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Identification of Bone Marrow Cell Subpopulations Associated With Improved Functional Outcomes in Patients With Chronic Left Ventricular Dysfunction: An Embedded Cohort Evaluation of the FOCUS-CCTRN Trial

Doris A. Taylor^{*}, Emerson C. Perin^{*}, James T. Willerson^{*}, Claudia Zierold[†], Micheline Resende^{*}, Marjorie Carlson[†], Belinda Nestor^{*}, Elizabeth Wise[‡], Aaron Orozco^{*}, Carl J. Pepine[‡], Timothy D. Henry^{§,¶}, Stephen G. Ellis[#], David X. M. Zhao^{**}, Jay H. Traverse[¶], John P. Cooke^{††,‡‡}, Robert C. Schutt^{††,‡‡}, Aruni Bhatnagar^{§§}, Maria B. Grant[‡], Dejian Lai^{¶¶}, Brian H. Johnstone^{##}, Shelly L. Sayre^{¶¶}, Lem Moyé^{¶¶}, Ray F. Ebert^{***}, Roberto Bolli^{§§}, Robert D. Simari^{†††}, and Christopher R. Cogle[‡] for the Cardiovascular Cell Therapy Research Network (CCTRN)

^{*}Texas Heart Institute, CHI St. Luke's Health, Houston, TX, USA

[†]University of Minnesota School of Medicine, Minneapolis, MN, USA

[‡]University of Florida College of Medicine, Gainesville, FL, USA

[§]Cedars-Sinai Heart Institute, Los Angeles, CA, USA

[¶]Minneapolis Heart Institute Foundation at Abbott, Minneapolis, MN, USA

[#]Cleveland Clinic Foundation, Cleveland, OH, USA

^{**}Wake Forest School of Medicine, Winston-Salem, NC, USA

^{††}Houston Methodist DeBakey Heart & Vascular Center, Houston, TX, USA

^{‡‡}Houston Methodist Research Institute, Houston, TX, USA

^{§§}University of Louisville, Louisville, KY, USA

^{¶¶}University of Texas School of Public Health, Houston, TX, USA

^{##}Indiana University School of Medicine, Indianapolis, IN, USA

^{***}National Heart, Lung, and Blood Institute, Bethesda, MD, USA

^{†††}University of Kansas School of Medicine, Kansas City, KS, USA

Abstract

In the current study, we sought to identify bone marrow-derived mononuclear cell (BM-MNC) subpopulations associated with a combined improvement in left ventricular ejection fraction (LVEF), left ventricular end-systolic volume (LVESV), and maximal oxygen consumption (VO₂ max) in patients with chronic ischemic cardiomyopathy 6 months after receiving transcatheter

injections of autologous BM-MNCs or placebo. For this prospectively planned analysis, we conducted an embedded cohort study comprising 78 patients from the FOCUS-Cardiovascular Cell Therapy Research Network (CCTRN) trial. Baseline BM-MNC immunophenotypes and progenitor cell activity were determined by flow cytometry and colony-forming assays, respectively. Previously stable patients who demonstrated improvement in LVEF, LVESV, and VO₂ max during the 6-month course of the FOCUS-CCTRN study (group 1, *n* = 17) were compared to those who showed no change or worsened in one to three of these endpoints (group 2, *n* = 61) and to a subset of patients from group 2 who declined in all three functional endpoints (group 2A, *n* = 11). Group 1 had higher frequencies of B-cell and CXCR4⁺ BM-MNC subpopulations at study baseline than group 2 or 2A. Furthermore, patients in group 1 had fewer endothelial colony-forming cells and monocytes/macrophages in their bone marrow than those in group 2A. To our knowledge, this is the first study to show that in patients with ischemic cardiomyopathy, certain bone marrow-derived cell subsets are associated with improvement in LVEF, LVESV, and VO₂ max at 6 months. These results suggest that the presence of both progenitor and immune cell populations in the bone marrow may influence the natural history of chronic ischemic cardiomyopathy—even in stable patients. Thus, it may be important to consider the bone marrow composition and associated regenerative capacity of patients when assigning them to treatment groups and evaluating the results of cell therapy trials.

Keywords

Bone marrow; Heart failure; Ischemic cardiomyopathy; Stem cells; Cell therapy

INTRODUCTION

Clinical investigations of heterogeneous bone marrow-derived mononuclear cells (BM-MNCs) to treat patients with acute myocardial infarction (MI) or chronic ischemic heart failure (HF) have not met early expectations for success¹⁻³. These variable findings have highlighted the need for studies aimed at improving our understanding of whether the mechanisms and pathways associated with endogenous cardiac repair involve BM-MNCs. In our recently reported study of an acute MI cohort, we found that improved patient outcomes were associated with higher frequencies of CD31⁺ BM-MNCs and with higher growth rates in colony-forming assays⁴, suggesting that both cell number and function can contribute to patient outcomes. However, in that study, we examined associations between bone marrow (BM) cell populations and patient outcomes only in the cell-treated group and only shortly after acute MI. It is unknown whether there are specific cell populations in the BM that affect cardiac repair independently of treatment, especially in patients with HF. In the current study, we sought to identify BM-MNC subpopulations associated with a rigorous definition of improved cardiac function in patients with HF. The FOCUS-Cardiovascular Cell Therapy Research Network (FOCUS-CCTRN) clinical trial, which assessed the effects of BM-MNC therapy in patients with chronic ischemic HF, showed negative results for all three primary endpoints [left ventricular end-systolic volume (LVESV), maximal oxygen consumption (VO₂ max), and reversibility on single-photon emission tomography (SPECT)], but a 2.7% difference in the exploratory endpoint left ventricular ejection fraction (LVEF) between the cell- and placebo-treated patients at 6 months^{1,5,6}. We regrouped

FOCUS-CCTRN patients according to outcome using a combined criterion of improved LVEF, LVESV, and VO₂ max with the goal of identifying associations between the BM-MNC profile and functional outcomes. Although a reversible defect by SPECT was originally one of the three primary endpoints chosen for the FOCUS study, it became obvious during the study that signal noise exceeded the expected bounds; accordingly, SPECT measurements were not included in our multiple parameter determination of functional improvement for this analysis.

