability to activate autophagy. Because of its known ability to reduce abnormal protein aggregations, trehalose was studied in several cellular and animal models of hereditary neurologic and muscular disorders. Three in vivo studies (Castillo et al (2013), Zhang et al (2014) and Li Y et al (2015)) demonstrated that trehalose is a potential protective agent in ALS. These studies were performed using the SOD1 mouse model with the G93T mutation or the G86R mutation. In mice, trehalose can be used orally because they lack intestinal trehalase, the enzyme that catalyzes conversion of trehalose to two glucose molecules. Treatment with trehalose both oral and intraperitoneal delayed onset of disease, prolonged survival, preserved motor function and motor neurons in the spinal cord. When trehalose is taken orally in humans, trehalase enzymes in the brush border of the gut actively cleave trehalose into 2 glucose molecules. The most effective way to ensure that adequate amounts of trehalose reaches the primary therapeutic target organs, brain and muscle cells, is to circumvent the extensive gut metabolism in humans by administering trehalose via IV infusion.

This is a randomized double-blind placebo-controlled trial. Participants who qualify will be randomized 3:1 to receive weekly infusions of Trehalose Injection, 90.5 mg/mL for intravenous infusion at a dose of 0.75g/kg or placebo (sodium chloride injection, 0.9%, USP) intravenous infusion at an equivalent weight-based volume.

### Allocation to Treatment Regimens

Participants must first be screened and determined to be eligible under the Master Protocol before they are randomized to a treatment regimen.

As soon as pre-defined criteria for futility for the regimen are met, or the target number of randomized participants has been reached, enrollment will stop in the regimen.

### Number of Planned Participants and Treatment Groups

The number of planned participants for this regimen is approximately 160.

There are 2 treatment groups for this regimen, active and placebo. Participants will be randomized in a 3:1 ratio to active treatment or placebo (i.e., 120 active: 40 placebo).

### Planned Number of Sites

Research participants will be enrolled from approximately 80 centers in the US.

### Treatment Duration

The maximum duration of the placebo-controlled treatment period is 24 weeks.

### Follow-up Duration

At the conclusion of the 24-week placebo-controlled treatment period of the study, all participants will either schedule a 28-day follow up phone call and end their participation in the

regimen or have the option to receive intravenous infusions of trehalose in the Open Label Extension (OLE) period of the study. The duration of the OLE period is planned for 52 weeks.

**Total Planned Trial Duration**

For participants completing the placebo-controlled treatment period of the study, the planned amount of time for a participant in the trial is up to 34 weeks, or about 8 months. This duration assumes a 6-week screening window, a 24-week placebo-controlled treatment period, and a 4-week safety follow-up period for those participants who do not enter the OLE. Participants will complete approximately 10 study visits during the placebo-controlled treatment period of the study, in addition to weekly infusions outside of these visits.

If the participant opts into the subsequent OLE, the total planned amount of time for a participant in the trial is approximately 86 weeks. This duration assumes a 6-week screening window, 24-week placebo-controlled treatment period, a 52-week OLE period, and a 4-week safety follow-up period. Participants will complete 10 study visits across the planned 52-week OLE period of the study, in addition to weekly infusions outside of these visits.

## SCHEDULE OF ACTIVITIES – PLACEBO-CONTROLLED PERIOD

As per the Schedule of Activities (SOA) below, visits must occur every 4 weeks and will be clinic-, phone- or telemedicine based, as applicable. In this regimen, participants receive a weekly infusion of trehalose or matching placebo. The infusions are performed at each site for at least the first 4 weeks then participant may be permitted, based on safety and tolerability assessments, to have infusions administered at home. There is a maximum 24-week duration of placebo-controlled treatment for a Regimen.

| Activity (page 1 of 2) | Master Protocol or Regimen-Specific | Master Protocol Screening[1] | Regimen Specific Screening[1] | Baseline[20] | Week 2[21] | Week 4 | Week 8 | Week 12[21] | Week 16[13] | Week 20[21] | Week 24 or Early Term. Visit | Follow-Up Safety Call[10] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Clinic | Clinic | Clinic | Phone | Clinic | Clinic | Phone | Clinic | Phone | Clinic | Phone |
| | | -42 to -1 Days[15] | -41 to 0 Day[15] | 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 after last dose ±3 days |
| Written Informed Consent[2] | Master | X | X | | | | | | | | | |
| Written Informed Consent - OLE | Master | | | | | | | | X | | | |
| Inclusion/Exclusion Review | Master | X | X[3] | | | | | | | | | |
| ALS & Medical History | Master | X | | | | | | | | | | |
| Demographics | Master | X | | | | | | | | | | |
| Physical Examination | Master | X | | | | | | | | | | |
| Neurological Exam | Master | X | | | | | | | | | | |
| Vital Signs[4] | Master | X | | X | | X | X | | X | | X | |
| Vital signs associated with infusion [5] | Regimen | | | | Weekly in conjunction with study drug administration from week 1 through week 24 | | | | | | | |
| Slow Vital Capacity | Master | X[14] | | X | | | X | | X | | X | |
| Muscle Strength Assessment | Master | | | X | | | X | | X | | X | |
| ALSFRS-R | Master | X | | X | | X | X | X | X | X | X | |
| ALSAQ-40 | Regimen | | | X | | | | | | | X | |
| CNS Bulbar Function Scale | Regimen | | | X | | | X | | X | | X | |
| 12-Lead ECG | Master | X | | | | | | | | | X | |
| Clinical Safety Labs[6, 17] | Master | X | | X | | X | X | | X | | X | |
| Biomarker Blood Collection | Master | | | X | | | X | | X | | X | |

| Activity (page 1 of 2) | Master Protocol or Regimen-Specific | Master Protocol Screening[1] | Regimen Specific Screening[1] | Baseline[20] | Week 2[21] | Week 4 | Week 8 | Week 12[21] | Week 16[13] | Week 20[21] | Week 24 or Early Term. Visit | Follow-Up Safety Call[10] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Clinic | Clinic | Clinic | Phone | Clinic | Clinic | Phone | Clinic | Phone | Clinic | Phone |
| | | -42 to -1 Days[15] | -41 to 0 Day[15] | 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 after last dose ±3 days |
| Biomarker Urine Collection[17] | Master | | | X | | | X | | X | | X | |
| PK Blood Collection[16] | Regimen | | | X | | | X | | | | | |
| DNA Collection[8] (optional) | Master | | | X | | | | | | | | |
| CSF Collection (optional) | Master | | | X | | | | | X[12] | | | |
| Concomitant Medication Review | Master | X | X | X | X | X | X | X | X | X | X | |
| Adverse Event Review[7] | Master | X | X | X | X | X | X | X | X | X | X | X |
| Document changes in health, medications, and infusion-related adverse events | Regimen | | | | Weekly in conjunction with study drug administration from week 1 through week 24 | | | | | | | |
| Columbia-Suicide Severity Rating Scale | Master | | | X | | X | X | | X | | X | |
| Assignment to the Regimen | Master | X | | | | | | | | | | |
| Randomization within the Regimen | Master | | X | | | | | | | | | |
| Adjust Dose as Needed[20] | Regimen | | | | | X | X | | X | | X[19] | |
| Administer/Dispense Study Drug | Master | | | X[9] | Weekly IV infusion from week 1 through week 24 | | | | | | X[10] | |
| Drug Accountability/Compliance | Master | | | | Weekly in conjunction with study drug administration from week 1 through week 24[22] | | | | | | X | |
| Exit Questionnaire | Master | | | | | | | | | | X | |
| Vital Status Determination[11] | Master | | | | | | | | | | X | |

