Clinical Track

Bone Marrow Characteristics Associated With Changes in Infarct Size After STEMI

A Biorepository Evaluation From the CCTRN TIME Trial


Rationale: Despite significant interest in bone marrow mononuclear cell (BMC) therapy for ischemic heart disease, current techniques have resulted in only modest benefits. However, selected patients have shown improvements after autologous BMC therapy, but the contributing factors are unclear.

Objective: The purpose of this study was to identify BMC characteristics associated with a reduction in infarct size after ST-segment-elevation–myocardial infarction.

Methods and Results: This prospective study comprised patients consecutively enrolled in the CCTRN TIME (Cardiovascular Cell Therapy Research Network Timing in Myocardial Infarction Evaluation) trial who agreed to have their BMCs stored and analyzed at the CCTRN Biorepository. Change in infarct size between baseline (3 days after percutaneous coronary intervention) and 6-month follow-up was measured by cardiac MRI. Infarct-size measurements and BMC phenotype and function data were obtained for 101 patients (mean age, 56.5 years; mean screening ejection fraction, 37%; mean baseline cardiac MRI ejection fraction, 45%). At 6 months, 75 patients (74.3%) showed a reduction in infarct size (mean change, −21.0±17.6%). Multiple regression analysis indicated that infarct size reduction was greater in patients who had a larger percentage of CD31+ BMCs (P=0.046) and in those with faster BMC growth rates in colony-forming unit Hill and endothelial-colony forming cell functional assays (P=0.033 and P=0.032, respectively).

Conclusions: This study identified BMC characteristics associated with a better clinical outcome in patients with segment-elevation–myocardial infarction and highlighted the importance of endothelial precursor activity in regenerating infarcted myocardium. Furthermore, it suggests that for these patients with segment-elevation–myocardial infarction, myocardial repair was more dependent on baseline BMC characteristics than on whether the patient underwent intracoronary BMC transplantation.

Clinical Trial Registration Information: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00684021.

Key Words: acute myocardial infarction • adult stem cell • coronary circulation • regeneration

Despite advances in emergency care and reperfusion therapy, segment-elevation–myocardial infarction (STEMI) remains an important cause of morbidity and mortality.1,2 In studies of cardiovascular cell therapy, harvested bone marrow mononuclear cells (BMCs), including hematopoietic and nonhematopoietic cell populations (eg, mesenchymal stromal

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Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMC</td>
<td>bone marrow mononuclear cell</td>
</tr>
<tr>
<td>CCTRN</td>
<td>Cardiovascular Cell Therapy Research Network</td>
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<tr>
<td>CFU-F</td>
<td>colony-forming unit fibroblast</td>
</tr>
<tr>
<td>CFU-Hill</td>
<td>colony-forming unit Hill</td>
</tr>
<tr>
<td>cMRI</td>
<td>cardiac MRI</td>
</tr>
<tr>
<td>ECFC</td>
<td>endothelial-colony forming cell</td>
</tr>
<tr>
<td>FC</td>
<td>flow cytometric</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-segment-elevation myocardial infarction</td>
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</table>

Several factors may explain the mixed responses seen in cell therapy clinical trials, including heterogeneity of BMC composition, differences in cell processing techniques, dose administered, timing and route of delivery, and patient characteristics. Although the study protocols were often designed to control for some of these factors, patients’ BMC characteristics were rarely assessed in relation to clinical outcomes. However, identifying the patterns in BMC characteristics associated with improved outcomes could lead to better patient selection and personalized enrichment of therapeutic cells in the product before transplantation and would enhance our understanding of the mechanisms involved in cardiovascular regeneration and repair. In this study, we sought to identify BMC characteristics associated with changes in infarct size after STEMI. Infarct size was chosen as the primary outcome for our analyses because it is an independent predictor of mortality in patients with coronary artery disease and measurements for this outcome are highly reproducible.

Methods

This prospective cohort study comprised patients consecutively enrolled in the Cardiovascular Cell Therapy Research Network (CCTRN) Timing in Myocardial Infarction Evaluation (TIME) trial who participated in the 6-month follow-up and provided written consent to have their excess BMCs stored for further analyses at the CCTRN Biorepository. These analyses were prespecified in the Ancillary Functional Studies for the CCTRN protocol. The design and rationale of the CCTRN TIME trial and the CCTRN Biorepository are described elsewhere. Briefly, CCTRN TIME was a multicenter, controlled, randomized, double-blind trial conducted at 5 clinical centers, their satellite facilities, and a data coordinating center. The trial protocol was approved by the local institutional review boards at each center, and participants provided written informed consent. Participants were randomized 2:1 to receive 150 million BMCs or placebo by intracoronary infusion on either day 3 or day 7 after primary percutaneous coronary intervention. With permission, the excess BMCs were sent to the CCTRN Biorepository for phenotype and functional analyses and storage.

Flow Cytometry Sample Preparation and Antibody Labeling

FC analysis was used to immunophenotype the BMC populations. Briefly, to lyse the red blood cells, we incubated the samples in ammonium-chloride-potassium lysis buffer (0.15 mol/L NH4Cl, 10 mmol/L KHCO3, and 0.1 mmol/L EDTA; pH, 7.2–7.4) for 5 minutes at room temperature. The remaining cells were then washed twice in phosphate-buffered saline+2.5% fetal calf serum (2.5% phosphate-buffered saline). Cell concentration and viability were determined by using the Guava ViaCount assay on a Guava PCA-96 system (Millipore Corporation, Billerica, MA). All antibodies were purchased from BD Biosciences (San Jose, CA) unless otherwise specified. Bone marrow cells were analyzed with 2 separate panels of antibodies and their respective isotype-matched controls: (1) CD45, CD34, CD133, KDR, and CD31 and (2) CD45, CD3, CD19, CD11b, CXCR4, and CD14 (Online Table I). Labeled cells were then incubated at room temperature for 20 minutes in the dark, washed twice in 2.5% phosphate-buffered saline, and resuspended to a final volume of 1 mL in 2.5% phosphate-buffered saline for FC analysis.

Flow Cytometry Instrument Set Up, Controls, and Fluorochrome Compensation

FC data were acquired by using a BD LSRII Flow Cytometer (BD Biosciences) with 4 fixed-aligned, air-cooled lasers: 20 mW UV laser (355 nm), 25 mW violet laser (405 nm), 20 mW blue laser (488 nm), and 17 mW red laser (635 nm). The lasers, photomultiplier tubes, dichroic long pass mirrors, band pass filters, and fluorochromes are listed in Online Table II, and the optical pathway configuration is depicted in Online Figure I. Before immunophenotyping was begun, instrument performance was validated by using BD Cytometer Setup and Tracking Beads (BD Biosciences). Six-peak Rainbow Calibration Particles (Spherotech Inc., Lake Forest, IL) were used to set and maintain the target median fluorescence intensity values throughout the study. Before data acquisition, hardware compensation was performed with cells stained with a single fluorochrome (Online Table III) by using the Compensation Setup feature of BD FACSDiva 6.0 software (BD Biosciences) and a compensation matrix.