It is rare to see HF patients in whom LVEF, LVESV, and VO₂ max improve at the same time; thus, by requiring favorable changes in all three of these cardiac functional parameters, we set a rigorous criterion for identifying patients who improved in the FOCUS-CCTRN trial. We reasoned that applying this criterion and comparing the BM profile of patients who improved to the BM profile of patients who did not could contribute to a better understanding of BM factors associated with positive functional changes. Accordingly, we identified the subgroup of participants in the FOCUS-CCTRN trial who showed improvement in LVEF, LVESV, and VO₂ max at the 6-month follow-up and then performed a cohort analysis to determine whether the composition of their BM-MNC samples at study onset differed from that of patients who did not improve in all three functional outcomes over the same period.

MATERIALS AND METHODS

Participants and Procedures

The FOCUS-CCTRN trial¹ was conducted at five clinical sites, and institutional review board approval was obtained from each site. The study complied with the Declaration of Helsinki, and informed consent was obtained for all patients. Of the 92 patients who were randomized and eligible for participation in this analysis, 78 had complete follow-up data and consented to have a portion of their BM-MNC product analyzed.

BM Collection and Analysis

BM samples were obtained and processed according to previously published methods¹. Briefly, the BM-MNC concentration was adjusted to produce a fixed dose of autologous cells, as described by Gee et al.⁷. The target dose of BM-MNCs was 100×10^6 . The treatment (BM-MNCs or placebo) was administered to the patient in 15 separate injections (0.2 ml each) to left ventricular (LV) endocardial regions identified as viable by electromechanical mapping on the day of BM collection within 12 h of aspiration¹. The excess fresh BM-MNC product was shipped to the Biorepository Core, where the functional activity (via colony-forming assays) and immunophenotype (via flow cytometry) were determined, as described previously⁸.

To characterize the hematopoietic stem cells, endothelial progenitor cells (EPCs), and immune cells in the BM, $1-5 \times 10^6$ viable cells were labeled with anti-CD45-PerCP-Cy5.5, anti-CD34-PE-Cy7, anti-CD133-PE (Miltenyi Biotec, Auburn, CA, USA), anti-CD306 vascular endothelial growth factor receptor (VEGFR2/KDR)-APC (R&D Systems, Minneapolis, MN, USA), anti-CD31-FITC, anti-CD3-PE-Cy7, anti-CD19-APC-H7, anti-

CD11b-APC, anti-CXCR4-PE, and anti-CD14-FITC or an isotype-matched control antibody. Cell surface markers were analyzed using FlowJo software 7.6.5 (Tree Star, Ashland, OR, USA). Data analysis was performed by gating the individual lymphocyte, monocyte, and granulocyte populations on the basis of their forward scatter (FSC-A) versus side scatter (SSC-A) properties. The frequencies of single-, double-, and triple-labeled cell populations (Table 1) and subsets were determined as a function of CD45 expression. Unless otherwise indicated, single-marker expression included CD45⁺ and CD45⁻ cells. Expression of CD14 and CD11b and secondary markers were assessed in the monocyte fraction, whereas the expression of CD19, CD3, CD31, CD34, KDR, and CXCR4 and secondary markers were assessed in the lymphocyte fraction. A modified version⁹ of the International Society of Hematotherapy and Graft Engineering (ISHAGE) sequential gating strategy¹⁰ was used to analyze the CD34⁺, CD133⁺, and KDR⁺ EPCs in the total nucleated cell fraction.

The gating strategies for the cell populations found to be significant are shown in Figures 1 and 2. In addition to cell populations expressing a given marker, certain subsets within those populations were also gated [e.g., negative^(neg), dim^(dim), intermediate^(intermed), or bright^(bright)] as indicated (Fig. 2D). The negative gate was always placed on the basis of the isotype control negative signal.

Endothelial cell function was quantified by using the endothelial colony-forming cell (ECFC) assay (reported as ECFC per 10⁸ cells) and the CFU-Hill assay (reported as CFU-EC) by using commercial kits according to the manufacturers' instructions (STEMCELL Technologies, Vancouver, Canada), as previously described⁸.

Embedded Cohort Analysis

The purpose of this analysis was to determine whether patients with a favorable cardiac functional outcome at 6 months had specific BM characteristics at study baseline that differed from those who did not improve. This evaluation of the putative relationships between BM-MNC immunophenotypes and LV function was prespecified in the FOCUS-CCTRN protocol. In the FOCUS-CCTRN trial, patients were randomized 2:1 to cell treatment or placebo. In the cell-treated patients, no significant beneficial effects of therapy were observed for LVESV or VO₂ max, whereas a small improvement in LVEF (2.7%, $p = 0.03$) occurred. We combined the BM-MNC and placebo groups into a single cohort characterized by chronic ischemic cardiomyopathy and HF, which increased the statistical power for the current analyses. A cohort approach was used to compare the baseline BM-MNC profiles of patients who improved in three available cardiac function indicators (LVEF, LVESV, and VO₂ max) to those of patients who did not improve. Left ventricular end-diastolic volume (LVEDV) was not an endpoint of this study and was not considered.