1 Master Protocol Screening procedures must be completed within 42 days to 1 day prior to the Baseline Visit. The Regimen-Specific Screening Visit and Baseline Visit should be combined, if possible.
2 During the Master Protocol Screening Visit, participants will be consented via the Platform Trial informed consent form (ICF). After a participant is randomized to a regimen, participants will be consented a second time via the regimen-specific ICF.
3 At the Regimen Specific Screening Visit, participants will have regimen-specific eligibility criteria assessed.
4 Vital signs include weight, systolic and diastolic pressure, respiratory rate, heart rate and temperature. Height measured at Master Protocol Screening Visit only. If an infusion will occur at the completion of the visit, vital signs should be collected within 30 minutes of the start of infusion to serve as the pre-infusion vital signs collection.
5 During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), 60 minutes after the start of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.
6 Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function and urinalysis. Hb A1C will be included in labs at baseline, week 16 and week 24. Serum pregnancy testing will occur in women of child-bearing potential at the Master Protocol Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.
7 Adverse events that occur after signing the master protocol consent form will be recorded.
8 The DNA sample can be collected after the Baseline Visit if a baseline sample is not obtained or the sample is not usable.
9 Administer first dose of study drug only after Baseline Visit procedures are completed.
10 Participants will only have a Follow-Up Safety Call at this time if they *do not* continue on in the OLE. Participants who continue into OLE will have a Follow-Up Safety Call after their last dose of investigational product during the OLE period.
11 Vital status, defined as a determination of date of death or death equivalent or date last known alive, will be determined for each randomized participant at the end of the placebo-controlled portion of their follow-up (generally the Week 24 visit, as indicated). If at that time the participant is alive, his or her vital status should be determined again at the time of the last patient last visit (LPLV) of the placebo-controlled portion of a given regimen. We may also ascertain vital status at later time points by using publicly available data sources as described in section 8.15 of the Master Protocol.
12 If the CSF collection is unable to be performed for logistical reasons, such as scheduling, at the Week 16 Visit, it may be performed at the Week 24 Visit.
13 Visit may be conducted via phone or telemedicine with remote services instead of in-person if this is needed to protect the safety of the participant due to a pandemic or other reason.
14 If required due to pandemic-related restrictions, Forced Vital Capacity (FVC) performed by a Pulmonary Function Laboratory evaluator or with a study-approved home spirometer, or sustained phonation using a study approved method may be used for eligibility (Master Protocol Screening ONLY).
15 Master Protocol Screening and Regimen Specific Screening visit windows are relative to Baseline (Day 0).
16 Participants completing the PK collection will have blood collected for PK analysis at Baseline (Day 0) and Week 8 (Day 56) at the following time points: pre-dose and 1 hour ± 5 minutes, 2 hours ± 5 minutes, and 4 hours ± 5 minutes post start of infusion (SOI). At each time point, 4mL of blood will be collected.
17 All urine samples must be collected prior to IP administration.
19 Dose is adjusted only for those participants continuing into the OLE.
20 The weight collected during each in clinic visit will be used to calculate the participant's dose. The participant will remain on a stable dose until the next in clinic visit (Weeks 4, 8, 16) when weight 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 section 5.6 Dosing Changes.
21 If the participant receives an in-clinic infusion within the visit window, this visit may be conducted while the participant is in clinic for their weekly infusion.
22 Drug accountability will not be done at phone visits. Compliance will be automatically calculated in the EDC.

## SCHEDULE OF ACTIVITIES – Open Label Extension (Optional)

| Activity (page 1 of 1) | Master Protocol or Regimen-Specific | Open Label Extension (Optional)[5] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 2[8] | Week 4 | Week 8[7] | Week 12[8] | Week 16[7] | Week 20[8] | Week 24[8] | Week 28[7] | Week 40[7] | Week 52 or Early Term. Visit[6, 7] | Follow-Up Safety Call[4, 6] |
| | | Clinic during Infusion | Clinic | Clinic | Phone | Clinic | Phone | Phone | Clinic | Clinic | Clinic | Phone |
| | | | Day 28 ±7 | Day 56 ±7 | Day 84 ±3 | Day 112 ±7 | Day 140 ±3 | Day168 ±3 | Day 196 ± 14 | Day 280 ± 14 | Day 364 ± 14 | 28 days after last dose ±3 days |
| Vital Signs[1] | Master | | X | X | | X | | | X | X | X | |
| Vital signs associated with infusion[9] | Regimen | Weekly in conjunction with investigational product administration from OLE week 1 through OLE week 52 | | | | | | | | | | |
| Slow Vital Capacity | Master | | X | X | | X | | | X | X | X | |
| ALSFRS-R | Master | | X | X | X | X | X | X | X | X | X | |
| ALSAQ-40 | Regimen | | | | | | | | X | | X | |
| CNS Bulbar Function Scale | Regimen | | | X | | X | | | X | X | X | |
| Clinical Safety Labs[2] | Master | | X | X | | X | | | X | X | X | |
| Biomarker Blood Collection | Master | | | | | X | | | X | | X | |
| Biomarker Urine Collection | Master | | | | | X | | | X | | X | |
| Concomitant Medication Review | Master | X | X | X | X | X | X | X | X | X | X | |
| Adverse Event Review[3] | Master | X | X | X | X | X | X | X | X | X | X | X |
| Document changes in health, medications, and infusion-related adverse events | Regimen | Weekly in conjunction with Investigational Product administration from OLE week 1 through OLE week 52 | | | | | | | | | | |
| Columbia-Suicide Severity Rating Scale | Master | | X | X | | X | | | X | X | X | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Adjust Dose as Needed[10] | Regimen | | X | X | | X | | | X | X | | |
| Administer/Dispense Investigational product | Master | Weekly IV infusion from OLE week 1 through week 52 | | | | | | | | | | |
| Drug Accountability/Compliance | Master | Weekly throughout study in conjunction with drug administration[11] | | | | | | | | | | |

1 Vital signs include weight, systolic and diastolic pressure, respiratory rate, heart rate and temperature. Height in cm measured at Master Protocol Screening Visit only.
2 Clinical safety labs include hematology (CBC with differential), complete chemistry panel, thyroid function and urinalysis. HbA1c will be added to the safety labs at weeks 16, 28 and 52. Serum pregnancy testing will occur in women of child-bearing potential at the Master Protocol Screening Visit and as necessary during the study. Pregnancy testing is only repeated as applicable if there is a concern for pregnancy.
3 Adverse events that occur after signing the master protocol consent form will be recorded.
4 Participants who continue into OLE will have a Follow-Up Safety Call (as described in the body of this RSA) after their last dose of investigational product during the OLE period.
5 The duration of the OLE is 52 weeks.
6 Participants who continue into the OLE and then withdraw consent or early terminate will be asked to complete an Early Termination Visit and Follow-Up Safety Call as described in the body of this RSA.
7 Visit may be conducted via phone or telemedicine with remote services instead of in-person if this is needed to protect the safety of the participant due to a pandemic or other reason.
8 If the participant receives an in-clinic infusion within the visit window, this visit may be conducted while the participant is in clinic for their weekly infusion.
9 During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), 60 minutes after the start of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of the 90-minute infusion.
10 The weight collected during each in clinic visit will be used to calculate the participant's dose. The participant will remain on a stable dose until the next in clinic visit (Weeks 4, 8, 16, 28, 40) when weight be collected, at which point the dose may be adjusted if the participant's weight has increased or decreased by 2kg. Additional information on dose adjustments is included in section 5.6 Dosing Changes.
11 Drug accountability will not be done at phone visits. Compliance will be automatically calculated in the EDC.

# 1. INTRODUCTION

## Regimen E: SLS-005 Trehalose

### 1.1 Trehalose Background Information

Trehalose is a disaccharide that is well known for its protein-stabilizing properties (Elbein 2003, Emanuele 2014) and its ability to activate autophagy (Rusmini 2019)). Because of its known ability to reduce abnormal protein aggregations, trehalose was studied in several cellular and animal models of hereditary neurologic and muscular disorders, especially those associated with CAG repeat expansion and polyalanine/polyglutamine accumulation (Tanaka 2004, Davies 2006). 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 activates lysosomal and autophagic activity by various pathways and increases expression of biomarkers of autophagy such as microtubule-associated protein 1A/1B-light chain 3 (LC-3 II). It was shown that the basis of activation of these pathways is cellular glucose starvation (Mardones 2016). However, the mechanism of lysosomal-autophagic activation by trehalose cannot be only glucose starvation. Trehalose also activates autophagy through the activation of Transcription Factor EB (TFEB), and relocation to the nucleus, a key factor in lysosomal and autophagy gene expression. (Rusmini 2019) Activation of TFEB is an emerging therapeutic target for a number of diseases with pathologic accumulation of storage material.

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. Oral ingestion of trehalose results in <0.5% absorption of trehalose. Seelos Therapeutics has developed IV trehalose to circumvent the breakdown of trehalose in the gut. In Study BBCO-001, an open-label Phase 2a study of 25 OPMD patients with dysphagia, weekly trehalose treatments at a dose of 27 grams (g) for 6 months was well tolerated. A single ascending dose study identified that clearance of trehalose is weight dependent and that the maximum tolerated dose (MTD) as 0.75 g/kg. This is the dose to be employed in this study.

