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EXPEDITED REVIEW

Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction

Final One-Year Results of the TOPCARE-AMI Trial

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OBJECTIVES The Transplantation of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial investigates both safety, feasibility, and potential effects on parameters of myocardial function of intracoronary infusion of either circulating progenitor cells (CPC) or bone marrow-derived progenitor cells (BMC) in patients with acute myocardial infarction (AMI).

BACKGROUND In animal experiments, therapy with adult progenitor cells was shown to improve vascularization, left ventricular (LV) remodeling, and contractility after AMI.

METHODS A total of 59 patients with AMI were randomly assigned to receive either CPC (n = 30) or BMC (n = 29) into the infarct artery at 4.9 ± 1.5 days after AMI.

RESULTS Intracoronary progenitor cell application did not incur any measurable ischemic myocardial damage, but one patient experienced distal embolization before cell therapy. During hospital follow-up, one patient in each cell group developed myocardial infarction; one of these patients died of cardiogenic shock. No further cardiovascular events, including ventricular arrhythmias or syncope, occurred during one-year follow-up. By quantitative LV angiography at four months, LV ejection fraction (EF) significantly increased (50 ± 10% to 58 ± 10%; $p < 0.001$), and end-systolic volumes significantly decreased (54 ± 19 ml to 44 ± 20 ml; $p < 0.001$), without differences between the two cell groups. Contrast-enhanced magnetic resonance imaging after one year revealed an increased EF ($p < 0.001$), reduced infarct size ($p < 0.001$), and absence of reactive hypertrophy, suggesting functional regeneration of the infarcted ventricles.

CONCLUSIONS Intracoronary infusion of progenitor cells (either BMC or CPC) is safe and feasible in patients after AMI successfully revascularized by stent implantation. Both the excellent safety profile and the observed favorable effects on LV remodeling, provide the rationale for larger randomized double-blind trials. (J Am Coll Cardiol 2004;44:1690–9) © 2004 by the American College of Cardiology Foundation

Post-infarction heart failure remains a major cause of morbidity and mortality (1). Although prompt reperfusion of the occluded artery has significantly reduced early mortality rates (2), contemporary reperfusion strategies using percutaneous coronary intervention were shown to be associated with modest improvements in global left ventricular (LV) function as evidenced by a 2% to 4% increase in ejection fraction (EF) six months after an acute myocardial infarction (AMI) (3,4). The major goal to improve post-infarction LV function further would be the stimulation of neovascularization and the enhancement of regeneration of cardiac myocytes within the infarct area.

Recent experimental studies suggested that bone marrow-derived progenitor cells (BMC) or circulating progenitor cells (CPC) might contribute to the regeneration of infarcted myocardium and enhance neovascularization of ischemic myocardium (5–8). Moreover, either intravenous infusion or intramyocardial injection of adult progenitor cells resulted in sustained improvement of cardiac function both after experimentally induced myocardial infarction (MI) (5–9) and in initial pilot studies in patients with acute and chronic ischemic heart disease (10–14). We initiated the Transplantation of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial as a comprehensive pilot study to document the clinical safety and feasibility of intracoronary progenitor cell infusion and to quantitate parameters of LV function and remodeling after MI in order to estimate potential effects of progenitor cell therapy necessary to adequately design subsequent double-blind controlled trials. The present report de-

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Abbreviations and Acronyms

AMI	= acute myocardial infarction
BMC	= bone marrow-derived progenitor cells
CPC	= circulating progenitor cells
EF	= ejection fraction
LAD	= left anterior descending
LV	= left ventricular
MI	= myocardial infarction
MRI	= magnetic resonance imaging
TOPCARE-AMI	= Transplantation of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction trial

scribes the final results of the TOPCARE-AMI trial including one-year follow-up of all patients.

METHODS

Patients. Between October 2001 and March 2003, a total of 59 patients with acute ST-segment elevation MI were recruited into the study at a single center. Because of the experimental nature of the treatment, the study protocol called for an interim analysis of the outcome data after the first 20 patients completed their four months' invasive follow-up. The data of these first 20 patients have been published previously (14). In addition, data on four-month magnetic resonance imaging (MRI) follow-up have been reported (15) in 26 of the patients.

Patients between 18 and 75 years of age were eligible for inclusion into the study if they had a first ST-segment elevation AMI, which was treated by coronary stenting using bare metal stents with glycoprotein IIb/IIIa blockade (tirofiban, eptifibatide, or abciximab) in the acute phase of MI. Exclusion criteria were the presence of cardiogenic shock (defined as systolic blood pressure <80 mm Hg requiring intravenous pressors or intra-aortic balloon counterpulsation), major bleeding requiring blood transfusion after acute reperfusion treatment, a history of leukopenia, thrombocytopenia, or hepatic or renal dysfunction, evidence for malignant diseases, or unwillingness to participate. The ethics review board of the Hospital of the Johann Wolfgang Goethe University of Frankfurt, Germany, approved the protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written, informed consent was obtained from each patient.

Study design. The primary aim of the present pilot trial was to assess the safety and feasibility of progenitor cell therapy after MI with a one-year follow-up after therapy. In addition, as a secondary aim, various quantitative parameters of myocardial and coronary vascular function were assessed in an exploratory fashion to generate hypotheses and to provide a rational basis for power calculation of further controlled trials. Quantitative parameters of the explorative analysis, invasively repeated in eligible patients at four months, included LV angiography and intracoronary Doppler measurements, whereas echocardiography and MRI were also repeated after one year in subsets of patients.

