AMG0001

Protocol AG-CLI-0209

A PHASE IIB PILOT STUDY TO CONFIRM THE FEASIBILITY AND TOLERABILITY OF A MODIFIED DOSAGE REGIMEN OF AMG0001 IN SUBJECTS WITH CRITICAL LIMB ISCHEMIA

March 3, 2016

Sponsor: AnGes, Inc.

STUDY SPONSOR:

AnGes, Inc.
10411 Motor City Drive, Suite 430
Bethesda, MD 20817
USA
(301) 990-1660

PRIMARY INVESTIGATOR:

Richard James Powell, MD
Section Chief, Vascular Surgery
Dartmouth – Hitchcock Medical Center
One Medical Center Drive
Lebanon, NH 03756
(603) 650-8677

SPONSOR SIGNATURE OF PROTOCOL & PROTOCOL AMENDMENT(s)

Protocol Number:	**AG-CLI-0209**
Protocol Title:	**A PHASE IIB PILOT STUDY TO CONFIRM THE FEASIBILITY AND TOLERABILITY OF A MODIFIED DOSAGE REGIMEN OF AMG0001 IN SUBJECTS WITH CRITICAL LIMB ISCHEMIA**
Protocol Version Date:	03 March 2016
Amendment Number:	04
Signatures:	Hiroyuki Suda President of AnGes, Inc. Irene Tennant Director of Clinical Operations Colleen Davenport, PhD Director of Regulatory Affairs and Pharmacovigilance

INVESTIGATOR AGREEMENT

Protocol Number:	**AG-CLI-0209**
Title:	**A PHASE IIB PILOT STUDY TO CONFIRM THE FEASIBILITY AND TOLERABILITY OF A MODIFIED DOSAGE REGIMEN OF AMG0001 IN SUBJECTS WITH CRITICAL LIMB ISCHEMIA**
Protocol Version Date:	03 March 2016
Amendment Number:	04

By signing this cover page I attest that I have read and understand the contents of the original Clinical Protocol AG-CLI-0209 and any subsequent amendments. I agree to adhere to the design, conduct and reporting requirements of the study as stated in the clinical protocol and to my obligations to The Sponsor as described in the protocol and executed contracts between myself, my Institution and The Sponsor.

Investigator's Signature: _____

Investigator's Name: _____

Institution: _____

Date: (DD/MM/YYYY format)_____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABI	Ankle Brachial Index
AE	Adverse Event
AKA	Above Knee Amputation
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASO	Arteriosclerosis Obliterans
AST	Aspartate Aminotransferase
bFGF	basic Fibroblast Growth Factor
BKA	Below Knee Amputation
BMI	Body Mass Index
BP	Blood Pressure
bp	base pairs
bpm	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations (U.S.)
CI	Confidence interval
Cl	Chloride
CLI	Critical Limb Ischemia
cm	Centimeter
CMH	Cochran-Mantel-Haenszel
CRF	Case Report Form
CRO	Contract Research Organization
CTA	Computed tomography angiography
CV	Curriculum vitae
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic Acid
EC	Ethics Committee
ECG	Electrocardiogram
ELISA	Enzyme Linked Immunosorbent Assay
ESRD	End Stage Renal Disease
ETDRS	Early Treatment Diabetic Retinopathy Study
FBG	Fasting Blood Glucose
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
FSH	Follicle-Stimulating Hormone
$HbA1_c$	Glycated hemoglobin
HBO	Hyperbaric Oxygen
HCC	Hepatocellular Carcinoma
HDL	High Density Lipoprotein
HGF	Hepatocyte Growth Factor
HR	Hazard Ratio
HUV	Human Umbilical Vein

IBC	Institutional Biosafety Committee
IC	Intermittent Claudication
IM	Intramuscular
IRB	Institutional Review Board
ITT	Intention To Treat
IV	Intravenous
K	Potassium
kg	Kilogram
KM	Kaplan Meier
LDH	Lactic Acid Dehydrogenase
LDL	Low Density Lipoprotein
LLOQ	Lower limit of quantification
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial Infarction
mm	Millimeter
mg	Milligram
mmHg	Millimeters of Mercury
MRA	Magnetic resonance angiogram
Na	Sodium
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities (Division of the National Institutes of Health)
PAD	Peripheral Arterial Disease
PAP	Papanicolaou
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PSA	Prostate-specific antigen
PTA	Percutaneous Transluminal Angioplasty
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
SAE	Serious Adverse Event
SD	Standard Deviation
SFA	Superficial Femoral Artery
TAO	Thrombo-angiitis Obliterans
TBI	Toe Brachial Index
TcPO2	Transcutaneous Oxygen Tension
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
VSMC	Vascular Smooth Muscle Cells
WBC	White Blood Cell

1. INTRODUCTION

Peripheral artery disease (PAD) affecting the lower limbs results from the consequences of atherosclerosis. Critical limb ischemia (CLI) is the severest form of PAD and occurs when tissue viability is jeopardized at rest. The chief manifestations of CLI include ischemic pain at rest (Rutherford class 4, Fontaine class 3) and/or tissue loss (Fontaine class 4 or Rutherford class 5 when tissue loss is minor and Rutherford class 6 when tissue loss is major) resulting in ischemic ulceration and gangrene.

The management of CLI includes risk factor modification including the treatment of hyperlipidemia, hypertension, diabetes, and smoking. In addition, analgesics (often opioids) are used for the relief of rest pain. Meticulous foot care and local wound care are important to prevent or manage tissue loss. Finally the majority of subjects are treated with antiplatelet therapy and a statin. The current management, in addition to the above, includes revascularization by surgery or by endovascular interventions. Up to 15% of subjects are unsuitable for either of these forms of revascularization procedures while others (~40%) are at high risk (poor candidates) for surgical revascularization. Even though revascularization procedures have improved survival and may delay the need for major amputation (amputation at or above the ankle), their effectiveness is often short-lived and surgery is associated with significant morbidity.

Subjects unsuitable for revascularization are candidates for major amputation. This procedure is associated with considerable morbidity, a poor quality of life, and complications including, in some cases, death.

Subjects with CLI have, overall, a poor prognosis with a 5-year mortality of 50%. Their quality of life is comparable to the quality of life of subjects with advanced cancer. No systemic drug treatment is available for the treatment of CLI. There is an urgent medical need for additional management options.

1.1. Rationale for the Current Study, AG-CLI-0209

In summary, the clinical data generated to date through AMG0001 early phase studies have shown efficacy of AMG0001 in improving tissue perfusion (increase in $TcPO_2$), ulcer healing, rest pain relief, toe pressure and TBI improvement. In addition, the safety data to date in the AMG0001 clinical program confirms that the product is well tolerated. Based on these results, a phase 3 study AG-CLI-0206 was planned as a registration study and received SPA approval by the FDA. It will be undertaken in subjects with CLI who have no option for revascularization and also subjects who have poor option (high risk) for revascularization by surgery. The study will confirm the safety and efficacy of AMG0001.

Prior to undertaking the phase 3 study, a pilot study to confirm the feasibility of the study-related activities in study 0206 and the tolerability of the dosage regimen will be undertaken in 8-10 subjects. In this pilot study, subjects will receive 4 sets of injections of HGF plasmid two weeks apart starting at Day 0 and again at Month 3 (first cycle) and at Month 9 and again at Month 12 (second cycle), see table below.

Table 1: Dosage Regimen for the AG-CLI-0209 Study

First Cycle	**Day 0, Day 14, Day 28, and Day 42** (4 sets of injections, 2 weeks apart; each set is 4 mg subdivided into 8 injections of 0.5 mg of HGF plasmid in 3 mL saline) **Month 3, Month 3+14 days, Month 3+28 days, and Month 3+42 days** (4 sets of injections, 2 weeks apart; each set is 4 mg subdivided into 8 injections of 0.5 mg of HGF plasmid in 3 mL saline)
Second Cycle	**Month 9, Month 9+14 days, Month 9+28 days, and Month 9+42days** (4 sets of injections, 2 weeks apart; each set is 4 mg subdivided into 8 injections of 0.5 mg of HGF plasmid in 3 mL saline or matching placebo) **Month 12, Month 12+14 days, Month 12+28 days, and Month 12+42 days** (4 sets of injections, 2 weeks apart; each set is 4 mg subdivided into 8 injections of 0.5 mg of HGF plasmid in 3 mL saline)

Note: for the purposes of this study a month is defined as 30 days; days 14, 28 and 42 are calculated from either Day 0, Month 3, Month 9 or Month 12.

2. STUDY OBJECTIVES

The primary objectives of the clinical trial are:

1. To confirm the feasibility of study-related activities and the tolerability of a modified dosage regimen of AMG0001 in CLI
2. To evaluate the safety of AMG0001

3. STUDY DESIGN

This is a pilot study of AMG0001 (HGF plasmid) in approximately 8-10 CLI subjects. Subjects eligible for the study are CLI subjects (due to atherosclerotic arterial disease of the lower limb) with no option for revascularization by surgical bypass or endovascular intervention, or have a poor option (high risk) for revascularization by surgical bypass but no option for an endovascular intervention (Type D lesions or worse using the TASC II classification). Clinic visits will occur for screening, Days 0, 14, 28, 42, Month 3, Month 3+14 days, Month 3+28 days, Month 3+42 days, Month 6, Month 9, Month 9+ 14 days, Month 9 + 28 days, Month 9 +42 days, Month 12, Month 12+14 days, Month 12+28 days, Month 12+42 days, Month 15, and Month 18. Safety follow-up data using a questionnaire will be obtained every 6 months for 1.5 years after the 18 month study visit. The total duration of the study will be up to 37 months. All subjects will receive AMG0001 intramuscularly in the muscles of the diseased limb based on the location of disease.

Note: for the purposes of this study a month is defined as 30 days; days 14, 28 and 42 are calculated from either Day 0, Month 3, Month 9 or Month 12.

3.1. Study Population

Subjects (40-90 years old) with Chronic Critical Limb Ischemia who have:

Severe ischemic pedal rest pain (nocturnal metatarsalgia, Rutherford 4) in the index lower limb with ankle and/or toe systolic pressures of less than 50 and 30 mmHg, respectively, classical features of rest pain and on opioids for pain relief. Classical features include pedal pain that is severe and typically occurs at night when the limb is no longer in a dependent position but in severe cases can be continuous. The pain is localized in the distal part of the foot. Partial relief may be obtained by the dependent position whereas cold and elevation of the lower limb aggravates the pain. Severe pain responds only to opioids.

OR

Ischemic ulcers /gangrene (Rutherford 5) in the affected lower limb with ischemic ulcer measurements $\leq$20 cm^2 in area ($\leq$10 cm^2 for ischemic ulcers at the heel) for the largest ischemic ulcer with or without rest pain, and ankle and/or toe pressures less than 70 and 50 mmHg, respectively.

IN ADDITION, SUBJECTS WILL HAVE:

No option for revascularization by endovascular intervention or bypass surgery (see definitions below).

No option is defined as the inability to receive an endovascular intervention or surgical bypass procedure due to inflow, conduit or outflow reasons or due to a severe and irreversible co-morbidity.

OR

Poor option (high risk) for revascularization by surgery (see definition below) but no option for revascularization by an endovascular procedure (Type D lesions or worse using the TASC II classification).[1]

Definition for poor option (high risk) for revascularization surgery:

Anatomic

- Need for a tibial level synthetic or cryoperserved bypass
- Need for use of great saphenous vein < 3mm in diameter for tibial level bypass based on venous duplex ultrasound
- Need for use of spliced vein or single piece of non-great saphenous vein < 3mm in diameter, for tibial level bypass
- Distal target unsuitable for bypass, e.g. < 2mm in diameter or circumferentially calcified

1Norgren, L., Hiatt, W.R., et al., Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II), Eur J. VascEndovascSurg, Vol 33, S1-S70; 2007.

- Open lower extremity wound that would preclude construction of bypass

OR

Medical

- Subjects with severe cardiac, respiratory, or renal disease who are at increased risk of complications from surgery or anesthesia, e.g., significant heart disease (NYHA class III or IV), severe Chronic Obstructive Pulmonary Disease (COPD), or severe renal disease (creatinine clearance 30-45 mL/minute).
- Subjects with ischemic rest pain (Rutherford Class 4) will be included if the rest pain is severe, requires opioids, has the classical descriptions, and the ankle and/or toe pressures are less than 50 mm Hg or 30 mm Hg, respectively.
- All subjects with severe Rutherford 4 who enter the study should have rest pain that is stable for at least 2 weeks prior to entry into the study. All Rutherford 5 subjects should have stable wounds with no infection.
- Subjects will have been on a statin (unless there is a contraindication) and an antiplatelet agent (unless there is a contraindication) for 2 weeks or more prior to Day 0.

3.2. Inclusion criteria

Subjects must meet the following inclusion criteria in order to be enrolled into the study.

1. Subjects with CLI (Severe Rutherford 4 and Rutherford 5) who have:
   - No option for revascularization by endovascular intervention or surgical bypass

     OR

   - Poor option (high risk) for revascularization by surgery and no option for an endovascular intervention (see Section 3.1 for full definition for appropriate inclusions).
2. Subjects 40-90 years of either gender who have signed an informed consent form either directly or through a legally authorized representative.
3. Subjects currently are taking a statin and an anti-platelet agent (e.g., clopidogrel, ticlopidine, aspirin, etc.) for 2 weeks or more prior to Day 0 as part of their standard of care, unless contraindicated. Subjects for whom these agents are contraindicated will have the reason for contraindication recorded in their case report form (CRF).
4. Only women who are post-menopausal, who are surgically sterile, or who have documented primary ovarian insufficiency (premature ovarian failure) are eligible for inclusion (Please see Appendix G for further information).
5. If a male subject is of reproductive potential, he must agree to use an accepted and effective (barrier) form of birth control starting with the first dose of study product and continue for 12 weeks from the last dose of study product. This applies to both courses of treatment.

6. Subjects with a previous medical history of myocardial infarction and/or stroke should have adequate management of risk factors to prevent secondary occurrence. (See Section 4.2 Medical History for guidelines on appropriate secondary prevention.)
7. Subjects should have the ability to understand the requirements of the protocol and agree to return for the required study visits and assessments.

The index leg will be the leg with the greater severity of CLI disease. Entry requirements apply to the index leg. The index leg may also be referred to as the treated leg or affected leg in the text of this protocol or other study documents. If the subject has two legs that have the same Rutherford classification (severe Rutherford 4 or Rutherford 5) and are both eligible for treatment, the leg with greater disease severity (based on more extensive necrosis or more extensive/deeper ulceration(s), difference in ABI or TBI ≥ 0.1, and/or more extensive vascular disease based on the angiogram) will be chosen as the index leg. If there is no clinical, hemodynamic or angiographic or other evidence to determine which leg has greater disease severity, the subject will be excluded from the study.