Flow Cytometry Data Acquisition and Data Analysis

FC data were acquired on a BD Biosciences LSRII flow cytometer (BD Biosciences) at a low flow rate within the first hour after sample preparation by using FACS DIVA 6.0 software. Events were triggered on the forward scatter signal. A minimum of 10^5 events were acquired for analysis. A trained operator blinded to the patient’s characteristics performed all the tests and analyzed the data throughout the study. Acquired data were analyzed using FlowJo software 7.6.5 (Tree Star Inc., Ashland, OR). Data analysis was performed by first gating around the individual lymphocyte, monocyte, and granulocyte populations, as determined on the basis of their forward scatter versus side scatter properties (Figure 1A).
Figure 1. Gating strategy used for analyzing CD31+ cells and CD31+ cell subsets. A, Representative dot plot showing the gates used to identify bone marrow mononuclear cell (BMC) populations based on forward scatter (FSC-A) versus side scatter (SSC-A), B, Representative histogram showing CD31+ cells within the lymphocyte gate. Blue indicates CD31+ cells, and red indicates the isotype control. C, Representative dot plot showing the CD45+CD31+ cells within the lymphocyte gate. D, Representative dot plot showing the CD45+CD31+ subset within the lymphocyte gate (gate for the subset shown in black). Percentages shown in (B–D) are based on the total lymphocyte population. All data presented are from a single patient.

Cell debris and small particles were omitted from the analysis by excluding events with low forward scatter. All analyses were performed on gated lymphocytes unless otherwise specified. Online Figure II shows representative single-color histograms comparing antibodies to the isotype-matched control antibodies. CD34+ and CD34+CD133+ cells in the BMC product were measured using the CD31+ population. All data presented are from a single patient.

Functional Analyses
Trypan blue exclusion was used to assess viability of the cells that underwent functional analysis. BMC function was evaluated with 3 separate assays, such as (1) the colony-forming unit Hill (CFU-Hill), (2) the endothelial-colony forming cell (ECFC), and (3) the colony-forming unit fibroblast (CFU-F) assays. The number of colony forming units, type of colonies, and percent confluency were recorded on days 7, 14, 21, and 28 for the ECFC and CFU-F assays and on days 4 through 9 for the CFU-Hill assay. Results from all 3 assays were assessed with the following 3 metrics, such as (1) slope of the best-fit linear curve for the percent confluency over time, (2) exponential constant of the best-fit exponential curve for the number of colonies over time, and (3) maximum number of colonies present during the entire culture period.

Measurement of Infarct Size
The outcome measure of interest, change in infarct size, was calculated by subtracting the baseline measurement taken 3 days after primary percutaneous coronary intervention from the measurement made at 6 months. Infarct size was assessed with cardiac MRI (cMRI) using delayed contrast-enhanced imaging where appropriate. To improve infarct size measurements for this study, we used a cMRI algorithm that corrected for left ventricular mass; this algorithm was not used in the original CCTRN TIME study. Infarct size and BMC measurements were made by laboratory personnel masked to all clinical data and treatment assignments. Further details about the trial protocol and assessment of individual outcomes can be found in a previous report.

Statistical Analyses
When assessing the relationships between change in infarct size from baseline to 6 months and demographic variables, we categorized infarct size as a dichotomous variable (ie, either as a decrease or an increase). For this set of analyses, associations with continuous variables were determined by using an unpaired t-test and associations with categorical variables were determined by using the Fisher exact test. When the relationships between BMC parameters (cell phenotype and function) and infarct size were assessed, infarct size was treated as a continuous variable. These associations were determined by using both unvariable and multivariable linear regression analyses, in which the dependent variable was change in infarct size. Covariates for the multivariable model were selected based on previously available data that suggested that these factors would be relevant and biologically plausible to model the hypothesized relationship between bone marrow parameters and change in infarct size. Covariates for the model included age, baseline infarct size, diabetes mellitus, angiotensin-converting enzyme inhibitor use, hypertension, smoking status, and treatment received in the clinical trial. In this first assessment of the relationships between BMC characteristics and infarct size, we conducted multiple statistical tests. Because this was an exploratory assessment, we prioritized knowledge discovery, and we did not use statistical techniques to reduce the familywise error rate (eg, Bonferroni correction).

Results
In the CCTRN TIME clinical trial, 120 patients with STEMI underwent bone marrow aspiration and were randomized to receive either BMCs or placebo. Complete infarct size measurements and BMC product analyses were available for 101 (84.0%) of these patients. Reasons for incomplete follow-up were reported previously. At the 6-month follow-up, 75 patients (74.3%) showed a reduction in infarct size from baseline, with a mean infarct size of 50.4±25.3 g at baseline and 29.4±17.0 g at 6-month follow-up (mean change, −21.0±17.6 g). The patients who showed an increase in infarct size (n=26) had a mean infarct size of 30.9±19.8 g at baseline and 41.3±20.3 g at 6-month follow-up (mean change, 10.4±12.1 g). In Table 1, we present the baseline characteristics of patients when stratified according to direction of change in infarct size. With the exception of initial infarct size, there were no significant differences at baseline between patients who showed a reduction in infarct size at 6 months and those who showed an increase in infarct size. The initial infarct size measurements taken at days 3 and 7 postmyocardial infarction were found to significantly correlate with ejection fraction at 6 months (day 3: r=−0.376, P<0.001; day 7: r=−0.601, P<0.001). Of the 75 patients who showed a reduction in infarct size at 6 months, 51 (68.0%) had been treated with BMCs and 24 (32.0%) had received placebo. Of the 26 patients who showed an increase in infarct size at 6 months, 15 (57.7%) had been treated with BMCs and 11 (42.3%) had received placebo. Consistent with the
Table 1. Demographics and Clinical Data of Patients Stratified by Change in Infarct Size at 6 Months

