Intracoronary Injection of Bone Marrow–Derived Mononuclear Cells Early or Late After Acute Myocardial Infarction: Effects on Global Left Ventricular Function

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Intracoronary Injection of Bone Marrow–Derived Mononuclear Cells Early or Late After Acute Myocardial Infarction

Effects on Global Left Ventricular Function

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Background—Intracoronary administration of autologous bone marrow–derived mononuclear cells (BM-MNC) may improve remodeling of the left ventricle (LV) after acute myocardial infarction. The optimal time point of administration of BM-MNC is still uncertain and has rarely been addressed prospectively in randomized clinical trials.

Methods and Results—In a multicenter study, we randomized 200 patients with large, successfully reperfused ST-segment elevation myocardial infarction in a 1:1:1 pattern into an open-labeled control and 2 BM-MNC treatment groups. In the BM-MNC groups, cells were administered either early (ie, 5 to 7 days) or late (ie, 3 to 4 weeks) after acute myocardial infarction. Cardiac magnetic resonance imaging was performed at baseline and after 4 months. The primary end point was the change from baseline to 4 months in global LV ejection fraction between the 2 treatment groups and the control group. The absolute change in LV ejection fraction from baseline to 4 months was −0.4±8.8% (mean±SD; P=0.74 versus baseline) in the control group, 1.8±8.4% (P=0.12 versus baseline) in the early group, and 0.8±7.6% (P=0.45 versus baseline) in the late group. The treatment effect of BM-MNC as estimated by ANCOVA was 1.25 (95% confidence interval, −1.83 to 4.32; P=0.42) for the early therapy group and 0.55 (95% confidence interval, −2.61 to 3.71; P=0.73) for the late therapy group.

Conclusions—Among patients with ST-segment elevation myocardial infarction and LV dysfunction after successful reperfusion, intracoronary infusion of BM-MNC at either 5 to 7 days or 3 to 4 weeks after acute myocardial infarction did not improve LV function at 4-month follow-up.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00355186. (***Circulation.* 2013;127:1968-1979.**)

Key Words: bone marrow–derived progenitor cells ■ magnetic resonance imaging ■ myocardial infarction ■ regeneration ■ ventricular remodeling

Progenitor cell–based therapy with the use of autologous bone marrow as a source has been suggested to improve left ventricular (LV) function in patients with acute myocardial infarction (AMI) if administered after successful percutaneous coronary intervention. Indeed, several published studies using bone marrow–derived unselected mononuclear cells (BM-MNC) showed an improvement in global LV function[1–3] or in regional LV function.[4] Others, however, could not confirm any beneficial effect of cell therapy on LV function.[5,6] Such controversial results could be attributable to different study designs, different cell isolation protocols potentially leading to differences in cell functionality, or the way that end points have been assessed. Furthermore, timing of cell administration may be an important factor influencing the treatment effect of progenitor cell–based therapy. Indeed, in most of the studies BM-MNC were administered within the first 7 days

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after AMI. Interestingly, in a prespecified subgroup analysis of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial, the beneficial effects of BM-MNC appeared to be more pronounced with later cell application (ie, 5–7 days).[2] The Cardiovascular Cell Therapy Network performed 2 trials investigating different time points of cell application: The recently published Transplantation in Myocardial Infarction Evaluation (TIME) trial[7] compared BM-MNC therapy at 3 days versus 7 days after AMI, whereas the LateTIME trial[8] tested BM-MNC application 2 to 3 weeks after AMI against placebo. In neither one of these trials, however, did BM-MNC therapy improve LV function. However, a direct comparison of the effects of early versus late BM-MNC application on LV function is still lacking. Thus, the Swiss Multicenter Intracoronary Stem Cells Study in Acute Myocardial Infarction (SWISS-AMI) was designed to prospectively investigate the optimal time of BM-MNC administration at 2 different time points: early or 5 to 7 days versus late or 3 to 4 weeks after AMI.

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Methods

Study Sample and Protocol

The study design with predefined inclusion and exclusion criteria has been described previously.[9] In brief, patients with acute ST-segment elevation myocardial infarction (STEMI) and successful percutaneous coronary intervention within 24 hours after symptom onset were eligible for enrollment into this multicenter randomized controlled trial provided that they presented with an estimated LV ejection fraction (LVEF) of <45% as assessed by an LV angiogram or transthoracic echocardiography the day of or after the AMI. After giving their informed consent to participate in the study, patients were randomly assigned in a 1:1:1 fashion to 1 open labeled control and 2 BM-MNC treatment groups. The control group received best medical management according to current guidelines,[10] including aspirin and clopidgrel or prasugrel, statins, β-blockers, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, as well as aldosterone antagonists, if indicated. The early BM-MNC treatment group received cells at 5 to 7 days and the late BM-MNC group at 3 to 4 weeks after primary percutaneous coronary intervention, on top of best medical management. All patients underwent cardiac magnetic resonance imaging (CMR) at baseline and at 4 months after AMI. The primary hypothesis was that the change in LVEF at 4 months compared with baseline would be more pronounced in both treatment groups compared with control patients. A total of 4 Swiss tertiary centers (University Hospital Zurich, Bern University Hospital, Cantonal Hospital Lucerne, and Fondazione Cardiocentro Ticino) participated in this trial. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the regional ethical committee of each participating center as well as by the federal authorities (Swissmedic and Federal Office of Public Health).

Bone Marrow Aspiration and Cell Processing

Bone marrow aspiration was performed under sterile conditions in patients randomly assigned to the BM-MNC treatment arms with negative serological testing for hepatitis B, hepatitis C, and HIV either 5 to 7 days or 3 to 4 weeks after AMI. The cell processing was done in a centralized, good manufacturing practice–certified facility (Cell Therapy Unit, Cardiocentro Ticino, Lugano, Switzerland). Between 60 and 80 mL of bone marrow was collected from the iliac crest under local anesthesia. Then 1 mL of a solution containing 1000 IU heparin was added to each 10 mL of bone marrow aspirate to prevent clotting. Then the aspirate and 20 mL of the patient's serum were sent at room temperature by courier to the cell-processing center. The BM-MNC cell suspension was shipped back to the participating hospital within 24 hours. Shipping protocols as well as the isolation of the mononuclear cell fraction were performed according to a standard protocol according to previous studies[2,11] with minor modifications as described.[9] Briefly, with the use of density gradient centrifugation, the mononuclear cell fraction was resuspended in 10 mL of serum-free medium with 20% of autologous serum added without any additional heparin. An aliquot of cell suspension was utilized for fluorescence-activated cell sorting analysis with the use of fluorochrome-conjugated antibodies against anti-human CD34 and CD133; cell viability was assessed by 7-AAD cell uptake, and sterility was assessed by the Bact/Alert rapid method. Release criteria of the BM-MNC were product sterility, a cell count between 5×10^7 and 5×10^8, and cell viability of ≥80%. Migration capacity of BM-MNC was measured in a modified Boyden chamber as described previously.[12] This parameter was expressed as percentage of mononuclear cells able to actively cross a membrane coated with extracellular matrix proteins and, with the use of an invasion index, indicating the ratio between the latter and the percentage of cells passively crossing the same membrane when uncoated.

