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PROGENITOR CELL RELEASE PLUS EXERCISE TO IMPROVE FUNCTIONAL PERFORMANCE IN PERIPHERAL ARTERY DISEASE: THE PROPEL STUDY

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Abstract

Functional impairment, functional decline, and mobility loss are major public health problems in people with lower extremity peripheral artery disease (PAD). Few medical therapies significantly improve walking performance in PAD. We describe methods for the PROgenitor cell release Plus Exercise to improve functionaL performance in PAD (PROPEL) Study, a randomized controlled clinical trial designed to determine whether granulocyte-macrophage colony stimulating factor

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(GM-CSF) combined with supervised treadmill walking exercise improves six-minute walk distance more than GM-CSF alone, more than supervised treadmill exercise alone, and more than placebo plus attention control in participants with PAD, respectively. PROPEL Study participants are randomized to one of four arms in a 2 by 2 factorial design. The four study arms are GM-CSF plus supervised treadmill exercise, GM-CSF plus attention control, placebo plus supervised exercise therapy, or placebo plus attention control. The primary outcome is change in six-minute walk distance at 12-week follow-up. Secondary outcomes include change in brachial artery flow-mediated dilation (FMD), change in maximal treadmill walking time, and change in circulating CD34+ cells at 12-week follow-up. Outcomes are also measured at six-week and six-month follow-up. Results of the PROPEL Study will have important implications for understanding mechanisms of improving walking performance and preventing mobility loss in the large and growing number of men and women with PAD.

Approximately eight million men and women in the United States have lower extremity peripheral artery disease (PAD) (1). Patients with PAD have greater functional impairment and more rapid functional decline compared to those without PAD (2-5). Currently, there are only two FDA-approved medications for improving walking performance in patients with PAD (6-9). Of these, recent data show that one of these medications, pentoxifylline, is not substantially better than placebo and the other medication, cilostazol, provides modest benefit with regard to improved walking performance in PAD (6-9). New therapies are needed to improve walking performance and prevent decline in walking performance in patients with PAD.

Preliminary evidence suggests that interventions that increase levels of circulating progenitor cells improve walking performance in PAD (10-12). For example, recent data suggest that therapeutic agents such as granulocyte-macrophage colony stimulating factor (GM-CSF) or similar agents may increase levels of circulating progenitor cells, such as CD34+ cells, and simultaneously improve walking performance in patients with PAD (11). In addition, supervised treadmill exercise, an effective intervention that improves walking distance in patients with PAD, also increases levels of circulating progenitor cells (10,12),

Endothelial progenitor cells (EPCs), including CD34+ cells, normally exist in low concentrations in peripheral blood (13-15). Mobilizing factors, such as GM-CSF, promote release of EPCs from the bone marrow, spleen, and other sources into the circulation (13-15). These circulating progenitor cells have the capacity to differentiate into mature endothelial cells and form new blood vessels (angiogenesis) (15-18). In a landmark study by Asahara et al, investigators isolated CD34+ cells from humans and injected them into a hindlimb ischemia rabbit model (16). The CD34+ cells isolated from humans migrated to sites of tissue ischemia and incorporated into developing blood vessels (16). Thus, interventions that increase circulating progenitor cells may promote angiogenesis and improve walking performance in people with PAD (16). However, results of studies conducted to date have been mixed (11,19-20). Therefore, it is currently unclear whether interventions that increase progenitor cell levels improve walking performance in patients with PAD.

Supervised treadmill exercise is known to significantly improve walking performance in people with PAD (21). Supervised treadmill exercise also promotes lower extremity ischemia while the patient participants in walking exercise. Under normal conditions, ischemia promotes release of progenitor cells into the peripheral circulation and homing of these cells to ischemic sites (13,14,18,19). However, patients with PAD have fewer circulating progenitor cells than people without PAD (22,23). Based on this evidence, that both walking- related ischemia and GM-CSF can mobilize progenitor cells in people with PAD (10-12), we propose that the combination of walking exercise and GM-CSF may be a

