Statistical Analysis Plan

Title: A Phase 2a, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study to Assess the Safety of Engensis in Participants with Amyotrophic Lateral Sclerosis

Protocol Number: VMALS-002-2

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[redacted]

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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
ALS	Amyotrophic lateral sclerosis
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating Scale
ALT	Alanine transaminase (SGPT)
AST	Aspartate transaminase (SGOT)
ATC Level	Anatomic Therapeutic Chemical
ATLIS	Accurate Test of Limb Isometric Strength
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CGIC	Clinical Global Impression of Change
CK	Creatine Kinase
CM	Centimeter
CS	Clinically significant
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
EDC	Electronic data capture system
GGT	Gamma-glutamyl transpeptidase
HEENT	Head, eyes, ears, nose, and throat
HHD	Handheld Dynamometer
ICF	Informed Consent Form
IM	Intramuscular
ITT	Intent to Treat Population
ISR	Injection site reaction
KG	Kilogram
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not clinically significant
PGIC	Patient Global Impression of Change
PT	Prothrombin time
PTT	Partial prothrombin time
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	(Statistical Analysis System) statistical software suite developed by SAS Institute

SOC	System Organ Class
SVC	Slow Vital Capacity
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
WHO	World Health Organization

1 INTRODUCTION

This study is a Phase 2a, double-blind, randomized, placebo-controlled, multicenter study designed to assess the safety of intramuscular (IM) administration of Engensis in Participants with Amyotrophic Lateral Sclerosis (ALS) as compared to Placebo. Study Participants will be randomized in a 2:1 ratio to Engensis or Placebo.

This document details the planned statistical analyses for the Helixmith Co., Ltd VMALS-002-2 study titled "A Phase 2a, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study to Assess the Safety of Engensis in Participants with Amyotrophic Lateral Sclerosis". This document covers all statistical analyses planned in the study.

The proposed analyses are based on the contents of the final protocol, version 3.0 (dated 26-Jan-2021).

2 STUDY OBJECTIVES

Primary objective:

- To evaluate the safety of intramuscular (IM) injections of Engensis in Participants with Amyotrophic Lateral Sclerosis (ALS) compared to Placebo

Exploratory objectives:

- To evaluate changes in muscle function following Engensis injections in ALS Participants compared to Placebo
- To evaluate muscle strength changes following Engensis injections in ALS Participants compared to Placebo
- To evaluate Quality of Life improvement following Engensis injections in ALS Participants compared to Placebo
- To evaluate Patient and Clinical Reported Outcome improvement following Engensis injections in ALS Participants compared to Placebo
- To determine whether IM administration of Engensis has effects on respiratory function in ALS Participants compared to Placebo
- To determine whether IM administration of Engensis has positive effects on survival in ALS Participants compared to Placebo

- To determine whether IM administration of Engensis has positive effects on muscle atrophy in ALS Participants compared to Placebo

3 ENDPOINTS

3.1 Primary Endpoint

- Incidence of treatment-emergent adverse events (TEAEs) and treatment-emergent serious adverse events (TESAEs) for Engensis compared to Placebo
- Incidence of injection site reactions for Engensis compared to Placebo
- Incidence of clinically significant laboratory values for Engensis compared to Placebo

3.2 Exploratory Endpoints

- Change from Baseline (Day 0) in total mean Revised Amyotrophic Lateral Sclerosis Function Rating (ALSFRS-R) scores at Day 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in ALSFRS-R subscores for Fine and Gross Motor Functions (sum of scores for items 4 to 9) and for Bulbar Function (sum of scores for items 1 to 3) on Days 30, 60, 84, 120, 144, and 180 for Engensis compared to Placebo
- Changes in the slope of the total ALSFRS-R score over time for Engensis compared to Placebo
- Change from Baseline (Day 0) in muscle strength assessed bilaterally by Handheld Dynamometry (HHD) in muscles in the upper and lower extremities on Days 30, 60, 84, 120, 144, and 18y0 for Engensis compared to Placebo
- Change from Baseline (Day 0) in the Accurate Test of Limb Isometric Strength (ATLIS) where available at Days 30, 60, 84, 120, 144, and 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in Quality of Life (QoL) using the ALS Assessment Questionnaire (ALSAQ; with 40 items, ALSAQ-40) on Days 84 and 180 for Engensis compared to Placebo
- Patient Global Impression of Change (PGIC) and Clinical Global Impression of Change (CGIC) at Days 84 and 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in Slow Vital Capacity (SVC) on Days 30, 60, 84, 120, 144, and 180 for Engensis compared to Placebo
- Time to tracheostomy for Engensis compared to Placebo
- Time to all-cause mortality by Day 180 for Engensis compared to Placebo

- Change from Baseline (Day 0) in biomarkers for muscle atrophy in biopsies collected before and after injections for Engensis compared to Placebo

4 SAMPLE SIZE

A sample size of 18 Participants was chosen for safety assessments and preliminary evaluation of exploratory endpoints.