### 1.2 Nonclinical and Clinical Data

#### *1.2.1 Nonclinical Experience*

The safety and tolerability of trehalose has been extensively investigated. A detailed review of safety in animals is presented in the trehalose Investigator's Brochure (IB).

Three-month IV Good Laboratory Practice (GLP) toxicology studies in rats and dogs at a dose of 3.6 g/kg administered twice weekly showed no organ toxicity. A 6-month GLP toxicology study in rats with doses of 2.7, 5.4, and 10.8 g/kg demonstrated no organ toxicity. An inflammatory response at the end of the indwelling catheter was noted in a dose-dependent manner. Although there were an increasing number of observations with the increasing dose it was not a linear increase and thus it was considered by the reporting pathologist to a procedural related change due to manipulation of the catheter and not drug related. There was no systemic inflammatory

response noted. A 9-month chronic toxicity study in dogs using the same doses of 2.7, 5.4, and 10.8 g/kg did not demonstrate any organ toxicity.

*1.2.2 Nonclinical Literature*

Three in vivo studies demonstrated that trehalose is a potential protective agent in ALS. These studies were performed using the SOD1 'classical' mouse model with the G93T mutation or the G86R mutation. In mice trehalose can be used orally because they lack intestinal trehalase.

In the work of Castillo et al (2013), trehalose was administered to animals with a G86R SOD1 mutation from age 35 days onward. The dose used was 3 weekly IP injections of 2 g/kg plus 3% trehalose in free drinking water. It was found that in female mice trehalose increased survival from a mean of 139 days in untreated animals to 154 days in trehalose treated animals. Male mice survival increased from 145 to 177 days. The level of SOD1 accumulation in the brain was lower when tested postmortem. In addition, there was preservation of motor neurons noted in the ventral horn of the spinal cord in trehalose treated animals.

In a similar study, Zhang et al (2014) administered trehalose to animals with the G93A SOD 1 mutation in the drinking water only (2% concentration) starting on day 60. Trehalose delayed mean onset of disease from day 95 to 112 (based on clinical observation in the animals). Mean survival was prolonged from 124 to 145 days of age; however, disease duration was unchanged.

The number of preserved motor neurons in the spinal cord was assessed on day 120. Trehalose-treated animals had increased numbers of preserved motor neurons compared with untreated control animals. Neuronal SOD1 aggregates and p62 accumulation were decreased with trehalose treatment. Furthermore, histologic studies of muscle showed an increase in mean fiber size and evidence of preserved neuromuscular junctions, indicating reduced denervation of muscle tissue.

Li Y et al (2015) evaluated 2 protocols for the study of trehalose in the SOD1-G93A mouse model (given in 2% solution in the drinking water). A short-term therapy protocol evaluated the effects after 30 days of treatment. Long-term therapy monitored the effects from onset to death. In both protocols, trehalose therapy was started on day 60. Disease onset, defined as the time when the animal dragged 1 leg, was significantly delayed by trehalose. Similarly, the time to reach grade 2 (2 affected limbs) was prolonged. Survival (defined as animal sacrifice time when it reached grade 4 of the disease) was delayed by 7 days, but this change did not reach statistical significance. In the short-term protocol motor function was improved as assessed by rotarod and hanging functional tests. There was a clear change in autophagy activation (levels of p62) in the spinal cord during the short-term protocol.

*1.2.3 Clinical Experience*

Previous clinical trials evaluating IV trehalose in healthy subjects and patients with OMPD and SCA3 are summarized below. Overall, administration of IV trehalose to adults appears safe and well tolerated.

A total of 59 subjects have been exposed to trehalose: 18 healthy subjects, 25 OPMD patients, 15 SCA3 patients, 1 adult with Sanfilippo Type B. Out of the 59, 11 patients received SLS-005 in the under expanded access use programs (10 subjects with OPMD and 1 with Sanfilippo type B). The healthy subjects received a single dose of 27, 54, or 81 g (6 subjects each dose), the OPMD patients received repeated weekly doses of 27 g trehalose for a duration of 6 to 18 months, the

SCA3 patients received repeated weekly doses of 13.5 or 27 g for a duration of 6 to 12 months, and the Sanfilippo patient is receiving 0.75 g/kg weekly, currently at over 4 months of treatment. Overall, trehalose was well tolerated.

Study BB-TRE-101 studied the safety, tolerability, and pharmacokinetics (PK) of single ascending doses of SLS-005 in healthy subjects. The study demonstrated proportional linear kinetics for increasing doses of trehalose from 27 g to 81 g. In addition, it demonstrated a need for weight-based dosing since clearance increased with increasing weight. One (1) treatment-emergent adverse event was assessed as possibly related elevated liver enzymes in 1 subject in the 81 g treatment group and was reported as a suspected unexpected serious adverse reaction (SUSAR). Based on the data from this study, along with supportive data from animal studies, the MTD was established as 0.75 g/kg.

Study BBCO-001, an open-label study in patients with OPMD, assessed the safety, tolerability, PK, and efficacy of SLS-005 in patients treated for 6 months with weekly infusions of 27 grams. SLS-005 was generally safe and well tolerated. The most common adverse event (AE) was elevated urine glucose that occurred in 52% of patients. The AE of glycosuria was only reported from 1 site and, although the patients were asymptomatic, the investigator assessed the laboratory result as clinically significant. Increased glucose in urine is an anticipated result because trehalose is metabolized to glucose, however serum glucose did not exceed normal range There were 3 serious adverse events (SAEs) occurring in 2 patients: urinary tract infection, aspiration pneumonia, and aspiration leading to death. None of the SAEs were considered related to study drug.

The subsequent Study BB-OPMD-301 was an open-label extension protocol to BBCO 001 that continued treatment of patients for more than 1 year. The safety profile in this study was similar to the initial study.

Study BB-MJD-201, a Phase 2 double-blind, dose-controlled study in SCA3 patients, was an open-label study of 15 patients with SCA3 that evaluated the safety and tolerability of 2 different IV doses of SLS-005, 13.5 and 27 g, administered weekly. All AEs were considered mild in severity. The most common AE was transient glucosuria occurring in 20% (n=3) of patients. SLS-005 treatment was generally safe and well tolerated in this patient population.

## 1.3 Trehalose Therapeutic Rationale

### *1.3.1 Rationale for the study*

The nonclinical data generated to date for trehalose support its potential to be used as an effective treatment for ALS. Through its effects on reducing proteinaceous aggregates/inclusions promoting autophagy and lysosomal pathways and reducing cell death, trehalose improved muscle strength and overall motor skills in animal models of OPMD, SCA3, and ALS. The clinical safety profile of trehalose and the nonclinical toxicology data support the investigation of trehalose as a potential treatment of ALS.

### *1.3.2 Rationale for the Dose, Dosing Regimen, and Route of Administration*

**Error! Reference source not found.**If administered orally < 0.5% of ingested trehalose is absorbed into the blood stream. Therefore, to achieve therapeutic blood levels of trehalose, it is necessary to circumvent the metabolism in the human gastrointestinal tract. As such, Seelos has

developed SLS-005 an IV formulation of trehalose for injection, 90.5 mg/mL to be evaluated in this study.

In the single ascending dose study of IV trehalose in healthy subjects, the mean weight in the 54 g dose cohort was 74.5 kg. At the dose of 54 g, subjects received 0.72 g/kg. The area under the curve to time infinity ($AUC_{inf}$) at that dose was 8,595±2,575 hour × µg/mL. Since clearance is dependent on body weight (i.e., clearance increases with increasing body weight), in order to achieve a consistent exposure in, at, or near the exposure at the MTD, 54 g/1 hour, a weight-based dose of 0.75 g/kg should achieve an exposure within the clinically acceptable safety range.

One of the proposed mechanisms of action of trehalose is to activate autophagy, the blockade of glucose receptors results in a starvation scenario within the cell (DeBosch 2016). Therefore, the MTD will be used to ensure significant exposure. Weekly dosing of IV trehalose is the only regimen to be evaluated in clinical trials to date (patients with OPMD and SCA3) and will be used in this trial.

*1.3.3 Rationale for the Patient Population*

The pathologic hallmark of the disease is aggregation of proteinaceous inclusions in motor neurons. The main composite of these inclusions is misfolded TDP43 (especially the phosphorylated form), which accumulates in the cytoplasm and is depleted in the nucleus. Patients with either sporadic or familial ALS accumulate TDP43 and patients with the SOD mutation accumulate SOD aggregates and thus have the potential to benefit from treatment with trehalose through activation of autophagy.

