PROTOCOL TITLE: **H**epatocyte Growth Factor to **I**mprove Functioning in **PAD**: The **HI-PAD** Study

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VERSION NUMBER: Version #19

VERSION DATE: 12/12/2022

OBJECTIVES & BACKGROUND:

Lower extremity peripheral artery disease (PAD) affects 10-15% of men and women age 65 and older and 15 to 20% of men and women age 75 and older (1-3). Older people with PAD have greater functional impairment, faster functional decline, and higher rates of mobility loss than people without PAD (1,4-10). Ischemia-reperfusion injury of calf skeletal muscle in PAD is associated with increased calf muscle oxidative stress and calf muscle fiber damage (11-15). We recently demonstrated impaired autophagy in calf muscle fibers from patients with PAD (16). Smaller size and reduced activity of calf muscle fibers are associated with greater functional impairment and increased mobility loss in people with PAD (16-20). However, no medical therapies have been identified that improve calf muscle perfusion or reverse skeletal muscle damage in people with PAD. Few medical therapies exist for improving functioning in PAD.

VM202 is a plasmid that contains cDNA and encodes two isoforms of hepatocyte growth factor (HGF) (21). VM202 is administered by injection into calf muscle where it locally produces HGF (21). HGF is a paracrine cellular growth factor that stimulates angiogenesis, promotes skeletal muscle regeneration, and improves autophagy (21-33). Preliminary evidence shows that VM202 improves the ankle brachial index (ABI), a measure of PAD severity, and promotes healing of lower extremity ulcers in PAD patients with critical limb ischemia (CLI) (21,34). We hypothesize that the favorable effects of HGF will improve walking performance and prevent functional decline in PAD patients age 55 and older without CLI.

Based on prior study (21,34), we will administer 4 mgs of VM202 or placebo to calf muscle of one or two legs (total up to 8 mgs per treatment) every 14 days for a total of four treatments. Our primary aim will test the hypothesis that VM202 improves six-minute walk performance in older PAD patients without CLI at six-month follow up. Our secondary aims will test the hypotheses that VM202 increases treadmill walking performance and calf muscle perfusion in older PAD patients. Our secondary aims will also test the hypotheses that VM202 improves calf muscle biopsy measures of capillary density, muscle regeneration, and autophagy. From this point forward, VM202 refers to the genomic cDNA hybrid of the HGF gene, which is the therapeutic intervention. The HI-PAD study will gather preliminary evidence to test our hypotheses. If our hypotheses are supported by this pilot study, results will be used to design a definitive randomized clinical trial of VM202 in older PAD patients without CLI.

SPECIFIC AIMS

Primary Aim. Among 36 PAD participants without critical limb ischemia (CLI), we will determine whether those randomized to unilateral or bilateral calf muscle VM202 injections have greater

improvement in the six-minute walk at six-month follow-up, compared to those randomized to calf muscle placebo injections. *We hypothesize that PAD participants randomized to* VM202 *will achieve greater increases or less decline in six-minute walk at six-month follow-up, compared to those randomized to placebo.*

Secondary Aim #1. Among 36 PAD participants without CLI, we will determine whether those randomized to unilateral or bilateral calf muscle VM202 injections have greater improvement in maximal and pain-free treadmill walking time, respectively, at 3-month follow-up, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to* VM202 *will achieve greater increases or less decline in pain-free and maximal treadmill walking time at 3-month follow-up, compared to those randomized to placebo.*

Secondary Aim #2. Among 36 PAD participants without CLI, we will determine whether participants randomized to receive unilateral or bilateral calf muscle VM202 injections have greater increases in calf muscle perfusion, measured by magnetic resonance imaging (MRI) at baseline and 3-month follow-up, compared to those randomized to placebo injections. *We hypothesize that PAD participants randomized to VM202 will achieve more favorable changes in MRI-measured calf muscle perfusion at 3-month follow-up, compared to placebo.*

Secondary Aim #3. Among 36 PAD participants without CLI, we will use calf muscle biopsy at baseline and at 3-month follow-up to determine whether participants randomized to unilateral or bilateral calf muscle VM202 injections have more favorable changes in calf skeletal muscle markers of regeneration (satellite cell number and activation state and centrally nucleated and embryonic myosin heavy chain-expressing fibers), capillary density, and calf muscle markers of autophagy (LC3, LAMP2) compared to those randomized to unilateral or bilateral placebo injections. *We hypothesize that PAD participants randomized to VM202 will achieve more favorable changes in each skeletal muscle measure, compared to those randomized to placebo.*

Secondary Aim #4. Among PAD participants without critical limb ischemia (CLI), we will determine whether those randomized to unilateral or bilateral calf muscle VM202 injections have greater improvement in the walking impairment questionnaire (WIQ) and SF-36 physical functioning score (SF-36 PF) at 3-month and at 6-month follow-up, compared to those randomized to calf muscle placebo injections. *We hypothesize that PAD participants randomized to* VM202 *will achieve greater improvement in the WIQ and SF-36 at 3-month and at 6-month follow-up, compared to those randomized to placebo.*

Secondary Aim #5. Among 36 PAD participants without CLI, we will determine whether those randomized to VM202 injections have greater improvement in the six-minute walk at 3-month follow-up, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to* VM202 *will achieve greater increases or less decline in the six-minute walk at 3-month follow-up, compared to placebo.*

Exploratory Aim. Among PAD participants without CLI, we will determine whether those randomized to VM202 injections have greater improvement in the six-minute walk at 12-month follow-up, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to* VM202 *will achieve greater increases or less decline in the six-minute walk at 12-month follow-up, compared to placebo.*

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria. All participants will be age 55 or above and will have PAD. All participants will have symptomatic PAD, defined as exertion-induced ischemic calf muscle symptoms during the six-minute walk, during the baseline exercise stress test, or during daily walking activities.

PAD will be defined as follows. First, an ankle brachial index (ABI) ≤0.90 at the baseline study visit is an inclusion criterion for PAD. Second, potential participants with an ABI > 0.90 who have vascular lab evidence of PAD or angiographic evidence of significant PAD may be eligible.

