CONFIDENTIAL

A Phase I, Dose-Escalation Study to Assess the Safety and Tolerability of VM202 in Subjects with Critical Limb Ischemia

Protocol Number: US 06-1-001

Sponsor: [redacted]

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Study Title:	A Phase I, Dose-Escalation Study to Assess the Safety and Tolerability of VM202 in Subjects with Critical Limb Ischemia
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Sponsor:	[redacted]
[redacted]	[redacted]
IND Number:	13158

By signing below I confirm that I have read this protocol and agree that it contains all necessary details for conducting this study. I will conduct the study according to the procedures described in this protocol.

Investigator Name (Print):	**Dr. Tim Henry**
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Investigator Signature:	
Date:	

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List of Abbreviations and Definitions of Terms

ABBREVIATION	DEFINITION
ABI	ankle-brachial index
AE	adverse event
ALT	alanine transaminase (SGPT)
AST	aspartate transaminase (SGOT)
AUC	area under the plasma concentration time curve
BUN	blood urea nitrogen
°C	degrees Celsius
CBC	complete blood count
cDNA	complementary deoxyribonucleic acid
CHF	congestive heart failure
CFR	Code of Federal Regulation
CLI	critical limb ischemia
Cm	centimeter(s)
C_{max}	maximum concentration of drug
CRF	case report form
CRO	clinical research organization
CVD	cardiovascular disease
D	day(s)
dL	deciliter(s)
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
ET	endothelin
°F	degrees Fahrenheit
FDA	Food and Drug Administration
G	gram(s)
GCP	Good Clinical Practices
HGF	hepatocyte growth factor
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
IDUR	investigational drug utilization record
IND	Investigational New Drug
INR	international normalized ratio
IRB	Institutional Review Board
IV	intravenous
IVRS	Interactive Voice Response System
Kg	kilogram(s)
LOCF	last observation carried forward
LV	left ventricular
Mg	milligram(s)
Min	minute(s)
mmHg	millimeters mercury

ABBREVIATION	DEFINITION
MRA	magnetic resonance angiography
MTD	maximum tolerated dose
N	number
Ng	nanogram(s)
NIH	National Institutes of Health
NYHA	New York Heart Association
O_2	oxygen
OBA	Office of Biotechnology Activities
PAD	peripheral artery disease
pH	hydrogen ion concentration
PHI	protected health information
PPG	photoplethysmograph
PTA	percutaneous transluminal angioplasty
QD	once daily
®	registered trademark
RBC	red blood count
SAE	serious adverse event
SAP	Statistical Analysis Plan
sDBP	sitting diastolic blood pressure
SE	standard error
SGPT	serum glutamic pyruvic transaminase (same as ALT)
SOC	System Organ Class
SVR	systemic vascular resistance
$t_{½}$	elimination half-life
t_{max}	time of occurrence for maximum (peak) drug concentration
™	trademark
TBI	toe-brachial index
$TcPO_2$	Transcutaneous Oxygen Pressure Assessment
VAS	visual analog scale
VEGF	vascular endothelial growth factor
vs.	versus
WBC	white blood count
WHO	World Health Organization

PROTOCOL SYNOPSIS

Protocol Number:	US 06-1-001
Title:	A Phase I, Dose-Escalation Study to Assess the Safety and Tolerability of VM202 in Subjects with Critical Limb Ischemia
Study Phase:	I
Treatment Duration:	Study drug administered on two separate occasions, Days 1 and 15. Each treatment is divided into 4 or 8 IM injections.
Study Duration:	30 months
Individual Subject Enrollment:	12 months
Name of Drug:	VM202
Dosage:	Cohort I-VM202 2 mg with the first half of the total dose given on Day 1 and the second half given on Day 15 Cohort II-VM202 4 mg with the first half of the total dose given on Day 1 and the second half given on Day 15 Cohort III-VM202 8 mg with the first half of the total dose given on Day 1 and the second half given on Day 15 Cohort IV-VM202 16 mg with the first half of the total dose given on Day 1 and the second half given on Day 15
Route of Administration:	Intramuscular (IM) injection into the muscle closest to the affected area. Volume determined by dose.
Study Objectives:	To evaluate VM202 administered by intramuscular injection in subjects with critical limb ischemia for the following: 1. Safety 2. Tolerability 3. Preliminary efficacy
Study Design:	This is a prospective, dose-escalation study to evaluate the safety and tolerability of intramuscular VM202 in subjects with critical limb ischemia. The study will consist of four (4) cohorts with a total of 3 subjects enrolled in each cohort to VM202. For each dose cohort, VM202 will be administered as a local intramuscular

injection in 2 divided doses with a 2-week interval between the injections. Preliminary efficacy (hemodynamic assessments), safety and tolerability will be evaluated at Baseline (screening) and at designated time points throughout the study.

After the first subject in each cohort completes day 30 (± 2 days), subsequent to day 15 dosing of the other 2 subjects in the same cohort, an interim safety evaluation will be performed with the submission of safety data to the Data Safety Monitoring Committee (DSMC). If the DSMC recommends continuing the study, the next dose cohort will be treated. This process will be repeated between the second and third dose cohort and between the third and fourth dose cohort. All 4 dose cohorts will be followed for one year from the time of the first dose of study drug administration. If a dose limiting toxicity is observed in one subject in any dose group, three additional subjects will be added to the dose cohort in which the toxicity was observed. If no additional DLTs are observed in the 6 subjects in this dose level, it will be considered the Maximum Tolerated Dose (MTD). If a DLT occurs in 2/6 subjects, then the preceding dose level will be considered the MTD.

Structure: Single center

Sample Size: Approximately 12-15 subjects

Study Population: Subjects selected for this study will have critical limb ischemia that has not responded to standard therapy with symptoms including pain at rest, and/or ischemic ulcers.

Study Assessments: The following assessments will be performed at screening and / or at designated time points throughout the study:

- Informed Consent
- Physical Exam
- Vital Signs
- Pregnancy Test
- Photograph and measurement of ulcer
- Mammogram
- Collection of concomitant medications
- Collection of medical history
- Assessment of CLI
- Collection of adverse events
- Ankle Brachial Index (ABI)
- Toe Brachial Index (TBI)

- Wave Form Analysis
- Transcutaneous Oxygen Pressure Assessment ($TcPO_2$)
- High resolution MRA
- Visual Analog Scale (VAS) for pain
- Analgesic usage
- Safety Laboratory Testing (Hematology, Chemistry, Urinalysis)
- Retinal fundoscopy
- 12-lead ECG
- Tumor marker testing (Alpha feto-protein, CEA, PSA [males only], CA19-9 & CA125)
- Infection testing (HIV, Hepatitis B, CMV, HTLV, VDRL)
- HGF levels
- Anti-HGF antibody levels
- VM202 DNA levels
- Endothelial progenitor cell assay
- Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)

Rationale: This Phase I study is designed to determine the safety and tolerability of intramuscular injections of VM202 using standard objective and subjective measurements. Assessment of safety will be accomplished through monitoring vital signs, monitoring signs and symptoms of acute and chronic hypersensitivity reaction and evaluating all observed and reported adverse events, retinal fundoscopy, clinical laboratory results, tumor lab testing, infection testing and urinalysis. There will also be a long-term follow-up to determine the extent of new onset of malignancy in treated subjects. CA-125 will be done in females and a PSA will be done in males to rule out any occult malignancy prior to first study drug administration and throughout the study.

While the primary objectives are safety and tolerability, preliminary efficacy evaluations will include evaluation of changes from Baseline in hemodynamics (ABI, TBI, Wave Form), $TcPO_2$, VAS pain assessment, analgesic use, and MRA measurements.

Procedures: After the study subject is identified, a written informed consent will be obtained and screening process will be initiated. Hemodynamic assessments, $TcPO_2$, retinal fundoscopy and high resolution MRA will be done at Baseline. For subjects with

ischemic ulcer, a photograph and measurement of the ulcer will be obtained. Safety laboratory (hematology, chemistry, urinalysis), tumor marker tests, infection tests, chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray), and ECG will also be done at Baseline.

Subjects will be administered two doses of study drug on Days 1 and 15 as a local intra-muscular injection in the affected ischemic limb. There will be 4 or 8 injections depending on the dose level administered slowly for 20~30 seconds at pre-determined sites on only one affected leg (mainly at the calf muscles) without local anesthesia. After treatment administration, subjects will be observed for 1 hour in a comfortable supine position.

Analgesic usage and Serum HGF will be assessed on Day 8 post first dose treatment.

After the second dose is administered (Day15) to the leg previously injected, hemodynamic assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, photograph and measurement of ischemic ulcer, VAS for pain, and safety labs will also be assessed on Days 28, 59, 91, 180 and 365. High resolution MRA with flow assessment will be done at Days 91 and 180.

After the first subjects in each dose cohort have completed the Day-30 (± 2 days) follow-up visit and subsequent to day 15 dosing of the other subjects in the same cohort, an interim safety evaluation will be performed with the submission of data to the DSMC. If the DSMC recommends continuing the study, the next cohort will be dosed. All subjects will be followed for up to 1 year from the time of first dose of study drug administration.

Pharmacokinetic assessment will be performed using VM202 DNA levels at selected time points. Pharmacodynamic assessments will be performed using anti-HGF antibodies, endothelial progenitor cell assay and serum HGF levels at selected time points.

Safety Monitoring: Monitoring and collection of adverse event information (nature, frequency and incidence of occurrence) will be performed throughout the study period.

Blood and urine samples for safety laboratory analyses will be performed at screening and at specified time points throughout the study period. Safety laboratory analyses will be completed at screening and at specified time points throughout the study.

	Physical examination, vital signs and retinal fundoscopy will also be performed as part of safety monitoring at screening and at specified time points throughout the study.
Efficacy Evaluation:	The study will attempt to gather preliminary efficacy information based on VAS score, MRA, $TcPO_2$ and hemodynamics. Exploratory analyses will include pharmacokinetic and pharmacodynamic evaluations. Pharmacokinetic evaluations will be based on VM202 DNA levels post dosing. Pharmacodynamic evaluation will be based on anti-HGF antibodies and serum HGF levels pre treatment and endothelial progenitor cell assay post treatment.
Sample Size and Power	This is a Phase I, dose-escalation study. The sample size is based on the feasibility to assess safety. No statistical analysis of efficacy is planned.
Statistical Analysis:	<u>Safety and Tolerability</u>: The incidence and nature of adverse events will be reported in tabulated format. Adverse events will be described according to severity and to its relationship with the study drug. Serious adverse events and adverse events leading to treatment discontinuation will be listed Descriptive statistics (N, mean, median, SD, minimum and maximum values, where applicable) of safety laboratory parameters to include tumor markers will be reported. Abnormalities will be summarized for low and high values. Frequency counts for normal/abnormal findings for retinal fundoscopy will be presented. <u>Efficacy</u>: Descriptive statistics (N, mean, median, SD, minimum and maximum values) of vascular assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, High resolution MRA, and VAS for pain will be reported. Frequency counts for analgesics use by dose cohort will be reported. <u>Pharmacokinetics</u>: Descriptive statistics (N, mean, median, SD, minimum and maximum values, where applicable) of VM202 DNA levels will be reported. <u>Pharmacodynamics</u>: Pharmacodynamics will assess change from Day 1 in anti-HGF antibodies and serum HGF levels at pre treatment and

	endothelial progenitor cell assay post treatment between all 4-dose levels.
Sponsor:	[redacted]

1.0 Background Information

1.1 Peripheral Artery Disease

Peripheral arterial disease (PAD) is a common disorder usually caused by atherosclerotic changes in the arteries supplying the lower extremities, resulting in ischemic limb disease. Clinical manifestations of lower extremity PAD are related to the severity of muscular ischemia, which depends on the degree of arterial stenosis. Intermittent claudication is a common, initial presentation. Critical limb ischemia denotes a condition of severe arterial obstruction due to advanced PAD, associated with breakdown of the skin or pain in the lower limb at rest (Appendix A). The natural history of critical limb ischemia has been well documented to have an inexorable downhill course, frequently leading to the amputation of the affected limb. Two main etiologies of PAD are known as atherosclerotic peripheral arterial disease and thromboangiitis obliterans (Buerger's disease).

Bypass surgery can be recommended for subjects with critical leg ischemia if the arterial circulation of the lower calf or foot includes a reconstituted artery, the outcomes of surgery involving small-size arteries are often unsatisfactory due to a high failure rate. Many subjects face amputation of their limb as the sole therapeutic option for the severe symptoms of critical limb ischemia, when PTA or bypass surgery fails to improve the symptoms. Psychological testing of such subjects typically shows quality-of-life indices similar to those of subjects with terminal cancer. It is estimated that about 150,000 subjects per year require lower-limb amputations for ischemic diseases in the United States alone (corresponding statistics is not available in Korea). Their prognosis after amputation is even worse. The perioperative mortality for below-knee amputation is 5 to 10 percent and for above-knee amputation 15 to 20%. Moreover, full mobility is achieved in only 50% of below-knee and 25% of above-knee amputees. These forbidding statistics result from the lack of efficacious drug therapy for PAD. Consequently, there is an urgent need for novel treatment strategies for subjects with critical limb ischemia.

1.2 Therapeutic Angiogenesis in Ischemic Limb Disease

In subjects with ischemic limb disease, some angiogenesis occurs, creating collateral vessels that appear in relation to a gradually developing stenosis or occlusion. These new collateral vessels provide tissue perfusion and relieve clinical symptoms. The term "therapeutic angiogenesis" was coined to describe intervention used to stimulate or induce neovascularization by administering angiogenic factors, either as recombinant protein or by gene transfer, to the ischemic sites. Therapeutic angiogenesis represents a novel strategy for the treatment of cardiac and vascular diseases. Recently, hepatocyte growth factor (HGF) gene therapy for therapeutic angiogenesis was introduced by

Morishita and others as a promising new strategy for treating ischemic cardiovascular diseases.[1]

Morishita's group first proposed a novel hypothesis that the administration of HGF is effective for the treatment of PAD by augmenting collateral vessel development and tissue perfusion in ischemic limb muscles.[2,3] They have published the results of various animal studies of HGF therapy.[4,5,6] Morishita and others are currently conducting a phase III clinical trial in Japan and a phase II trial in the U.S. to investigate a plasmid vector containing the HGF gene for the indication of PAD.[7]

A product, VMDA-3601, with the same plasmid backbone, pCK, as VM202, and containing the therapeutic gene expressing vascular endothelial growth factor (VEGF)165 protein, has been studied for the indication of PAD in a phase I clinical trial in Korea (Samsung Seoul Hospital) and is currently under phase II investigation.[8] Animal studies of HGF therapy indicate that HGF gene therapy can improve anatomical and physiological functions in animal models of ischemic cardiovascular disease. No growth-promoting effects on tumors have been observed. HGF gene therapy induces formation of collateral vessels, inhibition of fibrosis, and suppression of apoptosis of cardiac myocytes, improving physiological cardiac functions measured by increased blood flow, ejection fraction, intra-ventricular dimensions and anterior wall thickness of left ventricle. Moreover, HGF protein used in combination with VEGF demonstrated that the angiogenic effect of VEGF can be enhanced by HGF.[9] These results demonstrate the concept that HGF gene can be used for safe and effective treatment of ischemic cardiovascular diseases, and that HGF gene therapy might be a more effective and safer treatment for ischemic cardiovascular diseases, compared to gene therapies using VEGF.

VM202 contains a novel structure of the HGF gene as a therapeutic gene, HGF-X7. The key feature of HGF-X7 is that it was designed by inserting a series of intron sequences into certain sites of HGF cDNA so that both isoforms of HGF protein are expressed simultaneously and as efficiently as in the human genome. Because there was no change in the coding region of the HGF gene, HGF proteins generated from VM202 are identical to the wild-type human HGF proteins.

1.3 Clinical Experience with Neo-Angiogenesis Therapeutic Products

The DNA vector used in VM202 has been studied for the indication of PAD in a Phase I clinical trial in Korea and is currently under Phase II investigation. The Phase I study consisted of 3 cohorts with a total of 3 subjects enrolled in each cohort. For each dose cohort, VMDA-3601 gene therapy was administered as an intramuscular injection (2, 4 or 8 mg) in two divided doses with a 2-week interval between the injections. An increase in the number of vessels and reduction in pain were observed in 7 out of 9 subjects. The drug was well tolerated. There were no serious adverse events attributed to study drug administration.

2.0 Rationale

The gene product VM202 has demonstrated potential for stimulating angiogenesis in peripheral blood vessels in animal models. The development of new blood vessels may improve ischemic limb tissues with an increase in skin temperature and a reduction in pain both at rest and with exercise as well as numbness in the affected area. In addition, wound ulceration may be reduced or eliminated, need for amputation may decrease and exercise capacity may be increased. This trial is being performed as the first step in evaluating safety, tolerability and preliminary efficacy of VM202. Future trials will address dosing, pharmacokinetics and efficacy of VM202 in an expanded cohort of subjects with varying degrees of peripheral vascular disease.

3.0 Study Objectives

3.1 Primary Objective

The primary objective of this study is to evaluate the safety and tolerability of intramuscular VM202 as a vehicle for therapeutic angiogenesis in subjects with critical limb ischemia.

3.2 Secondary Objectives

The secondary objectives of the study will include the following exploratory evaluations:

- To investigate the effects of the intramuscular VM202 to improve pain at rest and/or to heal ischemic ulcers of the affected limb. The VAS assessment tool will be used for this evaluation.
- To evaluate the anatomic and physiological extent of collateral vessel development in subjects with critical limb ischemia as measured by MRA.
- To evaluate the change in serum HGF levels from Day 1 to Day 59.
- To evaluate the change in VM202 DNA levels from Day 1 to Day 21.
- To evaluate the immune response to HGF in subjects with CLI as measured by the change in anti-HGF antibodies from Day 1.
- To evaluate the ability of VM202 to stimulate endothelial progenitor cells as measured by the change in endothelial cell progenitor assay levels from screening to Day 59.

4.0 Trial Design

4.1 Primary and Secondary Endpoints, Pharmacokinetics and Pharmacodynamics

The primary endpoints of this study are the safety and tolerability of escalating doses of VM202. Preliminary effectiveness will also be assessed.

4.1.1 Primary Safety Endpoints

The primary safety endpoints include the following:

1. Incidence of adverse events through Day 365.
2. Change from Baseline in clinical chemistry, hematology, u/a at Days 15, 28, 59, 91, 180 and 365.
3. Injection site reactions, as measured by assessments at Days 1, 8, 15, 21, 28, and 59 using National Cancer Institute's Common Terminology for Adverse Events v3.0 criteria as follows:
   Grade 1=Pain, itching, erythema;
   Grade 2=Pain or swelling with inflammation or phlebitis;
   Grade 3=Ulceration or necrosis that is severe or operative intervention indicated.
4. Clinically significant changes in physical examination from Baseline at Days 15, 28, 59, 91, 180 and 365.