## 2. OBJECTIVES

### 2.1 Study Objectives and Endpoints

Primary Efficacy Objective:

To evaluate the efficacy of trehalose injection, 90.5 mg/mL for intravenous infusion as compared to placebo on ALS disease progression.

Secondary Efficacy Objective:

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

Safety Objective:

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

Exploratory Efficacy Objective:

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

Primary Efficacy Endpoint:

Change in disease severity as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) total score using a Bayesian repeated measures model that accounts for loss to follow-up due to mortality.

Secondary Efficacy Endpoints:

- Change in respiratory function as assessed by slow vital capacity (SVC).
- Change in muscle strength as measured isometrically using hand-held dynamometry (HHD) and grip strength.
- Survival.

Safety Endpoints:

- Treatment-emergent adverse 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 Efficacy Endpoints:

- Changes in the biofluid biomarkers of neurodegeneration specified in the SOA.
- Changes in the patient reported outcomes specified in the SOA.

## 3. RSA DESIGN

This study is a multi-center, randomized, placebo-controlled trial, testing active dose of trehalose injection, 90.5 mg/mL for intravenous infusion (0.75 g/kg), given weekly versus placebo.

Participants will be randomized 3:1 active: placebo.

### 3.1 Scientific Rationale for RSA Design

This RSA is designed to correspond with the design of the Master Protocol and the goals of the HEALEY ALS Platform Trial.

### 3.2 End of Participation Definition

A participant is considered to have ended his or her participation in the placebo-controlled period of the Regimen if they:

- Complete planned placebo-controlled period visits, as described in the SOA, including participants on or off study drug
- Early terminate from the study and complete the Early Termination Visit and Follow-Up Phone call as described in Section 6.9
- Withdraw consent to continue participation in the study, or are lost to follow-up

If a participant initiates open-label study drug in the OLE period, he or she is considered to have completed his or her participation in the OLE period of the Regimen if they choose to discontinue participation or if all planned OLE period visits, including the last visit or the last scheduled procedure shown in the SOA, have been completed.

### 3.3 End of Regimen Definition

The end of the placebo-controlled treatment period in a Regimen occurs when all randomized participants have completed their participation in the placebo-controlled period as defined in section 3.2.

The end of the OLE period in a Regimen occurs when all participants who initiated open-label investigational product in the OLE period have completed their participation in the OLE period as defined in section 3.2.

## 4. RSA ENROLLMENT

### 4.1 Number of Study Participants

Approximately one hundred-sixty (160) participants will be randomized to this Regimen.

### 4.2 Inclusion and Exclusion Criteria

In order to be assigned to a Regimen, participants must meet the Master Protocol eligibility criteria. In addition, participants meeting all of the following inclusion and exclusion criteria will be allowed to enroll in this Regimen:

*4.2.1 RSA Inclusion Criteria*

There are no additional RSA Inclusion Criteria from those described in the Master Protocol.

*4.2.2 RSA Exclusion Criteria*

1. Current diagnosis or healthcare professional-recommended treatment (medication, exercise or diet) of diabetes mellitus
2. Master Protocol screening glucose $\geq$140 mg/dl
3. Prior treatment with IV trehalose or known hypersensitivity to trehalose
4. Current use of oral trehalose (see prohibited medication Section 5.9)
5. Inability for participant to return to site for weekly drug administration, until approved for home infusions
6. Master Protocol screening body weight >144 kilograms

### 4.3 Treatment Assignment Procedures

Each participant who meets all eligibility criteria for the Regimen will be randomized to receive either 0.75 g/kg of trehalose or equivalent weight-based volume of placebo by weekly intravenous infusion in the study's 24-week placebo-controlled treatment period.

### 4.4 Open Label Extension Eligibility

Participants who complete the 24-week placebo-controlled treatment period on active or placebo study drug will be eligible to participate in the OLE.

## 5. INVESTIGATIONAL PRODUCT

### 5.1 Investigational Product Manufacturer

The investigational product is SLS-005 (trehalose injection, 90.5 mg/mL for intravenous infusion). This solution for IV infusion is administered once a week at a planned dose of 0.75 g/kg.

Trehalose is a stable, non-reducing disaccharide with 2 glucose molecules linked in an alpha 1,1 configuration. It contains no less than 97.0% and no more than 102.0% of C12H22O11, calculated on the anhydrous basis.

Trehalose Injection 90.5 mg/mL (an aqueous, sterile solution for IV infusion) is provided as a clear liquid in a 300 mL infusion bag. Each 300 mL bag contains 27.15 g of trehalose. See the IB for further details on trehalose.

Placebo is a sodium chloride injection, 0.9%, USP for intravenous infusion, provided as a clear liquid in a 300 mL infusion bag. Placebo is administered once a week in an equivalent weight-based volume as described for trehalose dosing.

### 5.2 Labeling, Packaging, and Resupply

Trehalose will be provided in a 1 bag per kit configuration. Further details are provided in the Pharmacy Manual.

Placebo will be provided in a 1 bag per kit configuration. Placebo has been manufactured by the drug product manufacturer in the same container closure (300-mL freeflex IV bag).

### 5.3 Acquisition, Storage, and Preparation

#### Acquisition

Prior to study treatment, study drug will be supplied to the study site's pharmacy by the Sponsor or its designee.

Shipment of study drug supplies for the study will be accompanied by shipment records describing the contents of the shipment, drug information, and other appropriate documentation. The shipment form will assist in maintaining current and accurate inventory records.

The investigator must ensure the acknowledgement of receipt of the clinical trial material at the site, including that the material was received in good condition.

#### Study Drug Storage

The investigational drug and matching placebo will be stored in the study site’s pharmacy, at room temperature (20 to 25 ºC) with excursions permitted 15 to 30 °C. The Sponsor or its designee should be notified for any deviation from the storage conditions.

#### Study Drug Preparation

Trehalose Injection 90.5 mg/mL (an aqueous, sterile solution for IV infusion) is provided as a clear liquid in a 300 mL infusion bag containing 27.15 grams of trehalose. No preparation is required for administration of the study drug.

Placebo is also provided as a clear liquid in a 300 mL infusion bag. No preparation is required for administration of placebo.

The dose of IV trehalose or placebo will be calculated based on participant body weight to determine the volume administered to each participant. Each participant's weight should be measured consistently at each in-clinic visit. Shoes and heavy winter clothes (e.g. coats, sweaters, boots) should be removed prior to weighing. If shoes cannot be removed, then weight should be assessed with the participant's shoes on at each clinic visit. Doses are adjusted based on changes in body weight (2 kg increase or decrease) noted during clinical trial assessments. More than 1 bag may be needed per infusion based on the participant's body weight. The intended SLS-005 total infusion time, excluding interruptions, is 60 minutes for infusion volumes up to 2 bags (i.e. up to 600mL) and should not exceed 70 minutes. For patients who require more than 2 bags (i.e. >600mL) of study drug the intended SLS-005 total infusion time should be increased to 90 minutes and not exceed 100 minutes. Management of deviations/excursions is described in the Study Drug MOP.

### 5.4 Study Medication/Intervention, Administration, Escalation, and Duration

The participant will receive weekly infusions of study drug (trehalose or placebo). Although for most participants the total infusion time of SLS-005 is set at 60 minutes (+ 10 minutes), participants who require more than 2 bags of study drug the infusion time should be increased to 90 minutes (+10 minutes) throughout the study. All infusions will be administered using an infusion pump and will be followed with an IV flush. The start calendar date and 24-hour clock time as well as the end calendar date and 24-hour clock time of each infusion will be recorded in the study database. If an infusion is interrupted due to infusion issues, i.e. loss of IV site, the infusion can be completed once access has been re-established unless > 30 minutes have elapsed. If 30 or more minutes have elapsed the infusion should not be restarted, and the volume infused prior to the interruption recorded in the CRF as the total dose administered.

If needed the use of an implanted venous access device (e.g. Mediport, PICC) or percutaneous indwelling catheter is permitted however small caliber indwelling catheters in peripheral vessels should only be inserted for single use. Participants are to be monitored for administration site reactions during study drug administration. Infusion site reactions or infusion reactions are to be recorded as AEs using the appropriate coding terms on the eCRF.

