

Original Investigation

Effect of Progenitor Cell Mobilization With Granulocyte-Macrophage Colony-Stimulating Factor in Patients With Peripheral Artery Disease

A Randomized Clinical Trial

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IMPORTANCE Many patients with peripheral artery disease (PAD) have walking impairment despite therapy. Experimental studies in animals demonstrate improved perfusion in ischemic hind limb after mobilization of bone marrow progenitor cells (PCs), but whether this is effective in patients with PAD is unknown.

OBJECTIVE To investigate whether therapy with granulocyte-macrophage colony-stimulating factor (GM-CSF) improves exercise capacity in patients with intermittent claudication.

DESIGN, SETTING, AND PARTICIPANTS In a phase 2 double-blind, placebo-controlled study, 159 patients (median [SD] age, 64 [8] years; 87% male, 37% with diabetes) with intermittent claudication were enrolled at medical centers affiliated with Emory University in Atlanta, Georgia, between January 2010 and July 2012.

INTERVENTIONS Participants were randomized (1:1) to received 4 weeks of subcutaneous injections of GM-CSF (leukine), 500 µg/day 3 times a week, or placebo. Both groups were encouraged to walk to claudication daily.


MAIN OUTCOMES AND MEASURES The primary outcome was peak treadmill walking time (PWT) at 3 months. Secondary outcomes were PWT at 6 months and changes in circulating PC levels, ankle brachial index (ABI), and walking impairment questionnaire (WIQ) and 36-item Short-Form Health Survey (SF-36) scores.

RESULTS Of the 159 patients randomized, 80 were assigned to the GM-CSF group. The mean (SD) PWT at 3 months increased in the GM-CSF group from 296 (151) seconds to 405 (248) seconds (mean change, 109 seconds [95% CI, 67 to 151]) and in the placebo group from 308 (161) seconds to 376 (182) seconds (change of 56 seconds [95% CI, 14 to 98]), but this difference was not significant (mean difference in change in PWT, 53 seconds [95% CI, -6 to 112], $P = .08$). At 3 months, compared with placebo, GM-CSF improved the physical functioning subscore of the SF-36 questionnaire by 11.4 (95% CI, 6.7 to 16.1) vs 4.8 (95% CI, -0.1 to 9.6), with a mean difference in change for GM-CSF vs placebo of 7.5 (95% CI, 1.0 to 14.0; $P = .03$). Similarly, the distance score of the WIQ improved by 12.5 (95% CI, 6.4 to 18.7) vs 4.8 (95% CI, -0.2 to 9.8) with GM-CSF compared with placebo (mean difference in change, 7.9 [95% CI, 0.2 to 15.7], $P = .047$). There were no significant differences in the ABI, WIQ distance and speed scores, claudication onset time, or mental or physical component scores of the SF-36 between the groups.

CONCLUSIONS AND RELEVANCE Therapy with GM-CSF 3 times a week did not improve treadmill walking performance at the 3-month follow-up. The improvements in some secondary outcomes with GM-CSF suggest that it may warrant further study in patients with claudication.

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Peripheral artery disease (PAD) affects more than 8 million individuals in the United States.¹⁻³ Although exercise, smoking cessation, antiplatelet therapy, cilostazol, statins, and revascularization are used to treat PAD, men and women with PAD have significantly greater functional impairment and faster functional decline than those without PAD.^{1,4-7} Stem and progenitor cell (PC) therapy that promotes neoangiogenesis is an emerging treatment modality in PAD.

Progenitor cells, particularly those of endothelial origin, are involved in vascular repair and regeneration.⁸ They originate primarily but not exclusively from the bone marrow, differentiate into endothelial and other vascular cells, and contribute to neovascularization during tissue repair by direct and paracrine mechanisms.^{8,9}

ABI ankle brachial index

COT claudication onset time

G-CSF granulocyte colony-stimulating factor

GM-CSF granulocyte-macrophage colony-stimulating factor

PAD peripheral artery disease

PC progenitor cell

PWT peak walking time

WIQ walking impairment questionnaire

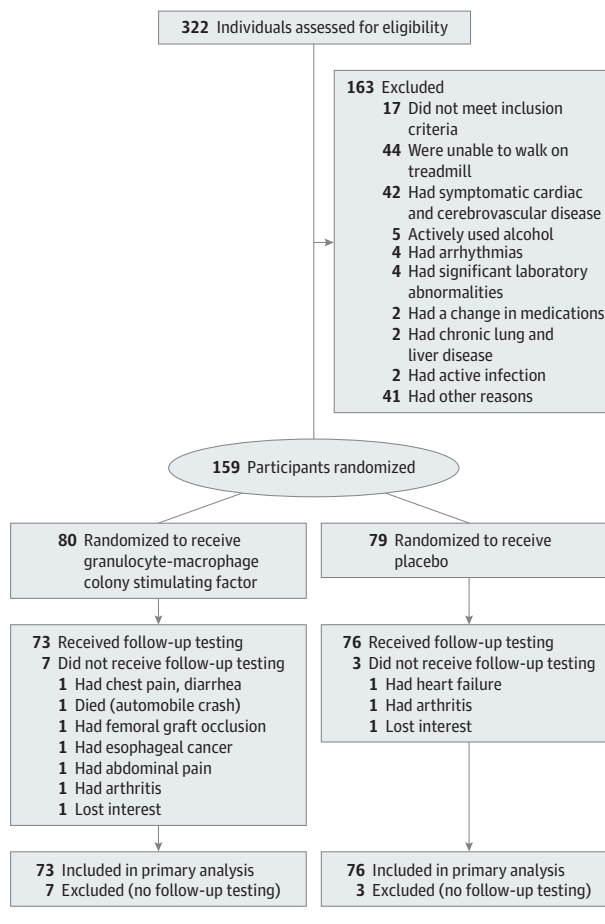
Endogenous, pharmacologically stimulated, and exogenous PCs contribute to reendothelialization and neovascularization. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulate mobilization of hematopoietic and other PCs from the bone marrow.^{10,11} In the murine hind limb ischemia model, GM-CSF administered by injection or by plasmid transfer augments circulating levels of PCs, increases capillary density, and promotes arteriogenesis.¹² Granulocyte-macrophage colony-stimulating factor also augments neoendothelialization of denuded arteries and promotes proliferation, differentiation, and survival of hematopoietic cells, monocytes, and macrophages.^{9,10,13,14}