5 RANDOMIZATION

Participants are to be randomized on Day 0 as close as possible to the time of the first Study Injections. Randomization will be conducted via an electronic data capture system (EDC) randomization module in a 2:1 ratio for Participants to receive either Engensis or Placebo.

6 PLANNED ANALYSES

No statistical analysis plan (SAP) prepared in advance of the data can be absolutely definitive and the final Clinical Study Report (CSR) may contain additional tables or statistical tests if warranted by the data obtained. The justification for any such additional analyses will be fully documented in the final CSR.

6.1 Analysis Populations

6.1.1 Enrolled Population

Enrolled Population will be used for presenting data on Participant disposition, it will consist of all screened Participants.

6.1.2 ITT Population

The Intent to Treat (ITT) Population consists of all randomized participants. Participants will be grouped according to their planned treatment. Exploratory endpoints will be analyzed in the ITT Population.

6.1.3 Safety Analysis Population

Safety Analysis Population will be used to provide tables and listings for safety analysis.

The Safety Analysis Population will contain all Participants who are randomized and receive at least one Study Injection. Participants will be grouped according to their actual treatment received, not according to their randomization assignment (as randomized). Participants treated with any

Engensis dose will be grouped in the Engensis group; Participants treated without any Engensis will be grouped in the Placebo group.

6.2 Derived Data

This section describes the derivations required for statistical analysis. Unless otherwise stated, variables derived in the source data will not be re-calculated.

6.2.1 Race

Where more than one race category has been selected for a participant, these race categories will be combined into a single category labeled "Multiple Race" in the summary tables. The listings will reflect the original selected categories.

6.2.2 Baseline

Baseline for safety parameters as well as for exploratory parameters is defined as the last non-missing value (either scheduled, unscheduled or repeat) before the participant receives first Study Injection.

6.2.3 Duration / Study Day / Time

Study day will be calculated as the number of days from first Study Injection.

- date of event – date of first Study Injection + 1, for events on or after first Study Injection
- date of event – date of first Study Injection, for events before first Study Injection

Duration of ALS diagnosis will be presented in months and calculated as (Date of Screening – Date of Onset of Symptoms) / 30.4375.

Time to tracheostomy will be calculated as time from date of first Study Injection to date of first occurrence of ALSFRS-R questionnaire with answer **"0 - Invasive mechanical ventilation by intubation or tracheostomy"** to question **"12. Respiratory insufficiency"** presented in days.

Time to all-cause mortality will be calculated as period from date of first Study Drug Injection to date of death within 180 days presented in days.

Both time-to-event endpoints will be censored by date of last visit.

6.2.4 Conventions for Missing and Partial Dates

All rules explained below for partial / missing dates will be followed unless contradicted by any other data recorded on the electronic Case Report Form (eCRF).

All dates presented in the individual participant listings will be as recorded on the eCRF (i.e., not completed as per the below rules).

6.2.5 Missing / Partial Start / Stop Date of Adverse Events (AE) and Concomitant Medications

Missing and partial start and stop date will be imputed for analysis purposes as follows.

Partial or missing stop date will be imputed as follows:

If the stop date is completely missing and the event has resolved, or the participant has stopped taking the concomitant medication, the stop date will be imputed as the date of the participant's last clinic visit in the study.

- If only the year is known, the stop date will be imputed as "31-Dec" of that year or as the date of the participant's last clinic visit in the study if in the same year.
- If the month and year are known, the stop date will be imputed as the last day of that month unless the stop date corresponds to the same month as the participant's last clinic visit in which case the date of participant's last clinic visit in the study will be used instead.

Missing start date will be imputed as follows:

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, the start date will be imputed as the date of the first dose of study drug.
- If the stop date occurs before the start of study drug, the start date of the event / concomitant medication will be imputed as the participant's screening date or the stop date of the event / concomitant medication whichever the earlier.

Partial start date (year present, but month and day missing)

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, and the year is the same as the year of first dosing the start date will be imputed as "01-Jan" of the same year or the date of the first dose of study drug whichever is latest. If the year is different from the year of first dosing "01-Jan" will be used.
- If the stop date occurs before the start of study drug, the start date of the event / concomitant medication will be imputed as the "01-Jan" of the same year.

