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Comparison of Different Bone Marrow–Derived Stem Cell Approaches in Reperfused STEMI

A Multicenter, Prospective, Randomized, Open-Labeled TECAM Trial

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ABSTRACT

BACKGROUND Stem cell–based therapy has emerged as a potential therapy in acute myocardial infarction (AMI). Although various approaches have been studied, intracoronary injection of bone marrow autologous mononuclear cells (BMMC) and the ability of granulocyte colony-stimulating factor (G-CSF) to mobilize endogenous cells have attracted the most attention.

OBJECTIVES This study compares, for the first time, the efficacy of BMMC injection, G-CSF mobilization, and the combination of both with standard treatment.

METHODS On Day 1 after primary percutaneous coronary intervention, 120 patients were randomized to a 1) intracoronary BMMC injection; 2) mobilization with G-CSF; 3) both (BMMC injection plus G-CSF); or 4) conventional treatment (control group). G-CSF, 10 μg/kg/day subcutaneously, was started Day 1 and maintained for 5 days. BMMC injection was performed on Days 3 to 5. Our primary endpoint was absolute change in 12-month left ventricular ejection fraction (LVEF) and left ventricular end-systolic volume (LVESV) relative to baseline measured by cardiac magnetic resonance.

RESULTS The mean change in LVEF between baseline and follow-up for all patients was 4 ± 6% ($p = 0.006$). Change in LVEF and LVESV over time did not differ significantly among the 4 groups. Patients actively treated with any stem cell approach showed similar changes in LVEF and LVESV versus control subjects, with a small but significant reduction in infarct area ($p = 0.038$).

CONCLUSIONS In our study, 3 different bone marrow–derived stem cell approaches in AMI did not result in improvement of LVEF or volumes compared with standard AMI care (Trial of Hematopoietic Stem Cells in Acute Myocardial Infarction [TECAM]; NCT00984178) (J Am Coll Cardiol 2015;65:2372–82) © 2015 by the American College of Cardiology Foundation.

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In the last decade, stem cell-based therapy has been evaluated as a potential therapeutic option for patients with acute myocardial infarction (AMI). Different routes of cell delivery have been evaluated, but intracoronary injection of autologous bone marrow mononuclear cells (BMMC) and mobilization of endogenous stem cells by granulocyte colony-stimulating factor (G-CSF) have attracted the most attention.

SEE PAGE 2383

Randomized clinical trials (RCTs) of these approaches have showed variable outcomes in terms of improved cardiac contractile function and suppressed left ventricular (LV) negative remodeling (1). However, the efficacy of these two specific approaches has never been compared nor has their combination been tested. The aim of this clinical trial was to compare the efficacy of intracoronary injection of BMMC, mobilization alone by G-CSF, or a combination of both therapies (intracoronary injection of BMMC plus G-CSF mobilization) with conventional treatment in the acute phase of AMI.

METHODS

The TECAM (Trial of Hematopoietic Stem Cells in Acute Myocardial Infarction) study was a randomized (1:1:1:1), multicenter, open-label, single-blind, controlled trial in patients with ST-segment elevation AMI (STEMI) who were successfully reperfused to compare the efficacy of 3 different approaches to cell therapy for preventing adverse ventricular remodeling compared with conventional therapy.

This study is an academic clinical trial. The study sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The corresponding authors had full access to all study data and were responsible for the decision to submit for publication.

PATIENT POPULATION. From November 2005 to January 2010, patients were enrolled from 8 Spanish hospitals. The patients were enrolled from the following institutions: Instituto de Ciencias del Corazón (n = 81), Hospital General Universitario Gregorio Marañón (n = 21), Hospital Universitario de Salamanca (n = 5), Hospital Río Hortega (n = 4), Complejo Hospitalario de León (n = 4), Hospital Río Carrión de Palencia (n = 3), Hospital de Segovia (n = 1), and Hospital General Yague de Burgos (n = 1). Inclusion criteria were as follows: age ≥18 years, AMI diagnosis with cardiac enzyme release and total summed ST-segment elevation ≥6 mm, akinesis or hypokinesis in the infarct-related artery area, successful reperfusion either with primary percutaneous coronary intervention (PCI) or post-fibrinolysis PCI with a final Thrombolysis In Myocardial Infarction 3 grade flow, rapamycin drug-eluting stent (DES) implantation in the infract-related artery, and adequate revascularization of the remaining coronary arteries before stem cell therapy. Exclusion criteria were as follows: cardiogenic shock; suspicion or evidence of infarct mechanical complication; history of sustained ventricular tachycardia or atrial fibrillation; patient with cardiac defibrillator or candidate for its potential implantation; investigational drug treatment in the previous 4 weeks; actual or potential use of antineoplastic drugs; oncology antecedents in the last 5 years; previous treatment with transmyocardial laser revascularization; women of childbearing potential; severe concomitant disease-modifying patient's survival during the study; active bleeding or major surgery within 2 weeks forbidding the use of heparin, abciximab, or antiplatelet therapy; previous malignant hematology disease or hypercoagulability disorders; previous known renal failure (creatinine >2.5 mg/dl); any kind of stroke in the previous year or any episode ever of hemorrhagic stroke; major surgery pending in the next year; previously known vascular disease that prevents catheterization; evidence of hypersensitivity to G-CSF treatment (filgrastim); or inability to give written informed consent.

Approval was obtained from national and institutional ethics committees and informed written consent was obtained from each patient. All patients were then randomized using a central telephone system. Blocking was used to generate the random allocation sequence. The block lengths were 4-, 8-, and successive 4-size blocks.

STUDY PROTOCOL AND STEM CELL APPROACHES. The day of infarct-related artery revascularization was defined as Day 0. All patients were reperfused with a rapamycin DES per protocol. On Day 1, patients were randomly assigned to either: 1) intracoronary injection of BMMC; 2) mobilization with G-CSF; 3) both therapies; or 4) conventional treatment of AMI (control group).

