PRELIMINARY COMMUNICATION

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Effect of the Use and Timing of Bone Marrow Mononuclear Cell Delivery on Left Ventricular Function After Acute Myocardial Infarction

The TIME Randomized Trial

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Context While the delivery of cell therapy after ST-segment elevation myocardial infarction (STEMI) has been evaluated in previous clinical trials, the influence of the timing of cell delivery on the effect on left ventricular function has not been analyzed.

Objectives To determine the effect of intracoronary autologous bone marrow mononuclear cell (BMC) delivery after STEMI on recovery of global and regional left ventricular function and whether timing of BMC delivery (3 days vs 7 days after reperfusion) influences this effect.

Design, Setting, and Patients A randomized, 2 × 2 factorial, double-blind, placebo-controlled trial, Timing In Myocardial infarction Evaluation (TIME) enrolled 120 patients with left ventricular dysfunction (left ventricular ejection fraction [LVEF] ≤ 45%) after successful primary percutaneous coronary intervention (PCI) of anterior STEMI between July 17, 2008, and November 15, 2011, as part of the Cardiovascular Cell Therapy Research Network sponsored by the National Heart, Lung, and Blood Institute.

Interventions Intracoronary infusion of 150×10^6 BMCs or placebo (randomized 2:1) within 12 hours of aspiration and cell processing administered at day 3 or day 7 (randomized 1:1) after treatment with PCI.

Main Outcome Measures The primary end points were change in global (LVEF) and regional (wall motion) left ventricular function in infarct and border zones at 6 months measured by cardiac magnetic resonance imaging and change in left ventricular function as affected by timing of treatment on day 3 vs day 7. The secondary end points included major adverse cardiovascular events as well as changes in left ventricular volumes and infarct size.

Results The mean (SD) patient age was 56.9 (10.9) years and 87.5% of participants were male. At 6 months, there was no significant increase in LVEF for the BMC group (45.2% [95% CI, 42.8% to 47.6%] to 48.3% [95% CI, 45.3% to 51.3%) vs the placebo group (44.5% [95% CI, 41.0% to 48.0%] to 47.8% [95% CI, 43.4% to 52.2%]) ($P = .96$). There was no significant treatment effect on regional left ventricular function observed in either infarct or border zones. There were no significant differences in change in global left ventricular function for patients treated at day 3 (–0.9% [95% CI, −6.6% to 4.9%], $P = .76$) or day 7 (1.1% [95% CI, –4.7% to 6.9%], $P = .70$). The timing of treatment had no significant effect on regional left ventricular function recovery. Major adverse events were rare among all treatment groups.

Conclusion Among patients with STEMI treated with primary PCI, the administration of intracoronary BMCs at either 3 days or 7 days after the event had no significant effect on recovery of global or regional left ventricular function compared with placebo.

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CELL THERAPY MAY EVENTUally become a therapeutic option for patients after acute myocardial infarction (AMI), potentially preventing the transition to end-stage heart failure for which cardiac transplantation is currently the only curative procedure available. Recent meta-analyses of bone marrow mononuclear cell (BMC) delivery to the infarct zone after AMI have shown small improvements in left ventricular function after successful reperfusion.[1] However, despite a growing number of trials, many fundamental questions such as optimal timing of BMC delivery remain unanswered.

Myocardium and bone marrow undergo important changes in the days to weeks after AMI that may affect stem or progenitor cell engraftment and survival.[2] This notion has support from the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial,[3] which determined in a prospectively specified analysis that delivery of BMCs 5 to 7 days after AMI resulted in greater improvement in left ventricular ejection fraction (LVEF) compared with earlier delivery. However, this important variable has never been evaluated in a prospective trial that randomly selects the day of cell delivery.

The National Heart, Lung, and Blood Institute established the Cardiovascular Cell Therapy Research Network to address mechanistic questions in cardiovascular cell therapy. The recently completed LateTIME[4] trial found BMC administration did not influence the ongoing postreperfusion recovery of either global or regional left ventricular function when delivered 2 to 3 weeks after AMI. Herein we present the results of a companion trial investigating the influences of the timing of cell delivery within the first week after AMI on the course of improving global and regional left ventricular function after reperfusion.

METHODS

Timing In Myocardial infarction Evaluation (TIME) was a randomized, 2×2 factorial, double-blind, placebo-controlled trial investigating the timing of intracoronary autologous BMCs within the first week after reperfusion in a high-risk cohort with ST-segment elevation myocardial infarction (STEMI).[5] Between July 17, 2008, and November 15, 2011, 120 patients with LVEF of 45% or less by echocardiography after primary percutaneous coronary intervention (PCI) with stenting were enrolled. Exclusions included previous bypass surgery or prior STEMI with residual left ventricular dysfunction (LVEF <55%).

Each clinical center and the data coordinating center had independent institutional review board approval and oversight. Briefly, all qualifying participants provided written informed consent and were randomized on a 1 to 1 ratio to receive therapy on either day 3 or day 7 after primary PCI with stenting.

Race/ethnicity was self-described by participants. Demographic and clinical variables were determined by interview and by review of the patient's medical record. All patients had cardiac magnetic resonance imaging (MRI) at day 3 (baseline), and those randomized to delivery on day 7 had another MRI on day 7 (baseline). Patients underwent bone marrow aspiration on the morning of their treatment day, and BMCs were isolated using a closed, automated Ficoll cell processing system (Sepax, Biosafe)[6] to ensure a uniform cellular product across centers.