Secondary Efficacy Endpoints

The Secondary Efficacy endpoints include the following:

1. Change from Baseline in hemodynamic measurements (Appendix B: ABI, TBI, and Wave Form Analysis) and $TcPO_2$ at Days 15, 28, 59, 91, 180 and 365.
2. Change from Baseline in subject-assessed pain, as measured by the VAS (Appendix C) at Days 15, 28, 59, 91, 180 and 365.
3. Change from Baseline in ulcer area, in square millimeters, as assessed by digital photograph and measurement of the largest contiguous ulcerated area, at Days 15, 28, 59, 91, 180 and 365.

Pharmacokinetic Evaluations

Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose at the first dose administration (Day 1). In addition, levels will be measured at Days 21, and 59 (if DNA is detected at Day 21).

Pharmacodynamic Evaluations

Pharmacodynamics will be assessed as the change from Day 1 in serum HGF level at Days 8, 15, 21 and 59. Potential immune response to HGF will be assessed as change

from Day 1 in level of neutralizing antibodies (anti-HGF antibodies) at Days 15, 28, 59 and 180. The ability of HGF to stimulate cell growth will be assessed as the change from Baseline in level of endothelial progenitor cell assay at Days 15, 28, 59 and 180.

Overall Study Design

The overall trial design includes: 1) a multi-dose Phase I safety and tolerability evaluation; 2) a preliminary efficacy, pharmacokinetic and pharmacodynamic evaluation; and 3) a safety review between dosing cohorts in order to meet defined safety criteria. The study will evaluate vascular assessments as primary measures of efficacy and adverse event collection and laboratory parameters as the primary monitored measures of safety (see Table 4-1).

Study Population

Subjects selected for this study will all have critical limb ischemia defined as partial or complete obstruction of a major artery perfusing either the upper or lower extremity that results in pain either at rest or with exertion, numbness, coldness and/or skin ulceration. They may also be a history of prior amputation, dry gangrene or atrophy associated with the above symptoms. Subjects will not be a candidate for either surgical revascularization or angioplasty.

Number of Study Subjects

There will 12-15 subjects selected for participation in this study with 3 subjects treated in each of four (4) study cohorts. There will be an additional 3 patients treated in a cohort if 1/3 subjects experiences a DLT and if the DSMC recommends continuing the trial.

Schedule of Assessments

The schedule of assessments is listed below in Table 4-1.

Table 4-1 Study Procedures Chart	Screen / Baseline	Treatment Period													
		First Dose		1st Follow-Up	Second Dose		2nd Follow-Up[5]								
	Day -30 to Day 1	Day 1 pre-dose	Day 1 post-dose	Day 8 (+/- 2days)	Day 15 Pre dose (+/- 3 days)	Day15 Post-dose	Day 16	Day 21 (+/- 3 days)	Day 28 (+/- 3 days)	Day 59 (+/- 7 days)	Day 91 (+/- 7 days)	Day 180 (+/- 10 days)	Day 365 (+/- 10 days)	Unscheduled Visit	Early Termination
Consent Form	X														
Confirm Eligibility		X													
Medical History	X														
Chest X-ray or CT scan of chest[1]	X												X		X
Physical Exam	X				X				X	X	X	X	X	X	X
Vital Signs (HR, BP, Respiratory)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Fundoscopy	X												X		X
Pregnancy Test	X				X[6]										
Photograph and measurement of ulcer	X	X			X				X	X	X	X	X		X
Clinical Lab Tests	X	X			X		X		X	X	X	X	X	X	X
Tumor markers[2]	X				X					X		X	X		
Infection tests[3]	X									X		X	X		X
Serum HGF		X		X	X			X		X					
anti-HGF antibodies		X			X					X		X		X	X
Blood VM202 DNA		X						X		X[7]					
12-lead ECG	X														
High Resolution MRA	X										X	X			X
Hemodynamic Assessment (ABI, TBI, Wave Form)	X	X			X				X	X	X	X	X	X	X
Pain VAS	X	X			X				X	X	X	X	X	X	X
Subject Analgesic use	X	X		X	X		X	X	X	X	X	X	X	X	
$TcPO_2$	X	X			X				X	X	X	X	X	X	X
Injection site reaction assessment			X	X		X	X	X	X	X					
Mammogram[4]	X														
Endothelial Progenitor Cell Assay	X		X			X			X	X		X	X	X	X
Concomitant Medications	X	X		X	X		X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 CT scan of the chest instead of chest X-ray will be performed if previous tobacco use
2 Tumor markers include: AFP, CEA,CA19-9 PSA (males) & CA125 (females)
3 Infection tests include: HIV, Hepatitis B, HCV, HTLV, CMV, VDRL5
4 If not performed in last 12 months prior to start of study
5 A serum test will be done at screening and a urine test on all other days
6 Complete draw if detected at Day 21

4.3 Design of the Trial

This Phase I multi-dose study will enroll a total of 12-15 subjects with documented critical limb ischemia (Appendix A). All subjects must meet inclusion and exclusion criteria as listed in Section 0. Each of 3 subjects in each of 4 cohorts will be evaluated as indicated following 2 IM doses of VM202 administered at 2-week intervals. Subjects in each of the four cohorts will all receive the same dose (2, 4, 8 or 16 mg) of VM202.

	Day															
	(-30) - (1)	1,15	30-37	39	69,84	99-106	108	138, 153	168-175	177	207, 222	237-245	365	434	503	572
Cohort I	Screen	Treat ment	Safety Review										End of Study			
Cohort II				Screen	Treat ment	Safety Review								End of Study		
Cohort III							Screen	Treat ment	Safety Review						End of Study	
Cohort IV										Screen	Treat ment	Safety Review				End of Study

Figure 4-1 Phase I Study Design

4.4 Dosing Cohort Assignment

A 3-digit subject number will be sequentially assigned to each study subject beginning with 101 when the informed consent is signed and any screening procedures are performed. Separate screening numbers will not be assigned. The subject will retain the original subject number assigned throughout the trial. If the subject is withdrawn from the study prior to receiving the first dose of study drug, that subject will be replaced but the subject number will not be reused.

A study pharmacist will be responsible to prepare the assigned study drug according to detailed instructions provided by the Sponsor or their designee.

4.5 Treatments

The test product used in this study is VM202, a plasmid DNA-based therapeutic product expressing HGF. The HGF gene, HGF-X7 is a genomic-cDNA hybrid HGF construct (HGF-X), which expresses both isoforms of HGF with high efficiency. VM202 has been developed using pCK DNA which has been safely used in previous human clinical trials with VEGF as the therapeutic vector.[8] The VM202 plasmid, also identified as pCK-HGFX7 with 7377 base pairs, is composed of a HCMV enhancer/promoter, a growth hormone polyadenylation terminator sequence, ColE1 originator, and the Kanamycin resistance gene, on a pCK backbone.

For each of the 4 cohorts a different dose level will be administered as follows:

Cohort I- 2 mg of VM202

Cohort II- 4 mg of VM202

Cohort III-8 mg of VM202

Cohort IV-16 mg of VM202

In the event that a dose limiting toxicity (DLT) is determined after the safety review between Cohort I and Cohort II, between Cohort II and Cohort III or between Cohort III and Cohort IV, there will be 3 additional subjects treated at the same dose. If a DLT occurs in 2/6 patients, then the preceding dose level will be considered the MTD.

4.6 Duration

Each subject will be screened up to 30 days prior to receiving the first dose of study drug. Following screening, subjects will receive study drug on Day 1 and Day 15 and will be followed up until Day 365 post first dose of study medication. There will be safety evaluations that will begin 30 days after the first dose of study drug (Day 30) for all cohorts. Safety evaluations to encompass 30 days post the first dose of the first subject and subsequent to day 15 dosing of the other subjects in the same cohort will be completed for each cohort and will be evaluated by the DSMC prior to dose escalation to the next cohort. This 30-day window will include a 2-week safety evaluation following each dose at Day 1 and 15. The study will take approximately 30 months to complete. The individual subject enrollment will be approximately 12 months.

5.0 Selection and Withdrawal of Subjects

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

5.1 Cancer Screening

All subjects participating in this trial must undergo routine cancer screening. The history and diagnosis of potential, or apparent malignant, and non-malignant diseases and neoplasms will be assessed through several diagnostic tests and procedures. Some diagnostic tests and procedures performed prior to study consent and documented in the patient's medical history may be acceptable where noted. Routine cancer screening includes the following:

1. Current fecal occult blood test and flexible sigmoidoscopy within 5 years prior to treatment. Colonoscopy within cancer screening guidelines. Patients presenting with positive fecal occult will require flexible sigmoidoscopy during screening
2. Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray) within 3 months prior to study entry
3. Mammogram (females only)-within 1 year prior to study entry
4. Papanicolaou (Pap) testing - women within 1 year prior to study entry
5. Prostate specific antigen (PSA) – men within 3 months prior to study entry
6. Current alpha-fetoprotein (AFP) – will be performed at screening
7. CA-125 – will be performed at screening (females only)

5.2 Inclusion Criteria

To participate in this study the subject must meet all of the following criteria:

1. Male or female, between 20 and 90 years of age
2. Have critical limb ischemia (Rutherford Class 4 and 5) and considered not a candidate for bypass graft surgery or percutaneous angioplasty due to co-morbid conditions, failure of previous surgical or interventional procedures or caliber of grafting arteries

Critical limb ischemia is defined as:

- Stable symptoms on standard therapy including anti-platelet agents, vascular rheologic agents, cilostazol, anticoagulant and pain medication for 30 days
- Pain at rest and/or ischemic ulcers for a minimum of 4 weeks

3. Have diagnostic angiography of the affected limb in the last 12 months demonstrating a significant occlusion of one or more of the following arteries: iliac, superficial femoral, popliteal, and one or more infra-popliteal arteries
4. Have a resting ankle systolic pressure (in either the dorsalis pedis or posterior tibial arteries) of ≤ 60 mmHg or a resting toe systolic pressure of ≤ 40 mmHg in the affected limb
5. Be willing to maintain current drug therapy for peripheral arterial disease throughout the course of the study including an anti-platelet and statin (CoA Reductase) inhibitor treatment
6. Be capable of understanding and complying with the protocol and signing the informed consent document prior to being subjected to any study related procedures.
7. Women who are surgically sterile or at least 1 year postmenopausal or who have been practicing adequate contraception for at least 12 weeks prior to entering the study. If the subject is of child-bearing potential, she must have a negative serum pregnancy test result prior to study enrollment and must agree to repeat pregnancy screening tests during the study.
8. If the subject or the subject's partner(s) is of child bearing potential, the subject and the subject's partner(s) must agree to use a "double barrier" method of birth control while participating in this study.

5.3 Exclusion Criteria

To participate in this study a subject may not meet any of the following criteria:

1. Subjects who have undergone a revascularization procedure or sympathectomy within 12 weeks prior to study entry that remains patent. A failed revascularization procedure in the previous 4 weeks is acceptable.
2. Subjects with grade 3 (hemorrhages, exudates) or grade 4 (papilledema) retinopathy
3. Subjects currently receiving immunosuppressive medications, chemotherapy, radiation therapy
4. Subject with aorto-iliac occlusion (greater than 75%)
5. Subjects that will require amputation within 4 weeks of randomization
6. Subjects with any co-morbid conditions likely to interfere with assessment of safety or efficacy or with an estimated life expectancy of less than 6 months
7. Subjects with history of drug (defined as illicit drug use) or a history of alcohol abuse (defined as regular or daily consumption of more than 4 alcoholic drinks per day) within the past 3 months

8. Subjects with a current history or new screening finding of malignant neoplasm except for basal cell carcinoma of the skin and squamous cell carcinoma of the skin (if excised and no evidence of recurrence)
9. Subjects with evidence of active infection (e.g., cellulitis, osteomyelitis) or deep ulceration exposing bone or tendon in the extremity planned for treatment
10. Subjects with a clinically significant abnormality in routine hematology, urinalysis, chemistry, liver function or other laboratory tests, including HIV, Hepatitis B (HepBSAg), Cytomegalovirus (CMV), Hepatitis C Virus (HCV), Venereal Disease Research Laboratory test (VDRL), prostate-specific antigen (PSA) and chorio-embryonic antigen (CEA), or signs of malignant neoplasm by radiological imaging tests, including chest radiograph at Screening or Day 1.

    Specific laboratory exclusion criteria include the following:
    - Hemoglobin less than 9.0 G/dl
    - WBC count less than 3,000
    - Platelet count less than 75,000
    - Fasting glucose greater than 250 mg/dl
    - AST and/or ALT greater than 3x upper limit of normal
11. Subjects with any other condition that in the opinion of the Investigator might put the subject at risk or interfere with his/her participation
12. Subjects unwilling or unable to comply with the protocol or to cooperate fully with the Investigator or site personnel
13. Subjects that have received any other investigational drug within the 30 days prior to study drug administration or will receive such a drug during the timeframe of this study
14. Subjects with uncontrolled hypertension defined as systolic blood pressure greater than 200 mmHg or diastolic blood pressure greater than 115 mmHg at Baseline evaluation
15. Subjects with advanced liver disease including decompensated cirrhosis, jaundice, ascites or bleeding varices

5.4 Subject Withdrawal Criteria

Subjects will be discontinued from the study prematurely if any of the following occur:

- The subject requests to be withdrawn from the study
- The Principal Investigator decides that it is in the subject's best interest

- The subject requires percutaneous or surgical intervention for treatment of acute limb ischemia.
- The subject is noncompliant with the protocol

If a subject withdraws from the study at any time, either at his request or at the Principal Investigator's discretion, the reason(s) for withdrawal will be recorded by the Principal Investigator on the Case Report Form (CRF). If the subject withdraws after receiving any study drug, the subject will be encouraged to continue to be monitored after withdrawal. In this regard, they will be informed that it is in their best interest to return for all follow-up visits to monitor for possible delayed safety effects from the investigational therapy. If possible, all final visit tasks will be completed for all subjects who withdraw from the study. Subjects who withdraw prior to receiving the first dose of study drug will be replaced. Subjects who have received the first dose of the study agent will not be replaced. Subjects withdrawn due to AEs will be monitored until resolution of the AE, as described in Section 8.6.

6.0 Treatment of Subjects

6.1 Treatments Administered

This study will be conducted using the dosing schedule shown in Table 6-1. Projected doses are based on an 80 kg human. The dose has been selected based upon assumptions that the allometric scaling of terminal t½ will apply.

Table 6-1 Treatments

Cohort	Number of Subjects	Total Dose (mg)	Number of Doses	Time of Dosing
I	3	2	2; each divided among 8 injection sites	Day 1, Day 15
II	3	4	2; each divided among 16 injection sites	Day 1, Day 15
III	3	8	2; each divided among 16 injection sites	Day 1, Day 15
IV	3	16	2, each divided among 16 injection sites	Day 1, Day 15

6.1.1 Treatment Regimen and Follow-Up

For each cohort, VM202 will be administered as either 8 or 16 local intramuscular injections to the affected limb in 2 divided doses with a 2-week interval between the injections. A total of 4 or 8 different injection sites will be arbitrarily chosen dependent on the dose and the location of the occluded vessels. The location of the injections sites will be documented on a leg diagram provided in the CRF. Each injection will contain a maximum of 2 ml (regardless of weight) and will be injected at each site slowly for about 20 to 30 seconds as described in Appendix D.

VM202 is provided in a sterile glass vial containing lyophilized 2.0 mg of study drug. Before use, it will be reconstituted with 4 ml of water for injection (WFI). Each reconstituted vial is only to be used for one patient. After the vial is reconstituted the following dosing information is applicable:

Cohort I has a total dose of 2.0 mg which is 1.0 mg per administration (0.25 mg per syringe in 0.5 ml for 4 injection sites per administration)

Cohort II has a total dose of 4.0 mg which is 2.0 mg per administration (0.25 mg per syringe in 0.5 ml for 8 injection sites per administration)

Cohort III has a total dose of 8.0 mg which is 4.0 mg per administration (0.5 mg per syringe in 1.0 ml for 8 injection sites per administration)

Cohort IV has a total dose of 16.0 mg which is 8.0 mg per administration (1.0 mg per syringe in 2.0 ml for 8 injection sites per administration)

VM202 will be administered in a 1.0 ml syringe for Cohorts I, II and III and a 3.0 ml syringe for Cohort IV. The volume of medication to be used for the four dose cohorts is as follows:

- Cohort I-fill 4 syringes with 0.5 ml per syringe
- Cohort II-fill 8 syringes with 0.5 ml per syringe
- Cohort III-fill 8 syringes with 1.0 ml per syringe
- Cohort IV-fill 8 syringes with 2.0 ml per syringe

The same volume will be used for both the first and the second doses (series of either 4 or 8 injections in only one affected leg) for each subject.

Injection volume by ml

Cohort	Dose	Volume/ administration	Volume/ injection site	Injections/Administration
I	1mg	2ml	0.5ml	4
II	2mg	4ml	0.5ml	8
III	4mg	8ml	1ml	8
IV	8mg	16ml	2ml	8

Injection volume by units

Cohort	Dose	Volume/ administration	Volume/ injection site	Injections/Administration
I	1mg	200units	50units	4
II	2mg	400units	50units	8
III	4mg	800units	100units	8
IV	8mg	1600units	200units	8

There will be a mandatory review of all safety data between the treatments of all dose cohorts (Cohorts I-IV). No subject may be enrolled in subsequent cohorts until the DSMC has reviewed the data and provided a written notice to the sponsor that the trial may continue.

In the event that a DLT is determined after the safety review in any cohort, there will be 3 additional subjects treated at the same dose level. If no further DLTs are observed in the 6 subjects and the DSMC approves dose escalation, then subjects can be enrolled to the

next higher dose level. If 2/6 subjects experience a DLT then the preceding dose level will be considered the MTD.

6.1.2 Study Agent Handling and Accountability

The vials will be labeled as required by all applicable regulations. The vials will be stored at 2-8ºC. The vials (containing lyophilized VM202) may be kept up to 6 hours at room temperature prior to injection. If the vial is not used by 6 hours at room temperature, it must be discarded.

The vials of VM202 reconstituted with WFI may be kept up to 6 hours at room temperature prior to injection. If the vial is not used by 6 hours at room temperature, it must be discarded.

The study pharmacist will maintain accurate records of receipt of all test articles, including dates of receipt. In addition, accurate records will be kept regarding when and how much test article is administered to each individual subject in the study. Reasons for deviation from the expected regimen must also be recorded. An Investigational Drug Utilization Record will be provided for this purpose and will be signed by the study pharmacist at the conclusion of the study. At completion of the study in order to satisfy regulatory requirements regarding drug accountability and destruction, the study pharmacist will return all used, unused, empty, and partially used vials, with dispensing records, to the Sponsor for final accountability, inventory, and destruction.