Based on the observations of improved neovascularization in experimental models with GM-CSF,⁹ and some equivocal clinical trials,¹⁵⁻¹⁷ we previously completed a phase 1 dose-escalation trial in patients with claudication, which demonstrated the safety of GM-CSF and its ability to mobilize PCs into the circulation. Additionally, we observed a strong trend toward an improvement in exercise duration after 3 months of treatment.¹⁸ In this larger phase 2 study, we tested the hypothesis that GM-CSF administration in patients with symptomatic PAD would improve walking performance. In exploratory analyses, we assessed the safety of GM-CSF for patients with claudication.

Methods

Between January 2010, and July 2012, 322 patients aged between 21 and 80 years with PAD were screened. Of these, 159 were enrolled from Emory Healthcare, Veterans Affairs hospitals in Atlanta, and the Medical College of Georgia (Figure). Practicing physicians at these hospitals were invited to refer potentially eligible patients with PAD.

Figure. Study Enrollment, Randomization, and Follow-up



Eligibility criteria required participants to have angiographically documented history of obstructive PAD, a 2-month or longer history of intermittent claudication (Rutherford category 1-3)¹⁹ in one or both lower extremities despite appropriate and stable medication regimen that included a statin for at least 3 months, a peak walking time (PWT) on a treadmill for at least 3 minutes, a peak walking time (PWT) on a treadmill between 1 and 12 minutes, and an ankle brachial index (ABI) of less than 0.85 in the symptomatic limb. At screening, participants had to exhibit stable and reproducible walking capacity, defined as a less than 20% variation in PWT measured by 2 screening treadmill tests within a 2-week period. If the ABI was greater than 0.85, a reduction of 20% in ABI measured within 1 minute of treadmill testing or a toe brachial index of less than 0.70 was required for eligibility.

Exclusion criteria included the presence of critical limb ischemia (Rutherford category 4-6); advanced diabetic retinopathy; current or planned participation in a structured exercise program; active infection; coronary or lower extremity revascularization within the past 4 months; history of acute coronary or cerebrovascular syndrome within the past 4 months; any history of myeloid malignancy, severe congestive heart failure, or chronic renal, hepatic, or other inflammatory diseases; or a life expectancy of less than 12 months. The trial was

approved by the institutional review boards, informed consent was obtained from participants, and an investigational new drug waiver was obtained from the US Food and Drug Administration.

Randomization and Study Interventions

Treatment assignments were generated in random, permuted blocks by the study pharmacy at Emory and stratified for diabetes to ensure equal randomization of participants with diabetes into the treatment groups.

Participants were randomized 1:1 to receive either GM-CSF, 500 µg (sargramostim [Leukine]; sanofi-aventis), or matching placebo (normal saline) by a self-administered subcutaneous injection thrice weekly on Monday, Wednesday, and Friday for 4 weeks. At the beginning of each week, 1 injection was administered under observation. Patients were advised to walk to claudication only at least 3 times a day during the 4-week treatment period to encourage “homing” of the mobilized PCs to the ischemic muscle bed in the GM-CSF group. The dose of study drug was modified by an investigator who was not involved directly with patients (E.K.W.) for adverse effects, including pain unresponsive to analgesics, rash on more than 25% of body surface area, fever greater than 38.5°C, splenomegaly, or significant leukocytosis (white blood cell count >35 000/µL) or thrombocytopenia (platelet count <75 000/µL). Participants and investigators involved in the clinical evaluation, implementation of the protocol, and analysis remained blinded to treatment assignment for the duration of the trial.

Measurements

During weekly visits at the end of each of the first 4 weeks, adherence was reinforced. Safety and adverse events were recorded at each visit (baseline; weeks 1, 2, 3, 4, and 5; and 3 and 6 months) and by telephone interview at 8 weeks. Measures of response—treadmill exercise, ABI, and symptom questionnaires—were recorded at baseline and 3 and 6 months. Measures of underlying mechanisms (PC counts) were recorded at baseline, 2 weeks, 4 weeks, 3 months, and 6 months. (Details on this timeline appear in the eFigure in the Supplement.)

Treadmill Testing

Treadmill exercise testing was conducted using the protocol by Gardner et al.²⁰ The modified Bruce protocol was used among those participants (n = 6) who developed claudication after exercising for 12 minutes on the Gardner et al protocol. Treadmill exercise testing was performed twice at each visit, with the maximum claudication onset time (COT) and PWT recorded. The COT was measured as the time to onset of the participant's typical claudication and the PWT as the maximum distance the patient could walk on the treadmill. Patients unfamiliar with treadmill exercise were allowed to familiarize themselves with the treadmill beforehand. Patients underwent 2 treadmill exercise tests to ensure reproducibility (<20% difference in PWT) and a third exercise test for cardio cardiopulmonary testing (Spectra 29; SensorMedics) to measure peak oxygen uptake. During the 3- and 6-month visits, patients underwent 2 exercise tests within a 1-week pe-

riod with cardiopulmonary testing during one test. The maximum COT and PWT from the tests was used for data analysis at each time point.