Figure 1 illustrates the study design. As previously described (14), patients were randomly assigned to receive intracoronary infusion of either BMC or CPC three to

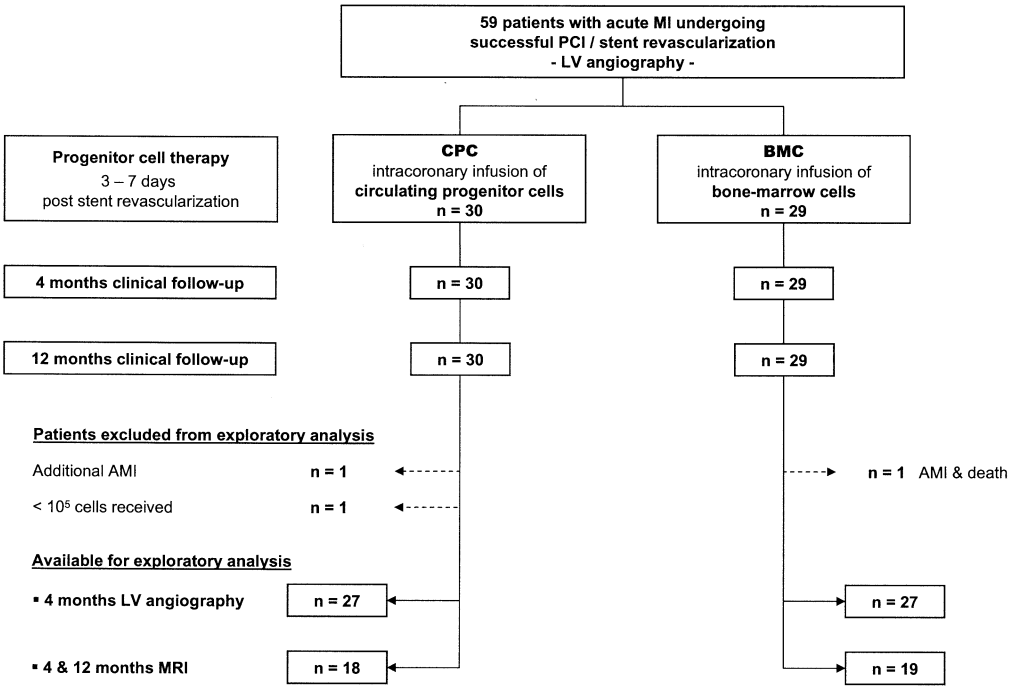

Figure 1. Trial design (see text for details). AMI = acute myocardial infarction; BMC = bone marrow-derived progenitor cells; CPC = circulating progenitor cells; LV = left ventricular; MI = myocardial infarction; MRI = magnetic resonance imaging; PCI = percutaneous coronary intervention.

seven days (median 4.9 days) after AMI. Two patients with repeated AMI were excluded from further exploratory analysis. One patient refused follow-up angiography, but underwent clinical assessment. One patient received an insufficient number of cells owing to failure of preparation of the syringe and, thus, was excluded from further exploratory analysis.

Transplantation protocol. In patients receiving BMC, bone marrow aspirates were obtained in the morning of the day of cell transplantation. In patients receiving CPC, 250 ml of venous blood was collected immediately after randomization (24 h after the AMI), mononuclear cells were purified and ex vivo cultured for three days, and then re-infused into the infarct artery. After arterial puncture, patients—pretreated with aspirin and clopidogrel—received 7.500 to 10.000 U of heparin, and a bolus of abciximab (0.25 mg/kg) was given in the majority of patients prior to cell therapy. Cells were infused via an over-the-wire balloon catheter advanced into the stent previously implanted during the acute reperfusion procedure and inflated with low pressure to completely block blood flow for 3 min to allow for adhesion and potential transmigration of the infused cells through the endothelium. This maneuver was repeated three times to accommodate infusion of the total 10 ml progenitor cell suspension, interrupted by 3 min of reflow by deflating the balloon to minimize extensive ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency, absence of embolization, and unimpeded flow of contrast material.

Preparation and characterization of progenitor cells. As previously reported (14,15), after aspiration of 50 ml bone marrow, BMC were isolated by Ficoll density gradient centrifugation. After two washing steps, cells were re-suspended in 10 ml X-vivo-10 medium (Biowhittaker, Apen, Germany). The cell suspension consists of heterogeneous cell populations including hematopoietic progenitor cells, which were determined by fluorescence-activated cell sorter analysis using directly conjugated antibodies against anti-human CD34 (FITC, Becton Dickinson, Heidelberg, Germany), anti-CD45 (Becton Dickinson), and CD133 (Miltenyi Biotech, Bergisch Gladbach, Germany), but also other cell types (e.g., side population cells, stromal cells, and so on). Overall, a mean value of $5.5 \pm 3.9 \times 10^6$ CD34/CD45-positive cells was infused per patient. To assess colony-forming units, BMC (1×10^5 per dish) were seeded in methylcellulose plates (Methocult GF H4535 including stem cell factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interleukin-3, interleukin-6; CellSystems, St. Katharinen, Germany). Plates were studied under phase-contrast microscopy, and colony-forming units of granulocyte-macrophage (colonies >50 cells) were counted after 14 days of incubation by two independent investigators.