Exclusion criteria.
1. Above- or below-knee amputation.
2. Critical limb ischemia, including individuals with gangrene and lower extremity ulcers.
3. Wheelchair-bound or requiring a cane or walker to ambulate.
4. Walking is limited by a symptom other than PAD.
5. Planned lower extremity revascularization, major orthopedic surgery, or other major surgery during the next six months.
6. Lower extremity revascularization, orthopedic surgery, cardiovascular event, coronary revascularization, or other major surgery in the previous three months and planned revascularization or major surgery during the next six months.
7. Major medical illness including renal disease requiring dialysis, lung disease requiring oxygen, Parkinson's disease, or a life-threatening illness with life expectancy less than six months. [NOTE: Participants who only use oxygen at night may still qualify]
8. History of cancer within the last 5 years or incomplete cancer screening as recommended by the American Cancer Society. Specifically, participants will be asked to provide documentation regarding screening history for colon cancer and breast and cervical cancer (women), according to the American Cancer Society guidelines. Screening for colon cancer may consist of stool testing for blood in the past year. Men must either provide documentation regarding prostate cancer screening history or indicate after a telephone or in-person discussion with Dr. McDermott that they have elected to decline prostate cancer screening. A chest computed tomography will be performed for participants 55 to 74 years old with ≥30 pack year history of smoking, unless they have not smoked within the past 15 years, to screen for lung cancer that may exclude them. The study team may also perform colon, breast, and/or cervical cancer screenings as part of study participation. The study team will provide stool testing for blood for colon cancer screening, mammogram for breast cancer screening, and a Pap test for cervical cancer screening according to the participant's eligibility for these screening tests, using the American Cancer Society guidelines. Men who elect to have prostate cancer screening who have not had this completed with their physician can have a prostate specific antigen (PSA) test performed by study investigators. Participants who have a history of non-melanoma skin cancer (i.e. basal cell carcinoma or squamous cell carcinoma of the skin) may still be eligible if the lesion was completely removed and there has been no evidence of recurrence in the past year.
9. Evidence of proliferative retinopathy. Participants who were treated for retinopathy at least 5 years prior to their baseline assessment who do not have evidence of proliferative retinopathy at the time of baseline assessment may still be eligible.
10. Positive test for active Human Immunodeficiency Virus (HIV), hepatitis B virus, hepatitis C virus or Human T-lymphotropic virus. Patients who have positive antibodies for HIV, hepatitis B, or hepatitis C who do not have detectable viral load will be eligible for participation.
11. Mini-Mental Status Examination (MMSE) score <23 or dementia.
12. Participation in or completion of a clinical trial in the previous three months. [NOTE: after completing a stem cell or gene therapy intervention, participants will become eligible after the final study follow-up visit of the stem cell or gene therapy study so long as at least six months have passed since the final intervention administration. After completing a supplement or drug therapy (other than stem cell or gene therapy), participants will be eligible after the final study follow-up visit as long as at least three months have passed since the final intervention of the trial.]
13. Increase in angina or angina at rest.
14. Premenopausal women.

15. Non-English speaking.
16. Visual impairment that limits walking ability.
17. In addition to the above criteria, investigator discretion will be used to determine if the trial is unsafe or not a good fit for the potential participant.
18. Potential participants who have had symptoms from peripheral artery disease for less than six months will be excluded.
19. Potential participants who, after being advised of therapeutic options available for people with PAD, prefer to return to their physician to discuss alternative treatment (e.g. supervised exercise or revascularization). Potential participants may participate in the study after 12 weeks has passed since their last supervised exercise session or revascularization if they meet inclusion criteria.
20. Potential participants with a baseline six-minute walk value < 595 or $\geq$ 1,520 feetwill be excluded.
21. Potential participants with the following laboratory values will be excluded: a hemoglobin value < 8.0 g/dL, a white blood cell count < 3,000 cells per microliter, platelet count < 75,000/mm^3, GFR < 20 mL/minute/1.73 M^2, AST or ALT value > 3 times the upper limit of normal, or any other clinically significant laboratory abnormality which, in the opinion of the investigator, should exclude the participant. Participants may undergo a serum electrophoresis and an immunofixation blood test if indicated to further evaluate abnormalities on the complete blood count if needed to assess study eligibility.
22. Potential participants started on cilostazol within the past three months will be excluded. They may be evaluated for eligibility once three months has passed since beginning cilostazol.

Vulnerable populations (fetuses, pregnant women, children, prisoners, and institutionalized persons) and adults unable to consent will not be included in the study.

STUDY TIMELINES:
Each participant will participate in the study for six months after randomization. We plan to enroll 39 participants over an 18-month period.

STUDY ENDPOINTS:
Our primary outcome is change in the six-minute walk at six-month follow-up. We will repeat six-minute walk testing at 12-month follow-up to assess durability of VM202 benefit. Secondary outcomes are pain-free and maximal treadmill walking distance, calf biopsy measures of skeletal muscle regeneration, capillary density, and autophagy, the WIQ and SF-36 PF scores, and MRI-measured calf muscle perfusion at three-month follow-up.

Performance sites. Northwestern University Feinberg School of Medicine is the lead site. Performance sites also include University of Florida (Principal Investigator (PI) Christiaan Leeuwenburgh MD), University of Kentucky (PI- Dr. Charlotte Peterson), and University of Virginia (PI- Dr. Christopher Kramer). Drs. Leeuwenburgh and Peterson will receive muscle specimens, labeled only with a study identification number, and will analyze them for our proposed muscle measures. Dr. Kramer will receive de-identified magnetic resonance images labeled only with a study identification number, and will analyze them for perfusion measures.

PROCEDURES INVOLVED:
Initial eligibility criteria will be assessed by telephone. Potential participants who remain eligible after the telephone screening will be scheduled for a baseline visit, where they will undergo additional testing to determine their eligibility for randomization.