Patients in the placebo and cell-treated groups were reassigned to three different groups based on their 6-month functional outcomes: (1) group 1 ($n = 17$): patients who showed improvement in all three endpoints (i.e., an increase in LVEF, a decrease in LVESV, and an increase in VO₂ max), (2) group 2 ($n = 61$): all other patients, and (3) group 2A ($n = 11$): a subset of group 2 that included only individuals who showed a decline in all three endpoints. We then determined whether the baseline BM-MNC immunophenotypes and functions of

the three groups differed at study onset. It is important to note that we grouped patients based on functional endpoints rather than treatment; therefore, the composition of the three groups differed in regard to treatment received. Group 1 (triple-positive functional outcomes) included 16 BM-MNC-treated and 1 placebo-treated patients, group 2 (all others) included 35 BM-MNC-treated and 26 placebo-treated patients, and group 2A (triple-negative functional outcomes) included 7 BM-MNC-treated and 4 placebo-treated patients.

Statistical Methods

Measures of central tendency and dispersion were compared. Patient demographic and clinical data were compared by using the Student's *t*-test for continuous variables and were reported by mean and standard error of the mean (SEM). Wilcoxon two-sample testing was used for the non-parametric evaluations [brain natriuretic peptide BNP(reg) and BNP(pro)]. Fisher's exact test was used to compare dichotomous variables. Differences in the New York Heart Association classification and the Canadian Cardiovascular Society classification were assessed by using the chi-square test. Differences in phenotype and function data were assessed by using the Student's *t*-test. Statistical comparisons with a value of $p < 0.05$ were considered significant with no corrections for multiplicity in this wide-ranging evaluation.

Heat Map

To visualize the distribution of cell phenotypes and changes present in group 1 versus groups 2 and 2A, we generated a heat map showing the relative change in frequencies of a given cell population or function. The signal-to-noise ratio (SNR), calculated as the delta percent difference in group 1 versus groups 2 and 2A divided by the standard error, was plotted for each cell population and function. Green represents a positive SNR (i.e., an absolute higher cell percentage or function), black represents no difference, and red represents a negative SNR (i.e., an absolute lower cell percentage or function).

RESULTS

Comparisons Between Group 1 and Group 2 Patients

By definition, at the 6-month follow-up, group 1 showed greater improvement in LVEF, LVESV, and VO_2 max, as compared to group 2 (Table 2). Other baseline demographic and clinical data for groups 1 and 2 are shown in Table 3. Patients in group 1 had significantly lower BNP levels and a significantly smaller percentage who underwent coronary artery bypass graft (CABG) surgery than in group 2. The age in groups 1 and 2 did not statistically differ, but there was a trend ($p = 0.067$) toward a lower age in group 1.

Flow cytometry showed that the frequencies of eight cell populations were significantly elevated in the BM of patients in group 1 when adjusted for age, as compared to the frequencies in group 2 patients (Table 4). The elevated populations included $CD11b^+$, $CD31^{dim}$, $CD19^+$, and $CXCR4^+$ cells and subsets thereof. In terms of absolute percentages, these cell populations were increased by 1–7% (Fig. 3A). It was not possible to adjust the results for baseline BNP since BNP was not measured in the same manner across sites. When the data were adjusted for CABG, the $CXCR4^+$ and $CXCR4^{intermed}$ cell populations were no longer significantly different between groups 1 and 2.

Comparisons Between Group 1 and Group 2A Patients

As a more constrained approach, we compared the patients with the best functional outcomes (triple-positive patients, group 1) to those with the worst outcomes (triple-negative patients, group 2A). Group 1 and group 2A membership was defined on the basis of changes in LVEF, VO₂ max, and LVESV and, therefore, had the expected differences in these variables (Table 2). The differences in LVEF, VO₂ max, and LVESV were greater when group 1 was compared with group 2A than when it was compared with group 2. A comparison of other baseline demographic and clinical variables showed that the patients in group 1 were significantly younger than those in group 2A and had significantly lower BNP levels (Table 3). Four hematopoietic/ immune cell populations (CD45⁺CD19⁺, CD19⁺CD11b⁺, CD19⁺CXCR4⁺, and CD45⁺CD11b⁺ cells) were significantly higher in the BM of group 1 than in group 2A (Table 5 and Fig. 3B), whereas both the percentage of CD45⁺CD31^{bright} cells and the number of ECFCs per 10⁸ cells were lower (Table 5 and Fig. 3C). This remained true after adjustment for age.

BM-MNC Characteristics Associated With Positive Functional Outcomes

The heat map shown in Figure 4 was designed to enable high-level comparisons between group 1 and groups 2 and 2A. These comparisons are shown for a total of 33 variables: 31 BM-MNC frequencies and 2 cell functions (CFU-EC and ECFC). A positive SNR was observed for 48% of these variables in the group 1 versus group 2 comparison and for 69% in the group 1 versus group 2A comparison. In the comparison of group 1 versus group 2, the primary cell populations with a positive SNR were hematopoietic and immune cells. However, approximately twice as many progenitor cell populations (12 vs. 7) showed a positive SNR in the comparison of group 1 versus group 2A. This difference suggests that higher endogenous levels of progenitor cells may predict a beneficial cardiac outcome.

DISCUSSION

The current study was designed to determine whether improvements in three important functional endpoints (LVEF, LVESV, and VO₂ max) in patients with ischemic cardiomyopathy were associated with the frequency of certain cell types in the patient's BM. The effect of treatment was outside the scope of this study, but of the 17 patients in group 1 (i.e., those who showed improvement for all three endpoints), 16 were treated with BM-MNCs. We found that HF patients who improved in a combination of LVEF, LVESV, and VO₂ max at the 6-month follow-up had higher levels of CD19⁺ B cells, CD11b⁺ monocytes, CD31^{dim} subsets, and CXCR4⁺ migratory cells and lower levels of CD31^{bright} lymphocyte subsets in their BM at baseline than patients who improved in fewer endpoints or whose condition worsened.

