Impact of preoperative left ventricular function and time from infarction on the long-term benefits after intramyocardial CD133⁺ bone marrow stem cell transplant

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Objective: Our objective was to elucidate long-term clinical and functional effects of intramyocardial stem cell transplant and to identify patients who will show sustained benefit.

Methods: Long-term outcomes of 35 patients after intramyocardial CD133⁺ bone marrow stem cell transplant during coronary artery bypass grafting were compared with those of a control group of 20 patients after coronary artery bypass grafting alone. Clinical effects were assessed with the New York Heart Association classification system and the Minnesota Living With Heart Failure questionnaire. Electrocardiography, 24-hour Holter monitoring, echocardiography, myocardial perfusion scanning, magnetic resonance imaging, and computed tomography were performed. Logistic regression analyses were used to identify prognostic factors for improvement in long-term left ventricular ejection fraction after stem cell treatment.

Results: The stem cell group revealed similar New York Heart Association and life quality scores to the control group. Myocardial perfusion score at the area of risk was significantly increased in the stem cell group after 36-month follow-up ($P = .024$ vs control). Multivariate logistic regression analysis revealed a 44-fold higher probability of at least 5% improvement in left ventricular ejection fraction for patients with preoperative left ventricular ejection fraction not greater than 40% than for patients with preoperative ejection fraction greater than 40% ($P = .018$). Furthermore, patients operated on between 7 and 12 weeks after myocardial infarction had a 56-fold higher chance of at least 5% improvement in left ventricular ejection fraction than patients treated later than 12 weeks after infarction ($P = .023$).

Conclusions: Intramyocardial stem cell therapy was safe but lacked significant lasting benefits beyond 6 months in our study cohort with a limited number of patients. Preoperative left ventricular ejection fraction and time since myocardial infarction may be critical parameters for selection of patients who can benefit most from intramyocardial stem cell treatment during coronary artery bypass grafting. (J Thorac Cardiovasc Surg 2011;142:1530-9)

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During the past decade, stem cells have emerged as a novel option for the repair of damaged tissues in various organ systems. The discovery of their predominantly paracrine effects on the myocardial regenerative capacity has spawned

numerous experimental and clinical investigations. Various stem cell lineages and delivery routes have been instituted, with encouraging results that have often been associated with improvements in left ventricular function and perfusion.¹⁻⁸ Among the cell lineages used, bone marrow stem cells (BMSCs) have been shown to contribute to functional myocardial recovery through induction of angiogenesis, limitation of apoptosis, and other positive effects on cardiomyocyte salvage pathways.^{4,9-13} Several clinical studies with BMSCs, including those from our group, have demonstrated functional benefits of myocardial treatment with stem cells during short- or medium-term follow-up.^{14,15}

In addition to application routes such as intracoronary, intraventricular, and transventricular approaches, patients undergoing coronary artery bypass grafting (CABG) have been preferentially included in clinical trials of stem cell treatment. The main reason behind this approach is the unlimited access of the cardiac surgeon to the site of the infarcted myocardium, which enables cells to be applied

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Abbreviations and Acronyms

ANCOVA	= analysis of covariance
BMSC	= bone marrow stem cell
CABG	= coronary artery bypass grafting
LVEDD	= left ventricular end-diastolic diameter
LVEDV	= left ventricular end-diastolic volume
LVEF	= left ventricular ejection fraction
MLHFQ	= Minnesota Living With Heart Failure Questionnaire
MRI	= magnetic resonance imaging
NYHA	= New York Heart Association

to the target region by direct intramyocardial injection. This strategy has significant advantages relative to intracoronary or endomyocardial delivery methods with respect to the precision of cellular targeting.

Since June 2001, we have used a protocol for intramyocardial injection of purified CD133⁺ BMSCs that involves their direct application into the diseased myocardium at the time of CABG. Medium-term follow-up results have already been published.^{15,16} Although short-term safety and functional results regarding cardiac stem cell treatment are encouraging, long-term safety and efficacy data are still lacking and greatly needed. First, we wanted to rule out possible side effects of stem cell therapy on the basis of longer-term follow-up data.¹⁷⁻¹⁹ Second, we found it necessary to examine the efficacy of this therapeutic modality as a sustained clinical and functional advantage. Finally, we aimed to identify the specific patient groups showing the most benefit from autologous intramyocardial BMSC transplant concomitant with CABG. We here provide follow-up out to 9 years with nearly complete results of the clinical assessment of both the stem cell and control groups. Furthermore, functional investigations on 20 stem cell-treated patients are presented together with data from a control group of 15 patients who also underwent long-term follow-up investigations.

MATERIALS AND METHODS

Patient characteristics are listed in Table 1. The study was approved by the institutional review board and ethics committee at the University of Ro-stock. Informed consent was obtained from each patient before enrollment. Inclusion criteria were as follows: (1) history of myocardial infarction at least 14 days before admission, (2) indication for bypass grafting on coronary arteries other than the infarct vessel, and (3) distinct area of akinetic left ventricular myocardium corresponding with the infarct localization.

Exclusion criteria were as follows: (1) debilitating chronic disease (malignancy or terminal renal failure), (2) emergency operation, (3) concomitant valve surgery, and (4) history of malignant ventricular arrhythmia. To accelerate recruitment, the inclusion criteria were slightly modified during the phase II trial as follows: (1) patients needing concomitant mitral valve repair for regurgitation were included, and (2) in the absence of a distinctly akinetic area within the left ventricular myocardium,

a globally reduced left ventricular ejection fraction (LVEF) was accepted. We did, however, exclude patients who underwent concomitant valve surgery from our long-term analysis. None of the patients in this evaluation had significant valvular pathology necessitating valvular intervention preoperatively or during the follow-up. Once it was determined that the main inclusion criteria had been met, enrollment was discussed with the patient and informed consent obtained. Next, the patient was referred for myocardial perfusion scintigraphy, Holter electrocardiography, and, recently, cardiac magnetic resonance imaging (MRI) as well. In the controlled study arm, random patient allocation to the control or treatment group was performed as described in Appendix E1.