Baseline testing. Participants will provide informed consent at baseline. An ankle brachial index (ABI) will be performed. Questionnaires will be administered and physical functioning tests will be performed. A treadmill exercise stress test and a physical exam will be performed. A blood sample will be obtained and height and weight and vital signs will be measured. Participants will be asked to undergo an MRI but will remain eligible if they are unable or unwilling to participate in the MRI portion of the study. If the participant agrees to the optional muscle biopsy, they will be asked to undergo a biopsy and return approximately seven days afterward for an incision site check. Baseline testing will require testing at multiple visits performed over multiple days. Research procedures are described in more detail below.

Participants will receive written material about therapeutic options available for people with PAD, including supervised exercise and revascularization. Potential participants will be advised that if they would like to consider these alternative options, they should return to their physician to discuss the alternative therapeutic options before evaluation for the HI-PAD Study.

Randomization. Participants will be randomized to VM202 vs. placebo using a SAS program. We will use block randomization with block sizes randomly selected from 4 and 6. Randomization will be stratified by:
1. Baseline functional performance, measured by a six-minute walk distance $\geq$ 1,100 feet vs. < 1,100 feet.
2. Eligibility for unilateral vs. bilateral calf muscle injections.

VM202 and placebo. Participants randomized to VM202 will receive bilateral calf muscle injections of VM202 if both legs have an ABI value $\leq$ 0.90. Participants randomized to placebo who have an ABI $\leq$ 0.90 in both legs will receive bilateral injections of placebo, containing no VM202. Participants who have an ABI $\leq$ 0.90 in only one leg will only receive injections in the leg with ABI $\leq$ 0.90. Participants included based on angiographic evidence of PAD will have injections in both legs if there is significant angiographic atherosclerosis in both legs. If the angiographic evidence demonstrates atherosclerosis in one leg alone, then only the leg with significant atherosclerosis will receive injections. Participants who were eligible based on a toe-brachial index (TBI) will receive study drug injections in legs with a TBI $\leq$ 0.70

Injections of VM202 (0.50 mg/ml) or placebo are administered by a physician in a double-blinded fashion after randomization on Day 0, Day 14, Day 28, and Day 42, for a total of four treatment days. On each treatment day, 16 injections per leg will be administered. Each injection contains 0.5 ml of liquid. Each VM202 injection contains 0.25 mg of VM202. Therefore, participants randomized to VM202 will receive 4 mgs of VM202 per calf muscle on each treatment day. Note that only legs with an ABI < 0.90 will be eligible for injection.

Injection windows will be +/- three days relative to Days 14, 28, and 42. For example, Day 14 injections would ideally be given 11 to 17 days after Day 0. However, if a participant is not able to come in for the injections within this window, the injections may be given up until the day before the next scheduled injection. For example, Day 14 injections may be given up until Day 27. Day 42 injections may be given up to 14 days later (i.e. Day 56).

The following methods will be used to ensure that the correct legs are injected. First, prior to the procedure, the leg(s) eligible for injection will be selected by the study manager, based on the ABI value in each leg, and the designated leg(s) will be confirmed by a second coordinator. The leg(s) to be injected will be noted on the study medical record and a copy of the ABI data collection form will also be attached to the medical record. Second, the day of the injection visit, both the clinician performing the injections and a study coordinator will separately initial to

confirm the leg(s) eligible to inject. The clinician performing the injections will mark the skin of the leg to inject if only one leg is eligible.

Injections are placed beginning 2 cm below the popliteal crease, and are administered in a pre-designed sequence and pattern, at a measured distance 2 cm apart.

Placebo is composed of only the excipient buffer formulation minus the VM202. Placebo consists of sucrose: 55mg (1.1%) and sodium chloride: 45 mg (0.9%). The placebo is liquid and no reconstitution is necessary.

For the injections, the syringe gauge is 27. The depth of the injection is to muscle fascia (depending on body habitus, typically 8 to 14 mm below the injection site). Injection sites are into the medial and lateral gastrocnemius. Negative pressure is applied on the syringe to ensure that there is no blood return, in order to avoid intravascular injection.

Three-month follow-up testing. Participants will be asked to return to the medical center after three months to repeat some or all testing performed at baseline. The three month follow-up time point is relative to the date of the first injection (not relative to the dates of baseline testing). Follow-up testing will ideally be performed after three months +/- seven days relative to Day 0 injections. However, follow-up testing will be performed outside of this window if necessary. In all instances, follow-up testing will be performed as close to the target window (+/- seven days relative to day 0) as possible.

Six-month follow-up testing. Participants will return six months after receiving their first study injections to repeat some or all testing performed at baseline. Participants with diabetes mellitus will have a second retinal examination at six-month follow-up.

Twelve-month follow-up testing. Participants will return 12 months after receiving their first study injections for final follow-up testing. Some or all of the tests performed at baseline will be repeated.

Some or all study measures may be repeated at baseline or follow-up for data quality (e.g. if a treadmill test must be stopped due to extremely high blood pressure before the patient completed the test is one potential example of why a measure may need to be repeated). In some cases, it may be necessary to take an additional, unscheduled blood pressure measurement. For instance, if a participant has high blood pressure during the ABI and investigators would like to double check their arm pressure measurement before performing the six-minute walk either at the same visit or at a subsequent visit. Determinations about blood pressure checks will be made on a case-by-case basis in consultation with Dr. McDermott or Dr. Lloyd-Jones or other qualified personnel.

Research procedures:
Ankle Brachial Index (ABI). After the participant rests supine for five minutes, the right brachial, dorsalis pedis (DP), posterior tibial (PT) and left DP, PT, and brachial artery pressures are measured using a hand-held Doppler probe. Pressures are measured twice. The ABI is calculated for each leg by dividing the average of the DP and PT pressures by the average brachial pressure. If an ankle brachial index result yields an equivocal result or suggests that a participant's lower extremity arteries are calcified, investigator discretion may be used to order a lower extremity artery test with toe pressures at Northwestern Memorial Hospital to determine eligibility for the trial. The test will repeat blood pressures in the arms and legs and will include a toe pressure measurement, which is performed by placing a small blood pressure cuff around

the great toe and attaching a plethysmography probe (circulatory sensor) on the bottom (ventral surface) of the tip of the great toe.

Questionnaire Administration. Participants will be administered IRB-approved study questionnaires by a trained and certified study coordinator.

