Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Nithin Chand Kurra Date

Racial Differences in the Effect of Progenitor Cell Mobilization with Granulocyte-Macrophage Colony-Stimulating Factor in Peripheral Artery Disease

By

Nithin C. Kurra
M.B.B.S., Dr. NTR University of Health Sciences, 2018

_____

Dr. Arshed Quyyumi, MD, FACC

Field Advisor

_____

Dr. Amit Shah, MD, MSCR

Faculty Thesis Advisor

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University in partial
fulfillment of the requirements for the degree of
Master of Public Health in
Epidemiology
2020

Racial Differences in the Effect of Progenitor Cell Mobilization with Granulocyte-Macrophage Colony-Stimulating Factor in Peripheral Artery Disease

By

Nithin C. Kurra
M.B.B.S., Dr. NTR University of Health Sciences, 2018

Field Advisor: Dr. Arshed Quyyumi, MD, FACC
Faculty Advisor: Dr. Amit Shah, MD, MSCR

An abstract of a thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University in partial fulfillment of the
requirements for the degree of
Master of Public Health in
Epidemiology
2020

Abstract

Racial Differences in the Effect of Progenitor Cell Mobilization with Granulocyte-Macrophage Colony-Stimulating Factor in Peripheral Artery Disease

Background: Peripheral Arterial Disease (PAD) is a progressive atherosclerotic disease of the peripheral arteries. The effect of GM-CSF on improving the walking capacity in patients with lower limb PAD is unclear based on current studies. Racial differences in the severity of lower extremity arterial atherosclerosis, degree of functional impairment, and complications secondary to PAD are well established, but racial differences in the effect of GM-CSF in patients with PAD is unclear.

Methods: One hundred and fifty-nine participants with PAD were enrolled at medical centers affiliated with Emory University in Atlanta, Georgia, in a double-blinded, placebo-controlled phase IIA trial. Participants were randomized into two treatment groups (1:1): GM-CSF and placebo. Participants would either receive $500\mu g$/day subcutaneous injections of GM-CSF (Leukine), three times a week for 4 weeks, or placebo. All participants were encouraged to walk at least 20mins/ day for 3 days/week. Peak walking time was recorded, and blood was collected to measure hemopoietic progenitor cell subpopulations. Differences in the impact of GM-CSF on peaking walking time between races were computed.

Results: Of the 159 participants, 79 (50.3%) were Black, and 78 (49.7%) were White with a mean age of 66 years and 62 years, respectively. Age, gender distribution, and total WBCs were significantly different between both the groups at baseline. Among Black participants, GM-CSF improved the peak walking time by +99.55 seconds (95% CI: +14.36, +184.73; p-value: 0.02) at 12 weeks, and +46.41 seconds (95% CI: -36.1, +128.93; p-value:0.27) at 24 weeks, compared to placebo. Among White participants, GM-CSF improved the peak walking time by +3.3 seconds (95% CI: -82.63, +89.23; p-value: 0.94) at 12 weeks, and +26.96 seconds (95% CI: -57.15, +111.07; p-value:0.53) at 24 weeks, compared to placebo. After adjusting for age, smoking, and baseline WBC count, there was an interaction of race on the effect of GM-CSF on PWT at 12-week follow-up (P=0.03).

Conclusions: GM-CSF did not result in a change in PWT in Whites with PAD at 12 weeks, but it improved the peak walking time in Blacks with PAD. Further investigations are needed to confirm the racial differences in the effect of GM-CSF in PAD.

Racial Differences in the Effect of Progenitor Cell Mobilization with Granulocyte-Macrophage Colony-Stimulating Factor in Peripheral Artery Disease

By

Nithin C. Kurra
M.B.B.S., Dr. NTR University of Health Sciences, 2018

Field Advisor: Dr. Arshed Quyyumi, MD, FACC
Faculty Advisor: Dr. Amit Shah, MD, MSCR

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University in partial fulfillment of the
requirements for the degree of
Master of Public Health in
Epidemiology
2020

Acknowledgments

I would like to thank Dr. Arshed Quyyumi for providing me this opportunity, and Dr. Amit Shah for his clinical expertise and guidance in Epidemiology concepts.

Also, I would like to thank Dr. Anurag Mehta for his continuous support and mentorship during the development of this thesis.

Lastly, I thank my parents Rajasekhara Rao and Jyothi, my brother Chaithanya, my uncle Vijay, my aunt Lavanya, my nephew, and niece Shruthi and Shravan for their love and support throughout this journey.

Table of Contents

List of Tables and Figures

Abbreviations

Abbreviation	**Description**
PAD	Peripheral Arterial Disease
MI	Myocardial Infarction
ABI	Ankle Brachial Index
G-CSF	Granulocyte Colony-Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
PC	Progenitor Cell
PWT	Peak Walking Time
HPC	Hemopoietic Progenitor Cell
WBC	White Blood Cell
CFU	Colony Forming Unit
SD	Standard Deviation

Introduction

Peripheral Arterial Disease (PAD) is a progressive atherosclerotic disease of the peripheral arteries. PAD is characterized by the stenosis and/ or occlusion of the large or medium-sized arteries in the extremities[4]. It is widespread globally, with a prevalence of more than 200 million across the world and approximately 10 million in the US. Older age groups (>40 years) constitute a substantial proportion of all the cases, and exposure to certain risk factors like smoking increases the likelihood of the disease. Diabetes, hypertension, and hypercholesterolemia accelerate the progression of the disease and increase the risk of complications.

Lower limbs are affected more than the upper limbs. Symptoms range from none to severe limb threating ischemia among which *claudicatio intermittens* (intermittent claudication), a reproducible pain in specific muscle groups which occurs with activity and relieved with rest, is the typical symptom of lower limb PAD[1] PAD is associated with higher incidence of other cardiovascular conditions like stroke and MI because of the common risk factors; furthermore, increasing severity of PAD is associated with worse outcomes.

The diagnosis can be established by the measurement of the ankle-brachial index (ABI). ABI is obtained by dividing the higher posterior tibial or dorsalis pedis systolic blood pressure in each leg by the higher of the systolic pressures of the right or left arm. An ABI of ≤0.90 is indicative of PAD[2]. Toe-brachial index, exercising testing, or Doppler waveform analysis are also used in some settings.

The management of PAD focusses on relieving symptoms and lowering the risk of cardiovascular disease progression and complications. Risk factor reduction includes smoking cessation, anti-hypertensive therapy, lipid-lowering therapy, antiplatelet therapy, and dietary modifications. Graded exercise-therapy, revascularization techniques like percutaneous angioplasty, vascular bypass grafting, endarterectomy are the main modes of treatment. Sometimes, amputation may be needed in the event of gangrene.

PAD causes significant morbidity and mortality in patients. The average medical expenses for patients with PAD annually are over $11,000, including inpatient care, outpatient hospital-based care, outpatient office-based care, and prescription medication[3].

