PROgenitor cell release Plus Exercise to improve functional performance in PAD: The PROPEL Study
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SPECIFIC AIMS

Preliminary evidence suggests that increasing circulating levels of CD34+ cells with granulocyte monocyte colony stimulating factor (GM-CSF) or other therapies may improve walking performance in patients with lower extremity peripheral arterial disease (PAD) (1,2). However, results of small clinical trials are mixed (1-4). The association of GM-CSF with improved walking performance in PAD is not definitively established. Preliminary data also suggest that lower extremity ischemia, induced during walking exercise, may increase circulating CD34+ cell levels, enhance homing of CD34+ cells to ischemic sites, and **augment** the ability of GM-CSF to improve walking performance in PAD (1,2). We propose a randomized controlled clinical trial (2 x 2 factorial design) of 240 participants with PAD randomized to one of four arms: Group A: GM-CSF + supervised exercise therapy; Group B: GM-CSF therapy + an attention control group; Group C: placebo + supervised exercise therapy; and Group D: placebo + attention control group. Our primary outcome is change in six-minute walk performance between baseline and 12-week follow-up. From this point forward, "GM-CSF combined with supervised treadmill exercise" refers to Group A; "GM-CSF therapy alone" refers to Group B, "supervised exercise therapy alone" refers to Group C, and no active therapy refers to Group D.

Primary Specific Aim.
1. We will determine whether GM-CSF combined with supervised treadmill exercise (Group A) achieves greater improvement in six-minute walk performance at 12-week follow-up, compared to GM-CSF alone (Group B) and supervised exercise alone (Group C), respectively. *We hypothesize that PAD participants randomized to Group A will achieve greater improvement in six-minute walk performance at 12-week follow-up, compared to those randomized to Group B and those randomized to Group C, respectively.*
2. We will determine whether GM-CSF therapy alone (Group B) significantly improves six-minute walk performance at 12-week follow-up, compared to Group D. *We hypothesize that PAD participants randomized to Group B will achieve greater improvement in the six-minute walk at 12-week follow-up than Group D.*
3. We will confirm that supervised treadmill exercise therapy alone (Group C) significantly increases six-minute walk performance at 12-week follow-up, compared to Group D. *We hypothesize that PAD participants randomized to Group C will achieve greater improvement in six-minute walk performance at 12-week follow-up, compared to those randomized to Group D*.

Secondary Specific Aim.
1. We will determine whether GM-CSF combined with supervised exercise (Group A) is associated with greater increases in brachial artery flow-mediated dilation (FMD) and maximal treadmill walking time at 12-week follow-up, compared to GM-CSF alone (Group B) and supervised exercise alone (Group C), respectively.
2. We will determine whether GM-CSF alone (Group B) is associated with significantly greater increases in brachial artery FMD and maximal treadmill walking time at 12-week follow-up, compared to Group D.
3. We will determine whether a supervised treadmill exercise intervention alone (Group C) is associated with greater increases in CD34+ cells at 12-week follow-up, compared to Group D.
4. We will confirm prior data demonstrating that supervised treadmill exercise alone (Group C) is associated with greater increases in brachial artery FMD and maximal treadmill walking time, compared to Group D.

In our Exploratory Aim, we will establish the <u>temporal trajectory</u> of favorable changes in study outcomes in response to GM-CSF. "Study outcomes" refers to six-minute walk performance, maximal treadmill walking time, and brachial artery FMD. We will also establish the temporal trajectory of increases in progenitor cells in response to supervised treadmill exercise. We will determine whether the degree of increase in progenitor cells is associated with the degree of improvement in study outcomes.

Exploratory Specific Aims.
1. We will determine whether GM-CSF combined with supervised treadmill exercise therapy (Group A) is associated with greater increases in study outcomes <u>at six-week follow-up</u> and at <u>six-month follow-up</u>, compared to GM-CSF therapy alone (Group B) and supervised exercise therapy alone (Group C), respectively.

2. We will determine whether GM-CSF alone (Group B) is associated with greater increases in study outcomes at six-week follow-up and at six-month follow-up, respectively, compared to Group D.
3. We will determine whether supervised exercise therapy alone (Group C) is associated with significantly greater increases in CD34+ cells and other progenitor cell measures at two-week follow-up, six-week follow-up and at six-month follow-up, compared to Group D.
4. Among participants receiving GM-CSF, we will determine whether the degree of increase in progenitor cell measures at two-week follow-up is associated with the degree of improvement in remaining study outcomes at six-week, 12-week, and six-month follow-up, respectively.
5. Among up to 30 participants with PAD and 15 consenting participants who are determined not to have PAD, we will determine whether progenitor cell measures increase acutely after a treadmill exercise stress test among participants with PAD and among those without PAD, respectively. We will further determine whether greater increases in progenitor cell measures after a treadmill exercise stress test are associated with better six-minute walk performance and higher ABI values at baseline, compared to lesser increases in progenitor cell measures after a treadmill exercise stress test.
6. Study participants will have the option of participating in a sub study that consists of a muscle biopsy of the medial head of the gastrocnemius muscle. The muscle biopsy will be performed by Dr. Robert Sufit, a board certified neurologist with more than 20 years of experience performing lower extremity muscle biopsies. The muscle biopsy will be performed in a clinical examination room using an open biopsy technique. A subcutaneous injection of lidocaine will be administered. For the lower extremity biopsy, an incision will be made in the lower extremity muscle (medial head of the gastrocnemius muscle and approximately 250 mgs of muscle tissue will be removed. Most or all of the sample will be frozen immediately in liquid nitrogen and stored at -80 degrees Celsius. Participants will be asked to return for a follow-up visit to check the site of the muscle biopsy approximately one week after the procedure. Participants may be asked to undergo a muscle biopsy at baseline and after 26-week follow-up. Thus, the total number of muscle biopsies a participant may be asked to have is two (one lower extremity biopsy at baseline and one at six-month follow-up). We may take photographs of the biopsy site and biopsy tissue before, during, and after the procedure. We may take photographs at the post-biopsy incision check-up as well to show the scar and healing. These images will not show any identifying information such as name or date of birth. These images may be used for grant proposals, manuscripts, or other research related activities. Muscle specimens will be sent to the Universities of Florida and Kentucky for analyses that include inflammatory biomarkers, markers of oxidative stress, measures of mitochondrial function and quantity, and muscle myofiber typing. Analyses may also be tested for protein measures of mitochondrial function, macrophages, satellite cells, and PCR/gene expression. Other measures related to skeletal muscle quality and function may also be performed.

In addition, in a subset of participants we will obtain a muscle biopsy from the left and the right leg, respectively. These bilateral biopsies could be obtained at baseline, at 26-week follow-up, or at both time points. The second (contralateral) biopsy will be performed at least six days after the first biopsy. In some cases, the second biopsy may take place when the participant returns for their incision site check after the first biopsy. It will be necessary for these individuals to remain off of their anti-platelet therapy for 7 days before each procedure (i.e. potentially for approximately 14 days continuously). Physician approval will be required.

A. RESEARCH STRATEGY- SIGNIFICANCE

A1. Lower extremity peripheral arterial disease (PAD) is common and is associated with functional impairment and functional decline. PAD affects eight million men and women in the United States, and will be increasingly prevalent as the U.S. population survives longer with chronic disease (5). Our work and that of others demonstrates that men and women with PAD have greater functional impairment and more rapid functional decline than those without PAD (6-10). The functional impairments documented in PAD are associated with loss of independence, increased mortality, and poor quality of life (11-13).

A2. Few medical therapies have been identified that improve functional impairment in PAD. Only two medications, pentoxifylline and cilostazol, are FDA-approved for treating PAD-associated walking impairment. Of these, pentoxifylline is usually ineffective and benefits from cilostazol are modest (14-17). New therapies are urgently needed to improve walking performance and prevent functional decline in patients with PAD.

A3. Interventions that increase circulating progenitor cells are among the most promising emerging therapies for patients with cardiovascular disease. Progenitor cells normally exist in low concentrations in peripheral blood. Interventions, such as GM-CSF, promote release of progenitor cells from bone marrow, spleen, and other sources into the circulation (18-20). These circulating progenitor cells can differentiate into mature endothelial cells and form new blood vessels, termed neoangiogenesis (21-24). In 1997, Asahara and colleagues demonstrated that CD34+ progenitor cells isolated from human peripheral blood differentiate into mature endothelial cells in vitro (22). In a rabbit hind limb ischemia model, these isolated CD34+ cells migrated to sites of tissue ischemia and incorporated into developing blood vessels (22). This work established that circulating progenitor cells isolated from humans can differentiate into mature endothelial cells and promote new blood vessel development in vivo. Progenitor cells also repair endothelial-cell injury and are associated with improved endothelial function (21-30). Mobilizing EPC reserves may improve walking performance in patients with PAD by increasing calf muscle perfusion through angiogenesis and by improving endothelial function and cardiovascular health. However, whether interventions that increase circulating progenitor cell levels improve functional performance in patients with PAD is not established.

A4. Therapeutic potential of CD34+ progenitor cells. The CD34+ cell marker clearly identifies functional progenitor cell populations that are released from bone marrow in response to tissue ischemia, differentiate into endothelial cells, and promote angiogenesis in vitro and in vivo (22,31,32). In addition to the work by Asahara and colleagues described in section A3 (22), Ishida and colleagues harvested CD34+ cells from peripheral blood and injected them into calf muscle of six patients with peripheral ischemia (five with thromboangiitis obliterans and one with critical limb ischemia). At six-week follow-up, participants experienced a 200% increase in treadmill walking distance (32). However, the sample size was small and did not include a control group. It is currently not established whether interventions that increase circulating levels of CD34+ cells can improve functional performance in patients with PAD.