Partial start date (month and year present, but day missing)

- If the stop date occurs on or after the start of study drug or the event / concomitant medication is ongoing, the start date will be imputed as the first day of the same month and

year unless this partial start date is in same month as the first dose of study drug in which case the date of first dose of study drug will be used.

- If the stop date occurs before the start of study drug, the start date will be imputed as the first day of the month and year of the partial stop date.

If the start time is missing it will be imputed only in the case where the start date of the concomitant medication / event corresponds to the date of the first dose of study drug. The time will be imputed as the same time as the first dose of study drug. In all other cases the time will not be imputed.

6.2.6 Missing Last Dates of Study Drug Dosing

Not applicable in this SAP.

6.2.7 Missing Diagnosis Dates

If the month and year are present but the day is missing, the diagnosis date will be set to first day of the relevant month. If only the year is recorded the diagnosis date will be set as "01-Jan" for that year.

6.2.8 Exposure to Study Drug

Exposure to study drug will be presented as the following:

- Number of performed injections for each performed dosing visit and overall
- Total dose(mg) received, only for Engensis group, calculated as number of performed injections * 0.25 mg.
- Time of study drug exposure calculated as the date of study discontinuation or the date of End of Study (whichever comes first) minus date of first day of injections + 1.

6.2.9 Compliance

Compliance will be calculated as 100% * [volume of administered injections] / [volume of planned injections]. Volumes of planned injections are:

- Each anatomic area for dosing day:
  - Abductor pollicis brevis, right: 1 mL
  - 2 Abductor pollicis brevis, left: 1 mL
  - 3 First dorsal interosseous, right: 1 mL
  - 4 First dorsal interosseous, left: 1 mL
  - 5 Biceps, right: 4 mL
  - 6 Biceps, left: 4 mL

  - 7 Deltoid, right: 4 mL
  - 8 Deltoid, left: 4 mL
  - 9 Extensor carpi radialis, right: 1 mL
  - 10 Extensor carpi radialis, left: 1 mL
  - 11 Flexor carpi ulnaris, right: 1 mL
  - 12 Flexor carpi ulnaris, left: 1 mL
  - 13 Flexor carpi radialis, right: 1 mL
  - 14 Flexor carpi radialis, left: 1 mL
  - 15 Quadriceps, right: 10 mL
  - 16 Quadriceps, left: 10 mL
  - 17 Gastrocnemius, right: 6 mL
  - 18 Gastrocnemius, left: 6 mL
  - 19 Tibialis anterior, right: 6 mL
  - 20 Tibialis anterior, left: 6 mL
- Each side for each dosing day: 32 mL
- Each dosing day: 64 mL
- Each anatomic area for treatment cycle:
  - Abductor pollicis brevis, right: 2 mL
  - 2 Abductor pollicis brevis, left: 2 mL
  - 3 First dorsal interosseous, right: 2 mL
  - 4 First dorsal interosseous, left: 2 mL
  - 5 Biceps, right: 8 mL
  - 6 Biceps, left: 8 mL
  - 7 Deltoid, right: 8 mL
  - 8 Deltoid, left: 8 mL
  - 9 Extensor carpi radialis, right: 2 mL
  - 10 Extensor carpi radialis, left: 2 mL
  - 11 Flexor carpi ulnaris, right: 2 mL
  - 12 Flexor carpi ulnaris, left: 2 mL
  - 13 Flexor carpi radialis, right: 2 mL
  - 14 Flexor carpi radialis, left: 2 mL
  - 15 Quadriceps, right: 20 mL
  - 16 Quadriceps, left: 20 mL
  - 17 Gastrocnemius, right: 12 mL

- 18 Gastrocnemius, left: 12 mL
- 19 Tibialis anterior, right: 12 mL
- 20 Tibialis anterior, left: 12 mL

- Each side for treatment cycle: 64 mL
- Each treatment cycle: 128 mL
- Each anatomic area for whole study:
  - Abductor pollicis brevis, right: 6 mL
  - 2 Abductor pollicis brevis, left: 6 mL
  - 3 First dorsal interosseous, right: 6 mL
  - 4 First dorsal interosseous, left: 6 mL
  - 5 Biceps, right: 24 mL
  - 6 Biceps, left: 24 mL
  - 7 Deltoid, right: 24 mL
  - 8 Deltoid, left: 24 mL
  - 9 Extensor carpi radialis, right: 6 mL
  - 10 Extensor carpi radialis, left: 6 mL
  - 11 Flexor carpi ulnaris, right: 6 mL
  - 12 Flexor carpi ulnaris, left: 6 mL
  - 13 Flexor carpi radialis, right: 6 mL
  - 14 Flexor carpi radialis, left: 6 mL
  - 15 Quadriceps, right: 60 mL
  - 16 Quadriceps, left: 60 mL
  - 17 Gastrocnemius, right: 36 mL
  - 18 Gastrocnemius, left: 36 mL
  - 19 Tibialis anterior, right: 36 mL
  - 20 Tibialis anterior, left: 36 mL
- Each side for whole study: 192 mL
- Whole study: 384 mL.