Intracoronary Infusion of BM-MNC

After arterial access was obtained (either via the common femoral artery or the radial artery), patients received 5000 IU of heparin intravenously. Then an over-the-wire balloon catheter was advanced via a guiding catheter in the segment of the former infarct-related vessel containing the stent. After inflation of the balloon with low pressure (2–4 bar) within the stented segment to obtain total occlusion of the vessel, BM-MNC were infused within 3 minutes to allow for adhesion and transmigration of the infused cells through the endothelium (stop-flow technique). This maneuver was repeated 3 times to allow for infusion of a total of 9 mL of progenitor cell suspension, interrupted by 5 minutes of reflow by deflating the balloon to minimize extensive ischemia and pain. Finally, coronary angiography was repeated to ascertain vessel patency.

Periprocedural safety of the BM-MNC infusion was monitored by assessment of serum cardiac enzymes including cardiac troponin the day after the intervention. Periprocedural myocardial infarction was defined as described previously.[13]

Cardiac Magnetic Resonance Imaging

Cardiac imaging was performed with the use of 1.5-T clinical MR systems. Dedicated cardiac phased-array receiver coils were used for signal reception. Patients underwent CMR studies at baseline (ie, during hospitalization for the AMI) and at 4 months of follow-up. After localizer acquisitions, the CMR studies assessed functional imaging of the LV by means of standard ECG-triggered steady state free precession acquisitions during repetitive breath-holds in 3 long-axis orientations and in contiguous short-axis orientations covering the entire LV. In the second part of the CMR examination, scar imaging was performed after administration of a bolus of a conventional extracellular gadolinium chelates contrast medium at a dose of 0.20 mmol/kg body wt by using an inversion-recovery fast gradient echo imaging sequence.[14,15] After determination of the inversion time nulling for normal myocardium, scar imaging was performed 20 minutes after administration of contrast medium in identical locations as functional data were acquired.

CMR data analysis was performed in a core laboratory (University Hospital Zurich) with the use of dedicated cardiac analysis software (GTVolume, Gyrotools Ltd, Zurich, Switzerland). LV end-diastolic and end-systolic volumes, LVEF, and LV mass were quantified for assessment of the primary end point (change of LVEF) and for assessment of ventricular remodeling over time in the 3 study groups.

Regional wall motion was assessed by measuring systolic and diastolic wall thickness in each of the 6 segments of all acquired slices, and thickening of each segment was calculated by subtracting systolic and diastolic values. A global thickening index was determined that took into account the median of all values of each segment. The same index was determined in the infarct territory, taking into account only the infarct-related segments.

The extent of microvascular obstruction, delineated as dark areas in the core of the necrotic zone in the late enhancement images, was quantified by manually contouring the dark core areas. Scar mass and tissue with microvascular obstruction were assessed in grams and milliliters (data not shown) and as a percentage of LV mass and of scar mass, respectively.

Changes over time (ie, 4 months versus baseline) of volumetric, functional, and scar parameters were compared between the groups to assess the influence of treatment on global and regional systolic function, LV remodeling, and scar mass.

End Points

The primary end point was the absolute change in global LVEF from baseline compared with 4-month follow-up between the control group and both BM-MNC therapy groups, respectively.

Secondary end points comprised the change in global LVEF from baseline to 4 months between control and a combined therapy group, as well as changes in LV end-diastolic and end-systolic volume, changes in infarct size, changes in the proportion of scar mass to total LV mass, and changes in global and regional myocardial thickening. To identify predictive markers, we analyzed treatment-marker interactions of the primary end point with infarct size, microvascular obstruction, baseline N-terminal pro-brain natriuretic peptide, and time from onset of chest pain to successful reperfusion therapy of the AMI. Prespecified clinical end points were analyzed including major adverse events (defined as all-cause death, recurrence of myocardial infarction, any coronary revascularization procedure, or rehospitalization for heart failure; all at 4 months) and time from STEMI to the occurrence of such events at 4 months. Only the first event for each patient was included in the analysis.

Statistical Analysis

In general, median and interquartile range (IQR) are presented for continuous variables. If criteria for normal distribution were fulfilled, variables are presented as mean and SD. Nominal variables are summarized in terms of frequencies and percentages.

For analysis of the primary end point, we performed ANCOVA, including LVEF values at 4 months as dependent variables and the associated baseline values and the factor treatment as independent variables. Estimates of the treatment effect are presented together with the 95% confidence interval (CI). Because comparisons of both experimental groups with control were considered the primary end point, *P* values were compared with the Bonferroni-corrected significance level α=0.025.

The same analysis as for the primary end point was repeated for the secondary end points (only ANCOVA analyses are shown). For analyses of the primary and secondary end points, only matched CMR data (baseline and 4 months) were used.

As an additional secondary end point, because we sought to find easily interpretable predictive markers for treatment success, prespecified continuous baseline markers were split at the median (in 1 case at a manually chosen cutoff). With this new variable, the *P* value for the interaction was then computed in an ANCOVA model with LVEF at 4 months as dependent variable and the associated baseline values, the factor treatment, the marker under consideration discretized at cutoff, and treatment-marker interaction as independent variables.

To compute regressions for LVEF at 4-month follow-up, further ANCOVA analysis involving adjustment for baseline LVEF was performed. A total of 21 binary, 7 nominal, and 40 continuous explanatory variables were analyzed separately.

Binary end points were compared between groups with the use of the χ^2 or Fisher exact test, depending on whether or not expected cell frequencies were <5. For continuous outcome independent samples, the *t* or Wilcoxon test was used.

Major adverse cardiac events were defined as the occurrence of 1 of the predefined clinical scenarios (death, myocardial infarction, coronary revascularization, or rehospitalization for heart failure) and were compared between groups at 4 months.

For the secondary end points, multiple comparisons were performed, and the analysis of the results shall be designated exploratory.