Stem cell preparation and the technique of stem cell transplant with concomitant CABG have already been described in our previous reports¹⁵ (Appendix E2). In brief, autologous CD133⁺ BMSCs were isolated from bone marrow aspirate before CABG with the Miltenyi Biotec CliniMACS system (Miltenyi Biotec Inc, Auburn, Calif). A mean of 6.0×10^6 cells (range, $1.0\text{--}10.0 \times 10^6$ cells) were injected into the border zone of the infarcted myocardium during on-pump CABG under cardioplegic arrest. Enrollment in the safety trial began in June 2001 and was completed in February 2003. The efficacy study began in May 2003 and was completed in February 2005. Long-term follow-up investigations were finalized in April 2010.

Long-Term Follow-up Investigations (Table E1)

Minnesota Living With Heart Failure Questionnaire.

Patient self-assessments were obtained with the Minnesota Living With Heart Failure Questionnaire (MLHFQ), a widely accepted disease-specific tool for the assessment of patients with heart failure in which a higher score indicates greater negative impact of the disease.²⁰ MLHFQ scores were not available preoperatively. The questionnaires were either sent to patients by conventional mail or filled out by the patients at the time of the inpatient long-term follow-up investigations.

New York Heart Association functional classification.

The New York Heart Association (NYHA) functional class was determined preoperatively and when patients were admitted for a long-term follow-up visit to the clinic or by mail correspondence according to widely accepted guidelines. The scores were compared between the stem cell and control groups in the long-term follow-up.

Transthoracic echocardiography. Transthoracic echocardiography was performed to measure global left ventricular contractility and dimensions. These measurements were conducted by 2 experienced echocardiographers who were blinded to the presence and region of stem cell injection. The LVEF, left ventricular end-diastolic diameter (LVEDD), and left ventricular end-diastolic volume (LVEDV) were determined. After the preoperative baseline evaluation, the patients underwent transthoracic echocardiography at 6 postoperative months, at 18 postoperative months, and at 65 ± 21 and 62 ± 9 postoperative months in the stem cell and control groups, respectively.

Myocardial perfusion scan. To determine myocardial perfusion in the area of risk, single-photon emission computed tomography (CT) was performed at rest. A bolus of 100-MBq thallous chloride TI-201 was injected intravenously, and scans were completed 5 minutes after injection with a 3-head gamma camera (Irix; Philips Nederland BV Medical Systems, Eindhoven, The Netherlands) in combination with a nonuniform attenuation correction. Images were assessed by quantitative measurements of the activity in the area at risk, the infarction zone, which was expressed as the ratio of postoperative to preoperative activity (arbitrarily set as 1.0). Myocardial perfusion scores were determined during the follow-up in all patients in whom stem cell injection had been performed, focusing on the peri-infarction area. The scores after 12 months and 36 months of follow-up were compared between the groups.

Twenty-four-hour Holter monitoring. Twenty-four-hour Holter monitoring was used for each patient to detect any sustained

TABLE 1. Baseline data of patients

Parameter	Control group	Stem cell group	P value
Age (y, mean $\pm$ SD)	63.5 $\pm$ 8.4	63.7 $\pm$ 9.6	.66
Sex (male/female)	16:4	30:5	.71
Infarct time (wk, median and range)	7.5 (2–830)	7.9 (2–1215)	.91
New York Heart Association functional class (No.)			
II	13 (65%)	22 (62.9%)	
III	7 (35%)	13 (37.1%)	
Coronary artery stenosis (no.)			
Left main	7 (35%)	14 (40%)	.75
Left anterior descending	20 (100%)	32 (91%)	.49
Left circumflex	17 (85%)	25 (71%)	.45
Right	18 (90%)	30 (86%)	.99
Target area for cell injection (no.)			
Anterior	NA	14 (40%)	
Posterior	NA	19 (54%)	
Apex	NA	9 (26%)	
Holter findings (no.)			
Relevant ventricular extrasystole	1	0	
Atrial fibrillation	0	0	
Intraoperative data			
No. of bypasses (mean $\pm$ SD)	3.6 $\pm$ 0.8	3.5 $\pm$ 0.9	.98
Left internal thoracic artery use (no.)	20 (100%)	29 (83%)	.07
Cell dose ($\times 10^6$ cells, median and range)	NA	6.0 (1.0–10.0)	
No. of injections	NA	10	
Postoperative data			
Need for inotropes (no.)	12 (60%)	23 (66%)	.77
Mechanical ventilation (h, mean $\pm$ SD)	6.0 $\pm$ 2.0	6.1 $\pm$ 2.4	.64
Creatine kinase (IU, median and range)	507 (195–1166)	466 (155–3698)	.79
Creatine kinase isoenzyme MB (IU, median and range)	28 (12–43)	25 (12–85)	.61
Ventricular extrasystole (% of all QRS, mean $\pm$ SD)	0.8% $\pm$ 0.01%	0.5% $\pm$ 0.009%	.21
Hospital stay (d, mean $\pm$ SD)	12 $\pm$ 2	14 $\pm$ 3	.18

NA, Not applicable.

arrhythmias both preoperatively and postoperatively during the follow-up visits at 3 months, 6 months, 18 months, and long-term follow-up for up to 5 postoperative years. The Lown classification was used for quantification.

MRI and CT. Cardiac MRI and CT were included in the long-term investigations to provide both functional and morphologic data.