6.2.10 Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)

Subscore for Fine and Gross Motor Functions will be calculated as sum of scores for items 4 to 9; subscore for Bulbar Function will be calculated as sum of scores for items 1 to 3.

6.2.11 Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40)

The questionnaire consists of 40 questions (Q1-Q40), which are converted to 5 subscales using the following rules (according to the ALSAQ User Manual):

PHYSICAL MOBILITY = ((Q1 + Q2 + Q3 + Q4 + Q5 + Q6 +Q7 + Q8 + Q9 + Q10)/40) x 100.

ACTIVITIES OF DAILY LIVING/INDEPENDENCE = ((Q11 + Q12 + Q13 + Q14 + Q15 + Q16 + Q17 + Q18 + Q19 + Q20)/40) x 100.

EATING AND DRINKING = ((Q21+Q22+Q23)/12) x 100.

COMMUNICATION = ((Q24 + Q25 + Q26 + Q27 + Q28 + Q29 + Q30) /28) x 100.

EMOTIONAL FUNCTIONING = ((Q31 + Q32 + Q33 + Q34 + Q35 + Q36 + Q37 + Q38 + Q39 + Q40)/40) x 100.

The following approaches will be used in case of missing or incorrectly filled questions:

- Worst values will be imputed
- Average value of correctly filled questions of the subscale

If a subscale of the questionnaire will have less than 50% correctly answered questions, it should be considered as missing.

Subscale values will be presented with 1 decimal place. Number of decimal places for descriptive statistics will be presented using rules in Section 6.3.1.

6.2.12 Inexact Values

In the case where a variable is recorded as "> x", "≥ x", "< x" or "≤ x", a value of x will be taken for analysis purposes.

6.2.13 Vital Signs

Body Mass Index (BMI) will be calculated as [weight in °KG] ÷ ([height in °CM] ÷ 100)^2.

6.2.14 Unscheduled Visits

Only scheduled post-baseline values will be tabulated. Post-baseline repeated, unscheduled and early termination assessments will not be included in tables, although these post-baseline assessments will be listed in the relevant appendices to the CSR.

6.2.15 Change from baseline

Change from baseline will be calculated as difference value on post-baseline visit – value on baseline visit. Percentage changes from baseline will be calculated as 100*(value on post-baseline visit – value on baseline visit) / value on baseline visit.

6.3 Conventions

All data listings, summaries, figures and statistical analyses will be generated using SAS version 9.4 or higher[1] unless otherwise noted.

Summaries will be presented by treatment group. Treatment group labels will be displayed as follows:

Engensis (N=XX)	Placebo (N=XX)	Overall (N=XX)

Listings will be sorted in the following order: treatment group, Participant, parameter, visit or cycle unless otherwise stated. All data will be listed.

Continuous variables will be summarized by the number of non-missing observations, mean, 95% confidence interval for mean, median, standard deviation, and minimum and maximum.

Categorical variables will be summarized by presenting the frequency and percent. Percentages will be based on the number of non-missing observations or the participant population unless otherwise specified. For each variable, all categories will be shown. Zero frequencies (but not the percent) within a category will be presented.

6.3.1 Decimal Places

Decimal places for derived data described in section 6.2 will be determined by the scale of measurement unless otherwise stated. No decimal places will be displayed if the smallest calculated value is ≥ 100; 1 decimal place will be displayed when the smallest value is within the interval (10, 100), with 10 being inclusive; 2 decimal places will be displayed when the smallest value is within (1, 10), with 1 being inclusive; and so on for even smaller scales of measurement.

Derived data where it is known in advance the result will be an integer for example day, month, year, number of days and total scores (for rating scales) will be presented with zero decimal places.

Means, 95% confidence intervals for mean, medians and percentiles will be displayed to one more decimal place than the data**,** dispersion statistics (e.g. standard deviation) will have two more

decimal places, and the minimum and maximum will be displayed to the same number of decimal places as reported in the raw data. Percentages will be displayed with one decimal place.

Rounding will be carried out according to the following rules: if the digits on the right start with 0, 1, 2, 3 or 4, then the rounding digit will remain unchanged, if the digits on the right begin with 5, 6, 7, 8 or 9, then the rounding digit will be increased by 1.