Our results suggest that B cells may either play a beneficial role in repair, or their numbers may serve as predictors of a favorable functional outcome in chronic ischemic cardiomyopathy patients. The increased frequencies of B-cell subsets (CD19⁺CXCR4⁺ and CD19⁺CD11b⁺) in the BM may be due to either an increase in B-cell production (for potential cardiac repair) or a decrease in B-cell mobilization from the BM. The latter

possibility seems less likely, given the increase in markers associated with migratory capability (CD11b and CXCR4) in the B-cell subsets.

Cell subsets expressing CXCR4, including CD19⁺ B cells, were also elevated in the BM-MNCs of patients who had superior functional outcomes. Several studies have identified CXCR4 and its ligand, stromal cell-derived factor-1 (SDF-1), as key molecular contributors to myocardial repair^{11,12}. Although the present study was not designed to, and therefore did not directly, assess cell recruitment or the incorporation of cells at cardiac injury sites, the association we found between higher numbers of CXCR4-expressing cells and functional improvement suggests that better cell migratory capacity in response to tissue injury or ischemia could contribute to improved outcome.

Several reports have suggested that monocytes and macrophages play a critical role both in cardiac repair and in angiogenesis and arteriogenesis¹³⁻¹⁵. In this study, we found that the BM frequency of CD45⁺CD11b⁺ monocytes was higher in group 1 than in groups 2 and 2A. Other studies have shown contradictory results about the role of CD11b on cardiac function. For instance, Frantz et al.¹⁶ demonstrated increased mortality in macrophage- and CD11b⁺Ly6G⁺ monocyte-depleted mice after MI compared with controls. However, Cogle et al.¹³ reported that increased levels of BM CD11b⁺ cells are associated with reductions in LVEF after acute MI, and Maier et al.¹⁵ showed that continuous CD11b⁺ cell infiltration is associated with chronic inflammation, fibrosis, and myocyte atrophy. Although the reason for these conflicting results is unclear, differences in disease severity, the fact that CD11b is expressed on a diverse array of immune cell populations (e.g., monocytes, granulocytes, natural killer, and B cells)¹⁷⁻¹⁹, and the recently described heterogeneity within monocyte/macrophage populations^{14,20} may be contributing factors.

Associations between cardiovascular disease outcomes and the frequency of CD31⁺ cells have been reported previously^{4,21}. The frequency of CD31^{dim} lymphocytes has been shown to be inversely correlated with cardiovascular risk factors, such as age, male sex, and CRP level, whereas CD31^{bright} monocytes have been found to be positively associated with cardiovascular risk factors²². We observed that the frequency of CD31^{dim} cells in the BM was significantly higher in group 1 than in group 2. In contrast, the frequency of CD45⁺CD31^{bright} cells in the BM was significantly lower in group 1 than in group 2A. It is currently unknown whether these BM CD31^{bright} and CD31^{dim} cells are positively associated with cardiovascular risk factors. However, the BM frequency and function of CD31^{dim} and CD31^{bright} BM subsets, as well as their relationship to cardiovascular risk factors, may warrant future study in candidates for stem cell therapy.

An unexpected finding in this study was the lower levels of BM ECFCs in group 1, as compared to the levels in group 2A. Peripheral blood ECFC levels have been reported to be low (0–3 ECFCs per 10⁸ mononuclear cells) in healthy individuals^{23,24} but to increase approximately 10-fold in patients with acute MI at 3 h after symptom onset, indicating increased mobilization of ECFCs to the circulation and ischemic tissue²⁵⁻²⁷. Our finding of lower ECFC levels in the BM of chronic HF patients is consistent with these previous studies in acute MI patients and may indicate greater mobilization of these cells to the ischemic myocardium.

A heat map analysis, which focused on cell population profiles, showed a high number of progenitor cell populations with a positive SNR in the group 1 versus group 2A comparison. This association, which occurred regardless of treatment type, supports the concept that the functional outcome is influenced by the patient's underlying progenitor cell profile/activity.

It is unknown why patients who were previously stable showed an improvement or decline in LV function. It is interesting to note that 16 of the 17 patients in group 1 received BM-MNCs. Furthermore, the difference in LVEF between group 1 and group 2A was greater than 10%, which is larger than the LVEF changes seen with many approved therapies in these patients. In addition, the fact that patients who showed improvement in LVEF, LVESV, and VO₂ max had a different BM profile than those who did not suggests that the underlying physiology of BM and its activation should not be ignored.

It has long been recognized that patient responses to treatments may differ substantially in a variety of clinical settings. Personalized medicine is based on the concept that optimal medical treatments may be tailored to the individual patient. In oncology, personalized therapy is already being used to select the most effective treatment option, including cell therapies, on the basis of the characteristics of the individual patient²⁸. A similarly personalized approach may be possible for cardiovascular cell therapy, if the specific variables influencing outcomes can be identified. The current findings suggest that both progenitor and nonprogenitor (e.g., hematopoietic and immune) cells are associated with increased endogenous regenerative capacity. Moreover, these results provide further rationale for identifying BM cell populations that enhance the repair process with the eventual hope of using this information as a tool to improve the stratification and randomization of patients for cell therapy trials.