A5. Preliminary evidence suggests that GM-CSF may improve walking performance in people with PAD by increasing CD34+ cells. Subcutaneous GM-CSF injections increase levels of circulating CD34+ cells by disrupting progenitor cell anchors in the bone marrow (33). In hind-limb ischemia animal models, increasing circulating levels of progenitor cells is associated with calf muscle capillary formation, improved calf muscle perfusion, and increased exercise capacity (22,31,32). However, three prior small clinical trials testing the ability of GM-CSF (or G-CSF) to improve walking performance in patients with PAD have shown mixed results (1,3,4). Of these three randomized controlled clinical trials (summarized in Table 1), one demonstrated improved maximal walking distance (1) and one demonstrated no change in treadmill walking performance in response to GM-CSF (3). While the third study demonstrated improved pain-free treadmill walking distance, the validity of this finding is questionable, since only four participants completed follow-up treadmill testing (4). Additional limitations of these studies follow. First, sample sizes were small. Second, generalizability of the findings to the typical patient with PAD is limited. Participants in the study by Arai and colleagues all had critical limb ischemia (4), those in the study by Van Royan and colleagues all had maximal walking distances < 200 meters (3), and those in the study by Subramaniyam and colleagues all had intermittent claudication (1). Yet most PAD participants will never have critical limb ischemia, many can walk > 200 meters on the treadmill, and most do not have symptoms of intermittent claudication (6,8,34,35). Third, no study assessed whether episodes of lower extremity ischemia, induced during supervised treadmill exercise, increases responsiveness to GM-CSF. Fourth, temporal trends in walking outcomes in response to the intervention were not evaluated in any study. The length of time required to achieve maximal benefit from GM-CSF and the duration of any therapeutic benefit is unknown. In summary, whether GM-CSF (or G-CSF) improves treadmill walking performance, with or without supervised treadmill exercise, remains unknown. A definitive trial is needed.

Table 1. Summary of Prior Small Clinical Trials Studying the Ability of G-CSF or GM-CSF Therapy to Improve Walking Performance in PAD

Study	Participants	Intervention(s)	Outcome Measure	Results	Study Limitations
Van Royen N et al (2005) (3)	40 with PAD and intermittent claudication (IC) who walked < 200 meters on the treadmill.	GM-CSF vs. placebo injections over 14 days.	Maximal treadmill walking time at 12-week follow-up.	No difference between the intervention vs. control group in increasing treadmill walking performance.	1. Small sample size. 2. Limited to PAD participants with IC and short maximal walking distance. 3. No supervised exercise therapy.
Arai M et al (2006) (4)	39 participants with PAD and critical leg ischemia.	G-CSF for 10 days (N=14) vs. bone marrow transplant and calf injection of mononuclear cells (N=13) vs. usual care (N=12).	Treadmill pain-free walking time, ABI, and transcutaneous oxygen saturation at 4-week follow-up.	G-CSF and bone marrow transplant were each associated with greater improvement in each outcome compared to usual care.	1. Small sample size. 2. Limited to participants with critical limb ischemia. 3. Only 4 participants completed treadmill test at follow-up.
Subramaniyam et al (2009) (1)	45 with PAD and intermittent claudication.	GM-CSF (N=29) vs. placebo injections (N=16) three times weekly for two weeks. All participants were instructed to exercise.	CD34+ cells, brachial artery FMD, and walking performance at 12-week follow-up.	GM-CSF was associated with significant increases in CD34+ cells, brachial artery FMD, and treadmill performance, compared to placebo.	1. Small sample size. 2. Participants exercised on their own. Exercise adherence was not measured and is known to be poor in PAD (36).

IC- Intermittent claudication. PAD- Peripheral arterial disease. ABI- Ankle brachial index. FMD-Flow mediated dilation.

A6. Supervised treadmill exercise may increase responsiveness to GM-CSF therapy in PAD. Treadmill exercise induces lower extremity tissue ischemia in patients with PAD. Experimental and animal research demonstrate that tissue ischemia and hypoxia promote release of progenitor cells from bone marrow and encourage homing of progenitor cells to ischemic sites (37-41). Homing of progenitor cells to ischemic sites is mediated by up-regulation of progenitor cell receptors in target tissues (i.e. calf muscle) and by up-regulation of tissue receptors on progenitor cells (37-43). Myocardial ischemia increases responsiveness to G-CSF in patients with coronary artery disease (40). Together, these data suggest that tissue ischemia, such as that induced during supervised treadmill exercise, may increase responsiveness to GM-CSF. However, whether GM-CSF combined with supervised treadmill exercise improves functional performance more than either GM-CSF alone or supervised treadmill exercise alone is unknown.

A7. Supervised treadmill exercise may improve walking performance in patients with PAD by increasing circulating progenitor cell levels. Our prior randomized controlled clinical trial (the Study to Improve Leg Circulation (SILC)) compared the ability of a supervised treadmill exercise intervention and a supervised lower extremity resistance training intervention, respectively, to improve functional performance and brachial artery flow-mediated dilation in 156 PAD participants, both with and without intermittent claudication symptoms (R01-HL073351) (44). The primary outcome measure was 6-month change in the distance achieved in the six-minute walk test. Results, summarized below, were published in JAMA (44).

Table 2. Results of our SILC randomized controlled clinical trial of supervised exercise interventions in PAD participants with and without symptoms of intermittent claudication (R01-HL073351) (44).

Absolute change between baseline and six-month follow-up	Treadmill Exercise (n=50)	Resistance Exercise (n=46)	Control group (n=47)
6 minute walk distance (meters)	**+21.28**[1]	-2.60	-15.02
Maximal treadmill walking time (minutes)	**+3.69**[1]	+2.41[2]	+0.51
Brachial artery FMD 60 seconds after cuff release (percent).	**+0.70%**[3]	+0.11%	-0.89%

[1] p<0.001 vs. control; [2] p =0.009 vs. control; [3] p=0.02 vs. control. FMD- flow mediated dilation.

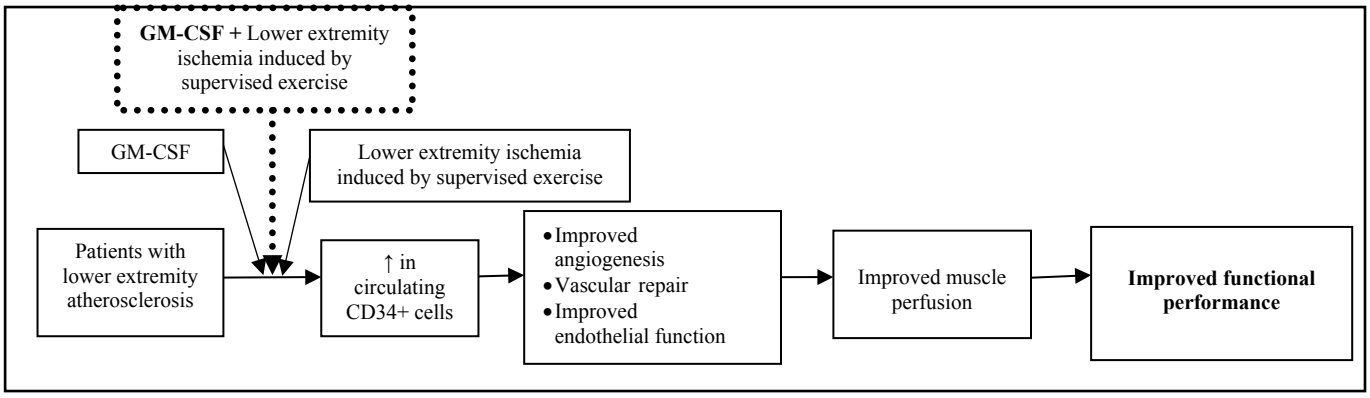
As shown in Table 2, our SILC trial demonstrated that supervised treadmill exercise improves walking performance and brachial artery flow-mediated dilation (FMD) in PAD participants with and without intermittent

claudication (44). SILC was the **first** randomized controlled trial to demonstrate that supervised treadmill exercise improves brachial artery FMD, a measure of endothelial function, in PAD (44). However, mechanisms of the favorable associations shown in Table 2 are unknown. We hypothesize that supervised treadmill exercise may improve outcomes by increasing circulating levels of CD34+ cells.

A8. Preliminary evidence suggests that walking exercise may increase circulating CD34+ cell levels in patients with PAD. Patients with PAD experience calf muscle ischemia during supervised treadmill exercise. Experimental and animal studies demonstrate that tissue ischemia promotes endogenous release of progenitor cells and increases homing of the progenitor cells to sites of tissue ischemia (37-43). Several small, uncontrolled, or non-randomized trials have demonstrated that exercise increases circulating progenitor cells **in patients without PAD** (21,44-48). However, people with PAD have fewer circulating progenitor cells and exercise at a lower intensity than people without PAD (49,50). Only one previous publication describes the association of exercise training with changes in circulating progenitor cells among patients with PAD (2). This publication reports results from two small randomized controlled trials. Study A included 18 patients with PAD and intermittent claudication without history of lower extremity revascularization. Study B included 18 patients with PAD and intermittent claudication with a history of lower extremity revascularization. Participants in Study A and Study B were randomized to either unsupervised treadmill exercise or no exercise for four weeks. The unsupervised treadmill walking was performed 30 times per week: ten times more frequently than the exercise currently recommended in clinical practice guidelines for PAD (2,35). At four-week follow-up, PAD participants randomized to exercise in Study A had significant increases in CD34+ cells, while PAD participants randomized to exercise in Study B had no increases in CD34+ cells. Neither control group experienced increases in CD34+ cells. This preliminary work demonstrates that unsupervised treadmill exercise may increase CD34+ cells in patients with PAD. However, the walking exercise intervention was performed 10 times more frequently than the exercise frequency recommended by PAD clinical practice guidelines (35). It is unknown whether supervised treadmill exercise, performed 3 times weekly, as recommended by current clinical practice guidelines, increases CD34+ cells in patients with PAD.

A9. Theoretical model for our proposed PROPEL Study.

Figure 1. Theoretical Model for Associations of Study Interventions with Improved Functional Performance in Participants with Peripheral Arterial Disease



As described in Sections A3 and A4 above, experimental and animal data demonstrate that progenitor cells released into the circulation can differentiate into endothelial cells, form new blood vessels (angiogenesis), repair injured endothelium, and improve endothelial function (22-24). Preliminary data also suggest that tissue ischemia, such as that induced by walking exercise in patients with PAD, may increase circulating progenitor cells and promote their homing to sites of tissue ischemia (37-43). We hypothesize that GM-CSF combined with supervised treadmill exercise maximizes circulating levels of CD34+ cells in patients with PAD and improves calf muscle perfusion, by promoting neo-angiogenesis, improving endothelial function, and promoting vascular repair. Figure 1 shows our proposed theoretical model for our study interventions.