6.4 Participant Disposition

The following data will be tabulated: number of screened participants, screen failures, participants in the Safety Analysis Population and cases of withdrawal/discontinuation by primary reason (Table 14.1.1.1). Participant visits will be presented in a separate table (Table 14.1.1.2).

The following data will be presented in a listing (Listing 16.2.1.1):

- Date of Informed Consent
- Original Consented Protocol Version
- Date of Discontinuation
- Primary reason for discontinuation
- Study completion (Yes/No)
- Date of last dose
- Date of last visit

6.5 Protocol Deviations

A listing of protocol deviations will be provided. The listing will include participant number, protocol deviation description and deviation comments (Listing 16.2.1.2).

6.6 Baseline Comparability

Demographics and baseline characteristics will be presented for the Safety Analysis Population.

The comparability of treatment groups with respect to participant demographics and baseline characteristics will be assessed in a descriptive manner, but no formal statistical testing will be performed.

Standard continuous or categorical variable summaries will be presented by randomized treatment group for the following variables:

- Demographic and Baseline Characteristics (Table 14.1.2.1)
    - Age

- Gender
- Child-bearing potential
- Race
- Ethnicity
- Weight
- Height
- BMI

All demographic and baseline characteristics, including also Coagulation Profile and Viral Screening will be presented in listings (Listings 16.2.4.1 - 16.2.4.4).

6.7 Medical History

Data on Medical History will be presented for the Safety Analysis Population. Medical History consists of the following parts:

- Amyotrophic Lateral Sclerosis (ALS) History
- Other Medical History
- Familial Cancer History

ALS History

ALS history consists of following data:

- ALS diagnosis, values are:
  - Clinically definite ALS
  - Clinically probable ALS
  - Clinically probable laboratory-supported ALS
- Site on Onset, values are:
  - Limb
  - Bulbar (includes respiratory)
  - Both limb and bulbar
  - Other
- Experiencing symptoms of lower motor dysfunction
  - Yes
  - No
- Experiencing upper motor neuron symptoms
  - Yes

- No

Other Medical History

Other Medical History consists of previous (Ongoing=No) and ongoing (Ongoing=Yes) conditions. Previous and ongoing conditions at screening will be presented separately. Conditions will be coded by Medical Dictionary of Regulated Activities (MedDRA, version 23.0, or later) primary system organ class (SOC) and preferred term (PT).

Familial Cancer History

Familial Cancer History consists of:

- Existence of cancer in the nearest relatives
  - Yes
  - No
- Description of cancer and relationship to a participant
  - Mother
  - Father
  - Sister
  - Brother
  - Maternal grandmother
  - Maternal grandfather
  - Paternal grandmother
  - Paternal grandfather
  - Aunt
  - Uncle
  - Other.

All data on Medical History will be presented in tables (Tables 14.1.3.1 – 14.1.3.4) and listings (Listings 16.2.4.5 – 16.2.4.7).

6.8 Prior and Concomitant Medications

Prior and concomitant medications will be presented for the Safety Analysis Population.

Prior and concomitant medications will be coded using WHO Drug dictionary version March 2020 (or later) and summarized using Anatomic Therapeutic Chemical (ATC) Level 3 and Preferred Term.

Separate tabulations will be produced for prior and concomitant medications presented by randomized treatment group. Prior medications are defined as all medications starting before the date of first dose of study drug. Concomitant medications are defined as medications taken on or after the date of first dose of study drug. Medications that started before the date of first dose of study drug and continuing after first dose of study drug will be counted in both categories.

All data on prior and concomitant medications will be presented in tables (Tables 14.3.8.1 and 14.3.8.2) and listings (Listings 16.2.4.8 and 16.2.4.9).

6.9 Exposure to Study Drug

Exposure to study drug will be presented for the Safety Analysis Population.

Number of performed injections and amount of performed injections by anatomic target area, side and total for each dosing day, each treatment cycle and for whole study will be presented by treatment group (Tables 14.3.5.1 and 14.3.5.2). Administered volume of Engensis will be presented in the same manner for Engensis group (Table 14.3.5.3).

All data on exposure will be presented in listings (Listings 16.2.10.1 and 16.2.10.2).

6.10 Treatment Compliance

Treatment compliance will be presented for the Safety Analysis Population.

Descriptive statistics for each side separately and total at each treatment day and treatment cycle as well as for whole study, as described in Section 6.2.9, will be presented by treatment group (Table 14.3.5.4).

Values of treatment compliance will be presented in listing (Listing 16.2.10.3).

