HELIXMITH

Protocol Title:	**A 6-Month Extension Study Following Protocol VMDN-003-2 – An Adaptive, Phase 3, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study to Assess the Safety and Efficacy of Engensis in Participants with Painful Diabetic Peripheral Neuropathy**
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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]

Date (DD-MMM-YYYY)

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1. Protocol Summary

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1.1. Synopsis

Protocol Title: A 6-Month Extension Study Following Protocol VMDN-003-2 – An Adaptive, Phase 3, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study to Assess the Safety and Efficacy of Engensis in Participants with Painful Diabetic Peripheral Neuropathy

Short Title: A 6-Month Phase 3 Extension of a Study to Assess Safety and Efficacy of Engensis in Painful Diabetic Peripheral Neuropathy

Rationale:

The purpose of this 6-month extension study (VMDN-003-2b) is to evaluate the durability of efficacy and long-term safety of intramuscular (IM) administration of Engensis or Placebo that was administered in the double-blind, randomized, VMDN-003-2 Placebo-controlled Phase 3 Study. No treatments will be administered in this VMDN-003-2b extension study. The combined overall duration of the VMDN-003-2 and VMDN-003-2b studies will be 12 months.

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 painful DPN.

In the VMDN-003-2 main study, Participants will have received Engensis or Placebo by IM injections in both legs (in the calf gastrocnemius muscles) during two Treatment Cycles (Treatment Cycle 1 on Days 0 and 14 and Treatment Cycle 2 on Days 90 and 104).

Data from Study VMDN-003-2 assessments at or prior to the Day 0, Day 104, and Day 180 Visits for Participants who enroll in Study VMDN-003-2b will be used as reference points for changes in efficacy parameters and for safety assessments during this extension study.

Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the efficacy of IM administration of Engensis on pain in participants with painful DPN in the feet and lower legs as compared to Placebo	• Change in the means of the Average Daily Pain Scores (ADPSs) from the full Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 365 Visit in the intent-to-treat (ITT) population

Objectives	Endpoints
Secondary Efficacy:	
• To evaluate the efficacy of IM administration of Engensis on the worst pain in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Change in the means of the Worst Pain scores from the full BPI-DPN from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
• To evaluate the efficacy of IM administration of Engensis on reducing pain in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Proportion of Responders (≥ 50% reduction in the ADPSs from the full BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
Secondary Safety:	
• To evaluate the safety of IM administration of Engensis in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Incidence of treatment-emergent adverse events (TEAEs) and treatment-emergent serious adverse events (TESAEs) for Engensis compared to Placebo • Incidence of clinically significant laboratory values for Engensis compared to Placebo
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]

Objectives	Endpoints
[redacted]	[redacted]

Overall Design

VMDN-003-2b is a 6-month extension study following Study VMDN-003-2, which is an adaptive, Phase 3, double-blind, randomized, placebo-controlled, multicenter study designed to assess the efficacy and safety of Engensis (containing the active pharmaceutical ingredient VM202) in Participants with painful DPN.

The purpose of this study is to assess the durability of efficacy and long-term safety of Engensis compared to Placebo as measured by changes in Average Daily Pain Score (ADPS) of the full Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN), Bedside Sensory Testing (BST), physical examinations, laboratory assessments, vital signs, treatment-emergent adverse events (TEAEs), treatment-emergent serious adverse events (TESAEs), and adverse events of special interest (AESIs).

Participants will be enrolled in the VMDN-003-2b study at completion of the Day 180 Visit of Study VMDN-003-2. Participants will continue to be identified by the same Participant number and the same treatment group (Engensis or Placebo) assigned by randomization in Study VMDN-003-2. No study drug or treatment will be administered in this VMDN-003-2b extension study. The double-blind treatment assignment from the prior study will be maintained for Investigators and Participants during this extension study.

Assessments to be conducted during this VMDN-003-2b extension study are as follows: vital signs, weight, BMI, physical examination, retinal fundoscopy (by an ophthalmologist), 12-lead electrocardiogram (ECG), laboratory assessments, HBA1c levels, concomitant medications and procedures, urine drug analysis, urine pregnancy test for females of childbearing potential, Accurate Pain Reporting (APR), Placebo Response Reduction (PRR), the Michigan Neuropathy Screening Instrument (MNSI), partial and full BPI-DPN, BST, and quality of life instruments (36-item Short Form Health Survey [SF-36] and EuroQol Health Utilities Index [EQ-5D]).

Assessments of TEAEs, TESAEs, AESIs, and clinically significant laboratory values will start as of the Day 180 Visit of Study VMDN-003-2 and continue throughout the VMDN-003-2b extension study through Day 365/ET.

During the last 7 days prior to the Day 270 and Day 365/ET Visits, Participants must complete the full BPI-DPN on an ePRO for determining the ADPS for at least 5 out of the last 7 days.

Follow-up Study Visits will be conducted on Days 270, 300, and 365/ET. At the Day 365/ET Visit (end of study), the following assessments will be conducted: the full BPI-DPN (performed during the 7 days prior to the Visit), MNSI, BST, Patient Global Impression of Change (PGIC), SF-36 and EQ-5D quality of life assessments, urine drug analysis, retinal fundoscopy, physical examination, concomitant medications and procedures, TEAEs, and AESIs. Blood will be drawn for determination of serum chemistry, lipid profile, pregnancy status, hematology, and HbA1c levels.

Study and Treatment Duration:

The timing for visits in the VMDN-003-2b study starts at the conclusion of the Day 180 Visit in Study VMDN-003-2. Participants in Study VMDN-003-2b will be followed from the Day 180 Visit (VMDN-003-2) to Day 365/ET. The VMDN-003-2b study duration is 6 months.

No study drug or treatment will be administered in Study VMDN-003-2b.

Visit Frequency:

There will be 3 visits to the Clinical Site during the extension study (after the Day 180 Visit from Study VMDN-003-2) on Days 270, 300, and 365/ET.

Intervention Groups and Duration:

Two treatment groups of Participants (Engensis or Placebo) from Study VMDN-003-2 will be followed in a double-blind fashion from Day 180 Visit (Study VMDN-003-2) through Day 365/ET of this extension protocol. No study drug (Engensis or Placebo) will be administered in the VMDN-003-2b study.

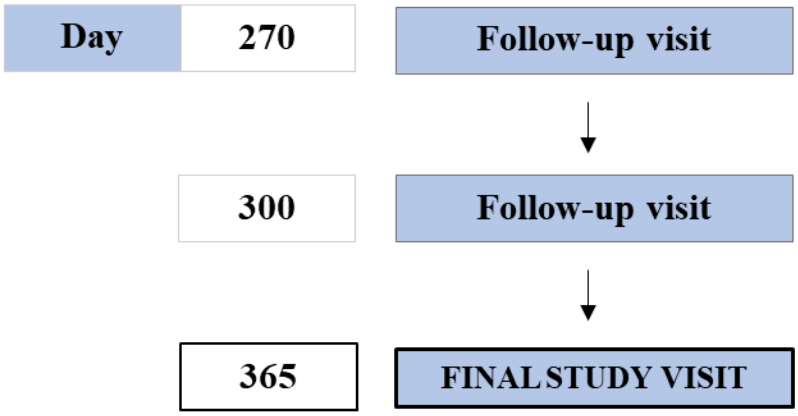
Number of Participants:

The number of Participants to be enrolled in this 6-month extension study depends upon the number of subjects who complete the Day 180 Visit in Study VMDN-003-2 and provide written informed consent for this extension protocol (prior to or during their Day 180 Visit in the VMDN-003-2 study).

Data Safety Monitoring Board:

The independent Data Safety Monitoring Board (DSMB) will periodically review a limited set of unblinded (noncomparative) tables and/or listings, including all reported TEAEs, TESAEs, and AESIs.

1.2. Study Schema



1.3. Schedule of Activities (SoA)

	Study Days[a]:	Follow-up Visits		End of Study/ET
		Day 270	Day 300	Day 365/ET
	Visit Window (days):	± 7	± 7	± 7
Informed Consent (obtained prior to or during the Participant's Day 180 Visit in Study VMDN-003-2)				
1	Vital Signs, Weight	X	X	X
2	Complete Physical Examination	X	X	X
3	Retinal Fundoscopy	X		X
4	12-Lead Electrocardiogram	X		X
5	Serum Chemistry, Hematology, Lipid Profile	X		X
6	Urine Pregnancy Test			X
7	Urine Drug Analysis	X	X	X
8	HbA1c Levels			X
9	Concomitant Medications and Procedures	X	X	X
10	Accurate Pain Reporting (APR), Placebo Response Reduction (PRR)	X	X	X
11	Full Brief Pain Inventory-Diabetic Peripheral Neuropathy (BPI-DPN)	X		X
12	Partial BPI-DPN (ePRO)	←	—	→
13	Quality of Life (SF-36, EQ-5D)	X		X
14	Patient Global Impression of Change (PGIC)	X		X
15	Treatment-emergent Adverse Events (TEAEs)	←	—	→
	Treatment-emergent Serious Adverse Events (TESAEs)	←	—	→
	Adverse Events of Special Interest (AESIs)	←	—	→
16	Michigan Neuropathy Screening Instrument (MNSI)	X		X
17	Bedside Sensory Testing (BST)	X		X

FOOTNOTES FOR SCHEDULE OF ACTIVITIES

***Schedule of Activities** includes activities during Screening and all Study Visits. Additional data from daily ePRO entries will be transferred directly into a study database.

Abbreviations: AESI = adverse event of special interest; APR = Accurate Pain Reporting; BMI = Body Mass Index; BPI = Brief Pain Inventory; BST = Bedside Sensory Testing; D = Day; DPN = diabetic peripheral neuropathy; ePRO = electronic diary; ET = Early Termination; EQ-5D = EuroQol Health Utilities Index; HbA1c = hemoglobin A1c; MNSI = Michigan Neuropathy Screening Instrument (MNSI); PGIC = Patient Global Impression of Change; PRR = Placebo Response Reduction; SF-36 = 36-Item Short Form Health Survey; TEAE = treatment-emergent adverse event; TESAE = treatment-emergent serious adverse event

[a] Study days for this extension protocol are based on the time (in days) from Day 0 in Study VMDN-003-2.

1. **Vital Signs and Weight:** Vital signs and weight will be measured on Days 270, 300, and 365/ET. 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.