Study Limitations

The analyses in this study only show associations between BM cell phenotype and functional outcome and do not identify a causal relationship. Potential confounders in this study were the BM harvest and the transendocardial route of administration. Previous studies have shown that both BM-derived stem cells and resident tissue stem cells actively participate in tissue repair in response to different types of injury^{27,29-31}. A consideration in cell therapy trials is that the harvest and delivery processes may themselves stimulate this pool of resident stem/progenitor cells, even in placebo-treated subjects, thereby contributing to repair independent of treatment type.

CONCLUSIONS

In this cohort of patients from the FOCUS-CCTRN trial, increased frequencies of B-cell subsets and migratory cells and decreased frequencies of both monocytes/ macrophages and ECFCs in the BM at baseline were associated with improvements in LVEF, LVESV, and VO₂ max, independent of treatment group. This study suggests that both progenitor and immune cell populations in the BM influence the natural history of chronic ischemic cardiomyopathy. When assessing a new therapy, all study groups should have the same baseline characteristics so that the treatment effects can be interpreted accurately. When designing and interpreting the results of cardiovascular disease studies, potential

confounding variables, such as age, disease severity, and current comorbidities, must be taken into account. Although we adjusted our findings based on age and CABG, other confounding variables such as BNP could not be addressed. Our study findings suggest that BM composition may be another important factor to consider, as it may affect the endogenous regenerative capacity of the patients.

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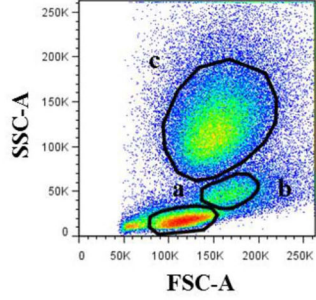
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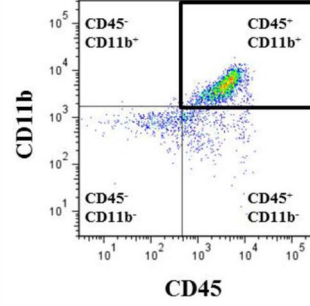
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A. Gated BMC Populations



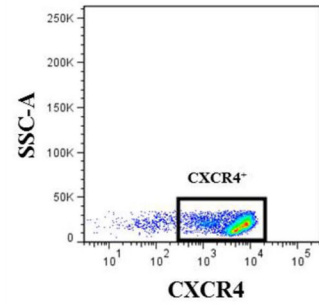
Cell Populations	% BMCs
Lymphocytes (a)	22.4
Monocytes (b)	7.08
Granulocytes (c)	46.7

B. CD45⁺CD11b⁺ Monocytes



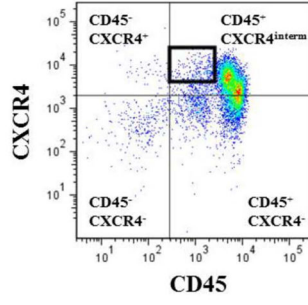
Cell Markers	% Positive
CD45 ⁻ CD11b ⁻	11.4
CD45 ⁺ CD11b ⁻	13.0
CD45 ⁺ CD11b ⁺	75.3
CD45 ⁻ CD11b ⁺	0.3

C. CD45⁺ CXCR4⁺ Lymphocytes



Cell Markers	% Positive
CXCR4 ⁺	20.3

D. CD45⁺ CXCR4^{interm} Lymphocytes



Cell Markers	% Positive
CD45 ⁺ CXCR4 ^{interm}	2.6

Figure 1. Gating strategy used for analyzing bone marrow-derived mononuclear cell (BM-MNC) populations that showed significant differences between group 1 and groups 2 and 2A. (A) Representative dot plot showing the gates used to identify BM-MNC populations based on forward scatter (FSC-A) versus side scatter (SSC-A). (B) Representative dot plot showing CD45⁺CD11b⁺ cells within the monocyte gate. (C) Representative dot plot showing CXCR4⁺ cells within the lymphocyte gate. (D) Representative dot plot showing CD45⁺CXCR4^{interm} cells within the lymphocyte gate.