3.3. Exclusion Criteria

1. Subjects whose CLI status is unstable (spontaneous marked improvement or marked worsening during the screening period) or who have excessive tissue necrosis that is unlikely to benefit from medication, or those poor option subjects requiring immediate revascularization by surgery. Stability of the CLI status will be confirmed by the Principal Investigator prior to randomization and retrospectively reviewed by the entry committee.
2. Subjects who may require a major amputation (amputation at or above the ankle) within 4 weeks of Day 0 (± 4 weeks of Day 0).
3. Subjects with ulcers with exposure of tendons, osteomyelitis or uncontrolled infection or with the largest ulcer that is greater than 20 cm^2 in area (>10 cm^2 area if on the heel).
4. Subjects with purely neuropathic or venous ulcers.
5. Subjects in Rutherford 6 class.
6. Subjects who have had revascularization by surgery or angioplasty within 3 months, unless the procedure has failed based on the anatomy or the hemodynamic measurements.
7. Subjects with a diagnosis of Buerger's disease (Thrombo-angiitis Obliterans).
8. Subjects currently receiving immunosuppressive, chemo or radiation therapy.
9. Evidence or history of malignant neoplasm (clinical, laboratory or imaging) except for successfully excised basal cell or squamous cell carcinoma, or successfully excised early melanoma of the skin. Subjects, who had successful tumor resection or radio-chemotherapy of breast cancer more than 10 years prior to inclusion in the study, and with no recurrence, may be enrolled in the study. Subjects, who had successful tumor resection or radio-chemotherapy of all other tumor types and have been in remission for more than 5 years prior to inclusion in the study, and with no recurrence, may be enrolled in the study. A dermatological exam will have ruled out any skin cancer.

10. Subjects who have proliferative retinopathy, or moderate or severe non-proliferative retinopathy, from any cause (ETDRS Score > 35), clinically significant macular oedema or previous panretinal photocoagulation therapy (Results from the Early Treatment Diabetic Retinopathy Study. Ophthalmology May 1991 Supplement 98: 823-833).
11. Females of child-bearing potential are excluded. This includes females who are either post-menopausal, surgically sterile (see Appendix G for further information of surgical sterility) or have documented primary ovarian insufficiency.
12. Subjects with severe renal disease defined as significant renal dysfunction evidenced by an estimated creatinine clearance of <30 mL/minute (calculated using the Cockroft Gault formula), or receiving chronic hemodialysis therapy.
13. A Stroke, TIA or MI within 3 months of entry into the study.
14. Subjects with known liver disease (e.g., hepatitis B or C or cirrhosis of the liver).
15. A subject with HIV, AIDS, or severe uncontrolled ulcerative colitis or Crohn's disease.
16. Subjects with a current, uncorrected history of alcohol or substance abuse.
17. Subjects that have been administered rhPDGF (e.g, becaplermin) or other growth factors locally within one month of randomization.
18. Subjects who have received another investigational drug within 30 days of randomization or have previously received any gene transfer therapy within 3 years of entering the study.

    Note: No waivers will be granted for subjects regarding Inclusion/Exclusion criteria.

3.4. Administration of Study Product

Study product will be administered to subjects who were confirmed eligible by the Principal Investigator (eligibility will be confirmed by an entry committee) and whose CLI is stable (see Exclusion Criteria Section 3.3, item #1).

The study product will be administered by eight (8) intramuscular injections delivered to the index leg (see Section 3.2 Inclusion criteria for definition of index leg) at each dosing visit (see Table 1 Dosage Regimen above). For each of the 8 injections, 3 mL of AMG0001 will be injected in about 3 seconds. The precise anatomical location for study product injections will be based on vascular anatomy. The site will receive a detailed schema from the above entry committee indicating where the injection sites should occur. Duplex scanning must be used during the injection procedure to further clarify the injection sites.

Subjects will not be eligible to receive the subsequent doses of study product if a major amputation of the index leg has occurred. If the subject has been revascularized in the index leg, the Investigator should ensure that the subsequent doses of study product are not administered less than 1 month post-revascularization.

Analgesics may be administered prior to treatment injections. Local anesthetics applied to the skin are recommended. An anti-anxiety agent may be administered pre-dose to relieve any anxiety surrounding treatment procedures. Administration of analgesics is at the discretion of

the Investigator, but cannot be given to the subject prior to VAS pain scale measurement or Quality of Life (VascuQol Questionnaire) measurements. All analgesics given prior to injection should be accurately recorded on the appropriate Concomitant Medication CRF.

4. DESCRIPTION OF STUDY PROCEDURES

4.1. Informed Consent

An Informed Consent template containing all the regulatory elements will be provided by the Sponsor to the site. The site may modify this document as long as the text relating to the FDA and/or other agency regulations are retained. A copy of the site's proposed Informed Consent document(s) must be submitted to the Sponsor or designee for review and comment prior to submission to the reviewing Institutional Review Board/ Ethics Committee (IRB/EC). The Informed Consent document must be approved by the IRB/EC and contain all elements required by FDA (21 CFR 50.25), ICH GCP, national, state, local and institutional regulations or requirements. If updates to the Informed Consent are required during the trial, the site must obtain IRB/EC approval of the new Informed Consent version as per national, state, local and institutional regulations or requirements and all active subjects must be re-consented.

It is the responsibility of the Investigator to provide each subject with full and adequate information using the IRB/EC approved Informed Consent document(s), including the objectives and procedures of the study, possible risks involved and alternative treatments, before inclusion in the study. Each subject must voluntarily provide written Informed Consent (including disclosure for the use and disclosure of research-related health information if applicable). The consent must be obtained prior to performing any study-related procedures that are not part of normal subject care. A copy of the signed Informed Consent must be given to the subject and an original copy of the Informed Consent must stay with the subject records at the site.

4.2. Medical History

The Investigator or designee will obtain a general medical history of past and present illnesses as well as medications currently being taken to address current illnesses. The medical history will include alcohol consumption, tobacco use, substance abuse, diabetic status, and status of any concurrent diseases. History of current symptoms and their severity will be obtained and recorded. History of drug allergies/reactions will also be obtained. See Section 4.7 Peripheral Vascular Intervention History (or Update) for medical history information collected at screening.

Subjects that have had previous myocardial infarction must have appropriate secondary prevention previously established and adequately documented in their medical record. Adequate secondary prevention includes:

- Lifestyle modification including the cessation of smoking, control of hypertension, and appropriate diet for optimum BMI management
- Aspirin therapy (72 mg to 162 mg every day) should be established unless contraindicated or the subject is receiving Plavix or a similar compound

- Treatment with statins for lipid abnormalities should be established unless contraindicated. (Note: Subjects will be receiving statins for PAD unless contraindicated.)
- An ACE-Inhibitor should be considered in all subjects unless contraindicated. As an alternative, subjects with Left-Ventricular Dysfunction maybe established on ARBs such as Valsartan.
- Beta-Blockers are recommended unless contraindicated

Subjects that have had a previous stroke should have appropriate secondary prevention previously established and adequately documented in their medical record. Adequate secondary prevention is defined as:

- Lifestyle modification to include the cessation of smoking, reduction in alcohol consumption, optimum BMI management, and increased moderate-intensity physical activity.
- Anti-hypertensive treatment is recommended, and treatment should be individualized according to the subject's characteristics.
- Subjects with diabetes should be under rigorous control of blood pressure and maintain blood glucose at near normal glucose levels. ACE-Inhibitors and ARBs are recommended for subjects with hypertension and diabetes mellitus to reduce the progression of renal disease. HbA1c should be targeted at < 7%.
- Statins are generally recommended as an agent for lowering lipid levels unless contraindicated. Subjects who have had an ischemic stroke or TIA, elevated cholesterol, co-morbid CAD or any evidence of atherosclerotic origin should be managed by NCEPIII guidelines. (Note: Subjects will be receiving statins for PAD unless contraindicated.)

4.3. Physical Examination

A complete general physical examination of the skin and major body systems (Eyes, Ears, Nose and Throat; Cardiovascular; Respiratory; Gastrointestinal; Musculoskeletal/Connective Tissue; Neurological; Endocrine/Metabolic; Hematopoietic/Lymphatic; Genitourinary) will be completed as part of the study procedures.

Any suspicious lesion found on the skin during the physical examination at Screening should be evaluated by a Dermatologist.

An abbreviated physical exam does not evaluate all systems, but those systems for which exams are deemed medically necessary based on symptoms (symptom-driven exam).

4.4. Concomitant Medications

Concomitant medication and the indication for its use must be clearly documented and recorded in the source documentation and CRF for each subject. Any medications taken from the date informed consent is signed and onward should be documented in the CRF.

The use of analgesic medications during the study should be carefully recorded. Investigators should refrain from making changes in the subject's use of analgesic medications after the baseline visit, unless the subject's medical condition warrants changes to their treatment. Changes in the use of analgesic medications after the baseline visit could confound the interpretation of endpoint assessments.

Topical wound medications must be captured as concomitant medications in the CRFs. **Wound care products such as Regranex® (rhPDGF), Apligraft®, Dermagraft®, Oasis®, as well as any other skin equivalents or topical growth factors are excluded for use during this trial. Hyperbaric oxygen (HBO) treatment is also excluded.**

All subjects should be on a statin and an anti-platelet agent (e.g., clopidogrel, ticlopidine, aspirin, etc.) as part of their standard of care, unless contraindicated. Subjects for which these agents are contraindicated must have the restriction reason recorded in their source documentation and CRF.

4.5. Electrocardiogram

A 12-lead electrocardiogram (ECG) will be performed during the Screening Period to document clinically significant anomalies (if not done up to 6 months prior to Day 0).

4.6. Angiogram

An aorto-iliac angiogram will be performed and will be used to determine the suitability of the subject for enrollment into the study and to determine injection site locations. The angiogram may have been completed up to 3 months prior to the start of screening, as long as the subject's clinical condition has not changed since the angiogram was performed or undergone a bypass or endovascular intervention within the previous 3 months. Acceptable forms of imaging for this trial also include Computerized Tomography Angiogram (CTA) and Magnetic Resonance Angiogram (MRA). An angiogram will also be required to be performed prior to a proposed surgical revascularization or major amputation.

4.7. Peripheral Vascular Intervention History (or Update)

During Screening

At Screening, the Investigator will perform a detailed assessment of all past peripheral vascular interventions (surgical bypass, an endovascular intervention or major/minor amputations). If the subject has never undergone a peripheral vascular intervention then the lack of these events will be documented as well. Information may include: date(s) of intervention, type(s) of intervention (e.g., PTA, surgical bypass, amputation, etc.), intervention site (e.g., iliac, femoro-popliteal, infrapopliteal, etc.), intervention side (right or left leg and index or non-index leg) conduit used (e.g., saphenous vein, polytetrafluoroethylene [PTFE], human umbilical vein [HUV], Dacron, etc.), and patency of most recent intervention prior to study start (including a description of the results from the most recent vascular imaging if available). Information concerning both major (defined as at or above the ankle) and minor (below the ankle) amputation should be collected. Specific information for each peripheral vascular procedure will be obtained and recorded on the

subjects' source documentation and CRF to allow independent review of the subjects' CLI status and suitability for entry into the study.

During Treatment and Follow up Period

During the study, detailed information must be documented in the subjects' source documentation and CRF on the subject's CLI status of the index leg including any treatment received for worsening CLI of the index leg, plan for major or minor amputations of the index leg, plan for revascularization procedures of the index leg, etc. Detailed information on the subjects' CLI status must be documented prior to planning a surgical revascularization or major amputation of the index leg. Data relating to the reasons for performing a surgical revascularization or major amputation of the index leg will be reviewed.

In no way will intervention that is deemed necessary be withheld for any subject.

4.8. Hemodynamic Measurements

The hemodynamic status of the affected lower limb will be evaluated to confirm the CLI diagnosis, and the subject's suitability for inclusion into the study. The ankle and toe pressures will be measured using Doppler or a photoplethysmograph (PPG) and the ankle/brachial index (ABI) and toe/ brachial index (TBI) will be estimated in the index leg, measuring both the posterior tibial and dorsalis pedis arteries for the ABI. The higher of the two readings will be used. The methodology for obtaining these measurements is outlined in Appendix B. A Vascular Report must be included in the source documentation to support the hemodynamic data.

During Screening hemodynamic measurements are required for both the index and non-index leg. Otherwise, at all other time points during the study, hemodynamic measurements are only required for the index leg.

If it is not possible to obtain the toe pressure because the big toe has been amputated, or is too deformed or necrotic to apply the cuff, then the second toe may be used. This should be noted in the source documentation and in the CRF.

A vessel with no detectable Doppler signal (complete occlusion) will be recorded as having a zero pressure ("0 mmHg"). Calcification of the media is common in diabetic subjects, rendering the tibial and peroneal arteries non-compressible, which causes falsely elevated ABI (>1.30). If the measurement cannot be assessed due to calcification then the non-compressible status of these arteries should be noted in the source documentation and CRF and the toe pressure used to diagnose and assess CLI.

4.9. Assessment of CLI and Ulcer Photography and Tracings

For study entry, the subject must be evaluated for clinical evidence of CLI according to the definition of the study population for this protocol found in Section 3.1 Study Population.

The Investigator must assign a baseline status to the subject's peripheral vascular disease based on the status of the index leg using the Rutherford Classifications (see Appendix C). In case of Rutherford Category 4 and Rutherford Category 5, the Principal Investigator must make sure that the inclusion criteria for subjects with Rutherford Category 4 and Rutherford Category 5 are

strictly followed for the subjects to be eligible to enter the study. (See Section 3.1 Study Population)

During the study, specific information on the subject's CLI status will be documented on the CRF and in the source documentation including information on ulcers/gangrene, rest pain, debridement/wound care, infection treatment, hospitalizations, minor and major amputations, revascularization by surgical bypass, etc.

In order to accurately assess ulcer size, the ulcer (s) must be cleaned and debrided to remove necrotic tissue, eschar, and callouses prior to photographs and ulcer tracings, as per the wound care guidelines (see Appendix D).

During Screening, ulcer photography and tracings of the largest ulcer (with ulcer debridement occurring prior to photography and tracing) must be performed and used as one of the evaluations to be made to confirm CLI stability for entry into the study. Ulcer photography at screening should include one photograph of the largest ulcer on the index leg and additional photographs of the index leg that capture the foot and lower leg to confirm the location of the largest ulcer, as well as all additional ulcers (and gangrene) and the overall status of the index leg at screening.