Six-minute walk. In the six-minute walk, participants walk back and forth along a 100-ft hallway for six minutes after standardized instructions to complete as many laps as possible. Distance covered in six minutes is recorded.

Treadmill testing. The Gardner graded treadmill exercise test is used to measure change in maximal treadmill walking time. Speed is maintained at 2.0 miles per hour (mph) and treadmill grade increases by 2.0% every two minutes. If patients cannot walk at 2.0 mph, treadmill speed is started at 0.50 mph and increased by 0.50 mph every 2 minutes until the participant reaches 2.0 mph, after which the treadmill grade is increased every two minutes while the speed remains at 2.0 mph. Participants with an abnormal cardiac stress test may be allowed to participate in the study if they do not have symptoms of unstable angina and their physician agrees that it is safe for them to participate, or if they have another stress test with imaging (or a cardiac catheterization) that shows no evidence of ischemia. The treadmill test will be performed at baseline and 3-month follow-up.

Written Questionnaire Administration. Participants will be asked to complete the WIQ and the SF-36 physical functioning score at baseline and follow-up. These questionnaires will be self-administered.

Blood collection and long-term storage. Blood will collected at baseline, Day 0 (first injection visit), 3-month follow-up, and 12-month follow-up. In addition, blood may be collected before and after one or more of the study injections (this additional blood testing was added to the protocol in July 2020). Please see the below table, “Schedule of Blood Test Evaluations”, for specific tests that will be performed at each visit.

Blood drawn for long-term storage will be processed and stored at -70 degrees Celsius. Ten percent of participants will be randomly assigned a quality control ID within REDCap. These participants will have an extra set of blood drawn for quality assurance. Stored blood will await later analyses for biomarkers and other emerging blood markers related to peripheral artery disease that may change in response to the intervention. Samples will be labeled with the participant’s study identification number and will not be stored with other health or identifying information. Information associated with the sample will be stored a secure database on password protected computers that are secured by Northwestern University firewalls. Access is limited to study staff. If the samples are shared with other researchers not part of the current study, the PI will grant permission to the other researchers to analyze the samples. Samples will be identified with a study identification number and the other researchers will not have access to PHI. Results of testing on the blood samples will not be shared with the study participants.

Approximately 55 mls of blood will be drawn at brawn at baseline, 10 mls at Day 0, 60 mls at three-month follow-up, and 10 mls at 12-month follow-up. Additional blood may be drawn before and after each injection visit (Day 0, 14, 28, 42) for a total of 25 ml per injection visit to test for the presence of VM202. On Day 0, the total amount of blood drawn will be up to 35 ml (10 ml for comprehensive chemistry panel and CBC + up to 25 ml additional blood). Quality control participants will have an additional 25 mls of blood drawn at baseline and three-month follow-up.

Schedule of Blood Test Evaluations.

	Baseline	Day 0	Day 14	Day 28	Day 42	3-month follow-up	12-month follow-up
HemoglobinA1C	X	X				X	
HGF						X	
VM202		X*	X*	X*	X*	X	
HTLV, HIV-1, HIV-2, Hepatitis B, Hepatitis C	X						
Comprehensive Chemistry Panel	X	X				X	X
CBC	X	X				X	X
Long-term storage	X					X	

*Blood for VM202 testing may be collected 2 hours +/- 1 hour before and after injection visits.

Calf muscle biopsies. Muscle biopsies will be performed by co-investigator Robert Sufit, MD, a board-certified neurologist with > 30 years of experience performing muscle biopsies or co-investigator Dr. Karen Ho. Dr. Sufit completed all biopsies for our pilot study. Muscle biopsies are obtained in the medial head of the gastrocnemius muscle in the leg with lowest ABI, at the point that is 67% of the distance between the medial malleolus and the medial aspect of the proximal tibia. The muscle biopsy will be obtained 1 cm away from one of the VM202 calf muscle injections. Muscle biopsies will be obtained prior to randomization and at 3-month follow-up, approximately 45 days after the final VM202 injection. The 3-month follow-up biopsy will be obtained adjacent to the baseline biopsy, indicated by the baseline biopsy scar. Anesthesia is achieved with subcutaneous lidocaine. Approximately 250 mgs of muscle tissue is obtained. Approximately 100 mgs is mounted and snap frozen in liquid nitrogen-cooled isopentane. The remainder, approximately 150 mgs, is frozen directly in liquid nitrogen and stored at -70 degrees Celsius.
Participants will be telephoned approximately seven days after the muscle biopsy. Participants who report any complaints about their biopsy site (such as significant pain or redness) will be scheduled for an evaluation of their biopsy site by a study physician. Participants who would like to have a muscle biopsy check may be scheduled for an evaluation of their biopsy site by a study physician.

Calf skeletal muscle regeneration and capillary density will be quantified on cryosections of muscle biopsies using immunohistochemistry by Dr. Peterson's laboratory at the University of Kentucky. Satellite cell number will be determined by counting Pax7+DAPI+ nuclei and activation state will be determined by counting MyoD+Pax7+ DAPI+ nuclei (16). Capillaries will be identified with the antibody against the endothelial marker CD31 and/or lection staining (35). Cell types will be expressed per muscle fiber. Regenerating fibers will be identified using an antibody against embryonic myosin heavy chain and by central nucleation to measure changes in response to VM202. LC-3 and LAMP-3 staining will be used to quantify autophagy, which we recently demonstrated is impaired in PAD subjects (16). All immunohistochemistry will be performed using well standardized, validated methods that have excellent test re-test reliability (16,35). RNA and protein will be isolated from the muscle and HGF abundance will be quantified.

Other measures related to skeletal muscle quality and function may also be performed at Northwestern or at other institutions (including University of Florida and University of Kentucky) as designated by the Principal Investigator.

Medications. Participants and their physicians will be asked not to change their medications during the study if possible. We will systematically record names and doses of medications and inquire about medication changes at follow-up. If we find differences in the use of specific medications between study groups (such as statins or cilostazol), we will adjust for these differences.