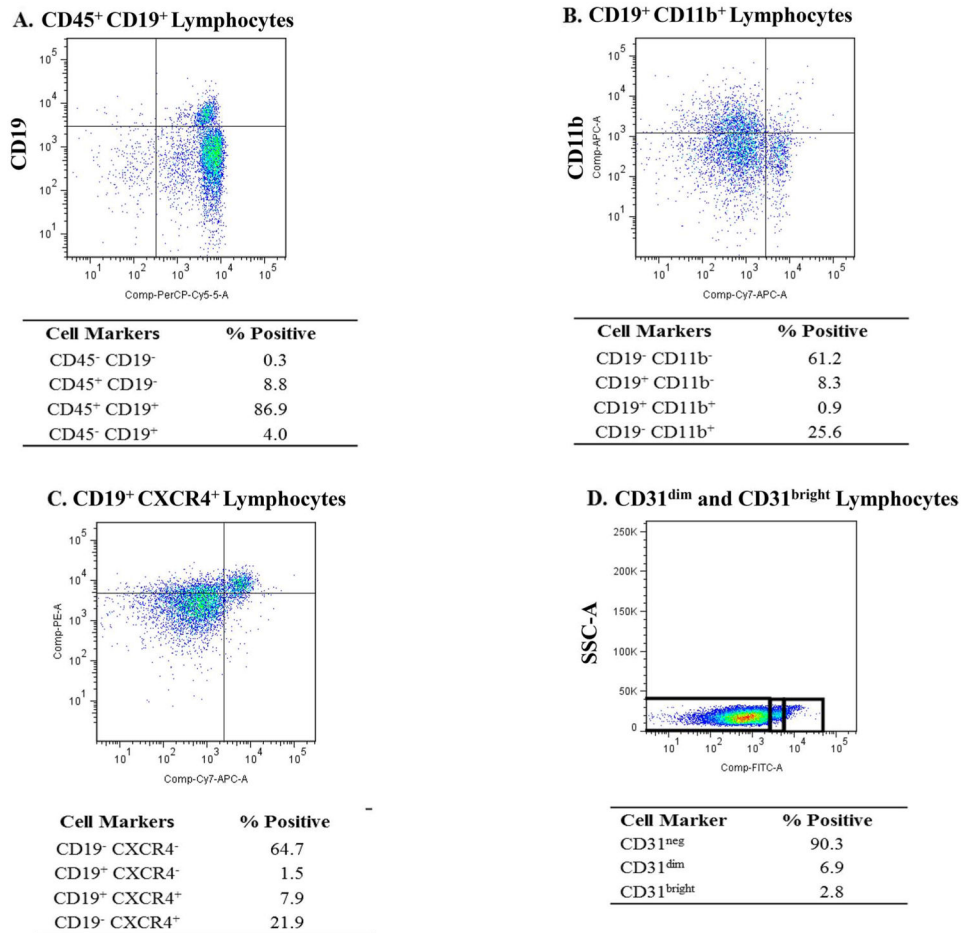


Figure 2. Gating strategy used for analyzing BM-MNC populations that showed significant differences between group 1 and groups 2 and 2A. (A) Representative dot plot showing CD45⁺CD19⁺ cells within the lymphocyte gate. (B) Representative dot plot showing CD19⁺CD11b⁺ cells within the lymphocyte gate. (C) Representative dot plot showing CD19⁺CXCR4⁺ cells within the lymphocyte gate. (D) Representative dot plot showing CD31^{neg}, CD31^{dim}, and CD31^{bright} cells within the lymphocyte gate.

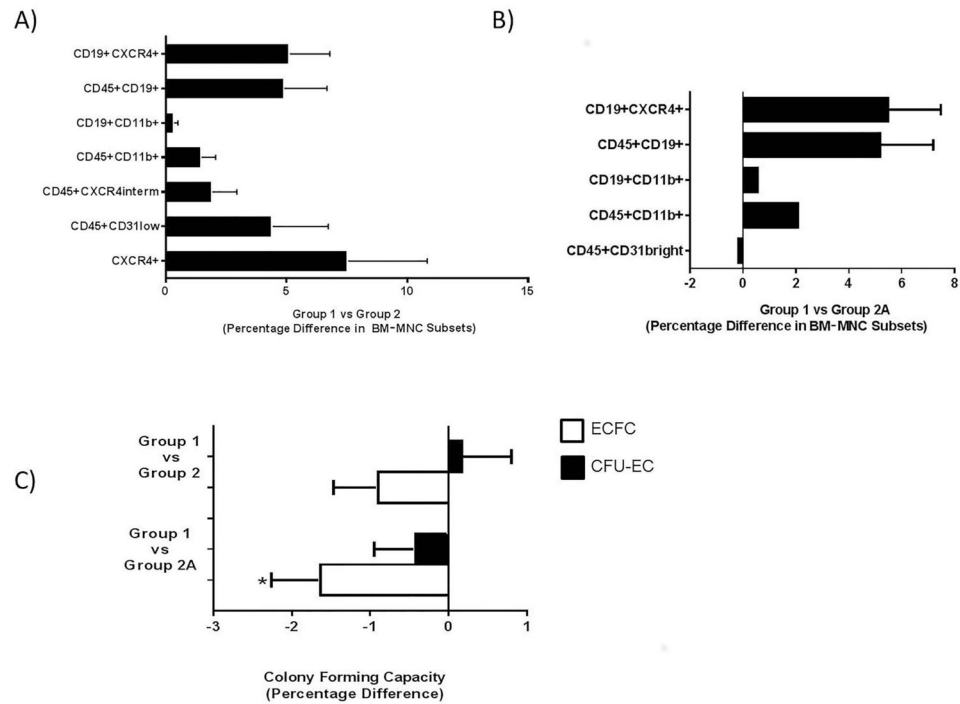


Figure 3. Significant differences in BM-MNC phenotype and function. Significant differences in BM-MNC subpopulation frequencies between (A) group 1 versus group 2 and (B) group 1 versus group 2A. (C) Percentage differences in BM-MNC function for group 1 versus groups 2 and 2A. * $p=0.016$.

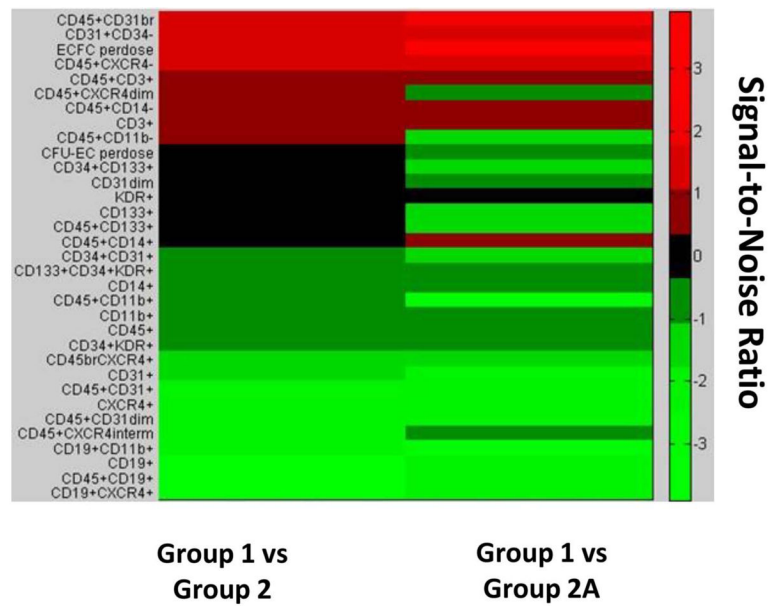


Figure 4.