In patients receiving CPC, 250 ml of venous blood was collected immediately after random assignment (24 h after the AMI). Mononuclear cells were suspended in X-vivo-15 medium (Biowhittaker) supplemented with 1 ng/ml carrier-free human recombinant vascular endothelial growth factor (R&D, Wiesbaden, Germany), 0.1 μmol/l atorvastatin (Pfizer, Freiburg, Germany), and 20% human serum drawn from each individual patient. Cells were seeded at a density of 6.4×10^5 cells/mm^2 at fibronectin-coated dishes (Roche, Grenzach, Germany). After three days of cultivation, cells were detached with 0.5 mmol/l ethylenediamine-tetraacetic acid, washed twice, and re-suspended in a final volume of 10 ml X-vivo-10 medium. The resulting cell suspension contains a heterogeneous population of progenitor cells. However, more than 90% of the cells show endothelial characteristics as demonstrated by Dil-acetylated low-density lipoprotein-uptake and lectin binding and the expression of typical endothelial marker proteins including vascular endothelial growth factor-R2 (KDR) (ReliaTech, Braunschweig, Germany), endoglin (CD105) (NeoMarkers, Asbach, Germany), von Willebrand factor (Oncogene, Schwalbach, Germany), and platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) (Dianova, Hamburg, Germany) (14,16–18). For both cell types, the percentage of migrating cells was determined as previously described (15).

Parameters of safety and feasibility. Clinical, medication, and safety laboratory data were prospectively collected by research nurses. Follow-up visits after two weeks, four months, and one year were performed by physicians. Procedural complications were defined as any ventricular arrhythmia, thrombus formation, distal embolization, or injury of the vessel associated with the progenitor cell infusion catheterization procedure. In patients undergoing bone-marrow puncture, potential bleeding complications were assessed. Laboratory safety parameters included C-reactive protein as a marker of inflammation and troponin T as a marker of myocardial necrosis, both assessed prior to cell therapy and after 24 h, and in a subset of patients at 14 days.

During hospitalization, any ventricular arrhythmias were documented. Telemetry was routinely performed for 24 h after the procedure in all patients. In addition, the following clinical events were recorded: infarct vessel stent thrombosis, target vessel, and non-target vessel revascularization, repeated MI (defined as creatine kinase elevation 2 × upper normal limit), cerebral infarction, and death of any cause.

During 12 months' clinical follow-up, the same parameters were assessed. Causes for potential rehospitalization during follow-up were verified by review of the discharge letters or charts of hospital stays. In addition, specific attention was paid to any potential signs or symptoms of arrhythmia such as syncope during follow-up. In 47 patients, 24-h Holter monitoring was performed at 12 months. At four months' invasive follow-up coronary angiography, the degree of luminal narrowing at the target lesion stent intervention was documented by quantitative angiographic analysis (Medis, Leiden, the Netherlands). In addition, any newly diagnosed cancer was documented.

LV angiography. The LV angiograms were obtained according to standard acquisition guidelines immediately after percutaneous stent revascularization of the infarct artery and at four months' follow-up. The LV EF and volumes were calculated with use of the area-length method (19), and regional wall motion was determined with use of the centerline chord method (20). After four months, left ventriculography was performed with identical projections according to standard acquisition guidelines. In one patient receiving CPC, LV angiography was not suitable for analysis, and one patient receiving BMC refused follow-up angiography, leaving 54 paired angiograms for exploratory analysis (Fig. 1).

MRI. Cardiac MRI (1.5-T system, Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany) was performed in a total of 37 patients after MI as well as at four months and one year after progenitor cell therapy. All images were acquired using a phased-array body surface coil with 4 to 12 elements during breath-holds (max 12 s) and were electrocardiogram-triggered. Cine images with a slice thickness of 8 mm were acquired throughout the entire left ventricle using contiguous two-dimensional True Fast Imaging in Steady state Precession sequences. The typical in-plane resolution was 2.2×1.3 mm^2. After intravenous application of gadolinium-diethylenetriaminepentaacetate (Gd-DTPA) (0.2 mmol/kg body weight), "late enhancement" imaging was performed with a delay time of 15 min. Contiguous inversion recovery two-dimensional Turboflash images (Turbo Fast Low Angle Shot; True Fast Imaging in Steady state Precession) using an individually optimized inversion time of 170 to 280 ms were acquired. Again, the slice thickness was 8 mm, and the in-plane resolution varied between 1.7×1.4 mm^2 and 1.4×1.4 mm^2. Using the ARGUS software (Siemens, Erlangen, Germany), LV function (EF), end-systolic and end-diastolic volumes as well as LV mass normalized to body weight, and the volumes of the regions revealing "late enhancement" were calculated from both examinations.

Stress echocardiography. At the day of cell transplantation, dobutamine stress echocardiography for the assessment of viable myocardium was carried out in a subset of 44 patients before intracoronary cell infusion. Both resting and dobutamine stress echocardiography tests were performed and analyzed as previously described (21,22). Studies were conducted with the patient in the left lateral position. Two-dimensional echocardiography using a phased-array electronic ultrasound system (System V, Vingmed, Norway) was performed in the four standard views: parasternal long- and short-views and apical four- and two-chamber views. A 12-lead electrocardiogram and blood pressure readings were recorded at baseline, at the end of each 3-min stage of increasing doses of dobutamine, at peak stress, and every 3 min during recovery for at least 6 min or until all stress-induced wall motion abnormalities resolved. Contractile reserve was defined as an improvement of regional wall motion score index during low-dose dobutamine infusion (10 μg/kg/min) in at least one segment.

Statistical analysis. Continuous variables are presented as mean ± SD and median. Categorical variables were compared with either the chi-square test or the Fisher exact test. Statistical comparisons between initial and follow-up data were performed in a non-parametric fashion using the Wilcoxon test. Non-parametric Mann-Whitney *U* or Kruskall-Wallis tests were used to compare continuous with categorical variables. Linear non-parametric correlation was calculated using the Spearman correlation. Statistical significance was assumed if $p < 0.05$. All statistical analysis was performed using SPSS (Version 11.5, SPSS Inc., Chicago, Illinois).