G-CSF injection started immediately after randomization, with a dose of 10 μg/kg/day subcutaneously and maintained for 5 days, both in the mobilization-alone and combined groups. Treatment with BMMC was performed on Days 3 to 5, again in

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

BMMC = bone marrow mononuclear cell(s)

CD = cluster of differentiation

CI = confidence interval

CMR = cardiac magnetic resonance imaging

DES = drug-eluting stent(s)

G-CSF = granulocyte colony-stimulating factor

HF = heart failure

LVEDV = left ventricular end-diastolic volume

LVEF = left ventricular ejection fraction

LVESV = left ventricular end-systolic volume

PCI = percutaneous coronary intervention

RCT = randomized clinical trial

STEMI = ST-segment elevation myocardial infarction

TECAM = Terapia Celular Aplicada al Miocardio

WMSI = wall motion score index

both the injection-alone and the combined group. A total of 50 ml of bone marrow was aspirated under local anesthesia from the iliac crest. The aspirate was filtered and centrifuged and isolated by Ficoll density separation. The interface was collected and washed twice with heparinized phosphate-buffered saline and resuspended at approximately 5 x 10^6 cells/ml in heparinized saline. Just before intracoronary injection, a small BMMC sample was collected for cytometry studies (FACScalibur, BD Biosciences, San Jose, California) for cluster of differentiation (CD) 34+, CD117+, CD133+, and cell viability analyzed using trypan blue reagent. An over-the-wire balloon catheter positioned at the site of stent implantation was inflated at 2 to 4 atm until complete block of blood flow. Then, the guidewire was retired and the BMMC suspension was infused with a pump at 1 to 2 ml/min during 3-minute periods of inflation and cell infusion alternating with 1 minute of deinflation and reperfusion until the total BMMC dose was given.

FOLLOW-UP. Follow-up included clinical evaluation at baseline, 30 days, and every 3 months up to 12 months; determination of creatine kinase, creatine kinase MB, and cardiac troponin T before and 24 h after transplantation; continuous electrocardiography monitoring from randomization to hospital discharge; echocardiography and cardiac magnetic resonance imaging (CMR) at baseline and 12 months; and cardiac catheterization at baseline (after randomization) and 12 months, including LV angiography and coronary angiography. Any of the following were regarded as major cardiac events: death of any origin, reinfarction, heart failure (HF), rehospitalization, target-vessel revascularization, and ventricular arrhythmias or syncope.

LV FUNCTION ASSESSMENTS. CMR was performed by means of scanners operating at 1.5-T. Image acquisition was done as previously described (2). In brief, global and regional LV function was assessed with breath-hold cineCMR in the cardiac short axis, vertical axis, and horizontal long axis. A 16-segment model was used, and each ventricular segment was given a score according to its motion: 1 = normal, 2 = hypokinetic, 3 = akinetic, and 4 = dyskinetic. Wall motion score index (WMSI) was calculated from the sum of the segmental scores divided by the segments visualized. A late gadolinium enhancement study was performed 15 min after intravenous administration of 0.2 mmol/kg body weight gadolinium-diethylene-triaminepentaacetate. Microvascular obstruction was defined as late hypoenhancement within a hyperenhanced region on late gadolinium enhancement images. Infarct size was identified as the zone of bright signal on late-enhanced images and was related to LV myocardial mass to calculate infarct area. LV volumes and ejection fraction were calculated with the use of Mass CMS software (Advion, Inc., Ithaca, New York).

LV angiograms were obtained in identical standard projections at baseline and at 12 months. LV ejection fraction (LVEF), LV end-systolic volume (LVESV), and LV end-diastolic volume (LVEDV) were calculated by the area-length method with the use of CMS software version 6.0 (Medism, Leiden, the Netherlands).

All CMR, coronary angiograms, and LV angiographies were analyzed at an independent central imaging core laboratory (ICICORELAB, Valladolid, Spain) blinded to patient treatment assignment.

STUDY ENDPOINTS. The primary endpoint was absolute change in global LVEF and in LVESV from baseline to 12 months, as measured by CMR. Secondary endpoints included changes in LVEDV, infarct size, infarct area, WMSI, and clinical events. Specified subgroup analyses were conducted to determine whether there was an interaction of the primary endpoint with baseline LVEF, time to PCI, infarct location, age, and microvascular obstruction. Based on our previous published data (2), we calculated that we would need a total of 88 patients (22 in each group) to detect a difference in global LVEF change of 5%, as measured by CMR, with an 80% power and a 2-sided alpha level of 0.05. We estimated follow-up loss and adjusted the sample size to 30 patients in each group.

DATA COLLECTION AND STATISTICAL ANALYSIS. Data were entered using a double-entry system and the accuracy of collected data was validated against medical records by an independent clinical research organization (Chiltern International Spain SA, Madrid, Spain). Data were then submitted to the data-coordinating center (Hospital Gregorio Marañón). Clinical outcome was adjudicated by an independent clinical events committee, blinded to study group assignment. A separate data and safety monitoring board, not affiliated with the study investigators, reviewed data periodically throughout the trial to identify potential safety issues and monitor study conduct.

Mean ± standard deviation, median, maximal, minimal, and number of observations were used to describe continuous variables, and frequencies were calculated for categorical variables. Differences between groups were assessed using 1-way breakdown analysis of variance for continuous variables or nonparametric tests when necessary. Categorical