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Δ Infarct Size&lt;0 (Decreased) (n=75)</th>
<th>Δ Infarct Size&gt;0 (Increased) (n=26)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.0±10.8</td>
<td>55.4±10.7</td>
<td>0.512</td>
</tr>
<tr>
<td>Female</td>
<td>12 (16%)</td>
<td>3 (11.5%)</td>
<td>0.754</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.76±0.10</td>
<td>1.75±0.08</td>
<td>0.666</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>94.9±19.5</td>
<td>93.2±23.1</td>
<td>0.725</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.5±5.2</td>
<td>30.2±6.6</td>
<td>0.855</td>
</tr>
<tr>
<td>Previous medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (18.7%)</td>
<td>7 (26.9%)</td>
<td>0.406</td>
</tr>
<tr>
<td>Hypertension</td>
<td>46 (61.3%)</td>
<td>13 (50%)</td>
<td>0.360</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>53 (70.7%)</td>
<td>16 (61.5%)</td>
<td>0.465</td>
</tr>
<tr>
<td>History of angina</td>
<td>16 (21.3%)</td>
<td>3 (11.5%)</td>
<td>0.386</td>
</tr>
<tr>
<td>Smoking</td>
<td>44 (58.7%)</td>
<td>18 (69.2%)</td>
<td>0.484</td>
</tr>
<tr>
<td>Cardiovascular and infarction details</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline infarct size, g</td>
<td>50.4±25.3</td>
<td>30.9±19.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate</td>
<td>80.1±15.7</td>
<td>82.2±11.2</td>
<td>0.524</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>119.8±19.7</td>
<td>111.3±16.7</td>
<td>0.053</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.6±14.2</td>
<td>73.2±13.5</td>
<td>0.889</td>
</tr>
<tr>
<td>LV end-diastolic volume index, mL/m²</td>
<td>74.4±17.3</td>
<td>73.2±16.9</td>
<td>0.753</td>
</tr>
<tr>
<td>LV end-systolic volume index, mL/m²</td>
<td>40.5±12.5</td>
<td>42.0±14.9</td>
<td>0.439</td>
</tr>
<tr>
<td>Screening EF (echocardiography)</td>
<td>37±6%</td>
<td>36±8%</td>
<td>0.719</td>
</tr>
<tr>
<td>Core laboratory EF (day 3) (cMRI)</td>
<td>46±10%</td>
<td>42±12%</td>
<td>0.137</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>14.1±2</td>
<td>13.4±1</td>
<td>0.142</td>
</tr>
<tr>
<td>High sensitivity CRP, mg/L</td>
<td>39.8±49.1</td>
<td>33.8±24</td>
<td>0.568</td>
</tr>
<tr>
<td>Peak CK, U/L</td>
<td>2970.8±2104.9</td>
<td>3180.0±2734.1</td>
<td>0.703</td>
</tr>
<tr>
<td>Peak CKMB, ng/mL</td>
<td>254.0±190.1</td>
<td>255.8±218.2</td>
<td>0.972</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>325.9±655</td>
<td>214.6±132.5</td>
<td>0.466</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>1428.2±1367.3</td>
<td>1440.6±1873.6</td>
<td>0.988</td>
</tr>
<tr>
<td>Troponin T (peak), ng/mL</td>
<td>9.3±7.6</td>
<td>9.2±6.9</td>
<td>0.963</td>
</tr>
<tr>
<td>Troponin I (peak), ng/mL</td>
<td>76.1±91</td>
<td>118.6±163.7</td>
<td>0.478</td>
</tr>
<tr>
<td>Drug eluting stent</td>
<td>63 (84%)</td>
<td>18 (69.2%)</td>
<td>0.151</td>
</tr>
<tr>
<td>LAD infarction</td>
<td>67 (89.3%)</td>
<td>23 (88.5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Preinfarction angina</td>
<td>14 (18.7%)</td>
<td>3 (11.5%)</td>
<td>0.548</td>
</tr>
<tr>
<td>Transferred after MI</td>
<td>33 (44%)</td>
<td>11 (42.3%)</td>
<td>1.000</td>
</tr>
<tr>
<td>PCI at nonstudy center hospital</td>
<td>6 (8%)</td>
<td>2 (7.7%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Discharge medications

| ACE inhibitor                                     | 62 (82.7%)                          | 23 (88.5%)                          | 0.756  |
| Aspirin                                           | 72 (96%)                            | 26 (100%)                           | 0.567  |
| β-blocker                                         | 73 (97.3%)                          | 26 (100%)                           | 1.000  |
| Clopidogrel or prasugrel                           | 73 (97.3%)                          | 23 (88.5%)                          | 0.106  |
| Statins                                           | 71 (94.7%)                          | 22 (84.6%)                          | 0.199  |
| Diuretic                                          | 16 (21.3%)                          | 5 (19.2%)                           | 1.000  |
| Coumadin or enoxaparin                            | 15 (20%)                            | 2 (7.7%)                            | 0.225  |

(Continued)

Table 1. Continued

<table>
<thead>
<tr>
<th>Cell product information</th>
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<tbody>
<tr>
<td>Time from PCI to infusion, d</td>
<td>5.1±2.3</td>
<td>4.6±2</td>
<td>0.245</td>
</tr>
<tr>
<td>Time from aspiration to infusion, h</td>
<td>8.7±2.9</td>
<td>8.6±1.5</td>
<td>0.846</td>
</tr>
<tr>
<td>Final volume, mL</td>
<td>149.7±1.9</td>
<td>144.2±28.6</td>
<td>0.097</td>
</tr>
<tr>
<td>Cell product viability, %</td>
<td>98.1±1.6</td>
<td>98.5±1</td>
<td>0.284</td>
</tr>
</tbody>
</table>

Values shown as the mean±SD or number (%). ACE indicates angiotensin-converting enzyme; BMI, body mass index; BP, blood pressure; CK, creatine kinase; CKMB, creatine kinase-MB; cMRI, cardiac MRI; CRP, C-reactive protein; EF, ejection fraction; LAD, left anterior descending; LV, left ventricular; MI, myocardial infarction; NT-proBNP, N-terminal pro-brain natriuretic peptide; and PCI, percutaneous coronary intervention.

results from the clinical trial, we found no significant difference in the change in infarct size over time between patients who received BMC therapy and those who did not (−13.3±20.1 g versus −12.2±23.9 g; P=0.802). In addition, when patients were stratified by both direction of change in infarct size and treatment type, no significant differences were found between the 4 groups for any of the demographic variables assessed (Online Table IV).

The BMC phenotype and functional assay results are summarized in Table 2. When univariable analysis was performed to compare the results for patients who showed a reduction in infarct size at 6 months and those who showed an increase in infarct size, no significant differences in cell frequency were found for the phenotypes assessed. However, a significant difference was observed in the BMC functional assessments. Specifically, the exponential constant for the ECFC assay was significantly higher in patients who showed a reduction in infarct size than in those who showed an increase in infarct size. In contrast, the CFU-Hill and CFU-F assays indicated no difference among patients who showed a reduction in infarct size at 6 months and those who showed an increase in infarct size. When patients were stratified by both direction of change in infarct size and treatment type for the phenotype and functional comparisons, a significant difference was found in the frequency of CD19+ cells (Online Table V).