Gadolinium-enhanced MRI was performed within long-term follow-up investigations to assess global left ventricular function and to detect the

presence of tissue changes. The patients were examined in the supine position with a 1.5-T system (Magnetom Avanto; Siemens Medical Solutions, Erlangen, Germany), and a phased-array cardiac coil was used for signal reception. All images were obtained during repeated breath-holding intervals and were electrocardiographically gated. MRI analysis was performed in a semiautomated fashion separately by observers who were unaware of patient group identity or timing of the scanning. LVEF at rest was calculated from the MRI cine short-axis views with commercially available software (Syngo Argus; Siemens Medical Solutions). In addition, the radiologists were asked to indicate the presence, location, and degree of any myocardial inflammation, scar, or tumor formation.

When the first reports about tissue changes after cell treatment were noted,¹⁹ the diagnostic long-term evaluation by cardiac CT was started for all treated patients to detect possible intramyocardial calcification caused by BMSC injections. This evaluation was performed only once during the follow-up as part of the long-term investigations.

The images were obtained with a 32-slice native CT scan (Toshiba Aquilion 32; Toshiba Medical Systems Corp, Tochigi, Japan) during 2 breath-holding intervals, and these images were electrocardiographically gated. The myocardium was scanned in sections of 5-mm width. The Vitrea 2 workstation (Vital Images, Inc, Minnetonka, Minn) was used for the assessment of myocardial calcifications. The blinded radiologists were asked to indicate the presence, location, and degree of any myocardial calcification and tissue changes.

Statistical Analysis

All data were stored and analyzed with the SPSS software package (version 15.0; SPSS Inc, an IBM Company, Chicago, Ill). Descriptive statistics were computed for variables of interest. The statistics computed included mean and SD for continuous variables and frequencies and relative frequencies for categorical factors. Results are presented as mean $\pm$ SD. With regard to possible baseline effects, the 2-sided hypothesis for the continuous primary variable of LVEF at different postoperative time points was provided by analysis of covariance (ANCOVA) with adjustment for baseline LVEF. Furthermore, we applied the generalized linear model repeated measures procedure for statistical analysis of the data to test the null hypotheses about the effects of both the between-subject factor (therapy group) and the within-subject factor (time). In dependence of distributions, the paired *t* test or the Wilcoxon test was used for the comparisons within the groups between 2 time points. For comparisons between more than 2 times, we performed repeated measures analysis of variance or nonparametric Friedman test. Comparisons between the study groups for categorical variables were done with the χ^2 test or Fisher's Exact test.

Logistic regression analyses were done to identify independent prognostic factors for an improvement in LVEF of at least 5%. Variables that tended to have a 1-dimensional effect on improvement ($P < .2$) were analyzed with a multivariate logistic regression model. Odds ratios and their 95% confidence intervals were calculated.

All *P* values resulted from 2-sided statistical tests.

RESULTS

Stem Cell Group

Fifteen patients were enrolled in the initial safety and feasibility trial. All but 4 patients are alive and well at the time of submission of this report. A 75-year-old man with cerebrovascular disease was unavailable for follow-up 9 months after surgery. Later investigation revealed that he had died of a stroke (no autopsy available). Three patients died at 62 postoperative months, 73 postoperative months, and 81 postoperative months. The reasons for their deaths are not available because of the lack of autopsies.

FIGURE 1. Kaplan-Meier survival curve for stem cell-treated group (blue) and control group (red).

In the efficacy study, 40 patients were randomly assigned to undergo either CABG and cell transplant or CABG alone. Twenty patients in the stem cell treatment group received a median dose of 7.2×10^6 CD133⁺ BMSCs. One patient was excluded because of postoperative deep sternal wound infection and poor compliance with the follow-up examinations. During the follow-up period, a 68-year-old female patient died of an unknown cause after a follow-up of 21 months. Another woman died of an unknown cause 57 months after surgery. As of this writing, all other patients are alive and well. Thus among the 35 patients who were treated with stem cell injection, one was excluded from the study and 6 deaths occurred as outlined here (Figure 1). The follow-up duration ranged between 32 and 99 months, with a mean follow-up of 65 ± 21 months, and encompassed a total of 1306 patient-months.

Control Group

In the control group with a total 20 patients, 2 patients were not available for the long-term follow-up because they decided not to attend further investigations. Three patients died during the follow-up: 2 male patients at 18 and 65 postoperative months and 1 female patient at 15 postoperative months (Figure 1). The male patient who died at 15 postoperative months had an echocardiographic LVEF of 24% and showed improved left ventricular performance in the 6-month follow-up investigation with LVEF of 50%. It was reported that he died of multiorgan failure after sepsis. The reason for the death of the other male patient

is unknown. The female patient had no change from the LVEF of 37% reported at discharge. She did not undergo further follow-up studies and had sudden cardiac death. Follow-up time for the group ranged between 48 and 76 months, with a mean follow-up of 62 ± 9 months and a total of 923 patient-months.

Adverse Events

In the stem cell-treated patient group, no myocardial infarctions or acute cardiac reinterventions were observed during the entire follow-up period. One male had to undergo mitral and aortic valve replacement as a result of acute bacterial endocarditis at 1 postoperative year. *Streptococcus bovis* was isolated from blood cultures, but no colon neoplasia could be detected. Other than serious neglect of dental hygiene, no risk factors for the endocarditis could be found. The occurrence of bacterial endocarditis was considered a major adverse cardiac event. Another male patient underwent elective coronary angioplasty as a result of worsening of his coronary heart disease and angina pectoris at 17 postoperative months. With respect to noncardiac events, a 79-year-old male patient survived a mild episode of pulmonary embolism resulting from deep venous thrombosis of the lower extremity during the late follow-up phase.