Ankle Brachial Index

With the patient lying supine in a temperature-controlled room, blood pressure cuffs were placed bilaterally on the upper arms and ankles and inflated to 30 mm Hg above the systolic pressure (Flo-Laboratory Model 2100; Parks Medical Electronics). Blood pressure in bilateral brachial, posterior tibial, and dorsalis pedis arteries was measured by Doppler. The ABI was calculated as the ratio of the higher of the 2 ankle pressures to the higher of the 2 brachial pressures.

Questionnaires

Participants completed the walking impairment questionnaire (WIQ), which enabled calculation of walking distance, walking speed, and stair-climbing scores. The 36-item Short-Form Health Survey (SF-36) was completed, and the physical composite score (PCS-36), mental composite score (MCS-36), and physical functioning subscore were calculated.

Progenitor Cell Counts

After an overnight fast, venous blood was collected in EDTA tubes and incubated with fluorochrome-labeled monoclonal antihuman mouse antibodies within 4 hours. Cell populations enriched for circulating PCs were enumerated using flow cytometry as CD45^{med+} cells coexpressing CD34⁺, CD133⁺, vascular endothelial growth factor receptor 2 (VEGFR2⁺), or CXCR4⁺ and their combination.²¹⁻²⁴

We incubated 300 µL of peripheral blood with 7 µL of FITC-CD34 (BD Biosciences), PerCP-CD45 (BD Biosciences), PE-VEGF-R2 (R&D System) and 5-µL APC-CD133 (Miltenyi), and 3-µL PE-Cy7-conjugated anti-CXCR4 (EBioscience, clone 12G5) in the dark for 15 minutes. Then 1.5-mL ammonium chloride lysing buffer was added to lyse red blood cells; 1.5-mL staining medium (PBS with 3% heat-inactivated serum and 0.1% sodium azide) was added to stop the lysing reaction. Prior to flow cytometry, 100 µL of AccuCheck Counting Beads (Invitrogen, No. PCB100) were added to act as an internal standard for direct estimation of the concentration of target cell subsets. At least 2.5 million events were acquired from the cytometer. Flow data were analyzed with Flowjo software (Treestar). Absolute mononuclear cell count was estimated as the sum of lymphocytes and monocytes using a Coulter ACT/Diff cell counter (Beckman Coulter). Progenitor cell populations are reported as cell counts per µL. In 20 samples that were analyzed on 2 occasions by the same technician, the coefficients of variation of the cell types were as follows: CD34⁺, 2.9%; CD34⁺/CD133⁺, 4.8%; CD34⁺/CXCR4⁺, 6.5%; and CD34⁺/CD133⁺/CXCR4⁺, 7.5%, CD34⁺/VEGF2R cells, 21.6%.

Statistical Analysis

The primary efficacy outcome was change in PWT from baseline at 3 months. Secondary efficacy outcomes considered were change in PWT at 6 months, change in COT at 3 and 6 months, and change in the WIQ and SF-36 survey scores. Safety end points included frequencies of adverse events, serious ad-

verse events, and discontinuations due to adverse events. All participants who were randomized and had end-point measurements at 3 or 6 months were included in the efficacy and safety analyses on an intention-to-treat basis. A post hoc per-protocol analysis was also performed after excluding protocol deviations.

Continuous variables are presented as mean (SD). Changes from baseline at 3 and 6 months are presented as mean (95% CI). Nonnormally distributed data are presented as median (interquartile range [IQR]) and categorical variables as proportions. Group comparisons involving categorical and continuous outcomes at baseline were evaluated with χ^2 tests and *t* tests, respectively. Statistical analyses of the primary and secondary outcomes were performed using a linear mixed-effects model, which provided separate estimates of the mean change by time on intervention and control groups. The model-based means are unbiased with unbalanced and incomplete data, provided that the missing data are noninformative (missing at random). A compound symmetry form in the repeated measurements was assumed for each outcome. Safety end points were analyzed by χ^2 tests. Data analysis was performed using SAS version 9.3 (SAS Institute). Statistical significance was based on 2-tailed tests, and a significance level of $\alpha = .05$ was used. Based on our phase 1 study, a target sample size of 80 patients in each group allowed us to detect a minimum difference in the improvement in PWT of 48.7 seconds between groups with power of 90% using $\alpha = .05$, assuming a common standard deviation of 94.4 seconds.¹⁸ No data are available regarding whether this difference of 48.7 seconds is consistent with a clinically meaningful change.

Results

From 322 screened participants with intermittent claudication, we randomized 159 participants, who had a mean age of 64 years and were 87% male, to receive either subcutaneous GM-CSF 3 times per week for 4 weeks ($n = 80$) or matching placebo ($n = 79$) (Figure). The age, sex, and risk factor prevalence did not differ significantly between the 2 groups (Table 1). Additionally, the treatment groups were matched for baseline symptoms, treadmill exercise duration, ABI, and PC counts. Of the 159 participants recruited, 10 did not undergo follow-up testing (Figure). Thus, the final intention-to-treat analysis was based on 149 participants who completed the 3-month follow-up, 6-month follow-up, or both. One hundred thirty participants had measurement of the primary end point at 3 months and 148 at 6 months.

Effect of GM-CSF on Circulating PCs

Treatment with GM-CSF resulted in leukocytosis with a peak increase in the total leucocyte count on week 3 of treatment (Table 2). Populations of PC-enriched cells were assessed by the expression of CD34, CD133, and CXCR4 epitopes on CD45^{med} cells. Therapy with GM-CSF resulted in an increase in PCs that peaked at the end of week 2 and returned to baseline by week 4. The peak increase in the median number of cir-

culating CD34⁺, CD34⁺/CD133⁺, and CD34⁺/CXCR4⁺ cells with GM-CSF ranged between 76% and 110% ($P < .001$ for all), but there was no increase in any of the leucocyte subpopulations in the placebo group (Table 2).