B. INNOVATION

B1. Innovative features of the proposed PROPEL Study. First, to our knowledge no prior studies have assessed whether the combination of GM-CSF and supervised treadmill exercise improves walking performance more than GM-CSF alone or supervised treadmill exercise alone in PAD. Yet, experimental and animal research demonstrate that tissue ischemia and hypoxia, such as that experienced by PAD patients during treadmill exercise, promote release of progenitor cells from bone marrow and encourage homing of progenitor cells to ischemic sites (37-43). Second, the time course of increases in progenitor cells in response to GM-CSF or supervised treadmill exercise among PAD participants is not defined. Although our primary outcome will be measured at 12-week follow-up, the PROPEL study will measure changes in CD34+ cells in response to study interventions at 2-week, 6-week, 12-week, and 6-month follow-up. Our results will help define the time-point of maximal improvement and the duration of improvement in study outcomes in response to study interventions and associated increases in progenitor cells. Third, to our knowledge no prior studies have directly assessed whether the degree of lower extremity ischemia achieved during treadmill exercise is associated with the degree of increase in circulating progenitor cells or the degree of improvement in study outcomes. We will measure lactate levels during treadmill exercise sessions to establish whether participants who achieve greater increases in lactate during exercise, indicating greater muscle ischemia, have greater increases in progenitor cells and greater improvement in study outcomes (see section C7).

B2. Distinctive features of this trial as compared to prior work. We are aware of one ongoing randomized clinical trial comparing the ability of GM-CSF vs. placebo to improve walking performance in patients with PAD (NCT number NCT0104141). Our proposal has many unique features as compared to this ongoing study. First, our proposal will test whether the combination of GM-CSF and supervised treadmill exercise is superior to GM-CSF alone and supervised treadmill exercise alone, respectively, for improving functional performance in PAD. Preliminary data support our hypothesis that GM-CSF may be most efficacious when delivered with supervised treadmill exercise, but this question is not being addressed by any study to our knowledge. Second, by measuring outcomes at six-weeks, 12-weeks, and six-month follow-up, our proposal will assess the temporal trajectory of favorable changes in response to GM-CSF with and without exercise therapy. Third, our proposal includes PAD participants both with and without classical intermittent claudication symptoms. This is important because most PAD patients do not have classic intermittent claudication symptoms. Asymptomatic PAD patients and PAD patients with atypical symptoms have significant functional impairment and faster functional decline than people without PAD (6,7,51). Fourth, whether supervised treadmill exercise, at the frequency and duration recommended by clinical practice guidelines (35), increases CD34+ cells in PAD participants is unknown. Our proposal will help establish whether increases in CD34+ cells mediate the favorable effects of supervised treadmill exercise in PAD. Fifth, we will use the six-minute walk test as our primary outcome measure. Our data and that of others demonstrate that the six-minute walk test is a better measure of community walking ability than treadmill testing in patients with PAD (see Section C12) (52-54).

B3. Additional innovative features. A unique feature of the proposed PROPEL study is the planned comparison in change in walking performance between two interventions that are each expected to increase circulating CD34+ cells. If supervised treadmill exercise and GM-CSF each increase circulating CD34+ cells, but only the groups receiving supervised treadmill exercise improve walking performance, this result will demonstrate that increasing circulating levels of CD34+ cells 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 would suggest a ceiling effect for the ability of interventions that increase CD34+ cells to improve walking performance in PAD. This proposed study is expected to identify biological pathways associated with improved functional performance in participants with PAD. In turn, this information is expected to lead to new therapies to help patients with PAD improve their functional performance and avoid decline. If our results demonstrate that increasing circulating levels of CD34+ cells is associated with improved walking performance, then future studies are expected to focus on methods that maximize circulating levels of CD34+ cells to improve walking performance in PAD.

C. METHODS

All data will be collected for research purposes only.

C1a. PRELIMINARY DATA. Our multidisciplinary investigative team includes internationally recognized experts in PAD, progenitor cells, exercise training, endothelial function, and functional performance. Since 1998, our observational work on functional impairment and decline in PAD has been funded by six R01 awards from the National Heart Lung and Blood Institute (NHLBI), led by Dr. McDermott. Our prior work establishes that participants with PAD, both with and without classic symptoms of intermittent claudication, experience significant declines in walking performance over time (7,51,55). Even asymptomatic PAD participants have greater impairment in functional performance and faster rates of functional decline compared to those without PAD (7,51,55-57). Since 1998, our group has completed more than 75 original research articles on PAD. Three or more of these original research manuscripts have been published in JAMA, Annals of Internal Medicine, Circulation, Journal of the American College of Cardiology, and other widely circulated journals.

Our ability to successfully complete this proposed study is demonstrated in part by our recently completed randomized controlled trial of exercise in PAD participants both with and without intermittent claudication symptoms (the SILC trial, R01-HL073351) (44). SILC was the first study to test the ability of an exercise intervention to improve walking performance in PAD participants with and without intermittent claudication symptoms. SILC was also the first randomized controlled trial to test the ability of an exercise intervention to increase brachial artery FMD in PAD participants. Results were published in JAMA (44) and are described in section A7. **Mechanisms** by which supervised treadmill exercise improves outcomes in PAD are not established. The current proposal builds on our prior work. We will assess whether supervised treadmill exercise increases the ability of GM-CSF to improve walking performance in PAD, establish whether supervised treadmill exercise increases circulating CD34+ cells in participants with PAD, and determine whether the degree of increase in circulating CD34+ cells correlates with the degree of improvement in walking performance and brachial artery FMD in participants with PAD. Our proposed PROPEL study is expected to identify biological pathways associated with improved functional performance in participants with PAD.

C1b. Test re-test reliability analysis of progenitor cell measures in our laboratory. In preparation for our proposed PROPEL study, we assessed test re-test reliability of our progenitor cell measures. Progenitor cells were measured in 29 participants with and without PAD on two separate days, 1-2 weeks apart. Progenitor cells were measured in Dr. Douglas Losordo's laboratory at Northwestern. Table 3 shows our results.

Table 3. Results of test re-test reliability analysis for progenitor cell measures in our laboratory (n=29)

Test	CD34+	ALDH+	CD34+/CD133+
Mean value (cells/ml)	635.3	812.0	509.4
Intra-pair standard deviation	72.8	75.2	60.7
Technical Error (%)	11.46	9.26	11.91

Table 3 results demonstrate excellent test re-test reliability of progenitor cell measures in our laboratory.

C1c. Associations of progenitor cell levels with six-minute walk performance. We also studied associations of circulating CD34+ cells with six-minute walk test performance among the 19 participants with PAD in our pilot study. As shown in Table 4, higher numbers of circulating CD34+ cells are associated with greater distance achieved in the six-minute walk among PAD participants.

Table 4. Associations of circulating progenitor cell concentrations with six-minute walk distance among PAD participants in our pilot study (n=19)*

	Correlation Coefficient with six-minute walk	P Value
CD34+ cell concentration	0.44	0.06
CD34+/CD133+ cell concentration	0.38	0.11

*Excludes pilot study participants without PAD.

In addition, among the 19 PAD participants, six-minute walk distance across tertiles of CD34+ cells were Tertile 1 (fewest CD34+ cells): 1,063 feet ± 438; Tertile 2: 1,306 feet ± 347; Tertile 3 (largest number of CD34+ cells): 1,394 feet ± 268, p trend = 0.18. In summary, our preliminary data demonstrate that higher concentrations of CD34+ cells are associated with better six-minute walk performance.

C1d. Additional evidence of our expertise with progenitor cells and PAD. Dr. Losordo recently completed a randomized controlled trial of 28 PAD participants with critical limb ischemia who were randomized to either a control group vs. CD34+ cell mobilization, apheresis, and re-injection of CD34+ cells into calf muscle. At six-month follow-up, amputation-free survival was significantly higher among those receiving CD34+ cell calf injections as compared to the control group (hazard ratio =0.26, p=0.016). This study provides additional evidence of our expertise and the benefits of CD34+ cells for patients with PAD.

Preliminary Data Summary. Our prior work has established that people with PAD, both with and without intermittent claudication symptoms, have greater functional impairment and faster rates of functional decline compared to those without PAD (6-8,51,55-57). Our SILC randomized trial of exercise demonstrated that a supervised treadmill walking exercise intervention significantly improves walking performance and brachial artery flow-mediated dilation in PAD participants both with and without symptoms of intermittent claudication (44). Our pilot data demonstrate that higher concentrations of CD34+ cells are associated with better walking performance in PAD. Our preliminary work demonstrates the validity, reliability, and feasibility of our methods.

C2. STUDY OVERVIEW. The PROPEL study design is a randomized controlled clinical trial of 240 participants with PAD who will be randomized to one of four study arms in a 2 x 2 factorial design: Group A: GM-CSF and supervised treadmill exercise; Group B: GM-CSF and attention control condition; Group C: placebo injections and supervised treadmill exercise; Group D: placebo injections and attention control condition (Figure 2). Potentially eligible participants will be required to successfully complete a two-week combined exercise/attention control run-in period prior to randomization. The run-in will help identify potential participants unlikely to adhere to study requirements (58).

Figure 2. Study Flow

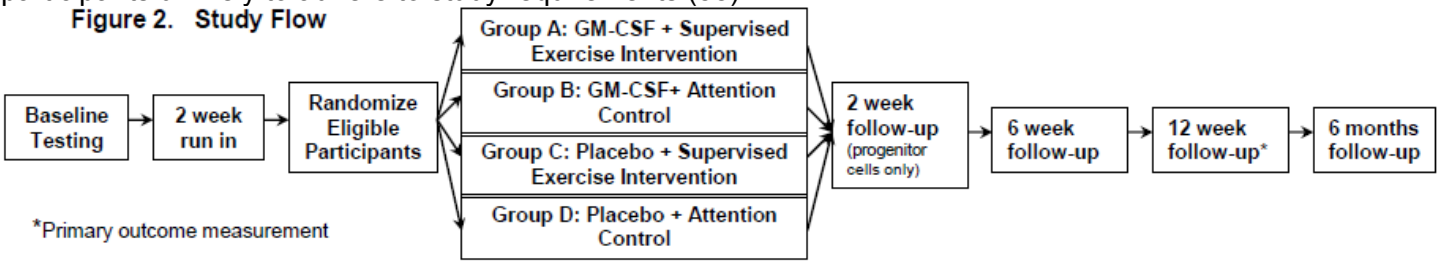


Table 5 summarizes characteristics of the four study intervention groups.