Heat map illustrating signal-to-noise ratios of bone marrow cell variables for group 1 versus groups 2 and 2A. Relative differences are shown for BM-MNC subpopulation frequencies and functions of group 1 versus group 2 (left) and group 1 versus group 2A (right). Green = higher relative levels in group 1, black = no difference in relative levels, and red = lower relative levels.

Table 1

Cell Surface Marker Expression Used to Report Phenotypes of Bone Marrow-Derived Mononuclear Cells (BM-MNCs)

Single-Positive Cell Surface Marker Profiles	Double-Positive Cell Surface Marker Profiles	Triple-Positive Cell Surface Marker Profiles
CD3 ⁺	CD45 ⁺ CD31 ^{bright}	CD133 ⁺ CD34 ⁺ KDR ⁺
CD31 ^{dim}	CD31 ⁺ CD34 ⁻	
KDR ⁺	CD45 ⁺ CD11b ⁺	
CD133 ⁺	CD45 ⁺ CXCR4 ⁻	
CD14 ⁺	CD45 ⁺ CD3 ⁺	
CD11b ⁺	CD45 ⁺ CXCR4 ^{dim}	
CD45 ⁺	CD45 ⁺ CD14 ⁻	
CD31 ⁺	CD45 ⁺ CD11b ⁻	
CXCR4 ⁺	CD34 ⁺ CD133 ⁺	
CD19 ⁺	CD45 ⁺ CD133 ⁺	
	CD45 ⁺ CD14 ⁺	
	CD34 ⁺ CD31 ⁺	
	CD34 ⁺ KDR ⁺	
	CD45 ^{bright} CXCR4 ⁺	
	CD45 ⁺ CD11b ⁻	
	CD45 ⁺ CD31 ⁺	
	CD45 ⁺ CD31 ^{dim}	
	CD45 ⁺ CXCR4 ^{interm}	
	CD19 ⁺ CD11b ⁺	
	CD45 ⁺ CD19 ⁺	
	CD19 ⁺ CXCR4 ⁺	

Table 2
 Change in Contractile Function (Baseline to 6 Months) in Groups 1, 2, and 2A

	Group 1		Group 2		Group 2A	
	Change	n	Change	n	Change	n
LVEF	3.8 ± 3.0 ^{*†}	17	0.3 ± 4.3	49	-6.8 ± 3.7	11
VO ₂ max	2.8 ± 2.5 ^{*†}	17	-0.5 ± 2.7	47	-2.0 ± 2.1	11
LVESV	-16.5 ± 10.2 ^{*†}	17	-1.6 ± 22.4	49	26.1 ± 22.3	11

Data are presented as mean ± standard deviation (SD).

^{*} Significant compared to group 2.

[†] Significant compared to group 2A.

Table 3

Baseline Characteristics of Groups 1, 2, and 2A

	Group 1 (n = 17)	Group 2 (n = 61)	Group 2A (n = 11)	p Value	
				Group 1 Versus Group 2	Group 1 Versus Group 2A
Age in years [mean (SD)]	60.4 (12.0)	65.2 (8.7)	69.53 (9.04)	0.067	0.022
Female	3 (17.7)	5 (8.2)	2 (18.18)	0.362	1.000
White	17 (100.0)	57 (93.4)	11 (100.00)	0.571	1.000
BMI [mean (SD)]	30.3 (5.5)	30.5 (5.9)	30.02 (7.29)	0.872	0.520
NYHA classification					
Class I	3 (17.7)	5 (8.2)	2 (18.18)	0.198	0.083
Class II	10 (58.8)	28 (45.9)	2 (18.18)		
Class III	4 (23.5)	28 (45.9)	7 (63.64)		
Class IV	0	0	0 (0.00)		
CCS classification*					
Class I	5 (29.4)	12 (23.5)	2 (20.00)	0.878	0.315
Class II	8 (47.1)	23 (45.1)	3 (30.00)		
Class III	4 (23.5)	15 (29.4)	4 (40.00)		
Class IV	0 (0.0)	1 (2.0)	1 (10.00)		
BP in mmHg [mean (SD)]					
Systolic	120.0 (23.9)	122.8 (17.1)	120.55 (16.82)	0.587	0.898
Diastolic	73.4 (14.9)	72.4 (10.3)	72.64 (10.38)	0.737	0.918
Laboratory evaluations					
BNP [median (interquartile range)] [†]	68.5 (51.0–128.1)	132.0 (81.0–278.0)	258.89 (185.11)	0.032	0.029
ProBNP [median (interquartile range)] [‡]	606.0 (171.0–2187.0)	863.5 (443.0–5334.0)	941.00 (384.67)	0.401	0.806
Medications at time of randomization					
ACEi/ARB	13 (76.5)	38 (62.3)	5 (45.45)	0.39	0.108
Aldosterone inhibitor	3 (17.7)	11 (18.3)	1 (9.09)	1	0.626
Antiarrhythmics	3 (17.7)	14 (23.0)	2 (18.18)	0.751	1.000
Aspirin	13 (76.5)	53 (86.9)	10 (90.91)	0.282	0.372
β-Blockers	16 (94.1)	59 (96.7)	11 (100.00)	0.527	1.000
Calcium channel blockers	1 (5.9)	10 (16.6)	1 (9.09)	0.439	1.000