Prior to dosing on Day 0, ulcer photography and tracing of the largest ulcer as at Screening (with ulcer debridement occurring prior to photography and tracing) will be completed again for establishing stability of the largest ulcer and of additional ulcers on the index leg prior to randomization. Ulcer photographs and photographs of the index leg will be taken at each visit (Day 14 through Month 18) and prior to any planned intervention of the index leg (surgical revascularization or major amputation). If the largest ulcer on the index leg heals during the study period, the ulcer must be re-evaluated 2 weeks after healing to confirm it has remained healed for a period of 2 weeks (this will require an additional visit to the site). Ulcer healing will be confirmed later via photographs by the study entry committee. *Ulcer and leg photographs must continue to be taken throughout the study period, regardless of the healing of the largest ulcer.*

4.10. Laboratory Tests

The following clinical laboratory tests will be performed:

1. Hematology: including hemoglobin, hematocrit, RBC count, WBC count (total and differential) and platelet count.
2. Urinalysis including protein, glucose, blood, ketones, bile, and microscopic examination.
3. Blood chemistry: glucose (fasting), uric acid, total cholesterol, HDL, calculated LDL, triglycerides, aspartate transaminase (AST), alanine aminotransferase (ALT), calcium, phosphorus, total bilirubin, alkaline phosphatase, total protein, albumin, globulin, lactic acid dehydrogenase (LDH), serum creatinine, creatinine clearance (estimated using the Cockroft Gault formula), blood urea nitrogen (BUN), and electrolytes (Na^+, K^+, Cl^-),).
4. Serum HGF protein assay and PCR for HGF plasmid and Anti HGF antibodies
5. HbA1c in diabetic subjects

6. FSH and estradiol levels in women 55 years or less (screening only)
7. Urine Pregnancy Test (screening only)

See Section 5 for screening period procedures, Table 3 "Schedule of Study Procedures" and Table 4 "Schedule of Sample Collection for the Serum HGF protein & PCR for HGF Plasmid Assays" for details on what visits these laboratory samples should be collected. Please see the Laboratory Procedure Manual for details regarding blood drawing, storage, processing, and transport.

Diabetic status: To determine the subjects' diabetic status at screening, all subjects will have their fasting blood glucose (FBG) measured. In subjects with known diabetes, supportive documentation will be required to confirm the diagnosis, e.g., evidence of raised blood glucose levels, treatment with insulin or oral anti-diabetic drugs. Diagnostic criteria for diabetes are in the table below.

Diabetic Status Criteria

If FBG < 7.0 mmol/L (126 mg/dL)	Non-diabetic
If FBG ≥ 7.0 mmol/L (126 mg/dL), or previous diabetes diagnosis	Diabetic

4.11. Ischemic Rest Pain Measurement

The severity of rest pain based on the average over the previous 7 days will be recorded using the 10 cm visual analog scale (VAS) (see Appendix E). The VAS score must be documented at all visits and prior to any planned intervention (revascularization or major amputation). In order to assess rest pain adequately, the use of pain medication/opioids must be specifically documented in the CRF during the VAS evaluation period. The subject should not be allowed to see his previous rest pain measurements nor should the score be revealed to him/her.

4.12. Post-Dosing Procedures

Blood samples will be obtained for measurement of serum HGF protein and HGF plasmid DNA four (4) hours after the injections are completed. These blood samples are required at certain dosing visits as per Table 4 "Schedule of Sample Collection for the Serum HGF protein & PCR for HGF Plasmid Assays".

All subjects should be observed and Adverse Events (AEs) recorded. Subject will be discharged from the clinic when clinically stable.

4.13. Adverse Event Review

The Investigator should start documenting adverse events after the first dose of study product administration, during study product administration, post treatment and throughout the study period (Month 18 Visit). See Section 8.0 Adverse Events for details.

Events relating to the worsening of CLI that occur in the index limb such as increased rest pain, new ischemic ulceration or increase the size of the ulcer, gangrene, cellulitis or osteomyelitis, and redness that are related to the study disease and are symptoms/signs of progression of study disease will not be recorded as adverse events (unless the speed or extent of the symptoms/signs are unexpected based on the natural history or bear a strong temporal relationship to the study product).Data on the subject's CLI status of the index limb will be recorded in detail on non-AE CRFs and is therefore not required to be documented as an AE or SAE.

In cases which any of the worsening of CLI events that occur in the index limb such as increased rest pain, new ischemic ulceration or increase in the size of the ulcer, gangrene, cellulitis or osteomyelitis, and redness that are symptoms/signs of progression of critical limb ischemia, **and that result in death**, the event should be reported as an SAE.

Systemic or metabolic complications that ensue subsequent to worsening CLI of the index leg should be reported as AEs or SAEs. Systemic complications include events such as septicemia, pneumonia, multi-organ failure and events resulting in death. These events should be captured on the AE or SAE section of the CRF. Metabolic complications include excessive increase in blood glucose or serum creatinine. Events that result in death should be reported as a SAE.

Limb specific events that occur in the **non-index limb** such as increased ischemic rest pain, new ischemic ulceration or increase in the size of the ulcer, gangrene, minor or major amputations, will be recorded as an AE or SAE on the AE CRF.

4.14. Retinopathy

A retinal exam will be performed by an ophthalmologist as part of the screening procedures (performed up to 6 months prior to Day 0) to exclude proliferative retinopathy, severe non-proliferative retinopathy from any cause (ETDRS retinopathy score > 35), clinically significant macular edema or previous pan retinal photocoagulation therapy. The ETDRS retinopathy score will be recorded. The exam will be repeated 0-14 days before the Month 12 and Month 18 visits. Subjects are not to receive study product at the Month 12 visit until the report has been reviewed.

5. OVERVIEW OF STUDY PROCEDURES

Table 2: List of Screening Procedures

Time: -30 days to Day -1	Comments
Subject Consent & Medical History	
Complete Physical Examination	
Peripheral Vascular Intervention History	Submit to the Entry committee to confirm eligibility
CLI Assessment	Submit to the Entry committee to confirm eligibility
Ulcer Tracings	Photos and tracing of largest ulcer on the index leg only. At least 2 photos should capture the largest ulcer and other picture(s) to capture the entire leg showing the location of the largest ulcer, all ulcers and status of the limb. Debridement should be performed prior to these evaluations. All photos and tracings must be submitted to the Entry committee to confirm eligibility.
Ulcer Photography	
Retinopathy Examination	Performed by an ophthalmologist up to 6 months prior to Day 0
12-Lead ECG	Up to 6 months prior to Day 0
HbA1C and FBG	
Angiogram Assessment	Submit to the Entry committee to confirm eligibility
Hemodynamic Measurements	Submit to the Entry committee to confirm eligibility
VAS (pain scale)	Submit to the Entry committee to confirm eligibility
Urine Pregnancy (Females)	Performed on all women under the age of 55 (regardless of surgical sterility)
FSH and Estradiol Serum Tests (see Appendix G)	Performed on all women under the age of 55
Hematology, Chemistry, CBC, Urinalysis	
Concomitant Medication Review	

The entry committee will be responsible for oversight of entry, confirming the stability of wound (post randomization) indicating the schema for injection sites and adjudicating on the justification for revascularization and major amputation.

Table 3: Schedule of Study Procedures

PROCEDURES	Dosing Visits: First Cycle								Follow Up Visit	Dosing Visits: Second Cycle								Follow Up Visits	
Time	Day 0	Day 14	Day 28	Day 42	Month 3	Month 3 Visit + 14 Days	Month 3 Visit + 28 Days	Month 3 Visit + 42 Days	Month 6	Month 9	Month 9 Visit + 14 Days	Month 9 Visit + 28 Days	Month 9 Visit + 42 Days	Month 12	Month 12 Visit + 14 Days	Month 12 Visit + 28 Days	Month 12 Visit + 42 Days	Month 15	Month 18
Vist Window	Baseline	± 2 days	± 2 days	± 3 days	± 7 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days	± 2 days	± 2 days	± 2 days	± 7 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days
Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18	Visit 19
Physical Examination	X(1)	X(2)	X(2)	X(2)	X(1)	X(2)	X(2)	X(2)	X(1)	X(1)	X(2)	X(2)	X(2)	X(1)	X(2)	X(2)	X(2)	X(1)	X(1)
Peripheral Vascular Intervention History/Update	X(3)*	X(3)	X(3)	X(3)	X(3)	X(3)	X(3)	X(3)	X	X(3)	X(3)	X(3)	X(3)	X(3)	X(3)	X(3)	X(3)	X	X
CLI Assessment (7)	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ulcer Tracings	X(4)*																		
Ulcer Photography (7)	X(4)*	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)	X(6)
Retinopathy Examination														X					X
Study product Administration	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Injection Site Photography	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Angiogram Assessment (7)														X					
TcPO2 (7)	X(3)				X(3)				X(3)	X(3)				X(3)				X	X
Hemodynamic Measurements (7)	X(3)*				X(3)				X	X(3)				X(3)				X	X
QOL (EQ-5D5L) (7)	X(3)*				X(3)				X	X(3)				X(3)				X	X
VAS (pain scale) (7)	X(3)*				X(3)				X	X(3)				X(3)				X	X
Urine Pregnancy	X(5)				X(5)					X(5)				X(5)					
Hematology, Chemistry, & CBC	X(3)				X(3)				X	X(3)				X(3)				X	X
Urinalysis Collection	X(3)				X(3)				X	X(3)				X(3)				X	X
Anti-HGF Antibodies Assay	X(3)				X(3)				X	X(3)				X(3)				X	X
Adverse Events Review (7)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Review (7)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*** Investigator must confirm stability of CLI prior to dosing between Day -2 and Day 0. These items must be submitted to the Adjudication Committee as a third party confirmation of CLI stability.**

(1) Complete physical exam should be performed. If a dosing visit, perform prior to dosing.

(2) Abbreviated physical exam should be performed. If a dosing visit, perform prior to dosing.

(3) Prior to dosing;

(4) Stability of CLI (≥ 2 weeks between the screening and the Day 0 assessments) and subject's ulcers should be confirmed prior to dosing, the subject must have a photo of the largest ulcer on the index leg as well as a photo(s) of the entire index leg to show all ulcer/gangrene locations. Tracings should be taken of the largest ulcer on the index leg only. These must be submitted to the Adjudication Committee. Debridement must be performed prior to these evaluations;

(5) Urine pregnancy tests will be performed prior to dosing on all women aged 55 years or less. For women who have been determined as premenopausal and are not surgically sterile, per the study requirements, will continue with the scheduled urine pregnancy testing throughout the course of the study.

(6) Photos of largest ulcer on the index leg as well as a photo or photos (if necessary) of the entire index leg to show all ulcer/gangrene locations. Debridement should be performed prior to these evaluations;

(7) Procedure must be performed prior to a planned interventional treatment (revascularization or amputation) while on study.

** For the purposes of this study a month is defined as 30 days. Days 14, 28 and 42 are calculated from either Day 0, Month 3, Month 9 or Month 12. All (non-dosing) follow-up visits should be calculated based on Day 0/Baseline.

Table 4: Schedule of Sample Collection for the Serum HGF Protein & PCR for HGF Plasmid Assays

Schedule of Sample Collection for the Serum HGF protein & PCR for HGF Plasmid Assays																
PROCEDURE	Dosing Visit: First Cycle	PK Sampling Visit	Dosing Visits: First Cycle							Follow Up Visit	Dosing Visits: Second Cycle			PK Sampling Visit	Dosing Visit: Second Cycle	Follow Up Visit
Time	Day 0	Day 4	Day 14	Day 28	Day 42	Month 3	Month 3 Visit + 14 Days	Month 3 Visit + 28 Days	Month 3 Visit + 42 Days	Month 6	Month 9	Month 9 Visit + 42 Days	Month 12	Month 12 Visit + 4 days	Month 12 Visit + 42 Days	Month 15
Vist Window	Baseline	0	± 2 days	± 2 days	± 3 days	± 7 days	± 2 days	± 2 days	± 2 days	± 7 days	± 7 days	± 2 days	± 7 days	0	± 2 days	± 7 days
Serum HGF protein Assay	X*	X	X*	X*	X*	X*			X*		X*		X*	X		X
PCR for HGF plasmid DNA Assay	X*	X	X*	X*	X*	X*	X*	X*	X*	X	X*	X*	X*	X	X*	X
*At Dosing Visits (indicated by the shaded columns), two (2) samples should be drawn one pre-dose and a second drawn four hours post-dosing.																

** The first 5 subjects who were randomized and received test medication will have blood samples drawn for PK 4 days following their Month 9 Visit.

5.1. Screening

Written informed consent must be obtained before the subject can begin any screening procedures that are not considered part of standard patient care. Once written informed consent is obtained, the subject must be assigned a screening number, after which the subject may begin the screening procedures for the study. The following screening procedures must be completed within a 30 day period (unless other time period windows have been documented for the procedure, for example ECG) prior to randomization and the receipt of study product on Day 0:

1. Subject Consent
2. Medical History including assessment of diabetes status, alcohol and tobacco use
3. Demographics
4. Physical Examination (see Section 4.3)
5. Concomitant Medication review
6. 12-Lead ECG (up to 6 months prior to Day 0)
7. Peripheral Vascular Intervention History
8. Assessment of CLI
9. Ulcer photographs of largest ulcer on the index leg and the index leg to include all ulcers/gangrene (at least 2 photos). Ulcer debridement, if necessary, should occur prior to photography.
10. Ulcer tracing of the largest ulcer of the index leg, with ulcer debridement occurring prior to tracing as necessary.
11. Hemodynamic measurements (ankle and toe pressures and estimation of Ankle Brachial Index (ABI) and Toe Brachial Index (TBI))
12. Aorto-iliac Angiogram Assessment (performed within the 3 months prior to screening unless subject has shown considerable deterioration in that time or subject has undergone a bypass or endovascular intervention within the previous 3 months).
13. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
14. Collect Laboratory Test samples for the following: Hematology, Chemistry, CBC, HbA1c, FBG, Urinalysis, FHS/estradiol for women 55 years and younger and Urine Pregnancy test (if applicable)
15. Retinopathy Examination (up to 6 months prior to Day 0)
16. Height, weight and vital signs assessment

5.2. Confirmation of Subjects' CLI Stability Pre-Dose

Sites must perform an assessment of the subject's CLI status in order to confirm stability prior to dosing. The stability assessment should occur on Day 0 prior to dosing (≥ 2 weeks between the screening and the Day 0 assessments), and should include ulcer and leg photography and tracings of the largest ulcer on the index leg (with ulcer debridement, if necessary, occurring prior to photography and tracing), hemodynamic measurements, and measurement of ischemic rest pain

severity. The assessment will confirm that the ulcer(s) and gangrene have not increased in size excessively so that the subject requires a surgical intervention or decreased in size excessively so that the subject's lesions will heal spontaneously without study product. The assessment will also confirm that hemodynamic measurements have not changed markedly (± > 0.1 ABI or TBI) and the ischemic rest pain has not increased or decreased greatly despite continuing the same dose of analgesics. Subjects with unstable CLI will not be randomized. They will be considered screen failures. The entry committee will verify the CLI stability post-randomization

5.3. Dosing Visit Study Procedures: Day 0

5.3.1. Pre-Dosing Procedures

1. Medical History Review
2. Physical Exam
3. Peripheral Vascular Intervention Update
4. Assessment of CLI status (associated measurements are listed separately)
5. Ulcer tracings as described in Section 5.2 Confirmation of Subjects' CLI Stability Pre-Dose
6. Ulcer photographs as described in Section 5.2
7. Hemodynamic measurements (ankle and toe pressures and estimation of ABI and TBI)
8. QOL Measurements (VascuQol Questionnaire)
9. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
10. Collect Laboratory Test samples for the following: Hematology, Chemistry, CBC, Urinalysis, Urine Pregnancy test, if applicable (see Appendix G), and anti-HGF antibody sample
11. Collect Pre-dose samples for the Serum HGF protein assay and the PCR for HGF Plasmid DNA assay
12. Concomitant Medication review

5.3.2. Dosing Procedures: Day 0

After successful completion of the pre-dose procedures and confirmation that the subject's CLI status is stable, the subject may receive the first dose of study product. Study product should be administered to the subject by 8 IM injections in the index leg utilizing the appropriate 21G needle and syringe as described in the Study Drug Administration Instructions. The 8 injection site locations will have been determined based on the location of the diseased vessels. Additionally, duplex scanning must be used during the injection procedure to further clarify the injection site locations (See Appendix A).