6.2 Study Procedures and Study Procedures Chart

Study procedures to be performed are described by visit in the following sections and summarized in Table 4-1. Prior to performing any study-related procedures, the Principal Investigator (or his/her designated staff) will obtain written informed consent from the subject or the subject's legally authorized representative. After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed at the time of the subject's signature by the personally dated signature of the person conducting the informed consent discussions. The Investigator will retain the original signed consent document. A copy of the signed consent document will be given to the subject or the subject's legally authorized representative. "Legally authorized representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the clinical study. The Investigator will not undertake any measures specifically required for the clinical study until valid consent has been obtained.

6.2.1 Screening Visit (Day -30 to Day 1)

Subjects will be assessed for all inclusion/exclusion criteria. The screening visit may occur up to 30 days prior to the Day 1 visit. Data for any screening subject, regardless of

their eventual eligibility to participate in the study, will be documented in the study case report forms. The following procedures will be completed:

- Obtain written informed consent
- Medical history
- Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)
- Full physical examination including stool for occult blood
- Photograph and measurement of ulcer
- Vital signs
- Retinal fundoscopy
- Pregnancy test (female subjects only)
- Clinical laboratory tests, including: chemistry, hematology, urinalysis
- Tumor markers assessment
- Infection tests
- 12-lead electrocardiogram
- High resolution MRA
- Hemodynamic assessments (ABI and TBI)
- $TcPO_2$
- Assessment of all concomitant medications
- Pain VAS
- Analgesic use
- Mammogram/pap smear (for females) – if not performed one year prior to study entry
- PSA (for males)
- CA-125 (for females)
- Endothelial progenitor cell assay

The subject will be screened for cancer and retinopathy to confirm that the patient is free of apparent neoplasms and proliferative retinopathy using any prior documentation of diagnostic tests and procedures performed prior to study consent and documented in the patient's medical history.

If the screening process is delayed and the assessments have fallen outside the screening period windows, then they must be repeated. If there are any questions regarding a subject's eligibility, please contact the Project Manager for clarification.

6.2.2 Day 1

Based on the subject meeting all of the screening procedures, the Investigator may administer the study agent to the patient following the Cohort assignment described above. If a subject is unable to receive the study agent because a clinically significant condition has evolved which, in the Investigator's opinion, represents a potential safety risk, the study drug will not be reassigned and the patient will be considered a screen failure. Therefore, it is important to determine the patient's medical status prior to the Day 1 visit and delay assignment until the condition resolves.

The following evaluations will be performed prior to study drug administration:

- Review all prior evaluations and confirm that subject meets all entry criteria
- Vital signs
- Digital photography and measurement of ulcer (if present)
- Clinical laboratory tests (chemistry, hematology, and urinalysis)
- Serum HGF
- Anti-HGF antibody level
- VM202 DNA level
- Hemodynamic assessments (ABI, TBI, Wave Form)
- $TcPO_2$
- Pain VAS
- Analgesic use
- Concomitant medications
- Adverse Events

If the subject meets all eligibility criteria, the following will be performed:

- Obtain study drug from the pharmacy
- Administer study drug in 8 separate injections as described above

After study drug administration, the subject will remain in a comfortable, supine position for observation for at least 60 minutes prior to being discharged. Any adverse reaction occurring during this period will be documented on the CRF.

Following study drug administration, the following evaluations will be performed:

- Vital signs (immediately after study drug administration and at 15, 30, and 60 minutes post-dose)
- Endothelial progenitor cell assay
- Concomitant medications
- Injection site reaction assessment

- Adverse events

The subject is instructed to call the Study Coordinator if any of the following occur after discharge from the clinic:

- Hives
- Fever
- Rash
- Difficulty breathing
- Itching
- Worsening of a leg ulcer
- Redness or flushing at the site of the test dose

If any of the above occurs, the subject will be instructed to call the clinic immediately and seek medical attention either at the clinic or to go to the nearest Emergency Room for immediate treatment and evaluation. No further study drug administration will occur until it is determined if this is a DLT or as directed by the treating physician.

6.2.3 Day 8 (± 2 days)

The following evaluations will be performed at Day 8 (± 2 days):

- Vital signs
- Serum HGF
- Injection site reaction assessment
- Analgesic use
- Concomitant medications
- Adverse events

6.2.4 Day 15 (± 3 days) Second Study Dose Administration

The second dose of study drug will be administered at Day 15 (± 3 days). Prior to the administration of the study agent, the following will be performed:

- Physical exam
- Vital signs (immediately after study drug administration and at 15, 30, and 60 minutes post-dose)
- Photograph and measurement of ulcer
- Urine Pregnancy test (females of childbearing potential)
- Clinical laboratory tests
- Tumor markers
- Serum HGF level

- anti-HGF antibodies
- $TcPO_2$
- Hemodynamic assessment
- Pain VAS
- Analgesic use
- Concomitant medications
- Adverse events

The study drug will be administered in the same manner and in the same dose as performed on Day 1. Following study drug administration, the following evaluations will be performed:

- Vital signs
- Injection site reaction assessment
- Endothelial progenitor cell assay
- Adverse events

6.2.5 Day 16 (post 2nd dosing)

The following procedures will be performed at the Day 16 visit:

- Vital signs
- Adverse events
- Clinical laboratory tests
- Analgesic use
- Injection site reaction assessment
- Concomitant medications

6.2.6 Day 21 (± 3 days)

The following procedures will be performed at the Day 21 (± 3 days) visit

- Vital signs
- Serum HGF
- VM202 DNA levels
- Injection site reaction assessment
- Analgesic use
- Concomitant medications
- Adverse events

6.2.7 Day 28 (± 3 days)

The following evaluations will be performed at Day 28 (± 3 days):

- Physical exam
- Vital signs
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS
- $TcPO_2$
- Endothelial progenitor cell assay
- Analgesic use
- Injection site reaction assessment
- Concomitant medications
- Adverse events

After all subjects in Cohort I have completed the Day 28 visit, enrollment will be temporarily suspended until the DSMC has reviewed all of the safety data for all subjects. If the DSMC recommends that the trial continue, enrollment in Cohort II will commence and all of the procedures from Day -30 through Day 28 will be repeated for Cohort II.

After all subjects in Cohort II have completed the Day 28 visit, enrollment will be temporarily suspended until the DSMC has reviewed all of the safety data for all subjects. If the DSMC recommends that the trial continue, enrollment in Cohort III will commence and all of the procedures from Day -30 through Day 28 will be repeated for Cohort III.

After the first subjects in Cohort III have completed the Day 28 visit and subsequent to the dosing of the other subjects in the same cohort, enrollment will be temporarily suspended until the DSMC has reviewed all of the safety data for all subjects. If the DSMC recommends that the trial continue, enrollment in Cohort IV will commence and all of the procedures from Day -30 through Day 28 will be repeated for Cohort IV.

6.2.8 Day 59 (± 7 days)

The following evaluations will be performed at Day 59 (± 7 days):

- Physical exam
- Vital signs
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Infection tests
- Tumor markers
- Serum HGF

- anti-HGF antibodies
- VM202 DNA levels (If DNA is detected at Day 21)
- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS
- $TcPO_2$
- Endothelial progenitor cell assay
- Analgesic Use
- Injection site reaction assessment
- Concomitant medications
- Adverse events

6.2.9 Day 91 (± 7 days)

The following evaluations will be performed at Day 91 (± 7 days):

- Physical exam
- Vital signs
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Hemodynamic assessments (ABI, TBI, Wave Form)
- $TcPO_2$
- Pain VAS
- High resolution MRA of lower extremity
- Analgesic use
- Concomitant medications
- Adverse events

6.2.10 Day 180 (± 10 days)

The following evaluations will be performed at Day 180 (± 10 days):

- Physical exam
- Vital signs
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Tumor markers
- Anti-HGF antibodies
- Infection tests
- $TcPO_2$

- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS
- Analgesic use
- Endothelial progenitor cell assay
- High resolution MRA of lower extremity
- Concomitant medications
- Adverse events

6.2.11 Day 365 (± 10 days)

The following evaluations will be performed at Day 365 (±10 days):

- Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)
- Physical exam
- Vital signs
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Tumor markers
- Retinal fundoscopy
- Infection tests
- $TcPO_2$
- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS
- Analgesic use
- Endothelial progenitor cell assay
- Concomitant medications
- Adverse events

6.2.12 Unscheduled Visits

The data collected during the entire study period are critical for the safety and efficacy assessments, therefore, every effort must be made to bring the subject back to the study site for all visits.

If a subject notifies the site that they have experienced an SAE, or experience an event such as peripheral bypass surgery, peripheral angioplasty or amputation during the follow-up period, they will be encouraged to return to the study site for additional assessments. These events will be reported immediately and the necessary

documentation will be collected. All adverse events that a patient experiences must be reported. The following procedures will be performed for all subjects who have an unscheduled visit:

- Physical exam
- Vital signs
- Anti-HGF antibodies
- Endothelial progenitor cell assay
- Clinical laboratory tests
- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS
- $TcPO_2$
- Analgesic Use
- Concomitant medications
- Adverse events

6.2.13 Early Termination Visits

Subjects who have had at least one dose of test material will return to the study site for study specific safety and efficacy assessment visits for the total study period (through Day 365), unless the subject withdraws study consent and refuses to participate. If the subject wishes to withdraw study consent, every effort must be made to encourage the subject to return to the study site for a termination visit assessment prior to withdrawing consent. The following procedures will be performed at the study termination visit:

- Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)
- Physical exam
- Vital signs
- Retinal fundoscopy
- Photograph and measurement of ulcer
- Clinical laboratory tests
- Infection tests
- Anti-HGF antibodies
- Endothelial progenitor cell assay
- High resolution MRA
- Hemodynamic assessments (ABI, TBI, Wave Form)
- Pain VAS

- $TcPO_2$
- Concomitant medications
- Adverse events

If a subject refuses to communicate with the study site, all attempts to contact the subject will be documented. The subject will be sent a certified letter to the subject's last known address.

6.3 Autopsy

In the event of the death of the study subject, the investigator will be required to request an autopsy from the family of the deceased. An autopsy will assist ViroMed in learning more about the safety and efficacy of gene transfer. If an autopsy is performed, the final autopsy report will be included in the subject's study file and a copy of the report will be sent to both ViroMed and the designated Clinical Research Organization (CRO). The refusal of the family to permit an autopsy will be documented in the study subject's file and on the CRF.

6.4 Concomitant Medications/Treatments

6.4.1 Concomitant Medications

All concomitant medications will be recorded on the CRF at each study visit. There are no prohibited concomitant medications except for other investigational products.

6.4.2 Replacement of Study Subjects

Subjects requiring percutaneous or surgical intervention for treatment of limb ischemia will be withdrawn from treatment. If the subject has received at least the first dose of study drug, the subject will not be replaced. If the subject has received one or both doses of study drug, the subject will remain in the study through the five year follow-up period.

6.4.3 Wound Care

Standard wound care techniques are acceptable (e.g., approved dressings, debridement). No un-approved pharmacological wound care therapies or experimental research materials may be used to treat the wounds during the study observation period. The application of approved growth factors or engineered skin replacements (e.g. Dermagraft®) is permitted. Patients who require hyperbaric treatment as part of their standard of care will be permitted to participate in the study.

6.4.4 Peripheral Vascular Procedures

The performance of any peripheral vascular procedures, including, but not limited to, peripheral bypass surgery, peripheral endarterectomy, peripheral angioplasty or amputation, are events that may occur during the course of the study. The Investigator will document all peripheral vascular interventions performed during the study period.

If the subject reports the occurrence of any disease-related events (e.g., amputation, revascularization bypass, angioplasty), every effort must be made to bring the subject back to the study site for full documentation of the event including sufficient information from the subject, hospital, any treating physician for each peripheral vascular procedure. This information is required by the Sponsor and will be added to the SAE CRF. Information will include:

- Date of intervention
- Type of intervention (e.g., PTA, peripheral bypass, amputation)
- Surgical location (e.g., iliac, femoro-popliteal, infra-popliteal; amputation: AKA BKA)
- Surgical materials (e.g., PTFE, HUV, Dacron)

6.5 Procedures for Monitoring Treatment Compliance

The study pharmacist, using study drug accountability documents (IDUR), will record the preparation and administration of each required dose of IM study drug. Any missed dose will be noted in the pharmacy source documents and on the subject's CRF. The Investigator is responsible for compliance with the protocol procedures and for monitoring the subject's compliance with the visit schedule.

6.6 Study Completion and Discontinuation

A subject may refuse test material 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. Due to the nature of the test material, the subject will be encouraged to maintain contact with the Investigator and report any SAEs experienced for the entire study period.

The Principal Investigator is permitted to discontinue the subject from test material treatment for medical reasons at any time. However the subject will continue to return for protocol specific safety and efficacy assessments visits for the remainder of the total study period (12-month post dose visit).

6.7 Subject Classification

6.7.1 Evaluable Subject

Any subject who receives at least one dose of study drug and has at least one post dose assessment.

6.7.2 Screen failure

Any subject who was consented and entered into the screening process appropriately, but subsequently did not meet the entry criteria in order to be treated. Subjects who fail

screening will not be followed for safety or efficacy assessment, and no other study procedures will be performed.

6.7.3 Lost to follow-up

A subject deemed to be lost to follow-up is any subject who received treatment, but who does not complete scheduled study visits. This includes those subjects who withdraw consent and refuse further study participation and all attempts to contact the subject are deemed unsuccessful.

7.0 Assessments and Procedures

7.1 Safety

7.1.1 Medical History

A complete medical history will be obtained at Baseline. All positive and negative findings will be carefully documented on the CRF. Any new finding discovered during the Baseline evaluation will be considered to be part of the medical history and will not be recorded as an adverse event.

7.1.1.1 Peripheral Vascular Intervention History

The Investigator will perform detailed assessment of all past peripheral vascular interventions prior to study enrollment. If the subject has never undergone a peripheral vascular intervention, then the lack of these events will be documented as well. Information will include: date(s) of intervention, type(s) of intervention (e.g., PTA, peripheral bypass, amputation), intervention site (e.g., iliac, femoro-popliteal, infra-popliteal; and patency of most recent intervention prior to study start (including a description of the most recent vascular imaging if available). Effort will be made to obtain sufficient information for each peripheral vascular procedure to confirm the subject's PAD status at Baseline.

7.1.2 Physical Examination

The Investigator will perform a full physical examination at Baseline and at Days 15, 28, 59, 91, 180 and 365. All changes from the Baseline will be documented on the CRF pages at the time of the follow-up physical examination.

7.1.2.1 Assessment of CLI

The lower extremities will be examined specifically for clinical evidence of CLI, such as ischemic rest pain, ischemic ulceration, gangrenous skin changes, or residual open wound(s) from minor amputation.

Subjects will be classified as having ischemic rest pain if the pain required analgesics for more than 2 weeks, and includes the following clinical characteristics:

1. Persistent or recurrent pain of the distal portion of the leg (usually toes, forefoot or ankle),
2. Pain begins or is increased, on elevation of the leg and improved by dependency.

Ischemic ulceration and gangrenous skin changes are equivalent necrotizing processes for the purpose of this study. In the case of residual open wound(s) from minor amputation, these will be considered ulceration(s) for the purposes of this assessment.

The Investigator will assess the number of individual ulcers and/or gangrenous tissue site(s) for location and size. Excess necrotic tissue and eschar will be removed when possible before taking measurements. Photographs of each ulceration/gangrene area will be taken for documentation purposes only. Two photographs of each ulceration/gangrene area will be obtained at each efficacy time point regardless of whether the area still contains the ulcer/gangrene, and regardless of how many doses of test material the subject received. During the monitoring visit, the monitor will retrieve one of the two photographs for each ulceration/gangrene area. The border of all photographs will be labeled with the photograph date, subject's initials, and the subject's study number assignment.

On efficacy assessment visits, the ulceration/gangrene area will be considered completely healed if assessed as having complete skin closure without drainage or dressing requirements. An ulceration/gangrene area that heals but re-appears and is unhealed at the assessment will be considered not healed. If the subject is discontinued from treatment, he/she will continue to be followed for ulceration/gangrene evaluations so that the subject's data can be included in analyses. If the subject is lost to follow-up without a proper ulceration/gangrene evaluation, the subject will be considered not to have healed.

7.1.3 Safety Laboratory Tests

Safety laboratory tests will include hematology, chemistry and urinalysis. A list of the specific safety and other laboratory tests (tumor markers and infection tests) required is provided in Table 7-1.

7.1.4 Retinal Fundoscopy

Proliferative retinopathy is defined as the presence of new proliferating blood vessels (neovascularization) arising from the retina or optic disc and growing on the retinal surface or into the vitreous cavity screened by an acceptable method (e.g. dilated retinal examination and retinal photographs) at Baseline and Day 365. An ophthalmology examination for proliferative retinopathy must be performed by an ophthalmologist. Results from such evaluations performed within 3 months of Baseline will be accepted.

Table 7-1 Study Specific Laboratory Procedures

Hematology	**Chemistry**
Red blood cell (RBC) count	BUN
Hemoglobin	Bicarbonate
Hematocrit	Albumin
Mean corpuscular volume	Creatinine
Mean corpuscular hemoglobin	Glucose
Mean corpuscular hemoglobin concentration	Potassium
White blood cell count	Sodium
Neutrophils	Chloride
Lymphocytes	Uric acid
Monocytes	Calcium

Table 7-1 Study Specific Laboratory Procedures	
Eosinophils	Phosphorous
Basophils	Total protein
Blood smear	**Tumor markers**
Platelet count	Prostate specific antigen (PSA)
Liver Function Tests	Alpha-fetoprotein
GGT	Cancer antigen 19-9 (CA 19-9)
ALT	CA125
AST	Carcinoembryonic antigen (CEA)
Alkaline phosphatase	**Infection tests**
LDH	HIV
Total bilirubin	HepBSAg
	HCV
Endothelial Progenitor Cell Assay (EPC)	HTLV
	CMV
	VDRL

7.1.5 12-Lead ECG

A screening ECG will be obtained at Baseline. All ECG recordings will be printed in duplicate. The site will provide the final interpretation of the Screening ECG.

7.1.6 Local Injection Site Reaction Assessment

Local injection sites reactions will be assessed using the grading defined by the National Cancer Institute's Common Terminology for Adverse Events v3.0. The grading categories are as follows and will be recorded on the CRF:

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Injection site reaction	Pain; itching; erythema	Pain or swelling, with inflammation or phlebitis	Ulceration or necrosis that is severe; operative intervention indicated		
Ulceration		Superficial ulceration <2 cm size; local wound care; medical intervention indicated	Ulceration ≥2 cm size; operative debridement, primary closure or other invasive intervention indicated (e.g. hyperbaric oxygen)	Life-threatening consequences; major invasive intervention indicated (e.g., complete resection , tissue reconstruction, flap, or grafting	Death
Allergic reaction/ hypersensitivity	Transient flushing or rash; drug fever <38˚C (<100.4˚F)	Rash; flushing ; urticaria; dyspnea; drug fever ≥38˚C (≥100.4˚F	Symptomatic bronchospasm, with or without urticaria; parenteral medications(s) indicated; allergy-related edema/ angioedema; hypotension	Anaphylaxis	Death

7.1.7 Incidence of Adverse Events

All adverse events and serious adverse events will be recorded on the CRF at each visit.