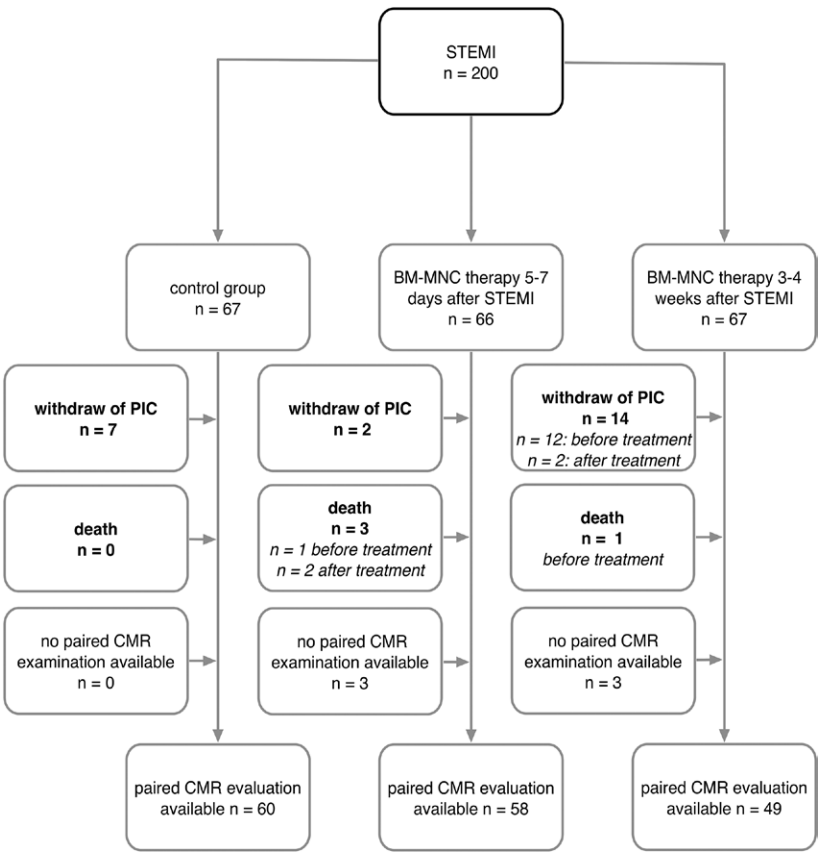

Figure 1. Flow diagram of patient enrollment until 4-month follow-up. BM-MNC indicates bone marrow–derived mononuclear cells; CMR, cardiac magnetic resonance imaging; PIC, patient informed consent; and STEMI, ST-segment elevation myocardial infarction.

Table 1. Baseline Characteristics of the Included Patients

	Control (n=67)	Early (n=65)	Late (n=63)	*P* Value
Age, median (IQR), y	56 (14.5)	55 (15)	62 (15)	0.70* 0.06†
BMI, median (IQR), kg/m^2	26.7 (4.4)	27.0 (6.1)	27.0 (4.4)	0.92* 0.89†
Male sex, %	83.6	86.2	82.5	0.18* 1.00†
Hypertension, %	43.3	49.2	38.7	0.60* 0.72†
Hyperlipidemia, %	44.8	40.0	41.9	0.60* 0.86†
Diabetes mellitus, %	17.9	7.7	9.7	0.12* 0.21†
Smoking (active/previous), %	62.7	67.7	40.3	0.60* 0.01†
Family history of CAD, %	35.8	26.1	24.2	0.26* 0.18†
1-/2 -/3-vessel disease, %	64/21/15	54/32/14	57/27/16	0.34* 0.73†
Previous PCI before AMI, %	3.0	3.1	1.6	1.00* 1.00†
Infarct treatment				
Primary PCI, %	94.0	98.5	100.0	0.37* 0.12†
Concomitant PCI other than infarct-related artery, %	18.2	12.3	11.1	0.47* 0.32†
Infarct vessel LAD/LCX/RCA, %	89/3/8	95/2/3	92/3/5	0.51* 0.89†
Pain to revascularization time, h	4.5 (5)	4.8 (5.4)	4.0 (4.8)	0.57* 0.53†
Stent diameter, mm	3.5 (0.5)	3.0 (0.5)	3.5 (0.5)	0.73* 0.89†
Drug-eluting stent, %	71.6	80.0	81.0	0.31* 0.23†
TIMI flow before PCI	0 (0)	0 (0)	0 (0)	0.31* 0.87†
TIMI flow after PCI	3 (0)	3 (0)	3 (0)	0.94* 0.81†
Use of glycoprotein IIb/IIIa inhibitors/bivalirudin, %	71.7	78.5	78.1	0.88* 0.20†
Maximal creatine kinase, median (IQR), U/L	3671 (3685)	4314 (3561)	3436 (3813)	0.22* 0.78†
NT-proBNP, median (IQR), ng/L	1103 (1848)	1450 (1442)	1581 (1912)	0.15* 0.10†
Intra-aortic balloon pump/other assist device, %	16.4	15.6	22.6	1.00* 0.18†
CMR characteristics of LV				
LVEF, median (IQR), %	39.6 (11.2)	34.6 (16.1)	35.6 (11.2)	0.07* 0.03†
LVEDV, median (IQR), mL	154 (44)	153 (49)	149 (47)	0.89* 0.96†
LVESV, median (IQR), mL	94 (35)	94 (41)	97 (38)	0.54* 0.41‡
Scar mass, median (IQR), g	39.1 (37.2)	37.7 (32.1)	33.9 (24.2)	0.94* 0.21†

(*Continued*)

Table 1. Continued

	Control (n=67)	Early (n=65)	Late (n=63)	*P* Value
Myocardial scar, median (IQR), %	28.3 (16.3)	28.1 (16.2)	26.6 (15.9)	0.78* 0.53†
Microvascular obstruction, median (IQR), g	0.27 (1.55)	1.08 (3.00)	0.64 (2.49)	0.11* 0.51†
Medication at discharge/after 4 mo, %				
Aspirin	98.5/98.4	98.4/98.4	98.3/98.2	1.00/1.00* 1.00/1.00†
Clopidogrel or prasugrel	100/100	100/100	100/98.2	1.00/1.00* 1.00/0.84†
ACE inhibitor or ATII receptor blocker	95.5/100	100/100	96.6/98.2	1.00/1.00* 1.00/0.84†
β-Blocker	86.4/85.2	91.9/88.5	93.2/92.6	0.40/0.79* 0.25/0.25†
Aldosterone antagonist	12.1/11.5	12.9/14.8	15.2/9.3	1.00/0.79* 0.79/0.77†
Statin	97.0/98.4	100/95.1	98.3/100	0.50/0.62* 1.00/1.00†

ACE indicates angiotensin-converting enzyme; AMI, acute myocardial infarction; ATII, angiotensin II; BMI, body mass index; CAD, coronary artery disease; IQR, interquartile range; LAD, left anterior descending coronary artery; LCX, left circumflex artery; LV, left ventricle; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume; NT-proBNP, N-terminal pro-brain natriuretic peptide; PCI, percutaneous coronary intervention; RCA, right coronary artery; and TIMI, Thrombolysis in Myocardial Infarction.

**P* value, control vs early group.

†*P* value, control vs late group.