The injection sites will be marked with a tattoo or similar pen prior to injecting the study product on Day 0. The procedure will be repeated on injections made on Day 14, Day 28, and Day 42 using a different color tattoo or similar pen to track the injections. The Day 14, Day 28, and Day 42 injection sites will be 1-2 cm initially proximal to the Day 0 injection then 1-2 cm distal and

subsequently 1-2 cm lateral, respectively. No more than 3 injections should be made into the anterior or lateral compartments on any dosing visit.

The subject should remain in a supine position to receive all injections. For each of the 8 injections, 3 mL of AMG0001 will be injected in about 3 seconds with a 21G needle. Each of the injection sites will be marked with a tattoo pen. At completion of study product administration, photographs must be taken of the injection sites. In the injection site photo, a ruler should be placed alongside the index leg to assist with the accuracy of the subsequent injection site locations in case one or more of the tattoo pen marks cannot be seen.

The subject must remain in a supine position for 15 minutes post injections. The time of the study product administration should be noted in the subject's source documentation.

5.3.3. Post-Dosing Procedures: Day 0

Adverse events, if any, should be documented during and subsequent to the injection of the study product.

A blood sample will be obtained four (4) hours after the study product injections are completed for measurement of serum HGF protein and HGF plasmid DNA

Subject will be discharged from the clinic when clinically stable.

5.4. PK Sampling Visit: Days 4

A blood sample will be obtained at the Day 4 Visit to measure the serum HGF protein and the HGF plasmid DNA. In the first 5 subjects studied, similar sampling will occur 4 days after the Month 9 Visit.

5.5. Dosing Visit Study Procedures: Days 14 (± 2 days), 28 (± 2 days), and 42 (± 2 days)

Pre-Dosing Procedures: Days 14 (± 2 Days), 28 (± 2 Days), and 42 (± 2 Days)

1. Medical History review
2. Abbreviated Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. Collect Pre-dose blood samples for the Serum HGF protein assay and the PCR for HGF Plasmid DNA assay
7. Adverse Event review
8. Concomitant Medication review
9. Weight and Vital Signs assessment

Dosing Procedures: Days 14 (± 2 days), 28 (± 2 days), and 42 (± 2 days)

Please Note: If subject experiences a significant intercurrent illness, the Investigator may postpone a subsequent injection of study product within or as near to the dosing visit window. If a subject experiences an acceptable adverse event (e.g., hematoma) the Investigator may postpone administration of the study product for a few days. If the adverse event is unacceptable the study product administration should be stopped but the subject should continue to be followed as per protocol. If there are no drug-related adverse events and no significant intercurrent illnesses, the subject will receive the subsequent doses of study product.

After successful completion of the pre-dose procedures, study product should be administered to the subject by 8 IM injections in the index leg utilizing appropriate 21G needle and syringe indicated in (see Appendix A) The 8 injection site locations must be determined based on the final diagram provided to the site by an independent vascular surgeon. Additionally, duplex scanning must be used during the injection procedure to further clarify the injection site locations (See Appendix A)

The injection sites that were marked with a tattoo pen on Day 0 will be identified. The injection procedure will be repeated on Day 14, Day 28, and Day 42 using a different color tattoo pen to track the injections. The Day 14, Day 28, and Day 42 injection sites will be 1-2 cm initially proximal to the Day 0 injection then 1-2 cm distal and subsequently 1-2 cm lateral, respectively. No more than 3 injections should be made into the anterior or lateral compartments on any dosing visit.

The subject should remain in a supine position to receive all injections. For each of the 8 injections, 3 mL of AMG0001 will be injected in about 3 seconds with a 21G needle.

At completion of study product administration, photographs must be taken of the injection site locations (with ruler). The subject must remain in a supine position for 15 minutes post injections. The time of the study product administration should be noted in the subject's source documentation.

Note: for the purposes of this study a month is defined as 30 days; days 14, 28 and 42 are calculated from either Day 0, Month 3, Month 9 or Month 12.

Post-Dosing Procedures: Days 14 (± 2 days), 28 (± 2 days), and 42 (± 2 days)

These post-dosing procedures are the same as the post-dosing procedures of Day 0 in Section 5.3.3

5.6. Dosing Visit Study Procedures: Month 3 (± 7 days)

Pre-Dosing Procedures: Month 3 (± 7 days)

1. Medical History Review
2. Physical Exam
3. Peripheral Vascular Intervention Update
4. Assessment of CLI status (associated measurements are listed separately)
5. Ulcer photographs
6. Hemodynamic measurements (ankle and toe pressures and estimation of ABI and TBI)
7. QOL Measurements (VascuQol Questionnaire)

8. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
9. Collect laboratory test samples for the following: Hematology, Chemistry, CBC, Urinalysis, Urine Pregnancy Test, if applicable (see Appendix G), and anti-HGF antibody sample
10. Collect samples for a pre-dose Serum HGF protein assay and a PCR for HGF Plasmid DNA Assay (See Section 4.10, #4)
11. Adverse Event review
12. Concomitant Medication review
13. Weight and Vital Signs assessment

Dosing Procedures: Month 3 (± 7 days)

The dosing procedures are the same as the dosing procedures of Days 14, 28, and 42 in Section 5.3.2 .

The Month 3 injection site should be the same injection site as Day 0.

5.6.1. Post-Dosing Procedures: Month 3 (± 7 days)

These post-dosing procedures are the same as the post-dosing procedures of Day 0 in Section 5.3.3

5.6.2. Dosing Visit Study Procedures: Month 3+14 days (± 2 days), Month 3+28 days (± 2 days), and Month 3+42days (± 2 days)

Pre-Dosing Procedures: Month 3+14 days (± 2 days), Month 3+28 days (± 2 days), and Month 3+42 days (± 2 days)

1. Medical History review
2. Abbreviated Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. Collect a pre-dose blood draw for the Serum HGF protein assay and the PCR for HGF Plasmid DNA assay,
7. NOTE: Collect samples for BOTH assays at Month 3+42 days
8. NOTE: Collect only one (1) sample for the PCR for HGF plasmid DNA assay at Month 3+14 and 28 days
9. Adverse Event review
10. Concomitant Medication review
11. Weight and Vital Signs assessment

Dosing Procedures: Month 3+14 days (± 2 days), Month 3+28 days (± 2 days), and Month 3+42 days (± 2 days)

These dosing procedures are the same as the dosing procedures of Days 14, 28, and 42 in Section 5.5.

The Month 3+14 days, Month 3+ 28 days, and Month 3+ 42 days injection sites should be the same injection sites as on Days 14, 28, and Day 42, respectively.

Post-Dosing Procedures: Month 3+14 days (± 2 days), Month 3+28 days (± 2 days), and Month 3+42days (± 2 days)

Adverse events should be documented during and subsequent to the injection of the study product.

Four hours after the injections are completed, a blood sample will be obtained for measurement of serum HGF protein and HGF plasmid DNA.

Please note: At the Month 3+14 and 28 day visits, only a sample for the HGF plasmid DNA will be obtained, but at the Month 3+42 day visit a sample for both assays should be obtained.

Subject will be discharged from the clinic when clinically stable.

5.7. Follow-Up Visit Study Procedures: Month 6 (± 7 days)

1. Medical History
2. Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI (associated measurements are listed separately)
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. Injection Site Photography/Marking (to ensure the markings will still be visible at the next dosing visit (Month 9)
7. Hemodynamic Measurements
8. QOL Measurements (VascuQol Questionnaire)
9. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
10. Collect Laboratory Test samples for the following: Hematology, Chemistry, and CBC, Urinalysis, and an anti-HGF antibody assay
11. Collect a blood sample for the PCR for HGF Plasmid DNA assay
12. Adverse Event review
13. Concomitant Medication review
14. Weight and Vital Signs assessment

5.8. Dosing Visit Study Procedures: Month 9 (± 7 days)

ALL PROCEDURES FOR THE MONTH 9 VISIT ARE THE SAME AS THOSE OF THE MONTH 3 VISIT. See Section 5.6.

5.9. Dosing Visit Study Procedures: Month 9+14 days (± 2 days), Month 9+28 days (± 2 days), and Month 9+42 days (± 2 days)

Pre-Dosing Procedures: Month 9+14 days (± 2 days), Month 9+28 days (± 2 days), and Month +42 days (± 2 days)

1. Medical History review
2. Abbreviated Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. **(ONLY AT MONTH 9+42 days VISIT)** Collect a pre-dose blood sample for the PCR for HGF Plasmid DNA assay
7. Adverse Event review
8. Concomitant Medication review
9. Weight and Vital Signs assessment

Dosing Procedures: Month 9+14 days (± 2 days), Month 9+28 days (± 2 days), and Month 9+42days (± 2 days)

These dosing procedures are the same as the dosing procedures of Days 14, 28, and 42 in Section 5.5.

The Month 9+14 days, Month 9+ 28 days, and Month 9+ 42 days injection sites should be the same injection sites as on Days 14, 28, and Day 42, respectively.

Post-Dosing Procedures: Month 9+14 days (± 2 days), Month 9+28 days (± 2 days), and Month 9+42days (± 2 days)

Adverse events should be documented during and subsequent to the injection of the study product.

ONLY FOR MONTH 9+42 DAY VISIT - Four hours after the injections are completed, a blood sample will be obtained for measurement of serum HGF protein and HGF plasmid DNA. Please note: At the Month 9+14 and 28 day visits, there is **no** requirement for a four-hour post dose blood draw.

Subject will be discharged from the clinic when clinically stable.

5.10. Dosing Visit Study Procedures: Month 12 (± 7 days)

ALL PROCEDURES FOR THE MONTH 12 VISIT ARE THE SAME AS THOSE OF THE MONTH 3 VISIT (See Section 5.6).

In addition to the procedures in Section 5.6, the following procedures should also be performed pre-dose:

- Retinopathy Exam

5.11. PK Sampling Visit: Month 12 + 4 Days

A blood sample will be obtained at the Month 12 + 4 Days Visit for subjects enrolled in the study to measure the serum HGF protein and the HGF plasmid DNA.

5.12. Dosing Visit Study Procedures: Month 12+14 days (± 2 days), Month 12+28 days (± 2 days), and Month 12+42days (± 2 days)

ALL PROCEDURES FOR MONTH 12 + 14, 28 and 42 DAY VISITS ARE THE SAME AS THOSE OF THE MONTH 9 + 14, 28 and 42 DAY VISITS. For a list of the procedures to be performed at these dosing visits (see Section 5.9)

5.13. Follow-Up Visit Study Procedures: Month 15 (± 7 days)

1. Medical History
2. Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI (associated measurements are listed separately)
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. Hemodynamic Measurements
7. QOL Measurements (VascuQol Questionnaire)
8. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
9. Collect Laboratory Test samples for the following: Hematology, Chemistry, CBC, Urinalysis, and an anti-HGF antibody
10. Collect samples for the Serum HGF protein assay, and the PCR for HGF Plasmid DNA assay
11. Adverse Event review
12. Concomitant Medication review
13. Weight and Vital Signs assessment

5.14. Follow-Up Visit Study Procedures: Month 18 (± 7 days)

1. Medical History
2. Physical Examination
3. Peripheral Vascular Intervention Update
4. Assessment of CLI (associated measurements are listed separately)
5. Ulcer/gangrene and Index Leg Photography (after debridement, if necessary)
6. Retinopathy Examination
7. Hemodynamic Measurements
8. QOL Measurements (VascuQol Questionnaire)

9. Ischemic Rest Pain severity measured using the VAS pain scale (see Appendix E)
10. Collect Laboratory Test samples for the following: Hematology, Chemistry, CBC, Urinalysis, and an anti-HGF antibody
11. Adverse Event review
12. Concomitant Medication review
13. Weight and Vital Signs assessment

5.15. Assessments to Complete Prior to a Planned Intervention (Surgical Revascularization or Major Amputation)

1. Details of planned Intervention (type, date, etc)
2. Assessment of CLI (associated measurements are listed separately)
3. Ulcer/gangrene Photographs of Index Leg (after debridement)
4. Angiogram Assessment
5. Hemodynamic Measurements
6. QOL assessment using VascuQol Questionnaire
7. Ischemic Rest Pain severity measured using the VAS pain scale
8. Adverse Event review
9. Concomitant Medication review
10. Weight and Vital Signs assessment

5.16. Interim Telephone Contacts

The data collected during the entire study period are critical for the safety and efficacy assessments. Every effort will be made to bring the subject back to the study site for all scheduled visits. Due to the long time period between the Month 6 and Month 9 visits and the Month 15 and Month 18 visits, Study Personnel must make and document a telephone contact with each subject at Month 7.5 (± 5 days) as well as at Month 16.5 (± 5 days).

5.17. Unscheduled Visits

If the site is notified (e.g., at an Interim Telephone Contact) that the subject has experienced an AE that appears to be serious (see Section 8.2.2) or a significant deterioration in CLI status an attempt will be made to evaluate the subject at an unscheduled visit by the study staff unless the subject has been hospitalized remotely. If the subject has been hospitalized remotely details of the illness and treatment will be obtained and documented in the CRF.

Subjects who require 2-week confirmation of largest ulcer healing should also be evaluated at an unscheduled study visit by the study staff.

Data from unscheduled clinic visits will be collected in the CRF.