If needed, remote services such as infusions at home can be considered after the participant has completed at least 4 weeks of in-clinic infusions, as long as the participant has had no adverse reaction to the infusions and all safety assessments are within acceptable limits. Decision to permit home infusions is at the discretion of the site investigator. Participants approved by their site investigator for weekly infusions outside of the study site will still be required to attend on-site study visits at Week 16 (unless completed remotely due to pandemic-related or other reasons) and 24, and their associated IP infusions at these timepoints can be done in-clinic following completion of the study visit. Telephone visits at Weeks 2, 12 and 20 may be conducted in clinic if the participant is receiving their weekly infusions at the study site and the infusion will occur during the visit window.

### 5.5 Justification for Dosage

Based on the preclinical and clinical safety and PK data trehalose injection 90.5 mg/mL should be dosed using a weight-based dosing up to a maximum of 0.75 g/kg. Participants should receive trehalose injection 90.5 mg/mL over 60 + 10 minutes, with the exception noted above for more than 2 bags, using an infusion pump.

### 5.6 Dosage Changes

If for any reason a participant is unable to receive a full dose this should be documented as per participant compliance. Doses are adjusted (increased or decreased) based on a weight change of 2kg or greater from the previous in-clinic volume. A dosing chart, with the volume of study drug to be administered based off body weight to achieve a dose of 0.75 g/kg, is included in the Study Drug MOP.

### 5.7 Participant Compliance

Participants should be encouraged not to miss infusions if at all possible, as it may affect the efficacy of the drug. The dosing window is 1 week ± 3 days. There should be no fewer than 4 days between study drug infusions.

Compliance will be automatically calculated in the EDC for each weekly infusion.

### 5.8 Drug Returns and Destruction

At each in person visit the steps outlined in the Manual of Procedures must be followed for study drug accountability and compliance, as well as study drug return and destruction.

### 5.9 Overdose

Certain safety events that occur in association with investigational product may require reporting. These safety events include, but are not limited to, the following:

- Overdose of the investigational product, where 'overdose' is defined as > 125% of the intended dose for a single treatment day infusion.
- Suspected abuse/misuse of the investigational product
- Inadvertent or accidental exposure to the investigational product
- Medication error involving study drug (with or without participant exposure to the investigational product, e.g., name confusion)
- The half-life of Trehalose Injection 90.5 mg/mL is approximately 1.5 hours, and there is no antidote. Trehalose is metabolized to 2 glucose molecules, there are no other metabolites. Supportive care with monitoring of vital signs, blood glucose levels, and liver enzymes should be considered. In 1 subject who received 81 g of Trehalose Injection 90.5 mg/mL, there was an increase in liver enzyme levels, but liver function remained normal and liver enzyme values returned to normal without treatment.

These safety events should be reported to the Coordination Center whether they result in an AE/SAE or not. Safety events associated with an AE/SAE should also be reported in the EDC. The SI should also contact the Medical Monitor within 24 hours of the SI's awareness.

### 5.10 Prohibited Medications

To date, there are no known pharmacokinetic drug interactions with trehalose. Participants must not take the following medications while on study drug in Regimen E:

- Oral trehalose is not permitted during the study.

## 5.11 Trehalose Known Potential Risks and Benefits

### *5.11.1 Known Potential Risks*

The safety profile from previous clinical trials of IV trehalose in adults shows no drug-related AEs other than mild, transient glycosuria which was asymptomatic and only 1 SAE, elevated liver enzymes, was considered possibly related to study drug due to temporal association of the changes and lack of alternative causation, however the event resolved without any intervention.

Risks of study participation also includes the risk of an infusion reaction or infusion site reactions. There have been no infusion reactions to date in patients administered IV trehalose; however, 1 patient had an infusion site reaction (erythema). Toxicology data suggests that small indwelling catheters may result in vessel changes after multiple infusions. The inflammatory reaction noted was not associated with any generalized inflammation and there was no organ toxicity, thus it was considered a procedural complication of the indwelling catheter. If needed the use of an implanted venous access device (i.e. Mediport, PICC) or percutaneous indwelling catheter is permitted however small caliber indwelling catheters in peripheral vessels should be single use. Additional risks include those risks related to venipuncture (e.g., hematoma, bleeding, pain, infection), which are low, and the risk of progression of disease for patients assigned to receive placebo.

### *5.11.2 Known Potential Benefits*

Given the relatively benign safety profile of trehalose noted thus far, and the lack of a highly effective treatment for ALS, treatment with trehalose for its potential to arrest or slow progression of ALS disease symptoms is justified.

# 6 REGIMEN SCHEDULE

In addition to procedures in the Master Protocol, the following regimen-specific procedures will be conducted during the study:

- ALSAQ-40
- CNS Bulbar Function Scale
- Serum PK parameter estimates
- CSF biomarker LC3-II and Neurofilament light chain
- HbA1c as part of the clinical safety labs

Participants may be required to reconsent to the regimen if new procedures or information is added in the future. Should a participant need to reconsent, this should occur during the participant's next in-person visit. If the participant's next in-clinic visit is conducted remotely, reconsent may also be completed remotely using the following procedures:

1. The site staff sends copy of the informed consent form to the participant.
2. The participant reads through the consent form but does not sign.
3. The Site Investigator, or other study staff member approved and delegated to obtain informed consent, calls the participant and reviews the informed consent form with the participant over the phone or via telemedicine.
4. The participant signs the informed consent form and mails the original signed consent form back to the site.
5. Once received at the site, the individual who consented the participant via phone signs the informed consent form.

### *Modifications to Regimen Schedule*

Designated visits in the Schedule of Activities (i.e. Week 16) may be conducted via telemedicine (or phone if telemedicine is not available) with remote services instead of in-person if needed to protect the safety of the participant due to a pandemic or other reason. If a planned in-clinic visit is conducted via telemedicine (or phone if telemedicine is not available) with remote services, only selected procedures will be performed. Instructions on how to document missed procedures are included in the MOP.

In addition to the procedures in the Master Protocol that should be conducted during the phone or telemedicine and remote visits, the following regimen-specific procedures should be completed:

- CNS Bulbar Function Scale

Details on collection of the CNS Bulbar Function Scale, dispensing IP during remote visits, and documenting subjects' willingness to participate in OLE are described in the MOP.

## 6.1 Baseline Visit

This visit will take place on Day 0. The following procedures will be performed for the regimen schedule:

- ALSAQ-40
- CNS Bulbar Function Scale
- Collect safety labs including for HbA1c evaluation
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit
- Confirm dosing volume based off Master Protocol Screening Visit weight
- Administer first infusion of study drug in clinic *after* all Baseline procedures have been completed

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

- For applicable participants, collect PK blood sample (Pre-dose, 1-hour ± 5 minutes, 2 hours ± 5 minutes, and 4 hours ± 5 minutes post start of infusion)

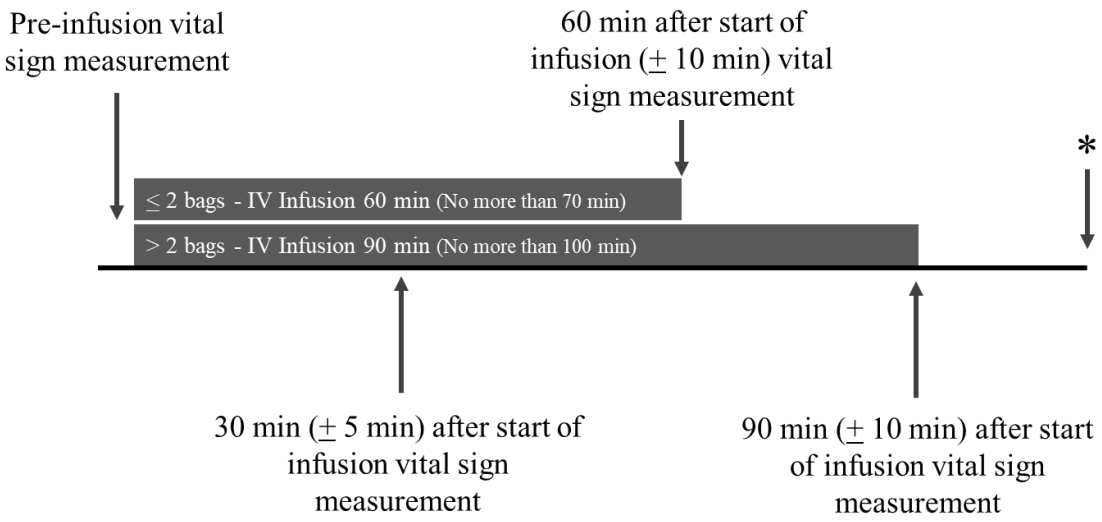
**6.2 Weekly Study Drug Infusion**

During the placebo-controlled treatment period, all participants will receive a weekly infusion of study drug (trehalose or placebo for 60 + 10 minutes, participants who require more than 2 bags of SLS-005 the infusion time is to be increased to 90 + 10 minutes) for the first 4 weeks of the study in clinic. Thereafter home infusions can be considered if the participant has had no adverse reaction to the infusion and all safety assessments are within acceptable limits.