Multiple regression analysis was also used to model the relationships between either cell phenotype or functional parameters and change in infarct size (results also shown in Table 2). After adjusting for age, history of diabetes mellitus, baseline infarct size, angiotensin-converting enzyme inhibitor use, hypertension, history of smoking, and therapy assignment in the clinical trial (BMC or placebo), patients with a higher percentage of CD31+ cells were shown to have a larger reduction in infarct size at 6 months (P=0.046). Additional gating analyses to explore this association (Figure 1) showed that a higher percentage of CD45+CD31+ cells (P=0.042), specifically CD45+CD31low cells (P=0.015), was associated with a larger reduction in infarct size. To further explore this
association, we show the changes in infarct size between day 3 and the 6-month follow-up stratified by the percentage of CD34⁺CD31⁻ cells in the BMC product (Figure 2). In multivariable regression analysis the percentage of CD19⁺ cells was not associated with change in infarct size (P=0.322). When multivariable regression analysis was performed to assess differences in BMC functional parameters, a higher exponential constant for the colony growth curve was found to be associated with a larger reduction in infarct size at 6 months in both the CFU-Hill and ECFC assays (P=0.030 and P=0.032, respectively). However, treatment assignment (BMCs or placebo) was not associated with a reduction in infarct size (P=0.706) at 6 months in the multivariate analysis.

**Table 2. BMC Phenotype and Functional Characteristics of Patients Stratified by Change in Infarct Size at 6 Months**

<table>
<thead>
<tr>
<th>Cell phenotype (% positive)</th>
<th>Δ Infarct Size&lt;0* (Decreased)</th>
<th>Δ Infarct Size&gt;0* (Increased)</th>
<th>Unadjusted P Value</th>
<th>Effect Size†</th>
<th>Adjusted P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3⁺</td>
<td>65.5 (58.0–73.9)</td>
<td>70.3(65.9–74.5)</td>
<td>0.871</td>
<td>0.03</td>
<td>0.456</td>
</tr>
<tr>
<td>CD11b⁺</td>
<td>72.0 (57.5–79.7)</td>
<td>69.9(63.7–76.0)</td>
<td>0.114</td>
<td>–0.02</td>
<td>0.638</td>
</tr>
<tr>
<td>CD14⁺ (% monocytes)</td>
<td>56.2 (46.6–68.6)</td>
<td>58.2 (48.1–65.4)</td>
<td>0.363</td>
<td>0.12</td>
<td>0.177</td>
</tr>
<tr>
<td>CD19⁺</td>
<td>11.4 (8.2–14.9)</td>
<td>9.0 (5.9–10.8)</td>
<td>0.122</td>
<td>–0.47</td>
<td>0.322</td>
</tr>
<tr>
<td>CD31⁺</td>
<td>40.5 (34.6–48.0)</td>
<td>37.6 (29.0–42.2)</td>
<td>0.154</td>
<td>–0.36</td>
<td>0.046</td>
</tr>
<tr>
<td>CD45⁺CD31⁻</td>
<td>39.3 (33.4–46.7)</td>
<td>36.5 (28.2–41.0)</td>
<td>0.184</td>
<td>–0.37</td>
<td>0.042</td>
</tr>
<tr>
<td>CD45⁺CD31⁻w</td>
<td>30.6 (25.5–36.3)</td>
<td>28.4 (21.1–33.0)</td>
<td>0.078</td>
<td>–0.52</td>
<td>0.015</td>
</tr>
<tr>
<td>CD34⁺</td>
<td>4.0 (2.7–6.3)</td>
<td>4.4 (3.3–5.4)</td>
<td>0.269</td>
<td>0.90</td>
<td>0.577</td>
</tr>
<tr>
<td>CD34⁺ (ISHAGE)</td>
<td>1.9 (1.4–2.7)</td>
<td>2.0 (1.5–2.7)</td>
<td>0.165</td>
<td>1.95</td>
<td>0.807</td>
</tr>
<tr>
<td>CD34⁺CD133⁻ (ISHAGE)</td>
<td>0.9 (0.6–1.5)</td>
<td>1.0 (0.7–1.3)</td>
<td>0.370</td>
<td>1.59</td>
<td>0.996</td>
</tr>
<tr>
<td>CD45⁺</td>
<td>94.6 (91.7–96.8)</td>
<td>95.0 (91.8–96.3)</td>
<td>0.461</td>
<td>0.02</td>
<td>0.593</td>
</tr>
<tr>
<td>CD133⁻</td>
<td>2.4 (1.7–4.3)</td>
<td>2.9 (2.2–3.7)</td>
<td>0.956</td>
<td>0.88</td>
<td>0.849</td>
</tr>
<tr>
<td>CXCR4⁺</td>
<td>42.6 (34.1–54.1)</td>
<td>38.7 (27.3–49.1)</td>
<td>0.723</td>
<td>0.01</td>
<td>0.939</td>
</tr>
<tr>
<td>KDR⁺</td>
<td>0.2 (0.1–0.3)</td>
<td>0.2 (0.1–0.5)</td>
<td>0.620</td>
<td>0.405</td>
<td>0.914</td>
</tr>
<tr>
<td>CD133⁺KDR⁺</td>
<td>0.011 (0.005–0.030)</td>
<td>0.011 (0.004–0.020)</td>
<td>0.090</td>
<td>–49.38</td>
<td>0.392</td>
</tr>
</tbody>
</table>

**Functional analysis**

| CFU-Hill (No. of exponential constant of colony) | 0.4 (0.0–0.7) | 0.2 (0.0–0.3) | 0.061 | –6.21 | 0.033 |
| CFU-Hill (No. of maximum colony)               | 4.5 (0.0–11.5) | 5.0 (2.0–15.0) | 0.661 | –0.17 | 0.259 |
| CFU-Hill (linear slope of confluency)           | 14.6 (10.6–19.3) | 14.9 (7.2–16.6) | 0.655 | 0.57 | 0.590 |
| ECFC (No. of exponential constant of colony)    | 0.1 (0.0–1.5) | 0.0 (0.0–0.1) | 0.003 | –8.66 | 0.032 |
| ECFC (No. of maximum colony)                   | 0.0 (0.0–14.0) | 0.0 (0.0–21.0) | 0.828 | –0.03 | 0.626 |
| ECFC (linear slope of confluency)               | 2.8 (0.7–3.8) | 2.5 (0.9–5.2) | 0.670 | –0.64 | 0.539 |
| CFU-F (No. of exponential constant of colony)   | 1.4 (0.0–1.6) | 0.0 (0.0–1.6) | 0.052 | –8.64 | 0.059 |
| CFU-F (No. of maximum colony)                  | 4.0 (1.0–8.0) | 6.0 (0.0–14.0) | 0.356 | 0.37 | 0.184 |
| CFU-F (linear slope of confluency)              | 2.2 (1.7–3.6) | 2.8 (1.2–3.4) | 0.119 | 2.86 | 0.493 |

All flow cytometric analyses were performed on gated lymphocytes unless otherwise specified. BMC indicates bone marrow mononuclear cell; CFU-F, colony-forming unit fibroblast; CFU-Hill, colony-forming unit Hill; ECFC, endothelial-colony forming cell; and ISHAGE, International Society of Hematotherapy and Graft Engineering.