MRI. We will use arterial spin labeling with cardiovascular magnetic resonance imaging to measure changes in calf skeletal muscle perfusion at 3 Tesla between PAD participants receiving VM202 vs. placebo. Co-investigator Christopher Kramer MD, from University of Virginia, is an internationally recognized expert in this measurement and will assist investigators with interpreting results. Results will be read blinded to group assignment. The procedure is performed as follows: A thigh cuff is inflated in the leg with lowest ABI up to 250 mm Hg. After five minutes, the blood pressure cuff is rapidly deflated. Seven control-tagged image pairs are acquired over 60 seconds using PASL pulse sequence with single-shot echo-planar imaging readouts (field of view 200x200 mm, matrix 64x64, repetition time 4000 ms, echo time 32 ms, slice thickness 10 mm). Perfusion is measured and quantified on a Siemens Healthcare workstation by selectively drawing regions of interest of the hyperemic areas on the perfusion maps. Images for calf muscle fat and skeletal muscle quantity may also be obtained.

CT scan. A chest computed tomography (CT) will be performed for participants age 55 to 74 with $\geq$30 pack year history of smoking, unless they have not smoked within the past 15 years, to screen for possible lung nodules that may exclude them. If the participant meets criteria for lung cancer screening based on their smoking history, and had a chest CT within the past year outside of the HI-PAD study, the results from that CT may be used to determine study eligibility.

Cancer screenings. If the participant is not up to date on all cancer screening examinations as recommended by the American Cancer Society or if the results cannot be obtained from their medical provider, investigators may perform fecal occult stool testing, a mammogram, a Pap test, or PSA testing.

Funduscopic examination. There is a hypothetical possibility that VM202 will exacerbate retinopathy. Therefore, a funduscopic examination will be performed at baseline by co-investigator Dr. Volpe or his associate, Dr. Paul Bryar, unless the participant can provide evidence of a full eye exam by an ophthalmologist from the previous year. Participants with evidence of proliferative retinopathy will not be included. Participants who were treated for retinopathy at least 5 years prior to baseline assessment who do not have evidence of proliferative retinopathy at the time of their baseline assessment may still be included. The funduscopic examination will be repeated at six-month follow-up in participants with diabetes. The funduscopic examination will include the use of eyedrops to dilate the pupils. Participants will be instructed that the drops will blur their vision for a short period of time and that driving may be difficult. The study will pay for taxi transportation home if the participant is unable to arrange pickup by a friend or family member. The dilating drops could cause a transient elevation of intraocular pressure. The transient increase in pressure can be clinically significant in patients with a history of closed or narrow angle glaucoma. The ophthalmologist will screen for closed or narrow angle glaucoma during the anterior segment exam and will not use dilating drops in these participants.

Other measures. Body mass index (BMI) will be assessed at baseline and follow-up by objectively measuring height and weight. Patient report will be used to document comorbidities. A four-meter walk test will be administered at usual and fastest pace at the baseline and follow-up visits. Participants will be asked to perform the usual paced four-meter walk at usual pace and the "fast paced" four meter walk at their fastest pace. Each of these short walks will be performed twice. Participants will be asked to complete a series of standing balance tests and chair stands. A physical exam will be performed and vital signs will be obtained.

COVID-19 Questionnaire. Currently enrolled and past participants may be called to see if they are willing to complete a questionnaire related to the COVID-19 pandemic. The questionnaire will be completed over the telephone and will help investigators determine how the pandemic is affecting older adults with PAD and how physical activity levels are affected during this time.

DATA AND SPECIMEN BANKING:
Muscle specimens and blood specimens for long-term storage will be stored in a freezer belonging to Dr. McDermott's research program at Northwestern University, in the freezer farm in the basement of Olson Pavilion. Specimens will be stored for up to 70 years, after which they will be destroyed.

Specimens will be coded; meaning that a key will exist that can link the codes back to the direct subject identifiers. Each participant will be assigned a unique study ID number that can be traced back to the study participant. The muscle specimens and the blood samples that are stored will be labeled with this unique identifier and the date and time of the blood collection.

Only Dr. McDermott has control over release of study data or specimens. Any investigators seeking to analyze blood or muscle specimens must contact Dr. McDermott for permission. Each request, if it occurs, will be considered on a case-by-case basis. Dr. McDermott will obtain IRB approval prior to releasing any blood or muscle specimens for analysis, other than those tests specifically named in this application.

DATA AND SPECIMEN MANAGEMENT:
Data is recorded using preprogrammed instruments and an electronic case report form using secure, HIPAA-compliant REDCap database software on servers maintained by Northwestern's Clinical and Translational Sciences Institute. We have substantial experience with REDCap.

POWER CONSIDERATIONS. Power calculations are based on a one-sided two-sample t-test with a significance level of 0.10. Our power calculations take into account a conservatively assumed 6% and 12% drop-out rate at 3-month and 6-month follow-up, respectively, based on our prior randomized trials of PAD participants (36,37). We will randomize 39 participants, with the aim to have at least 36 participants completing 3-month and 32 completing 6-month follow-up. For our Primary Aim, 32 participants at 6-month follow-up provides 70% power for detecting a difference of 0.65 SD in changes in six-minute walk distance at 6-month follow up, which is 33 meters. Therefore, the HI-PAD study has sufficient power for detecting a clinically meaningful change in six-minute walk distance at 6-month follow-up.