During the Week 8 in-clinic visit, PK assessments will occur prior to and after the infusion per the SOA. Therefore, if a participant transitions to home infusions after the first four weeks the site should coordinate the Week 8 infusion to occur in-clinic while the participant is in for the Week 8 in-clinic visit. The home infusion nurse will not collect any PK samples; therefore, the Week 8 PK samples would be missed if the Week 8 infusion does not occur in-clinic at the time of the Week 8 in-clinic visit. This would not result in a protocol deviation.

Concurrent assessments such as vital signs, reporting of any infusion related adverse events, and reporting of new concomitant medications is to be completed weekly in conjunction with the study drug infusion.

Blood pressure, respiration rate, and heart rate, should be conducted at four timepoints during each infusion: (1) Pre-dose, (2) midway through infusion = 30 ± 5 min post start of infusion, (3) end of infusion = 60 ± 10 min post start of infusion, and (4) 90 ± 10 min post start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.




**If a participant requires a 90-minute infusion duration due to receiving >2 bags of study drug an additional vital signs measurement should be done 30 minutes (± 10 min) after the end of infusion.*

Abbreviations: IV = intravenous

If the infusion is done at home, confirmation of infusion and information on infusion-related adverse events, and changes in health and medications will be provided to study site by the home infusion nurse via study provided source documents. Site staff will then enter the data into the EDC and follow up with the participant as needed to document any changes in AEs or concomitant medications in the EDC.

### 6.3 Week 2 Telephone Visit

This visit will take place 14 ± 3 days after the Baseline Visit. No regimen-specific procedures will be performed at this visit.

If the participant will receive their in-clinic infusion within the Week 2 visit window, this visit may occur in-clinic at the time of the infusion visit.

### 6.4 Week 4 and 8 Visits

Study drug should not be administered until *after* all study visit procedures are complete.

These visits will take place on Days 28 ± 7 and 56 ± 7 days, respectively. The following procedures will be performed for the regimen schedule:

- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit
- Collect safety labs
- CNS Bulbar Function Scale [Week 8 only]

- Adjustment of dosing volume based on most recent weight measurement as necessary
- Administer infusion of study drug in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
- During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

- For applicable participants, collect PK blood sample (Pre-dose, 1-hour ± 5 minutes, 2 hours ± 5 minutes, and 4 hours ± 5 minutes post start of infusion) [Week 8 only]

### 6.4 Week 12 Telephone Visit

This visit will take place 84 ± 3 days after the Baseline Visit. No regimen-specific procedures will be performed at this visit.

If the participant will receive their infusion in clinic and within the Week 12 visit window, this visit may occur in-clinic at the time of the infusion visit.

### 6.5 Week 16 Visit

Study drug should not be administered until after study visit procedures are complete.

This visit will take place on Day 112 ± 7 days. The following procedures will be performed for the regimen schedule:

- Document participant's willingness to participate in the OLE
  - If OLE consent is not obtained at Week 16, it may be obtained at Week 24.

- CNS Bulbar Function Scale
  - Collect safety labs including for HbA1c evaluation
  - Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit
- Adjustment of dosing volume based on most recent weight measurement as necessary
- Administer infusion of study drug in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
  - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a

participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

### 6.6 Week 20 Telephone Visit

This visit will take place 140 ± 3 days after the Baseline Visit. No regimen-specific procedures will be performed at this visit.

If the participant will receive their infusion in clinic and within the Week 20 visit window, this visit may occur in-clinic at the time of the infusion visit.

### 6.7 Week 24 Visit or Early Termination Visit

Study drug should not be administered until after study visit procedures are complete.

This visit will take place on Day 168 ± 7 days. The following procedures will be performed for the regimen schedule:

- ALSAQ-40
- CNS Bulbar Function Scale
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit
- Collect safety labs including for HbA1c evaluation
- Adjustment of dosing volume based on most recent weight measurement as necessary (only if participant is continuing in the Open Label Extension)
- Administer infusion (**Drug is only administered at this visit if the participant is continuing in the Open Label Extension.**)
- During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.
    - Note: The first OLE infusion should occur in-clinic following completion of the Week 24 visit. The participant's final infusion of the placebo-controlled treatment period should be scheduled and completed at least 4 days prior to the Week 24 Visit.

### 6.8 Follow-Up Safety Call

Participants will have a Follow-Up Safety Call 28±3 days after their last dose of study drug. Only those participants NOT continuing on in the Open Label Extension will have the Follow-Up Safety Call following the end of their participation in the placebo-controlled portion of the trial.

The following procedures will be performed:

- Assess and document AEs

## 6.9 Process for Early Terminations

Participants who early terminate from the study and do not complete the protocol per ITT will be asked to be seen for an in-person Early Termination Visit and complete a Follow-Up Safety Call.

The in-person Early Termination Visit should be scheduled as soon as possible after a participant early terminates. If the participant early terminates during the placebo-controlled portion of the Regimen, all assessments that are collected at the Week 24 in-clinic visit should be conducted. The Follow-Up Safety Call should be completed approximately 28 days after the last dose of study drug.

If the Early Termination Visit occurs approximately 28±3 days after the last dose of study drug, the information for the Follow-Up Safety Call can be collected during the Early Termination Visit, and a separate Follow-Up Safety Call does not need to be completed. If the in-person Early Termination Visit does not occur within 28±3 days of the last dose of study drug, the Follow-Up Safety Call should occur approximately 28 days after the last dose of study drug and the Early Termination Visit will be completed after the Follow-Up Safety Call.

If a participant decides to discontinue study drug, but will complete the protocol per ITT, an in-person Early Termination Visit and Follow-Up Safety Call is not necessary.

## 6.10 Open Label Extension

Participants who have completed the placebo-controlled portion of the trial on study drug, active drug or placebo, will be eligible to continue in the Open Label Extension (OLE) as outlined in the SOA. Participants will first be asked about their desire to continue in the OLE at the Regimen-Specific Screening Visit. Participants will consent to participate in the OLE at the week 16 visit, after the site investigator reviews the OLE procedures in detail. If OLE consent is not obtained during the week 16 visit, OLE consent may be obtained at the week 24 visit.

### *Modifications to Regimen Schedule*

Designated visits in the Schedule of Activities for the OLE (i.e. Week 8, Week 16, Week 28, and Week 40) may be conducted via telemedicine (or phone if telemedicine is not available) with remote services instead of in-person if needed to protect the safety of the participant due to a pandemic or other reason. If a planned in-clinic visit is conducted via telemedicine (or phone if telemedicine is not available) with remote services, only selected procedures will be performed. Instructions on how to document missed procedures are included in the MOP.

In addition to the procedures in the Master Protocol that should be conducted during the phone or telemedicine and remote visits, the following regimen-specific procedures should be completed:

- CNS Bulbar Function Scale
- ALSAQ-40

### *6.10.1 Weekly Investigational product Administration*

During the OLE all participants will receive a weekly infusion of investigational product (trehalose for 60 + 10 minutes, for participants who require more than 2 bags of SLS-005 the

infusion time is increased to 90 + 10 minutes) for the first 4 weeks of the study in clinic. Thereafter home infusions can be considered if the participant has had no adverse reaction to the infusion and all safety assessments are within acceptable limits. Concurrent assessments such as vital signs, reporting of any infusion related adverse events including Key Study Events (*see section 10.3 of Master Protocol*) and reporting of new concomitant medications should also be done weekly in conjunction with the investigational product infusion.

*6.10.2 Week 2 OLE Telephone Visit*

This visit will take place 14 ± 3 days the Week 24 Visit of the placebo-controlled portion of the trial. If the participant will receive their in-clinic infusion within the OLE Week 2 visit window, this visit may occur in-clinic at the time of the infusion visit.