RESULTS

The demographic, clinical, and angiographic characteristics of the study population as well as of the infused cells are summarized in Table 1. No significant differences existed in any of the baseline parameters.

Procedural safety. The procedure was well tolerated with usually no or only mild angina during the 3 min of balloon inflation for infusion of the cells in these patients shortly after MI. There were no procedural complications during cardiac catheterization related to intracoronary progenitor cell injections such as ventricular arrhythmias, new thrombus formation, embolization after cell infusion, or dissection due to balloon inflations (Table 2). However, one patient demonstrating a small residual thrombus at the proximal stent edge experienced embolic occlusion of the very distal vessel after balloon dilation. Because the event occurred before cell infusion, it was not judged to be related to progenitor cell therapy itself. Nevertheless, because repeated intracoronary instrumentation required for intracoronary cell infusion was most likely responsible for dislodgement of the thrombus and subsequent distal vessel occlusion, this complication was judged as procedure-related. In this patient, troponin T increased from 6.2 ng/ml before cell therapy to 7.3 ng/ml the next day, and creatine kinase increased from 120 U/l to 136 U/l (reference <80 U/l). In all patients, Thrombolysis In Myocardial Infarction (TIMI) blood flow did not worsen, and embolization after cell infusion could be ruled out at the final angiography. Overall, there was no evidence for myocardial damage induced by cell therapy: troponin T levels, obviously still elevated after MI, further declined 24 h after cell therapy (2.4 ± 2.0 ng/ml to 1.7 ±1.6 ng/ml; $p < 0.001$) and normalized at 14 days (0.025 ± 0.04 ng/ml) (Table 2). Likewise, there was no evidence for a systemic proinflammatory response to cell therapy, as overall C-reactive protein levels did not increase after cell therapy (3.1 ± 2.4 mg/dl to 2.9 ± 2.2 mg/dl; $p = 0.29$) and further declined to 0.97 ± 0.50 mg/dl at 14 days. In addition, there were no bleeding complications associated with bone-marrow puncture in patients undergoing maxi-

Table 1. Baseline Characteristics

	CPC (n = 30)		BMC (n = 29)		
	n	%	n	%	p Value
Risk factors					
Age, yrs	52 ± 10		52 ± 10		0.83
Male gender	27	90	26	90	1.0
Hypertension	18	60	15	52	0.52
Hyperlipidemia	19	63	17	59	0.71
Diabetes	6	20	8	28	0.44
Smoking	15	50	21	72	0.21
Family history for CHD	10	33	14	48	0.24
CAD					0.84
1-vessel disease	21	70	21	72	
2-vessel disease	9	30	8	28	
Infarct treatment					
Infarct-related vessel					0.22
LAD	19	63	16	55	
LCX	5	17	2	7	
RCA	6	20	11	38	
PCI of second vessel	2	7	5	17	0.25
During infarct treatment procedure	—		4		
During cell therapy procedure	2		1		
Time to reperfusion/stent (median) (h)	13 ± 22 (4.5)		27 ± 40 (14)		
TIMI flow post reperfusion (%)					1.0
Flow grade 2	4	13	4	14	
Flow grade 3	26	87	25	86	
Ejection fraction (visually estimated)	42 ± 11		40 ± 7.7		0.90
CPR during acute MI (n)	0		2	7	0.24
Creatine kinase max (U/l)*†	1,118 ± 908		919 ± 723		0.37
Creatine kinase-MB max (U/l)*	126 ± 96		101 ± 97		0.34
Cell therapy					
Time stent to cell therapy (days)	4.9 ± 1.1		4.5 ± 1.7		0.23
Number of injected cells ($\times 10^6$)	16 ± 12		213 ± 75		—
% Migrating cells	13 ± 14‡		5.5 ± 5.0§		0.10
% $CD34^+/CD45^+$ (absolute cell number $\times 10^{-6}$)			1.1 ± 0.9 (2.6 ± 2.5)		—
% $CD34^+/CD133^+$ (absolute cell number $\times 10^{-6}$)			0.12 ± 0.10 (0.28 ± 0.34)		—
Colony-forming units/well			123 ± 86		—
Discharge medication					
Aspirin (%)	30	100	28	100	
Clopidogrel (%)	30	100	28	100	
ACE inhibitor (%)	29	97	28	100	1.0
Beta-blocker (%)	30	100	28	100	
Statin (%)	30	100	28	100	

Values are expressed as mean ± SD. *Without CPR patients; †upper normal limit: 80 IU (men); 70 U/l (women). ‡Toward vascular endothelial growth factor, §towards stromal cell-derived factor-1.

ACE = angiotensin-converting enzyme; BMC = bone marrow progenitor cells; CAD = coronary artery disease; CHD = coronary heart disease; CPC = circulating progenitor cells; CPR = cardiopulmonary resuscitation; LAD = left anterior descending; LCX = left circumflex; MI = myocardial infarction; PCI = percutaneous coronary intervention; RCA = right coronary artery; TIMI = Thrombolysis In Myocardial Infarction.

mal anticoagulant and antiplatelet therapy for cardiac catheterization a few hours after the bone-marrow puncture.