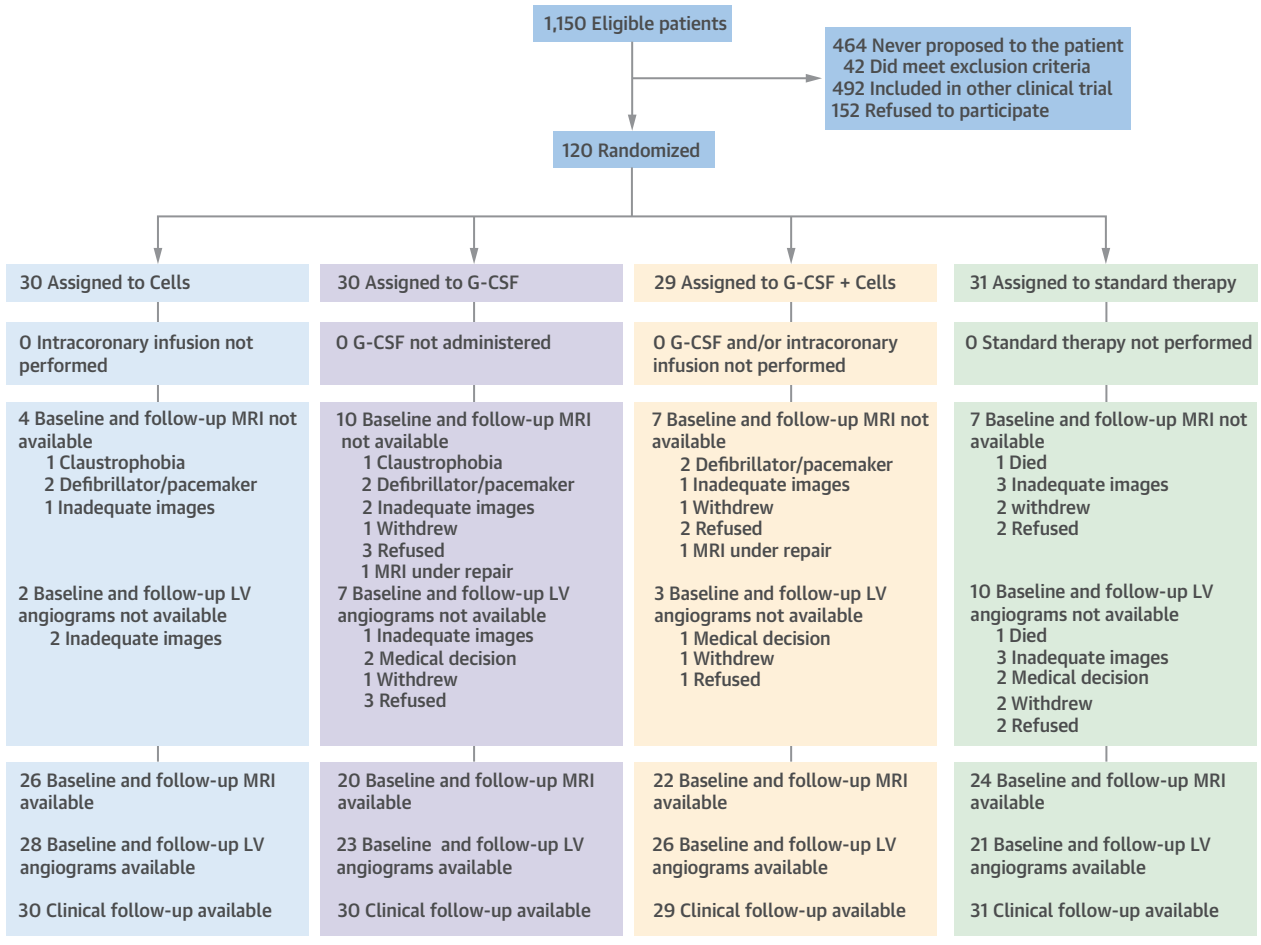
variables were compared using the chi-square and the Fisher exact tests when necessary. Confidence intervals (CIs) were calculated to estimate the difference between 2 means when necessary. A p value <0.05 was considered to indicate statistical significance. All reported p values are 2-sided. Statistical analyses were performed with PASW (SPSS) software version 18 (IBM Corporation, Armonk, New York).

RESULTS

ENROLLMENT AND BASELINE CHARACTERISTICS. Figure 1 shows the trial profile. A total of 120 patients with AMI successfully reperfused by means of rapamycin DES implantation gave written informed consent to participate in the trial. Thirty patients were randomly assigned to receive BMMC, 30 to G-CSF, 29 to BMMC plus G-CSF, and 31 to a control group where no aspiration or sham infusion was performed.

Baseline characteristics did not differ significantly among the 4 groups and revealed a typical distribution of risk factors and evidence-based medication (Table 1). Thirty-one (26%) patients were reperfused using primary PCI and 89 (74%) using post-fibrinolysis PCI; 37 patients underwent rescue PCI and 52 patients delayed PCI. All 4 groups were well-balanced in terms of reperfusion times, with a median time from symptom onset to initial reperfusion (primary PCI or fibrinolysis) <5 h. Delays were similar to those reported in our previous intervention trials in patients with STEMI (3,4). After PCI, all

FIGURE 1 Trial Profile



A total of 120 patients with reperfused acute myocardial infarction were randomized to receive cells (bone marrow mononuclear cells), granulocyte colony-stimulating factor (G-CSF), both (G-CSF plus cells), or standard therapy (no aspiration or sham infusion performed). The chart includes baseline and follow-up cardiac magnetic resonance imaging (MRI), left ventricular (LV) angiograms, and clinical follow-up available.

patients achieved Thrombolysis In Myocardial Infarction 3 flow grade.

With respect to cell transfer, median and range time from bone marrow collection to cell delivery was 21 h (16 to 24 h) with no significant difference among groups. Centrifugation and resuspension reduced cell volume from bone marrow harvest to a final volume of 10 ml, which we injected into the infarct-related coronary artery and which contained a median quantity of BMMC almost 7 times higher in patients previously treated with G-CSF versus those who did not receive G-CSF (560 vs. 83 x 10^6 cells; $p < 0.001$). The phenotypic characteristics of the injected cells are shown in Table 1. Of note, variability of bone marrow aspiration volume, number of injected cells, and percentage and absolute number of progenitors was high between patients.

LV FUNCTION VARIABLES. Paired (baseline and follow-up) LV assessments were available in 92 patients by CMR (n = 26, BMMC group; n = 20, G-CSF group; n = 22, combined group; and n = 24, control group) and in 98 patients undergoing LV angiography (n = 23, BMMC group; n = 28, G-CSF group; n = 26, combined group; and n = 21, control group). Reasons why 28 paired magnetic resonance imaging and 22 paired LV angiographies were not available are explained in Figure 1.