5.18. Ulcer Healing

Ulcer healing of the largest ulcer on the index limb (determined at Screening) will be assessed clinically by the Principal Investigator by direct visual inspection at each study visit. If the largest ulcer on the index leg is considered completely healed, photographs of the healed ulcer area must be captured. If the ulcer heals completely during the study period, the ulcer must be re-evaluated 2 weeks after healing to confirm it has remained healed for a period of 2 weeks. Confirmation of complete ulcer healing must be made by an outside physician unconnected with the study and nominated for this purpose

5.19. Diabetic control

Every effort will be made to ensure good diabetic control (HbA1c $\leq$ 7%), which will not only include maintaining ideal blood glucose levels, and appropriate foot care, but also managing diabetic complications such as diabetic retinopathy, neuropathy, and nephropathy. Ideally diabetic subjects should have their diabetes managed by a diabetic specialist (endocrinologist) throughout their participation in the study.

5.20. Documentation of Deaths

In cases where death is the outcome of a Serious Adverse Event (SAE), or occurs suddenly without apparent cause, appropriate documentation will be requested by the Sponsor.

All deaths including cases where death is due to subjects' worsening CLI, must be supported by source documentation such as a hospital discharge summary, death certificate, autopsy report or a doctor's report/letter. All serious adverse events with the outcome of death will be reported **immediately** to the Sponsor on the CRF via email or fax.

6. SCREEN FAILURES AND SUBJECT WITHDRAWALS

6.1. Withdrawals

A subject may refuse any or all treatment or study procedures at any time during the study. Participation is voluntary, and refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled. ***A subject who refuses to participate after receiving one or more doses of the study product should be encouraged to continue follow-up as per protocol including the safety follow-up program (See Section 10) Safety Follow-up Program regarding safety follow-up procedures).***

If the subject wishes to withdraw study consent, every effort must be made to encourage the subject to return to the study site for safety evaluation purposes for all remaining study visits. Subjects who wish to withdraw from the study prematurely and have had at least one dose of study product will be requested to return to the study site for study specific safety and efficacy assessments.

If the subject withdraws prior to Month 6, the procedures for the Month 6 visit should be completed as the exit visit. If the subject withdraws between Month 6 and Month 18, the procedures for the Month 18 visit should be completed as the exit visit. Additionally, every effort will be made to obtain and record information regarding revascularization by surgery, major

amputation, myocardial infarction, stroke, and death even if the subject does not return for an exit visit. In case any of these events have occurred, evidence from source documents will be obtained for confirmation. Prior informed consent will have been obtained from the subject to gain access to these documents. If the subject refuses any continued study participation, decline of study participation should be documented in writing. If the subject refuses to communicate with the study site, final attempts to contact the subject should be documented with a certified letter to the subject's last known address.

The Principal Investigator is permitted to discontinue the subject from receiving study product for medical reasons at any time. However, the subject will continue to return for protocol dictated follow-up visits for the remainder of the total study period (18 month visit) including the safety follow-up program.

6.2. Screen Failures

Screen failures will include any subject who was consented, but subsequently did not meet the entry criteria in order to receive study product. Subjects who fail screening will not be followed for safety or efficacy assessment, and no other study procedures will be performed. Appropriate screen failure data including reason for ineligibility will be collected on the CRF.

6.3. Study Suspension or Termination

The IRB, the Sponsor, the FDA, or other regulatory health agencies may choose to voluntarily suspend or terminate some or all activities of the study at any time if they judge that it is in the best interest of the subject. They may also suspend or terminate the study for various other reasons. They can do this without the consent of the subject.

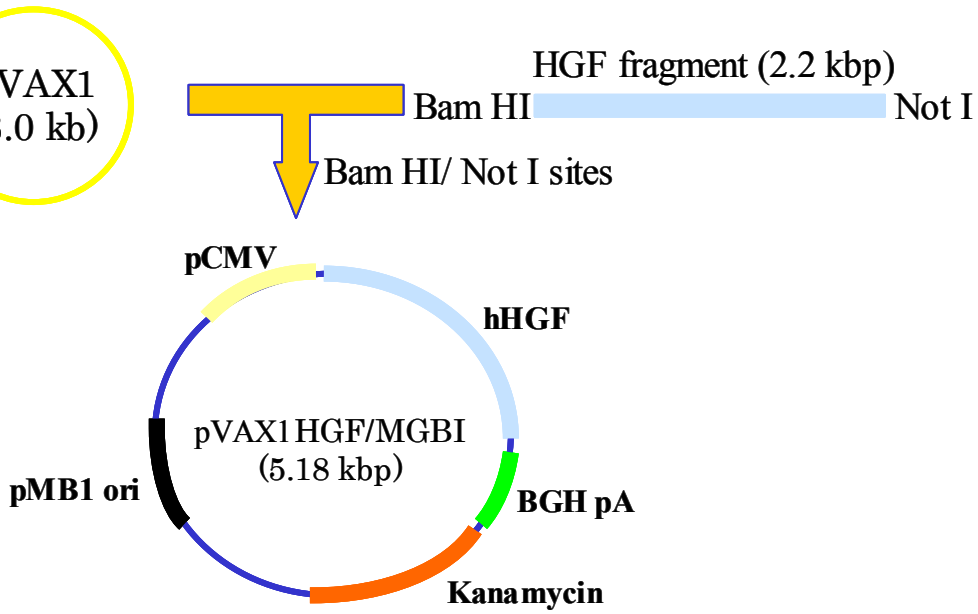
7. STUDY PRODUCT

7.1. Product Description

Active Drug: AMG0001 (Figure 1) is a clear liquid with a pH of approximately 4.5 to 7.5 containing 2.5 mg/mL AMG0001 (plasmid construct pVAX1 HGF/MGBI) dissolved in physiological saline in a 2.10 mL volume (5.25 mg plasmid).

Name: AMG0001 may also be named "HGF" or "HGF Plasmid"

Figure 1: Schematic representation of AMG0001



7.2. Packaging and Labeling

The study product will be provided in 3 mL glass vials. Each subject package and vial is labeled to indicate the unique vial number, vial volume, date of manufacture, and the following statement "CAUTION: New Drug - for investigational use only."

7.3. Storage

Details regarding storage of both undiluted and diluted investigational product are available in the Pharmacy Manual. Upon receipt the Investigational Product should immediately be stored in a freezer at -15^{0}C to -25^{0}C (5^{0}F to -13^{0}F). Freezers should not have an autodefrost feature and must have continuous temperature monitoring as described in the Pharmacy Manual. Once the vial is thawed at the clinical center, it should not be refrozen. Details for storage of the undiluted and diluted investigational product are available in Appendix A and the Pharmacy Manual.

7.4. Study Product Accountability

Upon receipt, the Investigator is responsible for taking an inventory of the study product. A record of this inventory must be kept and usage must be documented on study product accountability logs provided by the Sponsor. Study product accountability logs will be examined and reconciled by the study monitor.

In accordance with federal regulations (21 CFR 312.62), all Investigators are required to keep accurate records showing final disposition of all investigational drugs/study product. These records must be available to the Sponsor on request, showing accurate reconciliation of each and every shipment of study product and all activity of study product at the site (storage, dispensation to subjects, etc). The Investigator is also required to ensure adequate storage of study product, which includes appropriate documentation of such storage conditions (e.g., maintaining temperature logs, etc).

Ultimate accountability for the receipt, dispensation and record-keeping of the study product lies with the Investigator. Federal regulation requires that storage of the substance be in a secure enclosure, access to which is limited, to prevent theft or diversion. Neither the Pharmacist nor Investigator may supply the study product to any person outside of this protocol.

Dosing must be documented on the CRF for each subject in addition to the study product accountability logs.

Any unused vials must be retained at the study site until reconciliation by the study monitor. Opened (used) vials must be maintained until the study monitor confirms accountability. Once the study monitor completes accountability procedures, the used (opened) vials and any unused vials will be disposed of using each institution's recommended investigational drug and biohazard material disposition procedures.

The study product provided by the Sponsor for use in this study is intended for use only in the clinical trial outlined in this protocol and will be administered only to subjects appropriately enrolled in this trial. The use of the drug for other clinical or pre-clinical situations is strictly prohibited. AMG0001 is an investigational medication for use only in Clinical Trial AG-CLI-0209. The possession and use of AMG0001 must be closely controlled and monitored by Investigators. Upon completion of the study, the study monitor will arrange for return or destruction of all used or unused vials of study product.

8. SAFETY REPORTING

8.1. Adverse Events and Serious Adverse Events

The protocol has been designed with an understanding of the underlying cardiovascular disease and associated co-morbidities in the study population, and the unknown potential angiogenic effects of AMG0001. Although to date there are no indications that AMG0001 increases the incidence of serious nonfatal or fatal events, an evaluation of adverse events will be performed at each visit. The subject will be questioned in a non-leading manner to determine if any adverse events have occurred during product administration and during the post-treatment study period. **An event that occurs during the Screening Period (from the time informed consent to just prior to the first dose of study product administration) must be documented as part of the Medical History**.

Adverse Events should be documented and followed from the start of the first dose of study product (Day 0), until the final outcome is known or until the end of the study safety follow-up period. AEs (including SAEs) that have not resolved by the end of the study follow-up period should be recorded in the CRF as "ongoing". If it is not possible to obtain a final outcome for a SAE, the reason that a final outcome could not be obtained must be documented (e.g., completion of follow up, subject has moved and cannot be contacted, etc.).

Each Adverse Event or Serious Adverse Events should be adequately described in the subject's medical record. The description of AEs or SAEs will include the nature of the event, the time of onset, the duration and severity of each event, the Investigator's opinion regarding the causal relationship to study product or the subject's CLI, the action taken, and the outcome of the event.

All adverse events (excluding events related to the worsening of CLI as described below) will be reported and recorded in the subjects' source documentation and the appropriate CRF.

Any **serious adverse event** (see definition below) observed by the Investigator or study staff or reported by the subject that occurs within the study period will be recorded on the appropriate CRF. These events will be reported regardless of the perceived relationship of the event to the study product.

8.1.1. Adverse Events Associated with Worsening CLI

Adverse Events relating to the worsening of CLI that occur in the index limb such as increased rest pain, new ischemic ulceration or increase the size of the ulcer, gangrene, cellulitis or osteomyelitis, that are related to the study disease and are symptoms/signs of progression of study disease will not be recorded as adverse events (unless the speed or extent of the symptoms/signs are unexpected based on the natural history or bear a strong temporal relationship to the study product as determined by the Investigator). Data on the subject's CLI status of the index limb will be recorded in detail on non-AE CRFs and not required to be documented as an AE or SAE, unless the outcome of the event is death.

Systemic or metabolic complications (e.g., diabetic ketoacidosis, renal failure, etc.) that ensue subsequent to worsening CLI of the index leg should be reported as AEs or SAEs. SAEs due to systemic complications include events such as septicemia, pneumonia, multi-organ failure and events resulting in death. These events should be reported as an SAE.

Limb specific events that occur in the **non-index limb** such as increased rest pain, new ischemic ulceration or increase in the size of the ulcer, gangrene, minor or major amputations, will be recorded as an AE or SAE on the AE CRF

8.1.2. SAEs Associated with Worsening CLI

Serious Adverse Events relating to the worsening of CLI that occur in the index limb such as gangrene, cellulitis or osteomyelitis, are related to the study disease and are symptoms/signs of progression of study disease will not be recorded as serious adverse events (unless the speed or extent of the symptoms/signs are unexpected based on the natural history or bear a strong temporal relationship to the study product as determined by the Investigator).Data on the subject's CLI status of the index limb will be recorded in detail on non-AE CRFs and is not required to be documented as an SAE. Frequently worsening of CLI may lead to hospitalization followed by local or systemic treatment or surgical intervention (minor amputation, surgical revascularization or major amputation). The entries for the above events will be made in the CLI section of the CRF. However, in the case of serious intercurrent illness or systemic complications like septicemia, multi-organ failure, or pneumonia, these events will be captured in the AE or SAE section of the CRF.

8.1.3. Deaths Following Worsening of CLI

CLI-Related Adverse Events or CLI -Related Serious Adverse Events that result in death will be reported. The death will be captured on the SAE CRFs.

CLI-Related Deaths include but are not limited to the following:

- Deaths due to infected ulcers/gangrene that lead to septicemia/organ failure
- Perioperative Deaths following revascularization
- Perioperative Deaths following major amputation
- Worsening of CLI leading to death resulting from complications of Diabetic Ketoacidosis/Renal Failure

8.1.4. Deaths

All deaths will be documented in the CRFs in a specified section. In the event of death, the Investigator will seek permission for an autopsy examination. Information on deaths will be obtained during the Post-18 month follow-up via questionnaire. Hospital discharge reports or death certificates will be requested from the nearest relative.

8.2. Definitions

8.2.1. Treatment-Emergent Adverse Events

The following definition of a **treatment-emergent adverse event** will be used for this study: Any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding that is considered to be clinically significant), syndrome or disease which, either occurs during the study, having been absent at baseline, or if present at baseline, appears to have worsened in severity or frequency, whether or not the event is considered related to the study product.

It is the responsibility of the Investigator(s) to perform periodic assessments for adverse events. The Investigators and clinical staff will document all adverse events disclosed by the subject at baseline and during the treatment and follow-up periods. All clinical complaints volunteered by, or elicited from, the subject during the study or from findings on the subject's physical examination will be recorded in the clinical records and on the Adverse Event CRF. If any adverse event occurs, the subject will receive appropriate medical treatment and supervision. All adverse events will be followed until resolution where possible.

For the purpose of this study, index leg specific events that relate to the worsening of the subject's CLI will not be reported as Adverse Events unless applicable. (Please see Section 8.1).

8.2.2. Serious Adverse Event

A **serious adverse event** (21 CFR 312.32) is defined as any adverse event (excluding worsening CLI events if applicable) that suggests a significant hazard, contraindication, side effect or untoward medical occurrence that:

- Results in death,
- Is life-threatening,
- Requires (or prolongs) hospitalization,
- Causes persistent or significant disability/incapacity,
- Results in congenital anomalies or birth defects, or
- Other significant medical conditions which in the judgment of the investigator(s) or Sponsor represent significant hazards.

For the purpose of this study, index leg specific events that relate to the worsening of the subject's CLI will not be reported as Serious Adverse Events unless applicable. (Please see Section 8.1)

Any **serious and immediately life-threatening** adverse event, including death, occurring while the subject is receiving an investigational drug or control agent; within 18 months of the first administration of study product; and throughout the safety follow-up period, irrespective of the Investigator's opinion regarding drug relationship, will be reported by phone to the Sponsor and faxed **immediately**.

All serious adverse events will be reported by contacting the Sponsor/Safety Medical Monitor. The Safety Medical Monitor is available for medical consultation issues related to management of serious adverse events. Contact information regarding the Sponsor/Safety Medical Monitor can be found in the Study Reference Manual provided at the time of the Initiation Monitoring Visit.