After the cell product passed stipulated lot release criteria, a second randomization to either BMCs (2:1) or cell-free placebo occurred. Patients randomized to BMCs received a product containing 150×10^6 total nucleated cells (70%-80% of BMCs). Patients randomized to placebo received a cell-free product of 5% albumin in normal saline with 100 μL of autologous blood added to match color and consistency of the BMCs.

Within 12 hours of aspiration, patients received an infusion of BMCs or placebo in the infarct-related artery (Maverick balloon catheter, Boston Scientific) in 6 aliquots (5 mL each) using the stop-flow technique.[5] All patients received heparin during the procedure to achieve an activated clotting time of greater than 200 seconds and were treated with aspirin and 75 mg of clopidogrel in addition to other guideline-recommended post-MI medications.

Cardiac MRI of global and regional left ventricular function has been previously described.[4,5] Imaging using protocols developed by the MRI Core Laboratory (University of Florida) were performed using 1.5 T scanners that had been certified before study initiation.

The primary end points were change in global (LVEF) and regional left ventricular function (infarct and border zone) by MRI between baseline and 6 months when administered within the first 7 days after PCI and whether these changes were dependent on day of administration (day 3 vs day 7). The secondary end points included major adverse cardiovascular events as well as effects on left ventricular volumes and infarct size. Subgroup analysis for age, sex, race, hypertension, diabetes, statins, drug-eluting stent vs bare metal stent, and LVEF was prespecified. The distribution of participants across therapy groups precluded diabetes and statin analyses.

The statistical methods used have been detailed previously.[5] Briefly, global left ventricular function was assessed by MRI-derived LVEF, for which we assumed an effect size or a placebo-adjusted change (difference in the change over time in the BMC group minus the change in the placebo group) of $\delta = 5\%$ and a common group standard deviation of the difference of LVEF over time as $\sigma_{LVEF}(\Delta) = 7$ as derived from Wollert et al,[7] Lunde et al,[8] Schächinger et al,[9] and Janssens et al.[10]

Regional left ventricular function measure was defined as the change in wall motion over time in the infarct zone and in the infarct border zone. The infarct zone was defined as the segments with the largest 2-signal intensity-enhancement measures with gado-

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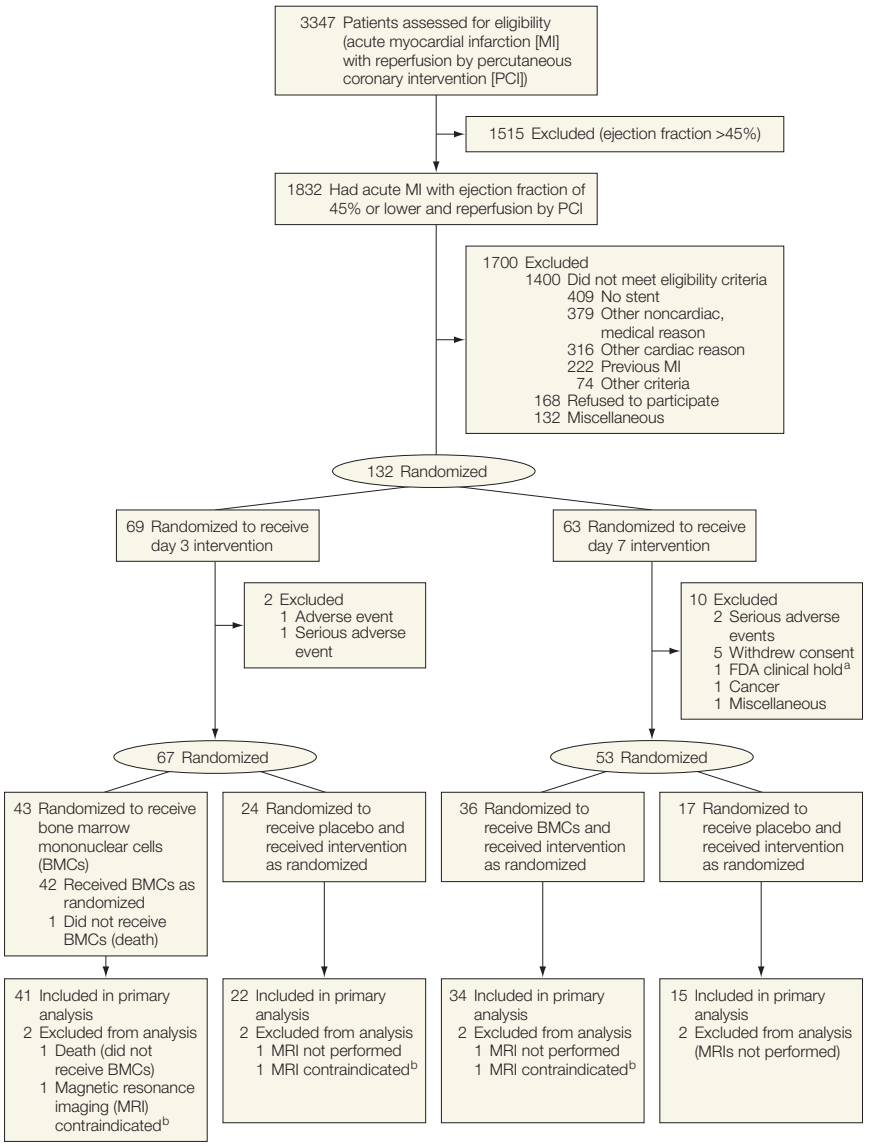
linium (using a 17-segment model). The border zone was defined as those regions adjacent to the infarct zone in which the signal intensity enhancement measures were in the 10% to 75% range of transmurality. For each of these measures of regional left ventricular function, we assumed an effect size of $\delta = 6.7$ mm and a common group standard deviation of $\sigma_{LVEF}(\Delta) = 9.5$.[7] Sixty patients each were required in an assessment of the effects of therapy on day 3 and day 7. This yield of 120 patients produced greater than 90% power for an overall assessment of therapy combining the day 3 and day 7 groups, as well as for the effect of therapy on day 3 vs the effect on day 7.