36 participants provides 70% power to detect a minimum difference of 0.61 standard deviations (SD) in change of pain-free and maximal treadmill walking time (secondary outcomes) at 3-month follow-up between VM202 and placebo. Using the estimated SD from our SILC trial, 0.61 SD represents a 31 meter difference for the six-minute walk distance. Prior studies have defined clinically meaningful changes in the six-minute walk as 30 meters (small meaningful difference)

and 50 meters (large meaningful difference) (38,39). Therefore, the HI-PAD study has sufficient power for detecting a clinically meaningful change in 6-minute walk performance. Furthermore, the observed difference in change in maximal treadmill walking time was 1.06 SD in our SILC exercise trial (36). The HI-PAD Study has power for a similar effect. We will construct the 90% confidence interval (CI) for the change in six-minute walk performance and maximal treadmill walking time by treatment group. The average length of the corresponding 90% CI is 0.82 SD, which corresponds to 42 meters in six-minute walking distance, which is sufficiently narrow to be informative (36). For Secondary Aims #2 and #3, 36 participants provide 70% power to detect a difference of 0.61 SD in change in each skeletal muscle outcome and in MRI-measured calf muscle perfusion between the VM202 and placebo groups. In a clinical trial in which obese men were randomized to resveratrol vs. placebo, Timmers et al observed greater increases in the resveratrol group in calf skeletal muscle citrate synthase activity and mitochondrial respiration of 3.17 and 2.12 SD, respectively (40). In an animal model, the observed treatment effects from ghrelin were more than 0.91 SD for citrate synthase and COX (41). If effects of VM202 on calf muscle measures are comparable to those of resveratrol and ghrelin, the power should be adequate. Similarly, for Secondary Aim #5, 36 participants provides 70% power to detect a minimum difference of 0.61 standard deviations (SD) in change of six-minute walk distance at 3-month follow-up between VM202 and placebo.

Statistical analyses will be performed using the intention to treat principle. Prior to analyses, the distributions of the outcome variables will be examined and necessary transformation will be performed. We will use one-sided two sample t-tests to compare changes in each outcome measure at 3-month and 6-month follow-up between the VM202 and placebo groups. We will also compare change in six-minute walk distance at 12-month follow-up. The balance of baseline characteristics (i.e. age, sex, race, and ABI) will be compared between the two groups using t-tests or Fisher's exact test. Nonparametric tests such as Wilcoxon rank test and permutation test may be used when appropriate. If there is any indication of major imbalance in baseline factors, the regression analysis of covariance will be used to adjust for imbalance. All statistical tests for the primary and secondary outcomes will be one-sided. The statistical significance level will be 0.10.
Changes made to the statistical analysis plan on 12/22/2022. The investigative team reviewed baseline characteristics of participants randomized to each study group on 11/23/2022. Because of the presence of some imbalances in baseline characteristics between the two study groups, the investigative team determined, prior to reviewing any outcome data, that analyses for study outcomes will adjust for age, race, former smoking status, and baseline performance on each outcome measure. Due to the COVID-19 pandemic, there were some additional missing data for the treadmill walking outcome. Because of missing data, analyses for the treadmill outcome will adjust only for baseline treadmill walking performance (and not for age, race, and former smoking). In a sensitivity analyses for pain-free walking distance, analyses will be performed with and without using maximal treadmill walking distance for individuals who did not report ischemic leg symptoms during the treadmill test.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS:
A Data and Safety Monitoring Board (DSMB) will monitor safety throughout the study. The DSMB will meet at least every six months during the study. The DSMB will review and approve the protocol prior to beginning data collection. They will decide on stopping criteria for the study. The biostatisticians will work with the DSMB to perform interim analyses. Adverse events will be monitored continuously throughout the study and will be reported to the DSMB in a timely manner according to pre-specified requirements. Analyses for each DSMB meeting will be completed according to the requests of the DSMB. Adverse event rates and interim study

results will be reviewed and discussed by the DSMB at the DSMB meetings. At least four categories of adverse events will be defined: a) death; b) cardiovascular events (myocardial infarction, stroke, and coronary arrhythmias); c) hospitalizations; and d) injury or illness causing chronic disability. We will report all serious adverse events to the DSMB in a timely fashion.

WITHDRAWAL OF PARTICIPANTS:
We anticipate that participant withdrawal from the research without their consent will be infrequent. However, a potential example is if a participant develops symptoms during the study and the principal investigator feels that the symptoms could make the study unsafe for the participant to continue. In this circumstance, the participant would be advised to follow-up with his or her physician. If the participant refuses to follow-up with their physician, it may be necessary for the participant to be withdrawn without their consent.

Participants may withdraw from the research at any time. If they decide to leave the research, they should contact the principal investigator, Dr. Mary McDermott. If they stop being in the research, already collected data may not be removed from the study database.

RISKS TO PARTICIPANTS:
Risks associated with VM202. VM202 has been studied in more than 160 patients in Phase I and Phase II studies of patients with peripheral neuropathy, PAD and CLI, and coronary artery disease. In general, VM202 has been safe and well-tolerated with infrequent side effects (21,34,42-44). Potential adverse events include pain at the injection site, local or systemic inflammatory response, infection at the injection site, venous thrombosis, retinopathy, and progression of tumor and cancer. Potential participants must be up to date on all cancer screening examinations recommended by the American Cancer Society, and have no history of cancer, in order to be eligible. Potential participants with a first degree relative with history of colon cancer before age 65 must have a colonoscopy for colon cancer screening. Baseline funduscopic examinations will be performed by an ophthalmologist at Northwestern Medicine. Potential participants with retinopathy will be excluded. Participants will be provided with a list of VM202 side effects and will be provided with Dr. McDermott's home and cellular telephone, in case the participant develops side effects or has questions.

Risks associated with the muscle biopsy. The muscle biopsy is associated with several potential risks. These include discomfort during the muscle biopsy procedure and for 1-2 days afterward, scarring from the muscle biopsy skin incision, bleeding (including a hematoma), and infection. Side effects of the tape used to bandage the incision site include a local allergic reaction. Side effects of lidocaine 1% without epinephrine may include pain at the injection site, an allergic reaction, potential emotional excitement, or temporarily lowered heart rate or blood pressure. In addition, potential participants who are asked to hold their anti-platelet therapy during the week leading up to the muscle biopsy procedure may experience a cardiovascular event related to the temporary discontinuation of the anti-platelet therapy. First, to minimize risk related to muscle biopsy, all participants undergoing muscle biopsy will receive a written handout regarding signs to watch for that may indicate wound infection. They will also be verbally instructed in this. Each participant will be instructed to call Dr. McDermott immediately if any signs of infection occur. Participants will be telephoned approximately seven days after the muscle biopsy. Participants who report any complaints about their biopsy site (such as significant pain or redness) will be scheduled for an evaluation of their biopsy site by a study physician. Participants who would like to have their biopsy site evaluated will also be scheduled for an evaluation by a study physician. Second, permission from the participant's physician will be required before participants are asked to discontinue anti-platelet therapy.