Table 5. Four Study Arms

	Supervised Treadmill Exercise	Attention Control Condition
GM-CSF	**Group A**. GM-CSF injections x 2 weeks + Supervised treadmill exercise x 6 months	**Group B**. GM-CSF injections x 2 weeks + Attention control condition x 6 months.
Placebo	**Group C**. Placebo injections x 2 weeks + Supervised treadmill exercise x 6 months.	**Group D**. Placebo injections x 2 weeks + Attention control condition x 6 months.

Table 6 shows the data collection planned at each time point. Our primary outcome is change in six-minute walk performance between baseline and 12-week follow-up.

Table 6. Data Collection Schedule for PROPEL Study

	Baseline	Two-week follow-up	Six-week follow-up	**Twelve week follow-up***	Six-month follow-up
Six-minute walk.	X		X	**X**	X
CD34+ cells and other progenitor cell measures	X	X	X	**X**	X
Brachial artery flow-mediated dilation.	X		X	**X**	X
Treadmill walking performance.	X		X	**X**	X

***Time point of primary outcome measurement**

We have not powered the study to test for an interaction between the two therapies. We are not necessarily looking for more than an additive benefit of GM-CSF with supervised treadmill exercise as compared to GM-CSF alone and supervised treadmill exercise alone. This is because any additional significant improvement in walking performance from combining GM-CSF and supervised treadmill exercise, compared to either therapy alone, will have significant clinical implications for PAD therapy.

C3. Inclusion Criteria. All participants will have PAD. PAD will be defined as follows. First, an ABI ≤ 0.90 at the baseline study visit is an inclusion criterion for PAD (60-63). ABI ≤ 0.90 is a well accepted standard for defining PAD (60-63). Second, potential participants who have an ABI > 0.90 but ≤ 1.00 and experience a 20% drop in ABI after the heel-rise exercise will be eligible. Third, potential participants who have an ABI > 0.90 but have a documented lower extremity revascularization procedure and experience a 20% drop in ABI after the heel-rise exercise will be eligible. Fourth, potential participants with an ABI > 0.90 who have vascular lab evidence of PAD will be eligible.

C4. Exclusion Criteria. Exclusion criteria and justification for each criterion follow. 1. Potential participants with below or above-knee amputation or critical limb ischemia, those who are wheelchair confined, those who use a walking aid other than a cane (i.e. people using walkers), those who are non-English speaking, those with significant hearing or visual impairment, and those unable to return to the medical center three times weekly will be excluded because these individuals will not be able to participate fully in our intervention; 2. Potential participants diagnosed with Parkinson's disease and individuals whose walking is limited by a symptom other than PAD will be excluded because our intervention is designed specifically for individuals whose walking is limited by leg ischemia. 3. Potential participants with > Class II New York Heart Association heart failure or angina, an increase in angina symptoms during the previous 6 months, angina at rest, severe aortic stenosis, coronary ischemia during the baseline exercise treadmill test, those with a left-bundle branch block or significant ST-T wave changes on the baseline ECG precluding interpretation of the baseline exercise treadmill test, those who stop during the treadmill stress test for shortness of breath, chest pain, hip pain, knee pain, or another symptom that may not represent ischemic leg pain, stopping during the six-minute walk test for symptoms other than ischemic leg pain, or a foot ulcer will be excluded because it may not be safe for them to enter an exercise program. 4. Potential participants who have had lower extremity revascularization during the past three months, those with major orthopedic surgery during the past three months, those with myocardial infarction, ischemic stroke, or coronary artery bypass grafting during the previous three months, and those contemplating lower extremity revascularization during the next six months will be excluded because these events may influence study outcomes, independently of the study interventions; 5. Potential participants with major medical illnesses including renal disease requiring dialysis or lung disease requiring oxygen will be excluded because these and other major medical illnesses may prevent their full participation in the study. Participants who use oxygen at night may still qualify. 6. Potential participants with a Mini-Mental Status Examination (MMSE) score < 23 (64) or disabling psychiatric illness will be excluded because these conditions may prevent their full study participation or ability to provide accurate responses to questionnaires; 7. Potential participants enrolled in cardiac rehabilitation during the past six months and those who are either currently enrolled in another clinical trial or who have been enrolled in another clinical trial during the past three months will be excluded because these interventions may alter outcome measures, independently of study interventions. However, for a clinical trial of stem cell or gene therapy intervention, potential participants will be potentially eligible immediately after the final study visit of the stem cell or gene therapy clinical trial, so long as at least six months has passed since the participant received their final treatment in the stem cell or gene therapy intervention. 8. Potential participants already walking for exercise at a level comparable to that targeted in the exercise intervention and those receiving G-CSF, GM-CSF, or erythropoietin within the past year will be excluded because these interventions may influence study outcomes independently of the interventions. 9. Pre-menopausal women will be excluded because cyclic estrogen changes can influence progenitor cell levels. 10. Potential participants with diabetes and documented proliferative retinopathy, those with history of myeloid malignancy, and those treated for late stage cancer during the previous three years will be excluded because GM-CSF may exacerbate these conditions. 11. Potential participants who fail to complete the study run-in requirements will be excluded because it may be difficult for them to adhere to the study requirements; 12. Potential participants who are deemed poor candidates for the study may be excluded at the discretion of the principal investigator. For example, potential participants who are argumentative or disruptive during study visits may not be well suited to the program.

C5. Recruitment. We will identify and randomize 267 PAD participants over 49-months, allowing for a 10% drop-out at 6-month follow-up (see timeline in our budget justification). This projected 10% drop-out rate is greater than the six-month drop-out rate of 7.7% in our recently completed SILC randomized controlled exercise clinical trial (44). Thus, our projected drop-out rate is reasonable. As in our previous clinical trials of patients with PAD, we will identify potential participants with the following methods: 1) Use of Northwestern's

Enterprise Data Warehouse to identify consecutive patients with PAD or patients at high risk for having PAD from Northwestern Medicine, including the Regional Medical Group in the western suburbs. We will contact physicians via phone, email, page, or fax prior to contacting their patients. If physicians do not reply within three weeks, up to five letters will be sent directly to the patient three weeks apart; 2) television, radio and newspaper advertising; 3) postcard mailings to men and women living in the wider Chicago area; 4) Mailed letters to participants in the Lifeline Screening program who were found to have PAD; 5) Study brochures and information flyers. 6) Mailed letters to University of Chicago patients with known PAD. A research coordinator at the University of Chicago will provide a list of patients with known PAD and NU coordinators will send letters, signed by a UofC physician, and make follow-up calls from UofC. 7) Mailed letters to Jesse Brown VA Medical Center patients with known PAD. A research coordinator with WOC status at JBVAMC will send letters to JBVAMC patients with PAD and make follow-up calls from JBVAMC. Participants recruited through these methods will sign a VA consent document and will undergo some study testing on-site at the VA. Study tests include questionnaires and functional performance measures. We have successfully used each of these methods in our prior or ongoing clinical trials of patients with PAD.

Evidence of our experience successfully recruiting PAD participants for NHLBI-funded studies: In the past five years we have randomized > 463 PAD participants into NHLBI-funded clinical trials. In addition, since 1998, we have enrolled more than 1,800 men and women with PAD into our NHLBI-funded studies (R01-HL58099, R01-HL073351, R01-HL071223, R01-HL073912, R01-HL083064, and R01-HL089198). We have accomplished this using methods described above and by partnering with other Chicago medical centers.

C6. Randomization. Participants will be randomized to one of four arms using a SAS computer program (see Table 5). Participants will be stratified by diabetes mellitus, since patients with diabetes have fewer progenitor cells than those without diabetes (49,50). Based on our prior work, we anticipate that approximately 25% to 30% of participants will have diabetes (44). Block randomization will be implemented to ensure balance between the four groups throughout recruitment. Block sizes will be randomly selected from eight and twelve.

C7. Supervised treadmill exercise program. Our exercise training protocol is based on prior studies, including our SILC clinical trial of exercise in PAD, and is consistent with current clinical practice guidelines for PAD patients (35,44,65-67). Our exercise intervention will be delivered three times weekly. In the first week, participants will be asked to exercise 15 minutes per session (excluding rest periods). Walking exercise duration will be increased by five minutes per session each week until 40 to 50 minutes of exercise per session is achieved (35). At this point, exercise intensity will be increased by alternately increasing treadmill speed and treadmill grade. Exercise intensity will be set to ensure that at each exercise session, participants experience ischemic leg symptoms within eight to ten minutes after onset of exercise. Participants will be asked to continue walking until they achieve leg symptom severity of “4 or 5” on a visual analog pain scale ranging from 1 to 5. After achieving leg symptom severity of “4 or 5” on the pain scale, participants will be allowed to rest until they are able to resume walking again (i.e. when pain reduces to a 2 or 1 on the scale). This method will help ensure that calf muscle ischemia is induced, releasing nitric oxide and vascular endothelial growth factor, which in turn promote release of progenitor cells (68,69). In the subset of participants without exertional leg symptoms (i.e. those with asymptomatic PAD), participants will be asked to exercise to achieve a Borg Rating of Perceived Exertion (RPE) score of 12-14 (70-73). To ensure that exercise intensity is sufficient to achieve an anaerobic state during exercise, we will measure lactic acid levels every two to four weeks at the beginning and end of exercise sessions. Participants whose lactic acid does not increase during exercise will have their exercise intensity increased at the next session and will be even more closely monitored for exercise intensity.

C8. Attention Control Group. Our attention control group will control 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 will attend weekly one-hour educational sessions at Northwestern University for six months. These educational sessions are on topics of interest to the typical PAD patient and are led by physicians and other health care workers. Topics include Medicare Part D, nutritional supplements, C-reactive protein, and hypertension. Sessions do not include information about exercise.