*Values represent median and interquartile range.
†Value is the coefficient from multivariate regression model and represents the modeled change in infarct size (g) per unit increase in the variable.
‡P value for the multivariable regression model that includes adjustment for age, baseline infarct size, history of hypertension, tobacco use, diabetes mellitus, angiotensin-converting enzyme inhibitor use, and treatment received (BMCs or placebo).

Discussion

The purpose of this exploratory study was to identify patterns in BMC characteristics associated with either an increase or a decrease in infarct size in patients with STEMI. To accomplish this, we analyzed data from patients in the CCTRN TIME trial who provided consent to have their BMC product analyzed by the CCTRN Biorepository laboratories. Multivariable regression analysis showed that an increased percentage of CD31⁺ cells in the bone marrow was associated with a greater reduction in infarct size at 6 months after STEMI. In addition, a greater reduction in infarct size was associated with a faster BMC growth rate in CFU-Hill and ECFC functional assays. Thus, our findings suggest that
Phenotype and functional assessments of bone marrow may be important for understanding individual responses to cell therapy in patients with acute STEMI. In addition, they may provide a means to prognosticate outcomes after STEMI and to evaluate the mechanisms underlying responses to myocardial injury.

CD31 (platelet endothelial cell adhesion molecule-1) is a cell-surface protein present on hematopoietic progenitor cells, myelomonocytic cells, and differentiated endothelial cells. It is known to regulate leukocyte adhesion and migration. In patients with STEMI, CD31 is highly expressed in the culprit plaque. Recently, it was demonstrated in both mice and humans that the CD31+CD45+ phenotype identifies a population of highly angiogenic and vasculogenic cells that express cell markers associated with hematopoietic stem/progenitor cells. In the same study, gene set enrichment analysis showed that the expression levels of proangiogenic genes are higher in bone marrow-derived CD31+CD45+ cells than in CD31− cells. It has also been reported that CD31+ cell therapy for myocardial infarction leads to efficient repair of ischemia in preclinical models. This finding in animals supports our current clinical study finding that a higher percentage of CD31+ cells in the BMC product was associated with a decrease in infarct size at 6 months after STEMI, possibly because of improved vascularization of the infarcted zone.

In our study, BMC function was assessed to determine whether ex vivo-derived metrics correlate with in vivo potential. BMC function was assessed with the CFU-Hill, ECFC, and CFU-F assays. Our results showed that the exponential constant for the CFU-Hill colony growth curve was associated with a reduction in infarct size after STEMI. These findings support those from previous studies showing that patients with a higher number of colonies cultured by the CFU-Hill assay have lower long-term cardiovascular risk. Our study also showed that rapid outgrowth of endothelial progenitor colonies, as measured by the ECFC assay, was associated with a greater reduction in infarct size at 6 months. In agreement with our findings, previous studies have demonstrated that ECFC cells are mobilized after myocardial infarction and that a higher ECFC frequency is associated with a reduction in infarct size after STEMI.

Interestingly, for both the ECFC and the CFU-Hill assays, we found that the exponential constant of the colony growth curve (determined by recording the colony number on days 7, 14, 21, and 28), but not the maximum number of colonies, was associated with a larger reduction in infarct size after STEMI, suggesting that the capacity for rapid cell growth is important for reducing infarct size.

Although CFU-Hill colonies were initially thought to be composed of endothelial progenitor cells, recent studies have shown that these colonies actually comprise a mixture of lymphocytes, monocytes, macrophages, and various subpopulations of endothelial cells. This agrees with our findings that patients whose BMCs produced higher numbers of CFU-Hill colonies also had a higher percentage of CD45+CD31+ cells (myelomonocytic cells, macrophages) in the bone marrow. Furthermore, the fact that we found these cell phenotypes and functions to be associated with a larger reductions in infarct size at 6 months is consistent with emerging data showing that monocytes play a key role in cardiac angiogenesis and collateral vessel formation, thereby contributing to cardiac repair after myocardial infarction. In fact, when a myocardial injury occurs, monocytes/macrophages show a biphasic response. Shortly after an injury, monocytes and type-1 (inflammatory) macrophages are recruited to the site via monocyte chemotactrant protein-1. However, within several days, type-2 (reparative) macrophages arrive to participate in injury resolution and contribute to cardiac angiogenesis and collateral vessel formation.

The CFU-F assay is a functional assessment of bone marrow-derived multipotent mesenchymal stromal cells, which are fibroblast-like cells capable of differentiating into bone, cartilage, adipose tissue, and fibrous tissue. There is increasing evidence that these cells may have a significant role in the response to injury in patients with ischemic cardiac disease. In this study, none of the metrics measured with the CFU-F assay were found to be significant (ie, \( P < 0.05 \)). However, this assay does show promise given that the results only narrowly missed our definition of statistical significance (unadjusted \( P = 0.052 \); adjusted \( P = 0.059 \)).
Therefore, we recommend that future studies evaluate the role of this assay in assessing response to myocardial injury.

Although BMC therapy was not shown to have a therapeutic effect in the original CCTRN TIME trial, there is still much information that can be gained by analyzing the BMC product from these patients with STEMI. First, our findings may provide new insight about the cellular subsets responsible for repair in patients with cardiovascular disease. Furthermore, our findings suggest that bone marrow composition may be a predictor of clinical outcomes. For the patients in this study, transplantation of autologous BMCs into the heart did not affect the clinical outcomes assessed, whereas the endogenous BMC characteristics at baseline were found to be associated with a reduction in infarct size. Both BMCs and progenitor cells are known to home to ischemic myocardium after an injury. Given that endogenous mechanisms for progenitor cell recruitment already exist, it may be more important to promote favorable bone marrow activity than to artificially translocate regenerative cells into the injured tissue. Therefore, our study may suggest important therapeutic targets for cardiovascular regenerative therapies.