7.1.8 Serum Sickness

Serum sickness is defined as low grade fever, joint pain, lassitude and myalgias within 72 hours of study drug administration. The incidence of serum sickness will be documented on the CRF.

7.1.9 Dose Limiting Toxicity (DLT)

Dose limiting toxicity will be defined as the occurrence of any of the following:

- Acute anaphylaxis including erythema, hives, wheals, wheezing, stridor or respiratory distress
- Temperature >102ºF with negative blood cultures within 24 hours of study drug administration
- Evidence for active tissue necrosis at the injection site within two weeks of study drug administration

Dose limiting toxicities will be recorded on the CRF. In the event of a DLT at any dose, 3 additional subjects will be added to the dose cohort in which the toxicity was observed. If no additional DLTs are observed in the 6 subjects in this dose level, it will be considered the Maximum Tolerated Dose (MTD). If a DLT occurs in 2/6 patients, then the preceding dose level will be considered the MTD.

7.2 Efficacy

7.2.1 Hemodynamic Assessments

7.2.1.1 Ankle-Brachial Index (ABI)

Ankle-Brachial index will be obtained using standard, calibrated instruments as described in Appendix B. The pressure in both arms with be measured, and the higher of the two pressures will be used. All values will be recorded at each assessment in the Case Report Form.

7.2.1.2 Toe-Brachial Index (TBI)

Toe-Brachial index will be obtained using standard, calibrated instruments as described in Appendix B. The pressure in both arms with be measured, and the higher of the two pressures will be used. All values will be recorded at each assessment in the Case Report Form.

7.2.1.3 Wave Form Analysis (PVR)

A copy of the arterial flow wave form will be obtained at each assessment. This will be placed in the subject's record and sent for analysis at the end of the study.

7.2.1.4 Transcutaneous Oxygen Pressure Assessment ($TcPO_2$)

During the screening period the $TcPO_2$ will be measured at pre-defined locations on the anterior and posterior calf and dorsum of the foot. The limb/chest $TcPO_2$ index will be calculated by using the lesser of the lower limb measurements. Transcutaneous oxygen pressure methodology is instrument dependent and therefore the investigator will follow the standard procedures defined by their instrument's manufacturer and the written instructions provided by the Sponsor to standardize this procedure.

At multiple time points prior to and after treatment (Days 1, 15, 28, 59, 91, 180 and 365), the subject will have $TcPO_2$ measured at pre-defined locations on the anterior and posterior calf and dorsum of the foot. For analysis purposed, it is important that placement of the sensor used for the $TcPO_2$ measurement is always on the same pre-defined limb locations for screening and follow-up visits unless it is not possible (site amputated, areas of ulceration/gangrene). Marking the site with indelible ink is recommended. If a sensor placement site is unavailable due to amputation or areas of ulceration/gangrene, sensor placement site will be assigned a clinically equivalent default

$TcPO_2$ value of zero pressure ("0 mmHg"). Hard copy instrumentation printout will be included in the source documentation to support the $TcPO_2$ data.

7.2.2 High Resolution MRA

Subjects will have High Resolution MRA at Baseline and at 3 months and 6 months post-treatment. Area of Ischemic Ulcer (Digital Photography and Ulcer Measurement)

If one or more ischemic ulcer is present on the subject's studied limb, a digital photograph will be taken of the largest contiguous area of ulceration with a target plate included in the image for calibration purposes. The wound perimeter will be traced and the area of ulceration will be calculated in square millimeters. If a MRA can not be done, a CT-Angio (CTA) can be performed on the subject.

7.2.3 Pain VAS Score

Pain intensity will be assessed by subjects marking a place on a 100 mm visual analog scale ranging from 0=no pain to 100=worst possible pain as described in Appendix C. The distance from 0 to the mark will be measured in millimeters (0 to 100) and recorded on the CRF as the VAS score.

7.2.4 Subject Analgesic Use

Analgesic use including generic name, dose and frequency will be collected at Baseline and each subsequent visit and will be recorded on the CRF. Any change from in the dose level or frequency of analgesics used will be documented.

8.0 Adverse Events and Serious Adverse Events

8.1 Adverse Event Definition

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. AEs will be graded according to the National Cancer Institute's Common Terminology for Adverse Events v3.0 criteria.

An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or abnormal results of an investigation (e.g., laboratory findings, electrocardiogram).

Therefore, an AE would include the following:

- Any event that is new (i.e., was not seen prior to the start of the study).
- A condition detected or diagnosed after the trial medication administration, even though it may have been present prior to the start of the study.
- A pre-existing intermittent event or condition that recurs with increased intensity and/or frequency after the start of the study.
- A pre- or post-treatment event that results from study participation (i.e., invasive procedures, modification of subject's therapeutic regimen)
- A change in blood pressure or heart rate that results in discontinuation of dosage or that exceeds protocol-specified criteria

An AE does not include the following:

- The disease or disorder being studied, or a sign or symptom of that disease or disorder, unless it is more severe than expected for the subject's condition.
- An overdose of the study medication if the subject is asymptomatic.
- A medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction). Depending on the trial design, such a procedure may be the result of an AE or may result in an AE, but is not an AE itself.
- A pre-existing condition or disease that does not worsen following study drug administration.
- Hospitalization for elective surgery for a pre-existing disease or condition that did not worsen or for cosmetic surgery.

In this study an AE can include an undesirable medical condition occurring at any time after screening procedures have been started until the final follow-up visit, even if no study treatment has been administered.

When an adverse event occurs, the Investigator must:

1. assess whether or not the test article caused the AE;
2. determine the intensity of the AE;
3. record the action taken regarding the test article;
4. indicate whether the AE led to discontinuation of the subject from the study; and
5. determine if the AE is serious.

All AEs that occur in any subject enrolled, before treatment, during treatment, or up to and including study Day 365 whether or not related to the study drug, must be recorded on the appropriate CRF form provided.

The following are definitions of levels of severity:

- Mild: an awareness of symptoms but easily tolerated
- Moderate: symptoms interfering with daily activities
- Severe: incapacitating, with inability to perform normal daily activities.

8.2 Definition of an SAE

For each AE, you must determine if it is a serious adverse event (SAE) according to the following definition.

An SAE is an AE, occurring at any dose, which fulfills one or more of the following criteria:

- An event that results in death.
- An event that is immediately life threatening. This does not include an event that, had it occurred in a more serious form, might have caused death.
- An event that results in persistent or significant disability or incapacity.
- An event that is a congenital abnormality/birth defect
- An event that requires in-subject hospitalization or prolongs an existing hospitalization. Complications that occur during hospitalization and do not meet at least one of the above criteria for seriousness are AEs, not SAEs. Hospitalization means that the subject was formally admitted to the hospital, and does not include presentation at an emergency room.
- An event that, though not included in the above list, is an important medical event that may jeopardize the safety of the subject or may require medical or surgical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment will be exercised in deciding whether a case is serious in those situations where important medical events may not be immediately life threatening or result in death, hospitalization, disability or incapacity. These include events that may jeopardize the subject or may require medical or surgical intervention to prevent one or

more outcomes listed in the definition of serious. Such events will usually be considered as serious. Examples of such events are:

- Angioedema not severe enough to require intubation but requiring intravenous hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment at the study site, in an emergency room, or at home for allergic bronchospasm
- Blood dyscrasias (e.g., neutropenia or anemia requiring blood transfusion) or convulsions that do not result in hospitalization
- Development of drug dependency or drug abuse (e.g., analgesics).

8.3 Laboratory AEs and SAEs

Abnormal laboratory findings (e.g., serum chemistry, hematology) or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the Investigator to be clinically significant must be recorded as AEs or SAEs if they meet the definition of an AE or SAE, as defined in Sections 0 and 8.2, respectively. Clinically significant abnormal laboratory or other assessment findings that are detected following study drug administration or that are present at Baseline and worsen following the start of the study constitute AEs or SAEs. The Investigator will exercise clinical judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

8.4 Detection of AEs and SAEs: Method, Frequency, and Time Period

The methods of detection for AEs and SAEs include, but are not limited to, the use of non-leading verbal questioning of the subject (e.g., how do you feel?), clinical observation by the Investigator or study staff, physical examinations, monitoring of vital signs, or laboratory assessments.

AEs will be recorded for each subject from the time the subject signs the informed consent form through the subject's final follow-up visit. AEs and SAEs that result from study participation and occur prior to the start of the study drug will be recorded.

8.5 Documentation of AEs and SAEs

All AEs occurring during the study must be recorded in the subject's medical records and on the AE page of the CRF, even if the AE is considered by the Investigator to be unrelated to the use of the study drug. SAEs that occur during the study must be documented in the subject's medical record, on the AE page of the CRF, and on the SAE Report Form.

A separate SAE CRF will be used for all SAEs. For SAEs which are related and within a specific time period, a single form can be used. An assessment of the relationship of each event to the study therapy will be added for each SAE on the form. Following initial reporting of the event, the Investigator will make every effort to establish a diagnosis of the event based on the presenting signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE and/or SAE and not the individual signs/symptoms.

Death will not be reported as a serious adverse event. Death will be reported as the outcome of the event. When a subject expires, please use the cause of Death (acute MI, CVA, sepsis) as the SAE and the outcome as Death. When no cause of Death is apparent, please use cardio-pulmonary arrest as the SAE.

If a clinically significant, abnormal laboratory finding or other abnormal assessment meets the definition of an AE or SAE, then the AE page of the CRF and the SAE Report Form must be completed, as appropriate. A diagnosis, if known, or clinical signs and symptoms if the diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, will be recorded on the AE page of the CRF and on the SAE Report Form, as appropriate. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding will be recorded. If an isolated laboratory abnormality is an SAE, then the following will be performed: 1) the laboratory data will be recorded in the SAE Report Form with the reference range and all preceding measurement(s) value(s), including Baseline value(s), and 2) copies of the laboratory reports and reference ranges will be sent with the SAE Report Form.

The SAE CRF used to report an SAE will be completed as thoroughly as possible and signed by the Investigator or designee before transmittal. The Investigator must provide the relationship of the event to the study drug (assessment of causality to the trial medication), action taken with the study drug and outcome (if known) at the time of the initial report. SAE CRFs will be sent via Datafax to: 760-268-6550.

8.6 Follow-up of AEs and SAEs

All AEs and SAEs must be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. The Investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as possible the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. ViroMed or their designee may request that the Investigator perform or arrange for the conduct of supplemental measurements and/or evaluations. If a subject dies during participation in the study or

during a recognized follow-up period, a copy of any post-mortem findings will be provided to ViroMed or their designee.

New or updated information will be recorded on the initial SAE Report Form. All corrections to the initial report will be dated and initialed. Each version will need to be sent to the designated CRO via DataFax.

In the event that a subject is lost to follow-up prior to the Investigator's being able to verify that an AE or SAE is resolved, stabilized, or otherwise explained, the site must document in the subject's medical records the repeated attempts (at least 3) to contact the subject and/or his/her care provider to retrieve the information; documentation will include date and mode of communication. In the case of "lost to follow-up." the event is considered as ongoing.

8.7 Immediate Reporting of SAEs

ANY SERIOUS AE THAT OCCURS DURING THIS INVESTIGATION (SEE SECTION 8.2.), WHETHER OR NOT RELATED TO THE STUDY MEDICATION, MUST BE REPORTED IMMEDIATELY TO THE SPONSOR AND/OR THE DESIGNATED CRO.

The reporting timeframes and the required documentation for any SAE occurring during the study are summarized in Table 2.

In the event of an AE leading to hospitalization, every effort will be made by the investigational site to obtain a hospital discharge summary.

In the event of a fatal AE, documentation of any available postmortem findings, including autopsy, will be provided to ViroMed or their designee. In any event, the Investigator will provide a narrative summary of circumstances, events related to the death, and cause of death, if known.

Table 8-1 SAE Reporting Requirements

	Initial Reports	Follow-up Reports
Type of SAE	Fatal/Life Threatening and all other SAEs	Any SAE
Timeframes	24 hours	As additional information become available.
Documents	**24 hours**: Completed initial SAE Report Form with minimum requirements (subject #, date of birth, event and start date)	Follow-up SAE Report Form and CRF documentation Hospital discharge summary Any relevant diagnostic test results/reports, including pathology reports Any postmortem findings/histopathology results

[a] Within 24 hours after the site learns about the event.
NOTE: SAE = Serious Adverse Event.

8.8 Transmission of SAE Reports

DataFax facsimile transmission of the SAE Report is the preferred method to transmit this information. The facsimile information for reporting of SAEs is:

DataFax number: 760-268-6550
Additional information beyond the SAE CRF will be faxed separately to 760-268-6500.

8.9 Regulatory Reporting Requirements

The Investigator must promptly report all SAEs in accordance with the procedures detailed in Section 7.3.8. ViroMed has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a drug under clinical investigation (21 CFR §312.32).

The Investigator must comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the Institutional Review Board (IRB) or Institutional Ethical Committee (IEC).

This protocol has been filed under an Investigational New Drug (IND) application with the US Food and Drug Administration (FDA). An SAE may qualify as an IND Safety Report (21 CFR §312.32) if the SAE is unexpected, serious, and there is a reasonable possibility that it may have been caused by the investigational drug. In this event, all Investigators filed to the IND (and associated INDs for the same medicinal product) will receive a copy of the IND Safety Report as submitted to the US FDA.

Upon receipt from ViroMed of an initial or follow-up IND Safety Report or other safety information (e.g., revised IDB), the Investigator must promptly notify his or her IRB or IEC.

8.10 Post-Study AEs and SAEs

Investigators are not obligated to actively seek AEs or SAEs beyond the reporting timeframes defined in Section 0. However, if the Investigator learns of any AE or SAEs occurring after the subject's last study visit and the event is deemed by the Investigator to be reasonably possibly related to the use of the study drug, he or she will promptly document and report the event to the safety monitor.

8.11 SAEs Related to Study Participation

An SAE considered related to study participation (e.g., procedures, invasive tests), even if it occurs during the pre- or post-treatment period will be reported promptly to the safety monitor in accordance with the procedures detailed above.

9.0 Statistics

The following sections provide an overview of the planned analysis. A statistical analysis plan (SAP) will be prepared separately from this protocol, which will provide detailed descriptions of the analysis population, statistical methods and models, and tested hypotheses to be analyzed. The SAP will serve as a companion to the protocol and as the *de facto* documentation of the proposed statistical evaluation.

9.1 Sample Size

This is a Phase I, dose-escalation study. The sample size is based on the feasibility to assess safety. No statistical analysis of efficacy is planned.

9.2 Population

Statistical analysis will be based on an Intent-to-Treat design, but subjects must receive at least one dose of study drug and have at least one post-dose assessment.

9.3 Statistical Analysis

Safety and Tolerability:

The incidence and nature of adverse events will be reported in tabulated format. Adverse events will be described according to severity and to its relationship with the study drug. Serious adverse events and adverse events leading to treatment discontinuation will be listed.

Descriptive statistics (N, mean, median, SD, minimum and maximum values, where applicable) of safety laboratory parameters infection tests and tumor markers will be reported. Abnormalities will be summarized for low and high values.

Retinal fundoscopy results per treatment group will be reported as change from baseline and subsequent evaluation at day 365.

Efficacy:

Descriptive statistics (N, mean, median, SD, minimum and maximum values) of vascular assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, High Resolution MRA, VAS for pain, analgesic use, and ulcer size will be reported. Frequency counts for analgesics use by dose cohort will be reported.

Pharmacokinetics:

Descriptive statistics (N, mean, median, SD, minimum and maximum values, where applicable) of VM202 DNA levels will be reported.

Pharmacodynamics:

Descriptive statistics (N, mean, median, SD, minimum and maximum values, where applicable) of endothelial progenitor cell assay levels, anti-HGF antibody levels and serum HGF levels will be reported.

Change in endothelial progenitor cell levels from Baseline will be described for each treatment dose at each selected time point.

9.4 Data Safety Monitoring Committee

A Data Safety Monitoring Committee will review all safety data available 30 days after the first subject in each cohort is dosed and subsequent to day 15 dosing of the other 2 subjects in the same cohort. The committee will operate via Guidance Document and will have pre-specified stopping rules. The committee will review all safety data including severity, relationship to study drug, action taken with study drug and outcome. Study drug administration for the next cohort cannot begin until the committee reviews all of the data and reports its findings to ViroMed or their designee. There will also be continuing review of safety events after the Day 59 visit for all subjects at defined intervals until all subjects have completed the 6 month and 12 month follow-up visit (Day 365).

10.0 Quality Control and Quality Assurance

10.1 Ethics

10.1.1 Responsibilities of the Investigator:

The procedures set out in this study protocol, pertaining to the conduct, evaluation and documentation of this study, are designed to ensure that the Sponsor and the Investigator abide by Good Clinical Practice (GCP) as described in 21 Code of Federal Regulations (CFR) Parts 50, 56 and 312 and the FDA Guidelines for the Monitoring of Clinical Investigations, January 1988. Compliance with these regulations also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki, Tokyo 2004.

Copies of these materials are available from ViroMed by request. The purpose of these regulations and legal obligations is to define the standards and principles for the proper conduct of clinical trials that have been developed by the medical, scientific and regulatory communities. They are not intended to impede or restrict clinical research.

The ethical standards defined within GCP are intended to ensure that:

- human subjects/subjects are provided with an adequate understanding of the possible risks of their participation in the study, and that they have a free choice to participate or not;
- the study is conducted with diligence and in conformance with the protocol in such a way as to insure the integrity of the findings;
- the potential benefits of the research justify the risks.

ViroMed is the Sponsor of the Investigational New Drug Application (IND) under which this study is being performed. ViroMed is responsible for the following:

- selecting qualified Investigators,
- providing Investigators with the information they need to properly conduct an investigation,
- ensuring proper monitoring of the investigation,
- ensuring that the study is conducted according to the general investigational plan and protocols contained in the IND,
- maintaining the IND, and
- ensuring that FDA and all participating Investigators are properly informed of significant new information regarding adverse effects or risks associated with the drug being studied.

As the Sponsor, ViroMed has delegated some responsibilities to the CRO.

The Investigator is responsible for ensuring that the study is conducted according to the signed Statement of Investigator (Form FDA-1572), the protocol, and all applicable regulations. The Investigator is responsible for protecting the rights, safety and welfare of subjects/subjects under his/her care, and for the control of the drugs under investigation.

10.1.2 Legal and Regulatory Considerations

10.1.2.1 Compliance with Law, Audit, and Debarment

The Investigator is required to maintain complete and accurate study documentation in compliance with current GCP standards and all applicable federal, state and local laws, rules, and regulations related to the conduct of a clinical study using recombinant DNA and gene transfer technology.

The Investigator is required to make all study documentation promptly available for inspection, review, or audit upon request by ViroMed, its representatives, or any appropriate regulatory agencies.