8.2.3. Expected Adverse Events

Adverse events described in the Investigator's Brochure or that have occurred previously in the course of the active studies for this product (unless the frequency/severity or consequences have changed) are considered expected adverse events.

8.2.4. Intensity or Severity of Adverse Events

Assignment of the grade of severity of adverse events will be based on intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of objective clinical signs or laboratory parameters. Adverse events will be graded according to their severity using the following criteria:

Mild: the adverse event is transient or minimal and either resolves spontaneously or no treatment is required. Symptoms do not interfere with subject's daily activities.

Moderate: the adverse event required only brief treatment with prescription medication and/or intervention but produced no sequelae and required no hospitalization; symptoms may interfere with daily activities; symptomatic, moderate change in activity; no change in social activities.

Severe: the event produced sequelae that required prolonged treatment with prescription medication, intervention and/or hospitalization; interrupts subject's usual daily activities; incapacitating, requires bed rest, absence from work and/or a decrease in social activities.

8.2.5. Relatedness of Adverse Event to an Intervention

An adverse event can be assessed as:

a. Likely Related - The adverse event is likely to be related to the study product(s)
b. Possibly Related - The adverse event may be related to the study product(s)
c. Likely Unrelated - The adverse event is unlikely to be related to the study product(s)

If an adverse event is evaluated as not related to the study product, there must be an alternative etiology in the Investigator's assessment for that event documented in the subject's medical records.

9. EVALUATIONS AND STATISTICAL METHODS

9.1. Evaluations

The following evaluations relating to tolerability, safety and clinical benefit will be made:

1. Adverse events (AEs) suspected to be related to injections of HGF plasmid
2. Discontinuations related to adverse effects from the injections of HGF plasmid
3. All AEs up to Month 3, Month 6, Month 12, and Month 18
4. Serious adverse events (SAEs) up to Month 3, Month 6, Month 12, and Month 18
5. Number of deaths and cause over the 18 month period up to Month 3, Month 6, Month 12, and Month 18
6. Number of subjects in whom the largest ulcer healed completely or gets smaller (photo confirmation) at 6, 12 and 18 months (cumulative)
7. Number of subjects in whom rest pain (based on the 100 mm VAS scale) reduces by 20 mm or more or was completely relieved at 6, 12 and 18 months (cumulative)
8. Change in VascuQol Questionnaire (QOL) score compared to the baseline at 6, 12 and 18 months
9. Number of subjects revascularized (bypass or endovascular intervention) up to Month 3, Month 6, Month 12, and Month 18
10. Number of subjects with minor and number of subjects with major amputations (at or above the ankle) up to Month 3, Month 6, Month 12, and Month 18

9.2. Statistical Methods

All summaries and analyses will be presented in tabular or graphical form. The study is not powered for the statistical inference and the test will be considered to be descriptive.

9.2.1. Disposition, Compliance, Demographic and Background Characteristics (Including Medical History)

The number subjects enrolled, and the number and percent of subjects who completed the study, prematurely discontinued, primary reason for discontinuation, and the compliance to the study product (number of subjects who received the first course of injections only, both the first and subsequent courses of injections) will be summarized.

The demographic data (age, weight, gender, race), and the prior and concomitant medication will be summarized at baseline and during the study. The use of selective concomitant medication such as medications for pain, hypertension, hyperlipidemia, antiplatelet agents will also be summarized.

9.2.2. Statistical Analysis for Efficacy and Safety

Categorical data will be summarized by the use of frequency tables showing the number and percentage of subjects falling within a particular category by visit, and detailed line listings of subjects will be provided.

Continuous data will be summarized by reporting number of observations, mean, standard deviation (SD), median, minimum and maximum by visit. Summary statistics will be provided for baseline and the change (with percent change) from baseline. A one-sample t-test will be performed on within group change-from-baseline for individual time point (Months 6, 9, 12, 15, and 18 with last observation data (assessed prior to intervention or worse score for deaths) carry-forward (LOCF)).

9.2.3. Windows for time points

Number of subjects followed up to 3 months: subjects with a follow-up visit at day 90 ±10 (days 80 to 100) will be counted.

For months 6, 9, 12, 15 and 18 evaluation: subjects with follow-up visits at day 182 + 15 (days 167 to 197), day 273 + 15 (days 258 to 288), day 365 + 30 (days 335 to 395), day 455 +35 (days 420 to 490), and day 548 + 45 (days 503 to 593) will be counted.

For 12 months and 18 months evaluations, events occurring up to day 395 and day 593 will be taken into account, respectively.

10. SAFETY FOLLOW-UP PROGRAM

Since this study uses a DNA plasmid for gene transfer, the Sponsor (AnGes, Inc.) is complying with regulatory guidance to conduct long-term safety follow-up to collect safety information on all participants who receive the study product. The long-term safety follow-up data will be collected every 6 months for an additional 18 months following the Month 18 Visit.

Adverse Events should be documented and followed from the start of the first dose of study product (Day 0), until the final outcome is known or until the end of the study safety follow-up period. AEs (including SAEs) that have not resolved by the end of the study follow-up period should be recorded in the CRF as "ongoing". If it is not possible to obtain a final outcome for a SAE, the reason that a final outcome could not be obtained must be documented (e.g., completion of follow up, subject has moved and cannot be contacted, etc.). Serious Adverse Events should be reported within 24 hours of site awareness.

Deaths, major amputations, revascularizations by surgical bypass, stroke, and myocardial infarction will be source verified. An appropriate request/permission for this safety follow-up program will be included in the informed consent.

The data collected during the follow-up period will consist of surgical revascularization, lower limb amputation, cancers, eyesight changes, bleeding/blood disorders, autoimmune diseases, neurological disorders, hospitalizations, unexpected or significant illnesses including stroke, myocardial infarction, and death. In the event of death, information regarding the above data (as best as possible) and date and cause of death will be requested from a spouse or nearest relative. The questionnaire is found in Appendix F.

Site staff should obtain information from the completed questionnaires via phone contact within ±7 days of the target follow-up contact date. If the subject has experienced any of the events types noted in the questionnaire additional information may need to be requested from the subject and/or supporting medical records may be required. In the event the subject experiences (CLI status of the index limb, revascularization of the index limb, amputation of the index limb, cancers, eyesight changes, bleeding/blood disorders, autoimmune diseases, neurological disorders, hospitalizations, and unexpected or significant illnesses including stroke, myocardial infarction, and death) data on relevant onset/resolution dates, treatments and confirmed diagnosis (as and where applicable) will be obtained.

All information collected by the questionnaire will be submitted periodically to the FDA and any other agencies as required.

All safety information collected by the questionnaire that is not included in the Biologics License Application (BLA) will be submitted periodically to the FDA and any other agencies as required. Subjects will not be identified in any report, publication, or submission. All subjects participating in the study will receive an explanation of these procedures, and their rights under HIPAA, at the time of Informed Consent.

11. ADMINISTRATIVE PROCEDURES

11.1. Institutional Review Board/ Ethics Committee

Regulatory Authorities require that an IRB/EC oversee all investigational drug studies (21 CFR 56). Each IRB/EC must conform to federal regulations and any country, state and local guidelines regarding such, will approve all aspects of the study, including but not limited to said protocol and written Informed Consent to be used, prior to initiation of the study. The Investigator will provide the Sponsor with a copy of the communication from the committee to the Investigator indicating approval of the protocol and consent form. All amendments to the protocol that affect the conduct of the study must be reviewed and approved prior to implementation, except where necessary to eliminate immediate hazards to human subjects.

As per IRB/EC requirements, the Investigator will be responsible for obtaining IRB/EC renewals; submitting expedited and other periodic safety reports to the IRB/EC for the duration of the study; and for following any additional reporting requirements by the IRB/EC approving study activity at his/her investigational site. Copies of all approvals, renewals and correspondence to and from the IRB/EC must be forwarded to the Sponsor.

11.2. Institutional Biosafety Committee (IBC)

The institution at which a gene transfer trial is being conducted shall establish an Institutional Biosafety Committee (IBC) whose responsibilities need not be restricted to recombinant DNA products. The IBC is responsible for conformance with the requirements set forth in the NIH Guidelines for Research Involving Recombinant DNA Molecules as defined in Section IV-B-2 for the US.

12. CONFIDENTIALITY

All information and data, including this protocol, and all data, clinical results and research conducted hereunder concerning the Sponsor's products and operations including the Sponsor's patent applications, formulas, manufacturing processes, basic scientific data and formulation information that has been supplied by the Sponsor and not previously published are considered confidential by the Sponsor and will remain the sole property of the Sponsor. The Investigator understands and agrees that said proprietary and/or confidential information disclosed to or produced by him/her there under is highly valuable to the Sponsor and will be used EXCLUSIVELY by the Investigator in accomplishing this study and will not use it for any other purposes without the Sponsor's prior written consent.

The Investigator agrees that he/she will not use any such proprietary and/or confidential information for any other purpose. The Investigator also understands and agrees that such disclosure will not be deemed to grant to the investigator a license for use of said proprietary and/or confidential information, except as expressly provided herein. It is understood by the Investigator that the information developed in the clinical study will be used by the Sponsor in connection with the development of AMG0001, therefore, may be disclosed and used solely by the Sponsor as required to such third parties and agencies as the Sponsor, in its sole discretion, warrants. In order to allow for the use of the information derived from the clinical studies, it is understood that there is an obligation to provide to the Sponsor complete test results and all data developed in this study. The Investigator agrees to promptly answer all inquiries from the Sponsor regarding completion, legibility or accuracy of trial data in the CRF.

APPENDIX A. Study Product Preparation &Administration

1. SELECTION OF INJECTION SITE LOCATIONS

AMG0001 injection site locations will be identified using images from contrast arteriograms, CTA, or MRA and prior to Day 0. Injection sites will be determined by the independent vascular surgeon. Injection sites will be chosen with the objective of inducing the formation of bridging collaterals from areas with normal blood flow. Desirable locations for injections, above and/or below the knee, are areas of musculature proximal and distal to the occluded sites and areas adjacent to the occlusion where blood flow through collateral blood vessels is identified on the pre-procedure angiogram. Eight optimal injection site locations will be identified for all subjects prior to the planned Day 0. Injection sites will be indicated on an anatomical diagram prior to the planned Day 0.

- Subjects with no apparent occlusion above the knee will receive all 8 injections below the knee. Injections will be distributed between musculature adjacent to the Anterior Tibial Artery, the Posterior Tibial Artery, and the Peroneal Artery. Areas with good collateral flow should be considered ideal for injection site locations.
- When Subjects do have occlusions noted on the screening angiogram, the number of injections administered to each artery will be distributed in proportion to the severity of the occlusions at each location. Injections will be located in the proximity of the occlusion.

Desirable locations for injection are areas of musculature proximal and distal to the sites of vessel occlusion, in areas where there is good blood flow. Areas of ischemia should be avoided as sites for injection. The goal of duplex guided injection is to be certain that injection of the study product is placed into the muscle in proximity to the diseased vessel and not into the peri-vascular space.

It is advised that AMG0001 Injections not be administered into the gastrocnemius muscle due to the close proximity of major arterial anatomy. See Section 4.4 of this Appendix (below) for Injection Site Location examples.

2. SYRINGE PREPARATION

Only the Principal Investigator or authorized personnel who have been instructed in the proper administration and accountability of the study product will administer the investigational study product. Study product intended for one subject must not be used for another subject. The Investigator must not use material provided for this particular study in another study.

The pharmacy should confirm that the subject has arrived in the clinic prior to preparation of study product.

Approximately 15 to 20 minutes prior to study product preparation remove the vial from the -20˚C (± 10˚C) storage and bring to room temperature. The vial should not be heated or microwaved. **The vial should not be shaken.** AMG0001 is stable at room temperature (25˚C) for up to 6 hours.

All preparation should be conducted using controlled and validated aseptic techniques under Biosafety Level 1 (BSL1) conditions, including Bio-Safety Cabinet II (BSC II) or Laminar Flow

Hood (Biosafety Class 1 or International Organization for Standardization (ISO) Class 5). Use of goggles, mask, sterile gloves and lab coat is recommended.

To prepare one administration of the AMG0001:

With a 21 Gauge needle, withdraw 1.7 mL contents of one AMG0001 into a syringe and dilute into a 30 mL sterile vial containing 23.7 mL sterile saline to provide 25.4 mL of a diluted 0.167 mg/mL solution. Slowly invert the 30 mL vial 4 times to provide gentle mixing of study product and diluent. Three (3) mL of the solution should be filled in each of 8 (3 mL) syringes provided by the Sponsor. Each 3 mL syringe of the diluted solution should be used for each of 8 injections during the administration. Syringes must be labeled with the subject number. The overage is provided to ensure that a delivered volume of 3 mL is injected and the residual undelivered volume in the vials and all 8 syringes is accounted for properly. Any residual material should be disposed of in an appropriate manner for biological waste products.

After dilution with sterile saline solution, the diluted study product is physically and chemically stable for 24 hours at 2°C-8°C. If the prepared syringes are refrigerated prior to being administered, they should be left to reach room temperature prior to the start of the injection procedure.

From a microbiological point of view, the product should be used immediately. The product is not intended to be stored after dilution unless this has taken place under controlled and validated aseptic conditions. If not used immediately, in-use storage times and conditions are the responsibility of the user and would normally not be longer than 6 hours up to 25°C or 24 hours at 2°C-8°C**.**

DO NOT DISCARD USED STUDY PRODUCT VIALS UNTIL AUTHORIZED BY THE STUDY MONITOR.

3. ADMINISTRATION OF INJECTIONS

Based on the injection site locations confirmed by the vascular surgeon, Investigators should mark the initial 8 injection sites with a tattoo pen prior to injecting the study product on Day 0. The procedure will be repeated on injections made on Day 14, Day 28, and Day 42 using a different color tattoo pen to track the injections. The Month 3, Month 9, and Month 12 injection site locations should mirror that of Day 0.

Subsequent injection site locations for the Day 14, Day 28, and Day 42 injection sites will be 1-2 cm initially proximal to the Day 0 injection then 1-2 cm distal and subsequently 1-2 cm lateral, respectively. The Month 3+14 days, Month 3+28 days, Month 3+42 days, Month 9+14 days, Month 9+28 days, Month 9+42 days, and Month 12 + 14 days, Month 12 + 28 days, and Month 12 + 42 days injection site locations should mirror that of Days 14, 28, and 42, respectively.

No more than 3 injections should be made into the anterior or lateral compartments on any dosing visit.

The Investigator should utilize duplex ultra-sound to guide the 8 IM injections.

Investigators must utilize a 21 Gauge, 7cm echotip® needle and the 3mL syringes for the IM injections of study product.

Investigators should trace blood vessels from the proximal portion while maintaining the probe at a right angle with the color Doppler and identify occluded areas. Investigators should select

injection sites as areas of musculature proximal and distal to the occluded sites and areas adjacent to the occlusion where blood flow through the collateral blood vessels.