When interpreting the results of this study, several factors should be taken into consideration. First, this study was limited to analyses of BMCs obtained at a single time point after myocardial infarction. However, information from 1 point in time is not likely to completely capture the dynamic nature of the injury response. Second, although the injury response may include BMCs, cells in the peripheral circulation, and local inflammatory responses, we only assessed BMC characteristics in this study. Despite this limitation, we were able to identify multiple associations with the outcome of interest. Third, although the analyses performed in this study were prespecified, this was a biologically guided exploratory study. In an attempt to balance this and focus on the characteristics with the potential to influence the future direction of cell therapy, we limited our discussion to the associations for which we limited our interaction to the statistical model to limit its potential for being a confounding factor. Fifth, in patients with an acute infarction, quantifying infarct size by using cMRI can be problematic because these measurements may include areas of myocardial necrosis, as well as areas of edema and inflammation. Therefore, changes in infarct size may reflect changes in inflammation in addition to changes in the pattern of necrosis. Characterizing patients on the basis of infarct size could generate a regression to the mean scenario in which patients with a small infarct size at baseline are unlikely to show much change, whereas those with a large infarct size at baseline may show a smaller infarct size at the 6-month follow-up. To help determine whether the observed changes in infarct size were in fact related to changes in myocardial necrosis, we explored correlations between the infarct size calculated by using cMRI and both creatine kinase-MB level and ejection fraction, 2 variables known to be associated with infarct size. In this analysis, the correlation between creatine kinase-MB level and day 3 infarct size was relatively weak ($R^2=0.35$), whereas the correlation between creatine kinase-MB level and day 7 infarct size was much stronger ($R^2=0.66$). The correlations between day 3 infarct size and day 3 ejection fraction ($R^2=0.27$) and day 7 ejection fraction ($R^2=0.45$) were both low to moderate. Overall, the correlations between the cMRI measurement of infarct size and the surrogate markers were moderate. Finally, because this was an exploratory study, any relationships found to be significant will have to be reassessed in a confirmatory study.

Conclusions

To our knowledge, this is the first comprehensive report of the correlation between BMC phenotype and functional assay results and infarct size in patients with STEMI. This type of analysis may be useful for generating hypotheses on the role of cellular subsets in cardiovascular repair. Furthermore, although the response to cell therapy with unselected BMCs was limited in the CCTRN TIME trial, this study may provide a means to significantly improve cell product composition, optimize patient selection for clinical trials, and personalize medical therapies for patients with heart disease.

Acknowledgments

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Disclosures

None.

References


**Novelty and Significance**

What Is Known?

- In cardiovascular cell therapy, harvested bone marrow mononuclear cells (BMCs) are isolated and reinfused into the heart by using intracoronary infusion, with the goal of repairing injured myocardium after segment-elevation–myocardial infarction (STEMI).
- Clinical trials of intracoronary autologous BMC transplantation in patients with STEMI have shown varied results.

What New Information Does This Article Contribute?

- Specific populations of BMCs were found to be associated with reduced infarct size after STEMI.
- Patients who had BMCs that showed faster growth of endothelial precursor cells in in vitro assays had larger reductions in infarct size after STEMI.
- Transplantation of autologous BMCs into the heart did not affect clinical outcomes; rather, endogenous BMC characteristics at baseline were found to be associated with reduced infarct size.

Because STEMI is a significant cause of cardiovascular morbidity and mortality, an important area of ongoing investigation is the identification of specific BMC subsets that are involved in myocardial repair. In this study, we evaluated the baseline BMC characteristics of participants in the Cardiovascular Cell Therapy Research Network Timing in Myocardial Infarction Evaluation trial, a randomized, controlled trial assessing the use of autologous BMC transplantation in patients with STEMI. Although infusion of autologous BMCs into the heart did not affect clinical outcomes, some endogenous BMC characteristics at baseline were found to be associated with a reduction in infarct size at 6 months after STEMI. Specifically, this study showed that patients with bone marrow containing a higher percentage of CD45+CD31low cells had larger reductions in infarct size. Additionally, patients with bone marrow containing cells that grew more rapidly in the CFU-Hill and the endothelial-colony forming cell assays had larger reductions in infarct size. Thus, it may be more beneficial for patients to have favorable bone marrow activity than to artificially translocate regenerative cells into the injured myocardial tissue. These findings suggest that optimizing patient selection and improving cell product composition are important directions for future investigation.
Bone Marrow Characteristics Associated With Changes in Infarct Size After STEMI: A Biorepository Evaluation From the CCTRN TIME Trial

for the Cardiovascular Cell Therapy Research Network (CCTRN)

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Supplemental Material

Online Table I. Staining Reagents Used for the Phenotype Analysis

<table>
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<th>Clone</th>
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*KDR refers to kinase insert domain-containing receptor, also known VEGFR-2 (vascular endothelial growth factor receptor-2) and CD309.

†CXCR4 refers to C-X-C chemokine receptor type 4, also known as fusin and CD184.
# Online Table II. Flow Cytometer Set-up

<table>
<thead>
<tr>
<th>Detector array (laser)</th>
<th>PMT (detector)</th>
<th>Long pass dichroic mirror</th>
<th>Band pass filter</th>
<th>Fluorochrome</th>
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<tbody>
<tr>
<td>Octagon (488-nm blue laser)</td>
<td>A</td>
<td>735 LP</td>
<td>780/60</td>
<td>PE-Cy7</td>
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<tr>
<td></td>
<td>B</td>
<td>685 LP</td>
<td>695/40</td>
<td>PerCP-Cy5.5</td>
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<td>Trigon (633-nm red laser)</td>
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<td>780/60</td>
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<td>B</td>
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<td>660/20</td>
<td>APC</td>
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<td>440/40</td>
<td>Pacific Blue</td>
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PMT, photo multiplier tube. LP, longpass filter, nm nanometer
Online Table III. Compensation Tubes Used for Creating a Compensation Matrix

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<tr>
<th>Laser (PMT-Detector)</th>
<th>488-nm D</th>
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Online Table IV. Demographics and Clinical Data of Patients Stratified by Change in Infarct Size at 6 Months and Treatment Received (BMCs or Placebo)