P values and 95% CIs are therefore shown without further Bonferroni correction. All computations were done with R (R Development Core Team, 2012).

Sample Size/Power Calculation

The study has been powered for evaluation of the primary end point depending on the presence of paired CMR data, assessed either at baseline or at 4-month follow-up.

As described previously[9] and according to recent studies,[1,2,16] in planning sample size we assumed a difference between LVEF improvements from baseline to 4 months of δ=3.5% with SD of 6% between control and both treatment groups. A sample size of n=58 per group was therefore needed to detect such difference with a power of 80% for a Bonferroni-corrected significance level of α=0.025. Taking into account a dropout rate of 10%, we planned to include n=64 per arm and n=192 for the entire trial. All computations were done with R (R Development Core Team, 2012).

Table 2. Characteristics of BM-MNC and Cell Treatment

	Early (n=62)	Late (n=52)	*P* Value (Between-Group Difference)
Cell characteristics, median (IQR)			
BM aspiration volume, mL	65 (15)	70 (15)	0.30
Total nucleated cells, 10^6 cells	159.7 (125.8)	139.5 (120.5)	0.18
Viability, %	93.6 (5.55)	93.33 (6.60)	0.98
% $CD34^+$ cells	1.02 (0.72)	1.31 (0.97)	0.01*
Total $CD34^+$ cells, 10^6 cells	1.6 (1.69)	1.45 (2.43)	0.68
% $CD34^+/133^+$ cells	0.81 (0.78)	0.87 (0.97)	0.34
Total $CD34^+/133^+$ cells, 10^6 cells	0.96 (1.46)	0.92 (2.06)	0.77
% Invasion	33 (18)†	26.5 (16.5)‡	0.18
Invasion index	50.88 (24.38)†	45.64 (22.10)‡	0.21
Timing of BM-MNC treatment			
Days after AMI	6 (2)	24 (7)	NA

AMI indicates acute myocardial infarction; BM, bone marrow; BM-MNC, bone marrow–derived mononuclear cells; IQR, interquartile range; and % invasion, percentage of total nucleated cells showing invasion capacity.

*Estimated Wilcoxon effect, −0.31; 95% confidence interval, −0.56 to −0.07.

†n=29.

‡n=30.

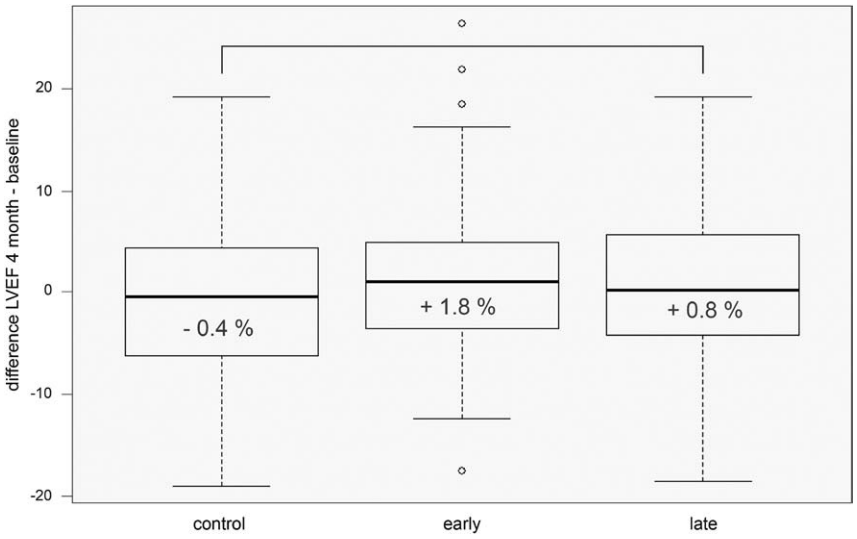

Figure 2. Descriptive statistic (box plot) of the mean of the differences between left ventricular ejection fraction (LVEF) at 4 months and LVEF at baseline for control vs early and control vs late bone marrow–derived mononuclear cell treatment.

Results

Patient Enrollment and Baseline Characteristics

Between October 2006 and January 2012, in the 4 Swiss cardiovascular centers, a total of 200 patients gave informed consent to participate in the trial. Of those, 66 patients were randomized to the early BM-MNC treatment group and 67 patients each to the control and late treatment groups, respectively. Because the dropout rate was higher than expected, the initially planned sample size had to be increased from 192 to 200 patients, thereby allowing us to attain at least 2 study arms with the necessary sample size. Dropouts were more common within the late treatment group. Patient flow from randomization until 4-month follow-up is shown in Figure 1. There was no statistically significant difference in baseline characteristics between the 3 groups apart from a higher age and a lower percentage of smokers in the late treatment group as well as a higher baseline LVEF in the control group (Table 1). Baseline CMR was performed at a median of 6 (IQR 4) days after the AMI.

Patients who withdrew informed consent or were unwilling to undergo repeat CMR examination; were generally older and had higher values of maximal creatine kinase at baseline, especially in the early treatment group but not in the late therapy group.

Characteristics of Index Myocardial Infarction

Overall, 92% of the patients had anterior STEMI attributable to left anterior descending coronary artery occlusion, and the median time from onset of chest pain to reperfusion therapy was 4.5 hours (IQR, 5.25). Seventy-six percent of the patients were treated with glycoprotein IIb/IIIa inhibitors or bivalirudin, and 78% received a drug-eluting stent with a median diameter of 3.5 mm (IQR, 0.5). The median of maximal creatine kinase plasma levels was 3919 U/L (IQR, 3664), and baseline LVEF, as assessed by CMR at a median of 6 (IQR, 4) days after the index AMI, was 37.4% (IQR, 13.2).

Characteristics of BM-MNC

A total of 153 (119) $\times 10^6$ nucleated cells were infused. Besides an impurity of granulocytes, the mononuclear fraction consisted mainly of lymphocytes, monocytes, and a small amount of functional hematopoietic, endothelial/angiogenic, and mesenchymal precursors (Table I in the online-only Data Supplement). Between 1% and 1.3% of the cells were $CD34^+$ cells. Complete cell processing data are shown in Table 2. There was no significant difference between the early and late therapy groups except for a higher percentage of $CD34^+$ cells in the late therapy group. For a subset of patients (n=59), we performed a functional test; the median (IQR) percentage of MNC exhibiting invasion capacity was overall 29% (19), and the overall invasion index was 49% (25), without any difference between early and late BM-MNC treatment groups.

Assessment of LV Function and Remodeling at Baseline and 4-Month Follow-Up

Primary End Point

The mean (SD) absolute change in LVEF at 4 months was −0.4% (8.8) in the control group, 1.8% (8.4) in the early group, and 0.8% (7.6) in the late group (Figure 2). With adjustment for baseline LVEF with an ANCOVA model, the estimated treatment effect averaged 1.25 (95% CI, −1.83 to 4.32; P=0.42 versus control) for the early therapy group and 0.55 (95% CI, −2.61 to 3.71; P=0.73 versus control) for the late therapy group (Table 3).