6.11 Efficacy Analyses

Not applicable in this SAP.

6.12 Pharmacokinetic Analyses

Not applicable in this SAP.

6.13 Pharmacodynamic Analyses

Not applicable in this SAP.

6.14 Safety Analyses

The safety analyses will be presented by the treatment received for the Safety Analysis Population.

6.14.1 Adverse Events

All study-related Adverse Events (AEs) will be collected starting after completion of the informed consent process at Screening to Day 0/randomization. Treatment-Emergent Adverse Events (TEAEs) and Treatment-Emergent Serious Adverse Events (TESAEs) will be collected after first injections on Day 0 through Day 180/End of Treatment or the last Study Visit up until the time that the Participant withdraws consent. All Serious Adverse Events (SAEs) will be collected from the completion of the informed consent process until Study Day 180 or the last Study Visit up until the time that the Participant withdraws consent. Any SAE occurring after consent and before the first injection on Day 0 should be recorded and reported only if associated with a protocol-specified procedure. Any AEs reported during Screening after completion of informed consent will be collected and recorded separately from TEAEs reported on Day 0 and through the end of the study.

Medical occurrences that begin before the start of study intervention and not related to required study procedures, but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the eCRF and not as AEs.

Adverse events with relationship equal to "Possibly related", "Probably related" and "Definitely related" will be considered as adverse events related to study medication. If an AE has missing relationship it is assumed to be related to the study drug for analysis purposes.

Adverse events with fatal outcome will be considered as fatal events. Maximum severity will be assumed for an AE with missing severity.

AEs will be coded using Medical Dictionary of Regulated Activities (MedDRA, version 23.0, or later) by System Organ Class (SOC) and preferred term (PT).

Adverse Event (AE) Definition

- An AE is any untoward medical occurrence in a patient or clinical study Participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.

- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Treatment-Emergent Adverse Event (TEAE) Definition

- An event that emerges during treatment, having been absent at pretreatment, or worsens relative to the pretreatment state, as defined in the E9 Guidance. AE will be considered as TEAE if it emerged or worsened on the day of first dosing or later to the end of study.

- The TEAE is not related to causality / drug relatedness. It may or may not be related to the drug but is considered a TEAE due to its appearance at or after the treatment has been administered.

Serious Adverse Event (SAE) Definition

An SAE is defined as any AE that, at any dose:

a Results in death

b Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the Participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the Participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

- Hospitalization for elective treatment of a pre-existing condition that did not worsen from

d Results in persistent disability/incapacity

- The term "disability" means a substantial disruption of a person's ability to conduct normal life functions.

- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle), which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e Is a congenital anomaly/birth defect

f Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the Participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers; intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Treatment-Emergent Serious Adverse Event (TESAE)

- A serious event that emerges during treatment, having been absent at pretreatment, or worsens relative to the pretreatment state, as defined in the EP Guidance.
- The TESAE is not related to causality / drug relatedness. It may or may not be related to the drug but is considered a TESAE due to its appearance at or after the treatment has been administered.

Adverse Events of Special Interests (AESI)

There are 4 main categories of AESIs that will be considered in the study:

1. Related to the angiogenesis potential of Engensis
   - Atherosclerosis
   - Cancer
2. Other medical problems in this patient population
   - Pulmonary Medical Problems
   - Progressive Muscle Weakness
   - Bulbar Disease
3. Injection site reactions
4. COVID-19 infections

The following tables will be presented:

- Summary of TEAE (Table 14.3.1.1)

- TEAEs organized by SOC and PT (Table 14.3.1.2)
- TESAEs organized by SOC and PT (Table 14.3.1.3)
- TEAEs organized by SOC and PT and Maximum Severity (Table 14.3.1.4)
- TEAEs organized by SOC and PT and Maximum Relationship (Table 14.3.1.5)
- TESAEs organized by SOC and PT and Maximum Severity (Table 14.3.1.6)
- TESAEs organized by SOC and PT and Maximum Relationship (Table 14.3.1.7)
- Incidence Injection site reactions by SOC and PT (Table 14.3.1.8)
- Incidence of clinically significant laboratory values (Table 14.3.1.9)
- AESIs organized by SOC and (Table 14.3.1.10)
- TEAEs leading to study discontinuation by SOC and PT (Table 14.3.1.11)
- TESAEs leading to study discontinuation by SOC and PT (Table 14.3.1.12)
- TEAEs leading to study drug withdrawal by SOC and PT (Table 14.3.1.13)
- TESAEs leading to study drug withdrawal by SOC and PT (Table 14.3.1.14)
- TEAEs related to study medication by SOC and PT (Table 14.3.1.15)
- TESAEs related to study medication by SOC and PT (Table 14.3.1.16)