more potent stimulus of progenitor cell release and homing to ischemic tissue than either intervention alone (Figure 1). The primary aims of the PROPEL Study are to determine whether GM-CSF combined with supervised treadmill exercise significantly improves six-minute walk distance at 12-week follow-up in people with PAD, as compared to GM-CSF alone and as compared to supervised treadmill exercise alone. PROPEL will also determine whether GM-CSF significantly improves six-minute walk distance at 12-week follow-up, as compared to placebo and will confirm previous studies that have demonstrated the ability of supervised treadmill exercise to improve six-minute walk distance in people with PAD (21,24-26). We hypothesize that the combination of GM-CSF + exercise will achieve greater gains in six-minute walk distance at 12-week follow-up as compared to GM-CSF alone and as compared to supervised treadmill exercise alone. We further hypothesize that GM-CSF will improve six-minute walk distance at 12-week follow-up as compared to placebo. No prior studies have assessed whether GM-CSF + exercise or whether GM-CSF alone significantly improves the six-minute walk. We will confirm previous studies demonstrating that supervised treadmill exercise improves six-minute walk distance as compared to placebo (21,24-26). If successful, the PROPEL Study will provide a novel therapeutic option to improve walking endurance in individuals with PAD.

METHODS

Overview

The Institutional Review Board at Northwestern University approved the protocol. All participants provide written, informed consent. The PROPEL Study plans to recruit 240 participants with PAD.

Eligibility

The inclusion criteria are an ankle brachial index (ABI) ≤ 0.90 or an ABI > 0.90 and ≤ 1.00 with a ≥ 20% drop in ABI following a heel-rise test. Potential participants with an ABI >0.90 are also eligible, if a certified vascular laboratory demonstrated prior lower extremity ischemia. Participants with prior lower extremity revascularization are eligible only if they have a 20% drop in ABI following a heel-rise test (27). Based on our experience in prior clinical trials of participants with PAD, fewer than five percent of all participants will be included because they have an ABI > 0.90 at baseline and meet one of these criteria (28). Exclusion criteria and justification for each criterion are listed in Table 1.

Recruitment

Potential participants are identified via newspaper and radio advertising, targeted mailings to community-dwelling men and women age 58 and over, posted flyers, and mailed letters to patients diagnosed with PAD and at high risk for PAD at Northwestern Memorial Hospital. Potential participants are first assessed for eligibility by telephone using a standardized interview. Those who remain eligible after telephone assessment are scheduled for a baseline study visit and return for a second and third baseline visit if they remain eligible after each visit. The initial baseline visit includes measurement of the ABI, six-minute walk distance, and height and weight. Medical history, race, and demographics are obtained using patient report. The second baseline visit consists of a physical examination and a treadmill stress test. The third baseline visit consists of the brachial artery flow-mediated dilation and CD34+ cell measurement.

Run-in period

Potential participants who may not be adherent to study interventions are excluded. To identify potential participants whose adherence to study interventions may be poor, all

participants are required to attend one treadmill exercise run-in session and one health education run-in session within a three week period. Participants who do not attend both sessions within three weeks are excluded. The run-in sessions are also used to ensure that potential participants are capable of engaging in treadmill exercise.

Outcome measures

The primary outcome of the PROPEL Study is change in six-minute walk distance at 12-week follow-up. Secondary outcomes include change in brachial artery flow-mediated dilation (FMD), change in maximal treadmill walking time, and change in CD34+ cells (Table 2).

Six-minute walk—The six-minute walk distance is a measure of walking endurance that is well-validated with excellent test re-test reliability. Our prior work shows that shorter six-minute walk distance and greater declines in six-minute walk distance are associated with higher rates of all-cause mortality, cardiovascular disease mortality, and mobility loss in people with PAD (29-31). As compared to a treadmill walking test, the six-minute walk test is more closely correlated with walking performance during daily life (32). In addition, treadmill walking, but not corridor walking such as the six-minute walk test, is associated with balance problems and anxiety, particularly in older men and women such as those with PAD (33-35). Treadmill walking performance is also associated with a significant learning effect (36-38). In clinical trials of supervised treadmill exercise, treadmill walking performance can increase in the exercise arm in part because participants regularly ‘practice’ treadmill walking activity and become more comfortable with treadmill walking. The six-minute walk is a valid and reliable measure of walking endurance in patients with PAD (39). Because participants randomized to treadmill exercise do not have opportunity to “practice” the six-minute walk as part of their intervention, the six-minute walk test is a more appropriate primary outcome measure for the PROPEL Trial than treadmill walking performance.

