HELIXMITH

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

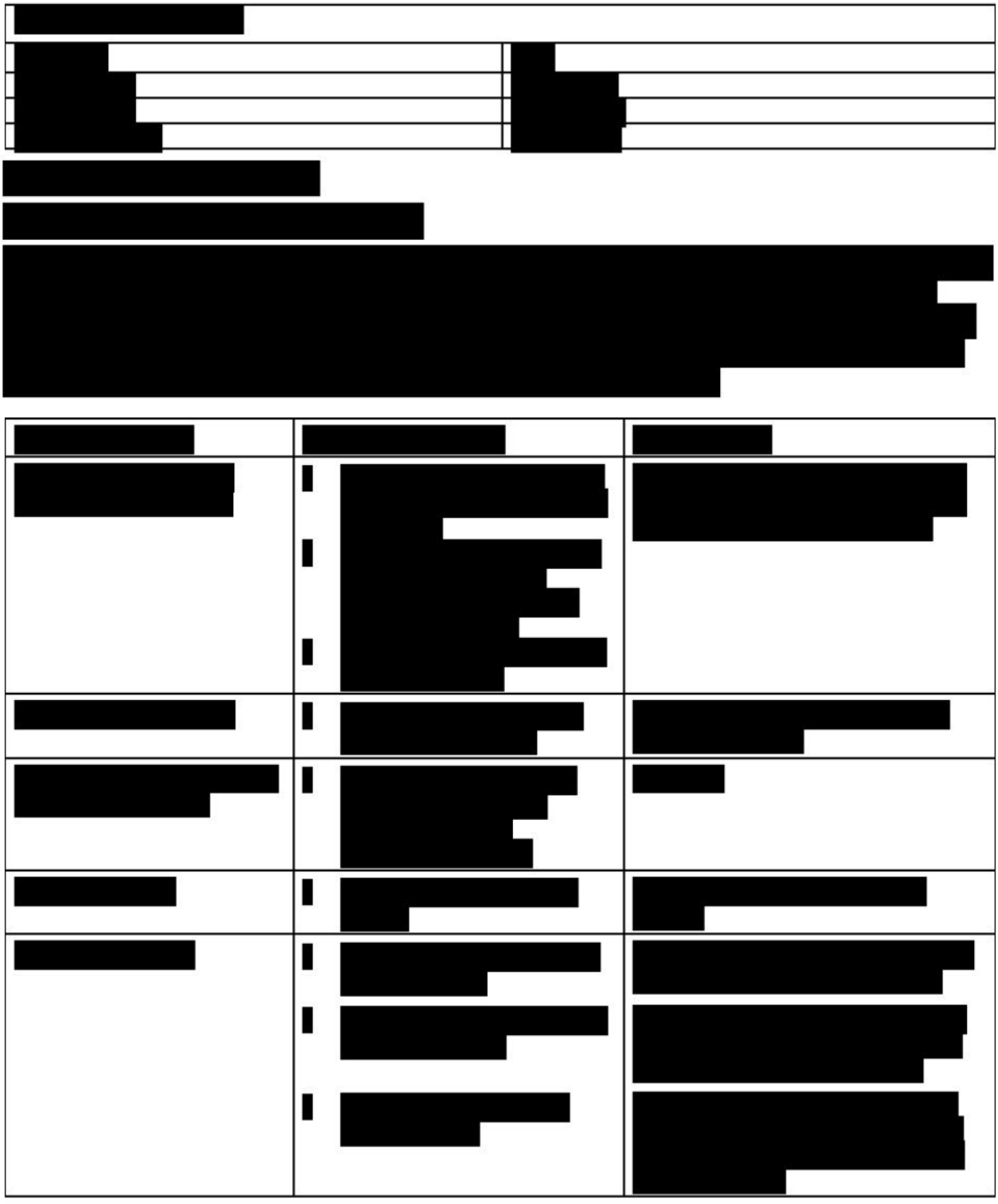
SIGNATURE PAGE

I, the undersigned, confirm that I have read and agree with the contents of this document.

Sponsor Signatory:

[redacted]

Protocol Amendment Summary of Changes Table



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1. PROTOCOL SUMMARY

The following sections present an overview of the study, study schema, and Schedule of Activities (SoA). A list of abbreviations is presented in Section 10.4, Appendix 4.

1.1. Synopsis

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

Short Title: Phase 2a Study to Assess the Safety of Engensis in Amyotrophic Lateral Sclerosis

Rationale:

The purpose of this study is to evaluate the safety of intramuscular (IM) administration of Engensis in Participants with Amyotrophic Lateral Sclerosis (ALS) as compared to Placebo. Safety will be assessed by incidences of treatment-emergent adverse events (TEAEs), treatment-emergent serious adverse events (TESAEs), injection site reactions (ISRs) and other adverse events of special interest (AESIs), and the clinically significant laboratory values after injections for Engensis compared to Placebo. Exploratory endpoints include assessment of muscle function using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) and ALSFRS-R subscores for Fine and Gross Motor Function; muscle strength by quantitative testing using Handheld Dynamometry (HHD) and the Accurate Test of Limb Isometric Strength (ATLIS) where available; quality of life using the ALS Assessment Questionnaire (ALSAQ-40); patient global impression of change (PGIC), and clinical global impression of change (CGIC); and evaluation of lung function using Slow Vital Capacity (SVC). Muscle biopsies will be performed during the study for future biomarker analyses.

Engensis contains the active pharmaceutical ingredient VM202, a novel genomic complementary deoxyribonucleic acid (cDNA) hybrid human hepatocyte growth factor (HGF) coding sequence (HGF-X7) expressing two isoforms of HGF, HGF_{728} and HGF_{723}, being developed for treatment of ALS.

Data from Helixmith's first clinical trial in ALS, the Phase 1/2 ALS study (VMALS-001), which included 18 Participants who were followed for 9 months, suggested that targeted delivery of HGF to motor neurons via intramuscular (IM) injections of Engensis was safe and well-tolerated. Safety assessments during the study included reports of 79 adverse events (AEs) in 17 Participants (94.4%), including 26 mild injection site reactions (Grade 1). Five SAEs were reported in 3 Participants. Only injection site reactions were considered related to study treatment. Following injections until Day 90, a plateau or a relative slowing in decline of the ALSFRS-R scores and muscle strength was noted, suggesting a slowing of disease progression. After 90 days, the plateau was no longer observed in the ALSFRS-R, with the notable exception of a trend toward better preservation of bulbar and breathing functions as measured by the ALSFRS-R.

Participants randomly assigned to Engensis will receive a total dose of 192 mg VM202 divided into 3 Treatment Cycles of 64 mg VM202 each. In this study, the per-injection amount (0.25 mg) of Engensis will be identical to that used in the Phase 1/2 ALS study and was also used in three diabetic peripheral neuropathy (DPN) studies, but the dosing schedule will differ. IM injections of Engensis or Placebo to muscles in hands, upper and lower arms, and legs will be divided into two Injection Visits scheduled two weeks apart during three Treatment Cycles: on Days 0 and 14 (Treatment Cycle 1), with retreatment on Days 60 and 74 (Treatment Cycle 2), and Days 120 and 134 (Treatment Cycle 3).

Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety of intramuscular (IM) injections of Engensis in Participants with Amyotrophic Lateral Sclerosis (ALS) compared to Placebo	• 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
Exploratory	
• To evaluate changes in muscle function following Engensis injections in ALS Participants compared to Placebo	• 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
• To evaluate muscle strength changes following Engensis injections in ALS Participants 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 180 for Engensis compared to Placebo

Objectives	Endpoints
	• 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
• To evaluate Quality of Life improvement following Engensis injections in ALS Participants 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
• To evaluate Patient and Clinical Reported Outcome improvement following Engensis injections in ALS Participants 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
• To determine whether IM administration of Engensis has effects on respiratory function in ALS Participants 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
• To determine whether IM administration of Engensis has positive effects on survival in ALS Participants compared to Placebo	• Time to all-cause mortality by Day 180 for Engensis compared to Placebo
• To determine whether IM administration of Engensis has positive effects on muscle atrophy in ALS Participants 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

Overall Design

VMALS-002-02 is a Phase 2a, double-blind, randomized, placebo-controlled, multicenter study designed to assess the safety of Engensis (containing the active pharmaceutical ingredient VM202) in Participants with ALS. Following the completion of the informed consent process, Screening activities (within 30 days prior to Day 0) will determine which Participants meet the eligibility criteria and who will be enrolled and randomly assigned in a double-blind fashion in a 2:1 ratio to either Engensis or Placebo, respectively.

During Screening, medical history, familial cancer history, demographics, vital signs, weight and height, complete physical examination, the ALSFRS-R, slow vital capacity (SVC), 12-lead electrocardiogram (ECG), laboratory assessments (coagulation profile, serum chemistry, and hematology), viral screening, a record of all concomitant medications and procedures, and urine pregnancy test for females of childbearing potential will be conducted. Adverse event (AE) assessment will start upon completion of the consent process at the start of Screening and treatment-emergent adverse event (TEAE) assessment, including injection site reactions, will start after the first injection of Engensis or Placebo (Day 0) and continue throughout the study.

Following randomization and prior to the first IM injections of Engensis or Placebo on Day 0, vital signs and weight, a complete physical examination, urine pregnancy test, SVC, concomitant medications and procedures, measurement of muscle strength by HHD and ATLIS (at clinical study sites where available), ALSFRS-R, muscle biopsy, and the quality of life assessment with the ALSAQ-40 will be completed.

All Participants will receive 0.5-mL (0.25 mg) IM injections (2 to 20 injections per muscle, depending on the anatomical location of the muscle) of Engensis or Placebo in target muscles at each of two dosing Visits during 3 Treatment Cycles: Treatment Cycle 1 on Days 0 and 14, Treatment Cycle 2 on Days 60 and 74, and Treatment Cycle 3 on Days 120 and 134. Vital sign measurements will be performed at 2 hours after completion of IM injections of Engensis or Placebo on Days 0, 14, 60, 74, 120, and 134.

Vital signs will be recorded at all Study Visits. On Day 0, a blood sample will be collected for future genetic testing. Muscle biopsies will be collected on Day 0, 84 (optional), and 144.

All Participants will receive 256 IM injections per Treatment Cycle (128 injections on each of 2 dosing days) as a divided dose to multiple injection sites in each muscle identified in the upper extremities (hands: first dorsal interosseous, abductor pollicis brevis; arms: biceps, deltoid, extensor carpi radialis, flexor carpi ulnaris, and flexor carpi radialis) and lower extremities (legs: quadriceps, gastrocnemius, and tibialis anterior). Each injection contains 0.25 mg Engensis/0.5 mL or 0.5 mL of Placebo. Participants assigned to Engensis will receive a total dose of 192 mg (3 cycles at 64 mg each) during the study.

Follow-up Study Visits will be conducted on Days 30, 84, 144, and 180 or early termination (ET). At the Day 180/ET Visit, the following assessments will be conducted: vital signs and weight, complete physical examination, 12-lead ECG, urine pregnancy test, ALSFRS-R, SVC, HHD and ATLIS (at sites where available), ALSAQ-40, PGIC, CGIC, concomitant medications, and TEAEs. Blood will be drawn for serum chemistry and hematology.

Study and Treatment Duration:

Screening will occur up to 30 days prior to Baseline (Day 0) and Participants will be followed from Day 0, the day of first Study Injections, to Day 180/ET.

Visit Frequency:

Consented Participants will be seen and evaluated for enrollment during Screening (up to 30 days prior to Baseline, Day 0). There are 10 Visits during the study from Day 0 to Day 180 for Study Injections and follow-up.

Intervention Groups and Duration:

Two treatment groups of Participants (Engensis or Placebo) will be in the study for 180 days.

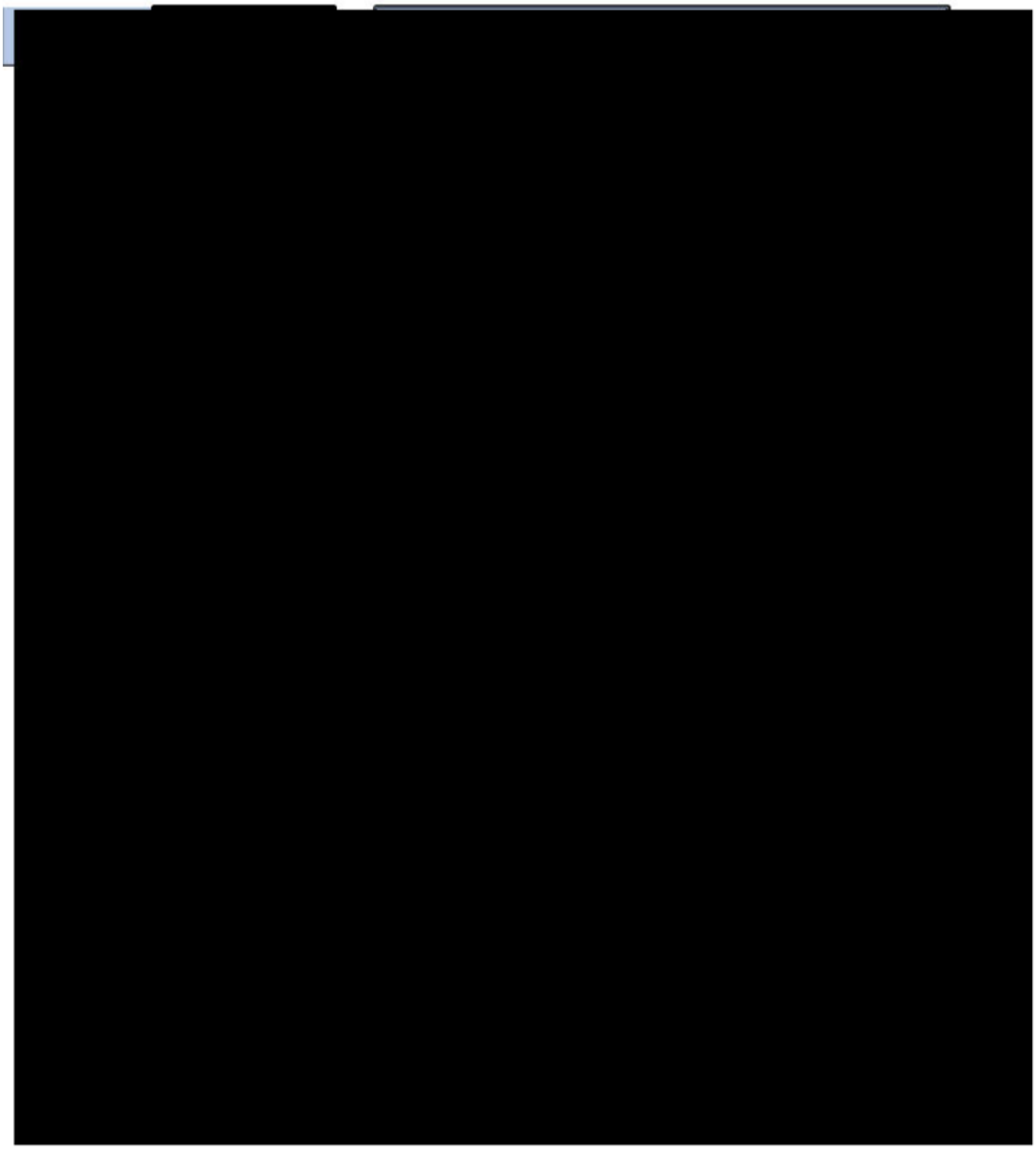
Number of Participants (N = 18):

The target sample size is 18 Participants who will be randomized in a 2:1 ratio to Engensis or Placebo (12:6).

Data Safety Monitoring Board:

The independent Data Safety Monitoring Board (DSMB) will periodically review, first on a limited set of noncomparative (blinded) tables and/or listings of all data and, if there is a concern, a comparative (unblinded) analysis will be conducted, including review of all reported TEAEs and TESAEs. The DSMB may perform multiple noncomparative analyses for safety. Comparative information will not be shared with the Sponsor or the Project Steering Committee, except in the event that the DSMB recommends a temporary suspension or termination of the study.

1.2. Study Schema



1.3. Schedule of Activities (SoA)

		Screen-ing	Treatment Cycle 1					Treatment Cycle 2					Treatment Cycle 3					End of Study/ ET
			1st Injections		2nd Injections			3rd Injections		4th Injections			5th Injections		6th Injections			
Study Day:		Days -30 to -1	Day 0[a]		Day 14[b]		Day 30	Day 60		Day 74[b]		Day 84	Day 120		Day 134[b]		Day 144	Day 180
Visit Window (days):					± 1		± 3	± 3		± 1		± 3	± 3		± 1		± 3	± 7
Procedure			pre-dose	post-dose	pre-dose	post-dose		pre-dose	post-dose	pre-dose	post-dose		pre-dose	post-dose	pre-dose	post-dose		
1	Informed Consent	X																
2	Demographics, Baseline Characteristics	X																
3	Medical History and Familial Cancer History	X																
4	Vital Signs, Weight, Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
5	Complete Physical Examination	X	X		X		X	X		X		X	X		X		X	X
6	12-Lead ECG	X										X						X
7	Coagulation Profile	X																
8	Serum Chemistry, Hematology	X						X					X					X
9	Blood Sample for Future Genetic Testing		X															
10	Viral Screening	X																
11	Urine Pregnancy Test	X	X															X
12	SVC	X	X				X	X				X	X				X	X
13	Concomitant Medications and Procedures	X	X		X		X	X		X		X	X		X		X	X

	Procedure	Screening Days -30 to -1	Treatment Cycle 1 1st Injections Day 0[a]		Treatment Cycle 1 2nd Injections Day 14[b]		Day 30	Treatment Cycle 2 3rd Injections Day 60		Treatment Cycle 2 4th Injections Day 74[b]		Day 84	Treatment Cycle 3 5th Injections Day 120		Treatment Cycle 3 6th Injections Day 134[b]		Day 144	End of Study/ ET Day 180
	Study Day:																	
	Visit Window (days):				± 1		± 3	± 3		± 1		± 3	± 3		± 1		± 3	± 7
	Procedure		pre-dose	post-dose	pre-dose	post-dose		pre-dose	post-dose	pre-dose	post-dose		pre-dose	post-dose	pre-dose	post-dose		
14	Randomization		X															
15	Study Injections		X		X			X		X			X		X			
16	ALSFRS-R	X	X				X	X				X	X				X	X
17	HHD		X				X	X				X	X				X	X
18	ATLIS		X				X	X				X	X				X	X
19	ALSAQ-40		X									X						X
20	PGIC and CGIC											X						X
21	Muscle Biopsy[c]		X									X[d]					X	
22	AEs, TEAEs, SAEs, TESAEs	X (AEs)	←						TEAEs									→
23	AESIs		←															→
24	AESI: Injection Site Reactions			X		X			X		X			X		X		

FOOTNOTES FOR THE SCHEDULE OF ACTIVITIES

Note: The **Schedule of Activities** includes activities during Screening and all Study Visits.