Background

In a world with a rapidly rising aging population, PAD is one of the significant health burdens contributing not only to mortality but also affecting the lower limb mobility and quality of life. Newer treatment modalities are necessary to fasten the treatment and reduce the incidence of complications. Stem and progenitor cell therapy is one such emerging modality.

Vascular networks adapt to chronic changes in blood flow by three primary mechanisms: Vasculogenesis, angiogenesis, and arteriogenesis. Vasculogenesis is seen in embryonic tissue and associated with the recruitment of endothelial progenitor cells. Angiogenesis, which is the branching and sprouting of new capillaries from preexisting vessels, is seen in response to tissue hypoxia. Arteriogenesis is the emergence of arteries and arterioles from preexisting microvessels with connection to an established vascular network[4]. Growing arterial collaterals are closely associated with the presence of activated tissue monocytes[5].

Apart from stimulating the proliferation of neutrophil and monocyte lineage cells, Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) also mobilize hematopoietic and other bone-marrow-derived progenitor cells into the peripheral circulation[6]. GM-CSF-treated animals showed a mild increase in capillary density and the number of intraparenchymal and leptomeningeal arterioles in rat models with bilateral carotid artery occlusion[7]. Endogenous, pharmacologically stimulated, and exogenous PCs contribute to reendothelialization and neovascularization.

Previously, a phase 1 dose-escalation trial was conducted, which demonstrated safety and the ability of GM-CSF to mobilize PCs into circulation. Also, improvement in exercise duration after three months of treatment was observed. We conducted a Phase-2 clinical trial, GPAD, which did not show a significant improvement in the peak walking time (PWT) with GM-CSF but showed improvements in some secondary outcomes. PROPEL, another Phase-II trial showed that GM-CSF did not have a significant effect on the 6-minute walk distance.

The prevalence of lower limb PAD is the highest in Blacks compared to Whites and Asians[8]. Also, Blacks are shown to have more severe lower limb atherosclerosis, higher rates of mobility loss, and greater functional impairment. The PROPEL study showed that GM-CSF improved the 6-minute walk distance in Whites with PAD but had no effect on Black participants. But, data about the racial differences in the effect of GM-CSF on the exercise capacity in patients with Peripheral Artery Disease (PAD) is insufficient. In another study, independent of other demographic and hematologic variables, Blacks demonstrated significantly better CD34+ HPCs mobilization responses to G-CSF than Caucasians[9]. But no information is available on the effect of GM-CSF.

In this post-hoc exploratory analysis, the effect of GM-CSF on the peak walking time among both Blacks and White participants of the GPAD-II is studied.

Methods

Informed consents were obtained from the participants, and the trial was approved by the institutional review boards at Emory University. An investigational new drug waiver was obtained from the US Food and Drug Administration.

Study Population and Data Sources: Three hundred and twenty-two patients aged between 21 and 80 years of age with symptoms of intermittent claudication were screened. Among them, 159 participants with PAD were enrolled in the study based on the inclusion and exclusion criteria. Practicing physicians at Emory Healthcare, Veterans Affairs hospitals in Atlanta, and the Medical College of Georgia were invited to refer potentially eligible patients with PAD.

Inclusion and exclusion criteria: Participants with an angiographically documented history of obstructive PAD, a peak walking time (PWT) on a treadmill between 1 and 12 minutes, a 2-month or longer history of intermittent claudication (Rutherford category 1-3)[10] in one or both lower extremities despite appropriate and stable medication regimen that included a statin for at least 3 months, and an ankle-brachial index (ABI) of <0.85 in the symptomatic limb were selected. Exclusion criteria included the presence of critical limb ischemia (Rutherford category 4-6); active infection; or a life expectancy of fewer than 12 months; advanced diabetic retinopathy; current or planned participation in a structured exercise program; coronary or lower extremity revascularization within the past four months; a history of acute coronary or cerebrovascular syndrome within the

past four months; any history of myeloid malignancy, severe congestive heart failure, or chronic renal, hepatic, or other inflammatory diseases.

Randomization: Random, permuted blocks of treatment assignments were generated, which were stratified for diabetes to ensure equal randomization of participants with diabetes into the treatment groups. Randomization was 1:1 to either GM-CSF group or matching placebo group. Double-blinding was followed for the duration of clinical evaluation, implementation of protocol, and analysis.

Interventions: Participants received either GM-CSF, 500µg (sargramostim [Leukine]; sanofi-aventis), or a matching placebo (normal saline). Treatment was administered subcutaneously thrice weekly on Monday, Wednesday, and Friday for four weeks. At the beginning of each week, one injection was administered under observation, and the remaining were self-administered. The dose of study drug was modified in participants with adverse effects, including pain unresponsive to analgesics, rash on >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).

Measurements: Safety and adverse events were recorded at baseline, week 1,2,3,4,5, and 8, and at three & six months. Measures of response-treadmill exercise, ABI were recorded at baseline, 3, and 6 months. PC counts were recorded at baseline, weeks 2 and 4, and 3 & 6 months. Gardner protocol was used to conduct the treadmill exercise testing and modified Bruce protocol for those (n=6) who developed claudication after exercising for 12 minutes[11]. PWT was measured as the maximum distance the patient could walk on the treadmill before the onset of the participant's typical claudication. At the 3rd and 6th month visits, 2 exercise tests were conducted within the 1-week period, and the maximum PWT value was used in the analysis. Venous blood was collected after an overnight fast and incubated with fluorochrome-labeled monoclonal antihuman mouse antibodies within 4 hours. Circulating PCs were identified using flow cytometry as $CD45^{med+}$ cells co-expressing CD^{34+}, CD^{133+}, vascular endothelial growth factor receptor 2 (VEGFR2+), or CXCR4+ and their combination.

Statistical Analysis: The primary outcome was the change in the PWT at three months, and the secondary outcome is the change in PWT at 6 months. All participants who had end-point measurements at 3^{rd} and 6^{th} month were included in the analysis on an intention-to-treat basis. All the participants with who are neither Blacks nor Whites (n=2) were excluded from the analysis. Baseline characteristics are presented as mean (95% SD) for continuous variables, and n (%) for categorical variables. Differences in the baseline characteristics between Blacks and Whites were compared using two-sample t-tests for continuous

variables, and χ^2 tests for categorical variables. Data analysis was conducted using SAS version 9.4 (SAS Institute). A significance level of $\propto = 0.05$ was used, and the statistical significance was based on 2-tailed tests. A compound symmetry form was assumed for each outcome.

Linear mixed-effects models were used to test the interaction between the effects of GM-CSF and race on the outcomes. These models provided separate estimates of the mean change by time on intervention and control groups. Age, gender, smoking status, and diabetes status were included in the model as they had significant baseline differences between Blacks and Whites and were potential confounders. The model-based means are unbiased with unbalanced and incomplete data, provided that the missing data are noninformative (missing at random).