C9. GM-CSF or placebo injections. GM-CSF or placebo will be administered subcutaneously by a registered nurse or physician, three times weekly for two weeks, in a double-blinded fashion (i.e. both investigators and

participants will be blinded to receipt of medication vs. placebo). GM-CSF will be used because the most promising data regarding the association of colony stimulating factors with improved walking performance in PAD participants used GM-CSF (1). In addition, GM-CSF may be associated with a lower risk of thromboses than G-CSF (1). The dose of GM-CSF will be $250\ ug/m^2/day$ subcutaneously three times weekly for two weeks (1). Higher doses of GM-CSF are not FDA approved. The most promising preliminary study of GM-CSF to improve walking performance in PAD patients used doses of 3, 6, and 10 ug/kg administered three times weekly (1). Although there was no clear linear dose-response association, the doses of 6 ug/kg and 10 ug/kg achieved the greatest number of circulating CD34+ cells, compared to the lower dose (1). The proposed dose of $250\ ug/m^2/day$ is expected to maximize benefit while minimizing side effects. To monitor for adverse effects, we will measure white blood count (WBC) once each week during the two weeks that the GM-CSF is administered. Additional WBCs might be measured at the discretion of the Safety Monitor, during the time period that study medication is administered. The study drug will be discontinued if the participant experiences an arterial thrombotic event or is hospitalized for a reason that may be related to the study drug. If a participant is hospitalized for a reason that may be related to the use of the study drug, the decision to reduce the drug dosage or discontinue the drug will be made by study safety officers Dr. Green or Dr. Lloyd-Jones on a case-by-case basis. In general, if a patient is suspected of acute coronary syndrome or a new significant arrhythmia, their study drug injections will be withheld until appropriate medical work-up is completed. If the participant is determined NOT to have an acute coronary syndrome, active coronary disease, or a significant arrhythmia, the participant may resume full does of the study medication. However, if the participant is determined to have an acute coronary syndrome or active, significant arrhythmia, then additional receipt of study drug, at full-dose or reduced does, will be made on a case-by-case basis by the study safety officers. The study nurse will have Tylenol available in the event of acute muscle pain following the study injection. The Tylenol will be provided by NMH pharmacy and administered at a maximum dose of 1000mg.

Monitored Walking Program: Potentially eligible study participants will be telephoned by study staff and invited to participate in this walking program. Interested participants will be asked to come to our walking exercise facility (i.e. Northwestern Memorial Hospital's Cardiac Rehab program) or to Northwestern Memorial Hospital where they will be provided with a device, an accelerometer and/or a pedometer that tracks their walking exercise activity. They will be shown how to use their device. They will be asked to use their device to monitor their activity, including walking exercise activity. They will be shown how to transmit the data from their accelerometer/pedometer back to the study coach. They will also be asked to record data on exercise time and distance using a paper tracking form. They may also be asked to send a text message to the study coach at the start and end of each walking exercise session, with details about their walking activity including the duration and intensity of each walking exercise bout.

In this monitored walking program, participants will be randomized to walk for exercise at low intensity (with minimal to no leg symptoms) or high intensity (with maximal leg symptoms during exercise activity). Participants will be asked to walk around the track at the exercise facility or up and down a hallway at Northwestern Memorial Hospital in order to learn the pace to walk according to their assignment to low intensity or high intensity exercise. Participants will then be helped to set targets (i.e. goals) for walking activity during the next week. They may be asked to return to the exercise center for additional coaching, for up to four additional sessions.

Participants will be provided with walking exercise "tracking" forms and asked to write down their goals for home-based walking activity each day for the next two weeks and record where and when they will exercise. They will be provided with "tracking' sheets on which to record their walking activity for each week that they are in the walking exercise program. After this initial on-site visit, study participants will be telephoned weekly for up to 14 weeks by a study coach who will review their walking exercise activity and provide feedback and encouragement. Participants may be visited at home or at their own exercise facility to monitor walking intensity. Each telephone call will last approximately 15 minutes. Participants will be asked to complete some study questionnaires before and after their participation in this program. They will be asked to return to the

medical center for final follow-up when they have completed the program. They will return their monitoring devices at their final visit.

Computerized Tablet or Computer Camera Study Visit: Potentially eligible study participants will be telephoned by study staff and informed of the option of returning for a visit where they will be taught how to operate a tablet or camera. Specifically, they will be taught how to link to video chat on the tablet or camera. They may be asked at a later date to participate in up to three video chat sessions, in which they use their tablet or computer camera at home to connect with a group leader and other study participants with peripheral artery disease. The video chat sessions will last one hour or less. Participants will be asked to return the tablets or cameras to investigators at the end of the pilot study.

MRI to measure lower extremity perfusion: To measure lower extremity blood flow or perfusion, we will use techniques in a subset of up to 50 participants who provide informed consent to this optional study element. In the MRI machine, we will ask participants to push against a plastic board with their feet (i.e. a plantarflexion motion) repeatedly at a rate of one push per second. A metronome will be used to monitor the rate of pushing. Participants will be asked to push for as long as they are able. When they are too tired to continue, they will rest and perfusion to the calf muscle will be measured using MRI. This test will be conducted on a Siemens Tim Trio 3 Tessalon MR or a Siemens Skyra 3 Tessalon scanner at the Center for Translational Imaging (CTI) in the Olson Pavilion at Northwestern. If the designated Tim Trio or Skyra 3.0 Tessalon scanner is unavailable, we will use a different 3.0 Tessalon scanner at Northwestern Memorial Hospital or the center for CTI. We may perform up to 3 perfusion MRI scans on individual participants, in order to conduct a test re-test reliability assessment. We will not use any contrast for this method.

OUTCOME MEASURES.

C10. Justification for measuring primary outcomes at 12-week follow-up. We recognize that the time point at which study outcomes are maximally improved following GM-CSF therapy is unknown. The half life of GM-CSF is 162 hours (75). 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 (1,74,75). However, available evidence supports measurement of our primary outcomes at 12-week follow-up. First, a previous uncontrolled clinical trial of 17 patients with PAD and critical limb ischemia demonstrated measurable improvement in toe brachial pressure index, TcPO2, and pain free walking distance four-weeks after CD34+ cells were injected into calf muscle of participants (76). These improvements persisted or even further improved at 24-week follow-up (76). In a separate study, 22 patients with bilateral PAD were randomized to receive calf injections of autologous bone-marrow derived mononuclear cells vs. peripheral blood derived mononuclear cells (77). Four-weeks after the injections, measurable improvements in transcutaneous oxygen pressure and rest-pain were observed in the leg receiving bone-marrow derived cells compared to the leg receiving peripheral mononuclear cells (77). These improvements were sustained at 24-week follow-up. Third, in the study by Subramaniyam and colleagues, two-weeks of GM-CSF therapy was associated with improved treadmill walking performance and brachial artery FMD at 12-week follow-up (1). Similarly, in studies of patients with coronary artery disease, improvements in cardiac ejection fractions or regional wall motion abnormalities are typically first observed four to 12 weeks after $\leq$ two weeks of GM-CSF therapy (78-80). These improvements are maintained or even increase during follow-up periods of up to one year (78-80). In summary, 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. Similarly, significant improvements in treadmill walking performance are observed 12-weeks after initiation of a supervised treadmill exercise intervention (35,65-67). Our exploratory aim will assess the time course of improved outcomes in response to GM-CSF therapy with and without exercise.

C11. Justification for measuring CD34+ cells as the primary progenitor cell outcome. We recognize that no single cell-surface marker is completely specific for endothelial progenitor cells (31). However, the CD34+ marker clearly identifies a population of functional progenitor cells that are released into the circulation in response to tissue ischemia, have the potential to differentiate into endothelial cells, and promote angiogenesis (22,31,32,76). Based on these prior data, CD34+ cells will be our primary progenitor cell

outcome measurement. Please see sections A3 and A4 for further discussion of this issue. In exploratory analyses, we will also measure CD133+ cells, KDR+ cells, ALDH bright cells, combinations of these cell markers, and progenitor cell colony forming units. It is important to point out that, compared to CD34+ cells, these exploratory progenitor cell measures have not been as consistently linked to favorable outcomes in patients with cardiovascular disease. **Progenitor Cell Measures.** CD34+ cell measurements will take place in Dr. Losordo's laboratory using methods implemented for our pilot study (see sections C1b and C1c). Total human peripheral blood mononuclear cells (MNCs) are isolated from the blood of participants by Ficoll density gradient centrifugation with Histopaque-1077 (Sigma), according to the manufacturer's instructions. Briefly, MNCs are aliquoted ($5x10^5$) to an Aldecount tube, incubated with BODIPY-AAD for 30 minutes at 37°C, and maintained on ice to prevent fluorescent byproduct exiting cells. Cells are washed in ALDH assay buffer at 4 degrees Celsius for 5 minutes and re-suspended in 90ul ALDH Assay buffer for further staining. ALDH labeled MNCs are incubated with FcR Blocking Reagent (Miltenyi Biotec) for 10 minutes followed by specific FACS antibodies for 30 minutes on ice to identify the CD34-PE Cy7 (Beckman Coulter) endothelial marker (primary outcome) and other cell surface markers (exploratory outcomes). These exploratory cell markers include CD133-APC (Miltenyi Biotec) and VEGF R2 / KDR-PE (R&D Biosystems). Dead and dying cells are excluded using staining with 7-AAD (1 μg/10^6 cells; Invitrogen). Directly conjugated isotype control antibodies are used to set baseline fluorescence levels. Flow cytometry is performed by Northwestern's Cancer Core Research facility, blinded to patient identity and group assignment using the DakoCytomation CyAn FACS instrument. Analyses are performed using the FlowJo software (Treestar). Gating mononuclear cells is performed on the basis of light-scattering properties. The labeled cells of interest (i.e. CD34+ cells) are expressed as percentages of live mononuclear cells. **Progenitor cell colony forming unit assay**. Total human peripheral blood mononuclear cells (PBMNC) will be isolated from blood by Ficoll density gradient centrifugation with Histopaque-1077 (Sigma) according to manufacturer instructions. PBMNC will be re-suspended in endothelial cell basal medium-2 (EBM-2, Clonetics) supplemented with 5% FBS, human vascular endothelial growth factor (VEGF)-A, human fibroblast growth factor-2, human epidermal growth factor, insulin-like growth factor-1, ascorbic acid, and antibiotics and then plated on fibronectin-coated 6 well plates at a concentration of 5 million cells per well. The endothelial colonies will be counted manually on day 7 by two investigators blinded to the study group assignments. Progenitor cells will be further characterized by dual-staining for DiI-Ac-LDL and Lectin and by the expression of endothelial marker proteins VEGFR-2, VE-cadherin, eNOS, and vWF.