The Fisher exact test was used for categorical variables, and the *t* test was used for continuous variables to assess the compatibility of baseline variables between groups. All hypothesis testing was 2-sided, and all effect sizes and their 95% confidence intervals were evaluated using the general linear model, adjusting for center and demographics. No adjustments for multiple comparisons were made in this phase 2 study. A *P* value of .05 was used to assess statistical significance. SAS version 9.2 (SAS Institute Inc) was used for the statistical analyses.

Figure 1. Flow Diagram of Timing In Myocardial infarction Evaluation Trial



[a] Indicates an order issued by the US Food and Drug Administration (FDA) to suspend an ongoing investigation; this hold was issued to ensure proper screening and monitoring of patients during the investigation by excluding those with left ventricular thrombus or atrial fibrillation who required anticoagulation therapy.
[b] All MRIs contraindicated because of implantable cardioverter-defibrillator placement.

RESULTS

Between July 2008 and November 2011, a total of 3347 patients were screened with almost half excluded due to having a LVEF greater than 45% (FIGURE 1). There were no statistically significant differences between the BMC and placebo groups in baseline characteristics except for higher peak creatine kinase and troponin levels among patients in the BMC group randomized to day 7 therapy and lack of diabetes among patients in the placebo group randomized to day 7 placebo therapy (TABLE 1). The qualifying LVEF (protocol-specified by echocardiography) within 48 hours of PCI ranged from 36.1% to 37.8%.

The mean time from PCI to bone marrow aspiration and cell processing was 3.3 days in the day 3 group and 7.5 days in the day 7 group. All BMC aspirates underwent automated cell processing at each center using Sepax. No patients experienced complications associated with the bone marrow harvesting.

The median time from bone marrow aspiration to infusion was 8.3 hours in the BMC group (Table 1); all patients received approximately 150 million total nucleated cells. The mean viability of the final BMC product was 98.2% and contained 2.2% of CD34 cells and 1.1% of cells that were both CD34 and CD133 cells (TABLE 2). The cell product was devoid of significant red blood cell contamination, contained only minuscule amounts of heparin (estimated at 0.01 U/mL), and most participants were infused within 1 hour of

Table 1. Baseline Characteristics of Patients in the Bone Marrow Mononuclear Cell (BMC) and Placebo Groups