Secondary End Points

When we combined the early and late groups into a common BM-MNC therapy group (n=107), the estimated treatment effect was 0.85 (95% CI, −1.75 to 3.44; P=0.52 versus control).

Negative remodeling occurred in all 3 groups. The median (IQR) LV end-diastolic volume increased from 154 (44) to 175 (76) mL in the control group, from 153 (49) to 185 (64) mL in the early treatment group, and from 149 (47) to 165 (73) mL in the late treatment group. Likewise, LV end-systolic volume increased from 94 (35) to 114 (68) mL in the control group, from 94 (41) to 105 (50) mL in the early treatment group, and from 97 (38) to 103 (54) mL in the late treatment group. Only for the late treatment group did ANCOVA testing reveal less negative remodeling (estimated treatment effect, −14.86; 95% CI, −28.98 to −0.74; P=0.04 for LV end-diastolic volume;

Table 3. ANCOVA of Primary and Secondary End Points, as Assessed by Cardiac Magnetic Resonance Imaging, at Baseline and 4-Month Follow-up

Variable		Group			Difference Between Treatment Groups and Control Group*	
		Control	Early	Late	Estimate (95% CI)	*P* Value
Primary end point†						
Global LVEF, %						
Baseline	Median (IQR)	39.6 (11.2)	34.6 (16.1)	35.6 (11.2)	1.25	0.42‡
	Mean (SD)	40.0 (9.9)	36.5 (9.9)	36.3 (8.2)	(−1.83 to 4.32)‡	0.73§
4 mo	Median (IQR)	38.7 (17.3)	40.1 (14.8)	37.8 (11.7)	0.55	
	Mean (SD)	39.6 (12.0)	37.9 (10.3)	37.4 (9.7)	(−2.61 to 3.71)§	
Secondary end points†						
LVEDV, mL						
Baseline	Median (IQR)	154 (44)	153 (49)	149 (47)	0.92	0.89‡
	Mean (SD)	153 (38)	156 (41)	157 (37)	(−11.95 to 13.78)‡	0.04§
4 mo	Median (IQR)	175 (76)	185 (64)	165 (73)	−14.86	
	Mean (SD)	180 (52)	183 (55)	167 (45)	(−28.98 to −0.74)§	
LVESV, mL						
Baseline	Median (IQR)	94 (35)	94 (41)	97 (38)	−2.06	0.72‡
	Mean (SD)	94 (33)	100 (36)	100 (29)	(−13.5 to 9.38)‡	0.08§
4 mo	Median (IQR)	114 (68)	105 (50)	103 (54)	−10.73	
	Mean (SD)	112 (46)	117 (51)	107 (40)	(−22.86 to 1.39)§	
Mass myocardial scar, g						
Baseline	Median (IQR)	39.1 (37.2)	37.7 (32.1)	33.9 (24.2)	−0.43	0.86‡
	Mean (SD)	45.3 (28.0)	44.0 (22.3)	38.5 (22.5)	(−5.17 to 4.31)‡	0.20§
4 mo	Median (IQR)	27.8 (17.2)	25.3 (19.7)	21.9 (14.4)	−2.99	
	Mean (SD)	29.2 (15.7)	28.9 (15.7)	24.3 (11.1)	(−7.64 to 1.66)§	
Myocardial scar, %						
Baseline	Median (IQR)	28.3 (16.3)	28.1 (16.2)	26.6 (15.9)	−1.15	0.45‡
	Mean (SD)	29.1 (13.1)	28.2 (11.7)	28.1 (11.9)	(−4.19 to 1.89)‡	0.41§
4 mo	Median (IQR)	24.3 (14.5)	22.2 (12.0)	22.9 (11.1)	−1.37	
	Mean (SD)	23.9 (10.4)	22.7 (9.4)	22.4 (8.5)	(−4.67 to 1.94)§	
Global wall thickening (average of all segments), mm						
Baseline	Median (IQR)	8.7 (2.7)	9.3 (3.5)	8.6 (3.1)	−0.21	0.34‡
	Mean (SD)	9.1 (2.3)	9.5 (2.3)	8.7 (2.2	(−0.66 to 0.23)‡	0.68§
4 mo	Median (IQR)	7.1 (1.9)	6.9 (1.4)	6.6 (2.0)	−0.10	
	Mean (SD)	7.2 (1.3)	7.1 (1.3)	7.0 (1.5)	(−0.60 to 0.39)§	
Wall thickening in infarct zone (average of all infarcted segments), mm						
Baseline	Median (IQR)	1.6 (1.2)	1.7 (2.0)	1.7 (1.6)	−0.075	0.72‡
	Mean (SD)	1.7 (1.2)	1.6 (1.2)	1.9 (1.2)	(−0.49 to 0.34)‡	0.79§
4 mo	Median (IQR)	2.0 (1.8)	2.1 (1.4)	2.1 (1.8)	0.064	
	Mean (SD)	2.2 (1.5)	2.2 (1.2)	2.2 (1.4)	(−0.41 to 0.54)§	

CI indicates confidence interval; IQR, interquartile range; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; and LVESD, left ventricular end-systolic volume. Example for analysis of the primary end point (control group=reference level; intercept = 9.37; LVEF baseline=0.76): For the early group, patients have on average a LVEF value at 4 mo that is 9.37+0.76×(the patient's baseline LVEF) +1.25. For a patient with a mean LVEF of 38%, a LVEF at 4 months of 9.37+0.76 ×38+1.25=39.5. CIs and *P* values are adjusted for baseline LVEF.

*Estimates of regression coefficient of treatment from the ANCOVA models.

†Descriptive statistic [median (IQR) and mean (SD)] of all available parameters at a given time.

‡*P* value, control vs early group.

§*P* value, control vs late group.

−10.73; 95% CI, −22.86 to 1.39; *P*=0.08 for LV end-systolic volume). In all groups, total scar mass uniformly decreased by >10 g, corresponding to a 4% to 5% decrease in the proportion of myocardial scar with respect to the entire myocardial mass.

Global LV thickening decreased slightly in all 3 groups. In contrast, myocardial thickening in the infarct-related segments showed only negligible changes. For both parameters, no treatment-related between-group difference was found. The entire results of CMR analyses at baseline and 4 months as well as ANCOVA testing are shown in Table 3.