Effect of GM-CSF on Treadmill Exercise Capacity

The PWT during treadmill exercise increased in patients in both the GM-CSF and placebo groups ($P < .01$ in both groups). The primary end point, comparing the increase in PWT from baseline to 3 months between the GM-CSF group (from mean [SD] 296 [151] to 405 [248] seconds, a change of 109 seconds [95% CI, 67 to 151], 30% increase) and placebo group (from 308 [161] to 376 [182] seconds, a mean change of 56 seconds [95% CI, 14 to 98], 21% increase), was not significant (difference in change between groups of 53 seconds [95% CI, -6 to 112]; $P = .08$) (Table 3). Changes in PWT at 6 months and COT were also not significant. The peak oxygen consumption (VO_{2max}) did not change significantly in either group (Table 2). Changes in PWT at 6 months and COT at both 3 and 6 months were also not significant.

Effect of GM-CSF on Other Secondary End Points

There was a greater improvement in the walking distance subscore of the WIQ at 3 months with GM-CSF compared with placebo; the difference in change between groups was 7.9 (95% CI, 0.2-15.7; $P = .047$) (Table 3). Similarly, there was a significantly greater improvement in the physical functioning subscale score of the SF-36 survey at 3 months with GM-CSF compared with placebo; the difference in change between groups was 7.5 (95% CI, 1.0-14.0; $P = .03$) (Table 3). There were no significant differences in changes in the WIQ speed or stair-climbing scores between the GM-CSF and placebo groups. There were no significant differences in change in the PCS-36 or MCS-36 scores between the GM-CSF and placebo groups. In addition, there were no significant differences in changes in the ABI or in oxygen consumption at the 3- or 6-month follow-up visits between the 2 groups.

Adverse Effects

Adjusting the GM-CSF dose was necessary for 1 patient who had a platelet count less than 75 000. There were 18 serious adverse events, 9 in the placebo and 9 in the GM-CSF group (eTable 1 in the Supplement). One patient in the GM-CSF group died in an automobile crash. Cancer was diagnosed in 2 patients treated with GM-CSF and 1 patient treated with placebo. Six of these participants were not followed up after their serious adverse events (Figure). Nonserious adverse effects that were more frequently observed in the GM-CSF group compared with the placebo group included headache, gastrointestinal symptoms, rash, and fatigue and were restricted to the first 4 weeks of therapy (eTable 2 in the Supplement). The most common adverse effect was rash appearing at the injection site, but none of these led to discontinuation or modification of therapy.

Exploratory Analyses

After excluding 4 participants with protocol deviations, including incomplete treatment due to incarceration, acci-

Table 1. Baseline Characteristics

	All (N = 159)	GM-CSF (n = 80)	Placebo (n = 79)
Age, mean (SD), y	63.9 (7.9)	64.3 (7.9)	63.6 (7.9)
Male sex, No. (%)	139 (87.4)	70 (87.5)	69 (87.3)
White, No. (%)	78 (49.1)	33 (41.3)	45 (57.0)
Black, No. (%)	79 (49.7)	45 (56.3)	34 (43.0)
Diabetes, No. (%)	58 (36.5)	29 (36.3)	29 (36.7)
Smoking, No. (%)	75 (47.2)	35 (43.8)	40 (50.6)
History, No. (%)			
Duration of claudication, mean (SD), mo	27.9 (26.5)	27.4 (25.8)	28.3 (27.3)
Previous peripheral revascularization	60 (37.7)	29 (36.3)	31 (39.2)
Stroke	38 (23.9)	18 (22.5)	20 (25.3)
Myocardial infarction	53 (33.3)	28 (35.0)	25 (31.6)
Coronary bypass graft surgery	37 (23.3)	19 (23.8)	18 (22.8)
Previous coronary angioplasty	49 (30.8)	25 (31.3)	24 (30.4)
Hypertension	153 (96.2)	78 (97.5)	75 (94.9)
Hyperlipidemia	134 (87.0)	70 (89.7)	64 (84.2)
Chronic kidney disease	12 (20.3)	8 (10.0)	4 (5.1)
Measurements, mean (SD)			
BMI ^a	29.8 (7.1)	29.6 (6.3)	30.1 (7.9)
Glucose, mg/dL	102.7 (31.6)	101.2 (26.3)	104.3 (36.2)
Creatinine, mg/dL	1.1 (0.4)	1.1 (0.4)	1.1 (0.3)
LDL, mg/dL	89.3 (31.5)	89.9 (32.5)	88.7 (30.7)
HDL, mg/dL	43.2 (12.8)	44.0 (12.5)	42.4 (13.1)
Peak walking time, s	305 (160)	303 (160)	308 (161)
Claudication onset time, s	153 (85)	152 (83)	155 (87)
Ankle brachial index	0.60 (0.15)	0.59 (0.15)	0.60 (0.16)
Medications, No. (%)			
Aspirin	101 (63.5)	52 (65.0)	49 (62.0)
Plavix	27 (17.0)	13 (16.3)	14 (17.7)
Statin	112 (70.4)	56 (70.0)	56 (70.9)
Cilostazol	40 (25.2)	24 (30.0)	16 (20.3)
Angiotensin antagonists (ACEi and ARB)	92 (57.9)	48 (60.0)	44 (55.7)
β-Blocker	79 (49.7)	38 (47.5)	41 (51.9)
Calcium channel blocker	51 (32.1)	31 (38.8)	20 (25.3)
WIQ scores, mean (SD) ^b			
Distance	27.5 (22.0)	25.2 (21.3)	29.7 (22.7)
Speed	36.1 (23.5)	36.0 (24.1)	36.2 (23.0)
Stair climbing	44.3 (25.2)	43.4 (25.4)	45.2 (25.0)
SF-36 scores, mean (SD)			
Physical functioning	40.5 (19.1)	40.1 (19.1)	41.0 (19.1)
PCS-36	31.7 (7.8)	31.1 (7.9)	32.3 (7.7)
MCS-36	48.4 (11.8)	49.3 (11.7)	47.6 (12.0)

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; GM-CSF, granulocyte-macrophage colony-stimulating factor; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MCS-36, mental composite score; PCS-36, physical composite score; SF-36, 36-item Short-Form Health Survey; WIQ, walking impairment questionnaire.