Following standardized instructions to complete as many laps as possible, participants walk back and forth over a 100-foot hallway for six minutes (28-32). Participants are instructed to walk continuously with the goal of covering as much ground as possible within the six minutes. Participants may stop and rest if needed. A research assistant walks with and slightly behind the participant, so that the research assistant does not pace the participant. Standardized words of encouragement are given at one-minute intervals, for example, “One minute has passed. You're doing well; keep up the good work.” The distance covered after six minutes and the distance at onset of leg symptoms are recorded.

Treadmill walking performance—Maximal treadmill walking distance and treadmill distance to onset of leg symptoms during treadmill walking are measured using the Gardner-Skinner protocol (36,37). The treadmill test is performed once for the baseline measurement and at scheduled intervals during follow-up (see Table 2). Treadmill speed is maintained at 2.0 miles per hour (mph). Treadmill grade begins at zero and increases by 2% every two minutes. Participants unable to walk at 2.0 mph complete a modified Gardner-Skinner protocol, in which treadmill speed begins at 0.5 mph and increases by 0.5 mph every two minutes until treadmill speed reaches 2.0 mph, after which the grade increases by 2% every two minutes (36,37).

Brachial artery flow-mediated dilation (FMD)—Brachial artery FMD is measured following a 12-hour fast by a registered cardiac sonographer using standard procedures (21,40). Participants are instructed to withhold medications, exercise, caffeine, and smoking prior to testing. The proximal brachial artery is imaged using B-mode and Doppler

ultrasound with a linear array vascular ultrasound transducer. A blood pressure cuff is applied proximal to the visualized brachial artery segment and is inflated for four minutes at 50 mm Hg above systolic pressure (minimum systolic pressure = 200 mmHg). Longitudinal images of the brachial artery and Doppler blood flow are obtained 60 and 90 seconds after cuff deflation. Images are interpreted by a single reader at the University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory, using established standards (21,40). FMD is defined as the ratio of the maximum brachial artery diameter after reactive hyperemia to the baseline, resting diameter, expressed as a percent.

CD34+ cells—CD34+ cells are the primary progenitor cell outcome measurement in the PROPEL trial. The CD34+ marker identifies a population of progenitor cells that are released into the circulation in response to tissue ischemia, have the potential to differentiate into endothelial cells, and promote angiogenesis (10,12,16,41-43). Human peripheral blood is collected from participants and red blood cells (RBCs) are lysed twice with freshly prepared lysis buffer (155mM NH4Cl, 10mM KHCO3, 0.1mM EDTA in deionized water) for 10 minutes at room temperature. Samples are subsequently washed 2x with PBS to remove any remaining lysis buffer. After the second wash, cell pellets were resuspended in PBS and cell counts are analyzed using the Countess® Automated Cell Counter (Life Technologies, NY). Cells are stained with LIVE/DEAD® Fixable Dead Cell Stains (Life Technologies, NY) for 20 minutes at room temperature protected from light allowing the detection of dead cells by flow cytometry. Fc receptors are blocked to limit non-specific antibody binding by incubating with FcR blocking reagent (Miltenyi, CA) for 10 minutes at 4°C. Samples are then stained with the following antibody cocktail: anti-CD34 VioBlue (Miltenyi, CA), anti-CD133-APC (Miltenyi, CA), anti-CD45 AlexaFluor 700 (BD biosciences, CA), and anti-CD31 (PECAM-1) APC-eFluor® 780 (Ebiosciences, CA) antibodies. Stained samples are washed once with MACS buffer (Miltenyi CA) to remove any unbound antibodies. Stained samples are acquired on a BD LSRII Flow Cytometer (BD Biosciences, CA) and data are analyzed using Flowjo software (Treestar, OR).

Additional measures

Ankle-brachial index—The ankle brachial index (ABI) is measured to determine eligibility. A handheld Doppler probe (Nicolet Vascular Pocket Dop II, Golden, CO) is used to measure systolic blood pressures after the participant rests supine for five minutes. Pressures are measured in the following order, and then repeated in reverse order: right brachial, dorsalis pedis, and posterior tibial arteries; left dorsalis pedis, posterior tibial, and brachial arteries. The ABI is calculated by dividing average pressures in each leg by the average of the four brachial pressures (21,29-31). When required for eligibility, a heel-rise test is performed. The heel-rise test consists of fifty heel rises at a rate of one per second followed by pressure measurement in both the right and left brachial, dorsalis pedis, and posterior tibial arteries. A 20% drop in ABI in either the left or right leg is needed to remain eligible for the PROPEL Study.