Table 2 shows baseline and follow-up CMR parameters. After 12 months, CMR showed no significant improvement in LVEF (baseline 48 ± 9%, follow-up 52 ± 10%, overall 4 ± 6%; 95% CI: 1.15 to 6.65; p = 0.006). The individual analysis for each group showed a significant increase of LVEF in comparison with baseline values only for the BMMC group (95% CI: 0.70 to 10.58; p = 0.026), with similar trends for the G-CSF group (95% CI: -3.99 to 8.49; p = 0.470), the combined group (95% CI: -1.61 to 9.15; p = 0.164), and the control group (95% CI: -1.43 to 9.09; p = 0.150). Of note, comparison of the absolute change in LVEF relative to baseline, 1 of our 2 specified primary endpoints, showed no significant difference among our 4 study groups.

LVESV and LVEDV remained almost unchanged from baseline to 12-month assessment for all patients; from 83 ± 27 to 80 ± 31 (p = 0.592) for LVESV and from 157 ± 31 to 166 ± 39 (p = 0.104) for LVEDV. Similarly, changes in LVESV and LVEDV did not differ over time for any individual randomized group. Neither the absolute change in LVESV relative to baseline, our second specified primary endpoint, nor the absolute change in LVEDV showed significant differences among the 4 comparison groups.

With respect to other CMR-determined LV efficacy parameters, mean infarct size significantly decreased from 21 ± 13 g at baseline to 14 ± 9 g at 12 months (4 ± 6% for all patients; 95% CI: -3.78 to -10.22; $p < 0.001$). The individual analysis for each group showed a significant decrease in infarct size versus baseline values for the BMMC group (95% CI: -0.40 to -12.62; p = 0.042), the G-CSF group (95% CI: -0.98 to -14.34; p = 0.026), and the combined group (95% CI: -3.79 to -15.25; p = 0.002), with no significant differences among control subjects (95% CI: -11.22 to 2.50; p = 0.207). However, comparison of the absolute reduction in infarct size relative to baseline showed no significant difference among the study groups. Also, all groups demonstrated similar overall infarct size.

Finally, regional contractility improved, as demonstrated by a significantly better WMSI of the infarcted wall, between baseline (1.56 ± 0.25) and follow-up (1.39 ± 0.24); for all patients, -0.18 ± 0.17 percentage points (95% CI: -0.11 to -0.25; $p < 0.001$). The individual analysis for each randomized group indicated a better WMSI of the infarcted wall over baseline values for BMMC injection (95% CI: -0.05 to -0.30; p = 0.007), G-CSF administration (95% CI: 0.01 to -0.35; p = 0.062), BMMC injection and G-CSF administration (95% CI: -0.08 to -0.35; p = 0.002), and standard of care (95% CI: -0.04 to -0.32; p = 0.021); but again, there was no significant difference based on absolute change in WMSI of the infarcted wall relative to baseline for any of the groups studied.

LV angiography reproduced exactly the findings observed by CMR for LVEF, LVESV, and LVEDV (Table 2).

PROCEDURAL SAFETY AND CLINICAL OUTCOMES. Two patients receiving intracoronary injection of BMMC (1 each in the BMMC and combined groups) presented periprocedural MI, defined as troponin elevation of at least 50% with respect to the troponin level before BMMC injection, with excellent clinical resolution. In patients treated with G-CSF, white blood cell count increased markedly during G-CSF administration, with no alteration in other rheology parameters, such as serum fibrinogen and blood viscosity. There was no evidence of transiently elevated body temperature or relevant bone pain, or any adverse events related to G-CSF administration.

Occurrence of major adverse cardiac events did not significantly differ among groups (Table 3). We noted no differences in treatment-related tachyarrhythmia on electrocardiography monitoring. Triplets (3 successive premature ventricular complexes and nonsustained ventricular tachycardias) were observed during hospitalization: 5 patients with BMMC injection, 5 patients with G-CSF, 3 patients from the combined group, and 6 control subjects. One BMMC