Results

Among all the patients screened with intermittent claudication, 159 participants were randomized into 2 groups. Of the 159 participants, 157 were either Black (n=79) or White (n=78) and were included in the analysis. The mean age of the 157 participants is 64 years comprising 87% males. The treatment groups were matched for baseline symptoms, treadmill exercise duration, ABI, and PC counts. One hundred and twenty-nine participants and 147 participants had a measurement of the primary endpoint at 12 weeks and 24 weeks, respectively. Among the White participants, 65 (83.3%) and 73 (93.6%) completed 12-week and 24-week follow-up, respectively. Among the Black participants, 64 (81%) and 74 (93.7%) completed 12-week and 24-week follow-up, respectively. None of the PWT values were missing at baseline. The number of participants missing data for each variable is given in Table1.

Baseline Characteristics: The mean age for White participants is 66 years, which differs significantly with the mean age of 62 years of Black participants. Among Whites, 74 (94.9%) were males while in Blacks, 63 (79.8%) were males. After randomization, 45 (56.96%) of Blacks received GM-CSF compared to 33 (42.31%) among Whites (p=0.07). Smoking, which is a major risk factor for PAD, did not differ significantly between Whites (39.7%) and Blacks (54.4%) (p=0.07). The average peak walking time in Whites is 285.38 seconds, and in Blacks is 316.66 seconds (p=0.21). Baseline glucose is higher in Whites (Mean: 108.38mg/dl)

compared to Blacks (p=0.03) while the baseline creatinine is higher in Blacks with a mean of 1.13mg/dl (p=0.02).

The total number of WBCs differed significantly between Whites (Mean: 8.3 x 10^6 cells/mm^3) and Blacks (Mean: 6.59 x 10^6 cells/mm^3) (p<0.001). However, significant baseline racial differences in the four leucocyte subsets were not observed (Table2&3).

Effect of GM-CSF on PWT: Among Black participants, at the 12-week follow-up, GM-CSF increased the PWT by +135.82 seconds (95%CI: +85.37, 186.27) while placebo increased the PWT by +41.19 (95%CI: -18.88, +101.26). A significant difference between the GM-CSF and placebo was observed at 12 weeks (p=0.02). At 24-week follow-up, GM-CSF increased the PWT by +117.51 seconds (95%CI: +68.85, +166.17) and placebo increased the PWT by +72.53 (95%CI: +16.86, +128.19). But a significant difference was not found between the GM-CSF and placebo groups (p=0.27) at 24 weeks.

Among the White participants, GM-CSF increased the PWT by +67.62 seconds (95%CI: +7.93, +127.32) at 12 weeks follow-up, while the placebo showed an increase of +77.76 seconds (95%CI: +27.39, +128.13). Statistically, a significant difference was not observed between the GM-CSF and placebo groups (p=0.94). At 24 weeks, GM-CSF increased the PWT by +110.41 seconds (95%CI: +52.95, +167.87) and placebo increased the PWT by +81.39 (95%CI: +33.21, +129.57). But a significant difference was not found between the GM-CSF and placebo groups (p=0.53) (Table4).

Statistical testing for interaction between race and GM-CSF was performed using the analyses of covariance to adjust for age, smoking, and baseline WBC counts. Interaction between race and the effect of GM-CSF was significant at 12 weeks (p=0.03), while the interaction term was not significant at 24 weeks (p=0.54).

Effect of GM-CSF on PCs: GM-CSF resulted in an increase in the number of all the subsets of leukocytes. The leukocytosis peaked at the end of week 2 and then dropped to the baseline by the end of week 24 from the start of GM-CSF therapy. There was a statistically significant difference between the baseline and week 2 counts of CD34+, CD34+/CD133+, and CD34+/CXCR4+ cells in both Blacks and White participants treated with GM-CSF which was not observed at 3 months and 6 months (Tables 5,6,7). Leukocyte counts did not change significantly in all the participants who were on placebo at any time period.

At week 2, statistically significant differences were also observed in the leukocyte subpopulations in participants given GM-CSF vs placebo in both the groups. This was not observed at 3 months and 6 months.

Blacks treated with GM-CSF were found to have significantly higher magnitudes of CD34+, CD34+/CD133+, and CD34+/CXCR4+ cells compared to Whites treated with GM-CSF at week 2 (p-values 0.003, 0.007, 0.0007 respectively) (Table8).

Discussion

In this exploratory analysis, the findings reveal that there is a significant improvement in the PWT at 3 months in Blacks when GM-CSF is given vs placebo. The peak walking time increased, on average, by 99.55 seconds (p=0.02). This significant difference was not seen in Whites at 3 months post-treatment (PWT increased by 3.3 seconds (p=0.94). But, significant differences in PWT between GM-CSF and placebo were not observed in either group at 6 months.

The observed difference in Blacks is seen at only one-time point i.e. 12 weeks and not later. Also, a significant difference was seen only in Blacks but not in Whites. The reasons for these are not clear. GM-CSF and placebo were administered under supervision at least once per week, and the rate of adverse events is comparable, which does not support the racial differences in the response. We can say that permanent physiologic changes in response to GM-CSF may not have happened as the difference could not be observed at 6-month follow-up, and no precise biological mechanism of action of the drug could be elicited.

One of the possible explanations could be the increase in counts of all the three circulating PCs (CD34+, CD34+/CD133+, and CD34+/CXCR4+ cells) in response to GM-CSF. There was a significant difference in the counts of the leukocyte subpopulations at week 2, but not at 3 and 6 months, with Blacks having higher counts. This is in accordance with another study which showed there was a greater mobilization of CD34+ cells, in response to G-CSF, in Blacks (n=215) compared to Whites (n=881)[9]. Though the baseline circulating PCs were slightly lower in

Blacks, significant differences in the leukocyte subpopulations were not present. But the total number of circulating WBCs were significantly lower in Blacks compared to Whites. Previous studies concluded that media conditioned by CD34+ cells were found to inhibit apoptosis and slightly stimulate the proliferation of other freshly isolated CD34+ cells; chemo-attract CFU-GM– and CFU-Meg–derived cells as well as other CD34+ cells; and, finally, stimulate the proliferation of human endothelial cells[9]. Though GM-CSF is not as potent as G-CSF in mobilizing PCs, it directly stimulates endothelial cells and monocytes, contributing to vasculogenesis[6,12-14].

Previous studies showed mixed effects of GM-CSF in PAD patients. The START trial, conducted on 40 patients, did not show any significant effect of GM-CSF vs placebo[15]. The PROPEL study, conducted on 210 patients, did not show any overall benefit of GM-CSF on walking distance. But, a significant increase in 6-minute walk distance at six weeks was observed in White participants (n=64) and not in Black participants (n=141). This difference was not seen at 12 and 26 weeks. Another study conducted on 45 PAD patients demonstrated significant improvement in treadmill walking time and CD34+ counts at 12 weeks, but racial differences were not studied[16].