Investigators should check pulsation with a color Doppler. The blood flow wave should also be examined with a color Doppler in order to exclude venous blood flow if arterial blood flow is poor around the selected injection sites.

Insert the needle while monitoring it under echographic guidance.

Confirm the injection into the muscle under B mode echography by injecting a small amount of the investigational product. If the injection is made into the muscle, the drug is shown to infiltrate in a spherical form. This observation cannot be seen if the injection is made into the fascia or interstitial tissues.

Inject the entire 3mL in the syringe in about 3 seconds. Immediately after completion of injection, lightly press the injection site with the finger in order to prevent reflux.

Do not massage the injection site.

The subject should remain in a supine position while receiving all 8 injections and for 15 minutes post injections.

The time and details of study product administration should be noted in the subject's source documentation and CRF.

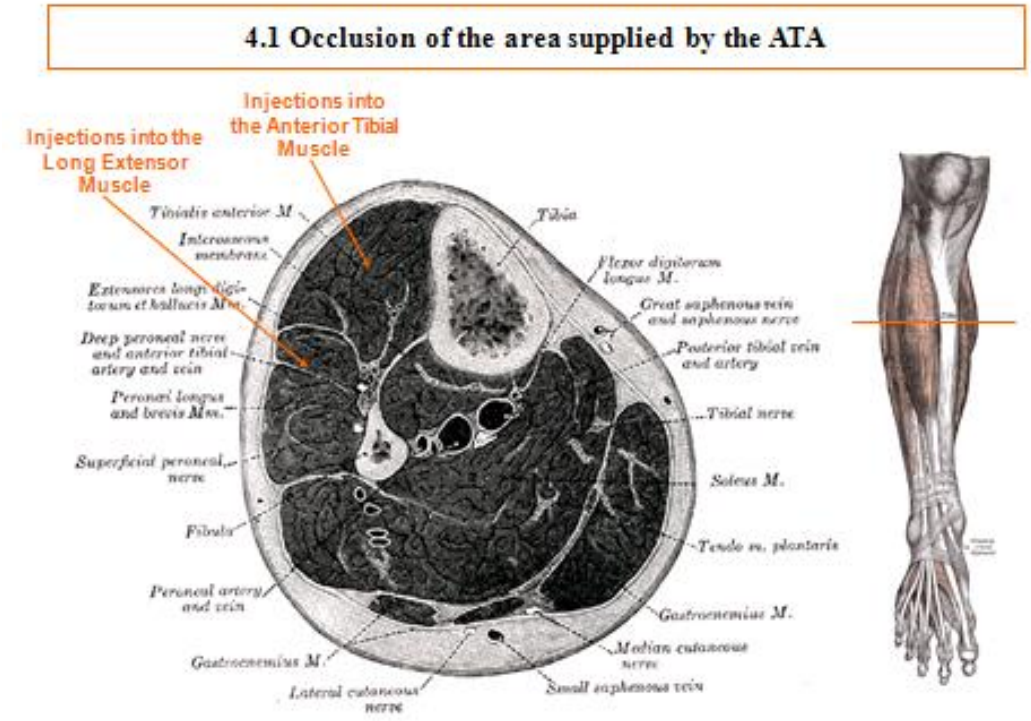
Photographs of the injection site locations (with ruler) must be taken at the completion of each dose.

4. ADMINISTRATION METHOD

Below are suggested needle approaches for the administration of study product to muscular anatomy supplied by commonly occluded arteries. The popliteal artery is branched into the anterior tibial artery (ATA), posterior tibial artery (PTA), and peroneal artery.

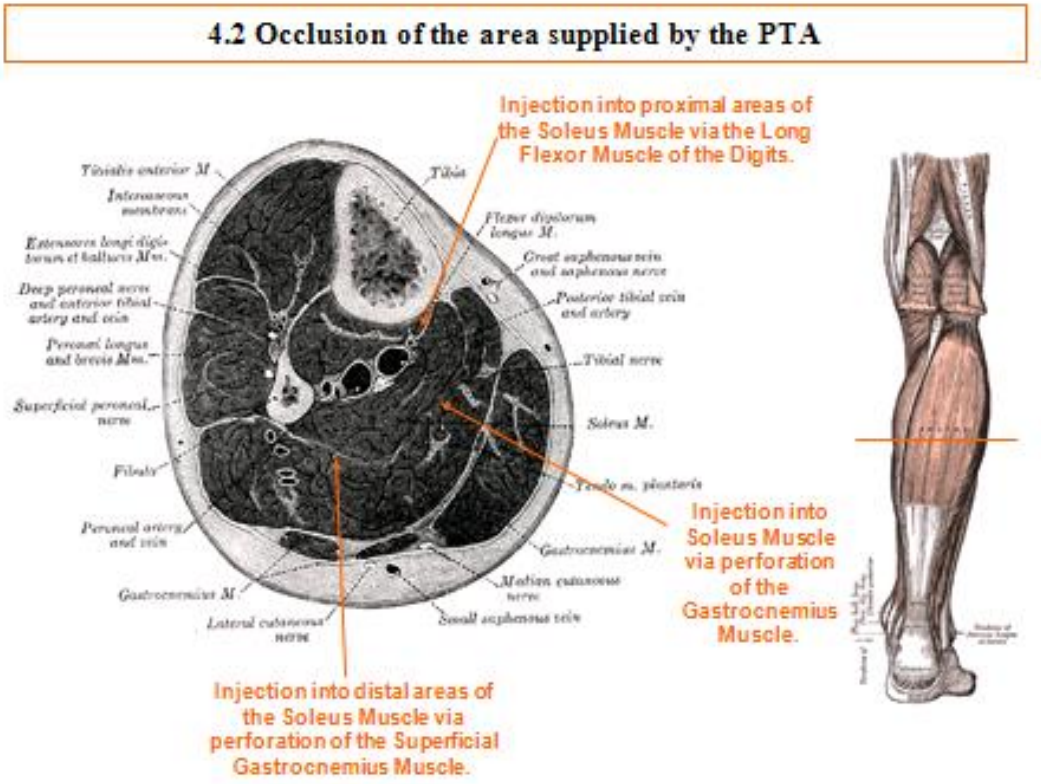
4.1 OCCLUSION OF THE AREA SUPPLIED BY THE ATA

ATA is distributed in a deep layer of compartments consisting of a group of extensor muscles such as the anterior tibial muscle and the long extensor muscle of the great toe. Collateral blood vessels are also distributed in these compartments. Apply the echo probe from the lateral site of the Tibia and make the injection into these extensor muscles. The ATA perforates the interosseous membrane right beneath the knee joint. If occlusion occurs in the area proximal to this perforation site, no efficacy can be expected even if injection is made into the component consisting of extensor muscles. It is recommended that injection not be made in such compartment.



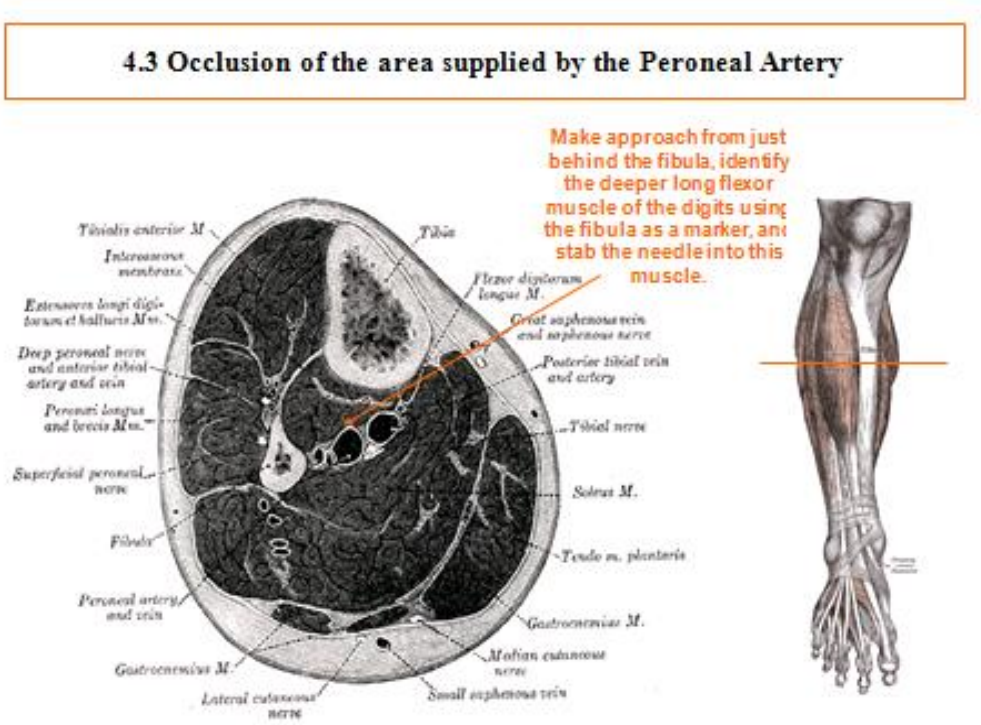
4.2 OCCLUSION IN THE AREA SUPPLIED BY THE PTA

The PTA is distributed between the soleus muscle and a deeper compartment. Collateral blood vessels maybe distributed in both compartments. The surface of the proximal portion of the crus (2/3) is covered with the gastrocnemius muscle. In order to inject the drug into the soleus muscle, the gastrocnemius muscle must be perforated with the needle. When a distal portion of the crus is targeted, checking based on ultra-sound guided images and injection are easy since the superficial gastrocnemius muscle is thin and the needling depth is shallow. In contrast, if the target tends to be in the deeper proximal areas and observation is difficult, make approach from just behind the tibia, i.e., from the medial side and stab the needle into the muscle such as the long flexor muscle of the digits.



4.3 STUDY PRODUCT PREPARATION &ADMINISTRATION

The peroneal artery is distributed into a layer deeper than the long flexor muscle of the digits. Make approach from just behind the fibula, identify the deeper long flexor muscle of the digits using the fibula as a marker, and stab the needle into this muscle.

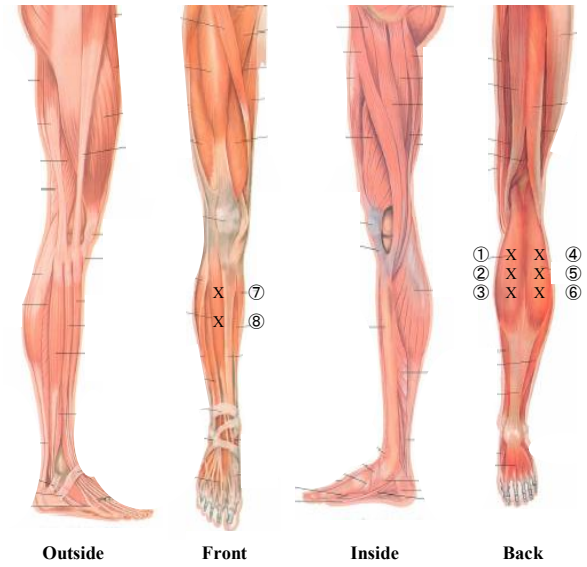


In 4.2 and 4.3 above, attention is required not to stab the needle into the gastrocnemius muscle. Because blood vessels are more branched in the gastrocnemius muscle than in the popliteal region, increases in collateral circuits in the gastrocnemius muscle compartment are not expected to contribute to increases in peripheral blood flow in the lower limbs.

Examples of Injection Site Location Scenarios are Given Below:

Injection sites

Patient ID:	Example #1- Meeting May 2005
Occlusion and/or Stenoses:	Anterior Tibial Artery and Posterior Tibial Artery

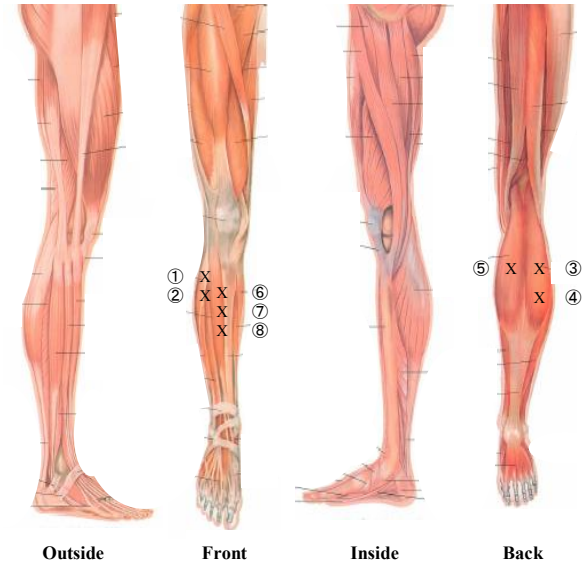


Target Muscle

① Soleus Muscle
② Soleus Muscle
③ Soleus Muscle
④ Soleus Muscle
⑤ Soleus Muscle
⑥ Soleus Muscle
⑦ Ant. Tibial Muscle
⑧ Ant. Tibial Muscle

Injection sites

Patient ID:	Pt. 1640 from AG-CLI-0205
Occlusion and/or Stenoses:	Anterior Tibal Artery, Posterior Tibial Artery, and Peroneal Artery

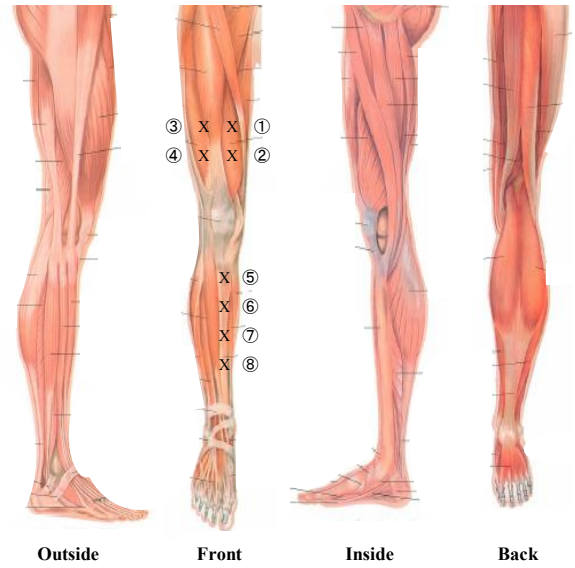


Target Muscle

① Peroneus Longus Muscle
② Peroneus Longus Muscle
③ Soleus Muscle
④ Soleus Muscle
⑤ Soleus Muscle
⑥ Ant. Tibial Muscle
⑦ Ant. Tibial Muscle
⑧ Ant. Tibial Muscle

Injection sites

Patient ID:	**Pt. 2333 from AG-CLI-0205**
Occlusion and/or Stenoses:	**Anterior Tibial Artery, Posterior Tibial Artery, Peroneal Artery, Femoral Artery, and Popiteal Artery**