TABLE 1 Baseline Characteristics

	BMMC (n = 30)	G-CSF (n = 30)	BMMC + G-CSF (n = 29)	Control Subjects (n = 31)	p Value
Age, yrs	54 ± 11	57 ± 9	56 ± 8	57 ± 11	0.591
Male	29 (97)	25 (83)	25 (86)	28 (90)	0.375
Hypertension	8 (27)	12 (40)	9 (31)	14 (45)	0.426
Diabetes mellitus	5 (17)	3 (10)	7 (24)	3 (10)	0.356
Hyperlipidemia	17 (57)	15 (50)	11 (38)	11 (35)	0.299
Smoking history	25 (83)	26 (87)	21 (72)	24 (77)	0.529
Family history of coronary disease	5 (17)	6 (20)	7 (24)	10 (32)	0.508
Previous history of coronary disease	2 (7)	3 (10)	1 (3)	1 (3)	0.644
Coronary artery disease					0.693
1-vessel disease	23 (77)	22 (73)	22 (76)	22 (71)	
2-vessel disease	6 (20)	8 (27)	7 (24)	7 (23)	
3-vessel disease	1 (3)	0 (0)	0 (0)	2 (6)	
Infarct-related artery					0.077
Left anterior descending coronary artery	20 (67)	20 (67)	24 (83)	21 (68)	
Left circumflex artery	0 (0)	4 (13)	0 (0)	1 (3)	
Right coronary artery	10 (33)	6 (20)	5 (17)	9 (29)	
PCI in noninfarct-related arteries	18 (60)	14 (47)	15 (52)	20 (65)	0.497
Time from symptom onset to primary PCI, h	4 (3-6)	3 (2-5)	4 (1-6)	3 (3-7)	0.818
Time from symptom onset to post-fibrinolysis PCI, h	14 (8-23)	18 (8-30)	16 (7-23)	17 (9-23)	0.779
Maximal creatine kinase, x ULN	3,641 ± 3,075	2,880 ± 1,494	3,838 ± 1,930	2,980 ± 2,179	0.247
Maximal creatine kinase MB, x ULN	337 ± 262	321 ± 198	350 ± 223	328 ± 209	0.972
Maximal troponin T, x ULN	11 ± 8	13 ± 30	22 ± 45	19 ± 37	0.514
NT-proBNP at randomization, x ULN	1,213 ± 1,444	1,178 ± 1,165	1,400 ± 698	1,836 ± 1,657	0.361
Time from reperfusion to G-CSF administration, h		39 (27-44)	44 (30-48)		0.251
Time from reperfusion to intracoronary infusion of cells, h	190 (164-216)		190 (178-212)		0.358
Bone marrow aspiration volume, ml	60 (31-65)		55 (20-70)		0.319
Number of injected cells, x 10^6	83 (60-117)		560 (351-915)		<0.001
Viability, %	89 ± 9		91 ± 6		0.321
CD34+, %	1.03 (0.41-1.08)		0.31 (0.19-1.14)		<0.001
CD34+, absolute number x 10^6	0.83 (0.33-2.07)		2.93 (0.69-22.6)		<0.001
CD133+, %	0.55 (0.24-0.85)		0.23 (0.08-0.41)		<0.001
CD133+, absolute number x 10^6	0.38 (0.15-1.1)		2.4 (0.4-14.3)		<0.001
CD117+, %	1.27 (0.67-2.04)		0.4 (0.19-1.6)		<0.001
CD117+, absolute number x 10^6	1.14 (0.37-2.78)		3.23 (0.81-32.7)		<0.001
Medication at discharge					
Aspirin	27 (90)	29 (100)	25 (93)	30 (97)	0.314
Clopidogrel	30 (100)	29 (100)	27 (100)	30 (97)	0.424
Beta-blocker	28 (93)	24 (83)	24 (89)	29 (94)	0.481
ACE inhibitor or AT II blocker	26 (87)	24 (83)	23 (85)	23 (74)	0.588
Diuretics	2 (7)	3 (11)	4 (15)	2 (7)	0.670
Statins	28 (93)	28 (97)	27 (100)	30 (97)	0.590
Medication at 12 months follow-up					
Aspirin	27 (90)	27 (93)	26 (96)	26 (87)	0.603
Clopidogrel	29 (97)	28 (97)	27 (100)	30 (100)	0.579
Beta-blocker	26 (87)	26 (89)	25 (93)	27 (90)	0.909
ACE inhibitor or AT II blocker	29 (97)	26 (90)	27 (100)	29 (97)	0.271
Diuretics	0 (0)	4 (14)	8 (30)	6 (20)	0.017
Statins	30 (100)	29 (100)	26 (96)	29 (97)	0.548

Values are mean ± SD, n (%), or median (range with the minimal and maximal observations).

ACE = angiotensin-converting enzyme; AT = angiotensin; BMMC = bone marrow mononuclear cell(s); CD = cluster of differentiation; G-CSF = granulocyte colony-stimulating factor; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention; ULN = upper limit of normal.

TABLE 2 Quantitative Measures of LV Function

	BMMC	G-CSF	BMMC + G-CSF	Control Subjects	p Value
CMR	26	20	22	24	
Global LV ejection fraction, %					
Baseline	49 ± 8	54 ± 9	45 ± 9	47 ± 8	0.008
Follow-up	55 ± 10	56 ± 10	48 ± 8	51 ± 10	0.050
Absolute difference	6 ± 6	2 ± 5	4 ± 7	4 ± 7	0.365
End-diastolic volume, ml					
Baseline	148 ± 26	159 ± 31	163 ± 32	161 ± 34	0.308
Follow-up	160 ± 46	159 ± 31	171 ± 39	174 ± 37	0.436
Absolute difference	12 ± 51	-0.1 ± 30	8 ± 25	12 ± 32	0.524
End-systolic volume, ml					
Baseline	77 ± 23	77 ± 25	91 ± 29	86 ± 29	0.230
Follow-up	74 ± 36	70 ± 24	89 ± 28	88 ± 32	0.103
Absolute difference	-3 ± 26	-7 ± 15	-2 ± 19	2 ± 24	0.362
Infarct size, g					
Baseline	20 ± 13	19 ± 12	28 ± 11	18 ± 14	0.068
Follow-up	13 ± 10	12 ± 9	18 ± 8	12 ± 9	0.110
Absolute difference	-6 ± 12	-9 ± 8	-10 ± 9	-4 ± 10	0.113
LV muscle mass, g					
Baseline	126 ± 22	127 ± 24	129 ± 20	136 ± 18	0.363
Follow-up	114 ± 20	105 ± 23	116 ± 22	120 ± 22	0.157
Absolute difference	-12 ± 15	-21 ± 11	-13 ± 16	-16 ± 17	0.216
Infarct size in relation to LV muscle mass, %					
Baseline	16 ± 11	16 ± 10	22 ± 7	14 ± 10	0.111
Follow-up	12 ± 9	11 ± 9	16 ± 7	12 ± 8	0.112
Absolute difference	-4 ± 10	-6 ± 5	-5 ± 7	-1 ± 7	0.062
Wall motion score index					
Baseline	1.51 ± 0.23	1.50 ± 0.26	1.71 ± 0.25	1.54 ± 0.21	0.012
Follow-up	1.34 ± 0.22	1.33 ± 0.29	1.50 ± 0.19	1.38 ± 0.24	0.065
Absolute difference	-0.17 ± 0.18	-0.17 ± 0.16	-0.21 ± 0.18	-0.16 ± 0.17	0.773
LV angiogram	28	23	26	21	
Global LV ejection fraction, %					
Baseline	50 ± 12	54 ± 14	47 ± 13	49 ± 12	0.268
Follow-up	58 ± 14	58 ± 14	50 ± 12	52 ± 12	0.051
Absolute difference	8 ± 12	5 ± 13	3 ± 12	3 ± 13	0.413
End-diastolic volume, ml					
Baseline	140 ± 34	132 ± 30	133 ± 30	127 ± 37	0.559
Follow-up	148 ± 32	141 ± 37	146 ± 37	137 ± 35	0.707
Absolute difference	8 ± 44	9 ± 37	13 ± 34	10 ± 45	0.963
End-systolic volume, ml					
Baseline	69 ± 19	62 ± 26	70 ± 21	67 ± 33	0.667
Follow-up	61 ± 26	60 ± 27	74 ± 29	66 ± 28	0.244
Absolute difference	-8 ± 24	-2 ± 21	3 ± 26	-1 ± 32	0.467