The difference in improvement between Whites and Blacks may be due to lower adherence to self-administration of the treatment in certain individuals or differences in the distribution of unknown risk factors contributing to the disease. Our Phase-I study showed a modest increase in the primary outcome at 2 weeks[16] and maximum leukocyte mobilization at 3 weeks, suggesting that treatment with

GM-CSF for 3 weeks may be sufficient for maximum response. Titrating to a higher dose or changing the duration of treatment may have varied effects in different groups.

There are a few limitations to our study. First, the study of the racial differences was exploratory, and so confirmation is required. Second, all the participants were encouraged to walk thrice weekly to increase the "homing" of PCs. The combined effect of GM-CSF and exercise may have contributed to the greater improvement in some participants, as demonstrated in studies using structured exercises for PAD patients[17]. Third, our study was not powered to evaluate the serious adverse events, although no significant changes were observed. Fourth, marked variability is seen in PWT, although the sample size for this study was based on phase I findings. So, this study may have been underpowered for the PWT at 12 weeks.

Conclusions

GM-CSF significantly improved the peak walking time at 12 weeks among Black participants with PAD but had no effect on White participants with PAD. This response can be supported by the significant increase in the leukocyte subpopulation in Blacks compared to Whites after two weeks. Further investigations are needed to confirm the variability in racial differences in response to GM-CSF and its clinical significance.

References

1. Criqui, M. H., & Aboyans, V. (2015). Epidemiology of peripheral artery disease. Circulation research, 116(9), 1509-1526.
2. Aboyans, V., Criqui, M. H., Denenberg, J. O., Knoke, J. D., Ridker, P. M., & Fronek, A. (2006). Risk factors for progression of peripheral arterial disease in large and small vessels. Circulation, 113(22), 2623-2629.
3. Scully, R. E., Arnaoutakis, D. J., Smith, A. D., Semel, M., & Nguyen, L. L. (2018). Estimated annual health care expenditures in individuals with peripheral arterial disease. Journal of vascular surgery, 67(2), 558-567.
4. Shu, J., & Santulli, G. (2018). Update on peripheral artery disease: epidemiology and evidence-based facts. Atherosclerosis, 275, 379-381.
5. Arras, M., Ito, W. D., Scholz, D., Winkler, B., Schaper, J., & Schaper, W. (1998). Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. The Journal of clinical investigation, 101(1), 40-50.
6. 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.
7. Schneider, U. C., Schilling, L., Schroeck, H., Nebe, C. T., Vajkoczy, P., & Woitzik, J. (2007). Granulocyte-macrophage colony-stimulating factor–induced vessel growth restores cerebral blood supply after bilateral carotid artery occlusion. Stroke, 38(4), 1320-1328.
8. Vitalis, A., Lip, G. Y., Kay, M., Vohra, R. K., & Shantsila, A. (2017). Ethnic differences in the prevalence of peripheral arterial disease: a systematic

review and meta-analysis. Expert review of cardiovascular therapy, 15(4), 327-338.

9. Panch, S. R., Yau, Y. Y., Fitzhugh, C. D., Hsieh, M. M., Tisdale, J. F., & Leitman, S. F. (2016). Hematopoietic progenitor cell mobilization is more robust in healthy African American compared to Caucasian donors and is not affected by the presence of sickle cell trait. Transfusion, 56(5), 1058-1065.
10. 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.
11. 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.
12. Buschmann, I. R., Hoefer, I. E., van Royen, N., Katzer, E., Braun-Dulleaus, R., Heil, M., ... & Schaper, W. (2001). GM-CSF: a strong arteriogenic factor acting by amplification of monocyte function. Atherosclerosis, 159(2), 343-356.
13. Seiler, C., Pohl, T., Wustmann, K., Hutter, D., Nicolet, P. A., Windecker, S., ... & Meier, B. (2001). Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. Circulation, 104(17), 2012-2017.
14. Hashimoto, S. I., Komuro, I., Yamada, M., & Akagawa, K. S. (2001). 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. The Journal of Immunology, 167(7), 3619-3625.

15. van Royen, N., Schirmer, S. H., Atasever, B., Behrens, C. Y., Ubbink, D., Buschmann, E. E., ... & Biemond, B. J. (2005). 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, 112(7), 1040-1046.
16. Subramaniyam V, Waller EK, Murrow JR, Manatunga A, Lonial S, Kasirajan K, Sutcliffe D, Harris W, Taylor RW, Alexander RW, Quyyumi AA. 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;158:53 60.e1.
17. McDermott, M. M., Liu, K., Guralnik, J. M., Criqui, M. H., Spring, B., Tian, L., ... & Tao, H. (2013). Home-based walking exercise intervention in peripheral artery disease: a randomized clinical trial. Jama, 310(1), 57-65.
18. Majka, M., Janowska-Wieczorek, A., Ratajczak, J., Ehrenman, K., Pietrzkowski, Z., Kowalska, M. A., ... & Ratajczak, M. Z. (2001). Numerous growth factors, cytokines, and chemokines are secreted by human CD34+ cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner: Presented at the 41st Annual Meeting of the American Society of Hematology, New Orleans, LA, December 3-7, 1999, and published in abstract form in Blood. 1999; 94

(suppl 1): 465a. Blood, The Journal of the American Society of Hematology, 97(10), 3075-3085.

Scholz, D., Cai, W. J., & Schaper, W. (2001). Arteriogenesis, a new concept of vascular adaptation in occlusive disease. Angiogenesis, 4(4), 247-257.

Table1. Number of observations with missing data by race and treatment group

Characteristic	Whites		Blacks	
	GM-CSF (N=33)	***Placebo (N=45)***	***GM-CSF (N=45)***	***Placebo (N=34)***
PWT, Baseline	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
PWT, week 12	6 (18.18%)	7 (15.56%)	7 (15.56%)	8 (23.53%)
PWT, week 24	3 (9.09%)	2 (4.44%)	3 (6.67%)	2 (5.88%)
COT, Baseline	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
COT, week 12	6 (18.18%)	7 (15.56%)	7 (15.56%)	8 (23.53%)
COT, week 24	3 (9.09%)	2 (4.44%)	5 (11.11%)	2 (5.88%)
Alcohol intake	0 (0.00%)	1 (2.22%)	0 (0.00%)	0 (0.00%)
BMI	1 (3.03%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
ABI	0 (0.00%)	1 (2.22%)	1 (2.22%)	0 (0.00%)
HDL	1 (3.03%)	1 (2.22%)	1 (2.22%)	0 (0.00%)
LDL	2 (6.06%)	3 (6.67%)	2 (4.44%)	0 (0.00%)
Leucocytes Subsets				
Total WBCs	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.94%)
CD34+/CD45med	0 (0.00%)	1 (2.22%)	2 (4.44%)	0 (0.00%)
CD34+/CD45med/CD133+	0 (0.00%)	1 (2.22%)	2 (4.44%)	0 (0.00%)
CD34+/CD45med/VEGF	0 (0.00%)	1 (2.22%)	2 (4.44%)	0 (0.00%)
CD34+/CD45med/CXCR4+	0 (0.00%)	1 (2.22%)	2 (4.44%)	0 (0.00%)