Individuals ineligible from conducting or working on clinical studies, including those ineligible as a result of debarment under the Generic Drug Enforcement Act of 1992, will not be allowed to conduct or work on studies sponsored by ViroMed. The Investigator is required to immediately disclose to ViroMed, in writing, if any person involved in the conduct of the study is debarred pursuant to a hearing by U.S. FDA under this anti-fraud law, or if any proceeding for debarment is pending, or is (to the best of your knowledge) threatened.

10.1.2.2 Protection of Human Subjects

The Investigator will be provided with a sample Informed Consent Form (ICF) for this study by ViroMed. It is requested that the study site use the sample form provided. However, the site may adapt the information to suit the needs of the study institution, if necessary. If changes are made by the site to the ICF, the modified ICF must be reviewed and approved prior to submission to the IRB. The final ICF must be approved by the Institutional Review Board (IRB) and accepted by ViroMed. The Investigator must provide ViroMed with an unsigned copy of the final ICF following approval by the IRB.

- The consent form must reflect the required elements of informed consent specified in 21 CFR Part 50.25.
- The Investigator must provide all subjects/subjects with all the information necessary to make an informed decision about their participation in the study, including the nature and intended purpose of the study, possible benefits and possible risks.

- All information in the informed consent form will be provided in language understandable to the subject.
- Before the subject/subject undergoes procedures specific to the protocol, the informed consent form must be signed and dated by the subject/subject (or the subject's legally authorized representative).
- If the subject/subject (or legally authorized representative) cannot read, the informed consent form must be signed and dated by an impartial witness.
- The person who discussed the informed consent information with the subject/subject must also sign and date the form.
- After all required signatures have been obtained, a copy of the informed consent form will be provided to the subject/subject, and the original must be kept on file at the site.

10.1.2.3 Institutional Review Board (IRB)

The Investigator is required to obtain initial and continuing review and approval by an IRB that complies with the requirements specified in 21 CFR Part 56. The protocol (and any amendments to it) and the ICF must be reviewed by the IRB. The IRB must provide the study site with written approval that must be forwarded to the CRO prior to study initiation. In addition, the IRB must also approve all advertising used to recruit subjects for the study. If the duration of the study is greater than one year, re-approval by the IRB must be obtained on a yearly basis (or at more frequent intervals if required by the IRB).

The Investigator must provide progress reports to the IRB, as well as any reports of SAE from the study site. The Investigator is also responsible for providing the IRB with reports of any IND Safety Reports from any other study site, or from any other study conducted with the study drug. ViroMed will provide this IND Safety Report to the study site.

10.1.2.4 Institutional Biosafety Committee (IBC)

The site at which this trial is being conducted will ensure that an Institutional Biosafety Committee is in place that is composed of at least 5 appropriately-qualified members. The IBC will ensure that the site conforms to the requirements set forth in the Section IV-B-2 of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, promulgated by the National Institutes of Health/Office of Biotechnology Activities (NIH/OBA).

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

10.1.2.5 Protection of Subject Data

The Investigator is responsible for keeping a record of all screened subjects including full names and last known addresses. All subjects will be identified in the CRF by initials, date of birth, gender, and race. An enrollment number will additionally identify subjects in the CRF.

The Investigator will inform the subject, in writing, about the possibility of audits by authorized representatives of ViroMed, the CRO and/or regulatory authorities. Such audits may require a review of those parts of the subject records relevant to the study. Confidentiality of subject data will be maintained in accordance with local laws. The informed consent form must contain a statement describing the extent to which confidentiality of the subject will be maintained.

10.2 Study Records, Data Handling, and Record Keeping

10.2.2 Investigator/Site Documentation

The Investigator must provide the CRO with the following documents prior to study initiation and retain a copy in the study file.

- A current and complete curriculum vitae for the Primary Investigator and each Sub investigator,
- Completed Form FDA-1572 signed and dated by the Primary Investigator,
- Approved ViroMed protocol (and any protocol amendments) signed and dated by the Primary Investigator,
- IBC membership list
- IRB membership list or assurance number,
- Informed Consent Form approved by the IRB and accepted by ViroMed,
- Written IRB approval of the protocol,
- Certifications and laboratory reference ranges for all local laboratories used for this study.

In addition to the documents listed above, the study site will also retain the following items:

- All original informed consent forms with required signatures
- All IRB approvals and correspondence (i.e., informed consent [including any approved revisions], protocol, AE, advertisements, newsletters)
- Copy of the Study Monitoring Log Sheet
- Clinical and non-clinical supply shipment forms
- Copies of all correspondence pertaining to the study (except budget issues) between ViroMed or the CRO and the site

- Copies of all SAE reports submitted to ViroMed or designated CRO
- Copies of all IND Safety Reports submitted to the site by ViroMed
- Copies of approved package labeling
- Study personnel signature log

The Clinical Study Agreement will be kept in a file separate from any study documents because this is a confidential agreement between the study site and/or the Principal Investigator and/or the study institution and ViroMed.

10.2.3 Financial Disclosure

Before the start of the study, the Investigator will disclose to the Sponsor any proprietary or financial interests he or she might hold in the investigational product or the Sponsor's company as outlined in the financial disclosure form provided by the Sponsor (or CRO). This will be documented in writing at the beginning and at the end of the trial. The Investigator agrees to update this information in case of significant changes during the study or within one year of its completion. The Investigator also agrees that where required by law or regulation, the Sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorizations.

10.2.4 Clinical Monitoring

The study will be monitored in compliance with Section 9 (Commitments) of FDA Form 1572, the relevant parts of 21 CFR, and according to the ICH GCP Guidelines (E6 Section 5).

A representative of the designated CRO will visit the institution prior to initiating the study and periodically thereafter to monitor acceptability of facilities, the agreement between CRF entries and original source documentation, adherence to the protocol, Good Clinical Practice (GCP) and to applicable FDA regulations and the maintenance of adequate clinical records. This representative is a Clinical Research Associate (CRA). The CRA will have access to subject records, medication sheets, laboratory data and other source documentation.

The frequency of monitoring visits will depend on subject enrollment. Once the study has been completed or terminated, a close-out or termination visit will be made. The Investigator and/or Study Coordinator will receive reasonable notification before each monitoring visit during the course of the study. At each visit, the Investigator will cooperate with the CRA for the review and verification of all CRFs, drug supply and inventory records, and any additional records requested for review.

All information contained in a subject's CRF must have corresponding source documentation. This source documentation includes, but is not limited to, notes taken at subject visits recording the date of the visit, vital signs, physical findings, adverse events,

or concomitant medications; laboratory reports; hospital records; and clinic records. All electronic progress notes must be printed, signed and dated by the principal Investigator.

The records of the study may be subject to audit by the Sponsor's representative or by government regulatory authorities (e.g., U.S. Food and Drug Administration or NIH). The Investigator must agree to allow access to the required subject records in the event of such an audit.

10.2.5 Recording of Data

The study site will be provided with multi-part CRFs for collecting subject data. All entries on these forms must be printed legibly using black ballpoint pen. All information recorded on the forms must be supported by documentation in the subject's file, with the exception of the subject's responses to self-administered assessments (e.g., SF-36). The site will be visited at regular intervals by a representative of the CRO called a Clinical Research Associate (CRA). The CRA will verify all CRF entries against subject records. CRFs and all supporting information will be readily available for review during scheduled monitoring visits.

The CRA is responsible for returning all completed forms to CRO. One copy must be retained at the site. CRF pages will not be mailed or faxed directly to the CRO unless the site has received specific permission to do so.

10.2.6 Database Management and Quality Control

Data items from the CRFs will be entered into a study database. Specific details of data collection and management procedures for this study will be provided in the Operations Manual for this trial.

Data Management staff, using error messages printed from validation programs and database listings, will systematically check the information entered into the database. The CRO will be responsible for the data management and will correct obvious errors in accordance with ViroMed-approved Data Management Guidelines. Other errors or omissions will be entered onto Data Clarification Forms (DCF), which will be returned to the investigational site for resolution. The site will retain a copy of the DCF. Once the original DCF is returned to the CRO the DCF will be reviewed, appropriate action will be taken, and the original signed DCF will be kept with the original CRFs. Quality control audits of all key safety and efficacy data in the database will be made prior to database lock according to CRO standard operating procedures (SOPs).

10.2.7 Investigator's Final Report

A summary report must be submitted to the Institutional Review Board and ViroMed or their designee within eight weeks after the study's completion or termination.

10.2.8 Record Storage and Maintenance

The Food and Drug Administration requires that an Investigator retain records for a period of two (2) years following the date a New Drug Application or Product License Application is approved for the drug for the indication for which it is being investigated; or, if no application or license is to be filed or, if the application or license is not approved for such indication, until two (2) years after the investigation is discontinued (21 CFR 312.62).

The Investigator will ensure that the following records are maintained:

- Subject files containing copies of completed case reports and supporting documentation and a copy of the signed, Informed Consent Form.
- Investigator files containing copies of the documents required for the initiation of the study (executed form FDA 1572, signed Investigator's Agreement, Curricula Vitae for the Principal and all Sub-Investigators, copy of the IRB approval of the Protocol and Informed Consent form), all approved versions of the protocol and informed consent form and copies of correspondence received from and sent to ViroMed. In addition to these records required by regulations, ViroMed requests that the Investigator keep a copy of the Financial Agreement between ViroMed and the Investigator.
- Pharmacy files containing copies of the Investigational Drug Utilization Records (IDUR) or an equivalent form approved by ViroMed, instructions for the use of the IDUR and package inserts and/or the Investigator's Brochure.

10.2.9 FDA/NIH Contact and Correspondence

The Investigator will notify ViroMed or the Medical Monitor within three days following FDA or NIH contact with the investigative site. The Investigator will provide ViroMed or their designee with copies of all correspondence with the FDA or NIH which may affect the review of the current study (e.g., Form 483, Inspectional Observations) or their qualification as an Investigator in studies conducted by ViroMed (e.g., warning letters). ViroMed reserves the right to be present at the site during any FDA or NIH inspection that involves this protocol.

10.2.10 HIPAA

The Health Insurance Portability and Accountability Act requires that health professionals with access to protected health information (PHI) maintain the confidence of this information. In this case, this includes the Principal Investigator if the PI has billed a third party for subject services. Pharmaceutical companies and CROs are not entities covered by HIPAA directly. The Investigator is aware that review of subjects' medical information by a CRO or its designees is permitted as long as the Investigator or physician caring for the subject gives permission to review the information and that the

information is not removed from the subject's medical record. The Sponsor will protect individual subject information to the full extent possible during this trial. At no time will a subject become identified in any publication or presentation. However, the subject may have to become identified in the event of an FDA audit or inspection in order to verify the accuracy of the data. The subject will remain blinded to the results of this trial for the duration of the trial and for a continuing period of time following completion of the study. Access to un-blinded subject information is at the discretion of the Sponsor and cannot occur prior to database lock or other specified events as determined solely in the discretion of the Sponsor.

10.2.10 Publication Policy

ViroMed has the right to publish, independently, the results of this clinical study. Authorship will depend on the degree of participation in protocol design, data analysis and manuscript preparation.

The Investigator may not publish the results from this clinical study without the prior written permission of ViroMed. Such permission will not be unreasonably withheld. If ViroMed grants such permission, the Investigator must submit a copy of any manuscript and/or abstract to ViroMed for review and comment at least 90 days prior to its submission for publication. ViroMed will provide the Investigator with comments within 90 days.

10.3 Handling of Investigational Drug

In accordance with federal regulations (21 CFR 312.62), all Investigators are required to keep accurate records showing final disposition of all investigational drugs. The study pharmacist in charge of the investigational drug may keep the records and sign an Investigational Drug Utilization Record (IDUR) or equivalent form. These records must be available to ViroMed on request, showing accurate reconciliation of each and every shipment of investigational drug. A separate IDUR (or the equivalent) must be made for each test material received.

The study pharmacist will be listed on the Site Delegation Log. The study pharmacist will be designated as the person responsible for the handling and dispensing of the test article. The study pharmacist will sign all forms related to study drug inventory, dispensing, storage and return.

Information regarding the handling of study drug will include the following:

Unique Vial Number: The unique vial number is indicated on the label applied to each container of the product.

Manufacture Date: The date the test material was manufactured will be listed on the label of the product

Date Shipment Received: including the number of vials and lot number.

Date Used: Date administered or dispensed to the subject.

Disposition of Material: Indicate if administered, destroyed, damaged in transit and destroyed, or other final disposition of material.

Unused Containers: Indicate any unopened units returned, as well as opened and partially used containers.

Date Returned: At the termination of the study, all study drug that is not assigned to a subject will be returned to ViroMed or their designee. Indicate the date when unused containers are returned (dd/mmm/yyyy). Any deviations from this procedure will be reported to the assigned Project Manager.

Disposition of Investigational Drug Utilization Records: When an IDUR (or the approved equivalent form) is complete for any given lot, it is to be signed by the study pharmacist in charge of the investigational drug. The CRA will make a photocopy of the original, which will be filed in the Investigator's file.

10.4 Disposition of Clinical Supplies

Ultimate accountability for the receipt, dispensation and record keeping of the test material 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 study pharmacist nor Investigator may supply the test material to any person outside of the protocol. (See previous section for details of the record keeping for the investigational material.)

The investigational drug provided by ViroMed 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. The use of the investigational drug by the Investigator, sub-Investigators or any third party, outside of the provisions stated in the protocol, without the express written permission of ViroMed is strictly prohibited. VM202 is an investigational drug for the use in Clinical Trial US 06-1-001. The possession and use of VM202 must be closely controlled and monitored by the Investigator. Upon completion of the study, the CRA will arrange for return of all used and unused VM202 to the Sponsor along with a copy of the drug supply and inventory records.

10.5 Investigator Confidentiality

All information and data, including this protocol, and all data, clinical results and research conducted hereunder concerning Sponsor's products and operations including Sponsor's patent applications, formulas, manufacturing processes, basic scientific data and formulation information that has been supplied by Sponsor and not previously published are considered confidential by Sponsor and will remain the sole property of Sponsor. The Investigator understands and agrees that said proprietary and/or confidential information disclosed to or produced by him/her is highly valuable to the Sponsor and will be used EXCLUSIVELY by the Investigator in accomplishing this study and will not be used for any other purpose 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 Sponsor in connection with the development of VM202. Therefore the information may be disclosed and used solely by Sponsor as required to such third parties and agencies as 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's representative (CRO) regarding completion, legibility or accuracy of trial data on the CRFs.

Appendix A

Clinical categories of chronic limb ischemia*

Grade	Category	Clinical description	Objective criteria
0	0	Asymptomatic – no hemodynamically significant occlusive disease	Normal treadmill or reactive hyperemia test
	1	Mild claudication	Completes treadmill exercise [1]; after exercise >50 mm Hg but at least 20 mm Hg lower than resting value
I	2	Moderate claudication	Between categories 1 and 3
II	3	Severe claudication	Cannot complete standard treadmill exercise[1] *and* AP after exercise <50 mm Hg
II[2]	4	Ischemic rest pain	Resting AP <40 mm Hg, flat or barely pulsatile ankle or metatarsal PVR; TP <30 mm Hg
III[2]	5	Minor tissue loss – non healing ulcer, focal gangrene with diffuse pedal ischemia	Resting AP <60 mm Hg, ankle or metatarsal PVR flat or barely pulsatile; TP <40 mm Hg
	6	Major tissue loss – extending above TM level, functional foot no longer salvageable	Same as category 5

AP, Ankle pressure; PVR, pulse volume recording; TP, toe pressure; TM, transmetatarsal.
Reference: Journal of Vascular Surgery. 1997;26(3).

[1] Five minutes at 2 mph on a 12% incline.

[2] Grades II and III, categories 4, 5, and 6, are embraced by the term chronic critical ischemia.

Appendix B

Hemodynamic Procedures

ABI and TBI Assessment

Explain the procedure and reassure the subject and ensure that he/she is lying flat and is comfortable, relaxed and rested with no pressure on the proximal vessels. Laying the subject supine reduces 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, 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 higher of the two values to calculate the ABPI.

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 or anterior tibial 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 arteries
5. Use the highest reading obtained to calculate the ABPI for that leg
6. Repeat for the other leg (At the screening visit only. The index leg will be used for future measurements).

Measure the toe systolic pressure:

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

   Note: The same method (Doppler or PPG) must be used on the patient 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 other toe artery
5. Use the highest reading obtained to calculate the TBI for that leg
6. Repeat ABI and TBI for the other leg

Calculate the ABI and TBI for each leg using the formula below:

ABI = Highest Ankle systolic pressure/ Highest Brachial systolic

TBI = Highest Toe systolic pressure/ Highest Brachial systolic

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. Ankle systolic pressure is not measured, pressure recorded is usually higher than ankle pressure
4. The pulse is irregular or the cuff is deflated too rapidly
5. 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,
6. Central systolic pressure may influence the 'normal' range for the ABPI

Appendix C

The Visual Analog Scale (VAS) Procedures

Pain Intensity Rating using a Visual Analog Scale (VAS)

The visual analog scale (VAS) is a 10-cm line, oriented horizontally, with one end indicating “no pain” and the other end representing “pain as bad as it can be”.

1. The subject is asked to mark a place on the line corresponding to the current pain intensity.
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).
3. The CRF will be faxed into DataFax and the measurement will be electronically recorded in the database

Appendix D

VM202 Test Material Preparation and Administration

1. **Selection of injection sites**

Identify occluded sites using contrast magnetic resonance (MRA) or computerized tomography (CTA) angiography. Select injection sites with the objective of inducing the formation of bridging collaterals from areas with normal blood flow and improving run-in. Therefore, areas adjacent to the occluded sites and areas where blood flow through good collateral blood vessels decreases are desirable as injection sites. Documentation of injections sites will be indicated by placing an **X** on the diagram provided in the CRF and shown below.

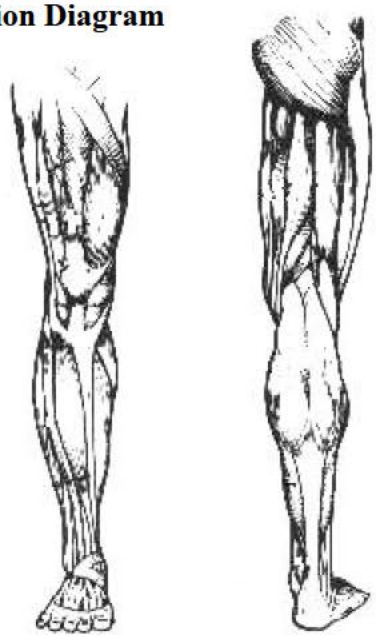
2. **Test material administration**

On Day 1 and Day 15 the subject will receive test material. The study treatment will be administered as up to 8 injections. A fine needle (e.g. 27 gauge, 1") suitable for IM injections will be used; 2.0 ml will be administered per injection site (total volume of 8 or 16 ml per administration, determined by cohort assignment). Inject the entire amount of the drug in about 20-30 seconds. Immediately after completion of injection, lightly press the injection site with the finger head in order to prevent reflux. Do not massage the injection site.