Randomization

Eligible participants are randomized to one of four study arms after completion of baseline testing and the study run-in using a randomly permuted block method (Figure 2). Randomization is stratified by the presence of diabetes mellitus, since patients with diabetes have fewer progenitor cells than those without (44,45).

Study interventions

Participants are randomized to one of four groups: 1) Supervised treadmill exercise + GM-CSF injections, 2) Supervised treadmill exercise + placebo injections, 3) Attention control +

GM-CSF injections, or 4) Attention control + placebo injections (Figure 2). All randomized participants are scheduled for six injections, one three times weekly, over a two-week period. Participants randomized to treadmill exercise begin their first exercise session two days after they receive their first study drug injection. The remaining exercise sessions take place on the same day as study drug injections. Exercise sessions continue for a total of six months. Participants randomized to the attention control group begin their sessions on the same day as their first study drug injection.

Supervised treadmill exercise group—Established methods for supervised exercised training are used in PROPEL (21). Participants randomized to the supervised treadmill exercise intervention attend exercise sessions three times weekly for six months. Participants begin with 15 minutes of exercise during the first week and increase to 40 to 50 minutes of exercise per session, excluding rest periods, by week eight. Between weeks eight and 26, exercise intensity is increased at least once weekly by increasing either treadmill speed or grade. Participants experiencing ischemic leg symptoms are encouraged to exercise to near-maximal leg symptoms (i.e. 4-5 on a scale of 1-5) before stopping to rest. This method helps to ensure that calf muscle ischemia is induced, thereby promoting the release of progenitor cells (13,14). Participants with PAD who report no exertional leg symptoms are encouraged to exercise at a level of 12-14 (moderately hard) on the Borg rating of perceived exertion scale (46).

Attention control group—The attention control arm controls for the possibility that regular contact with the study team may improve outcomes in participants randomized to supervised exercise. Participants randomized to the attention control group attend weekly health education sessions led by physicians and other healthcare professionals. Topics include education on hypertension, dementia, and medication safety. Sessions last sixty minutes and do not provide information on exercise or behavior change. Participants randomized to supervised treadmill exercise do not attend the attention control group sessions.

GM-CSF or placebo injections—Six doses of GM-CSF or placebo are administered in a double-blind fashion subcutaneously by a registered nurse or physician three times weekly over a two-week period. GM-CSF is administered at the maximum FDA-approved dose of 250 $\mu g/ m^2$ /day, which is expected to maximize benefit while minimizing potential side effects. Common side effects of GM-CSF include bone pain, muscle aches, headaches, and chills. Fluid retention, arrhythmias, and allergic reaction have also been reported. Participants are provided with a list of common side effects of GM-CSF. They are provided with the Principal Investigator's (MMM) telephone contact information, should they have any questions or concerns about symptoms. The white blood count (WBC) is measured on the day of the second and fifth injections. Injections are discontinued or drug dosage is reduced if the WBC $\geq$ 50,000/μL, an arterial thrombotic event occurs, or the participant is hospitalized for a reason that may be related to the study drug.

Statistical analysis

Baseline characteristics (age, sex, race, ABI, comorbidities, body mass index, smoking history, six-minute walk, and treadmill walking performance) will be compared between the four groups to ensure that baseline characteristics are balanced across the four groups. Variables with significant differences between groups will be adjusted for as covariates. For our primary specific aim, we will compare changes in six-minute walk distance between baseline and 12-week follow-up between groups using a two-sample t-test or analysis of covariance if necessary to adjust for potential imbalances in baseline characteristics. Analyses will be performed according to the intention to treat principle. We will repeat the

analyses for secondary outcomes. For both our primary and secondary outcomes, we will test the interaction between GM-CSF and supervised exercise with two-way ANOVA. In the absence of an interaction, we will estimate the additive effect of GM-CSF and supervised exercise simultaneously in all 240 participants, where the existence of model-based additive effects for GM-CSF and exercise is stronger than the targeted superiority of the combined treatments.