Table2. Baseline characteristics of Blacks vs White Participants

Characteristic	All (N=157)	Whites (N=78)	Blacks (N=79)	P-Value
Age, in years (Mean, SD)	63.95 (7.88)	65.56 (7.36)	62.36 (8.10)	0.01
Sex (no, %)				
Males	137 (87.26%)	74 (94.87%)	63 (79.75%)	0.005
Received GM-CSF	78 (49.68%)	33 (42.31%)	45 (56.96%)	0.07
Diabetes	58 (36.94%)	33 (42.31%)	24 (30.38%)	0.12
Smoking	74 (47.13%)	31 (39.74%)	43 (54.43%)	0.07
Alcohol intake	48 (30.57%)	21 (26.92%)	27 (34.18%)	0.35
History, No. (%)				
Previous peripheral revascularization	58 (36.94%)	30 (38.46%)	28 (35.44%)	0.69
Stroke	38 (24.20%)	21 (26.92%)	17 (21.52%)	0.43
Myocardial infarction	53 (33.76%)	29 (37.18%)	24 (30.38%)	0.37
Coronary bypass graft surgery	37 (23.57%)	22 (28.21%)	15 (18.99%)	0.64
Previous coronary angioplasty	49 (31.21%)	26 (33.33%)	23 (29.11%)	0.22
Hypertension	144 (91.72%)	68 (87.18%)	76 (96.20%)	0.1
Hyperlipidemia	132 (84.08%)	67 (85.90%)	65 (82.28%)	0.37
Chronic kidney disease	12 (7.64%)	3 (3.85%)	9 (11.39%)	0.08
Measurements, Mean (SD)				
BMI	29.85 (7.15)	30.00 (5.99)	29.70(8.16)	0.8
ABI	0.6 (0.15)	0.59 (0.15)	0.60(0.16)	0.84
Glucose	102.8 (31.77)	108.38(37.17)	97.28(24.35)	0.03
HDL	43.14 (12.76)	41.12(12.23)	45.10(13.04)	0.05
LDL	89.31 (31.68)	87.68(28.45)	90.86(34.58)	0.54
Creatinine	1.06 (0.36)	0.99(0.38)	1.13(0.34)	0.02
Peak Walking Time, s	301.12 (155.56)	285.38(155.0)	316.66(155.6)	0.21
Claudication Onset Time, s	149.41 (77.27)	136.55(66.1)	162.11(85.4)	0.04
WIQ scores, mean (SD)				
Distance	27.7 (22.06)	27.67 (21.0)	27.73 (23.2)	0.98
Speed	36.17 (23.34)	37.60 (22.6)	34.75 (24.1)	0.45
Stairclimbing	44.4 (25.06)	44.90 (25.2)	43.9 (25.1)	0.81
Leucocytes Subsets, x10^6, mean (SD)				
Total WBCs	7.44 (2.16)	8.30(2.36)	6.59(1.64)	<0.001
CD34+/CD45med	2.35 (1.41)	2.36(1.41)	2.34 (1.41)	0.83
CD34+/CD45med/CD133+	1.17 (0.78)	1.19 (0.78)	1.16 (0.79)	0.68
CD34+/CD45med/VEGF	0.05 (0.04)	0.05(0.04)	0.05 (0.04)	0.88
CD34+/CD45med/CXCR4+	1.17 (0.87)	1.18 (0.92)	1.16 (0.83)	0.94

Table3. Baseline characteristic of Blacks and Whites after randomization into treatment groups

Characteristic	Whites		Blacks	
	GM-CSF (N=33)	***Placebo (N=45)***	***GM-CSF (N=45)***	***Placebo (N=34)***
Age, in years	65.18(8.07)	65.83(6.87)	63.64(7.74)	60.66(8.36)
Gender				
Males	32 (96.97%)	42 (93.33%)	36 (80.00%)	27 (79.41%)
Females	1 (3.03%)	3 (6.67%)	9 (20.00%)	7 (20.59%)
Diabetes	17 (51.52%)	16 (35.56%)	12 (26.67%)	12 (35.29%)
Smoking	11 (33.33%)	20 (44.44%)	23 (51.11%)	20 (58.82%)
Alcohol intake	10 (30.30%)	11 (24.44%)	17 (37.78%)	10 (29.41%)
History, No. (%)				
Previous peripheral revascularization	12 (36.36%)	18 (40.00%)	15 (33.33%)	13 (38.24%)
Stroke	8 (24.24%)	13 (28.89%)	10 (22.22%)	7 (20.59%)
Myocardial infarction	13 (39.39%)	16 (35.56%)	15 (33.33%)	9 (26.47%)
Coronary bypass graft surgery	9 (27.27%)	13 (28.89%)	10 (22.22%)	5 (14.71%)
Previous coronary angioplasty	10 (30.30%)	16 (35.56%)	15 (33.33%)	8 (23.53%)
Hypertension	30 (90.91%)	38 (84.44%)	44 (97.78%)	32 (94.12%)
Hyperlipidemia	30 (90.91%)	37 (82.22%)	38 (84.44%)	27 (79.41%)
Chronic kidney disease	1 (3.33%)	2 (4.44%)	7 (15.56%)	2 (5.88%)
Measurements, Mean (SD)				
BMI	29.77(4.64)	30.16(6.84)	29.51(7.37)	29.96(9.22)
ABI	0.58(0.15)	0.61(0.15)	0.60(0.16)	0.60(0.17)
Glucose	109.79(32.98)	107.36(40.30)	94.96(18.93)	100.35(30.11)
HDL	42.28(11.24)	40.27(12.96)	45.09(13.19)	45.12(13.04)
LDL	86.13(27.94)	88.83(29.10)	92.72(36.10)	88.50(32.94)
Creatinine	0.95(0.44)	1.02(0.33)	1.13(0.32)	1.13(0.36)
Peak Walking Time, s	279.85(143.8)	289.44(164.2)	305.49(157.0)	331.44(154.8)
Claudication Onset Time, s	133.03(57.3)	139.13(57.3)	152.49(71.5)	174.85(100.7)
WIQ scores, mean (SD)				
Distance	28.64 (22.2)	26.96 (20.3)	23.47 (20.7)	33.38 (25.3)
Speed	38.27 (21.9)	37.11 (23.4)	34.49 (25.2)	35.12 (22.8)
Stairclimbing	44.24 (23.1)	45.38 (26.9)	43.16 (26.9)	44.91 (22.7)
Leucocytes Subsets, x10^6, mean (SD)				
Total WBCs	8.39 (1.92)	8.24 (2.53)	6.43 (1.77)	6.80 (1.45)
CD34+/CD45med	2.37 (1.15)	2.36 (1.60)	2.45 (1.38)	2.19 (1.44)
CD34+/CD45med/CD133+	1.15 (0.65)	1.21 (0.87)	1.23 (0.83)	1.07 (0.74)
CD34+/CD45med/VEGF	0.05 (0.04)	0.05 (0.04)	0.05 (0.03)	0.05 (0.06)
CD34+/CD45med/CXCR4+	1.31 (0.96)	1.08 (0.89)	1.16 (0.74)	1.16 (0.94)