2. **Complete Physical Examinations** will be performed on Days 270, 300, and 365/ET. The examination will include the following: head, eyes, ears, nose, and throat (HEENT); heart; lungs; abdomen; extremities; lymph nodes; neurological; 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 using appropriate medical terminology.
3. **Retinal Fundoscopy** will be performed by an ophthalmologist up to 30 days before the Day 270 and 365 Visits (and, if possible, upon Early Termination if the Participant discontinues prior to the Day 365 Visit). Results of the fundoscopy examinations at the Day 270 and Day 365 Visits compared to results at the Day 180 Visit (Study VMDN-003-2) will be assessed by the Investigator.
4. **12-Lead Electrocardiogram (ECG)** will be performed on Days 270 and 365/ET. The ECG recording and interpretation will be printed out and stored with the Participant's records. Any newly identified abnormalities are to be recorded as adverse events.
5. **Serum Chemistry, Hematology, and Lipid Profile:** These laboratory assessments will be evaluated on Days 270 and 365/ET.
    - **Chemistry** includes sodium, potassium, chloride, bicarbonate, calcium, inorganic phosphate, magnesium, glucose, amylase, lipase, creatine kinase, lactate dehydrogenase, and
        - Kidney function tests (blood urea nitrogen, creatinine)
        - 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
    - **Lipid Profile** includes total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides
6. **Urine Pregnancy Test:** Pregnancy tests for women of childbearing potential will be conducted in the clinic on Day 365/ET.
7. **Urine Drug Analysis** includes amphetamines (methamphetamine), barbiturates, benzodiazepines, buprenorphine, cannabinoid (THC), cocaine, oxycodone, methadone, opiates, phencyclidine (PCP), tricyclics (TCA), and ethyl alcohol. Cannabinoid detection is not an exclusion criterion but may be used in exploratory analyses. The urine drug analyses will be conducted on Days 270, 300, and 365/ET. Drug analyses will be performed by the central laboratory.
8. **HbA1c** levels will be measured on Day 365/ET.
9. **Concomitant Medications and Procedures**: All medications or vaccines, including over-the-counter or prescription medicines, vitamins, herbal supplements, and procedures that the Participant receives will be recorded on Days 270, 300, and 365/ET. For each medication, treatment, or procedure, the following information will be collected: Medication trade or generic name or type of procedure, indication, start date, stop date or ongoing, dose, units, frequency, and route.
10. **Accurate Pain Reporting (APR):** The Accurate Pain Reporting training will be conducted on Days 270, 300, and 365/ET.
    Placebo Response Reduction (PRR): The Placebo Response Reduction training will be conducted on Days 270, 300, and 365/ET.
11. **Brief Pain Inventory (BPI-DPN) – Full Questionnaire** will be administered through the ePRO daily starting at 7 days prior to Day 270 and Day 365/ET. The mean of the Average Daily Pain Scores (ADPSs, used for the primary endpoint) is the mean from a minimum of 5 ADPSs recorded in the 7 days prior to the Days 270 and 365/ET Visits. If the ePRO contains fewer than 5 daily entries over the 7 days prior to the Day 270 and Day 365/ET Visits, the visit must be rescheduled (up to 3 days later) so that the Participant can record 5 daily entries over the 7 days.
12. **Brief Pain Inventory (BPI) - Partial Questionnaire** (questions 3, 5, and 9a from the full questionnaire) will be completed on the ePRO every day that the full BPI is *not* conducted from the Day 180 Visit (VMDN-003-2, Initial Visit for this extension study) to the Day 365/ET Visit.

13. **Quality of Life Questionnaires (SF-36, EQ-5D)** will be administered on Day 270 and Day 365/ET. The study coordinator will check the questionnaires for completeness. Any omissions or ambiguous answers will be clarified by the Participant prior to leaving the clinic.
14. **Patient Global Impression of Change (PGIC)** will be administered on Days 270 and 365/ET.
15. **Treatment-emergent Adverse Events (TEAEs)** will be monitored throughout the study and will be recorded after from the Day 180 Visit (VMDN-003-2) until Day 365/ET in this extension study. Any TEAEs that started during Study VMDN-003-2 and were ongoing at the Day 180 Visit will continue to be followed after Day 180 during this extension protocol. Adverse events that occur in this protocol will be referred to as TEAEs.
    Treatment-emergent Serious Adverse Events (TESAEs) will be recorded and reported to the Sponsor within 24 hours of awareness of the TESAE by the Site.
    Adverse Events of Special Interest (AESIs): All AESIs will be continuously monitored throughout the study.
16. **Michigan Neuropathy Screening Instrument (MNSI)** will be administered on Day 270 and Day 365/ET for assessing disease progression during the study.
17. **Bedside Sensory Testing (BST)** will be administered on Day 270 and Day 365/ET to provide additional information that will allow exploratory analysis of BST sensory profiles.

2. INTRODUCTION

Engensis is a novel gene therapy treatment being developed for prolonged treatment of patients with painful diabetic peripheral neuropathy (DPN). 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 well as a neurotrophic factor promoting axonal growth and regeneration in diabetics in whom loss of microvasculature may accelerate neuronal loss (see the Investigator's Brochure [IB] for more detailed information).

Compared with alternate modes of delivery of exogenous HGF into the systemic circulation, which is impeded by rapid clearance by the liver, direct administration of the plasmid DNA in Engensis into target tissues is followed by expression of HGF in situ, which results in a lowered risk of genomic integration (particularly in skeletal muscle tissue) and apparent lack of immune responses (such as observed with viral vector delivery systems), making it an attractive option for local targeted delivery. In addition, Engensis contains biological agents (promoters and controllers of gene transcription), including the unique plasma vector pCK that makes the expression from skeletal muscle more efficient (see the IB for full description of additional activities identified for Engensis). Engensis is detectable and expresses HGF for up to 30 days after direct skeletal muscle injection.

2.1. Study Rationale

Diabetic peripheral neuropathy is a bilateral neuropathy. Based on the favorable safety profile of Engensis observed in the Phase 1 and Phase 2 critical limb ischemia (CLI) studies, preliminary efficacy data from the Phase 1/2 studies, and safety data from the Phase 1/2 studies and the Phase 3 studies of Engensis in Participants with DPN, we propose continued follow-up of bilateral treatment of the feet and lower legs in this Phase 3 extension study.

The purpose of this study is to further evaluate the durability of efficacy on pain and the long-term safety of IM administration of Engensis in Participants with painful DPN in the feet and lower legs, as compared to Placebo, in this 6-month extension in Participants who complete the Day 180 Visit in Study VMDN-003-2.

Engensis will be tested against Placebo. There will be no study drug administration in this VMDN-003-2b extension study.

2.2. Background

2.2.1. Pathophysiology of Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy (DPN) is a bilateral, particularly debilitating complication of diabetes characterized by pain that is burning, lancinating, tingling, or shooting (electric shock-like), occurring with paresthesia, and is typically worse at night. The pain can either be triggered by an external stimulus or be independent of external input. Unlike other painful sensations, which signal a warning in response to a harmful stimulus, neuropathic pain is maladaptive. Diabetic peripheral neuropathy accounts for significant morbidity by predisposing the foot to ulceration and lower extremity amputation.

Diabetic peripheral neuropathy manifests as three broad categories: sensory, motor, and autonomic, with the most prevalent form being somatic or sensorimotor neuropathy. Symptoms (e.g., burning, tingling, stabbing, or pins-and-needles) often exhibit a distal symmetric pattern, beginning at the base of the toes and ascending proximally up the lower leg as the disease progresses. Patients may also display muscle weakness, lack of coordination and ataxia, and loss of pain perception. Loss of protective sensation can lead to the formation of foot ulcerations, infections, and amputations.

The sequence of physiological events that result in DPN is poorly understood. The pathogenesis of diabetic neuropathy likely involves the interplay of hyperglycemia, ischemia, and oxidative stress. Figure 1 portrays the relationship of hyperglycemia to oxidative stress, metabolic alterations, vascular dysfunction, and neural damage.

Figure 1 The Neurodestructive Effects of Hyperglycemia

Increased polyol pathway flux. Hyperglycemia causes increased levels of intracellular glucose in nerves, leading to saturation of the glycolytic pathway. Extra glucose is shunted into the polyol pathway and converted to sorbitol and fructose by aldose reductase and sorbitol dehydrogenase, respectively. Accumulation of sorbitol and fructose leads to reduced nerve myoinositol, decreased membrane Na^+/K^+-ATPase activity, impaired axonal transport, and structural breakdown of nerves, causing abnormal action potential propagation.[1]

Non-enzymatic protein glycation. Advanced glycation end products occur because of nonenzymatic addition of glucose or other saccharides to proteins, lipids, and nucleotides. In diabetes, excess glucose accelerates advanced glycation end-product generation that leads to intracellular and extracellular protein cross-linking and protein aggregation. Activation of the advanced glycation end-product receptor alters intracellular signaling and gene expression, releases proinflammatory molecules, and results in an increased production of reactive oxygen species (ROS) that contribute to diabetic microvascular complications.

Oxidative Stress. Glucose can cause significant oxidative stress and damage when present in excess. Under physiological conditions, aerobic respiration is associated with the formation of a small number of free radicals or ROS. In a hyperglycemic state, however, particularly in endothelial cells, which do not have the ability to limit glucose entry into the cell, glucose accumulation exceeds the levels that glycolytic enzymes can handle.[2,3] The flood of excess glucose into endothelial cells is shunted into alternate metabolic pathways (e.g., polyol pathway, glycosylation, hexosamine pathway, the diacylglycerol activation of protein kinase C [PKC]). These pathways, particularly PKC, produce significantly larger amounts of ROS than aerobic respiration, overwhelming compensatory antioxidant mechanisms.[4,5,6,7,8,9,10,11] The resulting hyperglycemic oxidative stress contributes to endothelial dysfunction by inhibiting endothelial nitric oxide production and by initiating and promoting the deposition of modified lipids in the subendothelium. These factors accelerate atherosclerotic macrovascular disease and are associated with the development of apoptosis in neurons and supporting glial cells.[12]

Vascular Damage. Nerve tissue depends on adequate blood flow to deliver nutrients and remove metabolic waste. Normally, the capillary basement membrane allows the passage of nutrients into the cell and permits the removal of waste products. In patients with prolonged hyperglycemia, glucose is more likely to be deposited in the basement membrane, thus decreasing its permeability. Decreased permeability results in the buildup of toxic metabolites, resulting in poor cellular metabolism, further free radical formation, apoptosis, and a decline in vascularization of nervous tissues.

2.2.2. Current Treatment Options

Currently no approved drugs or interventional strategies are known to halt or reverse the progression of DPN. Treatments target pain reduction, physical function improvement, reduction of psychological distress, and quality of life improvements.

Long-term complications of both type 1 and type 2 diabetes can be reduced by tight glycemic control. To date, this is the only intervention specifically shown to arrest or postpone the onset and severity of peripheral neuropathy. The consequences of DPN can be ameliorated by the education of patients and their care providers as to foot care, proper footwear, and of hyposensitive areas and pressure points, all to prevent the occurrence of ulcers and to lower the risk of infection of soft tissue and bone.[13]

The incidence of neuropathy is also associated with potentially modifiable cardiovascular risk factors, including an elevated triglyceride level, a high body mass index (BMI), smoking, and hypertension.

Only three drugs have been approved by the Food and Drug Administration (FDA) specifically for the treatment of the symptoms of DPN: Nucynta ER (tapentadol), an opioid medication; Cymbalta (duloxetine), a serotonin and norepinephrine reuptake inhibitor; and Lyrica (pregabalin), an anticonvulsant drug. All are prescribed for the management of pain associated with DPN.

See the IB for presentation of guidelines issued by international organizations for DPN treatment options.