3. **Second test material administration**

Each administration will be given at least 2 cm from the previous injection site. It is recommended that an indelible marker be used to mark the location of previous injections to ensure that repeat injections are not delivered to exact same location with the subsequent administration.

Injection Documentation Diagram



Indicate on drawing by marking an X at each injection site

Appendix E

Reasons for Modifications

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Version 2.0 Modifications		
Cover page (C) Section 6.3 Autopsy (C) Section 8.1.5 Documentation of AEs and SAEs (C) Section 8.1.6 Follow-Up of AEs and SAEs (C) Section 8.1.7 Immediate Reporting of SAEs (C) Section 8.1.8 Transmission of SAE Reports (C) Section 10 Quality Control and QA (C) *Formerly:* [redacted]	Cover, 27, 41, 42, 43, 48, 49, 50, 51, 53	Updated the contact information for the medical monitor and CRO.
Signature Page (C) *Formerly:* Version Number: 1.0 *Modified to:* **Amended** Version Number: **2.0** *Formerly:* IND Number: *Modified to:* IND Number: **13158**	ii	Changed amended version number and added IND number.
Protocol Synopsis (D) *Formerly:* The Quality of Life questionnaire will be done on Days 28, 59 and 180 (± 2 days). Section 4.1.2 Secondary Efficacy Endpoints (D) *Formerly:* Change from Baseline in quality of life, as measured by the SF-36, at Days 15, 28, 59, 180 and 365. Section 4.2.3 Schedule of Assessments Table 4-1 (D)	x , xiii, xiv, xv, 6, 8, 18, 21, 22, 23, 24, 25, 36, 45	Deleted QOL because not statistically relevant for Phase I sample size.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Section 6.2.1 Screening Day (D) Section 6.2.5 Day 15 (D) Section 6.2.8 Day 28 (D) Section 6.2.9 Day 59 (D) Section 6.2.11 Day 180 (D) Section 6.2.12 Day 365 (D) Section 6.2.15 Early Termination Visits (D) *Formerly:* Quality of Life (QOL) Section 7.2.6 Quality of Life Score (D) *Formerly:* Quality of Life (QOL) will be measured using the Medical Outcomes Study short form (SF-36).[10] The survey should be self-administered by the subject or administered by the Investigator or designee. The survey should be administered the same way each time it is completed. Responses will be documented on the CRF. If SF-36 testing falls on a study drug administration day, the questionnaire will be taken before study drug administration so that study related discomforts do not influence the subject's health perception. Section 9.3 Statistical Analysis (C) *Formerly:* Descriptive statistics (N, mean, median, SD, minimum and maximum values) of vascular assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, High Resolution MRA, VAS for pain, analgesic use, ulcer size **and QOL response** will be reported. *Modified to:* Descriptive statistics (N, mean, median, SD, minimum and maximum values) of vascular assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, High Resolution MRA, VAS for pain, analgesic use, and ulcer size will be reported.		
Protocol Synopsis (C) *Formerly:* All subjects will be followed for two years from the time of the first dose of study drug administration. *Modified to:* All 4 dose cohorts will be followed for **up to five years**	xii, xiv, 8, 25, 28	Extend long-term follow-up from 2 to 5 years from the time of the first dose of study drug administration

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
from the time of the first dose of study drug administration. *Formerly:* All subjects will be followed for two years from the time of first dose administrations. *Modified to:* All subjects will be followed for two years from the time of first dose administrations **and will be in long term follow-up for a total of 5 years**. <u>Section 4.2.3 Schedule of Assessments Table 4-1 (C)</u> *Modified to include:* [6]Annual telephone follow-up contacts will continue for 4 years after day 365 *Delete:* Annual telephone follow-up at day 365 <u>Section 6.2.13 Long-term Follow-Up (C)</u> *Formerly:* In keeping with current recommendations by FDA to maintain a long-term follow-up on subjects who receive gene transfer therapy, safety data will be collected for **one (1)** additional year following the subject's completion of study-related procedures. *Modified to:* In keeping with current recommendations by FDA to maintain a long-term follow-up on subjects who receive gene transfer therapy, safety data will be collected for **up to 5 years (i.e., 4 additional years following the subject's completion of study-related procedures).** <u>Section 6.4.2 Replacement of Study Subjects (C)</u> *Formerly:* If the subject has received one or both doses of study drug, the subject will remain in the study through the two year follow-up period. *Modified to:* If the subject has received one or both doses of study drug, the subject will remain in the study through the **five** year follow-up period. <u>Protocol Synopsis (C)</u>	xii, 7, 10,	(requested by the FDA at the Oct. 31, 06 meeting). Clarification of the

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Formerly: If a dose limiting toxicity is observed at any dose group, three additional subjects will be added to the dose cohort in whom the toxicity was observed and to all lower dose cohorts and all evaluations will be repeated for these additional subjects. *Modified to:* If a dose limiting toxicity is observed in one subject in any dose group, three additional subjects will be added to the dose cohort in **which** the toxicity was observed. **If no additional DLTs are observed in the 6 subjects in this dose level, it will be considered the Maximum Tolerated Dose (MTD). If a DLT occurs in 2/6 subjects, then 3 additional subjects will be enrolled at the previous dose level. If no DLTs occur at this level it will be considered the MTD.** <u>Section 4.2.2 Number of Subjects (C)</u> *Formerly:* There will 12 subjects selected at one site for participation in this study with 3 subjects treated in each of four (4) study cohorts. *Modified to:* There will 12**-15** subjects selected at one site for participation in this study with 3 subjects treated in each of four (4) study cohorts. **There will be an additional 3 patients treated in a cohort if 1/3 subjects experiences a DLT and if the DSMC recommends continuing the trial.** <u>Section 4.5 Treatments (A)</u> *Addition:* **If a DLT occurs in 2/6 subjects then 3 additional subjects will be treated at the previous dose level and if no DLT occurs in that cohort, it will be considered the MTD.** <u>Section 6.1.1 Treatment Regimen and Follow-Up (C)</u> *Formerly:* In the event that a dose limiting toxicity (DLT) is determined after the safety review between Cohort I and Cohort II, between Cohort II and Cohort III or between Cohort III and Cohort IV, there will be 3 additional subjects treated at the same dose and at all	17, 35	standard 3+3 Phase 1 trial design (requested by the FDA at the Jan. 06 Pre-IND meeting).

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
lower doses (either 2 mg only, 2 mg and 4 mg or 2 mg, 4 mg and 8 mg) *Modified to:* In the event that a DLT is determined after the safety review **in any cohort**, there will be 3 additional subjects treated at the same dose **level**. **If no further DLTs are observed in the 6 subjects and the DSMC approves dose escalation, then subjects can be enrolled to the next higher dose level. If 2/6 subjects experience a DLT then the preceding dose level treatment group will be considered the MTD.** <u>Section 7.1.9 Dose Limiting Toxicity (DLT) (C)</u> *Formerly:* In the event of a DLT at any dose, 3 additional subjects will be treated at that dose and at all lower doses. *Modified to:* In the event of a DLT at any dose, **3 additional subjects will be added to the dose cohort in which the toxicity was observed. If no additional DLTs are observed in the 6 subjects in this dose level, it will be considered the Maximum Tolerated Dose (MTD). If a DLT occurs in 2/6 patients, then 3 additional subjects will be enrolled at the previous dose level. If no DLTs occur at this level it will be considered the MTD.**		
<u>Protocol Synopsis (C)</u> *Formerly:* Chest X-ray *Modified to:* Chest X-ray **or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)** *Formerly:* For subjects with ischemic ulcer, a photograph of the ulcer will be obtained. Safety laboratory (hematology, chemistry, urinalysis), tumor marker tests, infection tests, chest X-ray, and ECG will also be done at Baseline.	xiii, 8, 11, 18, 25, 27	Add CT scan of the chest to the List of Assessments as CT scan will be performed instead of the chest X-ray if the subject has a previous history of tobacco use (requested by the Institutional Biosafety Committee (Oct.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Modified to: For subjects with ischemic ulcer, a photograph of the ulcer will be obtained. Safety laboratory (hematology, chemistry, urinalysis), tumor marker tests, infection tests, chest X-ray **or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)**, and ECG will also be done at Baseline. Section 4.2.3 Schedule of Assessments Table 4-1 (C) *Formerly:* Chest X-ray *Modified to:* Chest X-ray **or CT scan of chest**[1] [1]CT scan of the chest instead of chest X-ray will be performed if previous tobacco use. Section 5.1 Cancer Screening (D) *Formerly:* Chest X-ray or CT scan of the chest within 3 months prior to study entry *Modified to:* Chest X-ray or CT scan of the chest **(if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)** within 3 months prior to study entry Section 6.2.1 Screening Day (C) *Formerly:* Chest X-ray (AP only) *Modified to:* Chest X-ray **or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)** Section 6.2.12 Day 365 (A) *Added:* **Chest X-ray or CT scan of the chest (if the subject has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray)** Section 6.2.15 Early Termination Visits (A) *Added:* **Chest X-ray or CT scan of the chest (if the subject**		06).

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
has a previous history of tobacco use, a CT scan will be performed instead of the chest X-ray) Protocol Synopsis (C) *Formerly:* There will be 8 injections administered slowly for 20~40 seconds at pre-determined sites (mainly at the calf muscles) without local anesthesia *Modified to:* There will be **4 or** 8 injections **depending on the on the dose level** administered slowly administered slowly for 20~40 seconds at pre-determined sites **on only one affected leg** (mainly at the calf muscles) without local anesthesia. *Formerly:* After the 2nd half of the total dose is administered (Day15), hemodynamic assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, photograph and measurement of ischemic ulcer, VAS for pain, and safety labs will also be assessed on Days 28, 59, 91, 180 and 365. *Modified to:* After the **second** dose is administered (Day15) **to the leg previously injected**, hemodynamic assessments (ABI, TBI, Wave Form Analysis), $TcPO_2$, photograph and measurement of ischemic ulcer, VAS for pain, and safety labs will also be assessed on Days 28, 59, 91, 180 and 365. Section 6.1.1 Treatment Regimen and Follow-Up (C) *Formerly:* The same volume should be used for both the first and the second doses (series of either 4 or 8 injections) for each subject. *Modified to:* The same volume **will** be used for both the first and the second doses (series of either 4 or 8 injections **in only one affected leg**) for each subject.	xiii, xiv, 16	Clarify that the doses are administered to only one leg (requested by the FDA at the Oct 31, 06 meeting).
Section 4.1.3 Pharmacokinetic Evaluations (D) *Formerly:* Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose and 60 minutes **and 24**	6, 8, 21	Deleted the time point of 24 hour post-drug administration for VM202 DNA

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
hours post-dose at the first and second dose administration (Day 1 and Day 15). *Modified to:* Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose and 60 minutes post-dose at the first and second dose administration (Day 1 and Day 15). <u>Section 4.1.3 Pharmacokinetic Evaluations (D)</u> *Formerly:* Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose and 60 minutes **and 24 hours** post-dose at the first and second dose administration (Day 1 and Day 15). *Modified to:* Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose and 60 minutes post-dose at the first and second dose administration (Day 1 and Day 15). <u>Section 4.2.3 Schedule of Assessments Table 4-1 (C)</u> *Formerly:* [3]Blood VM202 DNA level at 1 **and 24 hours** post study agent administration *Modified to:* [4]Blood VM202 DNA level at 1 hour post study agent administration <u>Section 6.2.3 Day 2 (D)</u> *Formerly:* 6.2.3 Day 2 The following evaluations will be performed at the Day 2 visit: · VM202 DNA level at 24-hours post dose · Adverse events <u>Section 4.6 Duration (C)</u> *Formerly:* There will be a 7-day safety evaluation that will begin 30 days after the first dose of study drug (Day 30) for cohort I. The safety evaluation (adverse events) must	10, 16	concentration level because 1 hour post-drug administration and day 8 time points are sufficient data points for this assessment. Clarification of the timing of the safety evaluation by the DSMC.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
be completed prior to the beginning of the next study drug administration for the following study cohort (II, III and IV). *Modified to:* There will be safety evaluations that will begin 30 days after the first dose of study drug (Day 30) for **all** cohorts. **Safety evaluations to encompass 30 days post the first dose will be completed for each cohort and will be evaluated by the DSMC prior to dose escalation to the next cohort. This 30-day window will include a 2-week safety evaluation following each dose at Day 1 and 15**. <u>Section 6.1.1 Treatment Regimen and Follow-Up (C)</u> *Formerly:* There will be a mandatory review of all safety data between the treatments of Cohort I and Cohort II, between Cohort III and Cohort IV. No subject may be enrolled in Cohort II, Cohort III or Cohort IV until the SRC has reviewed the data and provided a written notice to continue the trial to the Sponsor. *Modified to:* There will be a mandatory review of all safety data between the treatments of **all dose cohorts (Cohort I-IV).** No subject may be enrolled in **subsequent cohorts** until the DSMC has reviewed the data and provided a written notice to continue the trial to the Sponsor.		
<u>Section 5.3 Exclusion Criteria (C)</u> *Formerly:* Subjects with a current history of malignant neoplasm except for basal cell carcinoma of the skin and squamous cell carcinoma of the skin (if excised and no evidence of recurrence *Modified to:* Subjects with a current history **or new screening finding** of malignant neoplasm except for basal cell carcinoma of the skin and squamous cell carcinoma of the skin (if excised and no evidence of recurrence	12	Clarify that subjects will be excluded if a new screening finding of malignancy is observed (requested by the FDA at the Oct. 31, 06 meeting).
<u>Section 6.2.2 Day 1 (C)</u> *Formerly:* If any of the above occurs, the subject should be	20, 34	Clarify that subjects will contact the site if

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
instructed to immediately return to the clinic or to go to the nearest Emergency Room for immediate treatment and evaluation. No further study drug administration should occur in such cases. *Modified to:* If any of the above occurs, the subject **will** be instructed to **call the clinic immediately and seek medical attention either at the clinic or to** go to the nearest Emergency Room for immediate treatment and evaluation. No further study drug administration **will occur until it is determined if this is a DLT or as directed by the treating physician.** Section 7.1.9 Dose Limiting Toxicity (DLT) (C) *Formerly:* Evidence for active tissue necrosis at the injection site within 72 hours of study drug administration *Modified to:* Evidence for active tissue necrosis at the injection site within **two weeks** of study drug administration		pain, redness or discomfort occurs between day 1 and one week (requested by the FDA at the Oct. 31, 06 meeting).
Section 6.1.1 Treatment Regimen and Follow-Up (C) *Formerly:* The location of the injections sites will be documented **photographically** and on a leg diagram. Each injection will contain a maximum of 2 ml (regardless of weight) and will be injected at each site slowly for about 20 to 40 seconds. *Modified to:* The location of the injections sites will be documented on a leg diagram **provided in the CRF**. Each injection will contain a maximum of 2 ml (regardless of weight) and will be injected at each site slowly for about 20 to 40 seconds **as described in Appendix D**. Appendix D (C) *Formerly:* Precautions in administration Inject the entire amount of the drug in about 20-30 seconds. Immediately after completion of injection, lightly press the injection site with the finger head in order to prevent reflux. Do not massage the injection site.	15, 62	Specify general rules and descriptive schematic diagram in the protocol (Appendix D) and the CRF to serve as guidance for choosing injection sites on the leg (requested by the FDA at the Oct. 31, 06 meeting).

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Modified to: **Documentation of injections sites will be indicated by placing an X on the diagram provided in the CRF and shown below.** **Test material administration** **On Day 1 and Day 15 the subject will receive test material. The study treatment will be administered as up to 8 injections. A fine needle (e.g. 27 gauge, 1") suitable for IM injections will be used; 2.0 ml will be administered per injection site (total volume of 8 or 16 ml per administration, determined by cohort assignment).** Inject the entire amount of the drug in about 20-30 seconds. Immediately after completion of injection, lightly press the injection site with the finger head in order to prevent reflux. Do not massage the injection site. **Second test material administration** **Each administration will be given at least 2 cm from the previous injection site. It is recommended that an indelible marker be used to mark the location of previous injections to ensure that repeat injections are not delivered to exact same location with the subsequent administration.** **[Substituted figure with Injection Documentation Diagram]**		
<u>Section 6.7.3 Lost to follow-up (C)</u> *Formerly:* Any subject who is treated, but who does not complete all study visits through Day 365. This includes those subjects who withdraw consent and all attempts to contact the subject are unsuccessful. *Modified to:* **A subject deemed to be lost to follow-up is any** subject who **received treatment**, but who does not complete **scheduled** study visits. This includes those subjects who withdraw consent and **refuse further study participation and** all attempts to contact the subject are **deemed** unsuccessful.	30	Clarify definition of subjects who are lost to follow-up.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Section 7.3 Pharmacodynamics (D) *Formerly:* 7.3 Pharmacodynamics Pharmacodynamics will be evaluated based on HGF antibodies, endothelial progenitor cell assay and serum HGF levels present prior to and following injections. Endothelial progenitor cell levels will be evaluated for all subjects enrolled. Levels will be obtained at Baseline and at specific time points in the study as outlined in the schedule of assessments. Serum level of anti-HGF antibodies will be evaluated for all subjects enrolled. If anti-HGF antibodies are detected, cell-based assays will be performed to assess neutralization activity of the antibodies. Serum HGF levels will be evaluated at the intervals described in the section on study drug administration. 7.4 Pharmacokinetics Pharmacokinetics will be evaluated based VM202 DNA levels obtained at Baseline, 60 minutes and 24-hours after the first series of injections, 60 minutes and 24-hours after the second series of injections and at the intervals described in the section on study drug administration. While this is not a true pharmacokinetic evaluation, it will aid in determining if there is a correlation between VM202 DNA levels and other safety and efficacy evaluations.	37	Deleted Section 7.3 Pharmacodynamics and Section 7.4 Pharmacokinetics because both sections repeat the same information that appears in previous sections, specifically, Section 4.1.3 Pharmacokinetic Evaluations and Section 4.1.4 Pharmacodynamic Evaluations. Section 9.3 Statistical Analysis provides specifics on these evaluations.
Section 8.1 Adverse Event Definition (A) *Added:* AEs will be graded according to the National Cancer Institute's Common Terminology for Adverse Events v3.0 criteria.	38	Clarify that AEs will be graded according to the National Cancer Institute's Common Terminology for Adverse Events v3.0 criteria and will be provided for the purpose of DSMC review of all safety data (including AEs) to determine if dose escalation should occur

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
		(requested by the FDA at the Oct. 31, 06 meeting).
Section 8.1.5 Documentation of AEs and SAEs (C) *Formerly:* A ViroMed SAE Report Form should be used for each SAE. If at the time of initial reporting, multiple SAEs are present whether or not they are temporally and/or clinically related, they must be reported on separate ViroMed SAE report forms. Following initial reporting of the event, the Investigator should make every effort to establish a diagnosis of the event based on the presenting signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms. *Modified to:* A **separate** SAE **CRF will** be used for **all SAE's. For SAEs which are related and within a specific time period, a single form can be used. An assessment of the relationship of each event to the study therapy will be added for each SAE on the form.** Following initial reporting of the event, the Investigator **will** make every effort to establish a diagnosis of the event based on the presenting signs, symptoms, and/or other clinical information. In such cases, the diagnosis **will** be documented as the AE and/or SAE and not the individual signs/symptoms.	41	Clarify that an assessment of the relationship of each AE to the study therapy will be recorded and provided for the purpose of DSMC review of all safety data (including AEs) to determine if dose escalation should occur (requested by the FDA at the Oct. 31, 06 meeting).
Section 8.1.7 Immediate Reporting of SAEs (C) *Formerly:* Type of SAE - Fatal/Life Threatening Timeframe - 24 hours and 48 hours Documents - 24 hours: Telephone notification 48 hours: Fully completed initial ViroMed SAE Report Form and CRF Documentation Any available diagnostic test results *Modified to:* Type of SAE - Fatal/Life Threatening **and all other SAEs** Timeframe - 24 hour Documents - 24 hours: **Completed initial SAE**	43	Correct Table 8-1.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Report Form with minimum requirements (subject #, date of birth, event and start date)		
Section 9.0 Statistics (D) *Deleted:* The SAP will be completed and finalized prior to breaking the blind.	45	Delete reference to a blinded study.
Section 9.2 Population (C) *Formerly:* This study will have only an Intent-to-Treat population for the purposes of all statistical analyses. This population will include all subjects that signed the Informed Consent and received the test dose of study medication. *Modified to:* **Statistical analysis will be based on** an Intent-to-Treat **design, but subjects must receive at least one dose of study drug and have at least one post-dose assessment**.	45	Clarify definition of Intent-to-Treat in regard to statistical analysis.
Section 9.3 Statistical Analysis (C) *Formerly:* Retinal fundoscopy changes will be listed by frequency and treatment group only. *Modified to:* Retinal fundoscopy **results per** treatment group **will be reported as change from baseline and subsequent evaluation at day 365.**	45	Clarify statistical analysis of retinal fundoscopy.
Version 3.0 Modifications		
Protocol Synopsis (C) *Formerly:* If a DLT occurs in 2/6 subjects, then 3 additional subjects will be enrolled at the previous dose level. If no DLTs occur at this level it will be considered the MTD. *Modified to:* If a DLT occurs in 2/6 subjects, then **the preceding dose** level will be considered the MTD. Section 4.5 Treatments (C) *Formerly:*	xii, 11, 18, 35	Corrections overlooked in version 2 amendment of the protocol for the clarification of the standard 3+3 Phase 1 trial design (requested by the FDA at the Jan. 06 Pre-IND meeting).