In-hospital course. During 24-h telemetric monitoring, no ventricular arrhythmias were detected (Table 3). One patient (#19), who received CPC into the right coronary artery for inferior MI, and stent revascularization of a concomitant left anterior descending artery (LAD) stenosis at the time of cell therapy, experienced an additional anterior wall infarction due to stent thrombosis of the LAD (non-target vessel of prior cell infusion) three days after cell therapy, which was successfully treated by immediate recanalization of the LAD. It was discovered afterwards that this patient suffered from genetically determined severe antithrombin III deficiency and, in addition, had a non–insulin-dependent diabetes mellitus. Additionally patient (#25), with insulin-dependent diabetes mellitus, receiving BMC into the LAD after a large anterior infarction, experienced adverse events including an MI and subsequent death: three days after cell therapy with concomitant stent revascularization of a very proximal left circumflex stenosis, subacute stent thrombosis of the LAD (target vessel of prior cell infusion) occurred, which was successfully revascularized.

Table 2. Procedural Safety of Intracoronary Progenitor Cell Infusion

	CPC (n = 30)	BMC (n = 29)	Both Cell Groups (n = 54)
Procedural complications*	0	0	0
CRP (mg/dl)			
Before cell therapy	2.8 ± 2.2 (2.0)	3.5 ± 2.6 (2.6)	3.1 ± 2.4 (2.3)
24 h after cell therapy	2.6 ± 2.3 (1.8)	3.2 ± 2.0 (2.8)	2.9 ± 2.2 (2.3)
14 days after cell therapy (n = 48)	0.65 ± 0.54 (0.5)	1.1 ± 1.3 (0.6)	0.82 ± 0.97 (0.5)
4 months follow-up	0.49 ± 0.38 (0.3)	0.40 ± 0.18 (0.3)	0.44 ± 0.30 (0.3)
Troponin			
Before to cell therapy	2.3 ± 1.9 (1.7)	2.5 ± 2.1 (1.9)	2.4 ± 2.0 (1.85)
24 h after cell therapy	1.5 ± 1.4 (1.2)	1.9 ± 1.8 (1.5)	1.7 ± 1.6 (1.4)
14 days after cell therapy (n = 46)	0.02 ± 0.03 (0.01)	0.03 ± 0.04 (0.01)	0.03 ± 0.04 (0.01)

Values are expressed as mean ± SD (median). *Thrombosis, embolization, arrhythmia, or dissection related to cell infusion. CRP = C-reactive protein; other abbreviations as in Table 1.

However, at day 5, additional stent thrombosis of the left circumflex artery occurred and the patient developed cardiogenic shock, which subsequently led to death.

Clinical follow-up at 4 and 12 months. No further stent thrombosis, MI, or death occurred beyond the initial hospitalization period (Table 3). At one-year follow-up, target (infarct) vessel revascularization (excluding the one in-hospital stent thrombosis) was performed in a total of 12 patients (21%), 7 (23%) in the CPC group, and 5 of 28 (18%) patients discharged alive in the BMC group.

In addition, no syncope, ventricular arrhythmia, or cerebral infarction was observed. The 24-h Holter monitoring, performed in 47 patients at 12 months, revealed no evidence of ventricular tachycardia. No case of cancer was detected until 12 months' follow-up.

Thus, there were no delayed adverse events related to progenitor cell therapy. None of the treated patients were rehospitalized for treatment of heart failure. Thus, by Kaplan-Meier analysis, survival free of death, recurrent MI, or rehospitalization for heart failure was 97%. Figure 2 illustrates Kaplan-Meier curves for survival free of death, recurrent MI, and target (infarct) vessel revascularization.

Quantitative LV angiography at four months. In the 54 patients suitable for exploratory analysis of LV function four months after transplantation of progenitor cells, the LV EF had significantly increased from 50 ± 10% at baseline to 58.3 ± 10% ($p < 0.001$) (Table 4, Fig. 3). Detailed analysis of regional wall motion revealed the most prominent improvements in the border zone adjacent to the central infarct area (Table 4). The LV end-diastolic volume did not significantly change, whereas LV end-systolic volume was significantly reduced at four months' follow-up in both groups of patients.

Baseline LV EF ($r = 0.42$, $p = 0.002$) was the only significant univariate predictor of improvement in EF during the four months' follow-up, whereas female gender ($p = 0.07$), maximal creatine kinase elevation ($p = 0.10$), age

Table 3. Clinical Events

	CPC (n = 30)	BMC (n = 29)
In-hospital events		
Death	0	1*
MI	1†	1*
Infarct vessel stent thrombosis	0	1*
Non-target vessel stent thrombosis	1†	1*
Cerebral infection	0	0
Ventricular arrhythmia (monitoring)	0	0
12-month follow-up (cumulative)		
Death	0	1*
MI	1†	1*
Rehospitalization due to heart failure	0	0
Stent thrombosis after hospitalization	0	0
Infarct vessel revascularization‡	7	6
Coronary bypass surgery	0	0
Cerebral infarction	0	0
Cancer	0	0
Syncope	0	0
Documented ventricular arrhythmia	0	0
Ventricular tachycardia in 24-h Holter ECG (n = 47)	0	0
Cumulative death or MI	1	1
Cumulative death, MI, or rehospitalization due to heart failure	1	1
Cumulative death, MI, or infarct vessel revascularization	8	6

*Same patient, †same patient; ‡including revascularization due to in-hospital stent thrombosis and to restenosis.
ECG = electrocardiogram; other abbreviations as in Table 1.

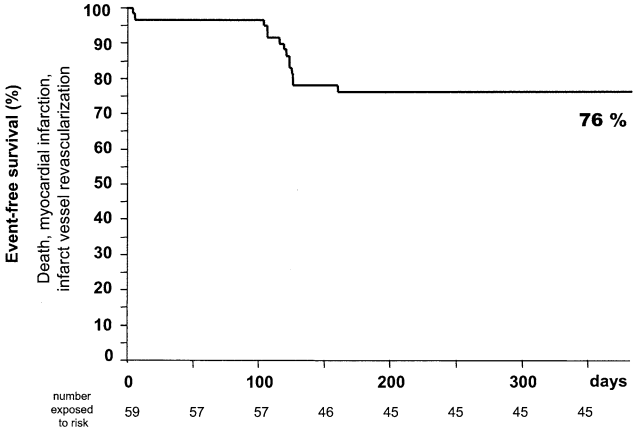

Figure 2. Event-free survival of death, recurrent myocardial infarction, or target vessel revascularization (Kaplan-Meier analysis).