Table4. Effects of GM-CSF on Peak Walking Time (PWT) over time by race and treatment group

	White		Black	
	GM-CSF	Placebo	GM-CSF	Placebo
Baseline	279.85(143.8) (n=33)	289.44(164.15) (n=45)	305.49(156.95) (n=45)	331.44(154.77) (n=34)
12 wk follow-up	342.07(193.61) (n=27)	378.47(195.4) (n=38)	446.55(276.65) (n=38)	371.65(165.09) (n=26)
24 wk follow-up	395.6(269.61) (n=30)	371.84(224.59) (n=43)	421.26(245.98) (n=42)	405.81(187.26) (n=32)
Within group change at 12 wk	+67.62(+7.93,+127.32)	+77.76(+27.39,+128.13)	+135.82(+85.37,+186.27)	+41.19(-18.88,+101.26)
Between group change at 12 wk	+3.3(-82.63,+89.23) p= 0.94		+99.55(+14.36,+184.73) p=0.02	
Within group change at 24 wk	+110.41(+52.95,+167.87)	+81.39(+33.21,+129.57)	+117.51(+68.85,+166.17)	+72.53(+16.86,+128.19)
Between group change at 24 wk	+26.96(-57.15,+111.07) p= 0.53		+46.41(-36.1,+128.93) p=0.27	

Table5. Effects of GM-CSF on CD34+/ CD45med+ cell counts over time by race and treatment group

CD34+/CD45med+	White		Black	
	GM-CSF	**Placebo**	**GM-CSF**	**Placebo**
Baseline	2.37 (1.15)(n=33)	2.36 (1.6)(n=44)	2.45 (1.38)(n=43)	2.19 (1.44)(n=34)
2 wk follow-up	4.2 (2.01)(n=32)	2.09 (1.19)(n=43)	5.39 (4.22)(n=40)	1.99 (1.01)(n=34)
12 wk follow-up	2.47 (1.73)(n=27)	2.13 (1.28)(n=39)	2.47 (1.65)(n=39)	1.78 (0.77)(n=29)
24 wk follow-up	2.53 (1.02)(n=30)	2.26 (1.28)(n=41)	2.42 (1.81)(n=41)	2.03 (1.23)(n=33)
Within group change at 2 wk	1.81 (0.28)	-0.22 (0.24)	2.86 (0.25)	-0.20 (0.27)
Between group change at 2 wk	2.01 (0.33) p<0.0001		3.04 (0.33) p<0.0001	
Within group change at 12 wk	0.02 (0.30)	-0.24 (0.25)	-0.08 (0.25)	-0.38 (0.29)
Between group change at 12 wk	0.25 (0.37) p=0.50		0.29(0.36) p=0.43	
Within group change at 24 wk	0.09 (0.29)	-0.13 (0.25)	-0.09 (0.25)	-0.17 (0.28)
Between group change at 24 wk	0.22 (0.35) p=0.54		0.09 (0.34) p=0.80	

*Within-group/Between-group changes given as Mean (SE)

**The CD34+/CD45med+ counts are given as mean (SD), $x10_6/mm_3$

Table6. Effects of GM-CSF on CD34+/ CD45med+/CD133+ cell counts over time by race and treatment

CD34+/CD45med+/CD133+	White		Black	
	GM-CSF	**Placebo**	**GM-CSF**	**Placebo**
Baseline	1.15 (0.65)(n=33)	1.21 (0.87)(n=44)	1.23 (0.83)(n=43)	1.07 (0.74)(n=34)
2 wk follow-up	2.14 (1.1)(n=32)	1.06 (0.6)(n=43)	2.79 (2.68)(n=40)	0.99 (0.51)(n=34)
12 wk follow-up	1.29 (1.07)(n=27)	1.06 (0.62)(n=39)	1.29 (0.91)(n=39)	0.92 (0.4)(n=29)
24 wk follow-up	1.32 (0.59)(n=30)	1.21 (0.69)(n=41)	1.29 (1)(n=41)	1.05 (0.71)(n=33)
Within group change at 2 wk	0.98 (0.17)	-0.13 (0.15)	1.54 (0.15)	-0.08 (0.17)
Between group change at 2 wk	1.09 (0.19) p<0.0001		1.61 (0.20) p<0.0001	
Within group change at 12 wk	0.10 (0.18)	-0.16 (0.15)	0.02 (0.15)	-0.12 (0.17)
Between group change at 12 wk	0.26 (0.22) p=0.22		0.14 (0.21) p=0.51	
Within group change at 24 wk	0.13 (0.17)	-0.03 (0.15)	0.03 (0.15)	-0.02 (0.17)
Between group change at 24 wk	0.17 (0.21) p=0.42		0.06 (0.20) p=0.77	

*Within-group/Between-group changes given as Mean (SE)

**The CD34+/CD45med+/CD133+ counts are given as mean (SD), $x10_6/mm_3$

Table7. Effects of GM-CSF on CD34+/ CD45med+/CXCR4+ cell counts over time by race and treatment

CD34+/CD45med+/CXCR4+	White		Black	
	GM-CSF	**Placebo**	**GM-CSF**	**Placebo**
Baseline	1.31 (0.96)(n=33)	1.08 (0.89)(n=44)	1.16 (0.74)(n=43)	1.16 (0.94)(n=34)
2 wk follow-up	2.07 (1.37)(n=32)	0.95 (0.56)(n=43)	2.88 (2.39)(n=40)	1.14 (0.68)(n=34)
12 wk follow-up	1.41 (1.43)(n=27)	0.96 (0.62)(n=39)	1.17 (0.67)(n=39)	0.93 (0.57)(n=29)
24 wk follow-up	1.28 (0.72)(n=30)	0.95 (0.56)(n=41)	1.36 (1.41)(n=41)	1.25 (1.08)(n=33)
Within group change at 2 wk	0.76 (0.21)	-0.12 (0.18)	1.70 (0.19)	-0.02 (0.21)
Between group change at 2 wk	0.85 (0.27) p=0.002		1.69 (0.27) p<0.0001	
Within group change at 12 wk	0.06 (0.22)	-0.12 (0.19)	-0.03 (0.19)	-0.19 (0.22)
Between group change at 12 wk	0.14 (0.28) p=0.62		0.13 (0.28) p=0.63	
Within group change at 24 wk	-0.05 (0.22)	-0.14 (0.19)	0.19 (0.19)	0.09 (0.21)
Between group change at 24 wk	0.07 (0.28) p=0.79		0.09 (0.27) p=0.73	