	Intervention on Day 3		Intervention on Day 7	
	BMC Group (n = 43)	Placebo Group (n = 24)	BMC Group (n = 36)	Placebo Group (n = 17)
Age, mean (SD), y	55.6 (10.8)	57.0 (12.4)	58.2 (11.3)	57.0 (8.0)
Female sex, No. (%)	5 (11.6)	3 (12.5)	5 (13.9)	2 (11.7)
Height, mean (SD), cm	177.0 (8.6)	173.5 (7.9)	175.0 (10.7)	176.0 (11.4)
Weight, mean (SD), kg	95.2 (18.6)	88.9 (22.6)	91.3 (20.3)	96.6 (16.2)
Body mass index, mean (SD)[a]	30.5 (5.4)	29.6 (6.8)	29.9 (5.5)	31.3 (3.3)
Race, No. (%)				
White	38 (88.4)	20 (83.3)	31 (86.1)	15 (88.2)
Nonwhite	5 (11.63)	4 (16.67)	5 (13.89)	2 (11.76)
Qualifying LVEF on echocardiogram, mean (SD), %	36.1 (6.1)	37.8 (6.6)	36.5 (6.3)	36.6 (4.1)
History, No. (%)				
Diabetes	10 (23.3)	8 (33.3)	4 (11.1)	0
High blood pressure	19 (44.2)	15 (62.5)	23 (63.9)	13 (76.5)
Hyperlipidemia	28 (65.1)	15 (62.5)	25 (69.4)	13 (76.5)
Angina	7 (16.3)	2 (8.3)	6 (16.7)	5 (29.4)
Smoking	28 (65.1)	17 (70.8)	19 (52.8)	11 (64.7)
Heart rate, beats/min				
At initial presentation in emergency department				
Mean (SD)	81.5 (14.2)	78.7 (13.6)	74.2 (15.3)	82.3 (17.7)
Median (range)	82.0 (70.0-91.0)	74.5 (70.0-90.5)	75.5 (65.0-82.5)	82.0 (75.0-89.0)
At discharge				
Mean (SD)	(n = 42) 76.8 (12.1)	79.5 (14.9)	75.8 (10.4)	78.1 (9.2)
Median (range)	74.5 (68.0-85.0)	76.5 (70.0-88.5)	77.5 (68.5-83.5)	78.0 (72.0-82.0)
Blood pressure, mean (SD), mm Hg	(n = 42)			
Systolic	115.2 (14.0)	115.4 (11.0)	111.5 (16.4)	112.0 (16.4)
Diastolic	70.2 (10.7)	68.3 (7.7)	68.7 (11.2)	69.5 (7.4)
Preinfarction angina, No. (%)	10 (23.3)	7 (29.2)	11 (30.6)	7 (41.2)
Laboratory results				
Hemoglobin, median (IQR), g/dL	(n = 38) 14.2 (13.6-14.9)	(n = 17) 12.6 (12.1-13.8)	(n = 29) 14.2 (13.3-14.9)	(n = 15) 14.4 (13.2-15.3)
High-sensitivity CRP, median (IQR), mg/L	(n = 39) 20.8 (8.9-52.2)	(n = 21) 38.8 (10.8-49.4)	(n = 33) 28.1 (9.5-48.6)	(n = 16) 34.0 (16.1-48.0)
Brain-type natriuretic peptide, median (IQR), pg/mL	(n = 34) 189.0 (90.0-394.0)	(n = 20) 205.5 (118.0-394.5)	(n = 30) 177.5 (139.0-238.0)	(n = 15) 150.0 (125.0-370.0)
Peak CKMB, median (IQR), ng/mL	(n = 29) 180.9 (42.1-1302.0)	(n = 19) 133.0 (62.0-432.7)	(n = 31) 402.0 (234.0-466.0)	(n = 15) 227.0 (76.0-442.0)
Peak troponin, median (IQR), ng/mL				
T	(n = 18) 6.2 (3.3-13.1)	(n = 12) 4.4 (2.4-9.4)	(n = 21) 11.3 (5.4-17.0)	(n = 14) 11.5 (3.3-15.3)
I	(n = 14) 26.9 (5.8-55.0)	(n = 4) 31.0 (22.0-70.2)	(n = 5) 181.5 (95.4-224.2)	(n = 1) 128.9 (128.9-128.9)
Myocardial infarction treatment				
Ischemic time, median (IQR), h	3.4 (2.4-7.6)	3.6 (2.2-8.6)	4.0 (2.1-6.5)	3.5 (2.2-11.8)
Door to balloon, median (IQR), h	(n = 42) 1.2 (0.7-1.7)	1.3 (0.6-2.4)	1.5 (1.0-1.9)	1.2 (0.6-2.2)
Transferred from outside hospital after PCI	5 (11.6)	2 (8.3)	2 (5.6)	0
Time from bone marrow aspiration to infusion, median (IQR), h	8.4 (7.9-9.2)	8.8 (8.0-9.5)	7.9 (7.5-8.9)	8.6 (7.8-9.0)
Time from PCI to infusion, median (IQR), d	(n = 42) 3.3 (2.8-3.8)	3.2 (2.5-4.1)	7.4 (7.0-7.9)	7.6 (7.0-8.3)
Drug-eluting stent, No. (%)	33 (76.74)	21 (87.50)	29 (80.56)	14 (82.35)
Stent region, No. (%)				
Left anterior descending coronary artery (LAD)	37 (86.0)	23 (95.8)	35 (97.2)	17 (100)
LAD only	35 (81.4)	22 (91.7)	33 (91.7)	17 (100)
LAD + left circumflex coronary artery	0	1 (4.2)	1 (2.8)	0
LAD + right coronary artery	2 (4.7)	0	1 (2.8)	0
Left circumflex coronary artery only	1 (2.3)	1 (4.7)	1 (2.8)	0
Right coronary artery only	5 (11.6)	0	0	0
Medications at time of randomization, No. (%)				
Angiotensin-converting enzyme inhibitor	35 (81.4)	19 (79.2)	33 (91.7)	13 (76.5)
Clopidogrel or plasugrel	42 (97.7)	23 (95.8)	33 (91.7)	17 (100)
Aspirin	42 (97.7)	24 (100)	34 (94.4)	17 (100)
β-Blockers	42 (97.7)	24 (100)	35 (97.2)	16 (94.1)
Statins	39 (90.7)	22 (91.7)	34 (94.4)	17 (100)
Diuretics	7 (16.3)	5 (20.8)	11 (30.6)	2 (11.8)
Warfarin or enoxaparin	4 (9.3)	3 (12.5)	11 (30.6)	3 (17.7)

Abbreviations: CKMB, creatine kinase–MB fraction; CRP, C-reactive protein; IQR, interquartile range; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention.
[a]Calculated as weight in kilograms divided by height in meters squared.