Table 4. Left Ventricular Angiography: Quantitative Global and Regional Left Ventricular Function

	CPC (n = 27)		BMC (n = 27)		Both Cell Groups (n = 54)		
	Cell Therapy	4 Months	Cell Therapy	4 Months	Cell Therapy	4 Months	p Value*
Ejection fraction (%)	51 ± 10 (50)	59 ± 10 (62)	49 ± 10 (50)	57 ± 10 (59)	50 ± 10 (50)	58 ± 10 (60)	<0.001
End-diastolic volume (ml)	107 ± 26 (102)	109 ± 33 (106)	111 ± 29 (111)	102 ± 31 (99)	109 ± 27 (107)	105 ± 31 (101)	0.45
End-systolic volume (ml)	52 ± 16 (53)	42 ± 18 (40)	56 ± 21 (53)	45 ± 21 (42)	54 ± 19 (53)	44 ± 20 (41)	<0.001
Regional wall motion (SD/chord)							
Infarct	−1.5 ± 0.28 (−1.43)	−0.69 ± 0.61 (−0.62)	−1.52 ± 0.26 (−1.53)	−0.60 ± 0.65 (−0.48)	−1.51 ± 0.26 (−1.45)	−0.64 ± 0.63 (−0.60)	<0.001
Infarct center	−1.59 ± 0.38 (−1.50)	−0.85 ± 0.71 (−0.96)	−1.71 ± 0.34 (−1.60)	−0.81 ± 0.66 (−0.65)	−1.65 ± 0.36 (−1.58)	−0.83 ± 0.68 (−0.86)	<0.001
Infarct border	−1.44 ± 0.20 (−1.43)	−0.54 ± 0.60 (−0.61)	−1.41 ± 0.19 (−1.35)	−0.44 ± 0.67 (−0.48)	−1.42 ± 0.19 (−1.41)	−0.49 ± 0.63 (−0.55)	<0.001
Infarct extension (hypokinetic chords)	38 ± 14 (39)	20 ± 16 (14)	40 ± 15 (40)	21 ± 18 (21)	39 ± 15 (39)	21 ± 17 (18)	<0.001

Values are expressed as mean ± SD (median). *Cell therapy versus 4 months for both cell groups.
Abbreviations as in Table 1.

above the median of 53 years (p = 0.49), presence of hypertension (p = 0.32), hypercholesterolemia (p = 0.88), diabetes (p = 0.87), extent of coronary artery disease (p = 0.51), time to revascularization therapy (p = 0.26), time from revascularization to progenitor cell therapy (p = 0.44), or occurrence of a restenosis (p = 0.91) did not predict improvement of LV EF. Figure 4 illustrates that those patients with the most severe impairment in LV function demonstrated the largest absolute improvements in global LV EF irrespective of the cell type infused (r = −0.42; p = 0.002). This correlation remains statistically significant, if the two outliers with the lowest initial EF and the best improvement of EF are excluded (r = −0.34; p = 0.014).

Finally, to investigate whether contractile improvement is confined to patients exhibiting the presence of contractile reserve prior to progenitor cell therapy, we determined any potential association between low-dose dobutamine-recruitable contractile reserve and improvement in global LV EF in a subset of 44 patients undergoing stress echocardiography before intracoronary progenitor cell infusion. As illustrated in Figure 5, improvements in LV EF at four months did not differ between patients with (n = 35) compared to those without evidence for contractile reserve (n = 9) before cell therapy.

LV function by MRI at 12 months. In a subset of 37 patients, contrast-enhanced MRI was serially performed 10 ± 5.9 days after AMI, at 4 months' and at 12 months' follow-up. As illustrated in Figure 6, MRI-determined global LV EF not only significantly improved within the first 4 months, but further increased between month 4 and month 12 resulting in a total increase of 9.3 ± 8.0% one year after progenitor cell treatment (Fig. 6A). At the same time, late enhancement volume as a measure of infarct size also significantly decreased from month 4 to month 12 (Fig. 6B) resulting in an overall reduction of late enhancement volume by −34 ± 34%. The MRI-determined LV mass (adjusted for body surface) decreased from 86 ± 15 ml/m^2 to 79 ± 15 ml/m^2 at 4 months and remained at 77 ± 15 ml/m^2 at 12 months. Thus, improved global LV function was observed despite the lack of reactive hypertrophy of non-infarcted myocardium, suggesting a favorable LV remodeling process following AMI.

DISCUSSION

The final results of the TOPCARE-AMI trial indicate that intracoronary infusion of CPC or BMC: 1) is feasible and safe in patients with AMI, 2) is associated with significant improvements in global LV function, and 3) show significant reductions in LV end-systolic volumes, suggesting a favorable LV remodeling process over one year following AMI.

Safety and feasibility. Progenitor cell therapy with either BMC or CPC after a median of 4.9 days following an MI was in general well tolerated without any complications related to the cell-infusion procedure itself. However, one patient suffered from a procedure-related thrombotic distal coronary vessel occlusion prior to intracoronary cell infusion. Importantly, there was no evidence for myocardial damage or aggravation of inflammatory responses incurred by intracoronary progenitor cell infusion. Although we cannot exclude the possibility that intracoronary cell infusion may have contributed to the two re-infarctions observed in the present study, the incidence of death and/or re-infarction (3.4%) compares favorably with recent trials using stent percutaneous coronary intervention for revascularization of AMI (3,4,23).