Values are n or mean ± SD.
CMR = cardiac magnetic resonance imaging; LV = left ventricular; other abbreviations as in Table 1.

patient with severely depressed LVEF presented with electrical storm and monomorphic sustained ventricular tachycardia 3 days after cell transplantation, even though there was no periprocedural complication. This patient received an implantable cardioverter-defibrillator after ruling out coronary reocclusion. During follow-up, 1 G-CSF patient presented with episodes of nonsustained ventricular tachycardias 3 months post-AMI. He received an implantable cardioverter-defibrillator after showing syncopal sustained ventricular tachycardias through programmed electrophysiological study. Three additional defibrillators were implanted during follow-up for primary prevention (1 BMMC patient, 2 combined group patients). One G-CSF patient required pacemaker implantation for complete auriculoventricular block development. In addition to the 2 previously described periprocedural myocardial infarctions, 1 BMMC patient presented with an acute in-stent thrombosis that was successfully treated with 2

additional DES. Finally, symptoms and signs of congestive HF requiring therapy were observed during follow-up in 10 patients: 1 with BMMC injection, 2 with G-CSF, 2 with BMMC plus G-CSF, and 5 control subjects. One control patient who developed HF died during follow-up.

Because patient number was limited and may have had an impact on LV outcome parameters, we analyzed the impact of any active cell therapy compared with the control group. At 12 months, there were no differences between active treatment and control subjects based on absolute changes in LVEF, LVEDV, LVESV, and WMSI. Although infarct size decreased over time in control subjects and in patients with active therapy, the absolute change in myocardial infarct area was significantly greater after active therapy, decreasing from 19 ± 10% at baseline to 13 ± 8% at 12-month follow-up, with an absolute change of -5 ± 7 percentage points (95% CI: -0.21 to -7.20; p = 0.038) (**Central Illustration**).

The lack of any additional active treatment effect on LVEF and remodelling was consistent across clinically relevant subgroups (baseline LVEF, time from bone marrow collection to cell delivery, time to PCI, infarct location, age, and microvascular obstruction).

TABLE 3 Clinical Events

	BMMC (n = 30)	G-CSF (n = 30)	BMMC + G-CSF (n = 29)	Control Subjects (n = 31)	p Value
Death	0 (0)	0 (0)	0 (0)	1 (3.2)	0.408
Recurrence of myocardial infarction	2 (6.7)	0 (0)	1 (3.4)	0 (0)	0.283
Heart failure	1 (3.3)	2 (6.7)	2 (6.9)	5 (16.1)	0.304
Rehospitalization	3 (10)	3 (10)	1 (3.4)	3 (9.7)	0.754
Ventricular arrhythmia or syncope	6 (20.0)	6 (20.0)	3 (10.3)	6 (19.4)	0.714
Any event	8 (26.7)	10 (33.3)	5 (17.2)	9 (29)	0.554

Values are n (%).
Abbreviations as in Table 1.

DISCUSSION

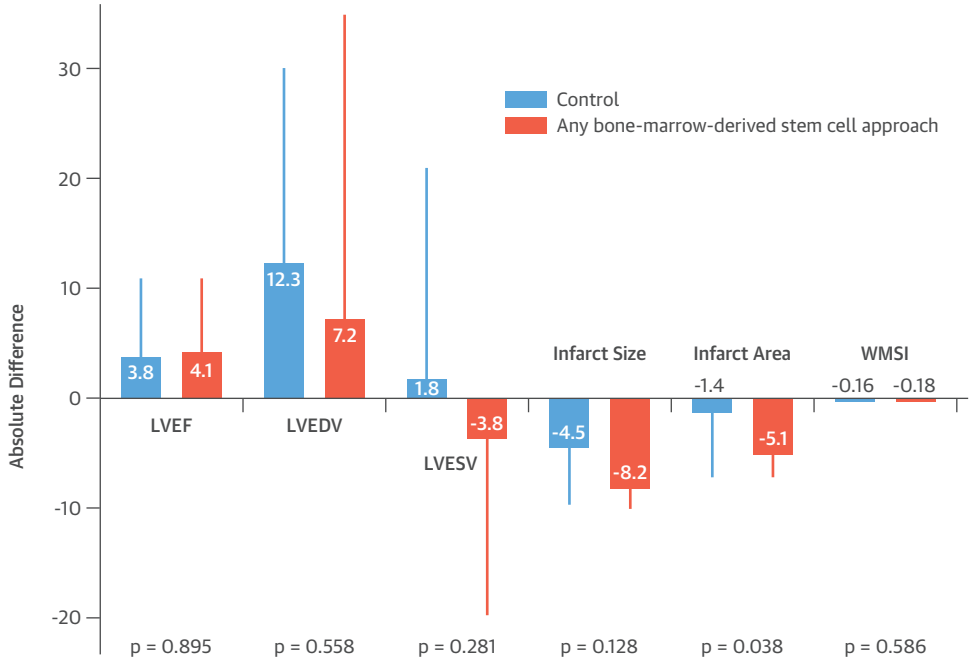
The randomized TECAM study shows a lack of relevant clinical benefit in global LV functional recovery after 12 months for patients with STEMI treated by 1 of 3 different approaches: intracoronary injection of BMMC, mobilization with G-CSF, or combined BMMC and G-CSF versus standard AMI care. Although a modest 4% improvement in LVEF was found overall, comparable with contemporary randomized reperfusion trials in patients with similar characteristics (3,4), none of the intervention groups showed greatly increased LVEF compared with control subjects. Furthermore, LV volumes did not differ among the treated groups at baseline or at 12-month follow-up, refuting our primary hypothesis that in timely reperfused AMI, any of the aforementioned cell therapies would significantly augment LV functional recovery, increasing LVEF or decreasing LVESV over time. Results were consistent for both imaging methods used: CMR and LV angiography.