Another treatment option is α-lipoic acid, a naturally occurring antioxidant compound found in spinach and broccoli. α-Lipoic acid was recently studied in a multicenter, placebo-controlled

study of Participants with type 2 diabetes and symptomatic neuropathy. One hundred eighty-one (181) Participants were given daily oral doses of 600, 1200, or 1800 mg of α-lipoic acid or placebo. After 5 weeks, neuropathic symptoms improved in those Participants who received α-lipoic acid. The 600-mg dose appeared to provide the optimum risk-to-benefit ratio.[14]

There are also invasive treatment options. One option is surgical decompression of the lower-extremity peripheral nerves in patients with DPN but this is still considered an experimental intervention (see IB for details). Pancreatic transplantation in patients with diabetes can stabilize neuropathy and, in some instances, improve motor, sensory, and autonomic function.

Other treatments include off-label use of several drugs and devices. Drugs commonly prescribed to manage pain symptoms include tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin–norepinephrine reuptake inhibitors (SNRIs). Other treatment strategies such as acupuncture, transcutaneous electrical nerve stimulation (TENS), capsaicin patches and creams, topical anesthetics, and isosorbide dinitrate (ISDN) spray may also be used. Each of these can alleviate some of the pain symptoms. None demonstrate any ability to improve the underlying neuropathy.

2.2.3. Unmet Clinical Need

Diabetic peripheral neuropathy is a serious complication of diabetes mellitus (DM), with painful DPN being a frequent manifestation of neuropathy. Tight glycemic control and treatment of cardiovascular risk factors are effective preventive measures, but DPN remains the most common complication of DM, affecting 60% to 70% of diabetics. Progressive DPN may result in loss of function in affected legs, serious infection complications, and amputation.

Current treatments provide symptomatic relief of the pain associated with DPN. A clear unmet medical need exists for treatments that address the underlying pathology of DPN and prevent the progressive destruction and loss of function associated with this disease.

2.2.4. Hepatocyte Growth Factor for the Treatment of Diabetic Neuropathy

Hepatocyte growth factor (HGF) is a multifunctional protein with highly potent angiogenic and neurotrophic properties produced by various cell types of mesenchymal origin such as fibroblasts, macrophages, and stromal cells.[15,16,17,18,19] Its biological function is mediated by its receptor c-Met, which is a member of the receptor tyrosine kinase family. In the peripheral nervous system and its associated tissues, c-Met is expressed in sensory neurons, Schwann cells, endothelial cells, and smooth muscle cells among others,[20,21] which all play important roles in the functions of the peripheral nerve and its regeneration after pathological insults. Indeed, HGF has been reported to act on sensory and motor neurons to enhance their survivability and neurite outgrowth, and also produces a neuroprotective effect in injured sensory neurons.[22,23,24,25,26] Local application of exogenous HGF has been shown to promote the growth of sympathetic neurons,[27,28,29,30,31,32] and to induce the formation of collateral vessels and increased blood flow both in diabetic and non-diabetic rat models.[33]

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 in rodent blood of less than 15 minutes.[34] One approach to maintaining a

durable supply of HGF in target tissues is to develop a gene transfer strategy that would allow for persistent expression of HGF protein in vivo. Consistent with this, liposome-mediated non-viral gene transfer of HGF effectively reversed various pathological changes including allodynia, hyperalgesia, and reduction of blood flow in rat diabetic and non-diabetic neuropathic models.[28,31] Unlike previous transfer models, Engensis does not contain any transfection agents. Although it is administered as a simple, naked plasmid DNA, IM injection of Engensis demonstrated both pain-relieving effects and nerve regeneration activities in animal models.

2.2.5. Engensis

[redacted]

administration of Engensis. Significant improvements in pain were detected in Participants with

[redacted]

2.3. Benefit/Risk Assessment

2.3.1. Risk Assessment

The risks for the study medication and study procedures are summarized in the following table.

Table 1 Potential Risks of Clinical Significance

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risks of Engensis		
Eye disorders	Eye disorders were observed in both Engensis and placebo Participants, including diabetic retinopathy, which was observed at a higher incidence in the placebo arm.	Presence of TEAEs will be assessed on Days 270, 300, and 365/ET to detect new or worsening prior eye disorders. Retinal fundoscopy will be performed on Days 270 and 365/ET

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Angiogenesis and promotion of tumor growth (cancer)	Because HGF has the potential to create new blood vessels (angiogenesis), there may be a risk of promoting tumor growth (cancer).	During Screening for Study VMDN-003-2, all Participants will have undergone cancer screening to minimize randomizing patients at increased risk for cancer or who have subclinical (no overt signs or symptoms) cancer.
	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.	During Screening, Participants diagnosed to have had a cerebrovascular accident or myocardial infarction within 3 months before Screening will be excluded in addition to Participants with Class 3 or 4 heart failure.
Risks of Study Procedures		
Complications of venipuncture (for blood draws for laboratory tests)	Venipuncture complications include minor bruising, hypotension, and syncope.	Incidences of adverse events of bruising, hypotension, and syncope will be closely monitored throughout the study.

Adverse Events in Prior Clinical Studies

Engensis has been well tolerated in all clinical studies conducted thus far. The preponderant adverse events have consisted of mild, transient injection site reactions, including itching, erythema, pain, and muscle spasms. Other TEAEs that have been reported were generally mild to moderate. Refer to the IB for a table of AEs that occurred in $\geq$ 2% of trial Participants.

Five serious AEs (SAEs) have been reported to date as possibly related to study treatment:

- colon cancer in the Phase 1 trial of CLI (assessed by the investigator to be possibly related to study treatment and, even though the sponsor noted that the Participant had a history of colon polyps, a relationship could not be ruled out)
- peroneal deep vein thrombosis (categorized as possibly related to the study injection) in the Phase 2 trial of CLI. The mechanism by which HGF or VM202 could be causative in the latter is not established

SAEs reported as possibly related in the VMDN-003 trial include:

- vitreous hemorrhage of the eye (categorized as possibly related to the study drug but was probably related to underlying disease)
- myocardial infarction (categorized by Helixmith as possibly related to the study drug, although categorized by the investigator as not related to the study drug)
- adenocarcinoma (categorized as possibly related to the study drug by the investigator and not related to the study drug by Helixmith)

2.3.2. Benefit Assessment

Diabetic peripheral neuropathy is one of the most-commonly encountered neuropathic pain syndromes and affects nearly half of all diabetic patients. Through its effects on motor or sensorimotor neurons, disabling consequences on function and quality of life predominate that include sleep disturbances, dependence on narcotic analgesics for pain, as well as trauma and infection from sensory impairment.

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 diabetic patients, as suggested by preceding studies.

2.3.3. Overall Benefit/Risk Conclusions

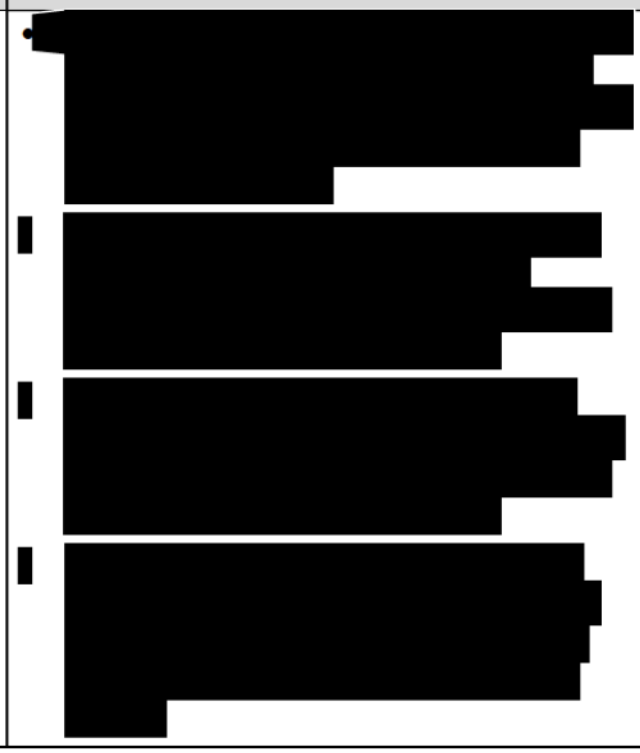
The overall profile of potential benefits and apparent safety for Engensis provide sufficient support to justify the conduct of the present study in its aim to meet the unmet needs of DPN patients.

3. OBJECTIVES AND ENDPOINTS

Data from Study VMDN-003-2 assessments at or prior to the Day 0, Day 104, and Day 180 Visits for Participants who enroll in Study VMDN-003-2b will be used as reference points for changes in efficacy parameters and for safety assessments during this extension study.

Objectives	Endpoints
Primary	
• To evaluate the efficacy of IM administration of Engensis on pain in participants with painful DPN in the feet and lower legs as compared to Placebo	• Change in the means of the Average Daily Pain Scores (ADPSs) from the full Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 365 Visit in the intent-to-treat (ITT) population
Secondary Efficacy:	
• To evaluate the efficacy of IM administration of Engensis on the worst pain in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Change in the means of the Worst Pain scores from the full BPI-DPN from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
• To evaluate the efficacy of IM administration of Engensis on reducing pain in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Proportion of Responders (≥ 50% reduction in the ADPSs from the full BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
Secondary Safety:	
• To evaluate the safety of IM administration of Engensis in Participants with painful DPN in the feet and lower legs as compared to Placebo	• Incidence of treatment-emergent adverse events (TEAEs) and treatment-emergent serious adverse events (TESAEs) for Engensis compared to Placebo • Incidence of clinically significant laboratory values for Engensis compared to Placebo

Objectives	Endpoints
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted] [redacted] [redacted] full BPI-DPN from the 7 days prior to the [redacted] [redacted]

Objectives	Endpoints
	

4. STUDY DESIGN

4.1. Overall Design

VMDN-003-2b is a 6-month, Phase 3, double-blind, randomized, placebo-controlled, multicenter, extension study to the VMDN-003-2 study and is designed to assess the durability of efficacy and the long-term safety of Engensis (containing the active pharmaceutical ingredient VM202) in Participants with painful DPN as compared to Placebo. No Participants in this VMDN-003-2b Study extension study will receive study drug. Refer to the SoA in Section 1.3 for timing of all assessments and treatments during this extension study.

4.2. Scientific Rationale for Study Design

VMDN-003-2 is designed as a well-controlled Phase 3 study, as it is a double-blind, randomized, placebo-controlled, and multicenter study that is sufficiently sized in numbers of Participants to evaluate the potential ability of Engensis to reduce neuropathic pain and disability in Participants with painful DPN. VMDN-003-2b is a 6-month follow-on to that study.

Collection of efficacy data will be conducted via e-Diaries, which will be used to remind Participants to record their efficacy data daily and in real time.

4.3. Participant Input into Design

Participant input into the design of this study was not collected, as the same methods were used in previous studies with Engensis and did not lead to unacceptable numbers of Participants discontinuing the studies. In addition, the methods used to assess pain reduction (BPI-DPN) and patient global impression of change (PGIC) have been validated and are the tools recommended by regulatory authorities to assess efficacy (pain reduction) and response (clinical benefit) in patients with painful DPN.

4.4. Justification for Dose

Participants will not be administered study drug in this VMDN-003-2b study.

4.5. 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 365/ET Visit or is discontinued.

4.6. Study Completion

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

4.7. Completed Participants

A Participant is considered to have completed the study if the Participant completes the Day 365/ET Visit and has at least 5 days of the most recent 7 days with electronic diary (eDiary) entries prior to the Day 365/ET Visit.