In a prespecified analysis for predictors of BM-MNC treatment effect, the entire patient sample was split according to the median value of selected variables. No significant interaction was found for infarct size and for microvascular obstruction (split at the median). However, an interaction with the treatment effect of BM-MNC was found for time from onset of chest pain to successful reperfusion therapy (*P*=0.0455 for early versus control; *P*=0.0035 for late versus control) as well as for the baseline value of N-terminal prohormone of brain natriuretic peptide (*P*=0.023 for early versus control; *P*=0.0097 for late versus control). Interestingly, early-reperfused patients (below the median value of 4.5 hours) demonstrated an importantly higher treatment effect of BM-MNC (Figure 3), injected either early or late after AMI, compared with controls (estimated treatment effect of 6.31; 95% CI, 0.13–12.48; *P*=0.046 for early BM-MNC therapy group; estimated treatment effect of 9.17; 95% CI, 3.08–15.26; *P*=0.004 for late BM-MNC therapy group). Likewise, patients with higher N-terminal prohormone of brain natriuretic peptide levels at baseline (above the median value of 1437 ng/L) demonstrated a higher effect of BM-MNC treatment in both therapy groups compared with controls (estimated treatment effect of 7.1; 95% CI, 1.00–13.20; *P*=0.023 for early BM-MNC therapy group; estimated treatment effect of of 9.02; 95% CI, 2.24–15.79; *P*=0.01 for late BM-MNC therapy group). In regard to the absence or presence of microvascular obstruction at baseline, results were inconsistent, showing significant interaction for the late but not the early therapy group.

Overall, regression analysis (Table II in the online-only Data Supplement) did not show an influence of cell-related parameters (total number of mononuclear cells, total number of $CD34^+$ and $CD133^+$ cells, proportion of $CD34^+$ and $CD133^+$ cells, and migratory capacity) or age on LVEF at 4 months, adjusted for baseline LVEF.

Clinical Follow-Up and Events

At 4 months, >75% of the patients were in New York Heart Association class I, and >92% were in Canadian Cardiovascular Society class I. There was no difference between groups (Table III in the online-only Data Supplement). The clinical event rates are shown in Table 4. Events occurring between randomization and cell therapy and the cumulative 4-month rates are presented separately. Overall mortality at 4 months was low (2%) despite the fact that high-risk patients with large AMIs were enrolled. No deaths were noted in the control group, whereas 1 patient (1.7%) died in the late treatment group and 3 (4.6%) in the early treatment group. Of note, however, 2 deaths (1 in each group) occurred between randomization and a scheduled BM-MNC treatment. There was no significant difference in the frequency of isolated serious adverse events at 4 months between the 3 groups or for the prespecified, combined clinical end point of death, recurrence

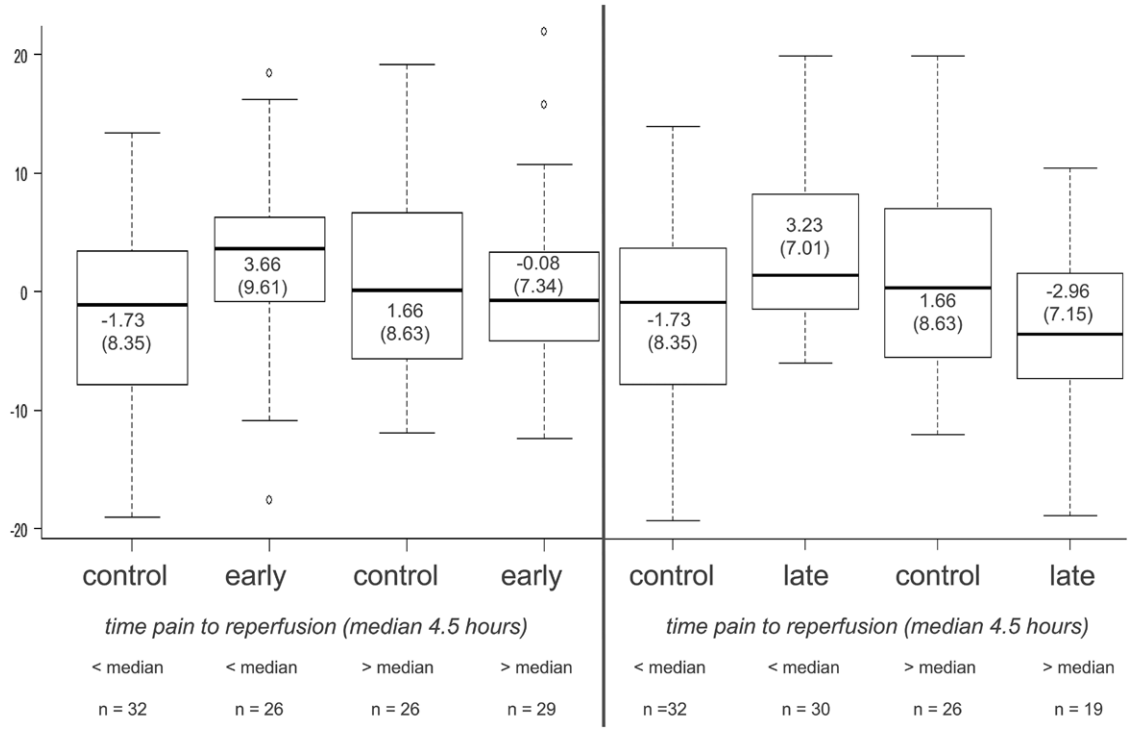

Figure 3. Descriptive statistic (box plot) of the mean of the differences between left ventricular ejection fraction at 4 months and left ventricular ejection fraction at baseline depending on time from onset of pain to reperfusion therapy (control vs early bone marrow–derived mononuclear cell group (**left**) and for control vs late bone marrow–derived mononuclear cell group (**right**).

Table 4. Clinical Events During Follow-up

	Control	Early	Late	*P* Value
Events between randomization and therapy				
Death	0	1 (3.1)	1 (1.7)	0.24* 0.48†
Events at 4-mo follow-up (cumulative)				
Death	0	3 (4.8)	1 (1.7)	0.24* 0.48†
Myocardial infarction	1 (1.6)	1 (1.6)	0	1.00* 1.00†
Rehospitalization for heart failure	2 (3.2)	0	2 (3.6)	0.50* 1.00†
Revascularization	3 (4.8)	3 (4.9)	2 (3.6)	1.00* 1.00†
Cerebral infarction	1 (1.6)	1 (1.7)	0	1.00* 1.00†
Combined events				
Death, myocardial infarction, revascularization, rehospitalization for heart failure	4 (6.4)	5 (7.9)	5 (8.8)	1.00* 0.74†
Death, myocardial infarction, revascularization, rehospitalization for heart failure, stroke	4 (6.4)	6 (9.5)	5 (8.8)	0.74* 0.74†

Values in parentheses are percentages.
**P* value, control vs early group.
†*P* value, control vs late group.

of myocardial infarction, repeated coronary revascularization, or rehospitalization for heart failure.