Risks associated with the six-minute walk test, treadmill stress test, four-meter walks, balance, and chair stands. The physical functioning tests may be associated with muscle fatigue or soreness. These symptoms typically resolve with rest. These tests may be associated with the risk of falling, coronary ischemia or dyspnea due to heart failure or lung disease, irregular heartbeat, chest pain, or heart attack. Rarely, falling may result in a fracture. However, the research assistant who will collect these data has been trained to prevent falling. The risk of a fracture secondary to a fall during the testing is less than 1 in 8,000. If a participant experiences chest pain, research assistants are trained to page Dr. McDermott immediately. If the chest discomfort does not immediately resolve with rest, participants are escorted to Northwestern's Emergency Department, which is located in the same building as the location of the tests. Dr. McDermott facilitates follow-up as appropriate, by contacting participants' physicians, for those who experience new chest discomfort during testing, for example. In our experience, the risk of chest discomfort is approximately 1 in 750. Symptoms or results of testing may lead to hospitalization, additional testing by the participant's physicians, or recommendations for procedures to improve blood flow to the heart.

Risks associated with ABI measurement. The ankle brachial index measurement consists of measuring systolic blood pressure in each extremity using a hand-held Doppler. The ABI is non-invasive, safe and does not have any known lasting side effects. During the ankle brachial index test, participants may experience discomfort from the inflated blood pressure cuff. However, this discomfort resolves immediately when the cuff is released.

Risks associated with questionnaire administration. Participation includes a risk of loss of confidentiality regarding personal health information. However, all research staff have undergone formal human subjects training. They are trained to protect the privacy of research subject participants.

Risks associated with drawing blood. The potential risks of drawing blood include a bruise at the site of vein puncture, inflammation of the vein, and infection. Participants undergoing a blood draw may experience lightheadedness, dizziness, or fainting.

Risks associated with MRI testing. The MRI scanner makes loud banging noises while doing a measurement. Participants will be provided with earplugs or earphones to protect against the noise. Participants will fill out an MRI screening form prior to participating in the MRI portion of the study to ensure they are safe for MRI testing. MRI testing can be difficult for people with claustrophobia. Participants who are claustrophobic will not be encouraged to participate in MRI testing. Participants may experience leg discomfort while the cuff is inflated.

Risks associated with CT testing. The cumulative radiation exposure from a CT scan is considered small. However, the effects of radiation add up over a lifetime. The radiation dose received for one chest CT scan (540 mrem) is approximately equal to 1.8 years of background radiation.

Risks associated with additional cancer screenings. Risks of a mammogram include an abnormal test result, including a false positive test result that could require additional imaging or a breast biopsy, mild discomfort during the exam, and radiation exposure. The cumulative radiation exposure from a mammogram is considered small. However, the effects of radiation add up over a lifetime. The effective dose from a typical routine mammography procedure (4 views) is 65 mrem and is approximately equal to 5.8 weeks of background radiation.

An abnormal Pap test, PSA, or fecal occult blood test may require additional testing or treatment. A Pap test may be associated with mild discomfort during the exam. An abnormal PSA test result may require a prostate cancer biopsy. In the event of an abnormal mammogram, PSA, Pap test, or fecal occult blood testing, the participant will be referred back to their own physician for follow-up testing. If a cancer screening test result is abnormal, none of the additional testing will be performed or paid for by the study.

In addition to these risks, this research may cause harm in ways that are unknown. These may be a minor inconvenience or may be so severe as to cause death.

POTENTIAL BENEFITS TO PARTICIPANTS:
Participants who are randomized to receive VM202 could experience improved walking performance or less decline in functional performance, if our hypotheses are correct.

SHARING OF RESULTS WITH PARTICIPANTS:
Participants will receive results of their ankle brachial index (ABI) test and will be provided with a "result letter" at the end of their baseline visit that includes the result of the ABI test. They will not be provided with other study results routinely. However, participants will be notified of abnormal stress test, blood, or blood pressure results.

SETTING:
Baseline and follow-up data collection will take place at Northwestern Memorial Hospital in the Galter Pavilion (675 N. St. Clair) or in the Feinberg Pavilion (251 E. Huron). MRI testing will take place in the Olson Pavilion (710 N. Fairbanks).

RESOURCES AVAILABLE:
Study coordinators who collect data are Bachelor's level or Master's level graduates who are Northwestern University employees. New study staff members are required to read study manuals and observe 1-2 study visits. Staff members are trained by senior, experienced staff in data collection and certification is performed by Dr. McDermott. A checklist is used for certification. Study staff receive feedback from Dr. McDermott on their certification performance. Staff members who are not fully ready to begin data collection are re-trained and then re-attempt certification with Dr. McDermott until data collection procedures are satisfactory as determined by Dr. McDermott. Staff members undergo re-certification, using detailed checklists, every six months. Dr. McDermott performs most of these re-certifications. Some of these re-certifications are performed by a project manager. Study staff members are directly supervised by a project manager and Ms. Kathryn Domanchuk, a Senior Clinical Research Associate, with more than 10 years' experience managing NIH-funded clinical trials.

RECRUITMENT METHODS:
Participants will be identified from among individuals with PAD who have participated previously in research conducted by Dr. McDermott and/or who have expressed an interest in participating in future studies conducted by Dr. McDermott. Participants who we screen for ongoing studies who may have PAD but are ineligible for that study and interested participating in a study may be screened for this study.

In addition, PAD participants may be identified from among consecutive patients diagnosed with PAD in the non-invasive vascular laboratory at Northwestern Medical Group (NMG). Dr. Mark Eskandari is medical director of the non-invasive vascular laboratory at NMG and will assist with identifying potential participants from the non-invasive vascular laboratory. As director of the vascular laboratory at NMG, Dr. Eskandari formally reads many of the non-invasive vascular

laboratory tests. He maintains all non-invasive vascular test results in his vascular laboratory. As director of the vascular laboratory, Dr. Eskandari could conceivably contact the patients whose test results are maintained in his laboratory. However, Dr. Eskandari prefers that the contact of potential participants in studies come from the physicians referring him for testing. Lists of patients who have undergone lower extremity arterial testing in the non-invasive vascular laboratory are generated monthly and e-mailed from NMG to Dr. McDermott. A research assistant will contact referring physicians of potential participants identified from the vascular laboratory via fax, phone, page, or electronic message (EPIC or e-mail), to ask for permission to contact their patient about the study. If a reply is not received within three weeks, up to five letters are mailed from Dr. McDermott about the research study. We have substantial experience with our recruitment methods for our previous or ongoing studies.