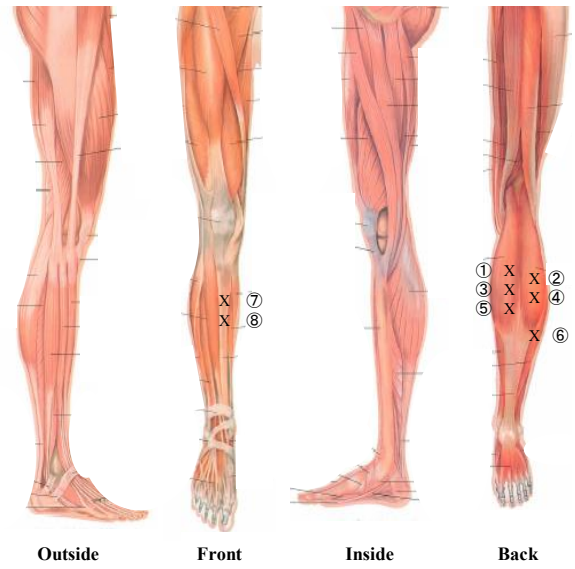


Target Muscle

① Vastus Medialis
② Vastus Medialis
③ Vastus Lateralis
④ Vastus Lateralis
⑤ Ant. Tibial Muscle
⑥ Ant. Tibial Muscle
⑦ Ant. Tibial Muscle
⑧ Ant. Tibial Muscle

Injection sites

Patient ID:	**Pt. AMG-01-37-01**
Occlusion and/or Stenoses:	**Anterior Tibial Artery, Posterior Tibial Artery, Popiteal Artery, Femoral Artery, Peroneal Artery, and Artery Distal Ankle Joint**



Target Muscle

① Soleus Muscle
② Soleus Muscle
③ Soleus Muscle
④ Soleus Muscle
⑤ Soleus Muscle
⑥ Flexor Hallucis Longus Muscle
⑦ Ant. Tibial Muscle
⑧ Ant. Tibial Muscle

APPENDIX B. HEMODYNAMIC PROCEDURES

ABI and TBI Assessment

Explain the procedure, reassure the subject, and ensure that he/she is lying flat and is comfortable, relaxed, and rested with no pressure on the proximal lower limb vessels. Laying the subject supine reduces the hydrostatic pressure inaccuracies. Reliability and ability to reproduce results both over time and between observers was tested and the index was found to vary by 0.06, which is considered acceptable. Measuring the pressure in both arms and using the higher of the two pressures increases the non-invasive accuracy of measurement of central systolic pressure but will not eliminate this potential limitation to the method.

Measure the brachial systolic blood pressure:

1. Place an appropriately sized cuff around the upper arm
2. Locate the brachial pulse and apply ultrasound contact gel
3. Angle the Doppler probe at 45 degrees and move the probe to obtain the best signal
4. Inflate the cuff until the signal is abolished then deflate the cuff slowly and record the pressure at which the signal returns being careful not to move the probe from the line of the artery
5. Repeat the procedure for the other arm
6. Use the highest of the two values to calculate the ABI

Measure the ankle systolic pressure:

1. Place an appropriately sized cuff around the ankle immediately above the malleoli having first protected any ulcer that may be present
2. Examine the foot, locating the dorsalis pedis pulse and apply contact
3. Continue as for the brachial pressure, recording this pressure in the same way
4. Repeat this for the posterior tibial artery
5. Use the highest reading obtained to calculate the ABI for that leg

Measure the toe systolic pressure:

1. Place an appropriately sized cuff around the hallux (1st toe) having first protected any ulcer that may be present. If the 1st toe is unavailable you may use other toes if appropriate, this should be documented in the source documents.
2. Examine the toe with the Doppler or PPG (photoplethysmograph), locating the pulse on the dorsal aspect and apply contact.

   Note: The same method (Doppler or PPG) must be used on the subject for all assessments through study completion.
3. Continue as for the ankle pressure, recording this pressure in the same way
4. Repeat this for the plantar toe artery
5. Use the highest reading obtained to calculate the TBI for that leg

Problems and errors may arise if:

1. The cuff is repeatedly inflated or inflated for long periods. This can cause the ankle pressure to fall.
2. The cuff is not placed at the ankle.
3. The pulse is irregular or the cuff is deflated too rapidly.
4. The true systolic pressure may be missed and inappropriately high reading will be obtained, if the vessels are calcified (associated with diabetes), the legs are large, fatty or edematous, the cuff size is too small, or the legs are dependent.
5. Central systolic pressure may influence the 'normal' range for the ABI.

For subjects with ankle and toe pressures in the affected limb that are not reliable due to non-compressible arteries or unattainable due to ulcers on ankle or great toe, there must be documentation of diagnosis of severe Rutherford category 4 or Rutherford category 5 and angiographic evidence of total occlusion of the superficial femoral, popliteal or at least one of the infra-popliteal arteries.

APPENDIX C. RUTHERFORD CLASSIFICATION

Rutherford Categories

Category	Clinical Description
	Asymptomatic
1.	**Mild claudication**
2.	**Moderate claudication**
3.	**Severe claudication**
4.	**Ischemic rest pain**
5.	**Minor tissue loss**
6.	**Major tissue loss**

APPENDIX D. WOUND CARE GUIDELINES

The purpose of these guidelines is to ensure that wound care during the study is consistent across all study subjects.

General Guidelines for Wound Care

All ulcers in both the treatment and control groups will receive wound care as summarized below for the duration of the study.

Debridement

Sharp or surgical debridement will be performed as deemed necessary by a wound care specialist (e.g. doctors/nurses at wound care clinics, orthopedic physicians, podiatrist, etc). If it is deemed necessary for any study subject, this procedure should always precede any photographic or ulcer tracing assessment. There are many debriding agents, but there is no consensus about the best agent. The method of debridement that is chosen will depend on the status of the wound, the resources of the healthcare provider and the overall condition of the subject. When chosen, this procedure should remove crusts as well as necrotic, devitalized tissue by surgical, enzymatic, mechanical, biological or autolytic debridement.

Treatment

Maintenance of a clean, moist ulcer environment is essential. Ideally, this should include daily dressing changes. Select a dressing that is cost-effective, appropriate to ulcer etiology, the setting and the provider. Dressing changes once daily or less often should be chosen where possible. Ulcer care products that can be utilized for study subjects include: saline, hydrogel, negative pressure dressings and topical antibiotics. Wound care products such as Regranex® (rhPDGF), Apligraft®, Dermagraft®, Oasis®, as well as any other skin equivalents or topical growth factors are excluded for use during this trial. Hyperbaric oxygen (HBO) treatment is also excluded.

Continuing education of the subject in regards to risk factor reduction should be addressed as frequently as necessary. This includes cigarette smoking cessation, control of any diabetes, elevated homocysteine levels, hyperlipidemia and hypertension. Additionally, where possible, exercise to increase arterial blood flow has been demonstrated to be helpful in long-term maintenance and arterial ulcer prevention.

Pressure relief by off-loading can also be pursued based on recommendations from the wound care specialist. Acceptable offloading methods include crutches, walker, wheelchair, custom shoes, depth shoes, shoe modifications, custom shoe inserts, custom relief orthotic walker, diabetic boots, forefront and heel relief shoes, and total contact casts.

Ref: Chronic Wound Care Guidelines, The Wound Healing Society, 341 N. Maitland Avenue, Suite 130, Maitland, FL 32751. (407) 647-8839. www.woundheal.org

APPENDIX E. THE VISUAL ANALOG SCALE (VAS) PROCEDURES - MEASURING REST PAIN

Pain Intensity rating using VAS

The visual analog scale (VAS) is a 10-cm line, oriented horizontally, with the left end of the line (the 0 mark) indicating "no pain" and the right end representing "pain as bad as it can be".

1. The subject is asked to mark a place on the line corresponding to the average pain intensity experienced in the last seven days.
2. The distance along the scale is then converted into a numeric reading by measuring the distance of the subjects mark in centimeters from the beginning of the scale (the 0 mark).

THE MEASUREMENT IS RECORDED IN CENTIMETERS FROM 00.0 TO 10.0 ONTO SOURCE DOCUMENTATION AND THE CRF.

The measurements will not be divulged to the subject. Additionally, the pain scale or VAS score from previous visits will not be shown to the subject.

APPENDIX F. POST 18-MONTH FOLLOW-UP QUESTIONNAIRE

Instructions: You may wish to seek your doctor's help in answering these questions.

Please consult your physician if you need help with any of these medical conditions.

"Diseased Leg" in this questionnaire refers to the diseased leg that received the injections of study product.

Diseased Leg (circle): RIGHT LEFT

Since your last visit or follow-up with this study, have you experienced any of the conditions mentioned below?

1. Any new or recurring cancer(s)? □ YES □ NO
   a. If yes, what type of cancer and when was it diagnosed?_____
   b. What cancer treatment did you receive? □None □Surgery □Chemotherapy □Radiation □Other (describe) _____

2. Any new problems with your eyesight? □ YES □ NO
   a. Did you see the eye doctor? □ YES □ NO
   b. If yes, what was the diagnosis and how were you treated? _____

3. Have you had any significant, unexplained bleeding from anywhere? □ YES □ NO
   a. If yes, give details of location, diagnosis and treatment._____

4. Have you been diagnosed with an autoimmune disorder (e.g., Rheumatoid arthritis, Lupus, Asthma)? □ YES □ NO
   a. If yes, please give details:_____
   b. How were you treated? _____

5. Have you been diagnosed with a blood disorder (e.g., anemia, platelet disorder, low white cell count)? □ YES □ NO

   If yes, please answer the following questions:
   a. When did it happen? _____
   b. What was the diagnosis? _____
   c. What treatment did you receive? _____
   d. What was the outcome? _____

6. Have you been diagnosed with a new nerve disorder or has there been a flare-up of an existing nerve disorder? □ YES □ NO

   If yes, please answer the following questions:

   a. What was the condition? _____
   b. When was it diagnosed? _____
   c. How was it treated? _____

7. Have you had any other unexpected or significant new illness? □ YES □ NO

   If yes, please describe the illness:_____

8. Have you been hospitalized? □ YES □ NO,

   If yes, please describe_____

9. Have you suffered from a stroke (transient or long-lasting)? □ YES □ NO

   If yes, please describe_____

10. Have you suffered from a myocardial infarction (heart attack)? □ YES □ NO

    If yes, please describe_____

11. Have you had any revascularization procedure performed on your diseased leg?

    □ YES □ NO

    If yes, please describe and specify which leg:_____

12. How are your symptoms relating to ulcers, gangrene and/or rest pain on your diseased leg? Improved Same Worse

13. Have you had an amputation above the ankle of either leg? □ YES □ NO

    a. If yes, please describe and specify which leg: :_____
    b. Please indicate date of amputation:_____

APPENDIX G. PREGNANCY PREVENTION AND REPORTING

AG-CLI-0209 is a study in with older population, however due to not having sufficient reproductive toxicity data for the study product, per (Guideline on Risk Assessment of Medicinal Products on Human Reproduction and Lactation: from Data to Labelling, EMEA/CHMP/203927/2005), proper pregnancy prevention and planning needs to be in place. Women who have recently been assessed as post-menopausal (<18 months) should have Follicle-stimulating hormone (FSH) and estradiol levels measured to confirm the post-menopausal state.

Pregnancy prevention

In accordance with good clinical practice, to avoid embryo-fetal exposure, AG-CLI-0209 has a risk evaluation and mitigation strategy to prevent pregnancy in subjects on study product. These measures help to minimize the possibility of fetal exposure to the investigational product due to the unknown risk to the health of the fetus and/or the pregnant woman.

Precautions include:

- Exclusion of women with child bearing potential on the AG-CLI-0209 study.
  - Women who are surgically sterile. For the purposes of this study, only women with hysterectomy or bilateral oophorectomy will be considered surgically sterile. Tubal ligation alone is not sufficient to be considered sterile.
  - Women who have documented primary ovarian insufficiency (premature ovarian failure) who are 55 years of age or younger will be given a urine pregnancy test within 7 days of Day 0, Month 3, Month 9 and Month 12 to ensure that they are not pregnant.
  - Confirmation of menopause by history of amenorrhea for 18 months or greater in the absence of other causes of amenorrhea, serum FSH (30 mIU/mL or higher) and serum estradiol (30 pg/mL or less) both consistent with menopause.
  - A pregnancy test should be conducted at screening. If pregnancy is confirmed prior to the start of the trial in general, the subject should not be enrolled in the trial.
- Male subject commitment to using a reliable method(s) of contraception, and/or abstinence, for the duration of therapeutic product exposure (up to 12 weeks post last study product dose). Please note that any pregnancy confirmed post that period will need to be documented up to the completion of the follow up period. (Reference Pregnancy Reporting below). As required, contraception should be extended beyond the last dose of the investigational product.

Contraception Methods

Male subjects must commit to use a highly effective method of contraception throughout treatment cycles through 12 weeks post last study dose. Effective methods include:

- Vasectomy
- Sexual Abstinence
- Barrier Methods (male or female condom with spermicide)

Pregnancy reporting

Subjects (female and male) should be advised to report, immediately, to the Investigator a suspected or confirmed pregnancy that occurs in the course of this clinical trial (including during any period of exposure that may exceed the length of the trial).

Pregnancy of a female subject or female partner of a subject must be reported within 24 hours of clinical research site awareness.

A female subject must discontinue study drug immediately and the investigator should report all pregnancies to the Sponsor, using the paper Pregnancy Report Form and SAE form. Please note that any pregnancy confirmed during investigational product exposure will need to be documented up to the completion of the follow up period.

Pregnancy of the female partner of a male subject that is within 12 weeks of last dose of study product should be reported to the investigator. The investigator should report the pregnancy to the Sponsor, using the paper Pregnancy Report Form and SAE form. Please note that any pregnancy confirmed during investigational product exposure will need to be documented up to the completion of the follow up period.

Pregnancy exposure to a female partner of a male subject after 12 weeks of last dose of study product should be reported to the investigator. The investigator should report the pregnancy to the Sponsor, using the paper Pregnancy Report Form only. This pregnancy should not be reported as an SAE.

Follow-up

Follow-up procedures regarding the course of the pregnancy should be discussed with the subject, as appropriate. Follow-up should be done by the investigator throughout the pregnancy and for an appropriate period thereafter. If the pregnancy is experienced by the female partner of a male subject, informed consent will be requested for follow up information.

The outcome of each pregnancy should be recorded and followed-up. For live births, longer term follow-up of a child is recommended, when possible and appropriate.

Miscarriages, Congenital anomalies, and birth defects should be reported as an SAE. Any complication occurring during pregnancy that meets the criteria for SAE should be reported in the SAE form.