In addition to measuring progenitor cells at the frequency shown in Table 6 above, in up to 50 participants with PAD we will measure progenitor cell levels before and immediately following the treadmill exercise stress test. We may also measure progenitor cell levels one hour post-exercise and one day post-exercise. The treadmill exercise stress test at which this may occur may be affiliated with any of the visits shown in Table 6. However, it is possible that the participant may be asked to undergo an additional treadmill exercise stress test in order to expedite collecting data on progenitor cell levels before and after an exercise stress test. Also- up to 30 participants who are determined ineligible for the study due to absence of PAD will be invited to participate in an exercise treadmill stress test. Levels of progenitor cells will be measured before and after the exercise treadmill stress test in these individuals without PAD as well as one hour post-exercise and one day post-exercise. These additional procedures will allow us to achieve our exploratory aim #5 above.

C12. Six-minute walk. Our primary outcome is change in the distance achieved in the six-minute walk test between baseline and 12-week follow-up. The six-minute walk is our primary outcome measure for several reasons. First, our work demonstrates that for PAD participants, performance on the six-minute walk test is more closely correlated with walking performance during daily life than treadmill walking performance (81). Secondly, for elderly patients, such as those with PAD, treadmill walking is associated with balance problems and anxiety (52-54). A previous study of 12 healthy elderly volunteers (ages 71-80 years) and 12 healthy young volunteers (ages 21-37) compared physiologic responses and steps per minute during a treadmill exercise test vs. the six-minute walk (corridor walking) performed on three separate occasions (52). The elderly group, but not the younger group, had consistently higher heart rates and lower step rates during the treadmill test than during the corridor walks (52). Third, treadmill walking is associated with a significant learning effect (82-84). Supervised treadmill exercise interventions, such as that proposed in the current study, can improve treadmill walking performance in part because of the "practice" associated with the three times weekly treadmill exercise sessions. 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 (44,81,85). Distance covered in six minutes is recorded. The intra-class correlation coefficient for the test-retest reliability of the six-minute walk test among 156 PAD participants in our SILC exercise trial was 0.90 ($p<0.001$) when two six-minute walk tests were completed one to two weeks apart (44).

C13. Treadmill testing. The Gardner graded treadmill exercise test is the standard, accepted treadmill protocol for measuring change in maximal treadmill walking time in response to interventions among PAD participants (82-84). The FDA requires treadmill evidence when evaluating therapeutic drugs for improving walking performance in PAD. In the Gardner exercise protocol, speed is maintained at 2.0 miles per hour (mph) and treadmill grade increases by 2.0% every two minutes (82-84). 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.

C14. Brachial Artery Flow-Mediated Dilation (FMD). As in our prior studies (44,86,87), brachial artery imaging will be performed by a Registered Diagnostic Cardiac Sonographer (44,86,87). Participants withhold vasoactive medications, refrain from smoking, and fast prior to testing (86,86). With the participant supine, a blood pressure cuff over the upper arm is inflated for four minutes. The inflation pressure is systolic blood pressure (SBP) + 50 mmHg. The brachial artery is imaged (B-mode and Doppler) 5 to 9 cm above the antecubital fossa using a linear array vascular ultrasound transducer (Siemens Medical Solutions, Sequoia Model #256, frequency 8 MHz). Three video sequences are obtained. The first verifies the location and baseline hemodynamic state of the brachial artery. The second begins 20 seconds before cuff inflation and continues for 10 seconds after inflation. The third begins 15 seconds before cuff release and continues for 90 seconds after deflation. Brachial artery FMD is calculated as the percent change in brachial artery diameter at 60 seconds after the release of the cuff. Because previous studies of coronary artery disease patients consistently show that brachial artery endothelial independent function does not change in response to interventions, we will not measure nitroglycerin-mediated vasodilation (89,90). Changes in brachial artery FMD will be read by Dr. James Stein's University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory. Images are read by a single reader blinded to participant characteristics. Measurement reproducibility in Dr. Stein's laboratory has a median FMD difference of 0.02% (inter-quartile range: -0.03–0.04).

C15. Other measures. As in our previous clinical trials, patient report will be used to document comorbidities. Patient reported comorbid disease is highly correlated with presence of comorbid disease measured with medical record review (91-95). In the National Hospital Discharge Survey of 122 elderly men and women, the percent agreement between patient report and medical record review was 85% for angina, 89% for cancer, 98% for diabetes, 94% for myocardial infarction, and 98% for stroke (92). Participants will be asked to bring their medication bottles for assessment of medication use at baseline and at follow-up visits. Patient-report is an accurate measure of cigarette smoking in PAD patients (96).

Some or all study measures may be repeated at baseline or follow-up for data quality (one potential example is if a treadmill test must be stopped due to extremely high blood pressure before the patient completed the test).

C16. Quality Control. As in our prior studies, health interviewers will be trained by a senior coordinator and certified by Dr. McDermott in each component of data collection, using a detailed checklist developed for the study (see Appendix A for example checklist). Health interviewers are rigorously evaluated for adherence to protocol, delineated in our manuals, prior to beginning data collection and every six months after initial certification. When deficiencies are identified, interviewers undergo additional training and re-assessment. Ten percent of participants are randomly identified for quality control. This subset has their ABI measured twice by independent examiners and has an extra blood sample obtained for quality control assessment of progenitor cells. The second blood sample is designated an arbitrary identification number to which the technician is blinded. Thus, quality control is monitored continuously. A randomly selected 10% subset of exercise session charts will be reviewed by Dr. McDermott to ensure protocol adherence. Dr. McDermott also attends one or more exercise sessions on a monthly basis to ensure adherence to protocol. Furthermore, during the first year

of the study, 10 participants will be selected for a second brachial artery FMD. These participants will be asked to return within two weeks (1-14 days, but not on the same day as the first FMD) after their baseline testing and before randomization for a repeat brachial artery FMD measurement. The purpose of this re-test is to assess the test re-test reliability of the brachial artery FMD measurement.

C17. Blinding for data collection. The health interviewer collecting outcome data will be blinded to the study group assignment. Participants are instructed not to reveal their group assignment. If a participant reveals their group assignment, another certified health interviewer is paged to continue the visit.

C18. Data Management. We will use methods in place for our previous and ongoing PAD studies to customize a data management system for the PROPEL Study. Data from baseline and follow-up visits will be acquired on paper and processed using the Teleform system by Cardiff Software. We have successfully used the Teleform system for over 6,000 patient visits in previous and ongoing studies. Teleform has three components. The first component, the Designer, functions as a page layout program with predefined tools for creating check boxes, bar code fields, and hand print fields. Form packets are assembled and individualized with ID labels prior to each visit. Completed forms are scanned using a high-speed scanner. The second component, the Reader, accepts scanned images and translates user responses (check marks, circles, or hand-printed text) into machine-readable responses. The third component, the Verifier, double-checks user responses prior to final acceptance. The Verifier is programmed with the data validation rules established when the form was created. Data are routinely backed-up and copied to CDROM for secure off-site storage.

C19. Data and Safety Monitoring Board (DSMB). As per NHLBI guidelines, the DSMB will be appointed by the NHLBI. The DSMB will review and approve the protocol prior to beginning recruitment. The DSMB will meet every six months. Adverse events will be monitored continuously throughout the study and will be reported to the DSMB and IRB promptly. See Section D2 below for more information about the DSMB.

C20. Statistical Power Considerations. As indicated above, the four study arms are defined as follows: Group A: GM-CSF combined with supervised treadmill exercise; Group B: GM-CSF therapy + an attention control group; Group C: placebo + supervised exercise; and Group D: placebo + attention control group. From this point forward, “GM-CSF combined with supervised treadmill exercise” refers to Group A; “GM-CSF therapy alone” refers to Group B, “supervised exercise therapy alone” refers to Group C, and placebo + attention control group (i.e. no active therapy) refers to Group D.

In our primary aim, we will determine at 12-week follow-up: (a) whether GM-CSF therapy combined with supervised treadmill exercise (Group A) significantly improves six-minute walk performance compared to GM-CSF therapy alone (Group B), (b) whether GM-CSF therapy combined with supervised treadmill exercise (Group A) significantly improves six-minute walk performance compared to supervised exercise therapy alone (Group C) and (c) whether GM-CSF therapy alone (Group B) significantly improves six-minute walk performance compared to placebo (Group D). We will also confirm the previously established association between supervised treadmill exercise (Group C) and improved six-minute walk performance, compared to no active therapy (Group D).

The final sample size in the PROPEL Trial, of 210 participants, was lower than the originally intended sample size of 240 participants. Prior to analyzing the data, investigators propose to alter the statistical analysis plan. The new statistical analysis plan allows us to address our original hypotheses but with greater statistical power, given the slightly lower sample size than originally intended. The revised statistical plan is as follows. The significance levels of the four primary comparisons will be determined using the Hochberg’s step up method rather than the conservative Bonferroni correction. Specifically, we will sort the p-values from the smallest to the biggest as $p_{(1)} < p_{(2)} < p_{(3)} < p_{(4)}$. For the purposes of the analysis plan, the “hypothesis” refers to the null hypothesis. Rejecting all four hypotheses indicates that the hypotheses specified in our specific aims above are correct. Under this assumption, we will

(1) reject all four hypothesis if $p_{(4)} \leq 0.05$;

(2) otherwise, we will reject hypotheses (1-3), if $p_{(3)} < 0.05/2$;

(3) otherwise, we will reject hypotheses (1-2), if $p_{(2)} < 0.05/3$;
(4) otherwise, we will reject the hypothesis (1), if $p_{(1)} < 0.05/4$

The Hochberg's step up method also controls the family-wise type one error and is more powerful than the Bonferroni adjustment.