SI conversion factors: To convert creatinine to μmol/L, multiply by 88.4; to convert LDL and HDL to mmol/L, multiply by 0.0259.

^a Calculated as weight in kilograms divided by height in meters squared.

^b The WIQ score quantifies walking difficulty on a 100-point scale, in which 0 indicates extreme difficulty and 100 indicates no difficulty with walking distance, walking speed, and stair-climbing elements.

dental lower limb injury, and 2 patients who enrolled in a rehabilitation program, we performed a post hoc per-protocol analysis (eTable 3 in the Supplement). In this analysis, the 113-second (95% CI, 73-152) increase (31%) in PWT from baseline to 3 months, and the 122-second (95% CI, 83-161) increase (35%) at 6 months in those receiving GM-CSF was significantly greater than the increase in the placebo group (44 seconds [95% CI, 4-84], 19%, $P = .02$, and 57 seconds [95% CI, 18-96], 21%, $P = .02$, between groups at 3 and 6 months, respectively).

In an exploratory analysis, we also investigated the relationship between the magnitude of PC mobilization and the improvement in exercise capacity with GM-CSF. Participants receiving GM-CSF who had a greater increase in PCs (>100% increase in CD34⁺/CD133⁺ cells, $n = 38$) at 2 weeks had a significantly greater increase in PWT at 3 months (131 seconds [95% CI, 74-187] vs 60 seconds [95% CI, 26-93], $P = .04$) and at 6 months (136 seconds [95% CI, 80-192] vs 70 seconds [95% CI, 37-103] seconds, $P = .048$) than those with less than 100% increase in these PCs.

Table 2. Changes in Leucocyte Subsets With GM-CSF and Placebo

	Mean (95% CI)							P Value ^a
	Baseline	Week 1	Week 2	Week 3	Week 4	3 mo	6 mo	
Leukocytes, ×10 ⁶ cells/mL								
GM-CSF	7.2 (6.8 to 7.7)	8.4 (7.7 to 9.0)	9.7 (8.8 to 10.6)	9.8 (8.9 to 10.7)	8.9 (8.1 to 9.7)	6.8 (6.4 to 7.2)	7.1 (6.5 to 7.6)	<.001
Placebo	7.6 (7.1 to 8.1)	7.1 (6.7 to 7.6)	6.9 (6.5 to 7.3) ^b	7.2 (6.7 to 7.6)	7.3 (6.8 to 7.8)	7.5 (7.0 to 7.9)	7.3 (6.8 to 7.8)	
Difference ^c		1.6 (0.9 to 2.2)	2.8 (2.2 to 3.5)	2.9 (2.3 to 3.6)	2.1 (1.5 to 2.7)	0.09 (-0.6 to 0.7)	0.2 (-0.4 to 0.9)	
CD34 ⁺ , cells/μL								
GM-CSF	2.4 (2.1 to 2.7)		4.9 (4.1 to 5.7) ^b		2.7 (2.3 to 3.1)	2.6 (2.1 to 3.0)	2.5 (2.1 to 2.9)	<.001
Placebo	2.3 (1.9 to 2.6)		2.1 (1.8 to 2.3)		2.3 (2.0 to 2.6)	2.0 (1.7 to 2.2)	2.2 (1.9 to 2.5)	
Difference ^c			2.6 (2.1 to 3.1)		0.1 (-0.3 to 0.6)	0.3 (-0.2 to 0.8)	0.2 (-0.3 to 0.6)	
CD34 ⁺ /CD133 ⁺ , cells/μL								
GM-CSF	1.2 (1.0 to 1.3)		2.5 (2.0 to 3.0) ^b		1.3 (1.1 to 1.5)	1.3 (1.1 to 1.6)	1.3 (1.1 to 1.5)	<.001
Placebo	1.2 (1.0 to 1.3)		1.0 (0.9 to 1.1)		1.2 (1.0 to 1.4)	1.0 (0.9 to 1.1)	1.1 (1.0 to 1.3)	
Difference ^c			1.4 (1.1 to 1.7)		-0.03 (-0.3 to 0.2)	0.2 (-0.1 to 0.5)	0.1 (-0.2 to 0.4)	
CD34 ⁺ /CXCR4 ⁺ , cells/μL								
GM-CSF	1.2 (1.0 to 1.4)		2.5 (2.0 to 3.0) ^b		1.3 (1.1 to 1.6)	1.4 (1.1 to 1.7)	1.3 (1.1 to 1.6)	<.001
Placebo	1.1 (0.9 to 1.3)		1.0 (0.9 to 1.2)		1.2 (1.0 to 1.3)	1.0 (0.8 to 1.1)	1.1 (0.9 to 1.3)	
Difference ^c			1.3 (1.0 to 1.7)		-0.006 (-0.4 to 0.4)	0.2 (-0.2 to 0.6)	0.2 (-0.2 to 0.6)	

Abbreviation: GM-CSF, granulocyte-macrophage colony-stimulating factor.

^b P < .001 from t tests comparing baseline at week 2.

^a P values refer to differences between GM-CSF and placebo groups by mixed-effects models.

^c Difference of changes from baseline between GM-CSF and placebo.