We also propose to obtain lists of consecutive patients with a diagnosis of lower extremity peripheral arterial disease and individuals at high risk for peripheral artery disease from Northwestern's Enterprise Data Warehouse (EDW). EDW lists will be provided by an individual who is employed by the Division of General Internal Medicine who has received training and permission to obtain the lists from the EDW.

Similar methods will be used as those described above, in which the patient's physician will be contacted via fax, telephone, page, or electronic message (EPIC or email) to ask for permission to contact their patient about the study. If a reply is not received within three weeks, up to five letters are mailed from Dr. McDermott about the research study.

In the recruitment letters, recipients are asked to call us if they are interested in participation or if they do not want to be contacted further. Potential participants who do not call us within three weeks of the first mailed recruitment letter may be telephoned by study staff and invited to participate.

In addition, we may use newspaper, television, and radio advertising to identify potential participants for this study. We may also use brochures, flyers, or posters that we will post in relevant office practices and public areas. We may use advertising on public transportation or online advertisements.

We will obtain a list of patients who live in the Chicago area from a mass mailing company. Using this, we will send postcards to those individuals on the list. The postcards will instruct people to call a study number if they are interested.

Recruitment materials associated with the above recruitment methods will be submitted to the IRB for approval before the recruitment methods are utilized.

We may also use CAPriCORN for recruitment. CAPriCORN is a PCORI-funded network of institutions in the Chicago area. The purpose of CAPriCORN is to assist investigators with recruitment for clinical trials. CAPriCORN has its own IRB (University of Illinois at Chicago) and uses ICD-9 codes and the electronic health record to identify potential participants who have PAD. These patients with PAD will be mailed a recruitment letter that describes the trial and invites the patient to participate.

We will use the University of Chicago for recruitment. The University of Chicago will obtain IRB approval at their site to identify potential participants who have PAD. Those patients will PAD will be mailed recruitment letters that describes the trial and invites the patient to participate. A

University of Chicago coordinator will screen potential participants over the telephone and schedule participants for baseline visits at Northwestern.

PAYMENT:
Participants will receive $25 after completing each injection visit and $25 after completing 3, 6, and 12-month follow-up for a total of $175 ($100 for injection visits and $75 for follow-up testing) if a participant completes all study measures.

In addition, if the participant undergoes the optional muscle biopsy portion of the study, they will receive $100 per muscle biopsy. If the participant undergoes an MRI, they will receive $25 per MRI.

Participants will be given assistance and/or reimbursement for expenses related to travel such as parking, bus/train fare, taxi or rideshare (i.e. Uber/Lyft) fare, and mileage, if requested. A receipt will be required for reimbursement over $40. Participants will be provided up to $90 per visit for travel reimbursement. If they require the use of our taxi or rideshare service, we will estimate the fare on www.taxifarefinder.com or on the rideshare website. A one-way fare estimate must be less than or equal to $45 (i.e. round trip of $90) in order for the study to provide taxi or rideshare service. In some instances, a participant's travel estimate may be within the $90 limit for their first visit, but may unexpectedly increase at a later visit due to price fluctuations with Uber/Lyft. In these instances, the study will continue to provide travel to participants and pay the increased travel fare. In addition, if after randomization, a participant becomes unable to attend study visits and requires transportation such as a shared ride service in order to continue participation, then the travel service will be provided, using investigator discretion, so that the randomized participant can continue in the trial. In these cases, the amount of travel using our taxi or rideshare service may exceed $90. This will help to prevent drop-out, thereby increasing trial integrity.

NUMBER OF LOCAL PARTICIPANTS:
We will identify and randomize 39 eligible participants. We anticipate that it will be necessary to consent many more than 39 participants to account for screen failures.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS:
Research staff undergo training (human subjects training) in the protection of participant confidentiality and privacy. Research staff have access to medical records only for the purpose of conducting research that is approved by the IRB.

Verbal questionnaires will be administered in an enclosed space by a trained and certified research assistant. Dr. McDermott certifies research assistants in data collection to help ensure that participants are treated with the highest level of professionalism.

COMPENSATION FOR RESEARCH-RELATED INJURY:
If the participant needs medical care because of taking part in this research study, they should contact the investigator and medical care will be made available. This care will be billed to the participant, their insurance, or other third party. Northwestern University has no program to pay for medical care for research-related injury.

CONSENT PROCESS:
Participants will be consented by a research assistant who has been trained and certified by Dr. McDermott in obtaining informed consent. A research assistant will explain the study to potential participants by telephone prior to their first study visit. When a potential participant arrives to the

medical center for study participation, the research assistant will explain the full details of the research study. The informed consent process will take place at the initial baseline study visit in a private area on Northwestern's medical campus.

Potential participants will be provided plenty of time to read the consent form. The research assistant will answer questions and Dr. McDermott or another study investigator at Northwestern is also available to answer any questions that participants may have about the research. If the participant would like more time to discuss the research study with their physician or family member before signing the consent document, they will be allowed to do so and the study visit will be rescheduled for a later date.

Potential participants who do not speak English, subjects who are not yet adults, cognitively impaired adults, and adults unable to provide written consent will not be eligible for study participation.

PROCESS TO DOCUMENT CONSENT IN WRITING:
Written consent will be documented on the IRB-approved informed consent document.

DRUGS OR DEVICES:
Northwestern Memorial Hospital's Investigational Pharmacy will receive randomization assignments from the study's data management team and will prepare identical-appearing syringes of VM202 or placebo according to the randomization assignment.

An IND was obtained on 9/8/2017.

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