The following listings will be provided:

- All AEs (Listing 16.2.7.1)
- AESIs (Listing 16.2.7.2)
- TESAEs (Listing 16.2.7.3)
- Injection Site Reactions (Listing 16.2.7.4)
- TEAEs leading to study discontinuation (Listing 16.2.7.5)
- TEAEs related to study medication (Listing 16.2.7.6)
- TESAEs leading to death (Listing 16.2.7.7)

6.14.2 Laboratory Data

Laboratory data consists of results of following examinations: serum chemistry, hematology, coagulations and urine pregnancy test.

Serum chemistry will include the following variables:

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Calcium
- Inorganic Phosphate
- Magnesium
- Glucose
- Amylase
- Lipase
- Lactate Dehydrogenase
- Blood Urea Nitrogen (BUN)
- Creatine Kinase (CK)
- Creatinine
- Aspartate Aminotransferase (AST)
- Alanine Aminotransferase (ALT)
- Alkaline Phosphatase
- Gamma-Glutamyl Transpeptidase (GGT)
- Total Bilirubin
- Total Protein
- Albumin

Hematology will include the following variables:

- White Blood Cells Count
- Hemoglobin
- Hematocrit
- Platelet Count
- Neutrophils Count

Serum chemistry and hematology analyses will be performed at Screening, Day 60 pre-dose, Day 120 pre-dose and End of Study.

Measured values of serum chemistry and hematology analyses and their changes from baseline, will be presented with descriptive statistics at each visit by treatment group (Tables 14.3.4.1 and 14.3.4.3). Incidence of clinically significant values and shift tables by the clinical significance based on laboratory assessments will be presented for each parameter (Tables 14.3.1.9, 14.3.4.2, 14.3.4.4). Percentages for each parameter will be based on the number of participants who have at least one measurement for at baseline and at corresponding post-baseline visit.

Result of urine pregnancy test is variable with two possible values: positive / negative. The test should be performed only for women with childbearing potential at Screening, Day 0 pre-dose and End of Study. Urinalysis will be presented only in a listing.

Results of serum chemistry, hematology and urine pregnancy tests will be listed (Listings 16.2.8.1 – 16.2.8.3).

6.14.3 Vital Signs

The following vital signs will be measured:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Pulse rate (bpm)
- Respiration rate (breath / min)
- Body temperature (degrees Celsius)
- Body weight (kg)
- Oxygen Saturation (%)
- Weight (kg)
- Height (cm)

Descriptive statistics for observed values and changes from baseline for vital signs, including weight, height (measured at screening only) and BMI will be presented by treatment group and visit (Table 14.3.6.1). Also frequency table by the clinical significance will be presented for each vital sign (Table 14.3.6.2).

All results of vital signs measurements will be listed (Listing 16.2.8.4).

6.14.4 Electrocardiogram Data

Electrocardiogram will be performed at Screening, Day 84 and End of Study. Descriptive statistics for electrocardiogram results: general interpretation, abnormal findings and clinical significance will be presented by treatment groups (Tables 14.3.7.1 and 14.3.7.2).

All electrocardiogram results will be listed (Listing 16.2.8.5).

6.14.5 Physical Examination

Complete physical examination will be performed at Screening, Day 0 pre-dose, Day 14 pre-dose, Day 30, Day 60 pre-dose, Day 74 pre-dose, Day 84, Day 120 pre-dose, Day 134 pre-dose, Day 144 and End of Study.

The following body systems will be examined:

- HEENT (Head, Eye, Ear, Nose, Throat)
- Heart
- Lungs
- Abdomen
- Extremities
- Lymph Nodes
- Neurological
- Musculoskeletal System
- Skin/Integumentary Systems

Each parameter will be presented with frequencies and percentages of values (Normal, Abnormal not Clinically Significant (NCS), Abnormal Clinically Significant (CS)) in shift tables based on baseline for each treatment group (Table 14.3.6.3). Percentages will be based on the number of participants who have measurement at baseline and at corresponding post-baseline visit.

A listing of physical examination results will be presented (Listing 16.2.8.6).

6.15 Exploratory Analyses

Exploratory analyses will be performed in the ITT population.

6.15.1 Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)

ALSFRS-R will be measured at Screening, Day 0 pre-dose, Day 30, Day 60 pre-dose, Day 84, Day 120 pre-dose, Day 144 and Day 180.

Total score and subscores for Fine and Gross Motor Functions and for Bulbar Function and their changes from baseline will be presented with descriptive statistics by visits and treatment group (Table 14.6.1, Listing 16.2.9.1).