Discussion

In this phase 2 study, we tested the hypothesis that administration of a myeloid cytokine that mobilizes bone marrow PCs into the circulation would improve walking performance in patients with claudication. In a placebo-controlled, double-blind study of subcutaneous injections of GM-CSF (500 μg per day self-administered 3 times a week for 4 weeks), there was no difference in change in PWT between the GM-CSF and placebo groups. In secondary analyses, there was improvement in the SF-36 physical functioning score and the WIQ distance score with GM-CSF as compared with placebo. However, there were no significant differences between the intervention and placebo groups with regard to change in WIQ walking speed or stair-climbing score, COT, PCS-36 or MCS-36 components of the SF-36, ABI, or oxygen consumption.

The crucial contribution of PCs to vascular repair, collateralization, and re-endothelialization of the vasculature after acute ischemic injury is well established.²⁵⁻²⁷ Although GM-CSF is a less potent mobilizing agent for leukocytes and hematopoietic progenitors compared with G-CSF, potential advantages of GM-CSF include selective mobilization of more primitive stem cells (CD34⁺/CD38⁻/HLA-DR⁺) and macrophages.²⁸ Moreover, GM-CSF has direct stimulatory effects on endothelial cells and monocytes, which may contrib-

ute to its vasculogenic properties.^{10,13-15,29-31} Mobilization of the bone marrow cells is relatively modest with GM-CSF, addressing potential safety concerns that have been raised with the use of G-CSF in atherosclerosis.^{29,32,33}

There may be several reasons for the lack of improvement in our primary outcome and most of our secondary outcome measures in this PAD population. First, aging and exposure to cardiovascular risk factors that are prevalent in our population are known to reduce the number and potency of endogenous PCs.^{34,35} The increase in leukocyte counts in our PAD population was modest in comparison with healthy cohorts, probably a reflection of senescence of the bone marrow in PAD.³⁶ Second, possible adherence issues with the self-injectable treatment in our study may have reduced effectiveness in some participants. Study drugs were injected under observation once each week. Whether titration to a higher dose of GM-CSF would have enhanced mobilization in those with relatively modest mobilization remains to be studied. However, in our phase 1 dose-escalation study, we did not observe a dose-response in the magnitude of PC mobilization with GM-CSF given in concentrations ranging from 3 to 10 μg/kg per day.¹⁸ Thus, it is possible that some participants were unable to mobilize sufficient PCs from their marrow for cytokine therapy to be effective or that stronger cytokines such as G-CSF may be appropriate for these individuals.

Table 3. Peak Walking Time, Claudication Onset Time, ABI, Peak Oxygen Consumption, WIQ and SF-36 Scores, and Their Changes at 3 and 6 Months With GM-CSF and Placebo^a

	GM-CSF			Placebo			Difference Between GM-CSF and Placebo			
	Baseline	3 mo	6 mo	Baseline	3 mo	6 mo	3 mo	P Value ^b	6 mo	P Value ^b
PWT, s	296 (151)	405 (248)	411 (253)	308 (161)	376 (182)	386 (209)				
Change		109 (67 to 151)	112 (71 to 153)		56 (14 to 98)	77 (36 to 117)	53 (-6 to 112)	.08	35 (-23 to 93)	.24
COT, s	146 (67)	230 (161)	233 (158)	155 (87)	220 (114)	216 (129)				
Change		81 (52 to 111)	93 (64 to 122)		61 (32 to 91)	61 (33 to 89)	20 (-22 to 62)	.35	32 (-8 to 72)	.12
ABI	0.59 (0.02)	0.63 (0.02)	0.61 (0.02)	0.60 (0.02)	0.64 (0.03)	0.65 (0.02)				
Change		0.04 (0.008 to 0.06)	0.02 (-0.003 to 0.05)		0.03 (0.004 to 0.06)	0.04 (0.01 to 0.06)	0.003 (-0.04 to 0.04)	.87	-0.01 (-0.05 to 0.02)	.45
VO _{2max} , L/min	1.25 (0.07)	1.24 (0.05)	1.21 (0.04)	1.25 (0.04)	1.27 (0.06)	1.34 (0.06)				
Change		-0.02 (-0.14 to 0.09)	-0.04 (-0.15 to 0.07)		0.04 (-0.07 to 0.15)	0.10 (-0.01 to 0.20)	-0.06 (-0.22 to 0.10)	.46	-0.13 (-0.29 to 0.02)	.09
WIQ scores ^c										
Distance	25.2 (21.3)	40.1 (27.6)	34.5 (27.4)	29.7 (22.7)	36.2 (24.8)	33.1 (24.4)				
Change		12.5 (6.4 to 18.7)	8.0 (2.1 to 14.0)		4.8 (-0.2 to 9.8)	3.7 (-0.9 to 8.3)	7.9 (0.2 to 15.7)	.047	4.9 (-2.4 to 12.1)	.19
Speed	36.0 (24.1)	47.3 (25.3)	47.5 (23.4)	36.3 (23.0)	43.2 (24.5)	44.7 (22.7)				
Change		9.1 (4.2 to 14.0)	9.9 (4.6 to 15.3)		7.5 (2.1 to 12.8)	8.5 (3.8 to 13.2)	1.5 (-5.7 to 8.8)	.68	1.1 (-5.7 to 7.8)	.76
Stair climbing	43.4 (25.4)	52.9 (26.4)	54.7 (25.9)	45.2 (25.0)	51.4 (28.8)	52.2 (24.3)				
Change		8.5 (3.0 to 13.9)	10.4 (4.9 to 15.9)		4.2 (-2.2 to 10.6)	6.6 (1.2 to 11.9)	3.6 (-4.4 to 11.6)	.38	3.5 (-4.0 to 11.0)	.36
SF-36 ^d										
PCS-36	31.1 (7.9)	35.5 (8.2)	36.3 (9.1)	32.3 (7.7)	34.3 (7.6)	35.7 (7.0)				
Change		4.1 (2.4 to 5.8)	5.2 (3.3 to 7.0)		2.1 (0.5 to 3.7)	3.1 (1.5 to 4.7)	2.3 (-0.1 to 4.6)	.06	1.9 (-0.4 to 4.1)	.11
MCS-36	49.3 (11.7)	50.4 (10.3)	49.5 (10.3)	47.6 (12.0)	51.3 (10.9)	48.6 (10.7)				
Change		0.7 (-1.5 to 2.9)	-0.2 (-2.4 to 2.0)		2.4 (-0.2 to 5.0)	1.1 (-1.0 to 3.2)	-1.4 (-4.6 to 1.8)	.39	-1.2 (-4.3 to 1.8)	.42
Physical functioning	40.1 (19.1)	52.9 (20.6)	55.1 (19.6)	41.0 (19.1)	46.6 (20.0)	49.4 (17.2)				
Change		11.4 (6.7 to 16.1)	14.3 (9.7 to 18.9)		4.8 (-0.1 to 9.6)	8.1 (3.7 to 12.4)	7.5 (1.0 to 14.0)	.03	5.9 (-0.3 to 12.1)	.06