Discussion

The SWISS-AMI trial, to the best of our knowledge, is the first randomized controlled clinical study that addresses both an early and a late time point of BM-MNC administration after AMI. Both therapy groups were compared with a control arm in a unique trial design, assuming an equal treatment effect. The study design and power calculation were based on the knowledge and data available in 2006 for the calculation of adequate sample size. Furthermore, standardized state-of-the-art cell processing and the best imaging modality (ie, CMR) were used to test this hypothesis. Surprisingly, the results of our study do not confirm any significant improvement of global LV function at 4-month follow-up, either with early (5–7 days) or with late (3–4 weeks) application of BM-MNC after a first and rather large AMI. Accordingly, the between-group mean difference between early cell therapy and control was 2.1% (SD >8%), which is far from the 3.5% improvement (SD 6%) that was assumed for sample size calculation. For none of the secondary end points could a consistent benefit of cell therapy for both the early and late therapy groups be found.

Among the 5 potential predictors for a significant treatment effect analyzed, we identified N-terminal prohormone of brain natriuretic peptide levels above the median and time from onset of chest pain to reperfusion therapy below the median of 4.5 hours as the most promising. A potential explanation for the latter finding may be that early reperfusion after AMI leads to lower infarct transmurality but not to a lower infarct size or to less microvascular obstruction, as shown previously.[17] In agreement with the results of a smaller trial,[18] we suggest that in patients with complete transmural scar, BM-MNC treatment may be less beneficial. Further analyses of the 12-month CMR data, currently under way, will reveal whether remodeling is favorably affected by BM-MNC therapy in the long run.

Our study fulfilled several prerequisites for a properly designed randomized clinical trial testing progenitor cell–based therapy. First, we managed to enroll mainly patients with a large myocardial infarction, as demonstrated by an overall median LVEF of 37% and a rather high peak plasma level of creatine kinase. This was not the case in most of the previous trials.[1–3] The size of AMI has been shown to favor the beneficial effects of cell therapy in subgroup analyses.[2,19] Thus, the patient population of the SWISS-AMI trial was uniquely suited to show a potential benefit of cell therapy. Second, the assessment of cardiac function and infarct parameters was based on CMR, which is currently considered the gold standard for the analysis of global and regional LV function[20,21] and is recognized as one of the most accurate techniques to quantify necrotic or fibrous tissue.[22–24] Moreover, the entire analysis was performed in a CMR core laboratory, blinded to the treatment assignment of the patients enrolled. In agreement with previous studies that used CMR to assess LV function, the changes in LVEF and remodeling were overall small or negligible.[4–6,8,19,25] Furthermore, for interpretation of the data, the time point of the baseline CMR study may be important. To account for the early improvement in LV function because of recovery of stunned myocardium, we performed the baseline examination at day 6 after AMI. This could explain the absence of improvement of LVEF in the control group from baseline to the 4-month follow-up, which was notable, for instance, in the REPAIR-AMI study.[2]

Third, the processing of BM-MNC was performed by an experienced[26] and certified core laboratory, with a standardized protocol used in previous trials.[2] Cell potency was assessed in vitro as described,[12] confirming appropriate BM-MNC viability. Furthermore, in vivo experiments using the mouse model of myocardial infarction recently described by us are currently ongoing.[27] Heparin, which potentially may abrogate the migration capacity of BM-MNC,[28] was not added directly to the cells. Like many of the research groups working in the field, we chose unselected MNC as treatment agents because they have been proven to be safe in previous clinical studies and are easy to obtain without complex purification and cultivation steps. Furthermore, selected MNC of any type have never been shown to be superior to unselected MNC in terms of neoangiogenesis if confronted directly in clinical trials. However, recent studies using selected, bone marrow–derived mesenchymal stem cells showed a promising increase in LVEF shortly after STEMI compared with placebo.[29] In addition, $CD34^+$ cells have been shown to successfully reduce refractory angina if injected directly into the ischemic myocardium.[30]

BM-MNC generally contain a small amount of progenitor cells including hematopoietic stem cells, mesenchymal stem cells,[31] endothelial progenitor cells,[32] multipotent adult mesenchymal progenitors,[33] and very small numbers of embryonic-like

stem cells.[34] Although which subsets of such progenitor cells may be responsible for the beneficial effects of BM-MNC in AMI has never been clarified, their mechanism of action is likely to involve paracrine effects mediated by cells contained in unselected BM-MNC, supporting the use of unselected rather than selected BM-MNC. Of note, in the SWISS-AMI trial, the proportion of CD34+ cells, which are thought to be particularly important for neoangiogenesis,[35] was 1% in the early and 1.3% in the late therapy group; thus, these results were comparable to results of previous trials. The total number of injected nucleated cells (overall median of 153 million cells) was lower than in the REPAIR-AMI or Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST)[1,2] trials but similar to the LateTIME trial[8] and the TIME trial.[7] According to published data,[36] doses of >150 million of injected mononuclear cells (as was used in the SWISS-AMI trial) have been shown to be sufficient to modify LV function. In addition, a clear relationship between total number of injected mononuclear cells or CD34+ cells and clinical efficacy has until now not been proven in prospective studies.

There are also several limitations of the study. The long duration between enrollment of the first and the last patients may lead to imbalances in the management of STEMI because during the study period new antiplatelet agents were introduced (such as bivalirudin and prasugrel). In addition, the dropout rate differed importantly between groups. Interestingly, the dropout rate was the lowest in the early treatment group, in which the patients received BM-MNC treatment during the initial hospitalization for AMI, whereas in the late treatment group it was much higher than expected. This may have led to a certain selection bias. Furthermore, baseline LVEF was somewhat higher in the control group than in both treatment groups. However, when the results of the subgroup analysis of 2 previous studies[2,19] are considered, this may have disadvantaged the results of the control group and thus cannot account for the overall disappointing results. Furthermore, in the analysis of the primary end point, the results were adjusted for baseline LVEF.

Besides the primary end point analysis, we performed a large number of statistical tests involving the secondary end points, which may increase the overall probability of a type I error. The results of the subgroup analysis must therefore be considered to be strictly hypothesis generating. Finally, when one considers the promising results of recent studies using selected cells[29,30] such as CD34+ cells or mesenchymal stem cells, the lack of cell selection in our study may also be seen as an important limitation.

The results of the SWISS-AMI trial may further reduce the euphoria that initially accompanied the clinical application of progenitor cell–based research. In the last decade, intracoronary injection of BM-MNC has been tested in several randomized controlled clinical trials, and improvement in global LV function has been reported by some[1–3] but not all of the investigators.[4–8,19] The results of the REPAIR-AMI trial[2] in particular were considerably promising and encouraging. Potential factors that may have influenced these controversial results in terms of LV remodeling may relate to cell functionality,[37] which has been shown to be abrogated by heparin[28] and which may be impaired in cardiovascular patients[27]; selection of patients, in particular differences in baseline LVEF and infarct size[2,19]; and choice of the optimal time point of cell application. For the latter, initial recommendations were based on the work of an expert committee[38] in the absence of any prospective data.