Order of Assessments during Visits (if assessment is to be performed): AEs/TEAEs, vital signs and weight, ALSFRS-R, HHD, ATLIS, pregnancy test, complete physical exam, SVC, ALSAQ-40, other questionnaires (PGIC and CGIC), blood sample collection for laboratory assessments, and muscle biopsy

[a] Day 0 is the Baseline Visit

[b] The Day 14 Visit will be scheduled 13-15 days after the Day 0 Visit, the Day 74 Visit will be scheduled 13-15 days after the Day 60 Visit, and the Day 134 Visit will be scheduled 13-15 days after the Day 120 Visit

[c] Muscle biopsies will be collected as the last procedure of the Visit. It is collected predose on Day 0.

[d] Day 84 muscle biopsy is optional.

Abbreviations: AEs = adverse events; AESI = adverse events of special interest; ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; ALSAQ-40 = Amyotrophic Lateral Sclerosis Assessment Questionnaire, 40 questions; ATLIS = accurate test of limb isometric strength; CGIC = Clinical Global Impression of Change; D = day; ET = early termination or withdrawal; HHD = Handheld Dynamometry; PGIC = Patient Global Impression of Change; SVC = Slow Vital Capacity; TEAEs = treatment-emergent adverse events; TESAEs = treatment-emergent serious adverse events

1. **Informed Consent** process is to be completed at Screening prior to conducting any study-specific procedures.
2. **Demographics and Baseline Characteristics** will include age, gender, race, and ethnicity and will be collected at Screening.
3. **Medical History and Familial Cancer History**: A medical history and familial cancer history will be obtained at Screening. The Investigator will confirm the diagnosis of ALS using the revised El Escorial / Airlie House diagnostic criteria. Participants will be confirmed as having clinically definite ALS, clinically probable ALS, or clinically probable laboratory-supported ALS ≤4 years.
4. **Vital Signs, Weight, and Height:** Vital signs will be measured at Screening, predose, and 2 hours (± 1 hour) post-dose at Study Injection Visits during the Treatment Cycle 1 (Days 0 and 14), Treatment Cycle 2 (Days 60 and 74), and Treatment Cycle 3 (Days 120 and 134). Vital signs will also be measured at Study Visits on Days 30, 84, 144, and 180/ET (Day 180 or Early Termination). After the Participant has rested in the seated position for 5 minutes, vital signs will be collected, including temperature, sitting blood pressure, heart rate, respiratory rate, and oxygen saturation on room air. The method of temperature measurement should be according to the Site's policy and should be consistently applied. Weight will be collected at Screening and predose at Study Injection Visits (Days 0, 14, 60, 74, 120, and 134); height will only be measured at Screening.
5. **Complete Physical Examination** will be performed at Screening, predose on Days 0, 14, 60, 74, 120, and 134, and at Study Visits on Days 30, 84, 144, and 180/ET. The examination will include the following: head, eyes, ears, nose, and throat (HEENT); heart; lungs; abdomen; extremities; lymph nodes; neurological; musculoskeletal system; and skin/integumentary systems. Any abnormalities should be categorized as clinically significant (CS) or not clinically significant (NCS), and the abnormalities should be recorded as adverse events, except at Screening.
6. **12-Lead Electrocardiogram (ECG)** will be performed at Screening, Day 84, and Day 180/ET. Any clinically significant findings at Screening will be recorded as part of the medical history. Clinically significant abnormalities are to be recorded as adverse events, except at Screening. The ECG recording must be stored with the Participant's records.
7. **Coagulation Profile** will be evaluated at Screening and will include prothrombin time (PT), partial prothrombin time (PTT), and International Normalized Ratio (INR).

8. **Serum Chemistry and Hematology:** Laboratory assessments will be evaluated at Screening (to establish eligibility) and on Days 60, 120, and 180/ET.

   Chemistry includes sodium, potassium, chloride, bicarbonate, calcium, inorganic phosphate, magnesium, glucose, amylase, lipase, creatine kinase, lactate dehydrogenase, and:

   a. Kidney function tests (blood urea nitrogen, creatinine)

   b. Liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, gamma-glutamyl transpeptidase [GGT], total bilirubin, total protein, and albumin)

   Hematology includes platelet count, hemoglobin, hematocrit, white blood cell count, and neutrophil count

9. **Blood Sample for Future Genetic Testing**: A blood sample will be collected at predose on Day 0 and stored.

10. **Viral Screening** is to be conducted at Screening and includes tests for human immunodeficiency virus (HIV), human T cell lymphotropic virus (HTLV) I/II antibody with reflex confirmatory assay, hepatitis B virus (HBV: IgM HBcAb, HBsAb, and HBsAg, and a quantitative HBV if needed clinically), and anti-hepatitis C virus (HCV) antibodies and a positive qualitative PCR if needed clinically. Testing will be performed at a local laboratory. All tests for HIV, HTLV, HBV, and HCV must be completed and negative prior to randomization.

11. **Urine Pregnancy Test:** Women of childbearing potential must have a negative urine pregnancy test at Screening, predose at Study Injection Visit on Day 0 and Day180/ET.

12. **Slow Vital Capacity (SVC)** will be measured at Screening, predose on Days 0, 60, and 120, and at Study Visits on Days 30, 84, 144, and 180/ET.

13. **Concomitant Medications and Procedures:** Record all medications or vaccines, including over-the-counter or prescription medicines, vitamins, herbal supplements, and procedures that the Participant was receiving during the 30 days prior to completion of the informed consent process at Screening, predose at Study Injection Visits on Days 0, 14, 60, 74, 120, and 134, and at Study Visits on Days 30, 84, 144, and 180/ET. For each medication, treatment, or procedure, the following information will be collected: Medication trade or generic name, type of procedure, indication, start date, stop date or ongoing, dose, units, frequency, and route.

14. **Randomization:** Completion of Screening activities, randomization, and first Study Injections (Treatment Cycle 1, Day 0) will occur on the same day (Day 0).

15. **Study Injections:** IM injections of Engensis or Placebo will be administered during Treatment Cycle 1 (1st and 2nd Study Injections on Days 0 and 14), Treatment Cycle 2 (3rd and 4th Study Injections on Days 60 and 74), and Treatment Cycle 3 (5th and 6th Study Injections on Days 120 and 134).

16. **Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)** will be performed at the Clinical Site at Screening, predose on the Days 0, 60, and 120, and on Days 30, 84, 144, and 180/ET.

17. **Handheld Dynamometry (HHD)** will be measured at the Clinical Site predose on Days 0, 60, and 120, and at Visits on Days 30, 84, 144, and 180/ET.

18. **ATLIS** will be measured (only at sites that have the ATLIS equipment) predose on Days 0, 60, and 120, and at Visits on Days 30, 84, 144, and 180/ET.

19. **Amyotrophic Lateral Sclerosis Assessment Questionnaire, 40 questions (ALSAQ-40)** will be measured predose on Day 0 and at Visits on Days 84 and 180/ET.

20. **Patient Global Impression of Change (PGIC) and Clinical Global Impression of Change (CGIC)** will be conducted at Visits on Days 84 and 180/ET.

21. **Muscle Biopsy**: Biopsies of the gastrocnemius muscle around the injection sites will be collected and stored at Day 0 predose, and Days 84 (optional) and 144 (approximately10 days after the second day of injections of Treatment Cycles 2 and 3).

22. **Adverse Events (AEs), Treatment-Emergent Adverse Events (TEAEs), Serious Adverse Events (SAEs) and Treatment-Emergent Serious Adverse Events (TESAEs):** AEs will be recorded after informed consent until the first injections on Day 0. After the start of the 1st Injections on Day 0 through Day 180, AEs will be recorded as treatment-emergent adverse events (TEAEs). Treatment-emergent serious adverse events (TESAEs) occurring after randomization and throughout the study will be recorded. SAEs occurring after the completion of the consent process and before the 1st Injections on Day 0 should be recorded and reported as SAEs only if associated with a protocol-specified procedure. All SAEs, and TESAEs occurring after the time of the 1st Injections through the last Study Visit on Day 180, should be recorded and reported to the Sponsor/designee within 24 hours of awareness of the SAE/TESAE by the Site.
23. **Adverse Events of Special Interest (AESIs):** All AESIs will be continuously monitored throughout the study.
24. **AESI: Injection Site Reactions (ISRs)** must be initially assessed immediately after all injections at an Injection Visit. The Investigator or designee will follow up with the Participant within 2 to 3 days after Study Injections to ask the Participant about reactions at any injection sites and, if any are noted, the worst reaction will be recorded as a TEAE and considered an AESI.

2. INTRODUCTION

Engensis is a novel gene therapy being developed for treatment of ALS. The active pharmaceutical ingredient of Engensis is VM202 (donaperminogene seltoplasmid), a plasmid deoxyribonucleic acid (DNA) designed as a gene transfer method to simultaneously express two isoforms of human hepatocyte growth factor (HGF), HGF_{728} and HGF_{723}, that are identical to wild-type human forms.

Hepatocyte growth factor is a potent angiogenic and vasculogenic growth factor stimulating the growth of endothelial cells and migration of vascular smooth muscle cells. As a multifunctional mesenchyme-derived cytokine, it has potent angiogenic and anti-apoptotic effects, including that of the lymphatic system. In addition, HGF upregulates the expression of vascular endothelial growth factor (VEGF) and other factors and demonstrates greater mitogenic activity than that of VEGF alone in human aortic endothelial cells in vitro. HGF is also important in the pathophysiology of insulin resistance, as a neurotrophic factor promoting axonal growth and regeneration in diabetics in whom loss of microvasculature may accelerate neuronal loss, more so than exogenous VEGF (see the Investigator's Brochure [IB] for more detailed information).

In this study Engensis will be administered by IM injection, which has distinct advantages over alternative delivery modes of HGF or Engensis. Intravenous infusion is impeded by rapid clearance of HGF by the liver and of Engensis by DNases, resulting in low tissue levels. The VM202 plasmid DNA expresses HGF in situ, allowing local targeted delivery. Plasmid DNA also has a lowered risk of genomic integration and of generating immune responses compared with viral delivery systems. Importantly, certain components of Engensis, including its unique plasmid vector pCK and promoters and controllers of gene transcription that make the expression from skeletal muscle more efficient (see the IB for full description), lead to expression of HGF that is detectable for up to 30 days after direct skeletal muscle injection.

2.1. Study Rationale

VMALS-002-2 is a well-controlled study to evaluate the safety of IM administration of Engensis to Participants with ALS, as compared with Placebo. Participants randomly assigned to Engensis will receive a total dose of 192 mg VM202 divided into 3 Treatment Cycles of 64 mg VM202 each. A dosing schedule developed from those used in the Phase 1/2 ALS study and in three diabetic peripheral neuropathy (DPN) studies will be used in this study. The IM injections to each target muscle group will be divided into three Injection Visits (equal halves), two weeks apart on Days 0 and 14, with retreatments at Days 60 and 74, and Days 120 and 134. Engensis will be delivered in a solution of 0.5 mg VM202/mL. See Section 4.3 for detailed information supporting the selection of doses and dosing regimen for this study.

Results from a Phase 1/2 ALS study suggest that targeted delivery of HGF to motor neurons via intramuscular injections of Engensis was safe and well tolerated, based on data from 18 enrolled Participants who were followed through 90 days after the first injection.[1] Following injections until Day 90, a plateau or a relative slowing in decline of the ALSFRS-R scores and muscle strength was noted, suggesting a slowing of disease progression. After 90 days, the plateau was no longer observed in the ALSFRS-R, with the notable exception of a trend toward better

preservation of bulbar and breathing functions as measured by the ALSFRS-R subscore, which appeared to persist out to 180 days. The change in ALSFRS-R total score over time or "slope" (> 0, indicating clinical stability or improvement) following Engensis treatment was greatest at 2 months in 50% of Participants. At 3 months following Engensis, this improvement was observed in 25% of Participants. Muscle strength was stable for the first 3 months following Engensis administration and then steadily declined in both upper and lower limbs at subsequent months. (See the IB for more information.)

2.2. Background

2.2.1. Pathophysiology of Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the loss of upper and lower motor neurons. The disease is diverse in its presentation, cause, and progression, but, in general, ALS presents clinically as asymmetric muscle weakness, wasting, spasticity, weight loss, dysphagia, and paralysis; in 10% to 15% of patients, cognitive impairment may arise before or following that of motor neuron dysfunction. Pathologically, ALS is characterized by progressive degeneration and loss of motor neurons in the spinal cord, brainstem, and cerebral cortex. From the time of diagnosis, median survival is 3 to 5 years; about 10% of patients can survive for ≥10 years. While the majority of ALS cases are sporadic (primary or idiopathic), about 5% to 10% of patients have a positive family history (familial ALS),[2,3] with a mutation in the Cu/Zn superoxide dismutase 1 (SOD1) gene accounting for 10% of familial ALS.[4]

Estimates of the incidence of ALS worldwide range from 1 to 3 cases per 100,000 people annually, with a higher incidence in Caucasians,[5,6,7,8,9] in people who smoke,[10,11] and in several geographic regions in which environmental toxins are suspected.[12,13,14,15] The ALS Association, the Centers for Disease Control and Prevention , and the National Institute of Neurological Disorders and Stroke estimate that as many as 30,000 Americans may have the disease at any given time, with approximately 5,600 newly diagnosed patients annually.[a]

[a] http://www.alsa.org/about-als/who-gets-als.html (accessed October 12, 2015); http://wwwn.cdc.gov/als/WhatisALS.aspx (accessed October 12, 2015); http://www.ninds.nih.gov/disorders/amyotrophiclateralsclerosis/detail_ALS.htm (accessed October 12, 2015)

undergo transcriptional dysregulation and abnormal ribonucleic acid (RNA) processing which,

[redacted]

[redacted] 1: **Molecular Mechanisms of Motor Neuron Injury in ALS**[25]

The caspase family. Regardless of the underlying metabolic pathway to degeneration, the end result in ALS is motor neuron death. The major executioners in the apoptotic process are proteases known as caspases (cysteine-dependent, aspartate-specific proteases). Caspases directly and indirectly orchestrate the morphologic changes of the cell during apoptosis. They exist as latent precursors, which, when activated, initiate the death program by destroying key components of the cellular infrastructure and activating factors that mediate damage to the cells.

The first evidence of the role of caspases in a neurodegenerative disease came from experiments in which the "ALS mouse" (SOD^{G93A}) was crossbred with a mouse expressing a mutant caspase 1 gene that inhibited caspase 1 in neurons. Mice expressing the mutant SOD1 transgene *and* the mutant caspase 1 transgene survived 9% longer, and disease progression was slowed by more than 50 percent.[26] Caspase inhibition in the same model was found to be neuroprotective and extended survival by 22%.[27]

The clinical relevance of these studies is supported by findings in ALS patients. Early activation of caspase-1 in ALS contributes significantly to toxicity.[28] However, neuronal death only occurs following upregulation and concomitant expression of caspase-3.[29,30] A therapy that could block

the upregulation of caspases could potentially improve survival and quality of Life (QoL) of ALS patients.

2.2.2. Current Treatment Options, Treatment Approaches, and Unmet Clinical Need

Rilutek (originally manufactured by Sanofi Aventis and approved as an orphan drug on December 12, 1995 [NDA 020599]) is now available in generic form as riluzole. The benefit of riluzole lies in extending early survival and/or time to tracheostomy, but without statistically significant difference in muscle strength or neurological function.[31]

Edaravone was approved by the Food and Drug Administration (FDA) on May 5, 2017 for patients with ALS. The drug is administered by IV infusion and believed to reduce oxidative stress. Edaravone was approved based on the results of a Phase 3 clinical trial in Japan and South Korea in which 137 ALS patients were given either edaravone or placebo. The group given edaravone experienced a 33% reduction in the decline of their physical abilities compared with the placebo group. Statistical significance was only reached in Participants diagnosed less than two years earlier and with rapid progression of symptoms, however; 2 other Phase 3 studies failed to show any clinical benefit of edaravone, and survival was not demonstrated to be prolonged with its use. Neither riluzole nor edaravone prevent or correct the underlying causes of ALS. Clearly, a therapy that could impede or reverse neurodegeneration is needed for patients with ALS. Engensis produces HGF, which acts as a neurotrophic factor that aids in the development and regeneration of peripheral nerves; it activated Schwann cells to become repair-type cells and enhanced the remyelination process, consequently leading to the proper regeneration of injured peripheral nerves in animal models (see the IB for more information).