In the control group, 1 female patient (as mentioned previously) had sudden cardiac death, which was considered a major adverse cardiac event. No more detailed information could be gained about this patient. A 78-year-old male patient survived global cardiac decompensation after the institution of anti-congestive heart failure therapy. Altogether, there was no significant difference in the frequency of adverse events between the 2 treatment groups (Fisher's Exact test, $P = .364$ between the stem cell and the control groups for the major adverse cardiac events and $P > .999$ for the mild adverse events).

Minnesota Living With Heart Failure Questionnaire

The stem cell-treated patients ($n = 26$) had an average cumulative score of 19.9 ± 17.3 on the MLHFQ, versus 28.4 ± 23.3 in the control group ($n = 14$; Figure 2). These results indicate that patients in the stem cell treatment group judged their quality of life with respect to their heart failure similarly to the patients in the control group ($P = .19$). In the analysis of the stem cell group in the safety trial alone, we encountered a score of 15 ± 7.9 at the long-term follow-up, whereas the stem cell group in the efficacy trial had a score of 23.3 ± 22.3 . The differences were still nonsignificant.

NYHA Functional Classification

The average NYHA functional class improved in the stem cell-treated patients ($n = 34$) from a preoperative value of 2.6 ± 0.3 to 1.6 ± 0.8 after a mean follow-up of 43 months (range, 24–69 months). The control group

FIGURE 2. Comparisons of functional outcomes. A, Minnesota Living With Heart Failure Questionnaire (*MLHFQ*) scores in the stem cell group ($n = 26$) and the control group ($n = 14$) at long-term follow-up. Preoperative Minnesota Living With Heart Failure Questionnaire scores of the groups were not available. B, New York Heart Association (*NYHA*) functional class values in the stem cell group ($n = 34$) and the control group ($n = 13$) both preoperatively (*Pre*) and at long-term follow-up (*LTFU*). Asterisk indicates $P < .05$ versus preoperative value.

($n = 13$) showed a similar improvement from 2.5 ± 0.2 preoperatively to 1.8 ± 0.9 in the long-term evaluation after a mean follow-up of 36 months (range, 21–48 months). The improvement in the NYHA functional class was statistically significant within each group when comparing the preoperative and long-term follow-up values (Wilcoxon test, $P < .001$ for stem cell group and $P = .025$ for control group). Figure 2 shows the change in NYHA functional class, with a comparison of stem cell and control groups. No statistically significant difference was observed between the stem cell and control groups in the long-term follow-up (ANCOVA with preoperative NYHA value as covariable, $P = .647$). When patients in the safety trial were considered separately, a similar estimate of NYHA functional class resulted. Patients in the safety trial ($n = 15$) had improvement from a preoperative NYHA functional class of 2.6 ± 0.3 to 1.5 ± 0.7 in the long-term follow-up. Similarly, patients in the efficacy trial ($n = 20$) had improvement in NYHA functional class from 2.6 ± 0.2 to 1.6 ± 0.8 in the long-term follow-up investigations.

Twenty-Four-Hour Holter Monitoring

In the stem cell group ($n = 20$), the Lown class had not changed after 41 ± 6 months of follow-up, with preoperative and long-term follow-up values of 0.9 ± 1.0 and 1.1 ± 0.9 , respectively ($P = .524$). No malignant ventricular arrhythmias or other life-threatening arrhythmias were recorded at any time during the entire follow-up. The Holter evaluation in the control group ($n = 15$) revealed a greater difference from the preoperative Lown class after 39 ± 5 months of follow-up, with values of 1.2 ± 0.5 and 1.5 ± 0.8 , respectively ($P = .356$).

Echocardiography

Long-term follow-up echocardiography was available for 20 stem cell-treated patients and 15 patients in the control

group. The echocardiographic analyses for the stem cell-treated patients and the control group for the long-term evaluation are given in Table 2. In the stem cell-treated patients, the preoperative LVEF was $41.1\% \pm 8.1\%$, and this value rose significantly to $49.9\% \pm 8.0\%$ at 6 months (paired t test, $P = .002$). LVEF values were determined to be $45.5\% \pm 10.2\%$ at 18 months and $46.9\% \pm 12.1\%$ after a mean of 65 ± 21 months of follow-up. In the control group, the preoperative LVEF value of $40.5\% \pm 10.3\%$ rose to $43.9\% \pm 7.0\%$ at 6 months (paired t test, $P = .032$), and LVEF values were $46.9\% \pm 5.8\%$ and $45.9\% \pm 11.9\%$ at mean follow-ups of 18 and 62 ± 9 months, respectively (Table 2).

For the LVEF value at 6 months, we observed a statistically significant difference between the stem cell and control groups (ANCOVA with the preoperative LVEF value as a covariable $P = .027$) but for the long-term follow-up there was no significant difference (ANCOVA with preoperative LVEF value as covariable $P = .802$). The changes in LVEF during the follow-up period are shown in Figure 3, A.