Power calculations

The minimum detectable difference (MDD) in the change between each pair of groups is estimated based on 80% power using a two sample, two-sided t-test with alpha=0.0125. The alpha=0.0125 was chosen as the level of significance to accommodate the multiple tests for the four planned pair-wise comparisons between study groups. With 60 participants in each of the four groups, the estimated MDD is 0.63 standard deviations (SD). Our previous clinical trial of supervised exercise in patients with PAD showed a difference in change in six-minute walk distance between the exercise group and the control group of 0.69 SD (21). This difference corresponded to 36.3 meters, which is less than the previously defined large meaningful difference for change in the six-minute walk (47).

DISCUSSION

The PROPEL Study will determine whether GM-CSF combined with supervised treadmill exercise therapy significantly improves six-minute walk distance in people with PAD at 12-week follow-up, as compared to GM-CSF alone and as compared to supervised treadmill exercise alone. PROPEL will also determine whether GM-CSF alone significantly improves the six-minute walk distance at 12-week follow-up as compared to placebo. PROPEL will confirm prior evidence that supervised treadmill exercise significantly improves six-minute walk distance in people with PAD (21,24-26). If the proposed hypotheses are correct, the PROPEL Study will identify a novel therapeutic option to improve walking endurance in people with PAD. By simultaneously measuring changes in progenitor cells, brachial artery flow-mediated dilation and walking performance at multiple time points during study interventions, the PROPEL Study will also identify mechanisms by which the study interventions may improve walking performance in people with PAD.

Preliminary evidence suggests that interventions such as GM-CSF or G-CSF that promote increases in circulating levels of CD34+ cells may improve walking performance in patients with PAD who do not have critical limb ischemia (14). However, results of two small clinical trials have been mixed (11,19). In 2005, Van Royen et al reported no difference in maximal treadmill walking time at 12-week follow-up after seven injections of GM-CSF at a dose of 10 μg/kg over 14 days versus placebo injections in forty participants with PAD and intermittent claudication who could walk no more than 200 meters on a treadmill at baseline (19). This study was limited by the small sample size and the fact that the study included only PAD participants who could walk no more than 200 meters on the treadmill. In 2009, Subramaniyam et al reported results of a randomized controlled trial that compared GM-CSF versus placebo injections administered three times weekly over a two-week period in 45 PAD participants with intermittent claudication (11). Participants in both the intervention and control groups were instructed to exercise on their own and outcomes were assessed at 12-week follow-up. The study showed that GM-CSF was associated with significant increases in CD34+ cells, brachial artery FMD, and treadmill performance, compared to placebo. At 12-week follow-up, CD34+ cells increased by 46% (P=0.035), flow-mediated dilation increased by 59% (P<0.01), and treadmill walking time increased by 55 seconds (P=0.016) in participants receiving GM-CSF, but these measures did not significantly change in the placebo group (11). The study by Subramaniyam et al is the first to combine walking exercise activity with GM-CSF and this characteristic of the intervention may have

contributed to the favorable findings. However, participants were instructed to exercise at home (11). Adherence to home exercise adherence was not measured and some previous studies, but not all, demonstrate that home-based exercise is not effective in people with PAD (28,48-50). The PROPEL trial will definitively determine whether the combination of supervised exercise training with GM-CSF significantly improves walking performance in patients with PAD. An ongoing trial of 160 PAD participants with intermittent claudication, the GPAD-2 study, will determine whether GM-CSF, administered three times weekly for four weeks, significantly improves treadmill walking performance in 160 PAD patients with intermittent claudication (NCT01041417). However, supervised treadmill exercise is not part of the intervention in GPAD-2. In addition, no prior studies have assessed whether GM-CSF significantly improves six-minute walk performance in people with PAD.