Table 2. Cell Characteristics of Bone Marrow Mononuclear Cell (BMC) and Placebo Groups[a]

	Intervention on Day 3		Intervention on Day 7	
	BMC (n = 43)	Placebo (n = 24)	BMC (n = 36)	Placebo (n = 17)
Total nucleated cells, mean (SD)/product × 10^6	146.6 (22.3)	149.5 (1.7)	146.2 (12.0)	145.4 (14.7)
Viability by Trypan blue exclusion, mean (SD), %	98.1 (1.7)	98.7 (1.0)	98.1 (1.4)	97.9 (1.6)
CD34 cells/product, mean (SD), %[b]	(n = 41) 2.4 (1.3)	(n = 23) 2.2 (1.0)	(n = 28) 1.6 (0.8)	(n = 16) 2.4 (0.9)
CD34 and CD133 cells/product, mean (SD),%[b]	(n = 41) 1.1 (0.7)	(n = 23) 1.2 (0.8)	(n = 28) 0.9 (0.6)	(n = 16) 1.2 (0.6)
Colony-forming units–Hill cells/product, median (IQR)[b]	(n = 30) 120 (0-330)	(n = 17) 120 (0-180)	(n = 25) 165 (0-390)	(n = 11) 330 (60-750)
Endothelial colony–forming cells/product, median (IQR)[b]	(n = 29) 0 (0-480)	(n = 16) 0 (0-265)	(n = 26) 0 (0-300)	(n = 10) 120 (0-420)

Abbreviation: IQR, interquartile range.
[a] The comparisons between the BMC and placebo groups were not statistically significant.
[b] A separate consent was used for the biorepository and 4 patients declined participation. Another 4 patients had insufficient product for the biorepository analysis and some analyzed data were unreportable.

Table 3. Clinical and Safety Outcomes at 6-Month End Point Window

	BMC (n = 79)	Placebo (n = 41)	Total Overall
Patients, No. (%)	13 (16)	7 (17)	20 (17)
Deaths	1	0	1
Reinfarctions	1	2	3
Repeat revascularizations	7	4	11
Target vessel	2	3	5
Nontarget vessel	5	1	6
Hospitalization for heart failure	4	1	5
ICD placements	3	3	6
Total events	**16**	**10**	**26**
Crude incidence rate	0.165	0.171	0.167
Relative risk (95% CI) for BMC vs placebo	0.96 (0.42-2.23)		
P value	.93		

Abbreviations: BMC, bone marrow mononuclear cell; ICD, implantable cardioverter-defibrillator.

completion of cell processing,[11] thereby avoiding concerns recently expressed in the literature.[12,13] In vitro and in vivo studies comparing the delivery of Sepax-derived BMCs with that of open Ficoll-selected BMCs demonstrated phenotypic equivalence and equal efficacy on hind limb recovery in a murine model of hind limb perfusion.

All patients received systemic heparin during treatment infusion (as in REPAIR-AMI and other trials using the stop-flow technique). No complications were associated with intracoronary infusion.

Despite a perceived high-risk cohort of patients with moderate to severe left ventricular dysfunction after large STEMIs, there were few clinical events (TABLE 3). One death occurred (due to subarachnoid hemorrhage) after randomization to the BMC group but before cell delivery was performed. Eleven patients underwent repeat revascularization and 6 received implantable cardioverter-defibrillators. There was no significant difference between the relative incidences of events comparing the BMC and placebo groups.

Follow-up MRIs were not performed in 8 patients because 1 had died, 3 had implantable cardioverter-defibrillator placements, and 4 declined for miscellaneous reasons (discomfort, anxiety, scheduling, or travel issues) (Figure 1).

When both BMC groups (n=75) were combined and compared with a combined placebo group (n=37), LVEF in the BMC group increased from 45.2% (95% CI, 42.8% to 47.6%) at baseline to 48.3% (95% CI, 45.3% to 51.3%) at 6 months while the combined placebo group increased from 44.5% (95% CI, 41.0% to 48.0%) to 47.8% (95% CI, 43.4% to 55.2%). Overall, there was no significant change in the difference between the 2 groups (–0.1% [95% CI, –4.1% to 3.9%]; *P*=.96). There was no significant difference between the change in regional wall motion in the infarct zone (–0.9 mm [95% CI, –3.0 to 1.2 mm]; *P*=.41) and the change in border zone (–0.5 mm [95% CI, –3.9 to 2.9 mm]; *P*=.78) (TABLE 4 and FIGURE 2).

A total of 41 patients in the BMC group and 22 patients in the placebo group had paired MRI data at baseline and at 6 months that were available for an analysis of global and regional left ventricular function in the day 3 group. The LVEF in the BMC group on the day of treatment was 46.1% (95% CI, 42.7% to 49.5%) and increased to 49.6% (95% CI, 45.3% to 53.9%) at 6 months, while the placebo group increased from 41.6% (95% CI, 37.4% to 45.8%) to 45.9% (95% CI, 40.1% to 51.7%) at 6 months. There was no significant difference between the change in LVEF of the BMC group compared with the

change in LVEF of the placebo group (–0.9% [95% CI, –6.6% to 4.9%]; *P*=.76).

Similarly, infarct zone wall motion in the BMC group on day 3 of treatment was 4.2 mm (95% CI, 2.6 to 5.8 mm) compared with 3.7 mm (95% CI, 1.9 to 5.5 mm) in the placebo group. The difference in the changes over 6 months in infarct zone wall motion between the 2 groups was not significant (–0.3 mm [95% CI, –3.3 to 2.7 mm]; *P*=.82). In the border zone, wall motion in the BMC group on day 3 of treatment was 16.7 mm (95% CI, 13.3 to 20.1 mm) vs 12.6 mm (95% CI, 8.0 to 17.2 mm) in the placebo group. The difference between the 6-month changes in both groups was not significant (–0.8 [95% CI, –5.6% to 4.0%]; *P*=.75).