Moreover, target vessel revascularization rate due to restenosis (21%) was within the range expected from bare metal stents used in patients with AMI (24). Thus, repeated

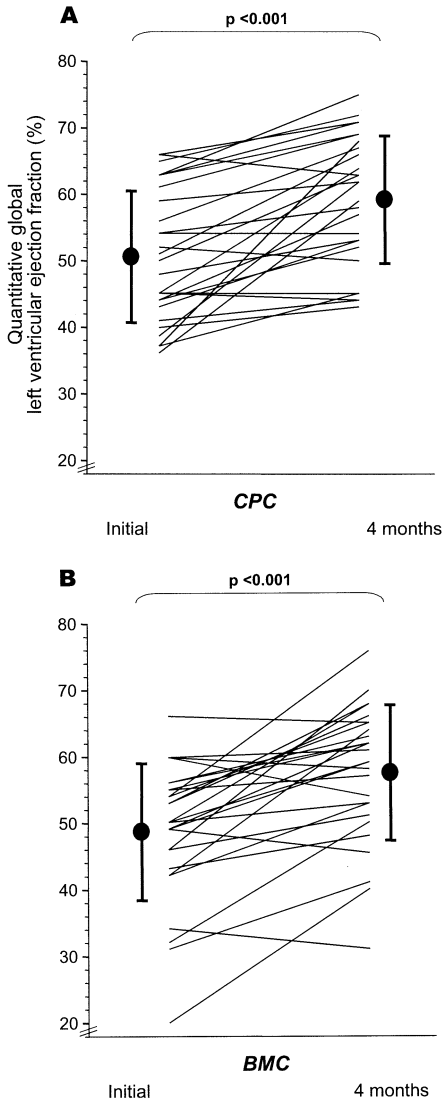

Figure 3. Left ventricular ejection fraction, measured by quantitative left ventricular angiography initially and at four-month follow-up for patients receiving circulating progenitor cells (CPC) or bone marrow-derived progenitor cells (BMC). **Bars** represent mean ± SD.

low-pressure balloon inflations within the previously implanted stent still not covered by endothelium—required to infuse the progenitor cells—a couple of days after AMI did not adversely affect four-month angiographic restenosis rates nor late (one year) target vessel revascularization rates.

Thus, the present data argue against recent speculations that progenitor cell therapy—combined with granulocyte colony-stimulating factor-induced mobilization—might enhance the restenosis process (MAGIC trial [25]). The 10 patients reported in the MAGIC trial were a heterogeneous population with previous MI varying from 2 to 270 days. Most importantly, granulocyte colony-stimulating factor treatment was initiated before percutaneous coronary intervention of the infarct-related stenosis, which might—by study design—enhance plaque inflammation, a well-established predictor of restenosis (26).

Finally, in the TOPCARE-AMI trial, neither during the

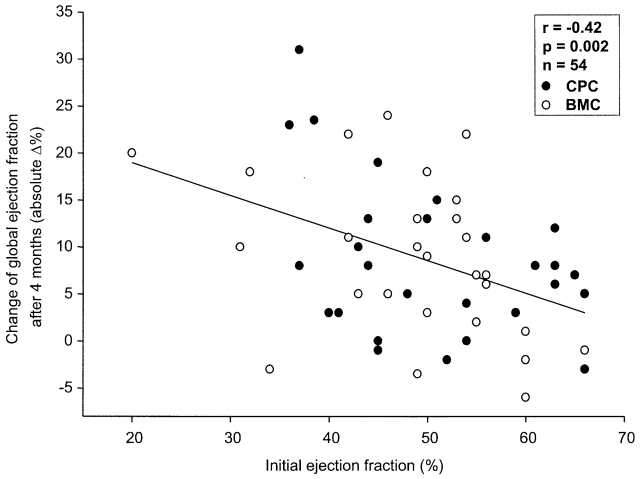

Figure 4. Correlation between initial global left ventricular ejection fraction and improvement of ejection fraction (difference between absolute values) during four-month follow-up (both assessed by quantitative left ventricular angiography). BMC = bone marrow-derived progenitor cells; CPC = circulating progenitor cells.

initial hospital stay nor during the 12-month follow-up period was there any evidence of malignant ventricular arrhythmias. In contrast, life-threatening ventricular arrhythmias have been reported following intramyocardial injection of skeletal myoblasts (27) in patients with chronic ischemic cardiomyopathy. Although patients with chronic ischemic cardiomyopathy are different from patients of the present study, one might speculate that intracoronary infusion of progenitor cells may favor homing of the cells into myocardial areas with preserved nutrient blood flow in contrast to injecting cells into scar tissue. Moreover, CPC, which ex vivo transdifferentiate into cardiomyocytes, were

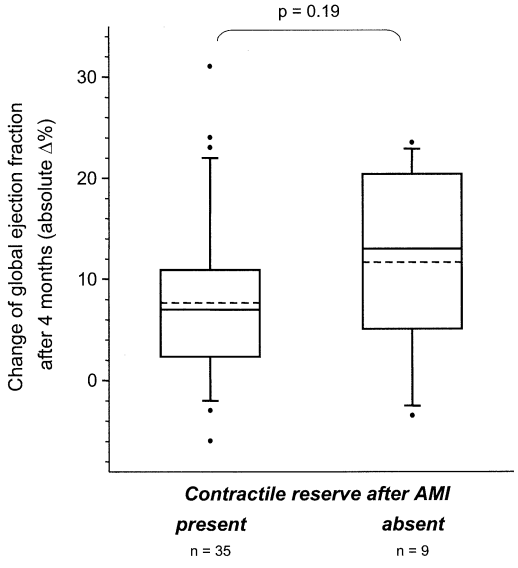

Figure 5. Improvement of global left ventricular ejection fraction after four months assessed by quantitative left ventricular angiography according to the presence or absence of contractile reserve by low-dose stress echocardiography before intracoronary progenitor cell infusion. AMI = acute myocardial infarction.