The following procedures should be performed:

- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)

*6.10.2 Week 4 OLE Visit*

This visit will take place in-person 28 ± 7 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect weight
- Perform SVC
- Administer ALSFRS-R questionnaire
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit.
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Adjustment of dosing volume based on most recent weight measurement as necessary
- Administer infusion of investigational product in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
    - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

*6.10.3 Week 8 OLE Visit*

This visit will take place in-person at 56 ± 7 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect weight
- Perform SVC
- Administer ALSFRS-R questionnaire
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit.
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Adjustment of dosing volume based on most recent weight measurement as necessary
- Administer infusion of investigational product in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
    - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

*6.10.4 Week 12 OLE Telephone Visit*

This visit will take place in clinic or via phone at 84 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. If the participant will receive their in-clinic infusion within the OLE Week 12 visit window, this visit may occur in-clinic at the time of the infusion visit.

The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)

### *6.10.5 Week 16 OLE Visit*

Investigational product should not be administered until after study visit procedures are complete.

This visit will take place in-person at 112 ± 7 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect weight
- Perform SVC
- Administer ALSFRS-R questionnaire
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs including Hb1Ac and, for WOCBP, for pregnancy test if applicable
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit.
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Collect blood sample for biomarker analyses
- Adjustment of dosing volume based on weight measurement as necessary
- Administer infusion of investigational product in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
    - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

### *6.10.6 Week 20 OLE Telephone Visit*

This visit will take place in clinic or via phone at 140 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. If the participant will receive their in-clinic infusion within the OLE Week 20 visit window, this visit may occur in-clinic at the time of the infusion visit.

The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications

- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)

### *6.10.7 Week 24 OLE Telephone Visit*

This visit will take place in clinic or via phone at 168 ± 3 days after the Week 24 Visit of the placebo-controlled portion of the trial. If the participant will receive an in-clinic infusion within the OLE Week 24 visit window, this visit may occur in-clinic at the time of the infusion visit.

The following procedures will be performed:

- Administer ALSFRS-R questionnaire
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)

### *6.10.8 Week 28 OLE Visit*

Investigational product should not be administered until after study visit procedures are complete.

The Week 28 OLE Visit will take place in-person 196 ± 14 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect weight
- Perform SVC
- Administer ALSFRS-R questionnaire
- ALSAQ-40
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs including HbA1c and, for WOCBP, for pregnancy test if applicable
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit.
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Collect blood sample for biomarker analyses
- Adjustment of dosing volume based on most recent weight measurement as necessary
- Administer infusion of investigational product in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
    - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-

infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 minutes (± 10 mins) after the end of infusion.

### *6.10.9 Week 40 OLE Visit*

The Week 40 OLE Visit will take place in-person 280 ± 14 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect weight
- Perform SVC
- Administer ALSFRS-R questionnaire
- CNS Bulbar Function Scale
- Collect blood samples for Clinical Safety Labs and, for WOCBP, for pregnancy test if applicable
- Collect urine, *prior to start of infusion*. If urine cannot be collected prior to infusion, no urine should be collected at this visit.
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)
- Administer the C-SSRS Since Last Visit questionnaire
- Adjustment of dosing volume based on weight measurement as necessary
- Administer infusion of investigational product in clinic after all study procedures have been completed, provided at least 4 days have passed since prior infusion.
    - During each infusion, vital signs associated with the infusion (systolic and diastolic pressure, respiratory rate, and heart rate) are to be measured pre-infusion, 30 minutes (± 5 mins) after the start of infusion (SOI), at the end of infusion (± 10 mins), and 90 minutes (± 10 mins) after the start of infusion. If a participant requires a 90-minute infusion duration due to receiving more than 2 bags of study drug an additional vital signs measurement is to be completed approximately 30 (± 10 mins) minutes after the end of infusion.

### *6.10.10 Week 52 OLE Visit*

The Week 52 OLE Visit will take place in-person 364 ± 14 days after the Week 24 Visit of the placebo-controlled portion of the trial. The following procedures will be performed:

- Collect vital signs including weight
- Perform SVC
- Administer ALSFRS-R questionnaire

- ALSAQ-40
- CNS Bulbar Function Scale
- Collect blood and urine samples for Clinical Safety Labs including HbA1c and, for WOCBP, for pregnancy test if applicable
- Review concomitant medications
- Assess and document AEs, including Key Study Events (*see section 10.3 of Master Protocol*)

  Administer the C-SSRS Since Last Visit questionnaire
- Collect blood sample for biomarker analyses

### *6.10.11 Follow-Up Safety Call*

Participants will have a Follow-Up Safety Call 28±3 days after their last dose of investigational product.

The following procedures will be performed:

- Assess and document AEs

### *6.10.12 Process for Early Terminations from the OLE*

Participants who early terminate from the study be asked to be seen for an in-person Early Termination Visit and complete a Follow-Up Safety Call.

The in-person Early Termination Visit should be scheduled as soon as possible after a participant early terminates. If the participant early terminates during the OLE period, all assessments that are collected at the OLE Week 52 in-clinic visit should be conducted. The Follow-Up Safety Call should be completed approximately 28 days after the last dose of investigational product.

If the Early Termination Visit occurs approximately 28±3 days after the last dose of investigational product, the information for the Follow-Up Safety Call can be collected during the Early Termination Visit, and a separate Follow-Up Safety Call does not need to be completed. If the in-person Early Termination Visit does not occur within 28±3 days of the last dose of investigational product, the Follow-Up Safety Call should occur approximately 28 days after the last dose of investigational product and the Early Termination Visit will be completed after the Follow-Up Safety Call.

# 7 OUTCOME MEASURES AND ASSESSMENTS

## 7.1 ALSAQ-40

The Amyotrophic Lateral Sclerosis Assessment Questionnaire-40 (ALSAQ-40) is a participant self-report health status patient-reported outcome. 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.

Participants will be handed the questionnaire and asked to write their answers themselves. Caregivers can also help, if needed.

## 7.2 Center for Neurologic Study Bulbar Function Scale

The Center for Neurologic Study Bulbar Function Scale (CNS-BFS) is a participant self-report scale that has been developed for use as an endpoint in clinical trials and as a clinical measure for evaluating and following ALS participants. The CNS-BFS consists of three domains (swallowing, speech, and salivation), which are assessed with a 21-question, self-report questionnaire.

Participants will be handed the questionnaire and asked to write their answers themselves. Caregivers can also help, if needed.

## 8.0 BIOFLUID COLLECTION

CSF Biomarker and serum PK samples will be collected as per the schedule of activities

### 8.1 CSF Biomarker Collection

The CSF for neurofilament light chain concentration and microtubule-associated protein 1A/-light chain 3 (LC3-II) will be obtained from participants who consent to lumbar puncture. Four cryovials containing 0.25 mL of CSF (for a total of 1.0 mL) will be provided from the Baseline and Week 16 Visits for analysis. Details regarding sample collection preparation and shipment will be provided in laboratory manual.

### 8.2 Serum Pharmacokinetic Assessments

Not all sites may have the capacity to collect PK for this regimen, therefore PK will only be collected on participants who are enrolled at a site capable of performing the PK collection procedures.

The first thirty-six (36) participants randomized within the regimen to reach the Week 8 visit at sites participating in the PK collection will complete the PK collection procedure. Any participant not able to complete Week 8 PK will be replaced.

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

# 9 REGIMEN-SPECIFIC STATISTICAL CONSIDERATIONS

## 9.1 Deviations from the Default Master Protocol Trial Design

The statistical design for this regimen will be in accordance with the default statistical design described in Appendix I of the Master Protocol with only one deviation. This regimen will not include interim analyses for early success.

## 9.2 Regimen Specific Operating Characteristics

Clinical trial simulation is used to quantify operating characteristics for this regimen (refer to the regimen SAP for further details).

## 9.3 Sharing of Controls from other Regimens

The primary analysis of this regimen will include sharing of all controls from the other regimens. This is justified by the minor differences in inclusion/exclusion criteria of the RSA, such that there are no expected systematic differences in the primary endpoint between the controls across regimens.

# APPENDIX I: THE ALSAQ-40

## ALSAQ-40

**Please complete this questionnaire as soon as possible.** If you have any difficulties filling in this questionnaire by yourself, please have someone help you. However it is **your** responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks.** There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please check the box that best describes your own experiences or feelings.**

**Please answer every question** even though some may seem very similar to others, or may not seem relevant to you.

All the information you provide is **confidential.**

The following statements all refer to difficulties that you may have had **during the last 2 weeks**.
Please indicate, by checking the appropriate box, how often the following statements have been true for you.