Treatment approaches based on anatomic delivery strategies have also been proposed for treatment of ALS or other neurodegenerative diseases.[32,33] For example, based on observations of retrograde neural transport of biotherapeutics following IM injection in animal models, a delivery approach of IM injections of stem cells has been applied in clinical trials of various adult stem cells populations, all with a limited number of injections and few muscle targets.[34,35,36] A much broader anatomic delivery strategy is being used in the present study.

2.2.3. Hepatocyte Growth Factor for Treatment of Amyotrophic Lateral Sclerosis

Hepatocyte growth factor (HGF) is a multi-functional mesenchyme-derived cytokine. It is a recognized potent angiogenic growth factor and anti-apoptosis agent, stimulating the growth of endothelial cells and the migration of vascular smooth muscle cells.[37,38,39,40] HGF stimulates DNA, RNA, and protein synthesis by endothelial cells in a dose-dependent manner, upregulates VEGF expression, and exhibits greater mitogenic activity than that of VEGF alone in human aortic endothelial cells *in vitro*.[41,42,43]

Although largely thought of as an angiogenic agent, HGF has been recently identified as a neurotrophic factor.[44,45,46,47,48,49,50] HGF and its cognate receptor (the c-Met receptor) are expressed in the peripheral nervous system as well as in various regions of the brain and spinal cord. Sun *et al.* (2002) found that local sustained HGF production in neural tissue in SOD^{G93A} mice (transgenic ALS model) alleviated the symptoms of ALS by direct neurotrophic activities on motor neurons and indirect activities on glial cells.[47] They found that HGF was neuroprotective. SOD mice transfected with a single copy of the HGF gene (G93A/HGF)

retained a significantly larger number of spinal motor neurons with a healthier morphology than SOD mice without the HGF gene (G93A single transgenic).

G93A/HGF mice also experienced improved motor performance, significantly delayed onset in paralysis, and survived longer than G93A mice. Researchers also found indirect evidence that HGF suppresses microgliosis and astrocytosis, both of which contribute to motor neuron degeneration by producing cytotoxic cytokines and eventual glial scar formation.[51,52]

It has been demonstrated that HGF can promote neurogenesis, angiogenesis, and synaptogenesis and that it can inhibit fibrotic changes in ischemic regions better than glial cell line-derived neurotrophic factor (GDNF).[53]

HGF may also have protective effects against excitotoxic injuries, because it has been shown to attenuate axonal degeneration by reducing excitotoxicity through modulation of the expression of the scaffolding protein of the N-methyl-D-aspartate (NMDA) receptor at the synapse.[54,55]

Finally, HGF may stave off cell death and halt the cascade of neighboring cell damage by inhibiting caspase signaling directly in motor neurons and indirectly by its effects on astrocytes.[56] In ALS patients, chronic, sublethal activation of caspase appears to mediate cell dysfunction, which precedes cell death.[57,58] Cell dysfunction of substantial magnitude, occurring before cell death, might result in symptomatic disease. Given that caspases may be active in individual neurons for a long period (potentially weeks to months), inhibition of caspase in these circumstances could reduce cell dysfunction and delay cell death.[54] Therefore, the neurotrophic action of HGF on motor neurons is, at least in part, promoted by preventing caspase-mediated cell death signals.

HGF overexpression also attenuates monocyte chemoattractant protein-1 (MCP-1) induction in astrocytes, which results in a reduction in microglial accumulation, a hallmark inflammatory process in ALS.[59]

The challenge associated with delivering a targeted sustained dose of exogenous HGF is in overcoming the instability of HGF in blood circulation and its rapid clearance by the liver; HGF has an in vivo half-life of less than 15 minutes.[60,61]

One approach to increasing HGF available to neurons is to develop a gene transfer strategy that would allow for persistent expression of HGF protein in vivo. Although plasmid DNA is one of the least efficient gene transfer systems currently in use, the fact that it is associated with limited persistence and no propensity for genomic integration (particularly in skeletal muscle tissue) makes it an attractive option for local targeted delivery.

2.2.4. Engensis and VM202

The investigational agent (study drug) being studied in this protocol is Engensis, which contains the active pharmaceutical ingredient VM202. VM202 is a plasmid DNA that contains the novel genomic complementary DNA (cDNA) hybrid human HGF coding sequence (HGF-X7) expressing two isoforms of HGF, HGF_{728} and HGF_{723} (Figure 2).

[redacted]

[redacted]

[redacted]

[redacted] has been evaluated for general toxicity following single IM and [redacted]

[redacted]

[redacted]

[redacted]

artery disease (Phase 2), one study in coronary artery disease (Phase 1), four studies (Phase 1/2, Phase 2, and two Phase 3) in Participants with painful DPN, a study (Phase 1/2) in ALS, and one (Phase 3) study in diabetic Participants with chronic nonhealing foot ulcers (NHU). Engensis was well tolerated, the most frequent reaction being pain at injection sites in 1-4% of Participants, which was similar to that observed in the placebo groups. No systemic effects have been attributed to Engensis.

2.2.6.1. Phase 1/2 Study in Participants with Amyotrophic Lateral Sclerosis

A Phase 1/2, open label, single center study in 18 Participants diagnosed with clinically definite, clinically probable, or clinically probable-laboratory supported ALS (VMALS-001; NCT02039401) lasting 9 months was completed.[62] Prior to injections on Day 0, Participants were assessed using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), the Medical Research Council (MRC) scale for muscle strength testing, dynamometry, forced vital capacity (FVC), and muscle circumference.

All Participants (18 Participants) received a total of 64 mg of VM202 IM in the upper limbs (abductor pollicis brevis, first dorsal interosseous, biceps, deltoid, extensor carpi radialis, flexor carpi ulnaris, and flexor carpi radialis) and lower limbs (quadriceps, gastrocnemius, and tibialis anterior).

Engensis was administered over the course of four visits: Days 0, 7, 14, and 30. As in previous Engensis studies, the final dose of VM202 for each target muscle group was divided and administered 2 weeks apart with injection of the upper limbs at separate visits from injection of the lower limbs.

Post-injection, ALSFRS-R, FVC, and muscle strength (as determined by the MRC scale) were assessed at Days 30, 60, and 90, and at 6 and 9 months. Muscle circumference and dynamometry were conducted on Days 60 and 90, and at 6 and 9 months. Participants were contacted through 36 months by phone to assess survival.

After Engensis or Placebo injections through Day 30, seventeen (17) Participants completed the 9-month follow-up; one Participant died prior to the 9-month visit. Three (3) Participants completed the study (36-month follow-up), while 14 Participants died following the 9-month visit; all deceased Participants died due to respiratory failure associated with ALS. Four additional serious adverse events (SAEs) that required hospitalization were reported. All deaths and SAEs were classified as unrelated to the study drug. Injection site reactions were limited to pain and/or bruising.

2.2.6.2. Other Engensis Clinical Studies

[redacted]

2.3. Benefit/Risk Assessment

2.3.1. Risk Assessment

[redacted]

Adverse Events in Clinical Studies

Engensis has been well tolerated in all clinical studies conducted thus far in more than 500 Participants. The preponderant adverse events have consisted of mild, transient injection site effects, including itching, erythema, pain, and muscle spasms. Other treatment-emergent adverse events (TEAEs) that have been reported were generally mild to moderate. Refer to the IB for a table of AEs that occurred in ≥ 2% of trial Participants.

2.3.2. Benefit Assessment

In ALS, sustained, local production of HGF in neural tissue may alleviate symptoms by direct neurotrophic and indirect neuroprotective activities on motor neurons. HGF has also been reported to play an important role in muscle damage and muscular atrophy; exogenous treatment of HGF ameliorated skeletal muscle atrophy and increased the generation of new muscle fibers.

Engensis induces relatively rapid and prolonged production of HGF, which, through its multiple mechanisms of actions on microvascular and neural repair, has the potential to improve outcomes in ALS patients, as suggested by preceding studies. If Engensis shows favorable outcomes in this study, it may be considered an important agent with a durable therapeutic effect for ALS patients.

2.3.3. Overall Benefit / Risk Conclusions

The overall profile of potential benefits and apparent safety for Engensis provide sufficient support to justify the conduct of the present study in its aim to meet the unmet needs of ALS patients.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To evaluate the safety of intramuscular (IM) injections of Engensis in Participants with Amyotrophic Lateral Sclerosis (ALS) compared to Placebo	• 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
Exploratory	
• To evaluate changes in muscle function following Engensis injections in ALS Participants compared to Placebo	• 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
• To evaluate muscle strength changes following Engensis injections in ALS Participants 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 180 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

Objectives	Endpoints
• To evaluate Quality of Life improvement following Engensis injections in ALS Participants 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
• To evaluate Patient and Clinical Reported Outcome improvement following Engensis injections in ALS Participants 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
• To determine whether IM administration of Engensis has effects on respiratory function in ALS Participants 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
• To determine whether IM administration of Engensis has positive effects on survival in ALS Participants compared to Placebo	• Time to all-cause mortality by Day 180 for Engensis compared to Placebo
• To determine whether IM administration of Engensis has positive effects on muscle atrophy in ALS Participants 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. STUDY DESIGN

4.1. Overall Design

VMALS-002-2 is a Phase 2a, double-blind, randomized, placebo-controlled, multicenter study designed to assess the safety of IM administration of Engensis in Participants with ALS as compared to Placebo. Study Participants will be randomized in a 2:1 ratio to Engensis or Placebo (control group). The study will include only patients with limb onset of ALS.

Screening. Screening activities (see SoA, Section 1.3) include assessments to determine study eligibility, assessments to establish Baseline results for use in analysis of endpoints, and Baseline safety assessments. All Screening activities will occur within the 30 days prior to Day 0.

Randomization. Participants who meet the eligibility criteria will be randomly assigned in a 2:1 ratio to one of the two treatment arms, Engensis or Placebo. Randomized treatment assignment will occur through the EDC randomization module in a double-blinded fashion. Blinding will be achieved by having the study medication (Engensis and Placebo) prepared by the unblinded Pharmacist or delegated staff.

Treatment Period. Participants will undergo three Treatment Cycles of Study Injections (Engensis or Placebo) at 60-day intervals (on Days 0 and 14, Days 60 and 74, and Days 120 and 134), with follow-up Visits at Days 30, 84, 144, and 180/ET.

4.1.1. Engensis and Placebo Treatment Cycles

Engensis is delivered in 0.5 mL IM injections of a solution of 0.5 mg/mL VM202. Eligible Participants will receive 3 Treatment Cycles of either Engensis or Placebo at 60-day intervals. During each Treatment Cycle, Engensis (64 mg) will be administered over the course of two dosing Visits: Days 0 and 14, Days 60 and 74, and Days 120 and 134, as presented in detail in Table 1, Table 2, and Table 3 for the First, Second, and Third Treatment Cycles, respectively.

All Participants will receive 256 injections of Engensis IM per Treatment Cycle (128 injections on each dosing day) as a divided dose in the upper extremities (abductor pollicis brevis, first dorsal interosseous, biceps, deltoid, extensor carpi radialis, flexor carpi ulnaris, and flexor carpi radialis) and lower extremities (quadriceps, gastrocnemius, and tibialis anterior). Each injection contains 0.25 mg/0.5 mL VM202 or 0.5 mL of Placebo. Participants randomly assigned to Engensis will receive a total dose of 192 mg of Engensis (3 cycles at 64 mg each) during the study.

Immediately before the first day of injections of the First, Second, and Third Treatment Cycles (Days 0, 60, and 120, respectively) assessments will be conducted in the following order:

1. Recording of TEAEs
2. Recording of concomitant medications/procedures
3. Vital signs and weight
4. ALSFRS-R
5. HHD

6. ATLIS
7. Pregnancy test (Day 0 only)
8. Complete physical examination
9. SVC (predose Day 0, 60, and 120)
10. ALSAQ-40 (predose on Day 0)
11. Blood draw for serum chemistry, and hematology (predose on Days 60 and 120)
12. Muscle biopsy (predose on Day 0)

A summary of the schedule of evaluations and Visits during the study can be found in the SoA.

4.1.1.1. Treatment Cycle 1

Participants will receive Engensis or Placebo by IM injections to the right and left sides of the specified muscles on Days 0 and 14 as shown in Table 1.

Table 1: Injection and Dosing Schedule: Treatment Cycle 1: Days 0 and 14

Anatomic Target Area	Number of Injections (Engensis or Placebo) and Dose per Treatment Cycle				
	Treatment Cycle 1: Day 0		Treatment Cycle 1: Day 14		Total per Treatment Cycle
	Right	Left	Right	Left	
	Number of Injections (Engensis or Placebo) 1 injection = 0.5 mL				
Hands					
Abductor pollicis brevis	2 injections	2 injections	2 injections	2 injections	8 injections
First dorsal interosseous	2 injections	2 injections	2 injections	2 injections	8 injections
Upper Arms					
Biceps	8 injections	8 injections	8 injections	8 injections	32 injections
Deltoid	8 injections	8 injections	8 injections	8 injections	32 injections
Lower Arms					
Extensor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi ulnaris	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Legs					
Quadriceps	20 injections	20 injections	20 injections	20 injections	80 injections
Gastrocnemius	12 injections	12 injections	12 injections	12 injections	48 injections
Tibialis anterior	6 injections	6 injections	6 injections	6 injections	24 injections
Total Number of Engensis or Placebo Injections	64 injections	64 injections	64 injections	64 injections	256 injections
Engensis Dose per Treatment Day/Cycle	**32 mg**		**32 mg**		**64 mg**

4.1.1.2. Treatment Cycle 2

Participants will receive Engensis or Placebo by IM injections to the right and left sides of the specified muscles on Days 60 and 74 as shown in Table 2.

Table 2: Injection and Dosing Schedule: Treatment Cycle 2: Days 60 and 74

Anatomic Target Area	Number of Injections (Engensis or Placebo) and Dose per Treatment Cycle				
	Treatment Cycle 2: Day 60		Treatment Cycle 2: Day 74		Total per Treatment Cycle
	Right	Left	Right	Left	
	Number of Injections (Engensis or Placebo) 1 injection = 0.5 mL				
Hands					
Abductor pollicis brevis	2 injections	2 injections	2 injections	2 injections	8 injections
First dorsal interosseous	2 injections	2 injections	2 injections	2 injections	8 injections
Upper Arms					
Biceps	8 injections	8 injections	8 injections	8 injections	32 injections
Deltoid	8 injections	8 injections	8 injections	8 injections	32 injections
Lower Arms					
Extensor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi ulnaris	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Legs					
Quadriceps	20 injections	20 injections	20 injections	20 injections	80 injections
Gastrocnemius	12 injections	12 injections	12 injections	12 injections	48 injections
Tibialis anterior	6 injections	6 injections	6 injections	6 injections	24 injections
Total Number of Engensis or Placebo Injections	64 injections	64 injections	64 injections	64 injections	256 injections
Engensis Dose per Treatment Day/Cycle	**32 mg**		**32 mg**		**64 mg**

4.1.1.3. Treatment Cycle 3

Participants will receive Engensis or Placebo by IM injections to the right and left sides of the specified muscles on Days 120 and 134 as shown in Table 3.

Table 3: Injection and Dosing Schedule: Treatment Cycle 3: Days 120 and 134

Anatomic Target Area	Number of Injections (Engensis or Placebo) and Dose per Treatment Cycle				
	Treatment Cycle 3: Day 120		Treatment Cycle 3: Day 134		Total per Treatment Cycle
	Right	Left	Right	Left	
	Number of Injections (Engensis or Placebo) 1 injection = 0.5 mL				
Hands					
Abductor pollicis brevis	2 injections	2 injections	2 injections	2 injections	8 injections
First dorsal interosseous	2 injections	2 injections	2 injections	2 injections	8 injections
Upper Arms					
Biceps	8 injections	8 injections	8 injections	8 injections	32 injections
Deltoid	8 injections	8 injections	8 injections	8 injections	32 injections
Lower Arms					
Extensor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi ulnaris	2 injections	2 injections	2 injections	2 injections	8 injections
Flexor carpi radialis	2 injections	2 injections	2 injections	2 injections	8 injections
Legs					
Quadriceps	20 injections	20 injections	20 injections	20 injections	80 injections
Gastrocnemius	12 injections	12 injections	12 injections	12 injections	48 injections
Tibialis anterior	6 injections	6 injections	6 injections	6 injections	24 injections
Total Number of Engensis or Placebo Injections	64 injections	64 injections	64 injections	64 injections	256 injections
Engensis Dose per Treatment Day/Cycle	**32 mg**		**32 mg**		**64 mg**

4.2. Scientific Rationale for Study Design

VMALS-002-2 is designed as a well-controlled Phase 2a study in ALS Participants, as it is a double-blind, randomized, placebo-controlled, and multicenter study that is sufficiently sized in numbers of Participants to evaluate the safety of Engensis in Participants with ALS.

4.3. Participant Input into Design

Participant input into the design of this study was collected via patient interviews. Prior to finalization of this protocol, ALS patients reviewed and provided direct input to the design of the clinical trial from a patient's perspective. Input included randomization ratio, injection methodology, and order of assessments during visits. Logistical input was also received and implemented to accommodate for potential inconvenience and discomfort for Participants.

4.4. Justification for Dose

Pharmacokinetic studies in animals and humans both indicate that VM202 (Engensis) has very limited systemic distribution. The plasmid DNA product is taken up into muscle cells within a radius of about 1 cm of each IM injection site. Only a small fraction of VM202 DNA is detectable in the systemic circulation following IM injection. VM202's short circulatory half is 2 to 5 minutes consequent to rapid clearance by circulating DNases and rapid uptake by the liver (reference Investigator's Brochure). The expression of human HGF after intracellular VM202 uptake continues for about 2 to 3 weeks following injection (ibid.). As HGF protein can bind to heparan sulfate, which is abundant in the extracellular matrix (ECM), longer-term retention of HGF protein results from entrapment within the ECM. Most HGF released into circulation, including from the ECM, is quickly degraded. The repeated injection of Engensis in study VMALS-002-2, with 6 treatment days over 19 weeks, is thus expected to provide enhanced HGF expression for approximately 5 to 6 months.