Abbreviations: ABI, ankle brachial index; COT, claudication onset time; GM-CSF, granulocyte-macrophage colony-stimulating factor; MCS-36, mental composite score; PCS-36, physical composite score; PWT, peak walking time; SF-36, 36-item Short-Form Health Survey; WIQ, walking impairment questionnaire.

^a Data are presented as absolute mean (standard deviation). For change from baseline, and for differences between groups, data are presented as model-based means (and 95% confidence intervals).

^b P values refer to differences between GM-CSF and placebo groups by mixed-effects models at 3 months and 6 months.

^c The WIQ quantifies walking difficulty on a 100-point scale, in which 0 indicates extreme difficulty and 100 indicates no difficulty with walking distance, walking speed, and stair-climbing elements.

^d The SF-36 PCS-36 and MCS-36 are summary measures derived from 8 scale scores, including the physical functioning subscale. Higher scores indicate better performance.

We observed a modest improvement in PWT in our phase 1 study after 2 weeks of therapy with GM-CSF.¹⁸ In this phase 2 study, we increased the duration of treatment to 4 weeks and selected a 500-µg dose of GM-CSF that was equivalent to a dose of approximately 6 µg/kg for an 80-kg individual. Leukocyte count data demonstrated that peak mobilization of bone marrow cells occurred at 3 weeks with a decline toward baseline at 4 weeks, suggesting that 3 weeks of thrice-weekly therapy may be enough to maximize marrow mobilization in PAD.¹⁸

In exploratory analyses, we excluded 4 participants with protocol deviations and performed a post hoc analysis. We observed a greater improvement in the peak treadmill time in participants treated with GM-CSF compared with placebo at 3

months that persisted for 6 months. The magnitude of increase in PWT on the treadmill with GM-CSF compared with placebo is similar to our phase 1 study findings and to those observed after direct intramuscular injection of PCs and with exercise training.^{18,37,38} In exploratory analyses, we also found that participants with a greater magnitude of mobilization of CD34⁺/CD133⁺ PCs had a greater therapeutic benefit, suggesting a link between the robustness of PC mobilization and the effectiveness of GM-CSF.

Our study had some limitations. First, our study design did not allow us to determine whether changes in the dose and duration and whether repeat administration enhance the therapeutic benefit of GM-CSF therapy. Second, our study popula-

tion consisted of patients with claudication who underwent angiography. Further study is needed to determine whether GM-CSF is beneficial in critical limb ischemia or to other people not eligible for this study. Third, our study design encouraged participants in both the GM-CSF and placebo groups to walk to claudication several times a day, to promote homing of PCs to the ischemic muscles.³⁹ However, this increased activity may have resulted in greater improvement in walking capacity in participants receiving placebo, as expected from studies demonstrating improvements with structured exercise in PAD.³⁸ Fourth, the magnitude of change in PWT that we used for our power calculations has not been demonstrated to represent a clinically meaningful change. The magnitude of improvement in PWT from GM-CSF was substantially less than that previously reported for ramipril therapy.⁴⁰ Fifth, although we had based our sample size for this study on our

phase 1 findings, we observed greater variability in PWT, and thus this study may have been underpowered for our primary end point. Sixth, although no significant differences were observed in the serious adverse event rates between groups, our study was not powered to evaluate this.

Conclusions

Therapy with GM-CSF 3 times a week did not improve treadmill walking performance at 3-month follow-up. The improvement in a subset of secondary outcomes observed with GM-CSF suggests that GM-CSF may warrant further study in patients with claudication. In addition, further investigation is needed to investigate the variability of responsiveness to GM-CSF and its clinical significance.

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REFERENCES

- Ostchega Y, Paulose-Ram R, Dillon CF, Gu Q, Hughes JP. Prevalence of peripheral arterial disease and risk factors in persons aged 60 and older: data from the National Health and Nutrition Examination Survey 1999-2004. *J Am Geriatr Soc*. 2007;55(4):583-589.
- Norgren L, Hiatt WR, Harris KA, Lammer J; TASC II Working Group. TASC II section F on revascularization in PAD. *J Endovasc Ther*. 2007;14(5):743-744.
- Go AS, Mozaffarian D, Roger VL, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke

statistics, 2013 update: a report from the American Heart Association. *Circulation*. 2013;127(1):e6-e245.

4. McDermott MM, Greenland P, Liu K, et al. Leg symptoms in peripheral arterial disease: associated clinical characteristics and functional impairment. *JAMA*. 2001;286(13):1599-1606.

5. McDermott MM, Liu K, Greenland P, et al. Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms. *JAMA*. 2004;292(4):453-461.