C21. Statistical Analyses Methods. Change in six minute walking distance at 12-week follow-up is our primary outcome. Changes in brachial artery FMD, maximal treadmill walking time, and CD34+ cells at 12-week follow-up are secondary outcomes. The distributions of each outcome will be examined and appropriate transformations will be performed if necessary. Prior to the analyses, baseline characteristics (i.e., age, sex, race, baseline six-minute walk, ABI, CD34+ cells, and other relevant variables) will be compared between the four groups, using a F-test or Chi-Square test to ensure that baseline characteristics are balanced across the four groups. Any variables that are significantly different between groups will be adjusted for as covariates.

For our Primary Aim, we will compare changes in six minute walk distance from baseline to 12-week follow-up between Groups A and B, between Groups A and C, between Groups B and D, and between Groups C and D using a two-sample t-test. Analysis of covariance will be used to adjust for baseline characteristics as necessary. For our Secondary Aim, we will compare changes in brachial artery FMD and maximal treadmill walking time from baseline to 12-week follow-up between Groups A and B, between Groups A and C, and between Groups B and D. We will also determine whether participants in Group C have greater increases in CD34+ cells at 12-week follow-up, compared to Group D. The two-sample t-test or analysis of covariance will be used for these comparisons. Efficiency augmentation methods for estimating the treatment effects using the distribution of baseline characteristics will be employed (96). The 95% confidence interval of the treatment effects will be constructed. Because the primary aim of the study is to demonstrate the superiority of Group A (GM-CSF + supervised exercise) as compared to Group B (GM-CSF alone) and Group C (supervised exercise alone), the commonly used two-way analysis of variance will be used only in supplementary analysis. Specifically, for the outcomes of interest in our primary and secondary aims, we will test the interaction between GM-CSF and supervised exercise with two-way ANOVA. In the absence of an interaction (i.e, the p value for the interaction term exceeds 0.25), we will further estimate the additive effect of GM-CSF and supervised exercise simultaneously in all 240 participants. Here, the existence of model-based additive effects for GM-CSF and exercise is much stronger than the targeted superiority of the combined treatments.

For our Exploratory Aims, we will use linear mixed effects regression analyses to compare changes in six-minute walk performance, maximal treadmill walking time, and brachial artery FMD between baseline and six-week follow-up and between baseline and six month follow-up between Groups A and B, Groups A and C, Groups B and D, and Groups C and D. We will use linear mixed effects regression analyses to compare changes in progenitor cell measures between baseline and six-week follow-up and between baseline and six-month follow-up between Groups C and D. Outcomes measured at baseline and each follow-up visit will be longitudinal response variables in the regression model. The regression coefficients for the interactions between group indicators and visit time are parameters of interest. The subject specific random intercept will be used to incorporate the within subject correlations. For our Exploratory Aim #4, we will employ linear mixed regression analysis to evaluate the association between changes in CD34+ cells and other progenitor cell measures from baseline to two-week follow-up with changes in six-minute walk distance, brachial artery FMD, and maximal treadmill walking time between baseline and six-week, twelve-week, and six-month follow-up visits. The responses are longitudinally measured outcomes of change between baseline and the six-week, twelve-week, and six-month visits, respectively. The independent variables of interest are changes in CD34+ cells and other progenitor cell measures from baseline to two-week follow-up. We will employ similar linear mixed regression analysis to evaluate associations between changes in progenitor cells between baseline and six-week, 12-week, and six-month follow-up with changes in six-minute walk distance, brachial artery FMD, and maximal treadmill walking time during the corresponding time period. The responses are longitudinally measured outcomes of change between baseline and the six-week, twelve-week, and six-month visits, respectively. Independent variables of interest are changes in progenitor cells from baseline to six-week, 12-week, and six-month follow-up. Finally, we will perform regression analyses among participants randomized to

the exercise group to determine whether the degree of increase in lactate levels during exercise sessions is associated with the degree of increase in progenitor cells.

C22. Intention to Treat. In all comparisons, we will employ the intention-to-treat principle. Participants will remain in their originally assigned groups for analyses, regardless of adherence to their assigned group. Based on our experience in our SILC clinical trial, the drop-out rate is expected to be <10% at six-month follow-up. Therefore, complete case analysis coupled with reasonable sensitivity analysis should be adequate to handle the problem of missing data. If the drop-out rate exceeds five percent, we will employ more sophisticated statistical methods such as multiple imputation and the pattern mixture approach to correct for the potential bias of estimates from simple complete-case analysis due to informative missing data. The study team has substantial experience employing these methods in prior studies (44,97,98). We will use the type one error value of 0.0125 to cope with multiple comparisons.

C23. Follow-up window. Follow-up testing will be completed within one week before or after the target follow-up date, at the 2-week, 6-week, 12-week, and 26-week follow-up time points after randomization. However, if the follow-up visit cannot be performed within the one week before or after the due date for each follow-up visit (e.g. if a participant is sick and unable to return to the center for testing within the follow-up window), we will obtain follow-up measures at any point up until the next scheduled follow-up test. For example, if we are unable to obtain the 12-week follow-up measures within the window, we will attempt to obtain the measures until the 26-week follow-up testing window begins.

D. HUMAN SUBJECTS.

D1. Risks to the subjects. As described in section C.2 above, subjects will be randomized to one of four groups: Group A: GM-CSF and supervised treadmill exercise; Group B: GM-CSF and attention control condition; Group C: placebo injections and supervised treadmill exercise; Group D: placebo injections and attention control condition (see Figure 2). Study participation will last for 6 months. Participants receiving GM-CSF will receive a GM-CSF injection three times weekly for the first two weeks after randomization. Participants receiving placebo injections (rather than GM-CSF) will receive a placebo injection three times weekly for two weeks. Participants randomized to supervised treadmill exercise will exercise three times weekly for six months with a trainer at the study exercise facility. Participants randomized to the attention control group will attend weekly educational sessions at Northwestern University Feinberg School of Medicine for six months. We will randomize a total of 267 participants with peripheral arterial disease (PAD). Based on our prior work, we expect that 240 participants will complete the study. Based on our previous work involving participants with PAD, we anticipate that the average age will be approximately 70 years, that at least 33% of participants will be minorities, and that approximately 50% will be women. For example, in our recently completed SILC randomized controlled clinical trial of exercise in patients with PAD (see section A7 above) (44), participants were average age 70.6 $\pm$ 10.3 and the average ABI was 0.61 $\pm$ 0.17. In SILC, participants included 52% women and 45% African-Americans. In general, patients with PAD have a relatively high prevalence of comorbid diseases, particularly coronary artery disease, cerebrovascular disease, diabetes mellitus, and pulmonary disease. Thus the patient population is likely to be of generally poorer health than that of similarly aged men and women without PAD in the general population. However, subjects must be willing to travel to the medical center three times weekly in order to participate. This requirement will necessarily eliminate some potential participants who are more frail. Inclusion and exclusion criteria are provided in sections C.3 and C.4 above. **Vulnerable populations (fetuses, pregnant women, children, prisoners, and institutionalized persons) will not be included in this study**.

Sources of material. Primary and secondary outcome measures that will be collected for this study are shown in Table 6 and include the six-minute walk test, treadmill walking performance, progenitor cell measures, and brachial artery flow-mediated dilation (FMD). We will also administer questionnaires to participants at baseline and at each follow-up visit to assess their medical history, including presence of comorbidities, medication use, smoking status, and other health characteristics. Data collection will not make use of existing records or data. The prevalence of comorbid disease will be measured based on patient report, based on previous study (91-95) (see section C.15 above).

Potential risks. GM-CSF is an FDA-approved medication, available commercially. GM-CSF is produced using recombinant DNA technology. Side effects of GM-CSF include myalgias, arthralgias, fever, fatigue, and headache (99, 100, 101). Splenic enlargement, usually subclinical, can occur. Splenic rupture related to GM-CSF therapy is rare (100). Additional rare but serious side effects, such as allergy or thrombotic events have also been observed (101).

The exercise program might be associated with an increased risk of heart attack, arrhythmia, or death. In addition, patients may develop ischemic chest pains during exercise. If participants develop chest pain during exercise, Dr. McDermott, Dr. Lloyd-Jones, or Dr. Losordo will be notified immediately. Dr. McDermott will oversee arrangement of appropriate follow-up (including immediate transport to the emergency room if appropriate). The exercise physiologist is certified in Cardiopulmonary Resuscitation (CPR) and use of an Automatic External Defibrillator (AED). Safety manuals and protocols will be developed prior to beginning the intervention. Chest pain symptoms during exercise may result in additional cardiac work-up that may lead to procedures to improve coronary blood flow. Subjects will be screened for active heart problems with a baseline exercise treadmill test prior to enrollment, according to currently recommended standards of screening PAD patients for coronary artery disease prior to their beginning an exercise program (35). These exercise stress tests will be performed as part of our protocol and interpreted by board-certified cardiologist and co-investigator Dr. Lloyd-Jones. Participants must have a normal 12-lead exercise stress test to be eligible. Abnormal baseline exercise stress tests may lead to additional cardiac work-up by the participant's physician that may lead to coronary angiography or coronary revascularization. If the baseline exercise stress test is equivocal or abnormal, the participant must demonstrate evidence of a recent (within the past six months) normal coronary perfusion test or coronary angiogram in order to be eligible for participation. The latter tests would be ordered by the participant's physician at their discretion.

Exercise may also be associated with muscle fatigue or soreness or dehydration which can result in fainting. This typically resolves with rest. The risks associated with baseline and follow-up testing include discomfort or pain in the extremities during the ankle brachial index test and falling during the walking tests. The ankle brachial index will be aborted if a participant experiences severe pain during the testing. The research assistant collecting these data has been trained to prevent falling. The risk of falling is less than 1 in 200. Falling during these tests may be associated with fracture. The risk of a fracture secondary to a fall during the walking tests is less than 1 in 5,000.

D2. Adequacy of protection against risk. Recruitment methods are described in section C5 above. Informed consent will be obtained at the time of the initial study visit. Subjects will be asked to read and sign the study consent form. A research assistant will administer the consent form and answer questions. The research staff has undergone human subjects training required by our institutional review board. This training includes information about the importance of maintaining confidentiality of personal health information. The study principal investigator or a co-investigator will be available to answer any questions as needed. Both the subjects and the individual administering the consent form will sign the consent form.