5. STUDY POPULATION

Participants for this 6-month extension study will come from the main study (Study VMDN-003-2) in which Participants complete the Day 180 Visit and give signed informed consent for the extension study.

The number of Participants who meet these qualifications will determine the number of Participants for this extension study.

5.1. Participant Identification

To maintain confidentiality, the name of the Participant should not be recorded on any study document other than the informed consent form and original medical records. All Participants who sign the informed consent form for the VMDN-003-2b study will continue to use the same unique identifier assigned to the Participant in the VMDN-003-2 study.

5.2. Lifestyle Considerations

5.2.1. Meals and Dietary Restrictions

There are no meal or dietary restrictions for this study.

5.2.2. Caffeine, Alcohol, and Tobacco

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

5.2.3. 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.

5.2.4. Activity

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

6. STUDY INTERVENTION

No study drug will be administered to Participants in this VMDN-003-2b extension study.

6.1. Measures to Minimize Bias

6.1.1. Randomization

There will be no randomization of Participants in this VMDN-003-2b extension study. All Participants who are entered into the VMDN-003-2b extension study will continue to be followed from the VMDN-003-02 study in a blinded fashion.

6.1.2. Stratification

There will be no stratification of Participants in the VMDN-003-2b extension study as all Participants who are entered into the VMDN-003-2b extension study are to continue in the stratification they were assigned in the VMDN-003-2 study.

6.1.3. Blinding

Participants, Investigators, Site staff, CRO staff, and Sponsor staff will remain blinded to treatment assignments except for the designated unblinded Clinical Research Associate (CRA) and the unblinded Medical Monitor.

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.

6.1.4. Maintenance of the Blind

The treatment assignment for each Participant that was determined by the Interactive Web Response System (IWRS) at randomization in the VMDN-003-2 study will be continued for each Participant in the VMDN-003-2b extension study.

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 designated unblinded 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.

6.2. Concomitant Therapy

All concomitant therapies should be captured in the Concomitant Therapy section of the eCRF.

6.2.1. Rescue Medicine

The only allowed rescue medication is acetaminophen in the VMDN-003-2b extension study. On Day 180 of the VMDN-003-2b extension study, the Site will dispense extra-strength acetaminophen (500 mg) provided by the Sponsor. The Participant will be instructed to take up to 2 caplets every 6 hours as needed for DPN pain for a maximum of 6 caplets (3 grams)/day. The study-issued acetaminophen is only to be used for DPN pain, and the Participant is not to use any other source of acetaminophen for DPN pain. The Participant may use up to 1 gram per day of a source of acetaminophen other than the study-issued Tylenol for conditions other than DPN pain. The Participant will be provided with an eDiary to record their pain level (NRS = 0-10 scale) before use, the date and time of use, and the amount of acetaminophen used. The Participant will be asked to return the eDiary and the unused rescue medication at each Visit. At each Visit, the Site will reconcile how many caplets were used, as documented in the eDiary, with how many caplets remain in the bottle(s). New bottle(s) of acetaminophen will then be dispensed by the Site to the Participant on Days 270 and 300 of this extension study.

6.3. Prohibited and Restricted Medications and Procedures

Prohibited medications and procedures are listed in Section 10.3, Appendix 3.

6.3.1. Medications That May Interfere with Engensis Bioactivity

Certain steroids (except inhaled, ocular, or intra-articular steroids) may interfere with the bioactivity of Engensis and are therefore prohibited from use during the study. Participants must agree to not take any of these drugs for the duration of the VMDN-003-2b extension study. A list of the prohibited medications in this category can be found in Section 10.3, Appendix 3.

6.3.2. Medications and Procedures That May Interfere with Assessment of Pain

Participants must refrain from use of these therapies for the duration of the VMDN-003-2b extension study:

- skeletal muscle relaxants
- opioids
- benzodiazepines (except for stable bedtime dose)
- injectable steroids
- capsaicin applied to the lower legs or feet
- local anesthetic creams and patches applied to the lower legs or feet (except local/topical anesthetic cream applied immediately prior to study drug injections
- ISDN spray applied to the lower legs or feet
- transcutaneous electrical nerve stimulation (TENS), acupuncture

A list of prohibited medications and procedures in this category can be found in Section 10.3, Appendix 3.

If not using a stable dose of duloxetine (Cymbalta), any antidepressants (e.g., amitriptyline and venlafaxine), or any other antiepileptics (e.g., valproic acid, carbamazepine, and vigabatrin)

during the VMDN-003-2 study, Participants must agree to not start these drugs during the VMDN-003-2b extension study. Participants on these medications on a stable dose during the VMDN-003-2 study are to continue these medications at the stable dose administered in the VMDN-003-2 study in the VMDN-003-2b extension study. Participants may not use pregabalin (Lyrica) or gabapentin (Neurontin) at any time during the VMDN-003-2b study.

6.4. Prior and Concomitant Medications, Treatments, and Procedures

Medications and treatments recorded at the Day 180 Visit of the VMDN-003-2 study will be migrated to the Medication and History eCRF of the VMDN-003-2b extension study. 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 VMDN-003-2b extension 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. Acetaminophen is the only rescue medication allowed in this study. Up to 3 grams per day will be allowed. Use of rescue medication will be recorded each day in the eDiary.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION

7.1. Participant Discontinuation/Withdrawal/Early Termination from the Study

Any Participant may voluntarily discontinue the VMDN-003-2b extension 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's 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, if possible, for Participants who discontinue prior to the VMDN-003-2b extension study Day 365/Early Termination Visit (ET; see Section 1.3, SoA).

- APR and PRR
- Full BPI-DPN
- MNSI
- BST
- PGIC
- SF-36 and EQ-5D
- Complete physical examination
- Vital signs, weight
- Retinal fundoscopy
- Serum chemistry, lipid profile, and hematology
- ECG
- Urine pregnancy test
- Urine drug analysis
- HbA1c
- Concomitant medications / procedures
- Adverse events

7.2. 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 365/ET (+7 days) in the VMDN-003-2b extension study.

The following actions must be taken 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 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

Study procedures and their timing are summarized in Section 1.3 (SoA).

Protocol waivers or exemptions are not allowed. Adherence to the protocol requirements, including those specified in the SoA, is essential.

8.1. Participant Training

8.1.1. Accurate Pain Reporting

The Accurate Pain Reporting (APR) training instructs Participants how to report pain scores accurately and reliably, and on the proper use of pain scales, with the aim of increasing the reporting accuracy of Participant pain. Participants will receive training on Days 270, 300, and 365/ET.

8.1.2. Placebo Response Reduction

The Placebo Response Reduction (PRR) training teaches the Participant about the appropriate expectations of personal benefit while participating in a clinical trial. The purpose is to provide Participants with truthful information that will neutralize the typically excessive expectations that drive high placebo responses in clinical studies. Participants will receive training on Days 270, 300, and 365/ET.

8.2. Efficacy Assessments and Procedures

8.2.1. Brief Pain Inventory for Diabetic Painful Neuropathy

The Brief Pain Inventory for Diabetic Painful Neuropathy (BPI-DPN) assessment tool (Section 10.5, Appendix 5) will be administered through use of an eDiary. The Investigator should ensure that the Participant is provided with instructions and is trained to use the eDiary and to complete the assessment tool properly before data collection begins. The BPI-DPN will be conducted using the Full BPI-DPN questionnaire and the Partial BPI-DPN questionnaire

Scores obtained from the Full BPI-DPN during the 7 days prior to the Day 270 and Day 365/ET Visits will be used to determine ADPSs. The ADPS is the mean of at least 5 daily pain scores recorded in the eDiary. If the eDiary contains fewer than 5 daily entries over the 7 days prior to the Day 270 or the Day 365/ET Visits, the Visit must be rescheduled so that the Participant can record 5 daily entries over the 7 days prior to the rescheduled Visit.

Participants will use the eDiary to rate their Average Daily Pain and Worst Daily Pain included in the Brief BPI-DPN on a daily basis from the Day 180 Visit (Study VMDN-003-2) to the Day 365/ET Visit of this extension study to allow an assessment of the level and duration of pain relief in the active treatment group versus Placebo.

8.2.2. Quality of Life/Participant Reported Outcome Measures

8.2.2.1. 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 an eDiary. The Investigator should ensure that the Participant is provided with instructions and is trained to use the eDiary and to complete the assessment tool properly before data collection begins (Section 10.6, Appendix 6). 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 Day 270 and Day 365/ET Visits.

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.

8.2.2.2. Short Form Health Survey

The 36-item Short Form Health Survey (SF-36; Section 10.7, Appendix 7) will be administered through use of an eDiary. The Investigator should ensure that the Participant is provided with instructions and is trained to use the eDiary and to complete the assessment tool properly before data collection begins to measure the Participant's perception of their overall health status on the Day 270 and Day365/ET Visits. This 36-question survey measures the Participant's perception of vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health.

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.

8.2.2.3. EuroQol Health Utilities Index

The EuroQol Health Utilities Index (EQ-5D) instrument (Section 10.8, Appendix 8) will also be administered through use of an eDiary. The Investigator should ensure that the Participant is provided with instructions and is trained to use the eDiary and to complete the assessment tool properly before data collection begins to measure the Participant's perception of their overall health status on the Day 270 and Day 365/ET Visits. The instrument has two parts – the descriptive system that measures mobility, self-care, usual activities, pain/discomfort, and anxiety/depression; and the visual analog scale (VAS) that records the Participant's self-rated health.

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.

8.2.3. Bedside Sensory Testing

Sensory tests will be performed at 3 predefined locations: dorsum of the foot, mid-shin, and mid-thigh, except low threshold mechanoreceptive function, which will be conducted at the bottom of the foot only on the Day 270 and Day 365/ET Visits. The Investigator should ensure that the Participant is seated comfortably before data collection begins.

The Bedside Sensory Testing is presented in Section 10.9, Appendix 9.

8.2.4. Michigan Neuropathy Screening Instrument

The Michigan Neuropathy Screening Instrument (MNSI) (Section 10.10, Appendix 10) will be administered through use of an eDiary (for the Participant) or an electronic tablet (for the Site) on Day 270 and Day 365/ET to track disease progression. The Investigator should ensure that the Participant is provided with instructions and is trained to use the eDiary and to complete the assessment tool properly before data collection begins. The MNSI comprises a Participant questionnaire (15 questions) and a physical evaluation that includes a foot inspection, vibration sensation testing, muscle stretch reflexes, and monofilament testing.

Upon completion of the MNSI 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. The Participant will be required to electronically sign and date the questionnaire.

8.3. Safety Assessments

8.3.1. Physical Examinations

Complete physical examinations (PEs) will be performed at the Day 270, Day 300, and Day 365/ET Visits; and at the last Visit if the Participant discontinues prior to Day 365. 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 TEAEs. Any physical examination abnormalities considered to be clinically significant (CS) should be added to TEAEs using medically appropriate terminology.

8.3.2. Medical History and Familial History of Cancer

Medical history for this extension study will be based on the medical history information recorded at Screening for Study VMDN-003-2.

Medication history will be based on recorded medications within 30 days prior to the Day 180 Visit of Study VMDN-003-2.

Elevated risks of cancer for type 2 diabetes or obesity are detailed in Section 8.4.5, Adverse Events of Special Interest.