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<th>Received placebo</th>
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<th>P value</th>
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<tr>
<td></td>
<td>Infarct size decreased (n=51)</td>
<td>Infarct size increased (n=15)</td>
<td>Infarct size decreased (n=24)</td>
<td>Infarct size increased (n=11)</td>
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<tr>
<td>Age</td>
<td>57.5 ± 11.2</td>
<td>53.0 ± 8.2</td>
<td>56.0 ± 10.1</td>
<td>58.7 ± 13.1</td>
<td>0.462</td>
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<tr>
<td>Female</td>
<td>8 (15.7%)</td>
<td>2 (13.3%)</td>
<td>4 (16.7%)</td>
<td>1 (9.1%)</td>
<td>0.938</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>1.77 ± 0.10</td>
<td>1.76 ± 0.10</td>
<td>1.75 ± 0.11</td>
<td>173 ± 0.06</td>
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<tr>
<td>Weight (kg)</td>
<td>94.5 ± 19.8</td>
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<td>95.7 ± 19.2</td>
<td>87.1 ± 22.2</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 5.2</td>
<td>31.5 ± 6.9</td>
<td>31.0 ± 5.3</td>
<td>28.5 ± 6.1</td>
<td>0.547</td>
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<tr>
<td>Prior medical history</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (19.6%)</td>
<td>3 (20%)</td>
<td>4 (16.7%)</td>
<td>4 (36.4%)</td>
<td>0.589</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28 (54.9%)</td>
<td>6 (40%)</td>
<td>18 (75%)</td>
<td>7 (63.6%)</td>
<td>0.158</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>36 (70.6%)</td>
<td>9 (60%)</td>
<td>17 (70.8%)</td>
<td>7 (63.6%)</td>
<td>0.853</td>
</tr>
<tr>
<td>History of angina</td>
<td>11 (21.6%)</td>
<td>2 (13.3%)</td>
<td>5 (20.8%)</td>
<td>1 (9.1%)</td>
<td>0.730</td>
</tr>
<tr>
<td>Smoking</td>
<td>30 (58.8%)</td>
<td>10 (66.7%)</td>
<td>14 (58.3%)</td>
<td>8 (72.7%)</td>
<td>0.799</td>
</tr>
<tr>
<td>Cardiovascular and infarction details</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline infarct size (grams)</td>
<td>47.4 ± 24.5</td>
<td>36.9 ± 26.4</td>
<td>43.6 ± 26.9</td>
<td>33.3 ± 17.3</td>
<td>0.251</td>
</tr>
<tr>
<td>Heart rate</td>
<td>79.9 ± 14.2</td>
<td>82.6 ± 10.2</td>
<td>80.5 ± 18.8</td>
<td>81.7 ± 12.9</td>
<td>0.929</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119.3 ± 20.8</td>
<td>112.3 ± 18.4</td>
<td>121.0 ± 17.4</td>
<td>110.0 ± 14.7</td>
<td>0.268</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73.3 ± 15.5</td>
<td>75.6 ± 13.6</td>
<td>74.4 ± 11.2</td>
<td>69.9 ± 13.4</td>
<td>0.763</td>
</tr>
<tr>
<td>Screening EF (echocardiography)</td>
<td>36.2 ± 5.9</td>
<td>36.0 ± 8.1</td>
<td>38.3 ± 4.3</td>
<td>36.8 ± 7.8</td>
<td>0.564</td>
</tr>
<tr>
<td>Core lab EF (day 3) (cMRI)</td>
<td>45.4 ± 9.9</td>
<td>44.9 ± 13.9</td>
<td>46.8 ± 9.3</td>
<td>38.7 ± 8.5</td>
<td>0.190</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.3 ± 1.8</td>
<td>13.6 ± 1.1</td>
<td>13.4 ± 2.2</td>
<td>13.1 ± 0.9</td>
<td>0.089</td>
</tr>
<tr>
<td>High sensitivity CRP (mg/L)</td>
<td>41.0 ± 54.8</td>
<td>26.5 ± 21.7</td>
<td>37.5 ± 35.8</td>
<td>46.0 ± 23.7</td>
<td>0.679</td>
</tr>
<tr>
<td>Peak CK (U/L)</td>
<td>3215.2 ± 2029.9</td>
<td>3593.6 ± 3006.1</td>
<td>2493.2 ± 2213.8</td>
<td>2600.9 ± 2326.3</td>
<td>0.442</td>
</tr>
<tr>
<td>Peak CKMB (ng/mL)</td>
<td>274.3 ± 174.8</td>
<td>262.6 ± 253.7</td>
<td>213.4 ± 216.6</td>
<td>245.8 ± 168.2</td>
<td>0.731</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>335.2 ± 779.7</td>
<td>191.0 ± 89.2</td>
<td>308.9 ± 341.9</td>
<td>247.0 ± 178</td>
<td>0.897</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>1691.4 ± 1414.8</td>
<td>550.3 ± 281.6</td>
<td>375.5 ± 207.2</td>
<td>2627.7 ± 2591</td>
<td>0.239</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
<td>p-value</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>----------</td>
</tr>
<tr>
<td>Troponin T (peak) (ng/mL)</td>
<td>10.9 ± 8.3</td>
<td>8.5 ± 7</td>
<td>6.9 ± 5.9</td>
<td>10.8 ± 7.6</td>
<td>0.349</td>
</tr>
<tr>
<td>Troponin I (peak) (ng/mL)</td>
<td>76.3 ± 96.2</td>
<td>214.1 ± 270.7</td>
<td>74.3 ± 77.2</td>
<td>54.9 ± 41.9</td>
<td>0.417</td>
</tr>
<tr>
<td>Drug eluting stent</td>
<td>42 (82.4%)</td>
<td>10 (66.7%)</td>
<td>21 (87.5%)</td>
<td>8 (72.7%)</td>
<td>0.380</td>
</tr>
<tr>
<td>LAD infarction</td>
<td>45 (88.2%)</td>
<td>12 (80%)</td>
<td>22 (91.7%)</td>
<td>11 (100%)</td>
<td>0.418</td>
</tr>
<tr>
<td>Preinfarction angina</td>
<td>10 (19.6%)</td>
<td>1 (6.7%)</td>
<td>4 (16.7%)</td>
<td>2 (18.2%)</td>
<td>0.704</td>
</tr>
<tr>
<td>Transferred after MI</td>
<td>20 (39.2%)</td>
<td>6 (40%)</td>
<td>13 (54.2%)</td>
<td>5 (45.5%)</td>
<td>0.663</td>
</tr>
<tr>
<td>PCI at non-study center hospital</td>
<td>6 (11.8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (18.2%)</td>
<td>0.112</td>
</tr>
<tr>
<td>Discharge medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>44 (86.3%)</td>
<td>14 (93.3%)</td>
<td>18 (75%)</td>
<td>9 (81.8%)</td>
<td>0.444</td>
</tr>
<tr>
<td>Aspirin</td>
<td>48 (94.1%)</td>
<td>15 (100%)</td>
<td>24 (100%)</td>
<td>11 (100%)</td>
<td>0.386</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>49 (96.1%)</td>
<td>15 (100%)</td>
<td>24 (100%)</td>
<td>11 (100%)</td>
<td>0.572</td>
</tr>
<tr>
<td>Clopidogrel or prasugrel</td>
<td>49 (96.1%)</td>
<td>13 (86.7%)</td>
<td>24 (100%)</td>
<td>10 (90.9%)</td>
<td>0.260</td>
</tr>
<tr>
<td>Statins</td>
<td>47 (92.2%)</td>
<td>13 (86.7%)</td>
<td>24 (100%)</td>
<td>9 (81.8%)</td>
<td>0.235</td>
</tr>
<tr>
<td>Diuretic</td>
<td>14 (27.5%)</td>
<td>1 (6.7%)</td>
<td>2 (8.3%)</td>
<td>4 (36.4%)</td>
<td>0.069</td>
</tr>
<tr>
<td>Coumadin or enoxaparin</td>
<td>12 (23.5%)</td>
<td>1 (6.7%)</td>
<td>3 (12.5%)</td>
<td>1 (9.1%)</td>
<td>0.316</td>
</tr>
<tr>
<td>Cell product information</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from PCI to infusion (days)</td>
<td>5.1 ± 2.2</td>
<td>5.0 ± 2.4</td>
<td>5.3 ± 2.5</td>
<td>3.9 ± 1.3</td>
<td>0.399</td>
</tr>
<tr>
<td>Time from aspiration to infusion (hours)</td>
<td>8.9 ± 3.5</td>
<td>8.6 ± 1.6</td>
<td>8.4 ± 1.1</td>
<td>8.6 ± 1.4</td>
<td>0.887</td>
</tr>
<tr>
<td>Final volume (mL)</td>
<td>150.0 ± 0</td>
<td>140.3 ± 37.7</td>
<td>149.0 ± 3.3</td>
<td>149.5 ± 1.8</td>
<td>0.146</td>
</tr>
<tr>
<td>Cell product viability (%)</td>
<td>98.1 ± 1.7</td>
<td>98.4 ± 1.2</td>
<td>98.2 ± 1.5</td>
<td>98.6 ± 0.8</td>
<td>0.710</td>
</tr>
</tbody>
</table>