*Within-group/Between-group changes given as Mean (SE)

**The CD34+/CD45med+/CXCR4+ counts are given as mean (SD), $x10_6/mm_3$

Table8. Comparison of the effect of GM-CSF on leukocyte subpopulations in Blacks vs Whites

WBC subpopulation	Black: GM-CSF	White: GM-CSF	Difference	P-Value
CD34+/CD45med+	5.39 (4.22)(n=40)	4.2 (2.01)(n=32)	+1.05 (0.35)	0.003
CD34+/CD45med+/CD133+	2.79 (2.68)(n=40)	2.14 (1.1)(n=32)	+0.56 (0.20)	0.007
CD34+/CD45med+/CXCR4+	2.88 (2.39)(n=40)	2.07 (1.37)(n=32)	+0.94 (0.27)	0.0007

*The difference is given as Mean (SE), x106/mm3

**The WBC subpopulation counts are given as mean (SD), $x10^6/mm^3$

Figure1. Change in the Peak Walking Time in different groups at different time points

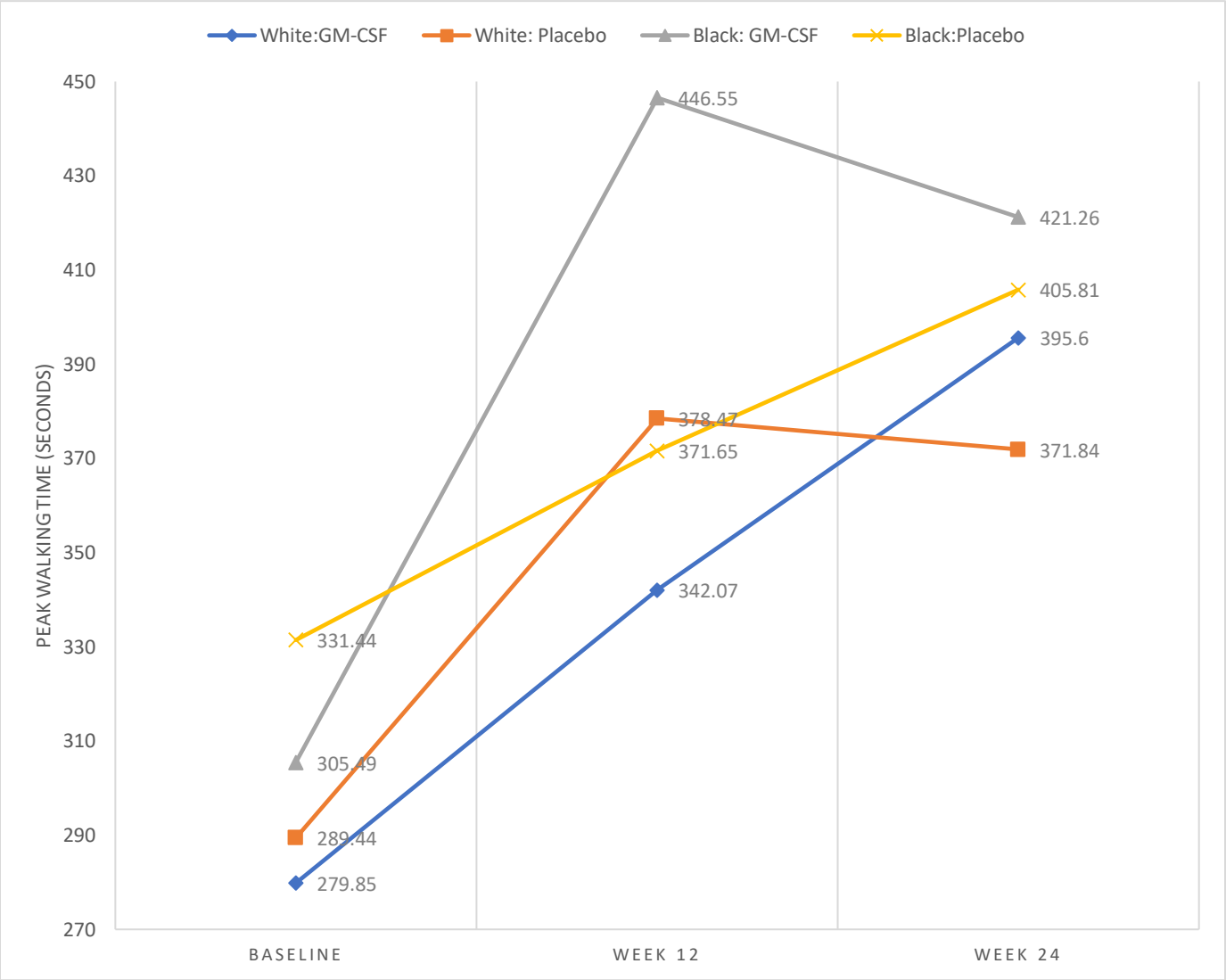

Figure2. Change in the CD34+/ CD45med+ cell counts in different groups at different time points