In addition, the favorable safety profile of Engensis from 10 clinical studies with over 500 total Participants who have received the drug indicates that the minimal safety risk of 3 cycles of treatment with Engensis over a 19-week period is justified for Participants with a life-threatening illness who may receive a potential benefit from increased neuromuscular activity and greater ability to exert self-control of voluntary muscle movements during their participation in study VMALS-002-2.

4.4.1. Support from Nonclinical Studies

Previous nonclinical GLP safety studies in rats and rabbits included single and repeat injections of VM202 (single injection day in a rat study; 4 injection days in a repeated-dose rat study; 5 injection days in a repeated-dose rabbit study). No treatment-related safety events were observed in any of the studies, so the no observed adverse effect level (NOAEL) dose was considered to be equal to or higher than the highest doses tested in each model system.

In the single-dose rat study, the maximum dose administered was 12.8 times the dose per injection day of 32 mg and 2.1 times the total cumulative dose of 192 mg to be administered in this study, assuming a human weight of 60 kg. In the repeated-dose rabbit study – in which rabbits were injected on Days 0, 7, 14, 21, and 28 – the total cumulative dose in the high-dose group was 1.9 times the total cumulative dose to be administered in this study. In the repeated dose rat study – in which rats were injected on Weeks 0, 4, 8, and 12 – the total cumulative dose in the high-dose group was 4.3 times the total cumulative dose to be administered in this study. These calculations indicate that the single daily dose and total human dose of Engensis to be used in study VMALS-002-2 is within the NOAEL established in these GLP studies.

4.4.2. Support from Clinical Studies

Previous clinical studies have used a variety of doses and timing periods for administration of Engensis (VM202). The dose-response for Engensis was evaluated in two painful DPN studies. In study VMDN-001 (Phase 1/2 open-label dose-escalation study in 12 DPN subjects), Participants received total doses of 4, 8, or 16 mg Engensis administered into the calf muscle of one leg. Half of each dose was administered at Day 0 and the other half was administered at Day 14. No dose-limiting toxicities, no SAEs, and no unexpected AEs were observed. One

subject experienced a minor injection site reaction (ISR) of transient erythema that resolved without treatment. Reductions in DPN pain were observed in 9 of the 12 subjects at 3 months after injection and in 10 of the 12 subjects at 6 and 12 months after injection.

In study VMDN-002 (Phase 2 double-blind placebo-controlled study in 103 subjects), Participants received total doses of 16 mg or 32 mg Engensis or Placebo in divided doses on Days 0 and 14. Doses were administered to both legs (total of 8 mg or 16 mg per leg). Safety and efficacy were followed for 9 months. All SAEs occurring in 10 subjects were classified as unrelated to study drug. The efficacy data showed that 16 mg was the optimal dose with significant analgesia lasting for up to 9 months following the treatments on Days 0 and 14.

Phase 3 DPN studies with Engensis doubled the treatment days and doubled the total dose of Engensis without a significant increase in AEs. Doses of 16 mg of Engensis were administered as divided doses at Days 0 and 14 (4 mg per leg per day), with a second treatment of 16 mg VM202 at 3 months administered as divided doses at Days 90 and 104, for a final total dose of 32 mg Engensis administered per subject. All doses in DPN studies have been administered to the gastrocnemius muscles, as nerves and muscles in the lower limbs are typically most affected by DPN pain in earlier stages of the disease.

In the initial VMALS-001 study in ALS subjects (Section 2.2.6.1), doses were administered to multiple muscle groups in both the lower and upper body. For convenience, doses were administered bilaterally to the lower limbs on Day 0, followed by doses to the upper limbs on Day 7, or vice-versa in two separate groups of Participants. The same series of injections were repeated at Days 14 and 21. Hence, a total of 256 injections were administered over 4 visits during two Treatment Cycles (64 injections, either 13 or 19 mg total dose per dosing day, depending on whether upper or lower limb muscles were injected; dosing on Days 0, 7, 14, and 30) with an overall total dose of 64 mg per Participant. Thus, the total dose in study VMALS-001 was double the total dose in the Phase 3 DPN studies, although the dose per individual injection (0.25 mg administered in 0.5 mL of solution) has remained constant since the earlier dose-ranging studies in DPN subjects. All participants tolerated 64 mg of VM202 well with no SAEs related to the drug.

In this VMALS-002-2 study, the total number of injection days was increased from 4 days to 6 days with both upper and lower limbs being injected on each dosing day. Therefore, a total of 768 injections is being administered over 6 visits (128 injections, 32 mg total dose per dosing day; Days 0, 14, 60, 74, 120, and 134) for an overall total dose of 192 mg Engensis per Participant. The dose of Engensis per individual injection and the number of muscle groups injected is unchanged from the earlier ALS study. Hence the dosing regimen per injected muscle remains the same on each treatment day as in the previous ALS study, but treatment days have been spread out over a longer period of time and the overall number of treatment days has been increased by 50%. This gradual escalation of dosing is consistent with the safety profile of Engensis both in animals and in humans, and with the extended, chronic debilitating disease process of ALS.

Most importantly, a favorable safety profile for Engensis has been demonstrated in over 500 recipients. Given the life-threatening nature of ALS, 3 administrations of Engensis, each separated by two months over a 6-month period, are justified for participants with such a negative prognosis and profoundly unmet need.

4.5. Randomization

Participants should be randomized as close as possible to the time of the first Study Injections. Randomization will be conducted via an EDC randomization module in a 2:1 ratio to receive either Engensis or Placebo.

4.6. End of Study Definition

The end of the study is defined as the date on which the last Participant in the study completes the Day 180 Visit or is terminated or discontinued early from the study (early termination).

4.7. Study Completion

The study will be considered complete upon Sponsor approval of the clinical study report.

4.8. Completed Participants

A Participant is considered to have completed the study if the Participant completes the Day 180 Visit.

5. STUDY POPULATION

Participants meeting the eligibility criteria will be enrolled and randomized.

5.1. Inclusion Criteria

1. Male or female Participants age ≥ 18 years, but ≤ 80 years, at time of completion of the informed consent process
2. Clinically definite or probable Amyotrophic Lateral Sclerosis (ALS) or laboratory-supported probable ALS as defined in the revised El Escorial/Airlie House diagnostic criteria
3. The site of onset of ALS symptoms is a limb and experiencing symptoms of lower motor dysfunction (e.g., weakness, atrophy, cramps, poor circulation, etc.) with upper motor neuron symptoms (e.g., brisk reflexes, spasticity)
4. Onset of ALS symptoms ≤ 4years
5. Slow Vital Capacity (SVC) ≥ 50% of predicted value at Screening
6. Not taking riluzole, or on a stable dose (defined as no noted toxicities) for at least 30 days prior to Screening and throughout the study
7. Not taking edaravone or on a maintenance cycle for at least 30 days prior to Screening and throughout the study
8. For females of childbearing potential, a negative urine pregnancy test at Screening and on Day 0
9. Male Participants and their female partners must agree to use double-barrier contraception during the study or provide proof of postmenopausal state (minimum 1 year) or surgical sterility
10. Male Participants must not donate sperm during the study
11. Female Participants must be nonpregnant, nonlactating, and either postmenopausal for at least 1 year, or surgically sterile for at least 3 months, or agree to use double-barrier contraception from 28 days prior to randomization (Day 0) and/or their last confirmed menstrual period prior to study randomization (whichever is longer) until the end of the study
12. Capable of complying and willing to comply with the requirements and restrictions in the informed consent form and this protocol
13. Willing to forgo new experimental ALS treatments for at least 6 months following randomization

5.2. Exclusion Criteria

1. Progressive or degenerative neurological disorder such as Alzheimer's disease, Parkinson's disease, vascular dementia, multiple sclerosis, and other neurological or vascular disorders felt by the Investigator to preclude participation
2. Requires tracheotomy ventilation or noninvasive ventilation related to bulbar function

3. Evidence by physical examination, history, or laboratory evaluation of significant concomitant disease with a life expectancy of < 6 months at Screening
4. INR values > 2.0
5. Platelet count < 100,000/μL
6. Inflammatory disorder of the blood vessels (inflammatory angiopathy or vasculitis, such as Buerger's disease)
7. Active infection (chronic infection or severe active infection that may compromise the Participant's wellbeing or participation in the study in the Investigator's judgment)
8. Chronic inflammatory disease (e.g., Crohn's disease, rheumatoid arthritis)
9. Positive human immunodeficiency virus (HIV) or human T-cell lymphotropic virus (HTLV) I/II test at Screening
10. Active acute or chronic hepatitis B
11. Active hepatitis C
12. Immunosuppression due to underlying disease (e.g., rheumatoid arthritis, systemic lupus erythematosus), or to currently receiving immunosuppressive drugs (e.g., chemotherapy, corticosteroids), or to radiation therapy
13. Stroke or myocardial infarction within 3 months prior to Screening
14. Active deep vein thrombosis
15. Recent history (< 3 years) or presence of cancer except basal cell carcinoma or squamous cell carcinoma of the skin that was excised and has shown no evidence of recurrence for at least 1 year
16. Major psychiatric disorder diagnosed in the past 6 months that has not been stabilized or in the Investigator's opinion would not allow the patient to participate in the scheduled procedures
17. Use of an investigational drug for the treatment of ALS in the past 30 days or 5 half-lives (if available), whichever is longer, or previous participation in a clinical study with Engensis
18. Stem cell administration for investigational treatment of ALS or other conditions in the 6 months prior to Screening

5.3. Number of Participants

The target sample size is 18 Participants who will be randomized in a 2:1 ratio to Engensis or Placebo (12:6).

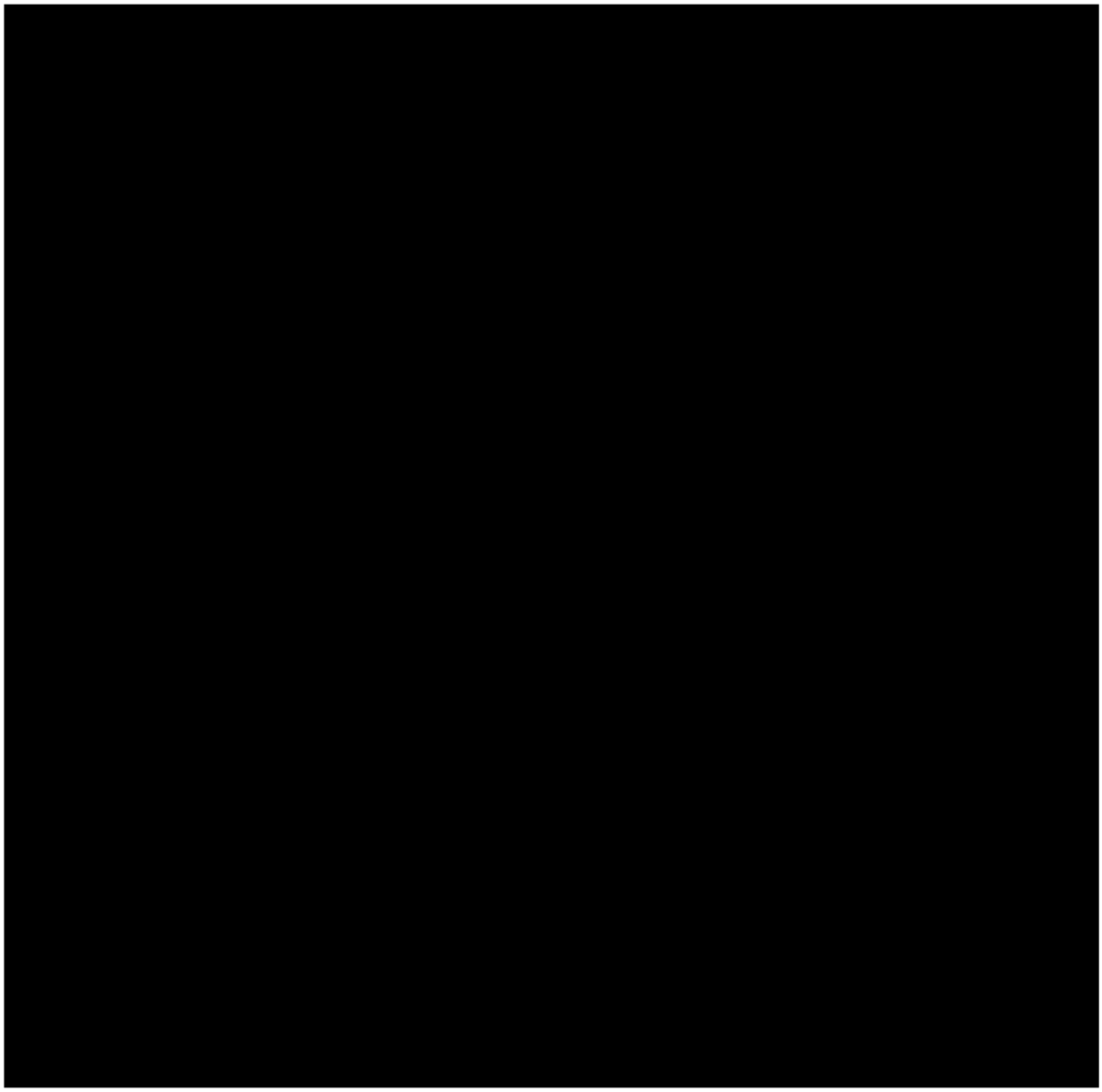
5.4. Enrollment, Randomization, and Treatment Assignment

5.4.1. Enrollment Adjudication Process

All Screening information for each Participant prior to randomization will be sent to an Adjudication Committee (Figure 3). The Adjudication Committee is composed of the Sponsor Medical Director, Medical Monitor, and Clinical Operations personnel. The Adjudication

Committee reviews the relevant clinical information of each Participant on a timely basis to ensure strict adherence to the Inclusion/Exclusion Criteria. The Committee either confirms the eligibility for randomization of an individual Participant or confers with the Investigator regarding the status of a Participant as a screen failure.

Figure 3: Randomization Process



5.4.2. Randomization and Treatment Allocation

Once a Participant is deemed to meet all Inclusion/Exclusion Criteria, blinded randomization will be conducted via an EDC randomization module in a 2:1 ratio to receive either Engensis or Placebo. The unblinded Pharmacist or delegated staff will prepare the Engensis or Placebo injections for that Participant prior to First Injections by the Investigator or qualified designee on Day 0.

5.5. Participant Identification

To maintain confidentiality, the name of the Participant should not be recorded on any study document other than the informed consent form. All Participants who sign the informed consent form will be assigned a unique identifier in the following format: XX-YYY where XX is the 2-digit Site number and YYY is the 3-digit sequential ID number starting with 001 for the first Participant consented at that Site. For example, the first Participant consented at Site 11 will be assigned 11-001. ID numbers for screen failures will not be reused. If a screen failure is later reconsented for rescreening, a new ID number will be assigned.

5.6. Screen Failures

A consented Participant who does not meet all eligibility criteria will immediately cease further study-related testing; the cessation will be communicated to the Sponsor or designee within 24 hours.

A minimal amount of screen failure information is required to ensure transparent reporting of screen failures to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes eligibility criteria, demographic data, screen failure details, and other pertinent materials.

5.7. Rescreening

Participants who provided informed consent but failed to meet all eligibility criteria may be rescreened once with Sponsor written permission via the adjudication process. Any screen failure may only be reconsented and rescreened once.

The following Screening assessments, if completed during the first Screening and found to meet eligibility criteria, may not need to be repeated when the Participant returns for rescreening:

- Viral Screening if performed within 3 months of signing the new informed consent

All other Screening assessments will need to be repeated.

5.8. Lifestyle Considerations

5.8.1. Meals and Dietary Restrictions

There are no meal or dietary restrictions for this study.

5.8.2. Caffeine, Alcohol, and Tobacco

There are no caffeine, tobacco, or alcohol restrictions for this study.

5.8.3. Supplements

There are no restrictions on herbal medicines or dietary supplements or changes in their use during the study. The use of any supplements during the study will be recorded on the Participant's medical record and the electronic case report form (eCRF) as concomitant medications.

5.8.4. Activity

There are no restrictions on activity. Participants should be advised to continue their normal activity level during the study.

6. STUDY INTERVENTION

6.1. Study Intervention(s) Administered

Refer to the Study Drug Injection Guidelines for administration of Engensis or Placebo.

6.1.1. Engensis

Engensis contains the active pharmaceutical ingredient VM202, a plasmid DNA containing a novel genomic cDNA hybrid human HGF coding sequence, HGF-X7, that expresses two isoforms of HGF, HGF_{728} and HGF_{723}. The plasmid has 7,377 base pairs with a human cytomegalovirus (HCMV) enhancer / promoter, a growth hormone polyadenylation terminator sequence, ColEl originator, and the kanamycin resistance gene on a pCK backbone.

The components of Engensis are provided in Table 4.

Table 4: Components of Engensis

Component	Function	Composition
VM202	Active pharmaceutical ingredient	2.5 mg
Sucrose	Suspension medium	55 mg (1.1%)[a]
Sodium Chloride	Suspension medium	45 mg (0.9%)[a]
Sterile Water for Injection	Suspension medium	5 mL

[a] Concentration after reconstitution in 5 mL water for injection.