Slope of the total score will be provided using graphical presentation of values by visit for both treatment group.

6.15.2 Handheld Dynamometry (HHD)

HHD will be measured at Day 0 pre-dose, Day 30, Day 60 pre-dose, Day 84, Day 120 pre-dose, Day 144 and Day 180.

Maximal values of strength of each muscle group of 3 trials and their changes from baseline will be presented with descriptive statistics for each visit by treatment group (Table 14.6.2, Listing 16.2.9.2).

6.15.3 Accurate test of limb isometric strength (ATLIS)

ATLIS will be measured (only at sites that have the ATLIS equipment) at Day 0 pre-dose, Day 30, Day 60 pre-dose, Day 84, Day 120 pre-dose, Day 144 and Day 180.

Values of muscle strength of each muscle group and their changes from baseline will be presented with descriptive statistics for each visit by treatment group (Table 14.6.3, Listing 16.2.9.3).

6.15.4 Amyotrophic Lateral Sclerosis Assessment Questionnaire, 40 questions (ALSAQ-40)

ALSAQ-40 will be measured at Day 0 pre-dose, Day 84 and Day 180.

Values of each question and their changes from baseline will be presented with descriptive statistics for each visit by treatment group (Table 14.6.4.1 and 14.6.4.2, Listing 16.2.9.4).

The subscales (physical mobility, activities of daily living/independence, eating and drinking, communication, and emotional functioning) will be presented with descriptive statistics for values on each visit and changes from baseline for each post-baseline visit (Tables 14.6.4.3 and 14.6.4.4).

6.15.5 Clinical Global Impression of Change (CGIC) and Patient Global Impression of Change (PGIC)

CGIC and PGIC will be measured at Day 84 and Day 180.

CGIC (Global improvement and Efficacy index) and PGIC (Impression of change and Degree of Change) will be presented with descriptive statistics for each visit by treatment group (Tables 14.6.5 and 14.6.6, Listings 16.2.9.5 and 16.2.9.6).

6.15.6 Slow vital capacity (SVC)

SVC will be measured at Screening, Day 0 pre-dose, Day 30, Day 60 pre-dose, Day 84, Day 120 pre-dose, Day 144 and Day 180.

Best vital capacity, second best vital capacity and third best vital capacity will be presented with descriptive statistics by visits and treatment group (Table 14.6.7). Also this data and other data collected (% predicted, number of trials and using face mask) will be listed (Listing 16.2.9.7).

6.15.7 Time to tracheostomy

Time to tracheostomy will be presented with descriptive statistics and corresponding survival curves using Kaplan- Meier analysis (Table 14.6.8, Listing 16.2.9.8). The questionnaire is planned to be filled at Screening, Day 0 pre-dose, Day 30, Day 60 pre-dose, Day 84, Day 120 pre-dose, Day 144 and Day 180.

6.15.8 Time to all-cause mortality

Time to all-cause mortality will be presented with descriptive statistics and corresponding survival curves using Kaplan-Meier analysis (Table 14.6.9, Listing 16.2.9.8).

6.15.9 Muscle Atrophy

Information about collected muscle biopsy will be presented in a listing (Listing 16.2.9.9).

7 INTERIM ANALYSIS

No interim analyses are planned.

8 DATA SAFETY MONITORING BOARD ANALYSIS

Data safety monitoring board (DSMB) analyses are described in DSMB Statistical Analysis Plan.

9 CHANGES TO PLANNED PROTOCOL ANALYSIS

There are no changes to the planned protocol analysis.

10 REFERENCES

1. SAS Institute Inc., Cary, NC, 27513, USA
2. ALSAQ User Manual
3. ICH Guidance E9: Statistical Principles for Clinical Trials
4. Willeke A. van den Beld, MD, MSc, Gitty A. C. van der Sanden, MSc, Rob C. A. Sengers, MD, PHD, Andre´ L. M. Verbeek, MD, PHD and Fons J. M. Gabreels (2006) "Validity and Reproducibility of Hand-Held Dynamometry in Children Aged 4-11 Years"; J Rehabil Med, 38: pp.57-64
5. Jeremy M. Shefner, PhD, Dawei Liu, PhD, Melanie L. Leitner, PhD, David Schoenfeld, PhD, Donald R. Johns, MD, PHD Toby Ferguson, MD, PhD, Merit Cudkowicz, MD, MSc (2016) "Quantitative Strength Testing In ALS Clinical Trials", Neurology 87 august 9

11 LIST OF TABLES, FIGURES AND LISTINGS

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