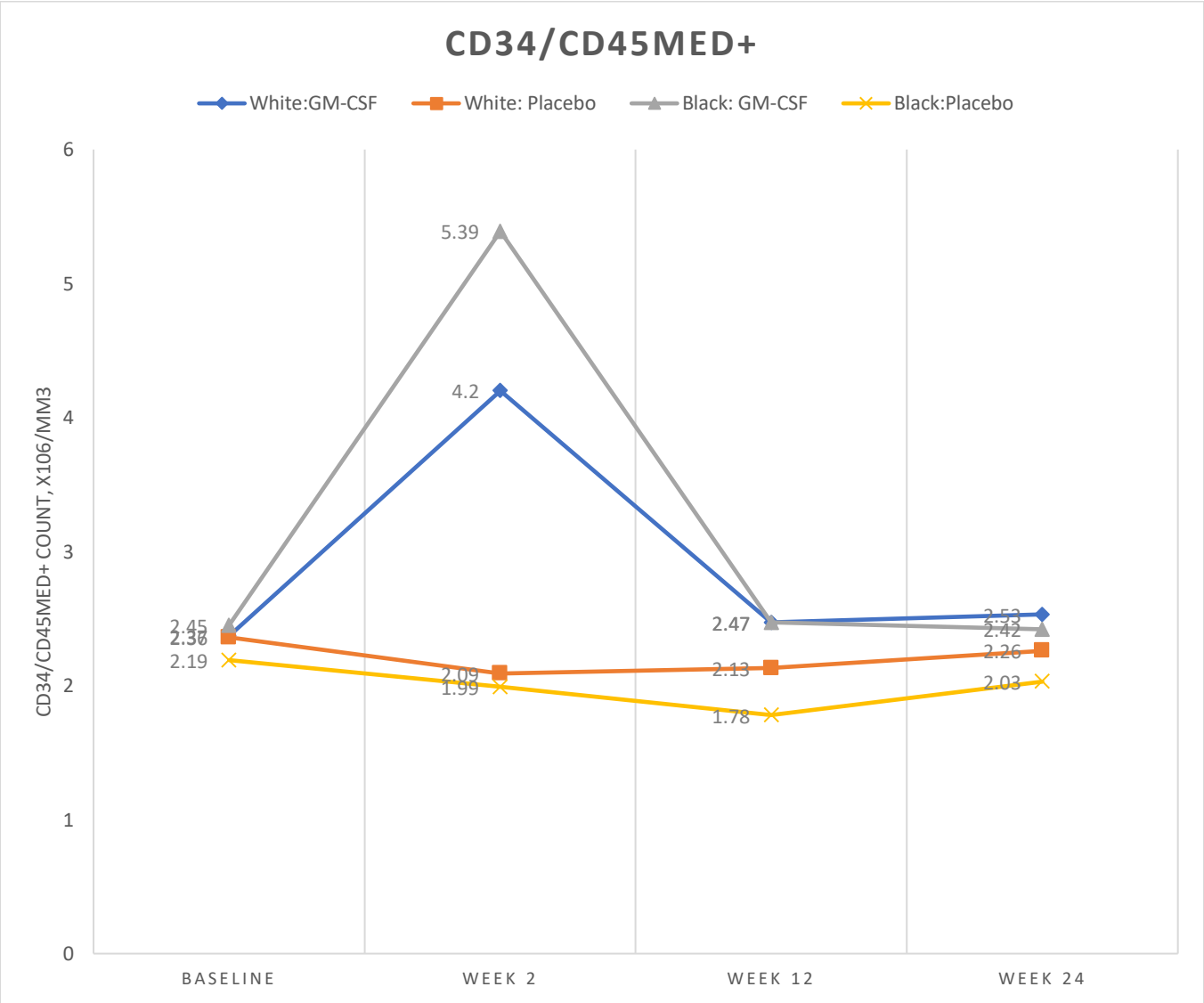

Figure3. Change in CD34+/ CD45med+/CD133+ cell counts in different groups at different time points

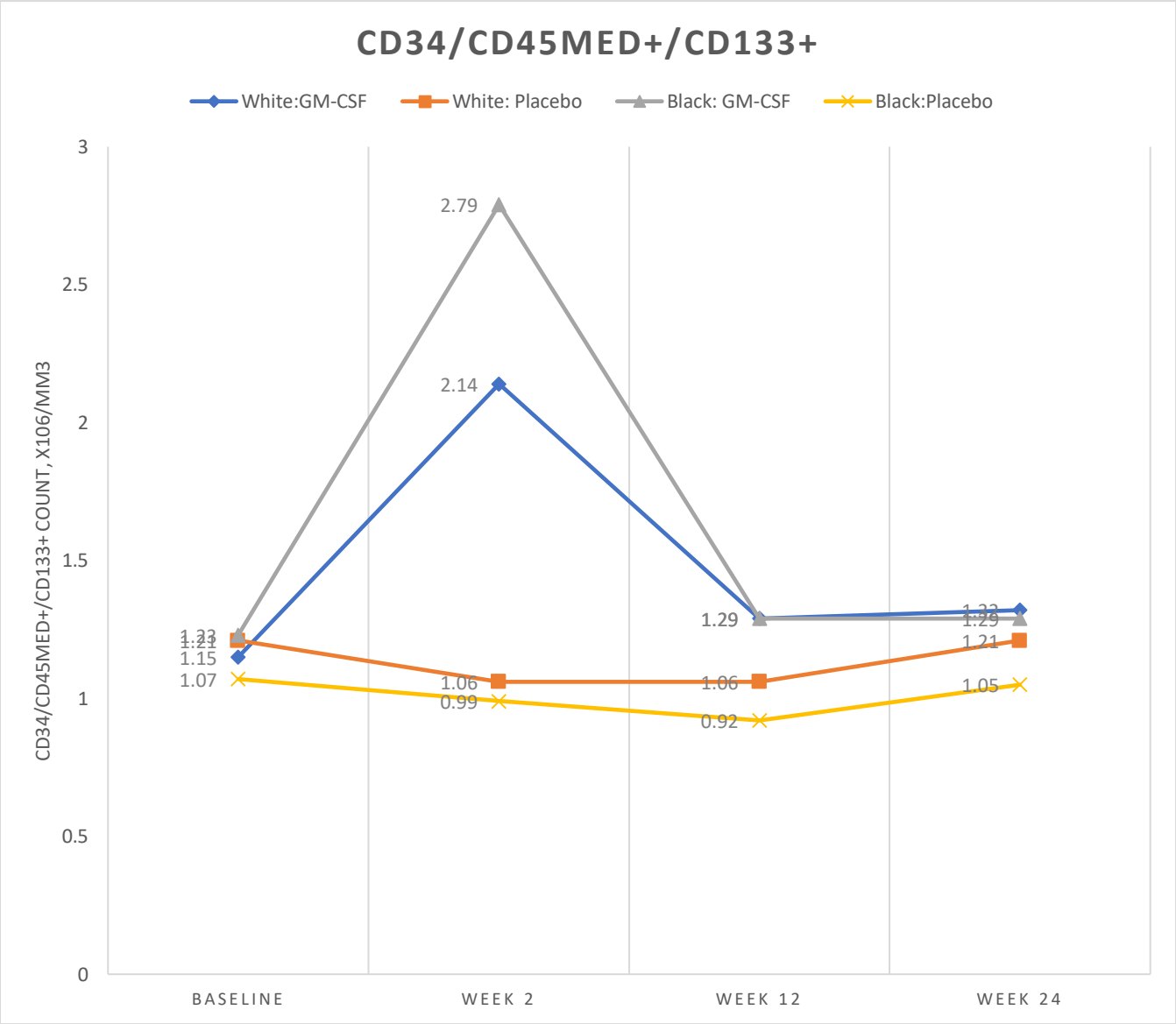

Figure4. Change in CD34+/ CD45med+/CXCR4+ cell counts in different groups at different time points