6.1.2. Placebo

Placebo will be sterile Engensis vehicle. The components of the Placebo are provided in Table 5. The Placebo is supplied in a sterile glass vial in liquid form. Each vial is only to be used for one Participant. Visually, the Placebo is indistinguishable from reconstituted Engensis. The Participant, Investigator, and study nurse will not be able to distinguish Placebo from Engensis.

Table 5: Components of the Placebo

Component	Function	Composition
Sucrose	Suspension medium	55 mg (1.1%)
Sodium Chloride	Suspension medium	45 mg (0.9%)
Sterile Water for Injection	Suspension medium	5 mL

[redacted]

[redacted]

6.3. Measures to Minimize Bias

6.3.1. Randomization

Participants are to be randomized on Day 0. Randomization will be conducted via an EDC randomization module in a 2:1 ratio for Participants to receive either Engensis or Placebo.

6.3.2. Blinding

Participants, Investigators, Site staff, clinical research organization (CRO) staff and Sponsor staff will be blinded to treatment assignments except for the designated Unblinded CRA, Sponsor Clinical Supplies Manager, and the Site's unblinded pharmacy staff.

[redacted]

The unblinded pharmacy staff are responsible for receiving, storing, preparing, and dispensing study drug per the Pharmacy Manual. An Investigator may be unblinded to the treatment assignment for a Participant with an SAE when the treatment decision requires knowledge of the treatment assignment.

To avoid potential bias, Investigators and study staff are expected to refrain from sharing safety and treatment outcomes with other participating Sites.

The DSMB, independent of the Sponsor, may review individual unblinded Participant narratives in the case of an SAE or multiple SAEs for which the DSMB Chair requests unblinding, but will not have access to unblinded tables and listings unless requested by the DSMB Chair when assessing a potential safety signal. While the study is ongoing and prior to database lock, the datasets will remain blinded with no preliminary summary of the study by the individual treatment arms except when requested by the DSMB Chair. The DSMB charter will specify its composition, frequency of meetings, guidelines for noncomparative or comparative data review, and range of recommendations that may be provided as a separate document accompanying the protocol.

6.3.3. Maintenance of the Blind

Once the Participant has been adjudicated and deemed eligible for potential randomization, the drug depot will prepare and ship both Engensis and Placebo vials to the Site pharmacy. The pharmacy will keep both Engensis and Placebo vials and the dose preparation worksheet in a secure location with access limited to unblinded pharmacy personnel responsible for preparing the syringes with assigned study treatment. The Investigator or designee will randomize the Participant using the EDC randomization module on Day 0 after confirming eligibility criteria.

The unblinded Pharmacist or delegated staff will receive a notification from the EDC randomization module indicating into which arm the participant has been randomized. The unblinded Pharmacist or delegated staff will prepare Engensis or Placebo per the Pharmacy Manual.

Blinding will be achieved by having study drug syringes prepared by the pharmacy staff and ensuring that the study drug vials are never left in a location where they could be seen by blinded study staff (e.g., study coordinator, study nurse, Investigator). The Engensis and Placebo vials are readily distinguishable from each other, even from a distance. However, because reconstituted Engensis is indistinguishable from Placebo, syringes containing Engensis are indistinguishable from syringes containing Placebo.

The pharmacy staff and select individuals such as the Unblinded CRA will be the only ones that will be unblinded to the treatment assignments. The Participant and study personnel, including study coordinators, study nurses, laboratory technicians, Investigators, and blinded CRAs, will remain blinded until all data has been entered into the database and the database is locked.

IN CASE OF EMERGENCY (i.e., SERIOUS ADVERSE EVENT [SAE]) AND ONLY WHEN THIS INFORMATION INFLUENCES THE PARTICIPANT'S MEDICAL MANAGEMENT, the Investigator may contact the Medical Monitor to request unblinding of the treatment assignment. The date and reason for unblinding will be documented in the electronic data capture (EDC) system.

6.4. Study Intervention Compliance

All Site personnel involved with the receipt, storage, preparation, administration, and destruction of study drug are required to document their understanding of the Study Drug Injection Guidelines. Refer to the Injection Guidelines.

All study drug will be administered under the supervision of the Investigator.

Compliance with study drug administration instructions will be assessed through the review of accountability and preparation logs as well as the reconciliation of used and unused vials, vials destroyed, and vials returned to the Sponsor.

6.5. Concomitant Therapy

Standard of care for ALS is presented in the American Academy of Neurology (AAN) guideline regarding management and care of the patient with ALS.[64] Recommendations are presented for multidisciplinary care, symptom management, and the treatment of cognitive/ behavioral impairment.

6.6. Dose Interruption

No intentional modifications of the number or concentration of doses will be permitted. An unintentional interruption of the number of injections (128 per Study Injection Visit) is only allowed if the Participant experiences an AE related to study drug administration or other health-related events (e.g., illness) during the study. If the full set of study injections cannot be administered during the normal Study Injection Visit, the Participant should be scheduled to receive the remaining injections within 48 hours. If this is not possible, then the remainder of the injections for that visit will not be administered.

6.7. Prior and Concomitant Medications, Treatments, and Procedures

Medications and treatments taken within 30 days prior to Day 0 will be recorded on the Medication and History eCRF at the Day 0 Visit. Any medication or vaccine, including over-the-counter or prescription medicines, vitamins, herbal supplements, and topical anesthetics for post-injection pain and procedures that the Participant is receiving at the time of enrollment or receives during the study must be recorded on the Concomitant Medications or Concomitant Procedures eCRF. For each medication or treatment, the following information will be collected:

- Medication trade or generic name, or procedure
- Indication for which the medication/procedure was given

- Dose/strength, route, and frequency of administration
- Date started and date stopped (or continuation at study exit)
- Reason for use

The medical monitor will be contacted if there are any questions regarding concomitant or prior therapy.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT WITHDRAWAL

7.1. Discontinuation of Study Intervention

In rare instances, it may be necessary to discontinue study drug for a Participant. If study drug is discontinued, the Participant will remain in the study to be evaluated for safety if the Participant agrees to continue participating in the study. See Section 1.3 (SoA) for any further assessments that need to be completed.

7.2. Participant Withdrawal/Discontinuation

Any Participant may voluntarily discontinue the study at any time without prejudice. The Investigator may discontinue a Participant from the study at any time if, in the Investigator's judgment, the Participant's health or safety would be compromised by remaining in the study.

Specific reasons for study discontinuation include the following:

- Participant decision
- Investigator's decision
- Adverse event
- Insufficient compliance with study requirements
- Lost to follow-up
- Other

The specific reasons for Participant discontinuation will be recorded.

The following assessments will be performed at an Early Termination (ET) Visit, if possible, for Participants who discontinue prior to the Day 180 Visit.

- Complete Physical examination
- Vital signs, weight
- 12-lead ECG
- Serum chemistry and hematology
- Urine pregnancy test
- SVC
- Concomitant medications
- Muscle strength by HHD
- ATLIS (where available)
- ALSFRS-R
- ALSAQ-40
- PGIC and CGIC
- Injection site reaction assessment if discontinued prior to Day 144
- Treatment-emergent adverse events

7.3. Lost to Follow-up

A Participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled Visits and is unable to be contacted by the Site by Day 180 (+7 days).

The following actions must be taken and recorded if a Participant fails to return to the clinic for a required study Visit:

- The site must attempt to contact the Participant and reschedule the missed Visit as soon as possible. The site must counsel the Participant on the importance of maintaining the assigned Visit schedule and ascertain whether or not the Participant wishes to and/or should continue in the study.
- If no response is obtained from the Participant, the Investigator is encouraged to contact one of the Participant's relatives or his/her general practitioner.
- Before a Participant is deemed lost to follow up, the Investigator or designee must make every effort to regain contact with the Participant, including at least three telephone calls and, finally, a certified letter to the Participant's last known mailing address. These contact attempts should be documented in the source records.
- Should the Participant continue to be unreachable, the Participant will be considered lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

All study procedures and assessments together with their timing are summarized in Section 1.3 (SoA).

Protocol waivers or exemptions are not allowed. Adherence to the protocol requirements is essential.

8.1. Screening (Days -30 to -1)

8.1.1. Screening Procedures

During Screening, and prior to randomization, Participant eligibility will be assessed, and the following Screening procedures will be conducted:

- Informed consent prior to any study-related procedures
- Evaluation of eligibility criteria
- Diagnosis confirmed for clinically definite or probable ALS or laboratory supported probable ALS as defined in the revised El Escorial/Airlie House diagnostic criteria
- Demographics, baseline characteristics, and medical history
- Concomitant medications and procedures
- ALSFRS-R
- SVC
- Vital signs, weight, and height
- Complete physical examination
- 12-Lead ECG
- Serum chemistry and hematology, and coagulation profile
- Urine Pregnancy Test (for women of childbearing potential only)
- Viral Screening – HIV, Anti-Human T-Cell Lymphotropic Virus (HTLV) I/II antibody with reflex confirmatory assay, Hepatitis B core antibody (IgM HBcAb), antibody to Hepatitis B antigen (HBsAb), Hepatitis B surface antigen (HBsAg) in addition to a quantitative HBV viral load if needed clinically, and Hepatitis C antibodies (Anti-HCV) and a positive qualitative PCR to assess HCV viral load

8.1.2. Revised El Escorial/Airlie House Criteria

According to the Revised El Escorial/Airlie House guidelines,[65] definitions for ALS are as follows:

- **Clinically definite ALS** is defined by clinical or electrophysiological evidence by the presence of lower motor neuron (LMN) as well as upper motor neuron (UMN) signs in

the bulbar region and at least 2 spinal regions or the presence of LMN and UMN signs in 3 spinal regions.

- **Clinically probable ALS** is defined on clinical or electrophysiological evidence by LMN and UMN signs in at least 2 regions with some UMN signs necessarily rostral to (above) the LMN signs.

- **Probable ALS–Laboratory Supported**, which is defined when clinical signs of UMN and LMN dysfunction are found in only 1 region but electrophysiological signs of LMN loss are observed in ≥2 regions.

8.2. Safety Assessments

8.2.1. Physical Examinations

A complete physical examination (PE) will be performed at the Screening, predose on Days 0, 14, 60, 74, 120, and 134, and on Days 30, 84, 144, and Day 180/ET. The complete PE will include the following: an examination of the skin/integumentary systems, general appearance, head, neck (including thyroid), eyes, ears, nose, throat, lymph nodes, chest (lungs), heart, abdomen, musculoskeletal system, neurological system, and any additional assessments needed to establish Baseline status or evaluate symptoms or AEs. Height will be recorded only during Screening. Any abnormalities should be categorized as clinically significant (CS) or not clinically significant (NCS), and the abnormalities recorded.

The Investigator or qualified medical personnel who routinely perform these evaluations in patients with ALS will conduct the examination, determine findings, and assess any abnormalities as to clinical significance.

8.2.2. Medical History

A detailed description of the Participant's current disease status, past medical conditions (including cancer), social history, and medication and treatment history will be recorded at the Screening Visit.

The Investigator will confirm the diagnosis of ALS using the revised El Escorial / Airlie House diagnostic criteria.

Social history includes familial, occupational, and recreational aspects of the Participant's personal life that have the potential to be clinically significant, including caffeine, nicotine, alcohol, and illicit drug use.

Medication and treatment history will also be collected at Screening, including riluzole and edaravone.

Cancer history will be collected for the 3 years prior to Screening. Familial cancer history will also be collected.

Any untoward medical events that occur from the time of consent and during the Screening period (prior to study drug administration) will be captured as AEs in the electronic case report form (eCRF). A change in medical status or medical history from time of signing of informed consent through first dose of study drug is to be reported as an AE or SAE (only if associated with a study specific procedure) as appropriate.

8.2.3. 12-Lead Electrocardiograms

12-Lead ECGs will be performed during Screening, Day 84, and Day 180/ET. Any clinically significant abnormalities are to be recorded. The ECG recording will be printed out and stored with the Participant's records.

8.2.4. Vital Signs

Vital signs will be measured at Screening and all subsequent study visits, including pre- and post-dose at Study Injection Visits during Treatment Cycle 1 (Days 0 and 14), Treatment Cycle 2 (Days 60 and 74), and Treatment Cycle 3 (Days 120 and 134), and at all other Study Visits (Days 30, 84, 144, and 180/ET). Weight will be collected predose at Study Injection Visits. After resting in the seated position for 5 minutes, vital signs will be recorded including temperature, sitting blood pressure, heart rate, respiratory rate, and oxygen saturation on room air. The method of temperature measurement should be according to the Site's policy and should be consistently applied.

8.2.5. Coagulation Profile

Blood samples for measurement of coagulation profile parameters (PT, PTT, and INR) will be collected at Screening.

8.2.6. Clinical Laboratory Assessments

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the Participant's condition.

All protocol-required laboratory assessments must be performed in accordance with the laboratory manual and the SoA.

8.2.6.1. Local and Central Laboratory Assessments

The laboratory assessments for safety and establishing eligibility will be performed at local laboratories at the time points noted in Section 1.3 (SoA). The Screening Local laboratory results from Screening will be entered into the EDC system by the sites. All assessments from Day 0 to 180/ET will be performed by the central laboratory; those for Screening will be done by a local laboratory.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study. The laboratory reports must be filed with

the source documents and uploaded into the EDC. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the Participant's condition.

All protocol-required laboratory assessments must be performed in accordance with the laboratory manual and the SoA.

8.2.6.1.1. Hematology

- White blood cell count
- Neutrophil (including calculated absolute neutrophil count)
- Hemoglobin
- Hematocrit
- Platelet count

8.2.6.1.2. Kidney Function Tests

- Blood urea nitrogen (BUN)
- Creatinine

8.2.6.1.3. Liver Function Tests

- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase
- Gamma-glutamyl transpeptidase (GGT)
- Total bilirubin
- Total protein
- Albumin

8.2.6.1.4. Clinical Chemistry

- Electrolytes (sodium, potassium, chloride, bicarbonate, calcium, inorganic phosphate, magnesium)
- Glucose (random collection, i.e., not necessarily fasting)
- Amylase
- Lipase
- Lactate dehydrogenase
- Creatine kinase

8.2.6.1.5. Additional Tests

- Urine pregnancy test

8.2.6.1.6. Viral Screening

- HIV
- HTLV I/II antibody with reflex confirmatory assay
- HBcAb (IgM)
- HBsAb
- HBsAg
- HBV viral load (if needed clinically)
- Anti-HCV antibodies and a positive qualitative PCR to assess HCV viral load

8.2.7. Slow Vital Capacity

Slow vital capacity is a pulmonary function test that quantifies the volume of air that can be slowly exhaled after slow maximum inhalation. SVC will be determined at Screening, predose on Days 60 and 120, and on Days 30, 84, 144, and 180/ET.

8.3. Other Assessments and Procedures

8.3.1. Revised Amyotrophic Lateral Sclerosis Functional Rating

The revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) is a validated rating scale for measuring the global function of patients with ALS. It provides a health professional-generated estimate of the patient's degree of functional impairment, which can be evaluated serially to objectively assess any response to treatment or progression of disease. The ALSFRS-R includes twelve questions that ask the physician to rate his/her impression of the patient's level of functional impairment in performing one of twelve common tasks, e.g., climbing stairs. Each task is rated on a five-point scale from 0 (can't do) to 4 (normal ability). Individual item scores are summed to produce a reported score of between 0=worst and 48=best. The ALSFRS-R will be conducted at Screening, predose on Days 0, 60, and 120, and on Days 30, 84, 144, and 180/ET. Details about the scale can be found in Section 10.6, Appendix 6.

8.3.2. Handheld Dynamometry

Muscle strength will be measured quantitatively by Handheld Dynamometry (HHD) and the change from baseline in HHD following Engensis injections as compared to Placebo. HHD is a procedure by which a dynamometer is applied to the body of an individual being tested.[66]

A non-AC powered dynamometer (manufactured by Hoggan Health Industries, Inc) will be used at all clinical sites. These measurements will be performed predose on Days 0, 60, and 120, and on Days 30, 84, 144, and 180/ET. Strength measurements will be evaluated by individual muscles.[67]

8.3.3. Accurate Test of Limb Isometric Strength

The Accurate Test of Limb Isometric Strength (ATLIS) is designed to evaluate muscle strength and measure the isometric strength of 12 muscle groups in the arms and legs and will be conducted at clinical sites that have ATLIS available and staff trained and available to administer the strength evaluation. ATLIS will be measured predose on Days 0, 60, and 120, and at Visits on Days 30, 84, 144, and 180/ET

8.3.4. Muscle Biopsies

Helixmith intends to use muscle biopsy samples of the gastrocnemius to evaluate biomarkers in order to gain further understanding of Engensis' mechanism of action and muscle atrophy in ALS. Testing results could provide information for future uses of Engensis in ALS. A biopsy of the gastrocnemius will be collected (and stored) at Day 0 predose, and on Days 84 (optional) and 144. See the Muscle Biopsy Guidelines for detailed information on the techniques and procedures to be used.

8.3.5. Quality of Life Assessment, Patient Reported Outcomes, and Clinical Reported Outcomes

8.3.5.1. Amyotrophic Lateral Sclerosis Assessment Questionnaire

The Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) is a disease-specific, Participant self-report health status, or electronic patient-reported outcome (ePRO). There are 40 questions in the ALSAQ-40 with 5 discrete scales: physical mobility (10 questions), activities of daily living and independence (10 questions), eating and drinking (3 questions), communication (7 questions), and emotional reactions (10 questions). The ALSAQ-40 will be measured predose on Day 0 and at Visits on Days 84 and 180/ET. See Section 10.7, Appendix 7, for the ALSAQ-40 questionnaire.

Participants are asked to think about the difficulties that they may have experienced during the last two weeks (e.g., "I have found it difficult to feed myself"). Participants are asked to indicate the frequency of each event by selecting one of 5 options (Likert scale): never, rarely, sometimes, often, always, or cannot do at all.