Because assumptions for a multivariate approach (equality of variance-covariance matrices) were proved to be false by the Box's M test ($P = .026$) we performed univariate LVEF approach of the generalized linear model repeated measures analysis. For testing the within-subjects factor (time) and the interaction of the within-subjects factor and the between-subjects factor (time $\times$ therapy group), validity of the assumption that variance-covariance matrix was circular was assessed by performing the test of sphericity by Mauchly with $P = .005$. Consequently, for testing the within-subjects factor (time) and the interaction of the within-subjects factor and the between-subjects factor (time $\times$ therapy group), we used the Greenhouse-Geisser modification of the F test. As a result, the time effect was found to be significant ($P = .024$) and the time $\times$ therapy group interaction not significant ($P = .431$). That means

TABLE 2. Echocardiographic data of patients who underwent long-term follow-up investigations

	LV ejection fraction (%)		LV end-diastolic volume (mm)	
	Control	Stem cell	Control	Stem cell
Preoperative	40.5% ± 10.3%	41.1% ± 8.1%	58.6 ± 5.5	55.7 ± 5.4
6 mo	43.9% ± 7.0%*	49.9% ± 8.0%*†	56.7 ± 5.6	53.8 ± 7.0
18 mo	46.9% ± 5.8%	45.5% ± 10.2%	57.1 ± 5.3	53.9 ± 6.3
Long-term follow-up	45.8% ± 11.9%	46.9% ± 12.1%	57.1 ± 5.3	56.6 ± 7.3

All data are mean ± SD. Long-term follow-up periods were 65 ± 21 months and 62 ± 9 months for the stem cell (n = 20) and control (n = 15) groups, respectively. LV, Left ventricular. *P < .05 versus preoperative. †P < .05 versus control group.

that on the one hand the hypothesis that for the whole data set the LVEF values at all 4 time points were equal is rejected, and on the other hand the hypothesis that deviations from equality over the 4 time points were the same for the 2 groups is not rejected. Consequently, the factor of group had no independent influence on course during the whole time period. Furthermore, we introduced contrasts among the within-subject variable in to study the levels of the within-subjects factor. We used the deviation contrast comparing a given level of time to the mean of all levels and tested 2 hypotheses. First, averaged over the 2 groups, the mean of the specified contrast was 0. Second, the means of the specified contrast were the same for the 2 groups.

As expected, for the first test, only for the contrast of the difference between the time point of 6 months and the grand mean of all 4 time points we could detect a significant increase averaged over the 2 groups ($P = .024$). Hypotheses that the means of a specified contrast were the same for the 2 groups were rejected only for the contrast of 6 months versus grand mean ($P = .038$).

For the parameter LVEDD, the generalized linear model repeated measures analysis offered no significant differences, neither for testing the within-subjects factor (time) and the interaction of the within-subjects factor and the between-subjects factor (time × therapy group) nor between the 2 therapy groups. When only the randomized

FIGURE 3. A, Comparison of all patients from the stem cell and control groups who underwent echocardiographic evaluation in the long-term follow-up (LTFU) with respect to left ventricular ejection fraction (LVEF) improvement during the follow-up period. B, Left ventricular ejection fraction (LVEF) course of a subgroup of patients in both groups with a preoperative left ventricular ejection fraction not more than 40% is compared between the stem cell group (n = 9) and the control group (n = 7). C, Comparison between the stem cell and control groups of the improvement in the left ventricular ejection fraction (LVEF) in the long-term follow-up relative to the preoperative left ventricular ejection fraction for the subgroup of patients with a preoperative left ventricular ejection fraction not greater than 40%. Long-term follow-ups were 65 ± 21 months and 62 ± 9 months for the stem cell group and the control group, respectively. Asterisk indicates $P < .05$ versus preoperative; dagger indicates $P < .05$ versus control group.

patients (stem cell $n = 12$, control $n = 15$) were analyzed, we could confirm similar results. Only for the contrast of the difference between the time point of 6 months and the grand mean of all 4 time points did we detect a significant increase averaged over 2 groups ($P = .007$). Hypotheses that the means of a specified contrast were the same for the 2 groups were rejected only for the contrast of 6 months versus the grand mean ($P = .011$).

We conducted further subgroup analysis of patients who had a preoperative LVEF no greater than 40%. Comparisons were realized by nonparametric tests to take into account the very small number of patients. Within this subgroup, the stem cell group patients ($n = 9$) had significant improvements from their preoperative mean LVEF of $33.0\% \pm 5.4\%$ to $50.6\% \pm 9.4\%$, $45.6\% \pm 11.8\%$, and $52.1\% \pm 14.8\%$ at 6 months, 18 months, and long-term follow-up, respectively (Friedman test $P = .001$). In comparison, the control group ($n = 7$) showed a nonsignificant improvement of the LVEF from $32.6\% \pm 9.7\%$ preoperatively to $37.6\% \pm 6.9\%$, $46.0\% \pm 7.7\%$, and $39.3\% \pm 14.0\%$ at 6 months, 18 months, and long-term follow-up. Within this subgroup of patients with lower preoperative LVEF, we detected significant differences at 6 months of follow-up exclusively between the stem cell group and the control group (Mann-Whitney test $P = .012$ for LVEF, $P = .016$ for LVEDD, 52.7 ± 7.4 mm and 60.3 ± 3.1 mm for the stem cell and control groups respectively). The LVEF differences in this subgroup of patients in the stem cell and control groups are shown in Figure 3, B and C.

Univariate logistic regression analysis identified the preoperative LVEF, elapsed time after myocardial infarction, and number of bypass grafts as significant factors to include in a multivariate logistic regression analysis (Table E2). Multivariate logistic regression revealed that patients with a preoperative LVEF not greater than 40% had a 44-fold higher chance of showing at least 5% LVEF improvement in the long-term follow-up ($P = .018$). Moreover, when patients were treated between 7 and 12 weeks after myocardial infarction, they had a 56-fold higher chance of at least 5% LVEF improvement relative to those who were treated beyond 12 weeks after myocardial infarction ($P = .023$). The number of bypass grafts constructed did not significantly influence functional improvement (Table E3).

Myocardial Perfusion Scan

Myocardial perfusion scans were available at mean follow-ups of 12 ± 5 and 36 ± 6 months from 24 stem cell-treated patients and 11 patients from the control group. The stem cell-treated patients did not score lower than the preoperative baseline value of 1.0 at 12 and 36 months, with values of 1.04 ± 0.15 and 1.13 ± 0.25 , respectively, whereas the patients in the control group did not show any differences relative to the preoperative levels with respect to the perfusion in the area of interest, with scores

of 0.98 ± 0.13 and 0.95 ± 0.13 after 12 and 36 months, respectively. The scores in both groups compared after a mean follow-up of 36 months revealed a significantly better perfusion score in the stem cell group ($P = .024$).