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

If you cannot walk at all
please check **Always/cannot walk at all.**

***How often during the last 2 weeks have the following been true?***

*Please check **one box** for each question.*

| | Never | Rarely | Some-times | Often | Always or cannot walk at all |
|---|---|---|---|---|---|
| **1. I have found it difficult to walk short distances, e.g. around the house.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **2. I have fallen over while walking.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **3. I have stumbled or tripped while walking.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **4. I have lost my balance while walking.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **5. I have had to concentrate while walking.** | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked **one box for each question** before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

*If you are not able to perform the activity at all please check* **Always/cannot at all**

***How often during the last 2 weeks have the following been true?***

*Please check* ***one box*** *for each question*

| | Never | Rarely | Some-times | Often | Always or cannot do at all |
|---|---|---|---|---|---|
| **6. Walking had worn me out.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **7. I have had pains in my legs while walking.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **8. I have found it difficult to go up and down the stairs.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **9. I have found it difficult to stand up.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **10. I have found it difficult to move from sitting in a chair to standing upright.** | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked* ***one box for each question*** *before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

*If you cannot do the activity at all*
*please check* **Always/cannot do at all.**

***How often during the last 2 weeks have the following been true?***

*Please check* ***one box*** *for each question*

| | Never | Rarely | Some-times | Often | Always or cannot do at all |
|---|---|---|---|---|---|
| **11. I have had difficulty using my arms and hands.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **12. I have found turning and moving in bed difficult.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **13. I have had difficulty picking things up.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **14. I have had difficulty holding books or newspapers, or turning pages.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **15. I have had difficulty writing clearly.** | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked* ***one box for each question*** *before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

*If you cannot do the activity at all*
*please check* **Always/cannot do at all.**

***How often during the last 2 weeks have the following been true?***

*Please check* ***one box*** *for each question*

| | Never | Rarely | Some-times | Often | Always or cannot do at all |
|---|---|---|---|---|---|
| 16. I have found it difficult to do jobs around the house. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 17. I have found it difficult to feed myself. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 18. I have had difficulty combing my hair or brushing and/or flossing my teeth. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 19. I have had difficulty getting dressed. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 20. I have had difficulty washing at the bathroom sink. | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked* ***one box for each question*** *before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

*If you cannot do the activity at all*
*please check* **Always/cannot do at all.**

***How often during the last 2 weeks have the following been true?***

*Please check* ***one box*** *for each question*

| | Never | Rarely | Some-times | Often | Always or cannot do at all |
|---|---|---|---|---|---|
| 21. I have had difficulty swallowing. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 22. I have had difficulty eating solid food. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 23. I have had difficulty drinking liquids. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 24. I have had difficulty participating in conversations. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 25. I have felt that my speech has not been easy to understand. | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked* ***one box for each question*** *before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

*If you cannot do the activity at all*
*please check* **Always/cannot do at all.**

***How often during the last 2 weeks have the following been true?***

*Please check* ***one box*** *for each question*

| | Never | Rarely | Some-times | Often | Always or cannot do at all |
|---|---|---|---|---|---|
| **26. I have stuttered or slurred my speech.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **27. I have had to talk very slowly.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **28. I have talked less than I used to do.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **29. I have been frustrated with my speech.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **30. I have felt self-conscious about my speech.** | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked* ***one box for each question*** *before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

***How often during the last 2 weeks have the following been true?***

*Please check **one box** for each question*

| | Never | Rarely | Some-times | Often | Always |
|---|---|---|---|---|---|
| 31. I have felt lonely. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 32. I have been bored. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 33. I have felt embarrassed in social situations. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 34. I have felt hopeless about the future. | ☐ | ☐ | ☐ | ☐ | ☐ |
| 35. I have worried that I am a burden to other people. | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked **one box for each question** before going on to the next page.*

**The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.**

***How often during the last 2 weeks have the following been true?***

*Please check **one box** for each question*

| | Never | Rarely | Some-times | Often | Always |
|---|---|---|---|---|---|
| **36. I have wondered why I keep going.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **37. I have felt angry because of the disease.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **38. I have felt depressed.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **39. I have worried about how the disease will affect me in the future.** | ☐ | ☐ | ☐ | ☐ | ☐ |
| **40. I have felt as if I have lost my independence** | ☐ | ☐ | ☐ | ☐ | ☐ |

*Please make sure that you have checked one **box for each question.***

***Thank you for completing this questionnaire.***

# APPENDIX II: THE BULBAR FUNCTION SCALE (CNS-BFS)

| BULBAR FUNCTION SCALE (CNS-BFS) | | | | | | |
|---|---|---|---|---|---|---|
| **SIALORRHEA** | **Does Not Apply (1)** | **Applies Rarely (2)** | **Applies Occasionally (3)** | **Applies Frequently (4)** | **Applies Most of the Time (5)** | |
| 1. Excessive saliva is a concern to me. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 2. I take medication to control drooling. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 3. Saliva causes me to gag or choke. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 4. Drooling causes me to be frustrated or embarrassed. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 5. In the morning I notice saliva on my pillow. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 6. My mouth needs to be dabbed to prevent drooling. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| 7. My secretions are not manageable. | ❍ | ❍ | ❍ | ❍ | ❍ | |
| | | | | **TOTAL Sialorrhea Score: _____** | | |
| **SPEECH** | **Does Not Apply (1)** | **Applies Rarely (2)** | **Applies Occasionally (3)** | **Applies Frequently (4)** | **Applies Most of the Time (5)** | **Unable to Communicate by Speaking (6)** |
| 1. My speech is difficult to understand. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| 2. To be understood I repeat myself. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| 3. People who understand me tell other people what I said. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4. To communicate I write things down or use devices such as a computer. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| 5. I am talking less because it takes so much effort to speak. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| 6. My speech is slower than usual. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| 7. It is hard for people to hear me. | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| | | | | **TOTAL Speech Score: _____** | | |

| **SWALLOWING** | **Does Not Apply (1)** | **Applies Rarely (2)** | **Applies Occasionally (3)** | **Applies Frequently (4)** | **Applies Most of the Time (5)** |
|---|---|---|---|---|---|
| ☐ Feeding tube is in place | | | | | |
| 1. Swallowing is a problem. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 2. Cutting my food makes it easier to chew and swallow. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 3. To get food down I have switched to a soft diet. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 4. After swallowing I gag or choke. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 5. It takes longer to eat. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 6. My weight is dropping because I can't eat normally. | ❍ | ❍ | ❍ | ❍ | ❍ |
| 7. Food gets stuck in my throat. | ❍ | ❍ | ❍ | ❍ | ❍ |
| | | | | **TOTAL Swallowing Score: _____** | |
| | | | | **OVERALL SCORE: _____** | |

## REFERENCES


1. Elbein AD, Pan YT, Pastuszak I, et al. New insights on trehalose: a multifunctional molecule. Glycobiology 2003;13(4):17R-27R.
2. Emanuele E. Can Trehalose Prevent Neurodegeneration? Insights from Experimental Studies. Current Drug Targets 2014;15(5):1-7.
3. Rusmini P, Cortese K, Crippa V, et al. Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration. Autophagy 2019;15(4):631-651.
4. Tanaka M, Machida Y, Niu S, et al. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. Nature Medicine 2004;10(2):148-154.
5. Davies JE, Sarkar S, Rubinsztein DC. Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. Human Molecular Genetics 2006;15(1):23-31.
6. Mardones P, Rubinsztein DC, Hetz C. Mystery solved: Trehalose kickstarts autophagy by blocking glucose transport. Science Signaling 2016;9(416):fs2.
7. Castillo K, Nassif M, Valenzuela V, et al. Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. Autophagy 2013;9(9):1308-1320.
8. Zhang X, Chen S, Song L, et al. MTOR-independent, autophagic enhancer trehalose prolongs motor neuron survival and ameliorates the autophagic flux defect in a mouse model of amyotrophic lateral sclerosis. Autophagy 2014;10(4):588-602.
9. Li Y, Guo Y, Wang X, et al. Trehalose decreases mutant SOD1 expression and alleviates motor deficiency in early but not end-stage amyotrophic lateral sclerosis in a SOD1-G93A mouse model. Neuroscience 2015;298:12-25.
10. DeBosch BJ, Heitmeier MR, Mayer AL, et al. Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. Science Signaling 2016;9(416):ra21.