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
If a DLT occurs in 2/6 subjects then 3 additional subjects will be treated at the previous dose level and if no DLT occurs in that cohort, it will be considered the MTD. *Modified to:* If a DLT occurs in 2/6 subjects, then **the preceding dose** level will be considered the MTD. <u>Section 6.1.1 Treatment Regimen and Follow-Up (D)</u> *Formerly:* If 2/6 subjects experience a DLT then the preceding dose level **treatment group** will be considered the MTD. *Modified to:* If 2/6 subjects experience a DLT then the preceding dose level will be considered the MTD. <u>Section 7.1.9 Dose Limiting Toxicity (DLT) (C)</u> *Formerly:* If a DLT occurs in 2/6 patients, then 3 additional subjects will be enrolled at the previous dose level. If no DLTs occur at this level it will be considered the MTD. *Modified to:* If a DLT occurs in 2/6 patients, then **the preceding dose level** will be considered the MTD.		
Version 4.0 Modifications <u>Signature Page (C)</u> ***Formerly:*** <u>Version Number: 3.0</u> <u>*Modified to:* Amended Version Number: 4.0</u>	ii	Changed amended version number
<u>Protocol Synopsis (Study Design) (C)</u> *Formerly:* Preliminary efficacy (hemodynamic assessments), safety and tolerability will be evaluated at Baseline (screening) and at designated time points **after administration of VM202**. *Modified to:* Preliminary efficacy (hemodynamic assessments), safety and tolerability will be evaluated at Baseline (screening) and at designated time points	xii,	Clarify time points of assessment.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
throughout the study. <u>Protocol Synopsis (Study Assessments) (A and C)</u> *Formerly:* The following assessments will be performed at screening and at **subsequent follow-up visits for one year**. *Modified to:* The following assessments will be performed at screening and **/ or** at **designated time points throughout the study:** • **Informed Consent** • **Physical Exam** • **Vital Signs** • **Pregnancy Test** • **Photograph and measurement of ulcer** • **Mammogram** • **Collection of concomitant medications** • **Collection of medical history** • **Assessment of CLI** • **Collection of adverse events**	xii	Clarify time points of assessments and add all study assessments in trial.
<u>Protocol Synopsis (Rationale) (A)</u> *Formerly:* CA-125 will be done in females and a PSA will be done in males to rule out any occult malignancy prior to first study drug administration. *Modified to:* CA-125 will be done in females and a PSA will be done in males to rule out any occult malignancy prior to first study drug administration **and throughout the study**.	xiii	Add to clarify time points of assessment.
<u>Protocol Synopsis (Protocol Synopsis and Section 4.6 Duration) (A)</u> Formerly: 19 months *Modified to:* 19 months **(Not to include the 4 year follow-up)**	xi, 11	Add to clarify duration.
<u>Protocol Synopsis (Protocol Synopsis)</u> (A) Formerly: Approximately 12 subjects *Modified to:* Approximately 12**-15** subjects	xii	Modify language to provided consistency.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Protocol Synopsis (Procedures) (C) *Formerly:* There will be 4 or 8 injections depending on the dose level administered slowly for 20~40 seconds at pre-determined sites on only one affected leg (mainly at the calf muscles) without local anesthesia. *Modified to:* There will be 4 or 8 injections depending on the dose level administered slowly for 20~**30** seconds at pre-determined sites on only one affected leg (mainly at the calf muscles) without local anesthesia.	xiv	Modify language per investigators brochure.
Protocol Synopsis (Procedures) (C) *Formerly:* Analgesic usage and **safety labs** will be assessed on Day 8 post first dose treatment. *Modified to:* Analgesic usage and **Serum HGF** will be assessed on Day 8 post first dose treatment.	xiv	Modify assessments to match study procedures chart.
Protocol Synopsis (Safety Monitoring) (C) *Formerly:* Safety laboratory analyses will be completed at screening and at **Day 28, 59, 91, 180 and 365**. A follow-up phone calls will be made at Day 365 in all subjects. **An additional** follow-up phone call will be made annually for up to five years after the first dose administration. *Modified to:* Safety laboratory analyses will be completed at screening and at **specified time points throughout the study**. Follow-up phone **calls** will be made **semi-**annually for up to 5 years after the first dose administration.	xiv-xv	Clarify time points of assessment.
Protocol Synopsis (Safety Monitoring) (C) *Formerly:* Physical examination, vital signs and retinal fundoscopy will also be performed as part of safety monitoring at screening and at **Day 91, 180 and 365 (retinal exam at screening and Day 365)**. *Modified to:* Physical examination, vital signs and retinal fundoscopy will also be performed as part of safety monitoring at screening and at **specified time points throughout the study**.	xv	Clarify time points of assessment.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Protocol Synopsis (Efficacy Evaluation) (C) *Formerly:* Pharmacodynamic evaluation will be based on anti-HGF antibodies, **endothelial progenitor cell assay** and serum HGF levels post treatment. *Modified to*: Pharmacodynamic evaluation will be based on anti-HGF antibodies and serum HGF levels **pre treatment and endothelial progenitor cell assay** levels post treatment.	xv	Clarify language to match study procedures chart.
Protocol Synopsis (Statistical Analysis) (C) *Formerly:* Pharmacodynamics will assess change from **Baseline** in anti-HGF antibodies, **endothelial progenitor cell assay** and serum HGF levels post treatment between all 4 doses. *Modified to:* Pharmacodynamics will assess change from **Day 1** in anti-HGF antibodies and serum HGF levels **at pre treatment and** endothelial progenitor cell assay post treatment between all 4 dose levels.	xvi	Modify assessment time point to match study procedures chart and clarify language.
Study Objectives (Secondary Objectives) (C) *Formerly:* To evaluate the immune response to HGF in subjects with CLI as measured by the change in anti-HGF antibodies from **Baseline**. *Modified to:* To evaluate the immune response to HGF in subjects with CLI as measured by the change in anti-HGF antibodies from **Day 1**.	5	Modify assessment time point to match study procedures chart.
Study Objectives (Secondary Objectives) (C) *Formerly:* To evaluate the ability of VM202 to stimulate endothelial progenitor cells as measured by the change in endothelial cell progenitor assay levels from **Day 1** to Day 59. *Modified to:* To evaluate the ability of VM202 to stimulate endothelial progenitor cells as measured by the change in endothelial cell progenitor assay levels from **screening** to Day 59.	5	Modify assessment time point to match study procedures chart.
Section 4.1.2 Secondary Efficacy Endpoints (A)	6	Add assessment

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Formerly: Change from Baseline in hemodynamic measurements (Appendix B: ABI, TBI, and Wave Form Analysis) and $TcPO_2$ at Days 15, 28, 59, 91**, and 180**. *Modified to:* Change from Baseline in hemodynamic measurements (Appendix B: ABI, TBI, and Wave Form Analysis) and $TcPO_2$ at Days 15, 28, 59, 91, 180 **and 365**.		time point to match study procedures chart.
<u>Section 4.1.4 Pharmacodynamic Evaluations (C)</u> *Formerly:* Pharmacodynamics will be assessed as the change from **Baseline** in serum HGF level at Days 8, 15, 21, 28 and 59. Potential immune response to HGF will be assessed as change from **Baseline** in level of neutralizing antibodies (anti-HGF antibodies) at Days 15, 28, 59 and 180. *Modified to:* Pharmacodynamics will be assessed as the change from **Day 1** in serum HGF level at Days 8, 15, 21, 28 and 59. Potential immune response to HGF will be assessed as change from **Day 1** in level of neutralizing antibodies (anti-HGF antibodies) at Days 15, 28, 59 and 180.	7	Modify assessment time point to match study procedures chart.
<u>Section 4.4 Dosing Cohort Assignment (C)</u> *Formerly:* A **sequential** 3-digit subject number will be assigned to each study subject **(**beginning with 101 **for Cohort I, 201 for Cohort II, 301 for Cohort III and 401 for Cohort IV)** when the informed consent is signed and **eligibility is confirmed. All patients will be assigned to each cohort sequentially beginning with Cohort I until the cohort group is enrolled. Cohorts II, III and IV will follow in the same manner. If the subject is withdrawn from the study prior to receiving the first dose of study drug, that subject will be replaced.** *Modified to:* A 3-digit subject number will be **sequentially** assigned to each study subject beginning with 101 when the informed consent is signed and **any screening procedures are performed**. **Separate screening numbers will not be assigned. The subject will retain the original subject number**	9	Clarify to match database assignment.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
assigned throughout the trial. If the subject is withdrawn from the study prior to receiving the first dose of study drug, that subject will be replaced but the subject number will not be reused.		
Section 4.5 Treatments (C) *Formerly:* **There will be four doses administered as follows:** *Modified to:* **For each of the 4 cohorts a different dose level will be administered as follows:**	9	Clarify language.
Section 5.1 Cancer Screening (A) *Added:* 7. **CA-125 –will be performed at screening (females only)**	11	Add to match study procedures chart.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	Affected Pages	Reason(s) for the Modification(s)
Section 4.1 Study Procedures Chart (C) *Formerly:*	8	Inconsistencies within the protocol.

	Screen / Baseline	Treatment Period										
		First Dose		1st Follow-Up	Second Dose		2nd Follow-Up					
	Day -30 to Day 1	Day 1 pre-dose	Day 1 post-dose	Day 8	Day 15 Pre dose	Day15 Post-dose	Day 21	Day 28	Day 59	Day 91	Day 180	Day 365
Consent Form	X											
Medical History	X											
Chest X-ray or CT scan of chest[1]	X											X
Physical Exam	X				X			X	X	X	X	X
Vital Signs (HR, BP, Respiratory)	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Fundoscopy	X											X
Pregnancy Test	X											
Photograph and measurement of ulcer	X	X			X			X	X	X	X	X
Clinical Lab Tests	X	X			X	X		X	X	X	X	X
Tumor markers[2]	X				X				X		X	X
Infection tests[3]	X								X		X	X
Serum HGF	X	X		X	X		X	X	X			
anti-HGF antibodies	X	X			X			X	X		X	
Blood VM202 DNA	X	X	X[4]	X	X	X[4]	X	X	X			
12-lead ECG	X											X
High Resolution MRA	X									X	X	
Hemodynamic Assessment (ABI, TBI, Wave Form)	X	X			X			X	X	X	X	X
Pain VAS	X	X			X			X	X	X	X	X
Subject Analgesic use	X	X		X	X	X	X	X	X	X	X	X
$TcPO_2$	X	X			X			X	X	X	X	X
Injection site reaction assessment			X	X		X	X	X	X			X
Mammogram[5]	X											X
Endothelial Progenitor Cell Assay	X	X			X			X	X		X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X
Telephone Follow-up[6]												

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	Affected Pages	Reason(s) for the Modification(s)

1 CT scan of the chest instead of chest X-ray will be performed if previous tobacco use
2 Tumor markers include: AFP, CEA, PSA, CA19-9 & CA125
3 Infection tests include: HIV, Hepatitis B, HCV, HTLV, CMV, VDRL
4 Blood VM202 DNA level at 1 hour post study agent administration
5 If not performed in last 12 months prior to start of study
6 Annual telephone follow-up contacts will continue for 4 years after day 365

Modified to:

	Screen / Baseline	Treatment Period													
		First Dose		1st Follow-Up	Second Dose		2nd Follow-Up[6]								
	Day -30 to Day 1	Day 1 pre-dose	Day 1 post-dose	Day 8 (+/- 2days)	Day 15 Pre dose (+/- 3 days)	Day15 Post-dose	Day 16	Day 21 (+/- 3 days)	Day 28 (+/- 3 days)	Day 59 (+/- 7 days)	Day 91 (+/- 7 days)	Day 180 (+/- 10 days)	Day 365 (+/- 10 days)	Unscheduled Visit	Early Termination
Consent Form	X														
Confirm Eligibility		X													
Medical History	X														
Chest X-ray or CT scan of chest[1]	X												X		X
Physical Exam	X				X				X	X	X	X	X	X	X
Vital Signs (HR, BP, Respiratory)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Fundoscopy	X												X		X
Pregnancy Test	X				X [7]										
Photograph and measurement of ulcer	X	X			X				X	X	X	X	X		X
Clinical Lab Tests	X	X			X		X		X	X	X	X	X	X	X
Tumor markers[2]	X				X					X		X	X		
Infection tests[3]	X									X		X	X		X
Serum HGF		X		X	X			X	X	X				X	X
anti-HGF antibodies		X			X				X	X		X		X	X
Blood VM202 DNA		X	X[4]	X	X	X[4]	X	X	X	X				X	X
12-lead ECG	X														
High Resolution MRA	X										X	X			X

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	Affected Pages	Reason(s) for the Modification(s)

Hemodynamic Assessment (ABI, TBI, Wave Form)	X	X			X				X	X	X	X	X	X	X
Pain VAS	X	X			X				X	X	X	X	X	X	X
Subject Analgesic use	X	X		X	X		X	X	X	X	X	X	X	X	
$TcPO_2$	X	X			X				X	X	X	X	X	X	X
Injection site reaction assessment			X	X		X	X	X	X	X					
Mammogram[5]	X														
Endothelial Progenitor Cell Assay	X		X			X			X	X		X	X	X	X
Concomitant Medications	X	X		X	X		X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 CT scan of the chest instead of chest X-ray will be performed if previous tobacco use
2 Tumor markers include: AFP, CEA,CA19-9 PSA (males) & CA125 (females)
3 Infection tests include: HIV, Hepatitis B, HCV, HTLV, CMV, VDRL5
4 Blood VM202 DNA level at 1 hour post study agent administration
5 If not performed in last 12 months prior to start of study
6 Telephone follow-up contacts will continue for 4 years after day 365
7 A serum test will be done at screening and a urine test on all other days

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Section 6.1.1 Treatment Regimen and Follow-Up (C) *Formerly:* Each injection will contain a maximum of 2 ml (regardless of weight) and will be injected at each site slowly for about 20 to **40** seconds as described in Appendix D. *Modified to:* Each injection will contain a maximum of 2 ml (regardless of weight) and will be injected at each site slowly for about 20 to **30** seconds as described in Appendix D.	15	Modify language per investigators brochure.
Section 6.1.2 Study Agent Handling and Accountability (C) *Formerly:* The vials will be labeled as required by all applicable regulations. The vials will be stored at **4**ºC. *Modified to:* The vials will be labeled as required by all applicable regulations. The vials will be stored at **2-8**ºC.	17	Modify language per investigators brochure.
Section 6.2.1 Screening Visit (Day -30 to Day 1) (C) *Formerly:* **Viral Tests** *Modified to:* **Infection tests**	18, 23, 24, 26	Clarify term throughout protocol language to match study procedures chart.
Section 6.2.1 Screening Visit (Day -30 to Day 1) (A) *Added:* CA-125 (for females) Endothelial progenitor cell assay	18	Add to match study procedures chart.
Section 6.2.2 Day 1 (C and D) *Formerly:* The following evaluations will be performed prior to study drug administration: **Viral Tests** **Endothelial progenitor cell assay** *Modified to:* Following study drug administration, the following evaluations will be performed: **Endothelial progenitor cell assay**	19	Clarify sentence and delete to match study procedures chart.
Section 6.2.4 Day 15 (± 3 days) Second Study Dose Administration (A and D) *Formerly:* Prior to the administration of the study agent, the following will be performed:	20	Delete to match study procedures chart and add pregnancy test per