Figure E1 is a representative image of myocardial perfusion in the area of interest in a patient of the stem cell group taken during follow-up at 3 years.

Magnetic Resonance Imaging

Functional data are available for 18 stem cell-treated patients ($n = 8$ from the safety trial, $n = 10$ from the efficacy trial) and 7 patients in the control group only within long-term follow-up. Preoperative data were not available. No adverse tissue changes were detected. The mean LVEF values detected by MRI were $52.8\% \pm 13.8\%$ in the stem cell-treated patients after a mean follow-up of 41 ± 4 months and $47.3\% \pm 11.5\%$ in the control group after a mean follow-up of 39 ± 3 months. The difference in the LVEF was not statistically significant ($P = .304$). Stem cell-treated patients had LVEDV index value of 68.1 ± 14.9 mL/m², whereas in the control group this value was 90.5 ± 14.3 mL/m². The left ventricular end-systolic volume index values were 31.6 ± 16.3 mL/m² and 53.7 ± 8.3 mL/m² for stem cell and control groups, respectively. The LVEDV and left ventricular end-systolic volume indices were significantly lower in the stem cell-treated patients than in the control group ($P = .017$ for both parameters).

Cardiac CT

Cardiac CT was available for 15 patients in the stem cell group after 49 ± 9 months of follow-up. In the control group, 7 patients underwent cardiac CT after 43 ± 5 months of follow-up. In the long-term follow-up investigations, no adverse tissue changes, such as intramyocardial calcification or tumor formation were recorded in the stem cell-treated patients enrolled in the phase I and phase II trials at the sites of intramyocardial BMSC transplant or in the control group patients from the phase II trial.

DISCUSSION

The main findings of our long-term follow-up investigations can be summarized as follows: (1) Intramyocardial autologous transplant of purified CD133⁺ BMSCs in a dose as high as 10×10^6 cells with concomitant CABG is feasible and safe, and it provides excellent long-term survival and safety according to our follow-up results out to 9 postoperative years. The results show limited and temporary true functional benefit for the myocardial performance, as determined by echocardiography in the long-term evaluation of this small cohort of patients. With regard to possible side effects of the treatment, no adverse tissue changes—neither tumor formation nor calcification at the implantation site—were recorded in the cardiac CT and MRI studies. Furthermore, no relevant malignant or sustained ventricular

arrhythmias were detected in the 24-hour Holter monitoring of the stem cell–treated patients, with no statistically significant changes in the preoperative Lown classification. (2) In the long-term evaluations of myocardial performance and volumetric measurements, cardiac function did not deteriorate significantly in comparison to the preoperative level when patients were analyzed irrespective of preoperative LVEF. Patients who had a moderately to severely compromised LVEF not greater than 40% seemed to gain more functional benefit from stem cell therapy (improvement of at least 5% in LVEF) than did patients with a preoperative LVEF higher than 40%. Only patients with LVEF not more than 40% had significant improvement in LVEF during the entire follow-up period. Moreover, patients who were treated between 7 to 12 weeks after myocardial infarction seemed to have a significantly higher chance of improvement than did those who were operated on later than 12 weeks after infarction.

The NYHA functional class and MLHFQ scores of our stem cell–treated patients seem to indicate a similar clinical course and comparable quality of life to those of the control group. Statistical analysis for these parameters did not detect any significant difference, perhaps as a result of the limited number of patients.

When stem cell and control groups were compared without selection of patients with respect to the preoperative myocardial function, a significant difference in myocardial function regarding LVEF could only be determined after 6 months of follow-up. In a subgroup of patients with a preoperative LVEF not greater than 40%, only those who underwent stem cell injection showed significant improvement in LVEF at 6 months, 18 months, and long-term follow-up, whereas no significant improvement was seen in the control group during the follow-up. Moreover, the LVEF and LVEDD in the stem cell group were significantly better than in the control group at 6 months. Further, we were able to show that preoperative LVEF not greater than 40% may be an independent predictor that significantly influences functional benefit from stem cell treatment beyond the anticipated LVEF improvement of at least 5% after CABG. Importantly, the interval between ischemic myocardial injury and the operation with concomitant stem cell treatment seems to boost the chance of a LVEF improvement of at least 5%, especially if this interval lies between 7 and 12 weeks. Experimental studies suggest cell delivery beyond the early phase of peak inflammatory response, with an optimal result around 3 weeks after acute event. Little guidance can be drawn from clinical studies to date, however, regarding the optimal timing of cell therapy.²¹

With cardiac MRI, we found the mean LVEF in the stem cell group to be higher than that in the control group in the long-term follow-up. In addition, LVEDV and left ventricular systolic volume indices in the stem cell group were also significantly lower than in the control group.