Because patient number was limited and may have impacted LV outcome parameters, we grouped all patients receiving active treatment and compared them with the standard care group. Although the absolute change in LVEF and volumes over time showed no significant differences, reduction of the infarct area over 12 months, as measured by serial contrast-enhanced CMR, was significantly greater with active treatment compared with control subjects. This additional reduction in myocardial infarct area suggests a potentially interesting biological effect of cell-based therapy on infarct remodeling and is consistent with previous RCTs of BMMC intracoronary injection (5) or G-CSF mobilization (6). However, without enhanced recovery of regional function in infarcted segments, we should be cautious not to overestimate this finding, which must be confirmed in future RCTs.

The TECAM trial is unique for several reasons. First of all, in patients with AMI, BMMC injection has been compared in 23 RCTs (5,7-28) and mobilization by G-CSF alone in 9 RCTs (6-14), but ours is the first RCT to explore combining the 2 in patients with AMI. A similar approach with G-CSF therapy and subsequent intracoronary infusion of collected peripheral blood stem cells improved LVEF and cardiac remodeling in patients with AMI (15,16). In our experience, the combined strategy did not improve global or regional function at 12 months compared with control subjects. Moreover, our use of CMR, currently considered the gold standard for primary endpoint measures of efficacy, has to be underlined. To date, 9 (39%) BMMC injection RCTs (5,9,14,20,22,24,25,27,28), and 3 (33%) G-CSF mobilization RCTs (6,10,11), used CMR. Because patient number may have limited our study and influenced LV outcome parameters, it is important to put into perspective the number of paired CMR studies performed in our actively treated patients (n = 68), which surpasses all but 3 previous studies of intracoronary BMMC injection, which analyzed 139 (17), 107 (28), and 75 (18) CMR studies. We also had more paired CMR studies than any of the previous G-CSF RCTs (10,11,6). Thus, our trial reaches one of the highest rates of paired CMR studies to date.

The TECAM trial confirms the lack of effect of intracoronary BMMC infusion on LV cardiac function seen in a meta-analysis of RCTs that used

CENTRAL ILLUSTRATION **Bone Marrow-Derived Stem Cell Treatment for Cardiac Remodeling**



San Roman, J.A. et al. J Am Coll Cardiol. 2015; 65(22):2372–82.

In comparing absolute differences in indices of left ventricular (LV) cardiac remodeling in patients with reperfused ST-segment elevation myocardial infarction, a bone marrow–derived stem cell approach only significantly improved infarct area (%) over control subjects; no differences were seen in ejection fraction (LVEF, %), end-diastolic volume (LVEDV, ml), end-systolic volume (LVESV, ml), infarct size (g), or wall motion score index (WMSI). Values are means **(bars)** and standard deviations **(lines)**.

CMR-derived endpoints (24). Furthermore, our neutral results for G-CSF therapy are consistent with previous RCTs using CMR (6,10,11), and a general meta-analysis (25). In contrast to previous studies (26,27), and in agreement with previous RCTs, there was no improvement in the recovery of LV function among patients with more depressed LVEF at baseline.

A positive correlation has been suggested between the BMMC dose infused and the LVEF effect measured by CMR. However, this observation was raised by a recent meta-analysis and by a post-hoc analysis (1,28), with the only RCT study to date designed to test that hypothesis (29), randomizing patients to either higher (10^8) or lower (10^7) BMMC injection, also failing to show any difference in LVEF or improvement in volumes. The TECAM trial represents an excellent opportunity to analyze whether the number of transplanted cells could be associated with an improvement in LV function. When comparing patients randomized to injection of BMMC alone (83×10^6 cells) with patients receiving injection of BMMC after G-CSF mobilization (560×10^6 cells; $p < 0.001$), no difference was seen in the absolute change in LVEF (5.6 ± 6.1 vs. 3.8 ± 7.2, respectively; $p = 0.347$) or LVESV (-3.0 ± 26 vs. -1.8 ± 19, respectively; $p = 0.856$) despite the fact the absolute numbers of CD34+, CD133+, and CD117+ progenitor cells injected were higher in the combined group.

One of the most controversial considerations with bone marrow-derived stem cell therapy is whether it affects clinical outcome in patients with AMI. The meta-analysis by Jeevanantham et al. (30) showed a reduction in all-cause mortality, cardiac mortality, recurrent AMI, hospitalization for HF, and in-stent thrombosis after BMMC transplantation. In contrast, the meta-analysis by de Jong et al. (24) described no beneficial effect on major adverse cardiac and cerebrovascular events. Active bone marrow-derived therapy in our RCT did not lead to a reduction in clinical outcomes at 12 months.

STUDY LIMITATIONS. There are several limitations and potential explanations why this study was neutral. First, the randomized TECAM trial had an open-label design. Also, patient numbers were

small and may have had an impact on LV outcome parameters. No adverse remodeling was observed in the control group, probably explained by well-established regional systems of STEMI care and goals for reperfusion therapy in our country (3,4). Also, because of our regional reperfusion systems, patients treated with cells presented no large necrosis with a baseline LVEF of 48 ± 9% where significant improvement is more difficult to evidence. Variability of innate donor BMMC diversity and bone marrow health also is high in BMMC RCTs, as it was in the TECAM trial. Finally, it has been suggested that the use of heparin in BMMC suspension might decrease cell homing (31). We used heparin for BMMC cell preparation and during intracoronary infusion in all cases.