Simultaneously with the SWISS-AMI trial, the Cardiovascular Cell Therapy Network started 2 similar cell therapy trials to prospectively address different time points of cell administration.[7,8] Surprisingly, none of these trials could confirm the relevant efficacy of BM-MNC to improve LV function at any of the tested time points. BM-MNC injection at 2 to 3 weeks after AMI failed to demonstrate improvement in LV function in the LateTIME trial compared with placebo.[8] Although an early treatment group was missing in the LateTIME trial, their results are in agreement with the results of the late therapy group of SWISS-AMI. In the latter trial, injection at 5 to 7 days after AMI showed a small, nonsignificant improvement in LVEF, comparable to the results of the respective subgroup of the TIME trial.[7] When all of the randomized clinical trials are considered, with the use of CMR to assess global and regional LV function, the effect of BM-MNC on LVEF seems to be rather marginal, as shown in a meta-analysis.[36]

In conclusion, in the SWISS-AMI trial in patients with STEMI and LV dysfunction after successful reperfusion therapy by primary percutaneous coronary intervention and intracoronary infusion of BM-MNC either 5 to 7 days or 3 to 4 weeks after AMI, we did not find improved LV function at 4 months. The question of whether the measurement of LVEF is the proper end point to assess the clinical utility of cell-based therapy remains open[25] and will await the results of upcoming large outcome trials.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Bone marrow–derived mononuclear cell (BM-MNC) treatment after acute myocardial infarction is appealing to patients and physicians because it represents a drug-free 1-time treatment to improve left ventricular (LV) remodeling. Harvest of bone marrow is relatively easy, and intracoronary injection of the BM-MNC has been demonstrated to be feasible and safe shortly after myocardial infarction. Many of the randomized controlled trials in the first years after the appearance of BM-MNC treatment showed a promising improvement in LV ejection fraction. In the last few years, enthusiasm is decreasing because many studies, using mainly cardiac magnetic resonance imaging to assess LV function, did not confirm those results, showing only marginal benefit in favor of BM-MNC treatment. As in 2 other recently published trials of similar study design, in the present series there was no significant benefit in favor of BM-MNC in terms of LV remodeling or global or regional LV function. Nevertheless, meta-analyses show a potential benefit in terms of prognosis. Whether BM-MNC are the right cell type to be used shortly after myocardial infarction cannot be answered by the present study, nor can the question of whether LV ejection fraction is the most appropriate surrogate end point. Finally, only the recently begun event-driven phase III studies will provide a definitive answer.

SUPPLEMENTAL MATERIAL

Table S1: BM-MNC characterization II

	Median (IQR)	n
Lymphocytes (% of total nucleated cells)§	46 (17)	116
Monocytes (% of total nucleated cells)§	8 (3)	105
CFC (colonies/10^6 cells)*	4050 (2638)	19
CFU-Hill (colonies/10^6 cells)**	11 (11)	6
CFU-F (colonies/10^6 cells)***	12 (11)	15

IQR: interquartile range; §differential cell counting by automated hematology cell analyzer; *CFC assay for the evaluation of hemopoietic cell precursors; **CFU-Hill assay for the evaluation of angiogenic potential; ***CFU-F for the evaluation of mesenchymal precursors.

Methods (not described in the main article):
Lymphocytes, Monocytes and Granulocytes were determined by Differential cell count using automated hematology cell analyzer (ABX Micros 60, Horiba medical).
Colony-Forming Cell (CFC) assay was performed plating cells in Methocult® (StemCell Technologies); after 14 days, plates were microscopically scored for the presence of hematopoietic colonies. For Colony-Forming Unit-Hill (CFU-Hill) assay, cells were suspended in Complete CFU-Hill medium (StemCell Technologies), then seeded in fibronectin coated 6 well plates; after 2 days, non adherent cell were collected, transferred in fibronectin coated 24 well plates and incubated for further 5 days; the wells were then fixed, stained and scored for the presence of colonies. For the Colony-Forming Unit-Fibroblast (CFU-F) assay, cells were plated in Mesencult®-XF (StemCell Technologies); after 14 days, the dishes were fixed, stained and scored for the presence of colonies.

Table S2: Analyses of covariance (ANCOVA) for LVEF, adjusted for baseline LVEF and for BM-MNC related variables

Variable	Median (IQR)	n	Estimate	95% CI	p
Bone marrow aspirate (ml)	70 (15)	105	0.03	-0.08 to 0.13	0.63
MNC (10^6 cells)	153 (119)	105	-0.003	-0.02 to 0.01	0.71
Cell Viability (%)	93.5 (6)	105	-0.04	-0.31 to 0.23	0.79
% CD34+ cells	1.12 (0.83)	105	-0.94	-2.63 to 0.76	0.27
Total CD34+ cells (10^6 cells)	1.55 (1.93)	105	-0.28	-0.91 to 0.35	0.37
% CD34+/CD133+ cells	0.84 (0.87)	105	0.02	-0.03 to 0.67	0.47
CD34+/CD133+ cells (10^6 cells)	0.94 (1.67)	105	-0.07	-0.80 to 0.65	0.84
% Invasion	29 (19)	55	0.08	-0.08 to 0.23	0.32
Invasion index	49.3 (25.5)	55	0.05	-0.07 to 0.16	0.42

Explanation: For each additional ml of bone marrow aspirate and adjusted for baseline LVEF, LVEF at 4 months is on average changed by 0.03% with 95% confidence interval [-0.08 to 0.13] and p-value 0.63, based on 105 complete observations.

% Invasion: percentage of total nucleated cells showing invasion capacity; MNC: Total nucleated cells

Table S3: NYHA and CCS Class at 4 months follow-up

NYHA class	**Control**	**Early**	**Late**	**p**
1	52 (85.2%)	46 (75.4%)	43 (76.8%)	
2	6 (9.8%)	13 (21.3%)	12 (21.4%)	0.23 *
3	2 (3.3%)	2 (3.3%)	1 (1.8%)	0.24 ‡
4	1 (1.6%)	0	0	
CCS class	**Control**	**Early**	**Late**	**p**
1	59 (96.7%)	56 (91.8%)	53 (94.6%)	
2	1 (1.6%)	3 (4.9%)	3 (5.4%)	0.51 *
3	1 (1.6%)	2 (3.3%)	0	0.35 ‡
4	0	0	0	

NYHA: New York Heart association; CCS: Canadian Cardiac society

*: p value control vs. early; ‡: p value control vs. late