It is conceivable that the functional benefits in the stem cell group may have been related to the improved myocardial perfusion score in the infarction area, which was statistically significant compared to the control group. The lack of preoperative MRI investigations, however, prevents drawing conclusions from these statistical results. Recently, in a short-term study van Ramshorst and associates²² showed improved myocardial perfusion after stem cell treatment in patients who were considered ineligible for myocardial revascularization. Moreover, Klein and coworkers²³ reported, besides in addition to safety, an improvement in MRI-determined LVEF and NYHA functional class in all patients with severely depressed left ventricular function after stand-alone treatment with autologous CD133⁺ BMSCs. In patients with only moderately depressed baseline myocardial function, only regional perfusion and regional contractile function were improved 4 months after intramyocardial BMSC transplant during CABG.²⁴

In a study that used an alternative stem cell delivery route but also intramyocardial injection, Tse and colleagues²⁵ reported long-term safety results of catheter-based autologous bone marrow mononuclear stem cell implantation in 12 patients. In that study, the injected cell number of CD34⁺ stem cells was considerably lower than that in our own trial. There was also no tumor or new scar formation, nor was there calcification at the site of cell implantation. Tse and colleagues²⁵ did not observe any new-onset ventricular arrhythmias. In these patients, however, LVEF was relatively normal at 60% ± 10%, in contrast to a mean preoperative LVEF of 41.1% ± 8.1% in our patient population. Furthermore, a significant number of major cardiovascular events took place, possibly because of the progression of coronary artery disease in nontargeted regions.

Patel and associates¹⁴ demonstrated comparable short-term results in a randomized study of 20 patients with intramyocardial injection of CD34⁺ autologous BMSCs and off-pump CABG surgery. A significant improvement in LVEF as great as 16.7% was observed at 6 months in the stem cell–treated group, in contrast to 6.5% in the control group. Furthermore, catheter-based intramyocardial injections of mononuclear bone marrow cell preparations also have been reported by Perin and colleagues⁷ and Fuchs and associates²⁶ to improve left ventricular function efficiently in chronic ischemic heart disease, partly after longer follow-up periods.

Some of the transcatheter approaches for infusion of bone marrow or circulating blood–derived progenitor cells have been reported to yield functional benefit in patients with acute myocardial infarction. One-year results of the TOPCARE-AMI pilot trial⁴ indicated, in addition to safety and feasibility, a favorable effect on left ventricular remodeling. In the scenario of chronic ischemic heart disease after myocardial infarction, a controlled, randomized study

showed a moderate improvement in left ventricular function in short-term follow-up after intracoronary application of BMSCs.⁵

Similarly, Bartunek and colleagues²⁷ showed improved left ventricular function and increased myocardial perfusion at 4-month follow-up after intracoronary delivery of enriched CD133⁺ progenitor cells. Although they showed the feasibility of intracoronary injection, the treatment was associated with an increased incidence of coronary events in the stem cell group. In contrast, Beitnes and co-workers²⁸ were able to show safety but no functional benefit with respect to left ventricular performance in 50 patients with ST-elevation myocardial infarction 3 years after treatment with intracoronary application of autologous BMSCs during percutaneous coronary intervention.

In a meta-analysis of BMSC studies, Abdel-Latif and associates²⁹ reported the safety data of the studies that predominantly used the intracoronary route for stem cell delivery with a mean follow-up time of 4 months (range, 3–18 months). There was also a lack of uniformity in the stem cell type used as well as the lack of a standard indication for stem cell treatment, such as acute and chronic ischemia. In this statistical analysis, transplant of BMSCs resulted in a 3.66% (95% confidence interval, 1.93%–5.40%) increase in LVEF; modest improvements in the scar size and left ventricular end-systolic volume were also evident. Furthermore, studies with follow-up duration reaching 18 months have failed to show any improvement in the LVEF. In a recent meta-analysis, Donndorf and colleagues³⁰ included only 6 randomized, controlled studies of intramyocardial stem cell transplant with CABG in the setting of chronic myocardial ischemia. They showed significant functional benefit, with a mean LVEF improvement of 5.4% (95% confidence interval, 1.36%–9.44%; $P = .009$) after a median follow-up of 6 months, whereas the incidence of major adverse cardiovascular events did not show any difference between the treatment arms.

Our analysis, however, has some drawbacks. There was no randomization of patients in the safety portion of our trial. Awareness of the study group thus may have affected the estimates of the NYHA functional class or quality of life assessments, although similar results were obtained when stem cell groups in the safety and efficacy trials were compared. In the controlled, randomized portion, difficulties were encountered after the first 12 patients because of the availability of the cell preparation facilities, which led to a change in our randomization plan (Appendix E2). We also are not able to provide graft patency data for our patients, because no follow-up angiography was performed. Even the fact that all patients underwent bypass grafting to the infarct or peri-infarct zone may have influenced myocardial function. Because of the unplanned withdrawal of patients, which led to incomplete follow-up testing of functional assessments, the patient numbers

were limited for the final analysis. This fact again may have caused a lack of significant difference in parameters despite obvious differences between the groups (eg, in long-term LVEF improvement). In addition, MLHFQ and MRI investigations were not available in the preoperative evaluation, so the results of these examinations should be accepted with caution.

Overall, we were able to demonstrate only a temporary and limited efficacy of surgical intramyocardial BMSC treatment for chronic myocardial ischemia in this small cohort. Importantly, we were able to detect a patient group that seems to gain the most long-term benefit with respect to the improvement of LVEF when autologous BMSCs are administered during a CABG procedure. These patients have a moderate to severe reduction of the LVEF as determined by preoperative echocardiographic analysis and are operated on between 7 to 12 weeks after myocardial infarction. It is of utmost importance for the future of cell-based therapies to identify those patients who might benefit from the regenerative stem cell effects to define any additional indications for a concomitant cell-based approach with surgical myocardial revascularization.

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APPENDIX E1. PATIENT RANDOMIZATION

For the prospective, controlled study arm (efficacy study), a randomization plan was generated with an open-access web-based tool (<http://www.tufts.edu/~gdallal/PLAN.HTM>) based on 100 subjects and 5 subblocks. This plan was followed for the first 12 patients. Because of the limited availability of the hematology class B procedure room, pursuing the trial became increasingly more difficult. The stratification strategy was therefore modified: Patients who were operated on during a week when the procedure room was available were allocated to the treatment group. When the hematology class B clean room was not available, the patient was stratified to the control group. Availability of the clean room was beyond the control of the investigators, resulting in the following random allocation sequence: 0100001100111001000101010100101011110111, where 0 represents coronary artery bypass grafting only and 1 represents coronary artery bypass grafting with cell injection. Patients, surgeons, and physicians were blinded to the study group in the in the prospective, controlled study arm.