8.3.3. 12-Lead Electrocardiograms

12-Lead electrocardiograms (ECGs) will be performed on Days 270 and 365/ET. Any clinically significant abnormalities are to be reported as adverse events. The ECG recording will be printed, and the investigator or delegated staff will initial and date the printout to indicate their review of the findings. The trace and the interpretation will then be stored with the Participant's records.

8.3.4. Vital Signs

Vital signs will be measured at the Day 270, 300, and 365/ET Visits. After resting in the seated position for 5 minutes, vital signs measured will include 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.

8.3.5. Clinical Laboratory Assessments

See Section 1.3 (SoA) for the timing and frequency.

8.3.5.1. Central Laboratory Assessments

All laboratory assessments at the Day 270 and 365/ET Visits will be performed by the central 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.

8.3.5.1.1. Hematology

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

8.3.5.1.2. Kidney Function Tests

- Blood urea nitrogen (BUN)
- Creatinine

8.3.5.1.3. Liver Function Tests

- Aspartate aminotransferase (AST)

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

8.3.5.1.4. 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
- HbA1c

8.3.5.1.5. Lipid Profile

- Total cholesterol
- High-density lipoprotein cholesterol (HDL-C)
- Low-density lipoprotein cholesterol (LDL-C)
- Triglycerides

8.3.5.1.6. Additional Tests

- Urine pregnancy test
- Urine drug analysis

8.3.6. Retinal Fundoscopy

Proliferative diabetic retinopathy, defined as the presence of new proliferating blood vessels (neovascularization) arising from the retina or optic disc and growing on the retinal surface or into the vitreous cavity, will be assessed by retinal fundoscopy at Day 270 and Day 365/ET. Retinal fundoscopy must be performed by an ophthalmologist up to 30 days before the Day 270 and Day 365 Visits and, if possible, upon ET. The same ophthalmology practice should be used for all measurements of individual participation. In cases where fundoscopy alone is deemed insufficient, fluorescein angiography may be performed.

8.4. Safety Assessments

8.4.1. Treatment-emergent Adverse Events and Serious Adverse Events

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

8.4.2. Time Period and Frequency for Collecting TEAE and TESAE Information

All TEAEs and TESAEs will be collected from the Day 180 Visit (Study VMDN-003-2) through Day 365/ET of VMDN-003-2b (see definition of TEAE, Section 10.2).

All TESAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.2.5. The Investigator will submit any updated TESAE data to the Sponsor within 24 hours of its availability.

Investigators are not obligated to actively seek TEAEs or TESAEs after conclusion of the study participation. However, if the Investigator learns of any TESAE, 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.

8.4.3. Method of Reporting TEAEs, TESAEs and AESIs

The method of recording, evaluating, and assessing causality of TEAEs, TESAEs, and AESIs, 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 identifying TEAEs and/or TESAEs. Open-ended and non-leading verbal questioning of the Participant is the preferred method to inquire about TEAE and TESAE occurrences.

8.4.4. TESAE Reporting to the Sponsor or Designee

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

8.4.5. Adverse Events of Special Interest

Categories of AESIs

There are three main categories of AESIs: 1) those considered to be related to the angiogenesis potential of Engensis, 2) other medical problems in this patient population, and 3) COVID-19 infections occurring in Participants during the study.

8.4.5.1. AESIs 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.[35] 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 or cerebral vascular disease from the Day 180 Visit (VMDN-003-2) to the Day 365/ET Visit (VMDN-003-2b) will be evaluated as AESIs.

Proliferative diabetic retinopathy

Neovascularization is an advanced stage of diabetic retinopathy that is due to relentless abnormal fibrovascular proliferation.[36] Because the angiogenic potential of Engensis may promote the development of or stimulate abnormal fibrovascular proliferation in the retina, new fundoscopic changes or symptoms of proliferative diabetic retinopathy that occur from the Day 180 Visit (VMDN-003-2) to Day 365/ET (VMDN-003-2b) are considered 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.[37] All types of cancer reported between the Day 180 Visit (VMDN-003-2) to Day 365/ET (VMDN-003-2b) will be deemed to be AESIs.

Diabetes mellitus and obesity (BMI ≥30) each increases the risk of several types of cancer.[38,39] In type 2 DM, there is a 2- to 3-fold increased risk for pancreatic cancer, a 2-fold increased risk for hepatobiliary and endometrial cancers, a 50% increased incidence of colorectal cancer, and a 20% increased risk for breast cancer.[40] Obese postmenopausal women have an increased risk of breast cancer,[41] and obesity is associated with a modestly increased risk of colorectal cancer,[42,43] pancreatic cancer,[44] and prostate cancer.[45]

8.4.5.2. AESIs Related to Other Medical Problems

Several medical problems are frequently observed in patients with diabetic peripheral neuropathy that should be considered AESIs. These medical problems are listed below by type of medical problem.

Infections and Infestations; Skin and Subcutaneous Disorders

Because patients with diabetic peripheral neuropathy are at an increased risk to develop skin lesions (e.g., blisters, ulcers) and various lower limb infectious complications (e.g., cellulitis, osteomyelitis) that may be associated with systemic infections (e.g., bacteremia, sepsis, or septic shock), all of these types of medical problems that occur between the Day 180 (VMDN-003-02) and the Day 365/ET (VMDN-003-2b) Visits will be considered AESIs. In addition, any amputations involving the lower extremity, development of Charcot foot, or other similar complications will also be considered AESIs.

Peripheral Arterial Disease

An overlap of DPN and peripheral artery disease (PAD) apparently exists in type 2 diabetic patients.[46] Because Participants with DPN may develop signs or symptoms of PAD between the Day 180 (VMDN-003-2) and the Day 365/ET (VMDN-003-2b) Visits, detailed evaluations of Participants considered to have PAD will be considered AESIs.

Diabetic Neuropathies

Because Participants in this study may develop other types of diabetic peripheral neuropathies while in the study, the following events will be considered to be AESIs in Participants with DPN: diabetic mononeuropathy, diabetic mononeuropathy multiplex, and diabetic polyradiculopathy. The occurrence of these other types of peripheral neuropathies in a Participant with DPN may indicate a more severe form of DPN.

Because Participants in this study may develop various manifestations of autonomic neuropathy (typically involves the gastrointestinal, genitourinary tract, cardiovascular systems) after randomization, the clinical manifestations of diabetic autonomic neuropathy will be considered to be AESIs.

Glycemic Control

Hypoglycemia, hyperglycemia, hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia are to be listed as AESIs based on the Common Terminology Criteria for Adverse Events (CTCAE) grading if they are Grades 3 or 4.

Metabolic complications of DM (such as diabetic ketoacidosis [DKA], hyperglycemic hyperosmolar non-ketotic coma, lactic acidosis, metabolic acidosis [overlaps with DKA]) are to be listed as AESIs.

8.4.5.3. COVID-19 Infections

A diagnosis of a COVID-19 infection occurring in participants will be recorded as an AESI and their disposition during the trial following the diagnosis of the COVID-19 infection will be tracked.

8.4.6. Follow-up of TEAEs and TESAEs

After the initial TEAE/TESAE report, the Investigator is required to proactively follow each Participant at subsequent Visits/contacts. All TEAEs, TESAEs, and AESIs (see Section 8.4.5) will be followed until resolution, stabilization, the event is otherwise explained, or the Participant is lost to follow-up (as defined in Section 7.2).

8.4.7. Regulatory Reporting Requirements for TESAEs

Prompt notification by the Investigator to the Sponsor of a TESAE 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 a TESAE or other specific safety information (e.g., summary or listing of TESAEs) from the Sponsor will review and then notify the IRB if appropriate according to local requirements.

8.4.7.1. Sponsor's Responsibility

All TEAEs and TESAEs 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 as 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, 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.

8.4.8. Pregnancy and Contraception

8.4.8.1. 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 28 days prior to randomization and/or their last confirmed menstrual period prior to study randomization (whichever is longer) until 2 months after study completion. Male Participants must also agree to use double-barrier contraception until 2 months after study completion. Male Participants must also agree to use double-barrier contraception until 2 months after study completion.

Double-barrier female contraception may include, but is not limited to, intrauterine device with spermicide, female condom with spermicide, diaphragm with spermicide, cervical cap with spermicide. The rhythm method and contraception by a partner are not considered acceptable methods of contraception.

For women of childbearing potential, a urine beta human chorionic gonadotropin (β-HCG) test will be performed on Day 365 or the last Study Visit to evaluate whether the Participant is pregnant.

8.4.8.2. 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.

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 a TEAE or TESAE, 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 TESAE 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 TESAE 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 TESAE through voluntary reporting.

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

8.4.9. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as TEAEs or TESAEs

Except for signs and symptoms of diabetic peripheral neuropathy (i.e., numbness, pain, tingling or burning sensation, cramps, and increased sensitivity to touch), all disease-related events or disease-related outcomes qualify as TEAEs or TESAEs.

9. STATISTICAL CONSIDERATIONS

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

9.1. Sample Size Determination

The Number of Participants to be enrolled in Study VMDN-003-2b after completion of the Day 180 Visit in Study VMDN-003-2 was based on the sample size estimated for enrollment into Study VMDN-003-2, which was based on the hypothesis for the primary efficacy endpoint in Study VMDN-003-2 (see Protocol VMDN-003-2).

9.2. Populations for Analyses

9.2.1. Intent-To-Treat Population

This subset includes all enrolled Participants in this study who start this extension study at the Day 180 Visit (Study VMDN-003-2). Participants in the ITT population will be analyzed according to original treatment assignment in Study VMDN-003-2, regardless of actual treatment received. The primary analyses of the primary and secondary efficacy endpoints will be based on this ITT population.

9.2.2. Safety Population

The safety analysis population will contain all enrolled Participants.

9.2.3. Modified ITT Population

The modified ITT (mITT) population includes all Participants who meet the following:

- Underwent (any) injections (Engensis or Placebo) in Study VMDN-003-2
- Correctly completed the BPI-DPN eDiary at Baseline and the 12-month follow-up in Study VMDN-003-2b

Participants will be grouped based on the randomly assigned treatments, not the actual treatment received. The mITT population will be used in the sensitivity analyses for the primary and secondary efficacy endpoints.

9.2.4. Per Protocol (PP) Population

The Per Protocol population is a subset of mITT. It includes all mITT Participants who met all the following criteria for Study VMDN-003-2 and Study VMDN-003-2b:

- The Participant met major protocol eligibility criteria in Study VMDN-003-2
- The Participant received all injections in Study VMDN-003-2, based on the randomized treatments, and had a Day 365/ET Visit, including a 7-day ADPS.

Additional criteria, if any, will be established before unblinding the randomization code by the independent CDRC that is masked to the treatment information of each Participant.

The PP population will be used in the sensitivity analyses for the primary and secondary efficacy endpoints.

9.3. Statistical Analyses

The Statistical Analysis Plan 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 and the statistical analyses.

9.3.1. Primary Endpoint(s)

As described in Section 3, the primary efficacy endpoint is:

- Change in the means of the ADPSs from the full BPI-DPN from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 365 Visit in the intent-to-treat (ITT) population.