	<i>p</i> Value			
	Group 1 (<i>n</i> = 17)	Group 2 (<i>n</i> = 61)	Group 2A (<i>n</i> = 11)	Group 1 Versus Group 2 Group 1 Versus Group 2A
Digitalis	1 (5.9)	5 (8.2)	2 (18.18)	1 0.611
Diuretics	11 (64.7)	44 (72.1)	7 (63.64)	0.56 1.000
Nitrates	8 (47.1)	41 (67.2)	8 (72.73)	0.16 0.260
P2 Y12 inhibitors	11 (64.7)	38 (62.3)	8 (72.73)	1 0.702
Medical history				
Diabetes	5 (29.4)	26 (42.6)	3 (27.27)	0.407 1.000
Hypertension	14 (82.4)	48 (78.7)	10 (90.91)	1 0.626
Hyperlipidemia	17 (100.0)	57 (93.4)	10 (90.91)	0.389 0.367
Angina	5 (29.4)	21 (34.4)	3 (27.27)	0.778 0.702
Obesity	7 (41.2)	18 (29.5)	3 (27.27)	0.559 0.443
History of myocardial infarction	15 (93.8)	55 (93.2)	11(100.00)	1 1.000
Prior revascularization	15 (88.2)	52 (85.3)	9 (81.82)	1 0.611
Prior CABG	7 (41.2)	53 (86.9)	9 (81.82)	<0.001 0.121
Prior hospitalization for CHF	12 (70.6)	38 (62.3)	3 (27.27)	0.582 1.000
History of TIA	1 (5.9)	7 (11.5)	2 (18.18)	0.678 0.126
History of stroke	0 (0.00)	5 (8.2)	2 (18.18)	0.58 0.672
Peripheral vascular disease	3 (17.7)	11 (18.0)	2 (18.18)	1 1.000
History of arrhythmia	10 (58.8)	29 (52.7)	5 (50.00)	0.783 1.000
Cardiac pacemaker	12 (70.6)	48 (78.7)	10 (90.91)	0.522 0.215
Smoking (former or current)	13 (76.5)	44 (72.1)	9 (81.82)	1 0.672
Cardiac function (baseline)				
LVEF (%)	35.3 (10.9)	33.5 (8.3)	35.8 (8.7)	0.97 0.870
LVESV (ml)	116.4 (53.2)	127.8 (45.7)	120.6 (46.2)	0.301 0.806
VO ₂ max (ml/kg/min)	15.1 (4.1)	14.7 (4.2)	15.6 (4.9)	0.999 0.74

Data presented as *n* (%), unless otherwise specified. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; BMI, body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; CABG, coronary artery bypass graft; CCS, Canadian Cardiovascular Society; CHF, congestive heart failure; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; NYHA, New York Heart Association; TIA, transient ischemic attack; VO₂ max, maximal oxygen consumption.

* Group 2: *n* = 51.

[†] Group 1: *n* = 10, group 2: *n* = 47, group 2A: *n* = 11; results reported as median (first quartile–third quartile).

[‡] Group 1: *n* = 7, group 2: *n* = 14, group 2A: *n* = 2; results reported as median (first quartile–third quartile).

Table 4

Significant Differences in Bone Marrow Cell Phenotype for Group 1 Versus Group 2

Cell Surface Marker Expression	Group 1 (n = 17)	Group 2 (n = 61)	p Value
CXCR4 ⁺	57.0 (11.3)	49.5 (13.8)	0.042
CD45 ⁺ CD31 ^{dim} *	30.9 (8.7)	26.5 (7.0)	0.033
CD45 ⁺ CXCR4 ^{interm} *	6.8 (3.9)	4.9 (2.9)	0.028
CD45 ⁺ CD11b ⁺ *	6.2 (2.0)	4.7 (2.5)	0.028
CD19 ⁺ CD11b ⁺ †	1.2 (0.6)	0.9 (0.5)	0.018
CD19 ⁺	15.0 (7.2)	10.0 (5.0)	0.001
CD45 ⁺ CD19 ⁺	15.8 (6.8)	10.9 (4.7)	0.001
CD19 ⁺ CXCR4 ⁺	14.4 (6.4)	9.30 (4.3)	<0.001

Data presented as mean (SD). Cell phenotype data shown as the cell frequency in the lymphocyte fraction of the bone marrow (BM), except for the CD45⁺CD11b⁺ population, which is shown as the cell frequency in the monocyte fraction of the BM.

* Group 2: n = 60.

† Group 2: n = 58.

Table 5

Significant Differences in Bone Marrow Cell Phenotypes and Function for Group 1 Versus Group 2A

Variable	Group 1 (n = 17)	Group 2A (n = 11)	p Value
Cell phenotype			
CD45 ⁺ CD31 ^{bright}	0.2 (0.1)	0.4 (0.3)	0.046
CD45 ⁺ CD11b ⁺	6.2 (2.0)	4.1 (2.0)	0.012
CD19 ⁺ CD11b ⁺	1.2 (0.6)	0.6 (0.2)	0.007
CD45 ⁺ CD19 ⁺	15.8 (6.8)	10.6 (5.4)	0.043
CD19 ⁺ CXCR4 ⁺	14.4 (6.4)	9.0 (4.9)	0.024
Cell function			
ECFC per dose [*]	85.0 (87.2)	248.9 (227.8)	0.016

Data presented as mean (SD). Cell phenotype data shown as the cell frequency in the lymphocyte fraction of the BM, except for the CD45⁺CD11b⁺ population, which is shown as the cell frequency in the monocyte fraction of the BM. ECFC, endothelial colony-forming cells.

^{*}Group 1: n = 16, group 2A: n = 9.