6. McDermott MM, Greenland P, Liu K, et al. The ankle brachial index is associated with leg function and physical activity: the Walking and Leg Circulation Study. *Ann Intern Med*. 2002;136(12):873-883.

7. McDermott MM, Guralnik JM, Tian L, et al. Baseline functional performance predicts the rate of mobility loss in peripheral arterial disease. *J Am Coll Cardiol*. 2007;50(10):974-998.

8. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275(5302):964-967.

9. Takahashi T, Kalka C, Masuda H, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med*. 1999;5(4):434-438.

10. Bussolino F, Wang JM, Defilippi P, et al. Granulocyte- and granulocyte-macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate. *Nature*. 1989;337(6206):471-473.

11. Bussolino F, Ziche M, Wang JM, et al. In vitro and in vivo activation of endothelial cells by colony-stimulating factors. *J Clin Invest*. 1991;87(3):986-995.

12. Sacramento CB, Cantagalli VD, Grings M, et al. Granulocyte-macrophage colony-stimulating factor gene based therapy for acute limb ischemia in a mouse model. *J Gene Med*. 2009;11(4):345-353.

13. Buschmann IR, Hoefler IE, van Royen N, et al. GM-CSF: a strong arteriogenic factor acting by amplification of monocyte function. *Atherosclerosis*. 2001;159(2):343-356.

14. Hashimoto SI, Komuro I, Yamada M, Akagawa KS. IL-10 inhibits granulocyte-macrophage colony-stimulating factor-dependent human monocyte survival at the early stage of the culture

and inhibits the generation of macrophages. *J Immunol.* 2001;167(7):3619-3625.

15. Seiler C, Pohl T, Wustmann K, et al. Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. *Circulation.* 2001;104(17):2012-2017.

16. Arai M, Misao Y, Nagai H, et al. Granulocyte colony-stimulating factor: a noninvasive regeneration therapy for treating atherosclerotic peripheral artery disease. *Circ J.* 2006;70(9):1093-1098.

17. van Royen N, Schirmer SH, Atasever B, et al. START Trial: a pilot study on STimulation of ARTeriogenesis using subcutaneous application of granulocyte-macrophage colony-stimulating factor as a new treatment for peripheral vascular disease. *Circulation.* 2005;112(7):1040-1046.

18. Subramaniam V, Waller EK, Murrow JR, et al. Bone marrow mobilization with granulocyte macrophage colony-stimulating factor improves endothelial dysfunction and exercise capacity in patients with peripheral arterial disease. *Am Heart J.* 2009;158(1):53-60.

19. Rutherford RB, Baker JD, Ernst C, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. *J Vasc Surg.* 1997;26(3):517-538.

20. Gardner AW, Skinner JS, Cantwell BW, Smith LK. Progressive vs single-stage treadmill tests for evaluation of claudication. *Med Sci Sports Exerc.* 1991;23(4):402-408.

21. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. *Blood.* 1996;87(1):1-13.

22. Berenson RJ. Transplantation of CD34+ hematopoietic precursors: clinical rationale. *Transplant Proc.* 1992;24(6):3032-3034.

23. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A.* 1992;89(7):2804-2808.

24. Miraglia S, Godfrey W, Yin AH, et al. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood.* 1997;90(12):5013-5021.

25. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107(11):1395-1402.

26. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med.* 2001;7(4):430-436.

27. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res.* 1999;85(3):221-228.

28. Fischmeister G, Kurz M, Haas OA, et al. G-CSF versus GM-CSF for stimulation of peripheral blood progenitor cells (PBPC) and leukocytes in healthy volunteers: comparison of efficacy and tolerability. *Ann Hematol.* 1999;78(3):117-123.

29. Lane TA, Ho AD, Bashey A, Peterson S, Young D, Law P. Mobilization of blood-derived stem and progenitor cells in normal subjects by granulocyte-macrophage- and granulocyte-colony-stimulating factors. *Transfusion.* 1999;39(1):39-47.

30. Lane TA, Law P, Maruyama M, et al. Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation. *Blood.* 1995;85(1):275-282.

31. Zbinden R, Vogel R, Meier B, Seiler C. Coronary collateral flow and peripheral blood monocyte concentration in patients treated with granulocyte-macrophage colony stimulating factor. *Heart.* 2004;90(8):945-946.

32. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood

stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet.* 2004;363(9411):751-756.

33. Shi Q, Rafii S, Wu MH, et al. Evidence for circulating bone marrow-derived endothelial cells. *Blood.* 1998;92(2):362-367.

34. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, et al. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation.* 2003;108(4):457-463.

35. Heiss C, Keymel S, Niesler U, Ziemann J, Kelm M, Kalka C. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol.* 2005;45(9):1441-1448.

36. Fischmeister G, Gadner H. Granulocyte colony-stimulating factor versus granulocyte-macrophage colony-stimulating factor for collection of peripheral blood progenitor cells from healthy donors. *Curr Opin Hematol.* 2000;7(3):150-155.

37. Tateishi-Yuyama E, Matsubara H, Murohara T, et al; Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet.* 2002;360(9331):427-435.

38. McDermott MM, Liu K, Guralnik JM, et al. Home-based walking exercise intervention in peripheral artery disease: a randomized clinical trial. *JAMA.* 2013;310(1):57-65.

39. Hristov M, Erl W, Weber PC. Endothelial progenitor cells: mobilization, differentiation, and homing. *Arterioscler Thromb Vasc Biol.* 2003;23(7):1185-1189.

40. Ahimastos AA, Walker PJ, Askew C, et al. Effect of ramipril on walking times and quality of life among patients with peripheral artery disease and intermittent claudication: a randomized controlled trial. *JAMA.* 2013;309(5):453-460.