Methods to minimize potential risks. *Minimizing risks related to GM-CSF*. The GM-CSF dose to be administered is an FDA-approved dose with demonstrated safety in participants with PAD (1). GM-CSF will be administered in our clinical research unit (CRU) by a registered nurse, who has experience administering GM-CSF, or a physician. Participants will be monitored in the CRU and during their return visits for side effects related to GM-CSF. Specifically, the blood pressure, temperature, and heart rate of each participant will be obtained before and after each injection of the study drug (GM-CSF) or placebo. Following the first injection, participants will be monitored for 2 hours, with blood pressure and pulse obtained every 15 minutes. If a participant is randomized to the treadmill exercise intervention, the participant will not attend the treadmill exercise session on the date of the first injection. Following injections 2 through 6, the participant will be monitored for 20 minutes. Participants will be educated about side effects to watch for and will be asked to contact Dr. McDermott promptly should any significant side effects develop. Dr. McDermott's pager and home telephone number will be provided to study participants. In addition, guidelines for dose reduction have been developed. For example, the dose of the study drug may be decreased by study investigator Dr. David Green or another un-blinded study physician based on the results of the white blood count.

Minimizing risks related to exercise. According to current clinical practice guidelines (35), all participants will undergo baseline exercise stress testing prior to randomization into one of the study groups. Potential participants with an abnormal baseline exercise stress test will be excluded. A physical examination will be completed by a physician during baseline assessments to help ensure that study participation is safe. In addition, participants will be monitored during exercise for development of chest discomfort, new dyspnea, or new fatigue during exertion. Dr. McDermott will be promptly notified when this occurs (by pager). Our exercise physiologists have significant experience working with populations of participants with peripheral arterial disease and have been trained in CPR, ACLS, and use of the automatic external defibrillator.

Minimizing risk related to baseline and follow-up testing. All study coordinators undergo baseline training and are certified by Dr. McDermott before beginning data collection. Training and certification involves ensuring that coordinators are trained in methods to help minimize falls. Dr. McDermott re-certifies coordinators every six months to ensure continued adherence to study protocol. Those who are not adhering to protocol undergo additional training followed by re-certification.

Minimizing risk related to loss of confidentiality. A number of methods will be employed to maintain confidentiality of participants. First, study recruitment letters will be mailed, using IRB-approved methods, only after receiving written permission from the participant's physician. The personal physician of each study participant will have the option of **not** allowing investigators to contact the potential participant. Lists of potentially eligible participants will be obtained by individuals who normally have access to these lists as part of their daily work requirements. Recruitment letters for potential participants identified from hospital and outpatient lists are prepared by research staff members whose job is to assist study investigators with recruitment. These research staff members have completed training in the ethical conduct of human subject research, including maintaining participant confidentiality. Recruitment letters to potential participants identified from medical center lists are mailed in sealed envelopes and addressed to the potential participant. All potential participants who receive mailed information about the study after the approval from their physician will have the opportunity to call a voice-mail system to ask NOT to be further contacted about this study. Secondly, only study investigators and key research staff will have access to the study database. Third, participants will be assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier will be used to distinguish participants in the database. Fourth, collected data will be maintained in locked computer files and file cabinets to which only study investigators have access. Collected data will be used only for research purposes. Any published data will not contain any individual identifiers.

Data and Safety Monitoring Board (DSMB). As per NHLBI guidelines, the DSMB will be appointed by the NHLBI. The DSMB will meet at least every six months during the study. They will meet to review and approve the protocol prior to beginning data collection. They will decide on specific stopping criteria for the study. However, because of the short duration of the study and because of our secondary and exploratory aims, there will be no criteria for stopping due to benefit. The biostatisticians and data manager will work closely with the DSMB to perform interim analyses. However, since the sample size is small and the follow-up is only six months, we do not plan to have any stopping criteria for beneficial effect. Rather, we will develop stopping criteria only for adverse effects.

Adverse events will be monitored continuously throughout the study and will be reported to the DSMB and IRB in a timely manner according to pre-specified requirements. 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) musculoskeletal outcomes (muscle soreness, foot ulcer, joint aches and pains); d) additional adverse symptoms and events (headache, fatigue, arthralgias, fever, allergy related to GM-CSF, splenic rupture related to GM-CSF). We will also monitor all hospitalizations. We will use a designated data collection form to record these events and they will be reported immediately to the Institutional Review Board and DSMB. Note that to date, however, exercise programs and GM-CSF have been demonstrated to be safe in patients with PAD (1,35,44).

The biostatisticians and data manager will work closely with the DSMB to perform interim analyses. However, since the sample size is small and the follow-up is only six months, we do not plan to have any stopping criteria for beneficial effect. Rather, we will develop stopping criteria only for adverse effects. For the proposed semi-annual DSMB meetings, data will be edited and cleaned contemporaneously to data collection. Analyses will be done according to the requests of the DSMB. In addition, for each major adverse event, the group assignment of the patient will be provided. In this way the DSMB can determine whether the event is intervention related.

D3. Potential benefits of the proposed research. Preliminary evidence suggests that increasing circulating levels of CD34+ cells with granulocyte monocyte colony stimulating factor (GM-CSF) or other therapies may improve walking performance in patients with lower extremity peripheral arterial disease (PAD) (1,2). However, results of small clinical trials are mixed (1-4). The association of GM-CSF with improved walking performance in PAD is not definitively established. Preliminary data also suggest that lower extremity ischemia, induced during walking exercise, may increase circulating CD34+ cell levels, enhance homing of CD34+ cells to ischemic sites, and **augment** the ability of GM-CSF to improve walking performance in PAD (1,2). However, whether the combination of GM-CSF and supervised treadmill exercise improve walking performance more than GM-CSF and supervised treadmill exercise, respectively, is currently unknown. This proposed clinical trial is needed to definitively establish a) whether the combination of supervised treadmill exercise and GM-CSF improves functional performance in PAD participants with and without intermittent claudication more than either intervention individually; b) whether GM-CSF improves functional performance in PAD patients with and without intermittent claudication as compared to placebo; c) whether supervised treadmill exercise increases circulating levels of CD34+ cells; d) whether the degree of increase in CD34+ cells is associated with the degree of improvement in six-minute walk performance; e) the temporal trajectory of improvement in study outcomes in response to study interventions and increases in progenitor cells. In addition to establishing the therapeutic benefit of GM-CSF with and without supervised treadmill exercise, this proposed study is expected to identify biological pathways associated with improved functional performance in patients with PAD. This information is expected to lead to new therapies to improve walking performance in patients with PAD.

D4. Importance of knowledge to be gained. Lower extremity peripheral arterial disease (PAD) is common. Currently 8 million men and women in the United States (U.S.) have PAD (5). The number of individuals with PAD is expected to increase as the U.S. population lives to older ages with chronic disease. Our prior work and that of others shows that patients with PAD have greater functional impairment, increased rates of functional decline, and increased mobility loss compared to persons without PAD (6-11). Older patients with functional impairment are less likely to remain independent in the community, have higher rates of hospitalization, and have poorer quality of life than those without functional impairment (12). Yet few therapies have been identified that improve lower extremity functioning or prevent functional decline or mobility loss in persons with PAD. Only two FDA-approved medications improve functional performance in patients with PAD. Identifying biological pathways associated with improved functional performance in PAD is expected to lead to new therapies to help patients with PAD preserve their functional performance and avoid decline. These findings will have important public health implications for preventing disability and nursing home placement for a large number of individuals.

D5. Collaborating sites. Brachial artery flow-mediated dilation results will be interpreted by Dr. James Stein's laboratory at the University of Wisconsin Medical School. Videotapes of the brachial artery flow mediated dilation measurement will be mailed to Dr. Stein's laboratory and results will be returned to Northwestern investigators. We have experience working with Dr. Stein in this capacity in our recently completed randomized controlled trial of exercise in patients with PAD (44). Dr. Lu Tian, assistant professor in the Department of Biostatistics at Stanford, will assist with statistical analyses. Dr. Tian was previously a faculty member at Northwestern University and has worked with Dr. McDermott, Dr. Liu, and other study investigators for more than five years. Drs. Michael H. Criqui (University of California at San Diego), Jack M. Guralnik (National Institute on Aging), and Luigi Ferrucci (National Institute on Aging) have worked with Dr. McDermott on PAD studies of functional impairment for over nine years and bring expertise in functional

assessment, PAD, and clinical trials to the study team. Drs. Brian Annex and Doris Taylor bring expertise in PAD, clinical trials, and/or progenitor cell measurement and interpretation to the study team.

E. Inclusion of Women/Minorities. PAD is common among older women and among African-Americans (102). We will ensure that our study population includes at least approximately 50% women and at least approximately 25% African-American (see enrollment table). We do not anticipate difficulty achieving these recruitment targets, since our recently completed randomized controlled clinical trial of exercise in participants with PAD included 52% women and 40% minorities (44). Our group has substantial experience successfully recruiting and enrolling women and minorities for our observational and clinical trial research in participants with PAD.

Methods to ensure adequate enrollment of women and minorities. To ensure that we achieve a study population that includes approximately 50% women and at least 25% minorities, we will make study participation as simple and enjoyable as possible for women and minority participants. For example, we can provide door-to-door transportation to study visits. We have also advertised our research opportunities on radio or in newspapers for which the audience includes a high proportion of minority populations. If we still have difficulty recruiting significant proportions of minorities, we will enlist minority leaders in the community to assist with recruitment and we will make a substantial effort to hire an African-American research assistant to assist us with recruitment.

Alternatives should the above methods be insufficient. If the above methods are not sufficient, then we will expand our recruitment of participants to hospitals in which the majority of patients are minorities. For example, colleague and vascular surgeon Dr. Walter McCarthy is director of the non-invasive vascular laboratory at Cook County Hospital in Chicago. Similarly, colleague and vascular surgeon Dr. Melina Kibbe is a vascular surgeon at the VA Chicago Medical Center. If necessary, we will work with Drs. McCarthy and Kibbe to identify additional participants from Cook County Hospital and VA Chicago, where many patients are members of under-represented minority populations.**F. Inclusion of Children.** PAD does not affect children. Therefore, children will not be included in the PROPEL study.

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