Values represent mean ± SD or number (%). BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; CK, creatine kinase; CKMB, creatine kinase-MB; CRP, C-reactive protein; EF, ejection fraction; LAD, left anterior descending; LV, left ventricular; MI, myocardial infarction; PCI, percutaneous coronary intervention.
Online Table V. BMC Phenotype and Functional Characteristics of Patients Stratified by Change in Infarct Size and Treatment Received (BMCs or Placebo)

<table>
<thead>
<tr>
<th></th>
<th>Received BMCs</th>
<th>Received placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infarct size decreased (n=51)</td>
<td>Infarct size increased (n=15)</td>
<td></td>
</tr>
<tr>
<td>Cell phenotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3⁺</td>
<td>64.7 (56.9-74.6)</td>
<td>70.4 (65.9-74.5)</td>
<td>0.149</td>
</tr>
<tr>
<td>CD11b⁺</td>
<td>72.5 (57.5-80.3)</td>
<td>72.4 (65.1-76.4)</td>
<td>0.978</td>
</tr>
<tr>
<td>CD14⁺ (% monocytes)</td>
<td>56.7 (44.8-69.3)</td>
<td>61.0 (55.9-67.8)</td>
<td>0.573</td>
</tr>
<tr>
<td>CD19⁺</td>
<td>9.3 (6.0-12.3)</td>
<td>7.9 (6.5-11.2)</td>
<td>0.038</td>
</tr>
<tr>
<td>CD133⁺</td>
<td>2.6 (1.8-4.4)</td>
<td>2.5 (1.5-3.4)</td>
<td>0.415</td>
</tr>
<tr>
<td>CD31⁺</td>
<td>40.3 (34.6-44.7)</td>
<td>39.4 (29.0-42.2)</td>
<td>0.592</td>
</tr>
<tr>
<td>CD34⁺</td>
<td>4.7 (2.7-7.2)</td>
<td>4.3 (3.0-5.4)</td>
<td>0.332</td>
</tr>
<tr>
<td>CD34⁺ (ISHAGE)</td>
<td>1.8 (1.2-2.8)</td>
<td>1.8 (1.4-2.2)</td>
<td>0.561</td>
</tr>
<tr>
<td>CD34⁺CD133⁺ (ISHAGE)</td>
<td>0.9 (0.5-1.5)</td>
<td>0.7 (0.7-1.2)</td>
<td>0.252</td>
</tr>
<tr>
<td>CD45⁺</td>
<td>94.4 (91.1-96.8)</td>
<td>93.9 (90.5-96.3)</td>
<td>0.784</td>
</tr>
<tr>
<td>CD45⁺CD31⁺</td>
<td>39.0 (33.4-43.4)</td>
<td>38.0 (28.0-41.0)</td>
<td>0.625</td>
</tr>
<tr>
<td>CD45⁺CD31low</td>
<td>30.6 (25.5-34.8)</td>
<td>28.4 (20.0-37.1)</td>
<td>0.541</td>
</tr>
<tr>
<td>Functional analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFU-Hill (exponential constant of colony #)</td>
<td>0.4 (0.0-0.7)</td>
<td>0.2 (0.0-0.3)</td>
<td>0.3 (0.0-0.6)</td>
</tr>
<tr>
<td>CFU-Hill (maximum colony #)</td>
<td>4.0 (0.0-11.0)</td>
<td>9.0 (4.0-17.0)</td>
<td>6.0 (0.0-14.0)</td>
</tr>
<tr>
<td>CFU-Hill (linear slope of confluency)</td>
<td>12.2 (8.8-14.9)</td>
<td>11.0 (3.6-16.4)</td>
<td>19.3 (16.1-24.8)</td>
</tr>
<tr>
<td>ECFC (exponential constant of colony #)</td>
<td>0.2 (0.0-1.6)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.2)</td>
</tr>
<tr>
<td>ECFC (maximum colony #)</td>
<td>3.5 (0.0-15.0)</td>
<td>0.0 (0.0-22.0)</td>
<td>0.0 (0.0-4.0)</td>
</tr>
<tr>
<td>ECFC (linear slope of confluency)</td>
<td>2.9 (2.3-4.6)</td>
<td>3.3 (1.8-7.1)</td>
<td>0.7 (0.0-3.8)</td>
</tr>
<tr>
<td>CFU-F (exponential constant of colony #)</td>
<td>1.4 (0.1-1.6)</td>
<td>0.1 (0.0-1.6)</td>
<td>0.1 (0.0-1.6)</td>
</tr>
<tr>
<td>CFU-F (maximum colony #)</td>
<td>4.0 (2.0-8.0)</td>
<td>6.0 (0.0-17.0)</td>
<td>2.0 (0.0-6.0)</td>
</tr>
<tr>
<td>CFU-F (linear slope of confluency)</td>
<td>2.5 (2.0-3.4)</td>
<td>3.1 (2.8-3.5)</td>
<td>2.0 (1.0-4.6)</td>
</tr>
</tbody>
</table>

All FC analyses were performed on gated lymphocytes unless otherwise specified. Values represent median and interquartile range.
Online Figure I. Flow cytometer optical pathway configuration

A. Blue Laser

B. Red Laser

C. Violet Laser

D. UV Laser
Online Figure 2. Flow cytometry histograms of BMC populations and isotype controls. A, Gated lymphocytes were evaluated for the presence of CD45, CD31, CD133, CD34, KDR (CD309), CD11b, CD3, CXCR4 (CD184), and CD19. B, Gated monocytes were evaluated for the presence of CD14. Flow cytometric gates are outlined in black for each dot plot. Antigen-specific signals are shown in blue, and signals for the respective fluorescent isotype IgG are shown in red.

A. Bone marrow lymphocytes

B. Bone marrow monocytes