CONCLUSIONS

We evaluated 3 different bone marrow-derived stem cell approaches (intracoronary injection of BMMC, mobilization with G-CSF, or both) in patients with AMI, but none resulted in improvement of LVEF or LV volumes versus standard AMI care, whether measured by CMR or angiography.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Intracoronary injection of bone marrow–derived autologous mononuclear cells alone or in combination with granulocyte colony-stimulating factor mobilization does not improve left ventricular function 12 months after primary percutaneous revascularization in patients with ST-segment elevation myocardial infarction.

TRANSLATIONAL OUTLOOK: Further studies involving different cells and delivery methods and longer-term clinical endpoints are needed to effectively modify adverse ventricular remodeling and improve survival and quality of life for victims of acute myocardial infarction.

REFERENCES

1. Clifford DM, Fisher SA, Brunskill SJ, et al. Stem cell treatment for acute myocardial infarction. Cochrane Database Syst Rev 2012;2:CD006536.

2. Fernandez-Aviles F, San Roman JA, Garcia-Frade J, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res 2004;95:742–8.

3. Fernandez-Aviles F, Alonso JJ, Pena G, et al. Primary angioplasty vs. early routine post-fibrinolysis angioplasty for acute myocardial infarction with ST-segment elevation: the GRACIA-2 non-inferiority, randomized, controlled trial. Eur Heart J 2007;28:949–60.

4. Sanchez PL, Gimeno F, Ancillo P, et al. Role of the paclitaxel-eluting stent and tirofiban in patients with ST-elevation myocardial infarction undergoing postfibrinolysis angioplasty: the GRACIA-3 randomized clinical trial. Circ Cardiovasc Interv 2010;3:297–307.

5. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006;367:113–21.

6. Achilli F, Malafronte C, Lenatti L, et al. Granulocyte colony-stimulating factor attenuates left ventricular remodelling after acute anterior STEMI: results of the single-blind, randomized, placebo-controlled multicentre STem cEll Mobilization in Acute Myocardial Infarction (STEM-AMI) Trial. Eur J Heart Fail 2010;12:1111–21.

7. Ince H, Petzsch M, Kleine HD, et al. Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. Circulation 2005;112:173–80.

8. Valgimigli M, Rigolin GM, et al. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. Eur Heart J 2005;26:1838–45.

9. Ellis SG, Penn MS, Bolwell B, et al. Granulocyte colony stimulating factor in patients with large acute myocardial infarction: results of a pilot dose-escalation randomized trial. Am Heart J 2006;152:1051.e9–14.

10. Zohlnhofer D, Ott I, Mehilli J, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. JAMA 2006;295:1003–10.

11. Ripa RS, Jorgensen E, Wang Y, et al. Stem cell mobilization induced by subcutaneous granulocyte-colony stimulating factor to improve cardiac regeneration after acute ST-elevation myocardial infarction: result of the double-blind, randomized, placebo-controlled stem cells in myocardial infarction (STEMMI) trial. Circulation 2006;113:1983–92.

12. Leone AM, Galiuto L, Garramone B, et al. Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the Rigenera Study). Am J Cardiol 2007;100:397–403.

13. Takano H, Hasegawa H, Kuwabara Y, et al. Feasibility and safety of granulocyte colony-stimulating factor treatment in patients with acute myocardial infarction. Int J Cardiol 2007;122:41–7.

14. Yang C, Deng Z, Jiang X, et al. Long-term effects of primary early granulo-monocyte colony-stimulating factor treatment on the left ventricular function and remodeling in patients with acute myocardial infarction with five-year follow-up. Int J Cardiol 2012;155:287–90.

15. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 2004;363:751–6.

16. Kang HJ, Lee HY, Na SH, et al. Differential effect of intracoronary infusion of mobilized

peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. Circulation 2006;114:I145–51.

17. Hirsch A, Nijveldt R, van der Vleuten PA, et al. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial. Eur Heart J 2011;32:1736–47.

18. Traverse JH, Henry TD, Pepine CJ, et al. Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA 2012;308:2380–9.

19. Traverse JH, Henry TD, Ellis SG, et al. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. JAMA 2011;306:2110–9.

20. Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199–209.

21. Traverse JH, McKenna DH, Harvey K, et al. Results of a phase 1, randomized, double-blind, placebo-controlled trial of bone marrow mononuclear stem cell administration in patients following ST-elevation myocardial infarction. Am Heart J 2010;160:428–34.

22. Wohrle J, Merkle N, Mailander V, et al. Results of intracoronary stem cell therapy after acute myocardial infarction. Am J Cardiol 2010;105:804–12.

23. Yao K, Huang R, Sun A, et al. Repeated autologous bone marrow mononuclear cell therapy in patients with large myocardial infarction. Eur J Heart Fail 2009;11:691–8.

24. de Jong R, Houtgraaf JH, Samiei S, et al. Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. Circ Cardiovasc Interv 2014;7:156–67.

25. Moazzami K, Roohi A, Moazzami B. Granulocyte colony stimulating factor therapy for acute myocardial infarction. Cochrane Database Syst Rev 2013;5:CD008844.

26. Huikuri HV, Kervinen K, Niemela M, et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008;29:2723–32.

27. Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355:1210–21.

28. Wohrle J, von Scheidt F, Schauwecker P, et al. Impact of cell number and microvascular obstruction in patients with bone-marrow derived cell therapy: final results from the randomized, double-blind, placebo controlled intracoronary Stem Cell therapy in patients with Acute Myocardial Infarction (SCAMI) trial. Clin Res Cardiol 2013;102:765–70.

29. Meluzin J, Mayer J, Groch L, et al. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. Am Heart J 2006;152:975.e9–15.

30. Jeevanantham V, Butler M, Saad A, et al. Response to letter regarding article, "Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis." Circulation 2013;127:e548.

31. Seeger FH, Rasper T, Fischer A, et al. Heparin disrupts the CXCR4/SDF-1 axis and impairs the functional capacity of bone marrow-derived mononuclear cells used for cardiovascular repair. Circ Res 2012;111:854–62.

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