A total of 34 patients in the BMC group and 15 patients in the placebo group had paired MRI data at baseline and 6 months available for analysis of global and regional left ventricular function in the day 7 group. Baseline LVEF measured on treatment day 7 was 44.0% (95% CI, 40.7% to 47.3%) in the BMC group and increased to 46.8% (95% CI, 42.7% to 50.9%) at 6 months vs baseline LVEF in the placebo group of 48.8% (95% CI, 43.3% to 54.3%) and increased to 50.4% (95% CI, 43.7% to

Table 4. End Point Analyses of Global and Regional Left Ventricular (LV) Function Between Baseline and 6 Months

	Bone Marrow Mononuclear Cell Group		Placebo Group		Between-Group Difference in 6-Month Change (95% CI)	*P* Value
	No.	Mean (SD) [95% CI]	No.	Mean (SD) [95% CI]		
Overall Effect of Therapy on Global LV Function						
Left ventricular ejection fraction, %						
Baseline	75	45.2 (10.6)	37	44.5 (10.8)		
Follow-up	75	48.3 (13.3)	37	47.8 (13.6)		
Within-group change	75	3.2 (10.3) [0.9 to 5.5]	37	3.3 (9.7) [0.2 to 6.4]	–0.1 (–4.1 to 3.9)	.96
Overall Effect of Therapy on Regional LV Function						
Infarct zone wall motion, mm						
Baseline	75	4.0 (4.7)	37	4.1 (3.7)		
Follow-up	75	5.7 (6.3)	37	6.7 (6.3)		
Within-group change	75	1.7 (5.5) [0.5 to 2.9]	37	2.6 (4.9) [1.0 to 4.2]	–0.9 (–3.0 to 1.2)	.41
Border zone wall motion, mm						
Baseline	75	15.2 (9.9)	37	13.1 (10.4)		
Follow-up	75	19.1 (11.8)	37	17.4 (13.0)		
Within-group change	75	3.8 (8.8) [1.8 to 5.8]	37	4.3 (8.0) [1.7 to 6.9]	–0.5 (–3.9 to 2.9)	.78
Global LV Function for Intervention on Day 3						
Left ventricular ejection fraction, %						
Baseline	41	46.1 (11.1)	22	41.6 (10.0)		
Follow-up	41	49.6 (14.2)	22	45.9 (13.8)		
Within-group change	41	3.5 (11.0) [0.1 to 6.9]	22	4.4 (10.6) [0 to 8.8]	–0.9 (–6.6 to 4.9)	.76
Regional LV Function for Intervention on Day 3						
Infarct zone wall motion, mm						
Baseline	41	4.2 (5.2)	22	3.7 (4.3)		
Follow-up	41	6.3 (6.9)	22	6.1 (6.7)		
Within-group change	41	2.1 (5.9) [0.3 to 3.9]	22	2.4 (5.3) [0.2 to 4.6]	–0.3 (–3.3 to 2.7)	.82
Border zone wall motion, mm						
Baseline	41	16.7 (11.2)	22	12.6 (11.0)		
Follow-up	41	20.2 (12.9)	22	16.9 (12.9)		
Within-group change	41	3.5 (9.3) [0.7 to 6.3]	22	4.3 (8.7) [0.7 to 7.9]	–0.8 (–5.6 to 4.0)	.75
Global LV Function for Intervention on Day 7						
Left ventricular ejection fraction, %						
Baseline	34	44.0 (9.9)	15	48.8 (10.9)		
Follow-up	34	46.8 (12.3)	15	50.4 (13.3)		
Within-group change	34	2.8 (9.7) [–0.5 to 6.1]	15	1.7 (8.2) [–2.4 to 5.8]	1.1 (–4.7 to 6.9)	.70
Regional LV Function for Intervention on Day 7						
Infarct zone wall motion, mm						
Baseline	34	3.8 (3.9)	15	4.7 (2.7)		
Follow-up	34	5.0 (5.5)	15	7.4 (5.9)		
Within-group change	34	1.2 (4.9) [–0.4 to 2.8]	15	2.8 (4.4) [0.6 to 5.0]	–1.6 (–4.5 to 1.4)	.30
Border zone wall motion, mm						
Baseline	34	13.5 (7.7)	15	13.8 (9.7)		
Follow-up	34	17.7 (10.3)	15	18.2 (13.6)		
Within-group change	34	4.2 (8.3) [1.4 to 7.0]	15	4.4 (7.2) [0.8 to 8.0]	–0.1 (–5.1 to 4.8)	.96

57.1%) with no overall change in differences between groups (1.1% [95% CI, –4.7% to 6.9%]; *P*=.70).