8.3.5.2. Patient Global Impression of Change

The Participant's impression of change after treatment will be measured with the Patient Global Impression of Change (PGIC) questionnaire through use of the ePRO. This questionnaire measures the Participant's perception of how treatment has affected their level of activity, symptoms, emotions, and overall quality of life. Each descriptor is ranked on an intensity scale of 1 = Very Much Improved; 2 = Much Improved; 3 = Minimally Improved; 4 = No Change; 5 = Minimally Worse; 6 = Much Worse; 7 = Very Much Worse. The test will be self-administered during the Days 84 and 180/ET Visits. The PGIC questionnaire is presented in Section 10.8, Appendix 8.

Upon completion of the questionnaire, the study coordinator will check the questionnaire for completeness. Any omissions or ambiguous answers will be clarified by the Participant prior to leaving the clinic.

8.3.5.3. Clinical Global Impression of Change

The Clinical Global Impression of Change (CGIC) is a validated instrument completed by observers as an assessment of QoL. The CGIC is a 7-point scale with scores range from 1 (very much improved) through 7 (very much worse). The test will be self-administered during the Days 84 and 180/ET Visits. The CGIC questionnaire is presented in Section 10.8, Appendix 8.

8.4. Blood Samples Stored for Future Genetic Testing

Blood samples will be collected from all Participants to be stored for future genetic testing at Screening.

8.5. Adverse Events and Serious Adverse Events

The definitions of AEs, TEAEs, SAEs and TESAEs can be found in Section 10.2, Appendix 2.

8.5.1. Time Period and Frequency for Collecting AE, TEAE, SAE, TESAE Information

All study-related AEs and SAEs will be collected starting after completion of the informed consent process at Screening to Day 0/randomization. TEAEs and TESAEs will be collected after first injections on Day 0 through Day 180/ET or the last Study Visit up until the time that the Participant withdraws consent. (see definition of TEAE, Section 10.2). 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.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.2.6, Appendix 2. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of its availability.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a Participant has been discharged from the study, and he/she considers the event to be possibly, probably, or definitely related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.5.2. Method of Detecting AEs, TEAEs, SAEs and TESAEs

The method of recording, evaluating, and assessing causality of AEs, TEAEs, SAEs and TESAEs, and the procedures for completing and transmitting SAE reports are provided in Section 10.2, Appendix 2.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the Participant is the preferred method to inquire about AE occurrences.

8.5.3. SAE Reporting to the Sponsor or Designee

All SAEs should be reported to the Sponsor or designee within 24 hours of knowledge of the SAE occurring.

8.5.4. Adverse Events of Special Interest

Categories of AESIs

There are three main categories of AESIs as presented below: 1) those considered to be related to the angiogenesis potential of Engensis, 2) other medical problems in this patient population, and 3) injection site reactions.

8.5.4.1. AESI Considered Related to Angiogenesis

Atherosclerosis

Hyperplasia of the vasa vasorum in the early stages of atherosclerosis is independent of angiogenesis, but the intimal neovascularization that follows the hyperplasia of the vasa vasorum is angiogenesis-dependent.[68] Angiogenesis increases oxygen and nutrients to the artery wall and supports initial plaque growth. Once the atherosclerotic plaque develops, intimal angiogenesis is thought to contribute to characteristics of an unstable plaque, plaque hemorrhage, and plaque rupture. Therefore, diagnoses suggestive of recent coronary artery disease since Baseline will be evaluated as AESIs.

Cancer

Angiogenesis plays an important role in the proliferation and metastatic spread of cancer as these processes are dependent on an adequate supply of oxygen and nutrients and removal of waste products.[69] All types of cancer reported after randomization will be deemed to be AESIs.

8.5.4.2. Other Medical Problems in ALS Patients

Several medical problems are frequently observed in patients with ALS that should be considered AESIs. These medical problems are listed below.

- Pulmonary Medical Problems: Because patients with ALS are at an increased risk to develop pulmonary medial problems such as aspiration of food or liquids. pneumonia,

and respiratory arrest, all events of this type that occur after randomization will be considered AESIs.

- Progressive Muscle Weakness: Because some patients with ALS may develop rapidly progressive muscle weakness that results in loss of ability to care for self, decubitus ulcers, or weight loss due to impaired deglutition, all events of this type that occur after randomization will be considered AESIs.

- Bulbar Disease: Participants may develop bulbar disease or worsening on bulbar disease following randomization manifested as challenges with dysarthria, facial weakness, impaired tongue pulsion or palate elevation, and impaired mastication and swallowing; all of these types of events that occur after randomization will be considered AESIs.

8.5.4.3. Injection Site Reactions

The most common ISRs in the prior Phase 1/2 study in ALS Participants were local pain and bruising at the site of injection. A follow-up notification for ISRs will occur within 2 to 3 days after each Study Injection Visit. The location and temporal relationship to the most recent injections will determine whether the event is an ISR and should be reported as a TEAE. For an AE to qualify as an ISR, the reaction must be located in close proximity to an injection site and be first observed and graded within 48 hours following Study Injections. ISRs that remain unresolved after 48 hours will continue to be assessed at subsequent Visits as ongoing AEs until resolved.

8.5.5. Follow-up of AEs, TEAEs, SAEs, and AESIs

After the initial AE/TEAE/SAE report, the Investigator is required to proactively follow each Participant at subsequent Visits/contacts. All SAEs and AESIs (see Section 8.5.4) will be followed until resolution, stabilization, the event is otherwise explained, or the Participant is lost to follow-up (as defined in Section 10.2.4, Appendix 2).

8.5.6. Regulatory Reporting Requirements for SAEs

Prompt notification by the Investigator to the Sponsor of an SAE is essential to ensure that legal obligations and ethical responsibilities towards the safety of Participants and the safety of a study drug under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs), and Investigators.

For all studies, Investigator Safety Reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator Safety Report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then notify the IRB if appropriate according to local requirements.

8.5.7. Sponsor's Responsibility

All AEs and SAEs will be reported on an annual basis to FDA in accordance with the IND regulation (21 CFR 312). Per the 2010 FDA Guidance Document for Industry and Investigators "Safety Reporting Requirements for INDs and BA/BE Studies," events categorized as "possibly" or "probably" related will be treated as "suspected adverse reactions." Events categorized as "definitely" related will be treated as an "adverse reaction."

All serious and unexpected study-drug-related or suspected adverse reactions will be reported to FDA and to all participating Investigators in an IND Safety Report within 15 calendar days of the event after the Sponsor determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32). Any unexpected fatal or life-threatening study-drug-related adverse reaction or suspected adverse reaction will be reported to the Agency within 7 calendar days after the Sponsor's initial receipt of the information.

The Sponsor will notify all participating Investigators of any new safety information that alters the current risk-benefit assessment of the study medication or that would be sufficient to consider changes in Engensis administration or in the overall conduct of the study.

8.5.8. Pregnancy and Contraception

8.5.8.1. Pregnancy Test (Women of Childbearing Potential Only) and Contraception

Female Participants must be nonpregnant, nonlactating, and either postmenopausal for at least 1 year, or surgically sterile for at least 3 months, or agree to use double-barrier contraception from 14 days prior to randomization and/or their last confirmed menstrual period prior to study randomization (whichever is longer) until 2 months after the last visit on Day 180. Double-barrier contraception may include, but is not limited to, intrauterine device with spermicide, female condom with spermicide, diaphragm with spermicide, cervical cap with spermicide; having a male sexual partner who agrees to use a male condom with spermicide; or having a sterile sexual partner.

For women of childbearing potential, a urine beta human chorionic gonadotropin (β-HCG) test will be performed during Screening and before randomization (Day 0); results of the test must be negative. The β-HCG test will also be performed at Day 180/ET to evaluate whether the Participant is pregnant.

The rhythm method is not considered acceptable method of contraception.

8.5.8.2. Pregnancy

The Investigator will collect pregnancy information on any female Participant who becomes pregnant while participating in this study. The initial information will be submitted to the Sponsor within 24 hours of learning of a Participant's pregnancy.

Further dosing will be discontinued for any female Participant who becomes pregnant during the study.

The Participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the Participant and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. A spontaneous abortion (occurring at < 22 weeks gestational age) or stillbirth (occurring at > 22 weeks gestational age) is always considered to be an SAE and will be reported as such. Other abnormal pregnancy outcomes (e.g., fetal death, congenital anomalies, ectopic pregnancy) are also considered SAEs and will be reported.

Any post-study pregnancy-related SAE considered possibly, probably, or definitely related to the study drug by the Investigator will be reported to the Sponsor. While the Investigator is not obligated to actively seek this information in former study Participants, he or she may learn of an SAE through voluntary reporting.

The Investigator will record a narrative description of the course of each pregnancy and its outcome.

8.5.9. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

Except for signs and symptoms of ALS (i.e., numbness, pain, tingling or burning sensation, cramps, and increased sensitivity to touch), all disease-related events or disease-related outcomes qualify as AEs or SAEs.

8.6. Study Completion

8.6.1. End of Study Definition

The end of the study is defined as the date on which the last Participant in the study completes the Day 180 Visit or is discontinued.

8.6.2. Completed Participants

A Participant is considered to have completed the study if the Participant completes all phases of the study including the last Visit on Day 180 or the last scheduled procedure shown in the SoA.

9. STATISTICAL CONSIDERATIONS

Detailed statistical methods are described in the Statistical Analysis Plan (SAP).

9.1. Sample Size Determination

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

9.2. Population for Analysis: Safety Population

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.

9.3. Statistical Analyses

The SAP will be finalized prior to database lock and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned endpoints.

9.3.1. Primary Endpoints

Safety analyses in this study will evaluate the safety profile of Engensis as compared with Placebo. The primary endpoints for safety are as follows:

- Incidence of TEAEs and 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

No formal statistical testing will be conducted for the safety analyses. All Participants in the safety subset will be included in these analyses. Participants will be grouped by treatment received. All summaries will be derived based on available data. No imputation will be performed for missing values. All safety analyses will be made on the Safety Population.

9.3.2. Exploratory Endpoints

The exploratory endpoints include:

- Change from Baseline (Day 0) in total mean 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 HHD in muscles in the upper and lower extremities on Days 30, 60, 84, 120, 144, and 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in the ATLIS tests, where available, at Days 30, 60, 84, 120, 144, and 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in QoL using the ALSAQ-40 on Days 84 and 180 for Engensis injections compared to Placebo
- PGIC and CGIC at Days 84 and 180 for Engensis compared to Placebo
- Change from Baseline (Day 0) in 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

9.4. Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will periodically review blinded (noncomparative) data as a limited set of unblinded tables and/or listings, including all reported AEs and SAEs. The objectives of the DSMB meetings are to review the safety outcomes of the study and provide guidance to study sponsor regarding the safety of Engensis. The data analyses for the DSMB meetings will be directly provided to the DSMB members, and no data will be released to the study sponsor or blinded designees. No adjustment will be made for multiple testing due to the DSMB data review. Further details of DSMB responsibilities are included in the DSMB Charter.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1. Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

10.1.1.1. Institutional Review Board

Prior to the initiation of the study, the protocol, the informed consent form, and Investigator's Brochure will be submitted to the IRB for approval. By signing the "Statement of Investigator" form (Form FDA 1572), the Investigator is ensuring that an IRB compliant with the requirements set forth in 21 CFR 56 will be responsible for the initial and continuing review of the proposed clinical study. A copy of the IRB approval letter for the protocol, the informed consent, and the protocol signature page must be submitted to the Sponsor prior to release of investigational supplies to the Study Site. The approval letter must refer to the specific protocol and the informed consent form. The Study Site must maintain an accurate and complete record of all reports, documents, and other submissions made to the IRB concerning this protocol. A list of the IRB members, their titles or occupations, and their institutional affiliation, or an IRB assurance number must be provided to the Sponsor prior to release of study supplies.

Health authority regulations require that all advertisements for Participant recruitment be approved by an IRB prior to implementation. The complete text and format must be submitted to the Sponsor for approval prior to IRB submission.

The Investigator is responsible for notifying the IRB of any SAEs as required by the IRB. A copy of the notification must be forwarded to the Sponsor or its designee.

Status reports must be submitted to the IRB at least once a year (or more frequently as required by the IRB), and the IRB must be notified of completion or termination of the study.

10.1.1.2. Informed Consent Process

The Investigator has the responsibility to inform each Participant, prior to the Screening evaluation, of the purpose of this clinical study, including possible risks and benefits, and document the informed consent process in the Participant's chart. An informed consent form containing the required elements of informed consent must be generated by the Investigator. After approval by the Sponsor, the informed consent form (ICF) must be submitted to and approved by an IRB. Prior to entry into the study or initiation of any study-related procedures, the Participant must read, sign, and date the ICF. The person executing the consent must also sign and date the IRB-approved ICF. One original ICF is to be retained by the Study Site, and a copy is to be given to the Participant. The informed consent process must be documented in the Participant's source/medical record.

The ICF must be written in a language in which the Participant is fluent. If a foreign language translation is required, a statement of certification of the translation must be issued. Regulations require that foreign language ICFs be submitted to the IRB for approval. The Investigator must

forward a copy of the ICF, the certified foreign language translation, and an IRB approval letter to the Sponsor.

The Investigator will explain the study purpose, procedures, and Participant's responsibilities to the Participant. The Participant's willingness and ability to meet the follow-up requirements will be determined and written informed consent will be obtained. The Participant will sign and date the ICF. The Investigator or a qualified designee will also sign and date the ICF. The original ICF will be retained with the Participant's records; a copy will be provided to the Participant.

If the ICF is amended during the study, the Investigator or a qualified designee must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IRB. For each new version of the ICF, the IRB will be consulted to determine if Participants who have not completed the study must be re-consented to the new ICF.

10.1.1.3. Obligations of the Sponsor and the Investigator

The Sponsor will select Investigators on the basis of multiple criteria that include their expertise in the area of ALS, their Site personnel's experience in conducting investigational clinical studies, their prospects for recruiting Participants to the study, and the proficiency with which he/she responds to the requirements of the Sponsor.

The Sponsor and Investigator must comply with all applicable regulations. In addition, the Investigator must follow local and institutional requirements pertaining, but not limited, to investigational product, clinical research, informed consent including the use and disclosure of the Participants' protected health information (PHI), and IRB regulations. The Sponsor will notify the Investigator of protocol and amendment approvals by regulatory authorities when applicable.

The Investigator and clinical research coordinator will be available to respond to reasonable requests and audit queries made by the authorized regulatory agency representatives. The Investigator and clinical research coordinator will provide the Sponsor with advance written notification if they plan to relocate to another institution.

Except where the Investigator's signature is specifically required, the term "Investigator" as used in this protocol and protocol-related documents is understood to refer to the Principal Investigator (PI) or appropriate Study Site personnel whom the PI designates to perform a certain duty. This delegation of authority needs to be documented appropriately and signed by the PI. The PI is ultimately responsible for the conduct of all aspects of the clinical study.

Sub-investigators or other appropriate Study Site personnel (e.g., listed on the Form FDA 1572) are eligible to sign for the PI on laboratory reports and may be designated to verify and electronically sign eCRFs.

10.1.2. Financial Disclosure

10.1.2.1. Conflict of Interest Policy

The independence of the study from any actual or perceived influence is critical. Therefore, any actual conflict of interest or financial interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this study should be disclosed. Furthermore, persons who have a perceived conflict of interest or financial interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this study.

10.1.2.2. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Participant Selection and Informed Consent Process

The Investigator will screen Participants who meet the eligibility criteria. The Investigator will not exercise selectivity, so that bias is prevented. All Participants must sign an ICF that has been approved both by the Sponsor and by the reviewing IEC/IRB. The informed consent should be in accordance with the current revision of the Declaration of Helsinki as well as current ICH and Good Clinical Practice (GCP) guidelines.

Prior to randomization, Participants will receive a comprehensive explanation of the proposed treatment including the nature and risks of the study, alternate therapies, any known AEs, the investigational status of the product, and the other elements that are part of obtaining proper informed consent. Potential Participants will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically any saved blood samples. Potential Participants will be allowed sufficient time to consider participation in the study after having the nature and risks of the study explained to them. The ICF must not include any exculpatory statements. The ICF and any separate Health Insurance Portability and Accountability Act (HIPAA) authorization form, if applicable, should be reviewed and approved by the Sponsor prior to IRB/IEC submission.

The Sponsor will provide to the Investigator, in writing, any new information that significantly bears on the Participants' risk in receiving the study drug. This new information will be communicated by the Investigator to Participants in accordance with IRB/IEC requirements. The ICF will be updated, and Participants will be re-consented, if necessary.

Site staff may conduct standard-of-care procedures and employ recruitment efforts prior to Participant consent; however, before any protocol-specified procedures are performed to determine protocol eligibility, an ICF must be signed. Participants will be given a copy of all consent forms that they sign.

By signing the ICF, the Participant agrees to complete all evaluations required by the study unless the Participant withdraws voluntarily or is terminated from the study for any reason.

10.1.4. Data Protection and Confidentiality

The data resulting from this study will be the proprietary information of the Sponsor and may be made public after all data have been analyzed and the study results are available. At the end of the study, a clinical study report will be written by the Sponsor.

10.1.4.1. Confidentiality

Participant confidentiality and privacy are strictly held in trust by the Investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to Participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies, or pharmaceutical companies supplying study product may inspect all documents and records required to be maintained by the Investigator including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for Participants. The Clinical Study Site will permit access to such records.

The Participant's contact information will be securely stored at each Clinical Site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB/IEC, applicable regulatory agencies, institutional policies, and/or Sponsor requirements.

Participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored by the Sponsor or their designee. This will not include the Participant's contact or identifying information. Rather, individual Participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by Clinical Sites and by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

10.1.4.2. Data Protection

In accordance with GCP and with the national data protection laws, all information concerning the Participants in the study must be treated as strictly confidential by all persons involved in the study.

The Investigator acknowledges that any and all information acquired from the Sponsor or developed or acquired in connection with the study is strictly confidential. The Investigator will not disclose any confidential information to any third party nor use confidential information for any purpose without first obtaining the consent of the Sponsor in writing. Such consent will be

deemed to have been given for disclosure to any person for whom the Investigator is responsible at his/her center, but only so far as required for the purposes of the study, and, in the case of disclosures to staff, only if such staff are bound by obligations of confidentiality no less strict than those set out herein.

Potential Participants must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the Participant who will be required to give consent for their data to be used as described in the ICF.

Potential Participants must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

10.1.5. Committee Structure

10.1.5.1. Data Safety Monitoring Board

An independent DSMB has been chartered for this study to review all safety data during the study and safeguard the interests of the Participants. The objectives of the DSMB meetings are to review the safety outcomes of the study and provide guidance to the Sponsor regarding the safety of Engensis. The DSMB will meet periodically and review a limited set of unblinded or noncomparative tables and/or listings of all reported AEs/TEAEs, AESIs, and SAEs/TESAEs. If a safety signal is identified during the noncomparative analysis, this will be followed by a comparative analysis as prompted by observations. The data analyses for the DSMB meetings will be directly provided to the DSMB members; no data will be released to the Sponsor or blinded designees. No adjustment will be made for multiple testing due to the DSMB data review.

The DSMB will consist of two physicians with expertise in clinical studies and one statistician. Members of the DSMB will be independent of study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflicts of interest. An independent biostatistician will be available for consultation.

The DSMB will operate under the rules of the charter, which have been reviewed at organizational meetings of the DSMB. Each data element that the DSMB requires to assess the safety of Engensis will be agreed upon prior to study start. Routine DSMB meetings will be regularly scheduled per the DSMB Chair to review safety and pooled all-cause mortality data, including unblinded Participant narratives. The DSMB Chair may request additional unblinded information at any time to further understand a safety trend. Ad hoc DSMB meetings can be convened at any time at the discretion of the DSMB Chair or the Sponsor. However, the DSMB Chair may not share any unblinded information with the Sponsor unless it is deemed necessary for the Sponsor to address a potential safety concern. The DSMB will provide recommendations to the Sponsor in accordance with the DSMB Charter.

10.1.5.2. Institutional Biosafety Committee

The Sites at which this study is being conducted will ensure that an Institutional Biosafety Committee (IBC) is in place. The IBC will ensure that the Site conforms to the requirements set forth in the Section IV-B-2 of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2019), promulgated by the NIH Office of Science Policy (OSP).

The Investigator will be responsible for petitioning the IBC and obtaining approval prior to enrolling any Participant in the study. The Investigator will also be required to obtain and follow all biohazard safety guidelines promulgated by the IBC, and to report all findings as required to the IBC and the OSP.

10.1.6. Data Quality Assurance

10.1.6.1. Clinical Monitoring

The Sponsor or designee will visit (in person or remotely, according to FDA Guidance on Conduct of Clinical Trials of Medical Product during COVID-19 Pandemic, March 2020, updated 03 Jun 2020) the Clinical Site for monitoring. The Sponsor's clinical monitor shall ensure that the Investigator understands the investigational status of the product, all protocol requirements, and his/her regulatory responsibilities as an Investigator. The clinical monitors will visit (in person or remotely) Clinical Sites at appropriate intervals to ensure compliance with the protocol and to verify the accuracy, completeness, and correctness of data reported and accountability of Engensis supplies.

The clinical monitor shall be available for consultation by the Investigator and serves as a liaison between the Clinical Site and the Sponsor. The clinical monitor or other authorized representatives of the Sponsor may inspect all data, documents, and records required to be maintained by the Investigator including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for Participants. The Clinical Site will permit access to such records. The Investigator will obtain, as part of the informed consent process, HIPAA-compliant authorization from Participants to use and disclose the requisite and relevant PHI and permission for authorized representatives of the Sponsor, or regulatory authorities including the FDA, to review, in confidence, any records identifying Participants in the clinical study.

10.1.6.2. Access to Study Documents and Study Monitoring

The Sponsor has designated a CRO to monitor the progress of this study. The clinical monitor, as a representative of the Sponsor, has the obligation to follow this study closely.

The Sponsor or its designee may meet with Investigators prior to the initiation of the study in order to review the adequacy of the Participant population, facilities, and equipment with respect to the needs of the study, and to familiarize the Investigator with the study protocol.

The Sponsor or its designee may meet with the Investigator(s) at the time that study Participants begin to be enrolled to ensure that Participants are being properly selected and that study data are being correctly recorded.

During the study, the clinical monitor will visit (in person or remotely) the study facilities regularly and use telephone and written communications on an ongoing basis to maintain current knowledge of the study. During periodic visits to the Clinical Site (in person or remotely), the monitor will review the source documents used for data entry in the EDC system to verify the accuracy and completeness of the information contained in those reports in preparation for retrieval. Source documents must contain all data entered in the EDC system. All data generated during this study and the source documents from which they originated are subject to inspection by the Sponsor or its representatives, and the FDA and other regulatory agencies.

Upon completion of the study, the clinical monitor will conduct a final visit (closeout) to the Site. The objectives of this visit are to ascertain that all regulatory records are complete, verify that study drug and other supplies have been accounted for, and ensure that the Investigator is aware of his/her responsibilities post-study.

10.1.6.3. Quality Assurance and Quality Control

Each Clinical Site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion.

Quality control (QC) procedures will be conducted within the EDC. Any missing data or data anomalies will be communicated to the sites for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical study is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP)).

The Clinical Site will provide direct access to all study-related source data documents and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities.

The Pharmacist must ensure that Engensis and Placebo are stored as specified in the protocol and Pharmacy Manual. The Pharmacist must maintain accurate records of the receipt of all Engensis and Placebo supplies and details of the dispensing and administration as specified in the Pharmacy Manual. The Engensis and Placebo must be administered only at the institution specified on the Form FDA 1572 for the Site.

The study will be conducted in accordance with the principles of GCPs: 21 CFR 50, 21 CFR 54, 21 CFR 56, and 21 CFR 312, Subpart D; the 2016 ICH Guideline on Good Clinical Practice (ICH E6(R2)); and HIPAA.

The Investigator at a Clinical Site must sign the Investigator Statement of Agreement.

10.1.6.4. Data Quality Assurance

The Sponsor's employees and/or their contracted representatives use SOPs designed to ensure that research procedures and documentation are consistently conducted/prepared to the highest quality standards. These SOPs also require compliance with Health Authority regulations and GCP guidance.

A quality assurance audit may be conducted by the Sponsor or its designee at any time during or after completion of a study. The Investigator will be given adequate notice if he/she is selected for an audit. The audit will include, but is not limited to, a review of all ICFs, source documents, medical records, and regulatory documentation; an assessment of study conduct and protocol compliance; and a review of the investigational drug accountability. At the conclusion of an audit, the auditor will conduct a brief meeting with the Investigator to review the noted observations.

All Participant data relating to the study will be recorded on electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate, correct, and complete by physically or electronically signing the eCRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

The Sponsor is responsible for the data management of this study including quality checking of the data.

The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized Site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of Participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.6.5. Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical study staff under the supervision of the Investigator. During the study, the Investigator will maintain complete and accurate documentation for the study. The Investigator should ensure that the Participant is provided with instructions and is trained to use the ePRO and to complete the assessment tool properly before data collection begins.

All eCRF data will be entered into a validated database compliant with 21 CFR Part 11. Laboratory data will be either manually entered or imported to the clinical database electronically. All data entry, verification, and validation will be performed in accordance with the current SOPs of the Sponsor or its designee. The database will be authorized for lock once no data queries are outstanding, all study data are considered clean, and all defined procedures are completed.

The Clinical Site will be provided with eCRFs and ePRO or electronic clinical outcome assessments (eCOA) devices in which to record all the protocol-specified data for each Participant. Entries made in the eCRF must be verifiable against source documents, or in certain circumstances as directed by the Sponsor, entries will have been directly entered into the eCRF; in such cases, the entry in the eCRF will be considered as the source data. Data reported in the eCRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. The Investigator will be responsible for reviewing all data and eCRF entries and will sign and date the designated pages in each Participant's eCRF, verifying that the information is true and correct. Queries generated by Data Management will be sent to the Clinical Site for resolution. The Investigator is responsible for the review and approval of all responses to eCRF queries.

10.1.6.6. Protocol Deviations

The Investigator will not deviate from this protocol for any reason without prior written approval by the Medical Monitor on behalf of the Sponsor except in the case of a medical emergency when the change is necessary to eliminate an apparent and immediate hazard to the Participant. Protocol deviations will be tracked through the EDC.

In the event of such an emergency, the Investigator will notify the Medical Monitor immediately by phone, notify the IRB, and confirm with the Medical Monitor in writing within 5 working days of the change being implemented.

10.1.6.7. Source Documents

As defined in the ICH Guidelines for Good Clinical Practice E6(R2), Section 1.52, source documents may include: original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, Participant's diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x rays, Participant files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study).

Source documents provide evidence for the existence of the Participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's Site.

The Investigator may need to request previous medical records or transfer records, depending on the study. Current medical records must also be available.

10.1.7. Data Management

All Participant data will be entered into a password-protected validated EDC system by authorized Site personnel according to the CRO's SOPs.

Data discrepancies identified via programmed edit checks, manual data review or discovered during data monitoring will be addressed and resolved. An audit trail in the EDC system will list all changes made to the data, with a date/time stamp and user initials. Upon database lock, occurring after data are declared clean and eCRFs have been approved by the Investigator, the CRO will provide SAS datasets to the Sponsor and designated Statistician for data analysis via secure data transfer specified in the Study Data Management Plan.

10.1.8. Recordkeeping and Retention

Data generated for the study should be stored in a limited-access file area and be accessible only to representatives of the Clinical Site, the Sponsor and its representatives, and FDA/relevant health authorities/regulatory agencies. All reports and communications relating to Participants will identify Participants only by Participant identification number. Complete Participant identification will be kept by the Investigator. This information will be treated with strict adherence to professional standards of confidentiality.

The Investigator must in reasonable time, upon request from any properly authorized officer or employee of FDA/relevant health authority/regulatory agency, permit such officer or employee to have access to requested records and reports, and copy and verify any records or reports made by the Investigator. Upon notification of a visit by the FDA/relevant health authority/regulatory agency, the Investigator will contact the Sponsor immediately. The Investigator will also grant the Sponsor's representatives the same privileges offered to FDA/relevant health authority or regulatory agents/officers/employees.

The Investigator must provide the Sponsor or its designee with the following documents prior to study initiation and retain a copy in the study file:

- A completed and signed Form FDA 1572. If, during the study, any changes occur that are not reflected on the current 1572, a new 1572 must be completed and returned to the Sponsor or its designee for submission to the FDA
- Current signed curricula vitae (within 2 years prior to study initiation) and current medical licenses for the Investigator and all co-Investigators listed on the 1572
- A copy of the original approval by the IRB for conducting the study. Renewals, with continuance of the study, must be submitted at yearly intervals or as required by IRB policy.
- A copy of the IRB-approved ICF
- The IRB member list and/or DHHS General Assurance Number (if the IRB has an Assurance number)

- A copy of the original approval by the IBC for conducting the study, if applicable. Renewals, with continuance of the study, must be submitted at yearly intervals or as required by IBC policy.
- Signed Financial Disclosure Forms for all personnel listed on the 1572 with a statement of non-voting by study staff
- The signature page of this protocol signed and dated by the Investigator
- The signature page of the IB signed and dated by the Investigator
- In addition to the documents listed above, the Clinical Site will also retain the following items:
    - Certifications and laboratory reference ranges for all local laboratories used for this study
    - A copy of delegation of authority log
    - Copies of the initiation visit report, the protocol, IB, screening/enrolment logs, subject ID list (not provided to sponsor/CRO), investigational product accountability records
    - All original ICFs with required signatures
    - All IRB correspondence (e.g., informed consent [including any approved revisions], protocol, AE, advertisements, newsletters)
    - All IBC correspondence
    - A copy of the Study Monitoring Log
    - Clinical and nonclinical supply shipment forms
    - Copies of all pertinent correspondence pertaining to the study (except budget issues) between the Sponsor, CRO, and the Site
    - Copies of all SAE reports submitted to the Sponsor
    - Copies of all Investigator Safety Reports submitted to the Site by the Sponsor
    - Copies of approved package labelling, if applicable

10.1.9. Study and Site Start and Closure

The study may be suspended temporarily or terminated prematurely if there is sufficient reasonable cause. Written notification documenting the reason for study suspension or termination will be provided by the Sponsor to Investigators and regulatory authorities. If the study is prematurely terminated or suspended, the Investigator will promptly inform Participants and the IRB and will provide the reasons for the termination or suspension. Participants will be contacted, as applicable, and be informed of changes to Study Visit schedules.

The Sponsor reserves the right to discontinue the study for any safety, ethical, or administrative reason at any time.

The Sponsor or designee reserves the right to close a Clinical Site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion.

The Investigator may initiate Clinical Site closure at any time, provided reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a Clinical Site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of Participants by the Investigator
- Discontinuation of further study drug development

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, IRBs, the regulatory authorities, and any CROs used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator will promptly inform the Participants and should ensure appropriate Participant therapy and/or follow-up.

10.1.10. Publication Policy

The study will be conducted in accordance with the publication and data sharing policies and regulations as defined in the agreement between the Sponsor and the institution. In addition, this study will be registered at www.ClinicalTrials.gov and in any other protocol registries required by the regions in which the study is conducted, and the results from this study will become publicly available.

None of the data resulting from this study will be allowed to be presented or published in any form by the Investigator or any other person without the prior written approval of the Sponsor.

10.1.11. Insurance

Matters relating to insurance for this study are to be defined in the agreement between the Sponsor and the institution.

10.2. Appendix 2. Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.2.1. Definition of AE

AE Definition
• An AE/TEAE is any untoward medical occurrence associated with the use of an investigational product or study procedure in a clinical study Participant, whether or not considered related to the study intervention. • NOTE: An AE/TEAE 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 or procedure.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, or clinical chemistry) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from Baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the Participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the Participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.2.2. Definition of TEAE

Treatment-Emergent Adverse Event is defined as:

- An event that emerges during treatment, having been absent at pretreatment, or worsens relative to the pretreatment state, as defined in the E9 Guidance.
- 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.

10.2.3. Definition of SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence 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 Baseline is not considered an SAE.

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.

10.2.4. Definition of TESAE

Treatment-Emergent Serious Adverse Event is defined as:

- A serious event that emerges during treatment, having been absent at pretreatment, or worsens relative to the pretreatment state, as defined in the E9 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.

10.2.5. Recording and Follow-Up of AE/TEAE and/or SAE/TESAE

AE and SAE Recording

- When an AE/TEAE or SAE/TESAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/TEAE or SAE/TESAE information in the eCRF.
- It is **not** acceptable for the Investigator to send photocopies of the Participant's medical records to the Sponsor or designee in lieu of completion of the AE/SAE eCRF page.

- Instances may arise when copies of medical records for certain cases are requested by the Sponsor or designee. In this case, all Participant identifiers, with the exception of the Participant number, will be redacted on the copies of the medical records before submission to the Sponsor or designee.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the Participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than that a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The Investigator will also consult the IB and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

- Situations may arise in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor or designee. However, the Investigator must always assess causality for every event before the initial transmission of the SAE data to the Sponsor or designee. Death or hospitalization are not to be specified as an SAE; these are criteria to determine seriousness.
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor or designee to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to the Sponsor or designee within 24 hours of receipt of the information.

10.2.6. Reporting of SAEs/TESAEs

SAE Reporting to the Sponsor or Designee via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the Sponsor or designee will be the electronic data collection tool.
- The Site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given Site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a Site receives a report of a new SAE from a study Participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the Site can report this information to the Medical Monitor or SAE coordinator by telephone.

10.3. Appendix 3. Grading of Injection Site Reactions

The following grading guidelines were compiled from sources from the FDA[70] and the National Cancer Institute[71] for Injection Site Reactions (Table 6). Injection sites should be assessed in each upper and each lower limb. A global assessment of all injection sites on each arm and on each leg is to be performed following each treatment. The injection site reaction with the most severe pain/tenderness, erythema/itching/urticaria, and bruising/swelling/bleeding on each arm and on each leg is to be used as the reference point for the injection site reaction grading following each treatment. Note that the most severe pain/tenderness injection site may not be the same injection site reaction with the most severe bruising/swelling/bleeding.

Table 6: Injection Site Reaction Grading

Reaction at Injection Site[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Pain, tenderness	Mild discomfort to touch, with or without warmth, erythema, itching	Symptoms with or without swelling and/or phlebitis	Symptoms with or without swelling or phlebitis or mild infection	Symptoms with severe infection or tissue injury or ulceration
	Does not interfere with daily activity	Interferes with daily activity	Precludes daily activity	Requires advanced medical and/or surgical care, including intravenous (IV) medications
	No or local treatment	Use of non-narcotic pain reliever	Use of narcotic pain reliever and/or oral antibiotics	
Erythema, itching, urticaria	Mild, localized itching, erythema or urticaria	Symptoms with skin changes beyond local injection site(s)	Symptoms with widespread skin changes	Bronchospasm, anaphylactoid, or anaphylactic reaction
	Does not interfere with activity	Interferes with movement and daily activity	Interferes with daily activity	Requires advanced medical care, including IV medication
	Use of topical treatment	Use of NSAIDs and/or antihistamines	Use of oral steroids or immuno-suppressives	
Bruising, swelling, bleeding	Mild, localized swelling, ecchymosis or hematoma	Moderate ecchymosis or hematoma	Extensive ecchymoses, large hematoma	Hypotension, life- threatening consequences
	Does not interfere with activity	Interferes with daily activity	Precludes activity	Requires transfusion, urgent intervention
	No treatment	Requires leg elevation, conservative treatment	Requires minimally invasive drainage	

Abbreviations: NSAID = non-steroidal anti-inflammatory drugs

[a] Injection site reactions are a category of AESIs and should be recorded as AEs using these grading guidelines.