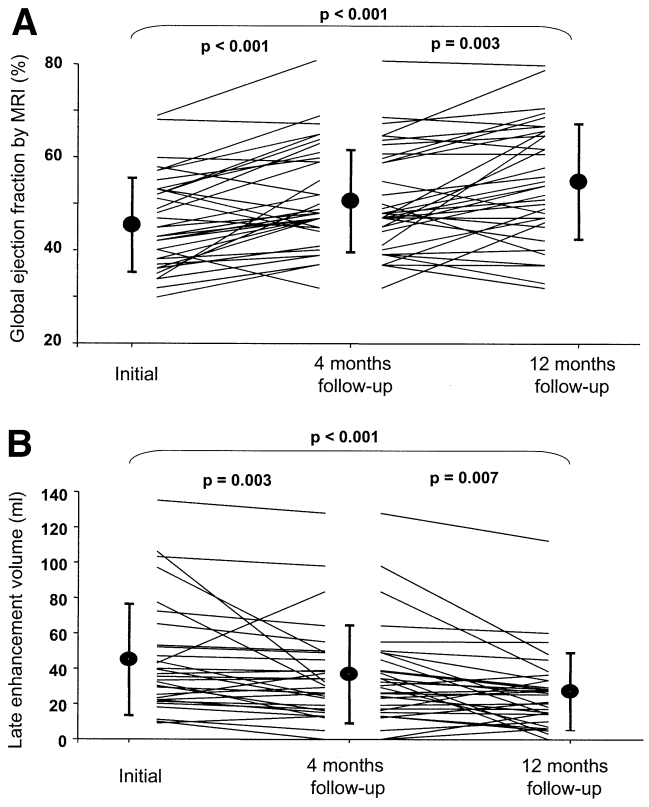

Figure 6. Magnetic resonance imaging (MRI)-determined improvement in global left ventricular ejection fraction **(A)** and reduction in late enhancement volume **(B)** after 4 and 12 months.

shown to develop gap junctions and to electrophysiologically integrate (28), in contrast to skeletal myoblasts (29).

LV function after progenitor cell therapy. The LV EF increased by an absolute 8.7 ± 8.3% four months following intracoronary progenitor cell infusion. However, we cannot exclude that ischemic preconditioning due to repeated balloon occlusions of the infarct artery required for intracoronary progenitor cell application 3 to 7 days after AMI may have contributed to the observed increase in global LV function.

Analysis of LV function by MRI one year after progenitor cell infusion not only documented a sustained improvement in global EF, but revealed the lack of infarct expansion as evidenced by reduced end-systolic volumes and unchanged end-diastolic volumes as well as absent reactive hypertrophy of non-infarcted segments as evidenced by a preserved significant reduction of LV mass between month 4 and month 12 compared to the acute phase post-MI. Normally, both end-diastolic and end-systolic LV volumes significantly increase by >20% over one year post-MI (30), a process termed "LV remodeling." Thus, transplantation of progenitor cells early after an AMI was associated with a favorable LV remodeling process over a one-year follow-up period.

Experimental studies suggested that improved ventricular function by progenitor cell transplantation following an MI is mainly due to stimulated neovascularization preventing late myocardial remodeling through enhanced blood flow, rescue of hibernating myocardium, reduction of myocardial fibrosis, and decreased apoptosis of hypertrophied myocytes in the peri-infarct region (6,7,31,32). In addition, intramyocardial injection of BMC was shown to lead to regeneration of significant amounts of contracting myocardium (5,33), although the capacity of purified hematopoietic progenitor cells to differentiate into cardiac myocytes has been questioned recently (34,35). Obviously, the present clinical study cannot disclose the cellular mechanisms associated with the observed improved LV contractility and remodeling processes. However, improvement in global LV EF was independent of the presence of contractile reserve, indicating that rescue of hibernating myocardium may not fully account for the observed beneficial effects. Moreover, improvement in LV function associated with a further reduction in MRI-determined infarct size observed between month 4 and month 12 suggests that intracoronary progenitor cell infusion may interfere with the endogenous infarct healing process. It is conceivable that homing of progenitor cells may modify post-infarction LV remodeling via the release of various growth factors attracting both circulating and tissue resident cardiac progenitor cells, thereby enhancing endogenous repair mechanisms of the myocardium (36,37).

In summary, results of the present study document that intracoronary infusion of either BMC or CPC is safe and feasible in patients after AMI successfully revascularized by stent implantation. Although the precise mechanisms of functional improvement remain to be elucidated, the excellent safety profile, combined with a favorable LV remodeling process following an MI provides the rationale for larger randomized, double-blind trials, like the ongoing Reinfusion of Enriched Progenitor Cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) multicenter trial, which will differentiate a potential progenitor cell associated effect from the effects of ischemic preconditioning due to repeated balloon occlusion of the infarct artery required for intracoronary progenitor cell application three to seven days after an AMI. Ultimately, based on the observations of the present trial, this novel form of regeneration enhancement therapy holds the promise to interfere with the development of post-infarction heart failure.

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