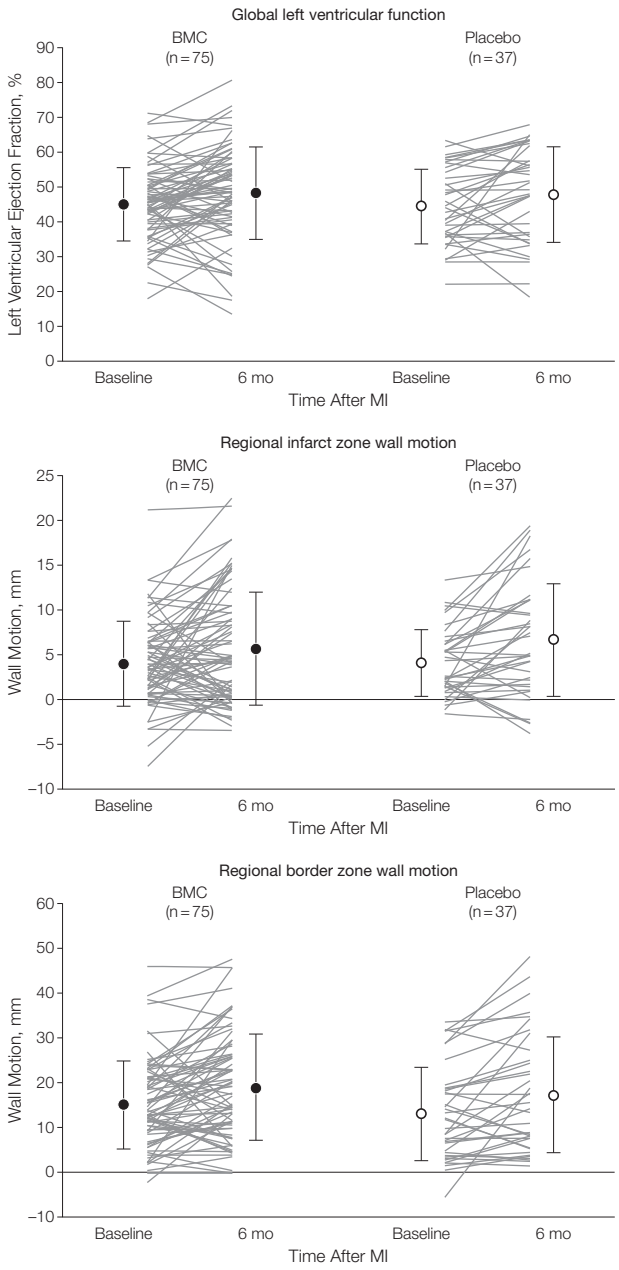
Regional wall motion in the infarct zone was 3.8 mm (95% CI, 2.5 to 5.1 mm) in the BMC group and 4.7 mm (95% CI, 3.3 to 6.1 mm) in the placebo group. Overall, there was no significant difference in changes in infarct wall motion from baseline to 6 months between the 2 groups (–1.6 mm [95% CI, –4.5 to 1.4 mm]; *P*=.30). Baseline border zone wall motion was 13.5 mm (95% CI, 10.9 to 16.1 mm) in the BMC group and 13.8 mm (95% CI, 8.9 to 18.7 mm) in the placebo group with no overall change in differences between the 2 groups over 6 months (–0.1 mm [95% CI, –5.1 to 4.8 mm]; *P*=.96).

For LVEF, the placebo-adjusted effect of BMC on day 3 was –0.9% (95% CI, –6.6% to 4.9%) and on day 7 was 1.1% (95% CI, –4.7% to 6.9%). The difference between the 2 groups was not significant (2.0% [95% CI, –6.3% to 10.2%]; *P*=.64). For infarct zone wall motion, the placebo-adjusted effect of BMC on day 3 was –0.3 mm (95% CI, –3.3 to 2.7 mm) and on day 7 was –1.6 mm (95% CI, –4.5 to 1.4 mm). This difference also was not significant (–1.2 mm [95% CI, –5.5 to 3.1 mm]; *P*=.57). For border zone wall motion, the placebo-adjusted effect of BMC on day 3 was –0.8 mm (95% CI, –5.6 to 4.0 mm) and on day 7 was –0.1 mm (95% CI, –5.1 to 4.8 mm). The difference between these was not significant (0.6 mm [95% CI, –6.3 to 7.6 mm]; *P*=.86).

Left ventricular end diastolic volume index increased by 11.7 mL/m^2 (95% CI, 7.4 to 16.0 mL/m^2) in the BMC group and by 10.9 mL/m^2 (95% CI, 5.1 to 16.7 mL/m^2) in the placebo group, which was not significantly different (change: 0.8 mL/m^2 [95% CI, –6.6 to 8.2 mL/m^2]; *P*=.83). Left ventricular end systolic volume index increased by 5.0 mL/m^2 (95% CI, 1.4 to 8.6 mL/m^2) in the BMC group and by 4.3 mL/m^2 (95% CI, –0.5 to 9.1 mL/m^2) in the placebo group (change: 0.7 mL/m^2 [95% CI, –5.5 to 7.0 mL/m^2]; *P*=.82). Infarct volumes uniformly decreased in both groups at both times but again, the differences between the BMC and placebo groups were not significant. Day of treatment did not influence the secondary end points. Models adjusting for center, age, diabetes, hypertension, hyperlipidemia, weight, infarct location, infarct size (peak creatine kinase level), and percentage of CD34 cells did not change the unadjusted results.