Preliminary data suggest that lower extremity ischemia, induced during walking exercise, may increase circulating CD34+ cell levels and enhance homing of CD34+ cells to ischemic sites, augmenting the ability of GM-CSF to improve walking performance in PAD (16-18). In support of this hypothesis, prior randomized trials of supervised treadmill exercise alone without GM-CSF show that as compared to a control group, supervised treadmill exercise significantly increases circulating EPCs and increases calf skeletal muscle capillary density in people with PAD (10,12,51,52). If supervised treadmill exercise and GM-CSF each increase circulating CD34+ cells, but only the groups receiving supervised treadmill exercise improve walking performance, this finding will demonstrate that increasing circulating levels of CD34+ cells alone is not sufficient to improve walking performance in PAD. Additionally, if supervised exercise alone and GM-CSF alone each improve walking performance, but their combined benefit does not exceed either intervention alone, this finding will suggest a ceiling effect for interventions that increase CD34+ cells to improve walking performance in PAD. To our knowledge, no prior studies have assessed whether the combination of supervised treadmill exercise and GM-CSF significantly improves walking performance in patients with PAD.

Although the landmark study by Asahara et al established that circulating progenitor cells can differentiate into mature endothelial cells and promote new blood vessel development, the method of injecting cells into the calf muscles is more invasive and costly than subcutaneous injections of GM-CSF (16). The subcutaneous injections given in the PROPEL Study are likely to be more acceptable to patients and will also be more cost effective than other alternatives for administering progenitor cells.

The PROPEL trial has some limitations. First, PROPEL does not include a measure of lower extremity muscle perfusion. Thus, PROPEL will not be able to determine the degree to which increases in lower extremity muscle perfusion improves walking performance in people with PAD. Second, the optimal time point for measuring improved outcomes in response to GM-CSF is not established. Peak levels of circulating CD34+ cells are observed 5-6 days after onset of GM-CSF therapy and subsequently decline even with continued GM-CSF injections. We selected 12-week follow-up as the time point of our primary outcome measure because previous study by Subramaniyam et al reported significant improvement in treadmill walking performance and brachial artery FMD at 12-week follow-up (11). Similarly, in studies of patients with coronary artery disease, improvements in cardiac ejection fractions or regional wall motion abnormalities are typically first observed one to three months after two weeks of GM-CSF therapy (53-55). These improvements are maintained or even increase during follow-up periods of up to one year (53-55). In addition, significant improvements in treadmill walking performance are observed 12-weeks after initiation of a supervised treadmill exercise intervention (56). Thus, although the optimal time point for measuring improved outcomes in response to GM-CSF is not established,

available data suggest that 12 weeks after therapy onset is a reasonable time point for measuring our primary outcomes.

In summary, if our results demonstrate that increasing circulating levels of CD34+ cells is associated with improved walking performance, with or without supervised treadmill exercise, then future studies should focus on methods that maximize circulating levels of CD34+ cells to improve walking performance in PAD. This information is expected to lead to new therapies for the large and growing number of patients with PAD who are debilitated by PAD-related walking limitations.

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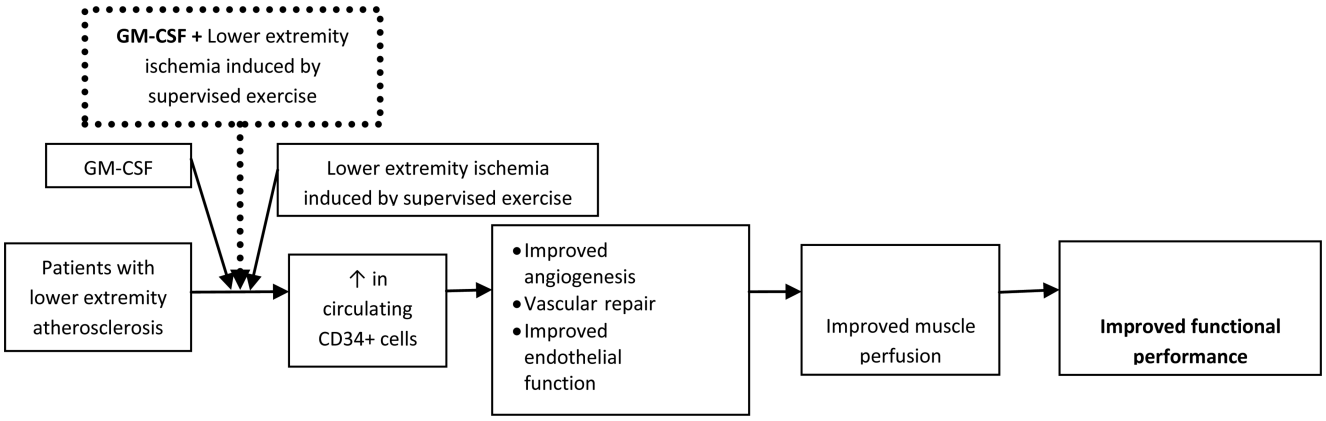

Figure 1.
Theoretical model by which interventions improve outcomes in the PROPEL Study.