9.3.2. Secondary Endpoints

The secondary efficacy endpoints include the following:

- Change in the means of the Worst Pain scores from the full BPI-DPN from the 7 days prior to the Day 0 Visit (Study VMDN 003 2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
- Proportion of Responders (≥ 50% reduction in the ADPSs from the full BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo

Secondary safety endpoints include:

- Incidence of TEAEs and TESAEs for Engensis compared to Placebo
- Incidence of clinically significant laboratory values for Engensis compared to Placebo

9.3.3. Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include:

- Patient Global Impression of Change (PGIC) on Day 270 and Day 365 for Engensis compared to Placebo
- Proportion of Responders (≥ 50% reduction in the ADPSs from the full BPI-DPN) during the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit who were Responders on Day 104 (final injection visit in Study VMDN 003 2) for Engensis compared to Placebo
- Change in the BST from the Day 0 Visit (Study VMDN 003-2) to Day 270 and to Day 365 Visit for Engensis compared to Placebo

- Proportion of Responders (≥ 20, 30, 40, 60, and 70% reduction in the ADPSs from the full BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN 003-2) to the 7 days prior to the Day 270 Visit and the 7 days prior to the Day 365 Visit for Engensis compared to Placebo
- Change in the means of the ADPSs from the full BPI-DPN from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to the 7 days prior to the Day 270 Visit for Engensis compared to Placebo
- Changes in the severity scores (from the full BPI-DPN) from the 7 days prior to the Day 0 Visit (Study VMDN-003-2) to 7 days prior to the Day 270 Visit and to 7 days prior to the Day 365 Visit for Engensis compared to Placebo
- Changes in MNSI assessments from the Day 0 Visit (Study VMDN 003 2) to Day 270 and Day 365 for Engensis compared to Placebo
- Changes in the SF-36 from the Day 0 Visit (Study VMDN 003 2) to Day 270 and to Day 365 for Engensis compared to Placebo
- Changes in the EQ-5D from the Day 0 Visit (Study VMDN-003-2) to Day 270 and to Day 365 for Engensis compared to Placebo
- Changes in the means of the daily use of rescue medication from the Day 180 Visit (Study VMDN-003-2) to Day 270 and to Day 365 for Engensis compared to Placebo

9.3.4. Safety Analyses

Safety analyses in this study will evaluate the safety profile of Engensis as 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.

9.3.5. Interim Analysis

There will be no interim analysis during this 6-month extension study.

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 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 (ICF) containing the required elements of informed consent must be generated by the Investigator. After approval by the Sponsor, the 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 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.

Participants will receive a comprehensive explanation of the study including the nature and risks of the study, 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 the 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 Data Safety Monitoring Board (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 tables and/or listings, including all reported TEAEs and TESAEs. The data analyses for the DSMB meetings will be directly provided to the DSMB members; no data will be released to the Sponsor and 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 12-week 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.

10.1.6. 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.

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 or its designee will 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). The objectives of this visit are to confirm that all regulatory records and reports are complete 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 implemented beginning with the data entry system, and data QC checks that will be run on the database will be generated. 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), Good Manufacturing Practices (GMP)].

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 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 the 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. At the conclusion of an audit, the auditor will conduct a brief meeting with the Investigator to review.

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

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

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 CRF 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.

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/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.

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.

Protocol deviations will be tracked through the EDC.

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 the 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 the 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 for the receipt 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
- All original ICFs with required signatures

- All IRB correspondence (e.g., informed consent [including any approved revisions], protocol, AEs, advertisements, newsletters)
- All IBC correspondence
- A copy of the Study Visit Log
- Copies of all pertinent correspondence pertaining to the study (except budget issues) between the Sponsor and the Site
- Copies of all TESAE 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 temporarily suspended or prematurely terminated 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

Note the definitions for AEs and SAEs presented below also apply to TEAEs and TESAEs in this protocol.

AE Definition
• An AE 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 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/TESAE 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 TESAE

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

A TESAE 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 AE.

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 TESAE 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. Recording and Follow-Up of TEAEs and/or TESAEs

AE and TESAE Recording

- When an AE/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 TEAE/TESAE information in the CRF.
- 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 TEAE/TESAE CRF 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 TEAE/TESAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each TEAE and TESAE 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 TEAE that is assessed as severe should not be confused with an TESAE. 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 TESAE, 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 TEAE/TESAE.
- 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 TEAE/TESAE, the Investigator **must** document in the medical notes that he/she has reviewed the TEAE/TESAE and has provided an assessment of causality.
- Situations may arise in which an TESAE 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 TESAE data to the Sponsor or designee. Death or hospitalization are not to be specified as an TESAE; these are criteria to determine seriousness.

- The Investigator may change his/her opinion of causality in light of follow-up information and send an TESAE 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 TEAE or TESAE 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 CRF. • The Investigator will submit any updated TESAE data to the Sponsor or designee within 24 hours of receipt of the information.

10.2.5. Reporting of SAEs

TESAE Reporting to the Sponsor or Designee via an Electronic Data Collection Tool
• The primary mechanism for reporting an TESAE to the Sponsor or designee will be the electronic data collection tool. • The Site will enter the TESAE 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 TESAE from a study Participant or receives updated data on a previously reported TESAE after the electronic data collection tool has been taken off-line, then the Site can report this information or to the Medical Monitor or TESAE coordinator by telephone.

10.3. Appendix 3. Prohibited Medications and Procedures

Table 2 Prohibited Medications – Steroids

Drug	Example of Common Name(s)	Maximum Dose Allowed During Study
Steroids		
Injected or oral corticosteroids[a]	prednisone, betamethasone, dexamethasone, cortisone, hydrocortisone, triamcinolone	None
Topical corticosteroids on the lower legs or feet	prednisone, betamethasone, dexamethasone, cortisone, hydrocortisone, triamcinolone	None on the lower legs or feet[b]

[a] Inhaled, ocular, and intra-articular steroids are allowed.
[b] Topical corticosteroid use is allowed on body surfaces other than the lower legs and feet.

Table 3 Prohibited Medications and Procedures – Opioids and Other Therapies

Drug or Procedure	Maximum Dose Allowed During Study
Acupuncture	None
Anesthetic creams (for use only during injections) and patches, including capsaicin, on lower legs and feet	None
Benzodiazepines	None, except for stable bedtime dose
Gabapentin, pregabalin	None
Isosorbide dinitrate spray on lower legs and feet	None
Opioids (e.g., morphine, oxycodone, tramadol, methadone, fentanyl)	None, except after trauma or surgery
Skeletal muscle relaxants (e.g., cyclobenzaprine, metaxalone, chlorzoxazone)	None
Transcutaneous electrical nerve stimulation	None

10.4. Appendix 4. Abbreviations and Definitions

10.4.1. Abbreviations

Abbreviation	Definition
ADPS	Average Daily Pain Score
AE	adverse event
AESI	adverse event of special interest
ALS	amyotrophic lateral sclerosis
ALT	alanine transaminase (SGPT)
APR	Accurate Pain Reporting
AST	aspartate transaminase (SGOT)
β-HCG	Beta human chorionic gonadotropin
BMI	body mass index
BP	blood pressure
BPI-DPN	Brief Pain Inventory for Diabetic Peripheral Neuropathy
BUN	blood urea nitrogen
BST	Bedside Sensory Testing
CABG	coronary artery bypass grafting
cDNA	complementary DNA
CDRC	Clinical Data Review Committee
CFR	Code of Federal Regulations
CKD-EPI	chronic kidney disease epidemiology collaboration
CLI	critical limb ischemia
CRF	case report form
CRO	clinical research organization
CS	clinically significant
CT	Computed Tomography
DIP	distal interphalangeal
DKA	diabetic ketoacidosis
DM	diabetes mellitus
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DPN	diabetic peripheral neuropathy
DSMB	Data Safety Monitoring Board

Abbreviation	Definition
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture system
eDiary	electronic diary
eGFR	estimated glomerular filtration rate
EQ-5D	EuroQol Health Utilities Index
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transpeptidase
GLP	Good Laboratory Practice
HbA1c	Hemoglobin A1c
HDL-C	high-density lipoprotein cholesterol
HEENT	head, eyes, ears, nose, and throat
HGF	hepatocyte growth factor
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IHD	ischemic heart disease
IM	intramuscular
IND	Investigational New Drug application
IRB	Institutional Review Board
ISDN	isosorbide dinitrate
ITT	intent-to-treat
IUD	intrauterine device
IWRS	Interactive Web Response System
LDL-C	low-density lipoprotein cholesterol
mITT	modified intent-to-treat
MNSI	Michigan Neuropathy Screening Instrument
NCS	not clinically significant
NHU	nonhealing foot ulcer
NIH	National Institutes of Health
NRS	Numerical Rating Scale

Abbreviation	Definition
OSP	Office of Science Policy
PAD	peripheral arterial disease
PCR	polymerase chain reaction
PCP	phencyclidine
PE	physical examination
PGIC	Patient Global Impression of Change
PHI	protected health information
PI	Principal Investigator
PKC	Protein kinase C
PP	per protocol
PRR	Placebo Response Reduction
QC	quality control
QoL	Quality of Life
ROS	reactive oxygen species
SAP	statistical analysis plan
SF-36	36-Item Short Form Health Survey
SoA	Schedule of Activities
SOP	standard operating procedure
SSRI	selective serotonin reuptake inhibitor
SUSAR	suspected unexpected serious adverse reaction
TCA	tricyclic antidepressants
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TENS	transcutaneous electrical nerve stimulation
THC	cannabinoid
VAS	Visual Analog Scale
VEGF	vascular endothelial growth factor

10.4.2. Definitions

Active Infection	Chronic infection or severe active infection that may compromise the Participant's well-being or participation in the study, in the Investigator's judgment
Average Daily Pain Score (ADPS)	The mean of at least 5 daily pain scores recorded for Question 5 of the BPI-DPN (Section10.5) in eDiary during the 7 days prior to the Visits on Days 270 and 365/ET
Worst Daily Pain Score	The mean of at least 5 worst daily pain scores recorded for Question 3 of the BPI-DPN (Section 10.5) in eDiary during the 7 days prior to the Visits on Days 270 and 365/ET
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.
Drug Abuse	The habitual taking of addictive or illegal drugs
Drugs of Abuse	Amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, fentanyl, meperidine, methadone, opiates, oxycodone, oxymorphone, phencyclidine, and tramadol
EMLA Cream	A mixture of lidocaine 2.5% and prilocaine 2.5%
First-degree relative	Parent, sibling, or child
Highly Effective Contraception Method	See Section 8.4.8
Investigator	The PI or appropriate Study Site personnel whom the PI designates to perform a certain duty
Lower Leg	The part of the leg between the knee joint and the ankle joint. Does not include the foot.
Medical Monitor	The Responsible Medical Officer or designee
Participant	An individual who has signed informed consent to be entered into the study
Placebo	Placebo comprises 45 mg sodium chloride and 55 mg sucrose in 5 mL of water for injection. Engensis vials contain the same excipients.
Safety Analysis Population	All Participants who receive a Study Injection
Site	A clinical research facility participating in the VMDN-003-2b study
Sponsor	Helixmith Co., Ltd. and its representatives contracted to provide services for study conduct
Stable Dosing Regimen	≤ 50% change in total daily dose relative to dosing at baseline of any medication
Stage 4 or 5 Kidney Disease	eGFR < 30 mL/min/1.73 m^2
Study Drug	Engensis or Placebo

10.5. Appendix 5. Brief Pain Inventory for Participants with Diabetic Peripheral Neuropathy (BPI-DPN)

The paper version of the BPI-DPN Short Form is shown below. Participants will be using an ePRO version, which will be developed prior to study startup.