10.4. Appendix 4. List of Abbreviations and Definitions

10.4.1. Abbreviations

Abbreviation	Definition
AE	adverse event
AESI	Adverse event of special interest
ALS	amyotrophic lateral sclerosis
ALT	alanine transaminase (SGPT)
ALSAQ-40	Amyotrophic Lateral Sclerosis Assessment Questionnaire
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating Scale
Anti-HCV	hepatitis C antibodies
APB	Abductor pollicis brevis
AST	aspartate transaminase (SGOT)
ATLIS	Accurate Test of Limb Isometric Strength
β-HCG	beta human chorionic gonadotropin
BUN	blood urea nitrogen
cDNA	Complimentary deoxyribose nucleic acid
CDRC	Clinical Data Review Committee
CFR	Code of Federal Regulation
CGIC	Clinical Global Impression of Change
CLI	critical limb ischemia
CONSORT	Consolidated Standards of Reporting Trials
CRA	Clinical Research Associate
CRO	clinical research organization
CS	clinically significant
CTCAE	National Cancer Institute: Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DPN	diabetic peripheral neuropathy
DSMB	Data Safety Monitoring Board
EAAT2	excitatory amino acid transporter 2
ECG	electrocardiogram
eCOA	electronic clinical outcome assessment
eCRF	electronic case report form
EDC	electronic data capture system
ePRO	electronic patient reported outcome
ET	early termination from the study
FDA	Food and Drug Administration
FDI	first dorsal interosseus
FVC	forced vital capacity

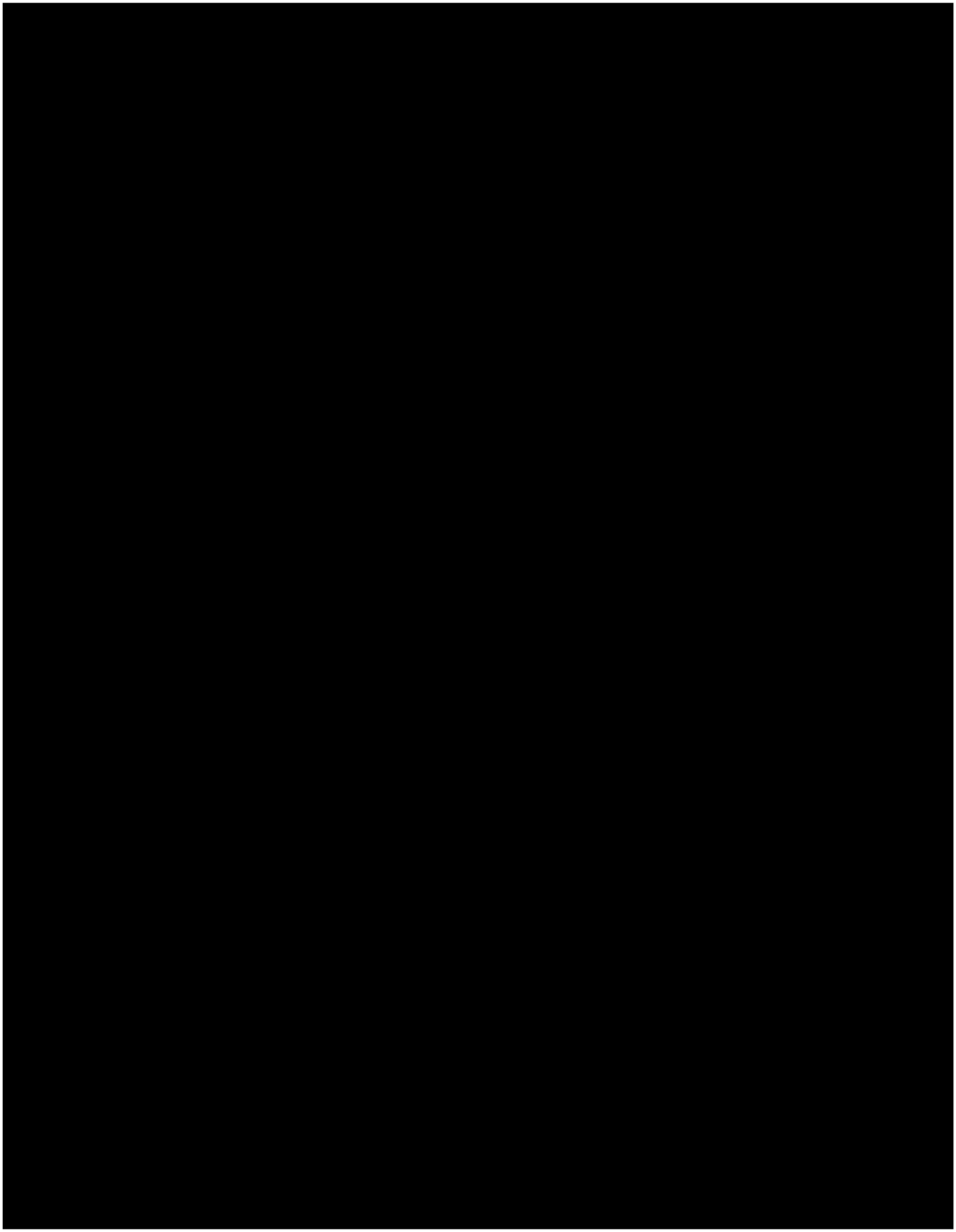
Abbreviation	Definition
GCP	good clinical practices
GGT	gamma-glutamyl transpeptidase
GLP	good laboratory practices
HBV	hepatitis B virus
HBcAb	hepatitis B core antibody (IgG and IgM)
HBsAb	antibody to Hepatitis B surface antigen
HBsAg	hepatitis B surface antigen
HCG	human chorionic gonadotropin
HCV	hepatitis C virus
HCMV	human cytomegalovirus
HDLC-C	High-density lipoprotein cholesterol
HEENT	head, eyes, ears, nose, and throat
HGF	hepatocyte growth factor
HGF-X7	hybrid hepatic growth factor coding sequence expressing two isoforms of HGF, HGF_{728} and HGF_{723}
HHD	Handheld Dynamometer
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HTLV	human T cell lymphotropic virus
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ID	identifier
IL-6	interleukin 6
IM	intramuscular
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ISR	Injection site reaction
IV	intravenous
LLOQ	lower limit of quantitation
LMN	lower motor neurons
MCP-1	monocyte chemoattractant protein 1
MRC	Medical Research Council
NCS	not clinically significant
NDA	New Drug Application
NHU	nonhealing foot ulcers
NIH	National Institutes of Health
NMDA	N-methyl-D-aspartate

Abbreviation	Definition
NO	nitrous oxide
NOAEL	No observed adverse event level
NSAID	non-steroidal anti-inflammatory drug
OSP	Office of Science Policy, National Institutes of Health
PAD	peripheral arterial disease
PE	physical examination
PGE2	prostaglandin E2
PGIC	Patient Global Impression of Change
PHI	protected health information
PRO	patient reported outcome
PT	prothrombin time
PTT	partial prothrombin time
QC	quality control
QoL	Quality of Life
RNA	ribonucleic acid
SAE	serious adverse event
SAP	Statistical Analysis Plan
SoA	Schedule of Activities
SOD1	superoxide dismutase 1
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
SVC	Slow Vital Capacity
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TNF	tumor necrosis factor
UMN	upper motor neuron
VEGF	vascular endothelial growth factor
WFI	water for injection

10.4.2. Definitions

Active Infection	Chronic infection or severe active infection that may compromise the Participant's wellbeing or participation in the study, in the Investigator's judgment
Day 14, etc.	Day 14 (for example) refers specifically to the actual day of the Visit designated as the Day 14 Visit, and not to the 14th day of the study for a Participant.
End of Study	Completion of the last Visit or procedure shown in the Schedule of Activities for the last Participant remaining in the study

Highly Effective Contraception Method	See Contraceptive Guidance
Injection Site Reaction	An AE located in close proximity to the injection sites on the target muscles and first observed within 48 hr following Study Injections
Investigator	The PI or appropriate study site personnel whom the PI designates to perform a certain duty
Placebo	Placebo comprises 45 mg sodium chloride and 55 mg sucrose in 5 mL water for injection. Engensis vials contain the same excipients.
Safety Analysis Population	All Participants who receive at least one Study Injection
Sponsor	Helixmith Co., Ltd. and its representatives contracted to provide services for study conduct
Study Drug	Engensis or Placebo
Study Injection	Injection of Engensis or Placebo
Participant	Anyone who is consented for Screening
Treatment Arm	The group of Participants assigned to receive Study Drug or the group assigned to receive Placebo during the first 6 months of treatment
Treatment Cycle	Two sets of Study Injections 14 days apart comprising a single dose
VM202	The active pharmaceutical ingredient of Engensis: a novel genomic complementary deoxyribonucleic acid (cDNA) hybrid human hepatocyte growth factor (HGF) coding sequence (HGF-X7) expressing two isoforms of HGF, HGF_{728} and HGF_{723}



10.6. Appendix 6. Revised Amyotrophic Lateral Sclerosis Functional Rating Scale

Bulbar	Fine Motor	Gross Motor	Breathing

1. Speech
4. Normal speech processes
3. Detectable speech disturbance
2. Intelligible with repeating
1. Speech combined with nonvocal communication
0. Loss of useful speech

2. Salivation
4. Normal
3. Slight but definite excess of saliva in mouth; may have nighttime drooling
2. Moderately excessive saliva; may have minimal drooling
1. Marked excess of saliva with some drooling
0. Marked drooling; requires constant tissue or handkerchief

3. Swallowing
4. Normal eating habits
3. Early eating problems-occasional choking
2. Dietary consistency changes
1. Needs supplemental tube feeding
0. NPO (exclusively parenteral or enteral feeding)

4. Handwriting
4. Normal
3. Slow or sloppy; all words are legible
2. Not all words are legible
1. Able to grip pen but unable to write
0. Unable to grip pen

5a. Cutting Food / Handling Utensils
4. Normal
3. Somewhat slow and clumsy, but no help needed
2. Can cut most foods, although clumsy and slow; some help needed
1. Food must be cut by someone, but can still feed slowly
0. Needs to be fed

5b. Cutting Food / Handling Utensils (Alt. for patients with Gastrostomy)
4. Normal
3. Clumsy but able to perform all manipulations independently
2. Some help needed with closures and fasteners
1. Provides minimal assistance to caregiver
0. Unable to perform any aspect of task

6. Dressing and hygiene
4. Normal function
3. Independent and complete self-care with effort or decreased efficiency
2. Intermittent assistance or substitute methods
1. Needs attendant for self-care
0. Total dependence

7. Turning in bed
4. Normal
3. Somewhat slow and clumsy, but no help needed
2. Can turn alone or adjust sheets, but with great difficulty
1. Can initiate, but not turn or adjust sheets alone
0. Helpless

8. Walking
4. Normal
3. Early ambulation difficulties
2. Walks with assistance
1. Non-ambulatory functional movement only
0. No purposeful leg movement

9. Climbing stairs
4. Normal
3. Slow
2. Mild unsteadiness or fatigue
1. Needs assistance
0. Cannot do

10. Dyspnea
4. None
3. Occurs when walking
2. Occurs with one or more of the following: eating, bathing, dressing (ADL)
1. Occurs at rest, difficulty breathing when either sitting or lying
0. Significant difficulty, considering using mechanical respiratory support

11. Orthopnea
4. None
3. Some difficulty sleeping at night due to shortness of breath. Does not routinely use more than two pillows
2. Needs extra pillow in order to sleep (more than two)
1. Can only sleep sitting up
0. Unable to sleep

12. Respiratory insufficiency
4. None
3. Intermittent use of BiPAP
2. Continuous use of BiPAP
1. Continuous use of BiPAP during the night and day
0. Invasive mechanical ventilation by intubation or tracheostomy

10.7. Appendix 7. Amyotrophic Lateral Sclerosis Assessment Questionnaire

The following is an example of the ALSAQ-40

Please complete this questionnaire as soon as possible. If you have any difficulties filling in the questionnaire by yourself, please get someone else to help you with it. 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 tick the box which best describes your own experience or feelings.

Please try to answer every question even though some may seem rather similar to others or may not seem relevant to you.

All the information you give will be treated in the strictest confidence.

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

Clinical Impression of Change Scale

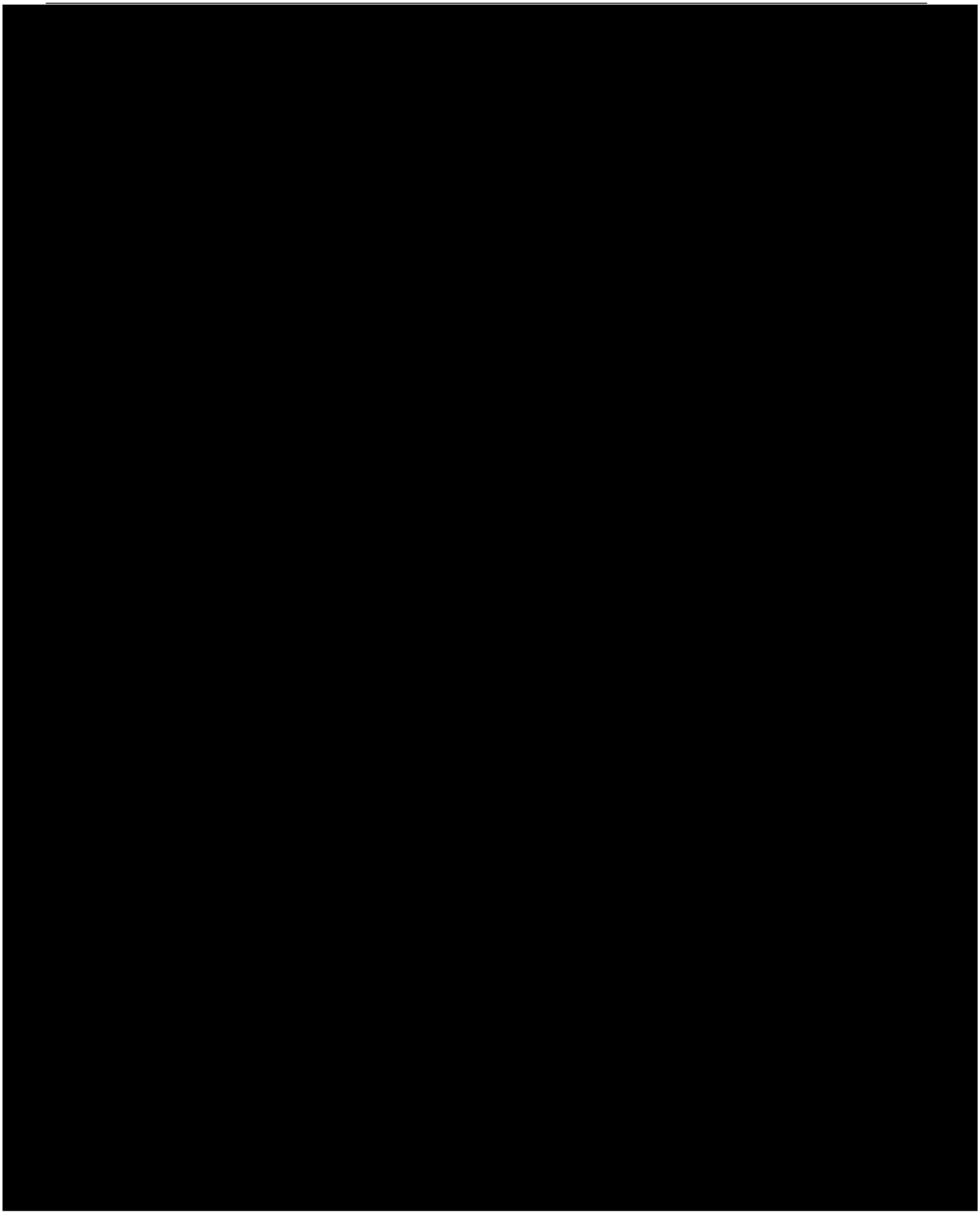
1. CGIC - Global Improvement: Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment. Compared to his/her condition at admission to the project, how much has he/she changed?

0 = Not assessed
1 = Very much improved
2 = Much improved
3 = Minimally improved
4 = No change
5 = Minimally worse
6 = Much worse
7 = Very much worse

2. Efficacy index: Rate this item on the basis of **drug effect only**.
Select the terms which best describe the degrees of therapeutic effect and side effects and record the number in the box where the two items intersect.
EXAMPLE: Therapeutic effect is rated as 'Moderate' and side effects are judged 'Do not significantly interfere with patient's functioning'.

Therapeutic effect		**Side effects**			
		None	*Does not significantly interfere with patient's functioning*	*Significantly interferes with patient's functioning*	*Outweighs therapeutic effect*
Marked	Vast improvement. Complete or nearly complete remission of all symptoms	01	02	03	04
Moderate	Decided Improvement. Partial remission of symptoms	05	06	07	08
Minimal	Slight improvement which doesn't alter status of care of patient	09	10	11	12
Unchanged or worse		13	14	15	16

Not assessed = 00



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