Several predetermined subgroup analyses were performed in the BMC group. In contrast to previous studies,[14,15] there was no improvement in the recovery of left ventricular func-

Figure 2. Global Left Ventricular Function and Regional Infarct and Border Zone Wall Motion



BMC indicates bone marrow mononuclear cell; MI, myocardial infarction.

tion among patients with more depressed LVEF at baseline (LVEF <45% via MRI). No difference was observed in global or regional function in patients stratified by ischemic time.

COMMENT

To our knowledge, TIME is the first cardiovascular cell therapy trial that was specifically designed to determine whether the timing of BMC administration after primary PCI influences left ventricular functional recovery. There was no overall effect of BMC treatment on this ongoing improvement at 6 months vs placebo despite previous supportive clinical data.[1,3] Additionally, the day of cell delivery did not demonstrate an effect on the recovery of left ventricular function or on left ventricular volumes or infarct size.

The design of TIME was based on previous data that the timing of cell delivery may be critical.[1,3,5] During the initial days to weeks after STEMI, there are significant temporal changes in the release of cytokines, such as stromal-derived factor 1,[16] and growth factors such as vascular endothelial growth factor and insulin-like growth factor 1, that may support stem cell homing and angiogenesis leading to improved cell survival and engraftment.

Conversely, reactive oxygen species and inflammatory cytokines such as interleukin 1 and tumor necrosis factor released by myocardium and circulating inflammatory cells may adversely affect the bone marrow and stem cell function and/or survival. These inflammatory mediators may impair the quality of cells harvested from the bone marrow as observed in a recent preclinical study demonstrating that BMCs are more potent several weeks after STEMI compared with those harvested a few days after STEMI as a result of inflammatory changes in the bone marrow mediated by interleukin 1.[17] The relative role of these potential positive and negative influences on cell therapy is uncertain.

TIME was developed shortly after early randomized trials suggested that autologous, intracoronary BMCs may improve left ventricular function after AMI.[7,9] Although several subsequent trials did not observe improved left ventricular function,[8,10,18,19] a Cochrane meta-analysis suggested small improvement in LVEF (mean change, 1.8% [95% CI, 0.3% to 3.3%) when measured by MRI as used in TIME.[1] A study to detect such a difference in LVEF would require 875 patients and would imply that this difference is biologically important. While these findings do not exclude this suggested effect size (for overall effect: 95% CI, –4.1 to 3.9; for day 3 effect: 95% CI –6.6 to 4.9; and for day 7 effect: 95% CI, –4.7 to 6.9), it is reasonable to critically examine some possible contributing aspects so that future studies in this area may proceed from an enlightened position.

Going forward it is crucial to understand how well this cohort did with contemporary management. In the age of aggressive primary prevention and rapid and successful primary PCI, identifying patients with significant left ventricular dysfunction after a first MI is challenging. The centers screened 3347 patients (of which about half did not have moderate or severe left ventricular dysfunction) to identify 132 patients who were randomized.[20] Among those qualifying with moderate or severe left ventricular dysfunction, ischemic time was remarkably brief (median, 3-4 hours), all received PCI with stenting, and guideline-based medications were highly used. This management was associated with recovery of left ventricular function, yielding an aggregate LVEF at 6 months exceeding 48%. As has been reported elsewhere, existing data indicate that LVEF would be expected to continue to increase at 18 and 36 months in half of the cohort and links with mortality are no longer apparent when LVEF exceeds 45%.[21] Since initiation of TIME and LateTIME, the Cardiovascular Cell Therapy Research Network has observed only a single cardiovascular-related death (subarachnoid hemorrhage prior to receiving study product) among 207 patients with moderate to large anterior STEMIs.

However, there is likely considerable heterogeneity among the cohort and it would be of interest to identify a population at greatest risk that might benefit (eg, those at risk for LVEF <45% at 6 months). If prospective cohorts cannot be identified, then an alternative approach is to recruit patients who have already demonstrated incomplete recovery at later time points and/or to consider novel cell types.[22] The development of novel and sensitive measures of left ventricular function to serve as surrogate end points continues to be a requirement in this field.

In addition, the phenotype and functionality of the BMC product in this population may be an issue. Bone marrow mononuclear cells from patients with ischemic cardiomyopathy have reduced colony-forming unit capacities and impaired migration to stromal-derived factor 1 and vascular endothelial growth factor that translate into reduced blood flow in the ischemic hind limb model.[20] Endothelial progenitor cells from patients with coronary artery disease also have impaired CXCR4 signaling with diminished neovascularization.[23] Cytokine production from BMCs is reduced compared with other bone marrow and adipose-derived cell types.[24] These considerations suggest that an autologous cell product derived from patients with coronary artery disease (as in TIME) may have less regenerative capacity vs allogeneic products obtained from younger, healthy donors.[25]

Although the field of cell therapy in cardiovascular disease has potential for identifying beneficial treatments, our study is consistent with the possibility that BMCs are not effective at improving left ventricular function when delivered into the immediate post-STEMI myocardial environment. However, long-term follow-up of these patients and the development of new composite end points may still reveal a role for this cell type after AMI. Recent and ongoing studies continue to assess the role of BMCs in other areas

such as heart failure and critical limb ischemia.[26]

CONCLUSIONS

Overall, the delivery of BMCs at 3 or 7 days after a STEMI and primary PCI did not affect subsequent improvement in left ventricular function at 6 months compared with placebo. These data should inform the future development of cell therapies for STEMI.

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