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Endothelial progenitor cell assay *Modified to:* Prior to the administration of the study agent, the following will be performed: **Urine Pregnancy test (females of childbearing potential)**		inclusion criteria.
Section 6.2.4 Day 15 (± 3 days) Second Study Dose Administration (A) *Added:* Following study drug administration, the following evaluation will be performed: **VM202 DNA level** **Endothelial progenitor cell assay**	21	Add to match study procedures chart.
Section 6.2.1.2 Long-term Follow-Up (C) *Formerly:* If living-hospitalizations **in the last six months** *Modified to:* If living-hospitalizations **since the previous contact**	25	Modify language to capture all data.
Section 6.2.1.2 Long-term Follow-Up (A) *Formerly:* Any subject who received VM202 will be asked to provide contact information and will be contacted annually by mail or telephone by **ViroMed**. Subjects will be informed of this follow-up requirement of the study during the informed consent process. Written instructions will be provided to each subject at the final visit that describe how to contact **ViroMed** if they experience and adverse event that could be related to participation in the study. Subjects will be asked to notify **ViroMed** should their contact information change. *Modified to:* Any subject who received VM202 will be asked to provide contact information and will be contacted annually by mail or telephone by ViroMed **or a ViroMed representative**. Subjects will be informed of this follow-up requirement of the study during the informed consent process. Written instructions will be provided to each subject at the final visit that describe how to contact ViroMed **or a ViroMed representative** if they experience and adverse event that could be related to participation in the study. Subjects will be asked to notify **ViroMed or**	25	Add to allow representative to conduct follow-up contact.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
a ViroMed representative should their contact information change.		
Section 6.2.1.2 Long-term Follow-Up (A) *Formerly:* Questionnaires will be developed for the annual contacts for **two** years after Day 365 **and annual contacts** that will elicit updates on any cancer, neurological, autoimmune, or hematological disorder that has appeared or changed since the last contact. *Modified to:* Questionnaires will be developed for the **semi-**annual contacts for **four** years after Day 365 that will elicit updates on any cancer, neurological, autoimmune, or hematological disorder that has appeared or changed since the last contact. Information will also be collected on any unexpected health problems including hospitalizations or new medications.	25	Add to clarify assessment time points per Sponsor request.
Section 6.2.1.4 Early Termination Visits (A) *Formerly*: Subjects who have had at least one dose of test material will return to the study site for specific safety and efficacy assessment visits for the total study period, unless the subject withdraws study consent and refuses to participate. ***Modified to:*** Subjects who have had at least one dose of test material will return to the study site for specific safety and efficacy assessment visits for the total study period **(through Day 365**), unless the subject withdraws study consent and refuses to participate.	26	Add to clarify time point
Section 6.2.1.4 Early Termination Visits (D) *Deleted:* **12 Lead ECG**	27	Delete to match study procedures chart
Section 6.4.4 Peripheral Vascular Procedures (C) *Formerly:* This information is required by the Sponsor. *Modified to:* This information is required by the Sponsor **and will be added to the SAE CRF**. *Deleted:* • **Diagnostic findings (e.g., angiographic,**	28	Modify to clarify how Peripheral Vascular Procedures will be captured as an SAE.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
segmental BP tests). • **Physical findings at time of procedure** ▪ **Infection (e.g., cellulitis, sepsis, osteomyelitis) that was refractory to antibiotics and/or had not responded to more conservative treatments (e.g., drainage, debridement)** ▪ **tissue loss due to necrosis** ▪ **uncontrollable rest pain that did not respond to more conservative standards of treatment (e.g., opiate analgesics).**		
<u>Section 7.2.2 High Resolution MRA (D)</u> *Formerly:* At each assessment, quantitative measures of blood flow in the target occluded artery(s) will be determined. In addition, volumetric analysis of newly developed vessels will also be described. *Deleted:* **At each assessment, quantitative measures of blood flow in the target occluded artery(s) will be determined. In addition, volumetric analysis of newly developed vessels will also be described.**	35	Deleted to match CRF.
<u>Section 8.5 Documentation of AEs and SAEs (C)</u> *Formerly:* 760-268-65**32** *Modified to:* 760-268-65**50**	39	Modify to correct telephone number.
<u>Appendix B (A)</u> *Formerly:* Repeat for the other leg. *Modify to:* Repeat for the other leg **(At the screening visit only. The index leg will be used for future measurements).**	56	Add to clarify procedure.
<u>Appendix C (C)</u> *Formerly:* The **measurement is** recorded **from 00.0 to 10.0 onto the CRF.** *Modified to:* The **CRF will be faxed into DataFax and the measurement will be electronically** recorded **in the database.**	58	Modify to match the CRF.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Version 5.0 Modifications Protocol Title (C) *Formerly:* A Phase I, Dose-Escalation**, Single Center** Study to Assess the Safety and Tolerability of VM202 in Subjects with Critical Limb Ischemia *Modified to:* A Phase I, Dose-Escalation Study to Assess the Safety and Tolerability of VM202 in Subjects with Critical Limb Ischemia	i, ii	Changed to allow the option to use multi -centers to increase patient enrollment.
Signature Page (C) *Formerly:* Version Number: 4.0 Date: **September 13**, 2007 *Modified to:* Amended Version Number: 5.0 Date: **November 29**, 2007	ii	Changed amended version number and date.
Protocol Synopsis: Study Design (C) *Formerly:* After **all subjects in the first dose cohort have completed the 30-day (± 2 days) follow-up visit following the 1st dose of study drug,** an interim safety evaluation will be performed with the submission of safety data to the Data Safety Monitoring Committee (DSMC). If the DSMC recommends continuing the study, the **second** dose cohort will be treated. *Modified to:* After **the first subject in each cohort completes day 30 (± 2 days), subsequent to day 15 dosing of the other 2 subjects in the same cohort,** an interim safety evaluation will be performed with the submission of safety data to the Data Safety Monitoring Committee (DSMC). If the DSMC recommends continuing the study, the **next** dose cohort will be treated.	xii	The cumulative safety of VM202 demonstrated in the first 2 cohorts allows advancement to future cohorts in a prudent and expeditious timeframe.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Protocol Synopsis: Procedures (C) *Formerly:* After **all** subject in each dose cohort have completed the Day -30 (+ 2 days) follow-up visit, an interim safety evaluation will be performed with the submission of data to the DSMC. *Modified to:* After **the first** subject in each dose cohort have completed the Day -30 (+ 2 day) follow-up visit **and subsequent to day 15 dosing of the other subjects in the same cohort** an interim safety evaluation will be performed with the submission of data to the DSMC.	xiv	The cumulative safety of VM202 demonstrated in the first 2 cohorts allows advancement to future cohorts in a prudent and expeditious timeframe.
Sample Size and Power (C) *Formerly:* This is a Phase I, Dose-Escalation**, Single Center** Study. *Modified to:* This is a Phase I, Dose-Escalation Study.	xv	Changed to allow the option to use multi -centers to increase patient enrollment.
Protocol Synopsis: Pharmacokinetics (C) *Formerly:* **Pharmacokinetic evaluation will determine AUC_{0-8}, C_{max}, T_{max}, $T_{1/2}$ for all 4-dose levels of VM202 DNA** *Modified to:* **Descriptive statistics (N, mean, median, SD, minimum and maxium values, where applicable) of VM202 DNA levels will be reported.**	xv	VM202 disappears from the body 4 hours after injection. Analysis can not be done.
Section 3.2 Secondary Objectives (C) *Formerly:* To evaluate the change in VM202 DNA levels from Day 1 to Day **59**. *Modify to:* To evaluate the change in VM202 DNA levels from Day 1 to Day **21**.	5	Changed to account for eliminated laboratory test
Section 4.1.3 Pharmacokinetic Evaluations (D) *Formerly:* Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose and 60 minutes post-dose at the first **and second** dose administration (Day 1 **and Day 15**). In addition, levels will be measured at Days **8**, 21, **28** and 59.	6	Deleted laboratory test

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Modify to: Pharmacokinetics of VM202 will be assessed using blood VM202 DNA concentration levels from samples obtained pre-dose at the first dose administration (Day 1). In addition, levels will be measured at Days 21 and 59 **(if DNA is detected at day 21).**		
Section 4.1.4 Pharmacodynamic Evaluations (D) *Formerly:* Pharmacodynamics will be assessed as the change from Day 1 in serum HGF level at Days 8, 15, 21**, 28** and 59. *Modify to:* Pharmacodynamics will be assessed as the change from Day 1 in serum HGF level at Days 8, 15, 21 and 59.	6	Deleted laboratory test
4.2.3 Number of Study Subjects (C) *Formerly:* There will 12-15 subjects **selected at one site** for participation in this study with 3 subjects treated in each of four (4) study cohorts. *Modify to:* There will 12-15 subjects selected for participation in this study with 3 subjects treated in each of four (4) study cohorts.	7	Changed to allow the option to use multi -centers to increase patient enrollment.

Type of Modification to Protocol (A: Added, C: Changed, D: Deleted)	**Affected Pages**	**Reason(s) for the Modification(s)**
Section 4.1 Study Procedures Chart (C) *Formerly:*	8	Deleted laboratory tests

	Screen / Baseline	**Treatment Period**													
		First Dose		**1st Follow-Up**	**Second Dose**		**2nd Follow-Up[6]**								
	Day -30 to Day 1	**Day 1 pre-dose**	**Day 1 post-dose**	**Day 8 (+/- 2days)**	**Day 15 Pre dose (+/- 3 days)**	**Day15 Post-dose**	**Day 16**	**Day 21 (+/- 3 days)**	**Day 28 (+/- 3 days)**	**Day 59 (+/- 7 days)**	**Day 91 (+/- 7 days)**	**Day 180 (+/- 10 days)**	**Day 365 (+/- 10 days)**	**Unscheduled Visit**	**Early Termination**
Consent Form	X														
Confirm Eligibility		X													
Medical History	X														
Chest X-ray or CT scan of chest[1]	X												X		X
Physical Exam	X				X				X	X	X	X	X	X	X
Vital Signs (HR, BP, Respiratory)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Fundoscopy	X												X		X
Pregnancy Test	X				X [7]										
Photograph and measurement of ulcer	X	X			X				X	X	X	X	X		X
Clinical Lab Tests	X	X			X		X		X	X	X	X	X	X	X
Tumor markers[2]	X				X					X		X	X		
Infection tests[3]	X									X		X	X		X
Serum HGF		X		X	X			X	X	X				X	X
anti-HGF antibodies		X			X				X	X		X		X	X
Blood VM202 DNA		X	X[4]	X	X	X[4]	X	X	X	X				X	X
12-lead ECG	X														
High Resolution MRA	X										X	X			X
Hemodynamic Assessment (ABI, TBI, Wave Form)	X	X			X				X	X	X	X	X	X	X

Pain VAS	X	X			X				X	X	X	X	X	X	X
Subject Analgesic use	X	X		X	X		X	X	X	X	X	X	X	X	
$TcPO_2$	X	X			X				X	X	X	X	X	X	X
Injection site reaction assessment			X	X		X	X	X	X	X					
Mammogram[5]	X														
Endothelial Progenitor Cell Assay	X		X			X			X	X		X	X	X	X
Concomitant Medications	X	X		X	X		X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 CT scan of the chest instead of chest X-ray will be performed if previous tobacco use
2 Tumor markers include: AFP, CEA,CA19-9 PSA (males) & CA125 (females)
3 Infection tests include: HIV, Hepatitis B, HCV, HTLV, CMV, VDRL5
4 Blood VM202 DNA level at 1 hour post study agent administration
5 If not performed in last 12 months prior to start of study
6 Telephone follow-up contacts will continue for 4 years after day 365
7 A serum test will be done at screening and a urine test on all other days

Modified to:

Table 4-1 Study Procedures Chart	**Screen / Baseline**	**Treatment Period**													
		First Dose		**1st Follow-Up**	**Second Dose**		**2nd Follow-Up[6]**								
	Day -30 to Day 1	**Day 1 pre-dose**	**Day 1 post-dose**	**Day 8 (+/- 2days)**	**Day 15 Pre dose (+/- 3 days)**	**Day15 Post-dose**	**Day 16**	**Day 21 (+/- 3 days)**	**Day 28 (+/- 3 days)**	**Day 59 (+/- 7 days)**	**Day 91 (+/- 7 days)**	**Day 180 (+/- 10 days)**	**Day 365[5] (+/- 10 days)**	**Unscheduled Visit**	**Early Termination**
Consent Form	X														
Confirm Eligibility		X													
Medical History	X														
Chest X-ray or CT scan of chest[1]	X												X		X
Physical Exam	X				X				X	X	X	X	X	X	X
Vital Signs (HR, BP, Respiratory)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Fundoscopy	X												X		X
Pregnancy Test	X				X[6]										
Photograph and measurement of ulcer	X	X			X				X	X	X	X	X		X
Clinical Lab Tests	X	X			X		X		X	X	X	X	X	X	X
Tumor markers[2]	X				X					X		X	X		
Infection tests[3]	X									X		X	X		X
Serum HGF		X		X	X			X		X					

anti-HGF antibodies		X			X					X		X		X	X	
Blood VM202 DNA		X						X		X[7]						
12-lead ECG	X															
High Resolution MRA	X										X	X			X	
Hemodynamic Assessment (ABI, TBI, Wave Form)	X	X			X				X	X	X	X	X	X	X	
Pain VAS	X	X			X				X	X	X	X	X	X	X	
Subject Analgesic use	X	X		X	X		X	X	X	X	X	X	X	X		
$TcPO_2$	X	X			X				X	X	X	X	X	X	X	
Injection site reaction assessment			X	X		X	X	X	X	X						
Mammogram[4]	X															
Endothelial Progenitor Cell Assay	X		X			X			X	X		X	X	X	X	
Concomitant Medications	X	X		X	X		X	X	X	X	X	X	X	X	X	
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	

1 CT scan of the chest instead of chest X-ray will be performed if previous tobacco use
2 Tumor markers include: AFP, CEA,CA19-9 PSA (males) & CA125 (females)
3 Infection tests include: HIV, Hepatitis B, HCV, HTLV, CMV, VDRL5
4 If not performed in last 12 months prior to start of study
5 Telephone follow-up contacts will continue for 4 years after day 365
6 A serum test will be done at screening and a urine test on all other days
7 Complete draw if detected at Day 21

4.6 Duration (C) *Formerly:* Safety evaluations to encompass 30 days post the first dose will be completed for each cohort and will be evaluated by the DSMC prior to dose escalation to the next cohort. *Modify to:* Safety evaluations to encompass 30 days post the first dose **of the first subject and subsequent to day 15 dosing of the other subjects in the same cohort will be completed for each cohort and** will be evaluated by the DSMC prior to dose escalation to the next cohort.	10	The cumulative safety of VM202 demonstrated in the first 2 cohorts allows advancement to future cohorts in a prudent and expeditious timeframe.
5.4 Inclusion Criteria (C) *Formerly:* Male or female, between 20 and 85 years of age *Modified to:* Male or female, between 20 and **90** years of age	11	Changed based on safety data from the first two cohorts. There are no safety concerns with raising the age limit to 90 years of age.
6.2.2 Day 1 (D) *Delete:* VM202 DNA levels at 60 minutes post dose	19	Deleted laboratory test
6.2.3 Day 8 (± 2 days) (D) *Delete:* VM202 DNA levels	20	Deleted laboratory test
6.2.4 Day 15 (± 3 days) Second Study Dose Administration (D) *Delete:* VM202 DNA levels	20	Deleted laboratory test
6.2.4 Day 15 (± 3 days) Second Study Dose Administration (D) *Delete:* VM202 DNA levels at 60 minutes post dose *Delete:* VM202 DNA levels	20	Deleted laboratory test
6.2.5 Day 16 (post 2nd dosing) Administration (D) *Delete:* VM202 DNA levels at 24 hours post dose	21	Deleted laboratory test
6.2.7 Day 28 (± 3 days) (D) *Delete:* Serum HGF *Delete:* VM202 DNA levels *Delete:* Anti-HGF Antibodies	22	Deleted laboratory test
6.2.8 Day 59 (± 7 days) (C)	22	Change due to

Formerly: VM202 DNA levels *Modified to:* VM202 DNA levels **(If DNA is detected at Day 21)**		deleted laboratory tests on Day 21
6.2.13 Unscheduled Visits (D) *Delete:* Serum HGF levels *Delete:* VM202 levels (if visit is before Day 59)	25	Deleted laboratory test
6.2.14 Early Termination Visits (D) *Delete:* Serum HGF levels Delete: VM202 levels	26	Deleted laboratory test
9.4 Data Safety Monitoring Committee (C) *Formerly:* A Data Safety Monitoring Committee will review all safety data **after each cohort of 3 subjects has completed the 30-day follow-up period after the first dose of study drug has been administered**. *Modified to:* A Data Safety Monitoring Committee will review all safety data **available 30 days after the first subject in each cohort is dosed and subsequent to day 15 dosing of the other 2 subjects in the same cohort**.	43	The cumulative safety of VM202 demonstrated in the first 2 cohorts allows advancement to future cohorts in a prudent and expeditious timeframe.
Version 6.0 Modifications		
Signature Page (C) *Formerly:* Version Number: 5.0 Date: **November 29**, 2007 *Modified to:* Amended Version Number: **6.0** Date: **August 31**, 2009	ii	Changed amended version number and date.
Global Protocol Change - Study Duration *Formerly:* 19 month plus **4** year follow-up after Day 365	xi, xii,xiv,8, 12, 26	Changed due to no indication of long term persistence of plasmid in non-target tissues and no evidence of off-target angiogenesis in any of our preclinical and clinical studies of VM202 to date.
Modified to: **30 months**	xi	This is the actual

6.2.12 Long Term Follow-Up *Add:* **Reason for hospitalizations, if applicable**	27	study duration Added to clarify hospitalization question on CRF.

Reference

[1] Morishita R, Sakaki M, Yamamoto K, et al. Impairment of collateral formation in lipoprotein (a) transgenic mice: therapeutic angiogenesis induced by human hepatocyte growth factor gene. Circulation 2002;105(12): 1491-6.

[2] Taniyama Y, Tachibana K, Hiraoka K, et al. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Therapy 2002;9(6):372-80.

[3] Taniyama Y, Morishita R, Hiraoka K, et al. Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes. Circulation 2001;104(19):2344-50.

[4] Aoki M, Morishita R, Taniyama Y, et al. Angiogenesis induced by hepatocyte growth factor in non-infarcted myocardium and infarcted myocardium: up-regulation of essential transcription factor of angiogenesis. Gene Therapy 2000;7(5):417-427.

[5] Matsuki A, Yamamoto S, Nakagami H, et al. No influence of tumor growth by intramuscular injection of hepatocyte growth factor plasmid DNA: safety evaluation of therapeutic angiogenesis gene therapy in mice. Biochemical and Biophysical Research Communications 2004;315(1):59-65.

[6] Taniyama Y, Morishita R, Aoki M, et al. Angiogenesis and anti-fibrotic action by hepatocyte growth factor in cardiomyopathy. Hypertension 2002;40(1):47-53.

[7] Morishita R, Aoki M, Hashiya N, et al. Safety evaluation of clinical gene therapy using hepatocyte growth factor to treat peripheral arterial disease. Hypertension 2004;44(2):203-9.

[8] Kim HJ, Jang SY, Park JI,et al. Vascular endothelial growth factor-induced angiogenic gene therapy in patients with peripheral artery disease. Experimental and Molecular Medicine 2004;36(4):366-44.

[9] Xin X, Yang S, Ingle G, et al. Hepatocyte growth factor enhances vascular endothelial growth factor-induced angiogenesis in vitro and in vivo. American Journal of Pathology 2001;158(3):1111-20.

[10] Ware JE, Sherbourne CD: The MOS 36-item short-form health survey (SF-36): 1. conceptual framework and item selection. Med Care 1992;30(6):473-483.

[11.] Morishita R. Recent progress in gene therapy for cardiovascular disease. Circulation J 2002;66(12):1077-86.