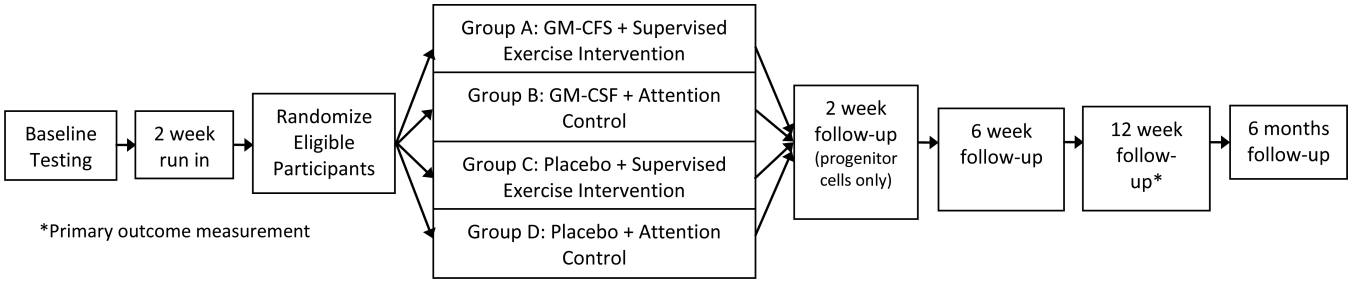

Figure 2.
Overview of data collection and interventions for the PROPEL Study

Table 1

Exclusion criteria for the PROPEL Study.

Exclusion Category/ Justification for Exclusion	Specific Exclusion criteria
Exclusion criteria selected because they may interfere with the participant's ability to participate fully in the study interventions.	Below or above-knee amputation Critical limb ischemia Wheelchair confinement Use of a walking aid (excluding canes) Unable/unwilling to return to medical center at required visit frequency Failure to complete study run-in phase Walking impairment primarily limited by a cause other than PAD Stopping during treadmill/six-minute walk tests for symptoms other than leg ischemia Significant visual or hearing impairment Poor fit for study based on investigator judgment Non-English speaking
Exclusion criteria selected because they may influence study outcomes independently of study participation	Lower extremity revascularization in the previous 9 months Major orthopedic surgery during the previous 6 months Myocardial infarction, stroke, or coronary artery bypass grafting during the previous 3 months Major surgery or lower extremity revascularization planned within the next 6 months Major medical illness including renal disease requiring dialysis during previous 12 months Current participation in other clinical trial or participation in another clinical trial during the previous 3 months Completion of cardiac rehabilitation within the last 6 months Walking at a level of exercise comparable to the level targeted by the exercise intervention Recipient of G-CSF, GM-CSF, or erythropoietin within the previous year Parkinson's Disease Requires oxygen with activity or exercise Mini-Mental Status Examination score < 23 Disabling psychiatric illness Pre-menopausal
Exclusion criteria selected because study participation may not be safe for individuals meeting these criteria.	Diagnosis of diabetes with proliferative retinopathy History of myeloid malignancy Treatment for late-stage cancer during previous three years >Class II New York Heart Association heart failure or angina Increase in angina symptoms during previous 6 months or angina at rest Severe aortic stenosis Coronary ischemia during the exercise stress test Left-bundle branch block or significant ST-T wave changes on baseline ECG

Table 2

Data collection plan for primary, secondary, and exploratory outcomes in the PROPEL Study

	Measurement time point				
	Baseline	**Two-week follow-up**	**Six-week follow-up**	**12-week follow-up**	**26-week follow-up**
Six-minute walk	X		Tertiary outcome measure	Primary outcome measure	Exploratory outcome measure
Brachial artery FMD	X		Tertiary outcome measure	Secondary outcome measure	Exploratory outcome measure
Treadmill walking performance	X		Exploratory outcome measure	Secondary outcome measure	Exploratory outcome measure.
CD34+ cells	X	Exploratory outcome measure	Exploratory outcome measure	Secondary outcome measure	Exploratory outcome measure