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Brief Pain Inventory (Short Form) - DPN

Date:____/____/____ Time:_____

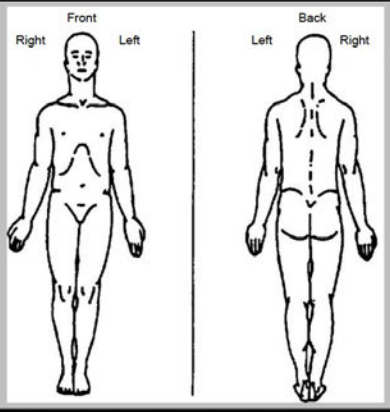
Name:_____ _____ _____

Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

   1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain due to your diabetes by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain due to your diabetes by circling the one number that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

5. Please rate your pain due to your diabetes by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain due to your diabetes by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

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STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Date:___/___/____ Time:_____

Name:_____ _____ _____

Last First Middle Initial

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
No Relief										Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain due to your diabetes has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

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10.6. Appendix 6. Patient Global Impression of Change

Patient Global Impression of Change (PGIC) Scale

Since the start of the study, my overall status is: Check (✓) one box only:

Very Much Improved	1	☐
Much Improved	2	☐
Minimally Improved	3	☐
No Change	4	☐
Minimally Worse	5	☐
Much Worse	6	☐
Very Much Worse	7	☐

Participant's Initials:_ Date: _____

10.7. Appendix 7. Short Form Health Survey (SF-36) Example

Short Form 36 (SF-36) Quality of Life Scale

SF-36 QUESTIONNAIRE

Name:_____ Ref. Dr:_____ Date: _____

ID#: _____ Age: _____ Gender: M / F

Please answer the 36 questions of the Health Survey completely, honestly, and without interruptions.

GENERAL HEALTH:
In general, would you say your health is:
◯ Excellent ◯ Very Good ◯ Good ◯ Fair ◯ Poor

Compared to one year ago, how would you rate your health in general now?
◯ Much better now than one year ago
◯ Somewhat better now than one year ago
◯ About the same
◯ Somewhat worse now than one year ago
◯ Much worse than one year ago

LIMITATIONS OF ACTIVITIES:
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
◯ Yes, Limited a lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Lifting or carrying groceries
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing several flights of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing one flight of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Bending, kneeling, or stooping
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking more than a mile
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking several blocks
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking one block
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Bathing or dressing yourself
◯Yes, Limited a Lot ◯Yes, Limited a Little ◯No, Not Limited at all

PHYSICAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

Cut down the amount of time you spent on work or other activities
◯Yes ◯No

Accomplished less than you would like
◯Yes ◯No

Were limited in the kind of work or other activities
◯Yes ◯No

Had difficulty performing the work or other activities (for example, it took extra effort)
◯Yes ◯No

EMOTIONAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

Cut down the amount of time you spent on work or other activities
◯Yes ◯No

Accomplished less than you would like
◯Yes ◯No

Didn't do work or other activities as carefully as usual
◯Yes ◯No

SOCIAL ACTIVITIES:
Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

◯Not at all ◯Slightly ◯Moderately ◯Severe ◯Very Severe

PAIN:
How much bodily pain have you had during the past 4 weeks?

◯None ◯Very Mild ◯Mild ◯Moderate ◯Severe ◯Very Severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

◯Not at all ◯A little bit ◯Moderately ◯Quite a bit ◯Extremely

ENERGY AND EMOTIONS:
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

Did you feel full of pep?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you been a very nervous person?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt so down in the dumps that nothing could cheer you up?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt calm and peaceful?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Did you have a lot of energy?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you felt downhearted and blue?
- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Did you feel worn out?
- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Have you been a happy person?
- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

Did you feel tired?
- All of the time
- Most of the time
- A good Bit of the Time
- Some of the time
- A little bit of the time
- None of the Time

SOCIAL ACTIVITIES:
During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little bit of the time
- None of the Time

GENERAL HEALTH:
How true or false is each of the following statements for you?

I seem to get sick a little easier than other people
○Definitely true ○Mostly true ○Don't know ○Mostly false ○Definitely false

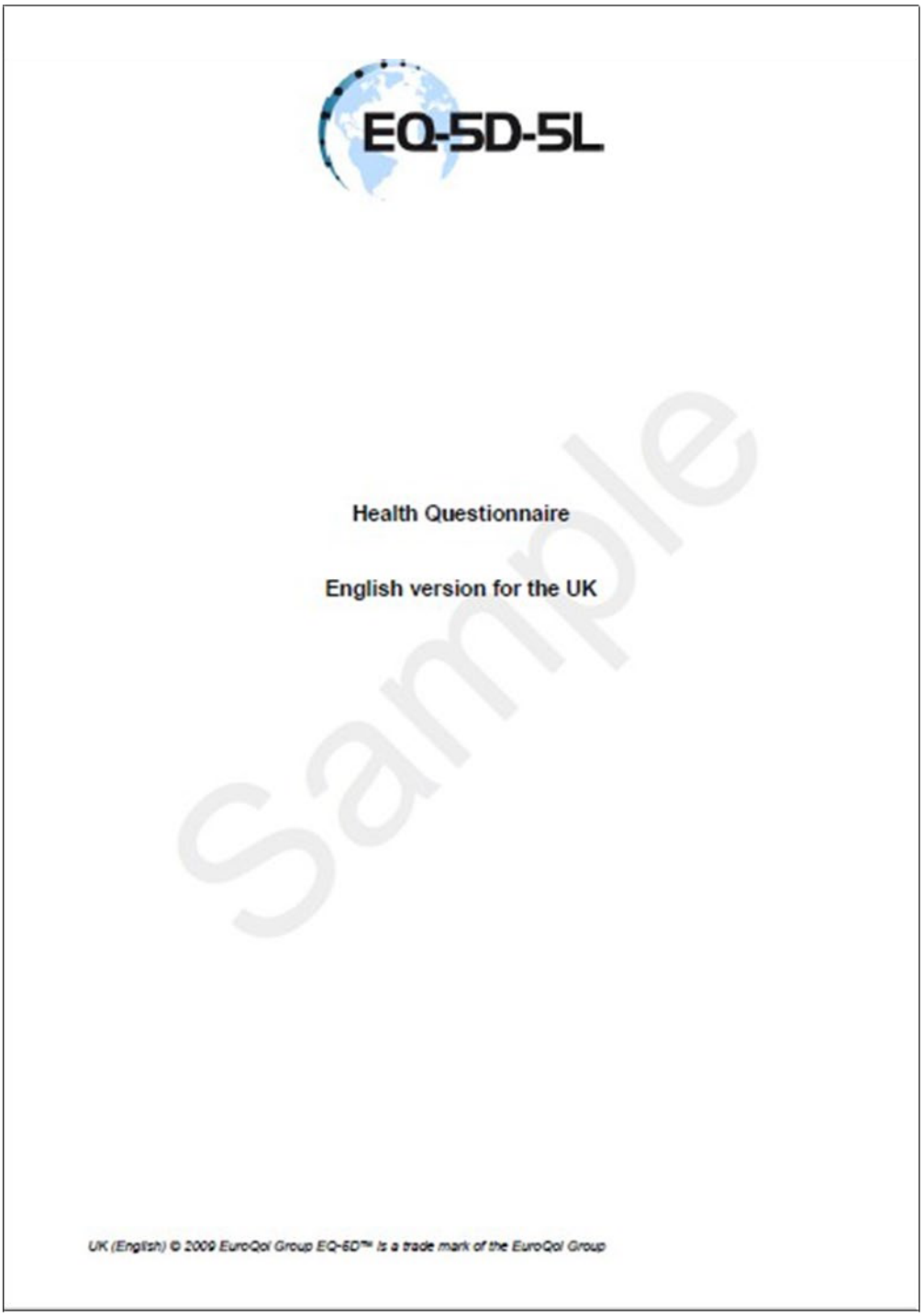
I am as healthy as anybody I know
○Definitely true ○Mostly true ○Don't know ○Mostly false ○Definitely false

I expect my health to get worse
○Definitely true ○Mostly true ○Don't know ○Mostly false ○Definitely false

My health is excellent
○Definitely true ○Mostly true ○Don't know ○Mostly false ○Definitely false

10.8. Appendix 8. EuroQol Health Utilities Index (EQ-5D)

European Quality of Life (EQ-5D) Quality of Life Scale



Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ☐
I have slight problems in walking about ☐
I have moderate problems in walking about ☐
I have severe problems in walking about ☐
I am unable to walk about ☐

SELF-CARE

I have no problems washing or dressing myself ☐
I have slight problems washing or dressing myself ☐
I have moderate problems washing or dressing myself ☐
I have severe problems washing or dressing myself ☐
I am unable to wash or dress myself ☐

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ☐
I have slight problems doing my usual activities ☐
I have moderate problems doing my usual activities ☐
I have severe problems doing my usual activities ☐
I am unable to do my usual activities ☐

PAIN / DISCOMFORT

I have no pain or discomfort ☐
I have slight pain or discomfort ☐
I have moderate pain or discomfort ☐
I have severe pain or discomfort ☐
I have extreme pain or discomfort ☐

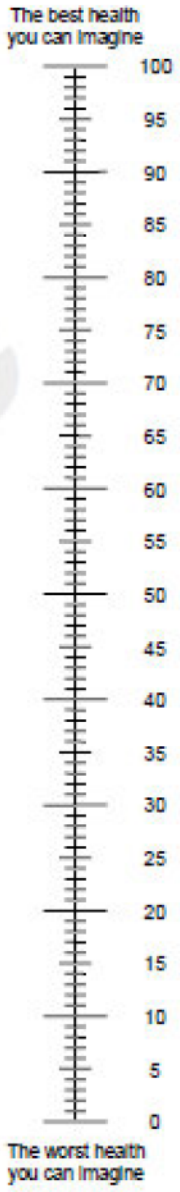
ANXIETY / DEPRESSION

I am not anxious or depressed ☐
I am slightly anxious or depressed ☐
I am moderately anxious or depressed ☐
I am severely anxious or depressed ☐
I am extremely anxious or depressed ☐

2

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



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10.9. Appendix 9. Bedside Sensory Testing (BST)

wcg ANALGESIC SOLUTIONS

BEDSIDE SENSORY TESTING KIT
Source Worksheet

To be completed by PI or Sub-PI	
Sponsor:	**Protocol Number:**
Site Number:	**Subject Number:**
Date of Assessment: (DD-MMM-YYYY) __ __ - __ __ __ - __ __ __ __	

1. **Neuropen Test - Low Threshold Mechanoreceptive Function**
Apply the tip of the Neuropen filament to ten random locations on the bottom of the subject's foot with enough force to bend the filament. Record either a *'Yes'* or *'No'* in response to whether the subject felt the Neuropen stimulation. Check one response below for each of the 10 locations.

1) Yes ☐ No ☐ **2)** Yes ☐ No ☐ **3)** Yes ☐ No ☐ **4)** Yes ☐ No ☐ **5)** Yes ☐ No ☐

6) Yes ☐ No ☐ **7)** Yes ☐ No ☐ **8)** Yes ☐ No ☐ **9)** Yes ☐ No ☐ **10)** Yes ☐ No ☐

2. **Foam Brush Test - Dynamic mechanical allodynia**
Stroke each area four times making a cross shape, then ask for pain score once at each location.

0-10 NRS: Foot (pain rating): _____ Shin (pain rating): _____ Thigh (pain rating): _____

3. **Safety Pin Test - Punctate Hyperalgesia**
Gently tap the sharp tip of the safety pin against the anatomic site 5 times using caution to avoid puncturing the skin. Record the pain ratings reported by the subject in response to each of the five pin pricks at each location. After recording the pain ratings at each site, ask the remaining questions regarding sharpness.

0-10 NRS: Foot (pain ratings): 1 _____ 2 _____ 3 _____ 4 _____ 5 _____

Was the pin sharp? Yes ☐ No ☐

0-10 NRS: Shin (pain ratings): 1 _____ 2 _____ 3 _____ 4 _____ 5 _____

Was the pin sharp? Yes ☐ No ☐

0-10 NRS: Thigh (pain ratings): 1 _____ 2 _____ 3 _____ 4 _____ 5 _____

Was the pin sharp? Yes ☐ No ☐

BSTK v2.0
Source Worksheet
Page 1 of 2

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BST Staff Source Worksheet (Continued)

wcg ANALGESIC SOLUTIONS

BEDSIDE SENSORY TESTING KIT
Source Worksheet

To be completed by PI or Sub-PI	
Sponsor:	**Protocol Number:**
Site Number:	**Subject Number:**
Date of Assessment: (DD-MMM-YYYY) __ __ - __ __ __ - __ __ __ __	

4. **Von Frey Filament Test – Temporal summation**
Apply the tip of the Von Frey filament to the anatomic site with enough force to bend the filament, record the five pain ratings reported by the subject at each of the three locations.

0-10 NRS: Foot (pain ratings): 1_____ 2_____ 3_____ 4_____ 5_____

0-10 NRS: Shin (pain ratings): 1_____ 2_____ 3_____ 4_____ 5_____

0-10 NRS: Thigh (pain ratings): 1_____ 2_____ 3_____ 4_____ 5_____

5. **Tuning Fork Test – Cold Allodynia**
Gently apply the circular end of the tuning fork against the anatomic site and hold it there for 10 seconds. Record the pain rating reported by the subject in response to application of the tuning fork at each location.

0-10 NRS: Foot (pain rating): _____ Shin (pain rating): _____ Thigh (pain rating): _____

—

To be completed by PI or Sub-PI	
Print Name:	**Title:**
Signature:	**Date: (DD-MMM-YYYY)** __ __ - __ __ __ - __ __ __ __

Administering Bedside Sensory Testing (BST)

Administration. An Investigator should administer BST on Days 225, 270, 315, and 365/ET. All sensory tests are being performed at 3 predefined locations: dorsum of the foot, mid-shin, and mid-thigh, except low threshold mechanoreceptive function, which will be conducted at the bottom of the foot only.

The Participant should be seated comfortably during all five procedures of the BST.

Procedure 1: Neuropen Test. The Neuropen test allows assessment of the extent of damage of the low threshold mechanoreceptive small fibers.

Method of Administration. Application of the von Frey filament (10 g) to ten random locations on the bottom of the foot

Summary Score. Frequency count of von Frey stimuli detected (range 0-10; 0 = none detected; 10 = all detected) for bottom of the foot; then reverse score frequency count to align with interpretation of other sensory tests.

Procedure 2: Foam Brush Test. Allodynia, a phenomenon in which exposure to a non-painful stimulus is perceived as painful, is associated with small fiber neuropathy. The foam brush test allows for assessment of Dynamic Mechanical Allodynia.

Method of Administration. Stroke each anatomical location with brush making plus shape.

Summary Score. Single 0-10 NRS pain intensity rating for each anatomical location

Procedure 3: Safety Pin Test. Hyperalgesia, a phenomenon in which exposure to a painful stimulus is perceived as more painful than it should be, is associated with small fiber neuropathy. The safety pin test allows for the assessment of punctate hyperalgesia.

Method of administration. Tap safety pin five times within each anatomical location

Summary Score. Average of five 0-10 NRS pain intensity ratings in each anatomical location

Procedure 4: Von Frey Filament Test. Temporal Summation is a phenomenon in which exposure to the application of consecutive repeated stimuli is perceived as more painful than exposure to a single stimulus. The Von Frey filament test allows for quantification of Temporal Summation.

Method of Administration. Apply von Frey filament five times in rapid succession within each anatomical location

Summary Score. Delta between first and last 0-10 NRS pain intensity rating in each anatomical location

Procedure 5: Tuning Fork Test. Allodynia, a phenomenon in which exposure to a non-painful stimulus is perceived as painful, is associated with small fiber neuropathy. The tuning fork test allows for assessment of Cold Allodynia.

Method of Administration. Apply tuning fork for 10 seconds at each anatomical location.

Summary Score. Single 0-10 NRS pain intensity rating for each anatomical location

10.10. Appendix 10. Michigan Neuropathy Screening Instrument

Patient Version

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

A. History (To be completed by the person with diabetes)

Please take a few minutes to answer the following questions about the feeling in your legs and feet. Check yes or no based on how you usually feel. Thank you.

1.	Are you legs and/or feet numb?	☐ Yes	☐ No
2.	Do you ever have any burning pain in your legs and/or feet?	☐ Yes	☐ No
3.	Are your feet too sensitive to touch?	☐ Yes	☐ No
4.	Do you get muscle cramps in your legs and/or feet?	☐ Yes	☐ No
5.	Do you ever have any prickling feelings in your legs or feet?	☐ Yes	☐ No
6.	Does it hurt when the bed covers touch your skin?	☐ Yes	☐ No
7.	When you get into the tub or shower, are you able to tell the hot water from the cold water?	☐ Yes	☐ No
8.	Have you ever had an open sore on your foot?	☐ Yes	☐ No
9.	Has your doctor ever told you that you have diabetic neuropathy?	☐ Yes	☐ No
10.	Do you feel weak all over most of the time?	☐ Yes	☐ No
11.	Are your symptoms worse at night?	☐ Yes	☐ No
12.	Do your legs hurt when you walk?	☐ Yes	☐ No
13.	Are you able to sense your feet when you walk?	☐ Yes	☐ No
14.	Is the skin on your feet so dry that it cracks open?	☐ Yes	☐ No
15.	Have you ever had an amputation?	☐ Yes	☐ No

Total: _____

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

B. Physical Assessment (To be completed by health professional)

1. Appearance of Feet

	Right	Left
a. Normal	☐ 0 Yes ☐ 1 No	Normal ☐ 0 Yes ☐ 1 No
b. If no, check all that apply:		If no, check all that apply:
Deformities	☐	Deformities ☐
Dry skin, callus	☐	Dry skin, callus ☐
Infection	☐	Infection ☐
Fissure	☐	Fissure ☐
Other	☐	Other ☐
specify:	_____	specify: _____

	Right		Left	
	Absent	Present	Absent	Present
2. Ulceration	☐ 0	☐ 1	☐ 0	☐ 1

	Right			Left		
	Present	Present/ Reinforcement	Absent	Present	Present/ Reinforcement	Absent
3. Ankle Reflexes	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

	Right			Left		
	Present	Decreased	Absent	Present	Decreased	Absent
4. Vibration perception at great toe	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

	Right			Left		
	Normal	Reduced	Absent	Normal	Reduced	Absent
5. Monofilament	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

Signature:_____ Total Score _____/10 Points

How to Use the Michigan Neuropathy Screening Instrument

History. The history questionnaire is self-administered by the Participant. Responses are added to obtain the total score. Responses of "yes" to items 1-3, 5-6, 8-9, 11-12, 14-15 are each counted as one point. A "no" response on items 7 and 13 counts as 1 point. Item #4 is a measure of impaired circulation and item #10 is a measure of general aesthenia and are not included in scoring. To decrease the potential for bias, all scoring information has been eliminated from the Participant version.

Upon completion of the questionnaire, the study coordinator will check the questionnaire for completeness. Note, the Participant will be asked to initial and date the questionnaire.

Physical Assessment. For all assessments, the foot should be warm (> 30°C).

Foot Inspection: The feet are inspected for evidence of excessively dry skin, callous formation, fissures, frank ulceration or deformities. Deformities include flat feet, hammer toes, overlapping toes, hallux valgus, joint subluxation, prominent metatarsal heads, medial convexity (Charcot foot) and amputation.

Vibration Sensation: Vibration sensation should be performed with the great toe unsupported. Vibration sensation will be tested bilaterally using a 128 Hz tuning fork placed over the dorsum of the great toe on the boney prominence of the distal interphalangeal (DIP) joint. Participants, whose eyes are closed, will be asked to indicate when they can no longer sense the vibration from the vibrating tuning fork. In general, the examiner should be able to feel vibration from the hand-held tuning fork for 5 seconds longer on his distal forefinger than a normal Participant can at the great toe (e.g., examiner's DIP joint of the first finger versus Participant's toe). If the examiner feels vibration for 10 or more seconds on his or her finger, then vibration is considered decreased. A study should be given when the tuning fork is not vibrating to be certain that the Participant is responding to vibration and not pressure or some other clue. Vibration is scored as 1) present if the examiner senses the vibration on his or her finger for < 10 seconds, 2) reduced if sensed for ≥ 10 or 3) absent (no vibration detection.)

Muscle Stretch Reflexes: The ankle reflexes will be examined using an appropriate reflex hammer (e.g., Trommer or Queen square). The ankle reflexes should be elicited in the sitting position with the foot dependent and the Participant relaxed. For the reflex, the foot should be passively positioned, and the foot dorsiflexed slightly to obtain optimal stretch of the muscle. The Achilles tendon should be percussed directly. If the reflex is obtained, it is graded as present. If the reflex is absent, the Participant is asked to perform the Jendrassic maneuver (i.e., hooking the fingers together and pulling). Reflexes elicited with the Jendrassic maneuver alone are designated "present with reinforcement." If the reflex is absent, even in the face of the Jendrassic maneuver, the reflex is considered absent.

Monofilament Testing: For this examination, it is important that the Participant's foot be supported (i.e., allow the sole of the foot to rest on a flat, warm surface). The 10-gram filament should initially be pre-stressed (4-6 perpendicular applications to the dorsum of the examiner's first finger). The filament is then applied to the dorsum of the great toe midway between the nail fold and the DIP joint. Do not hold the toe directly. The filament is applied perpendicularly and briefly, (< 1 second) with an even pressure. When the filament bends, the force of 10 grams has been applied. The Participant, whose eyes are closed, is asked to respond yes if he/she feels the filament. Eight correct responses out of 10 applications are considered normal, one to seven

correct responses indicate reduced sensation, and no correct answers translates into absent sensation.

10.11. Appendix 11. Protocol Amendment History

There are no amendments to this protocol.

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