APPENDIX E2. SURGICAL PROCEDURE

All patients were operated on under conditions of cardiopulmonary bypass and cardioplegic arrest. The left internal thoracic artery was used routinely, and saphenous vein

grafts or radial artery grafts were harvested as needed. All coronary arteries with relevant stenosis and sufficient diameter were grafted. All patients received the same cardioplegic solution. Once the coronary artery anastomoses had been completed, the infarct area was visualized and 10 0.2-mL aliquots of cell suspension were injected in the infarct border zone whenever this could be clearly visualized. Otherwise, cells were injected in an area of myocardium that corresponded to the localization of the perfusion defect on scintigraphy and disturbed wall motion on echocardiography and left ventricular angiography. A swab was used to occlude the injection channel for several seconds to minimize reflux of cell suspension. Immediately after the cell injection, the aortic clamp was removed, and the operation was completed as usual. In the control group of patients undergoing coronary artery bypass grafting alone, no sham injection was performed. After the stay in the intensive care unit and intermediate care unit, patients recovered on the surgical ward for at least 12 days, or they were transferred to the referring cardiology unit earlier. Standard postoperative medication included aspirin (INN acetylsalicylic acid, 100 mg daily), β -blockers, statins, and angiotensin-converting enzyme inhibitors and was adjusted by the cardiologist caring for the patient during follow-up as needed.

FIGURE E1. Representative single-photon emission computed tomographic scans of a patient from the stem cell group who underwent bypass grafting to the left anterior descending, circumflex, and right coronary arteries, as well as the injection of 6×10^6 CD133-selected CD34⁺ cells in the posteroinferior area of myocardial infarction. At discharge (2 weeks), tracer activity in the infarct area was still diminished; however, perfusion had virtually returned to normal at 3 months and showed sustained superior perfusion at 3 years relative to preoperative activity in the area of stem cell injection.

TABLE E1. Numbers of patients who had undergone follow-up investigations for each investigation and time point

	Preoperative	3 mo	6 mo	12 mo	18 mo	36 mo	Long-term follow-up
MLHFQ							SC 26, Control 14
New York Heart Association class	All patients		NP	NP	NP		SC 34, Control 13
Transthoracic echocardiography	All patients			SC 20, Control 15	SC 20, Control 15		SC 20, Control 15
Myocardial perfusion scan				SC 24, Control 11		SC 24, Control 11	
Holter	All patients	NP	NP		NP		SC 20, Control 15
Magnetic resonance imaging							SC 18, Control 7
Computed tomography							SC 15, Control 7

MLHFQ, Minnesota Living With Heart Failure Questionnaire; SC, stem cell group; NP, not presented (follow-up investigation was performed but is not presented in this article).

TABLE E2. Factors included in the univariate and multivariate regression analyses

Parameter	Univariate logistic regression			Multivariate logistic regression		
	<i>P</i> value	Unadjusted OR	95% CI	<i>P</i> value	Adjusted OR	95% CI
Therapy						
Treatment vs control	.589	0.679	0.167–2.77			
Age						
>60 vs ≤60 y	.384	1.86	0.461–7.48			
Sex						
Female vs male	.317	3.25	0.323–32.8			
Postinfarct time (wk)						
≤6 vs >12	.208	3.60	0.491–26.4	.307	4.34	0.260–72.3
7–12 vs >12	.029	12.0	1.29–111	.023	56.4	1.75–1812
New York Heart Association functional class						
2 vs 3	.819	0.750	0.064–8.83			
2.5 vs 3	.860	1.17	0.210–6.48			
Bypass grafts						
≥ 4 vs 3	.168	2.70	0.657–11.1	.858	0.791	0.061–10.3
Preoperative left ventricular end-diastolic diameter (mm)						
55–60 vs ≤55	.951	0.952	0.200–4.54			
>60 vs ≤55	.852	1.19	0.190–7.46			
Preoperative left ventricular end-diastolic volume (mL)						
141–170 vs ≤140	.141	0.268	0.046–1.55			
>170 vs ≤140	.876	0.857	0.124–5.94			
Preoperative left ventricular ejection fraction						
≤40% vs >40%	.004	14.0	2.37–82.7	.018	43.9	1.92–1006

OR, Odds ratio; CI, confidence interval.

TABLE E3. Comparison of left ventricular ejection fraction between stem cell and control groups in subgroups of patients with preoperative left ventricular ejection fraction not greater than 40% and greater than 40% and in subgroups of patients who were treated between 7 and 12 weeks after infarction and treated beyond 12 weeks after infarction

	Preoperative	6 mo	18 mo	Long-term
Preoperative LVEF ≤40				
Control	32.6% ± 9.7%	37.6% ± 6.9%	46.0% ± 7.7%	39.3% ± 14.0%
Stem cell	33.0% ± 5.4%	50.6% ± 9.4%	45.6% ± 11.8%	52.1% ± 14.8%
Preoperative LVEF >40				
Control	47.5% ± 3.7%	49.5% ± 3.0%	47.6% ± 4.5%	52.1% ± 6.9%
Stem cell	47.2% ± 3.8%	49.3% ± 7.1%	45.5% ± 9.7%	42.6% ± 8.5%
Treated 7–12 wk after myocardial infarction				
Control	40.5% ± 7.4%	44.3% ± 6.4%	53.0% ± 2.8%	46.8% ± 8.0%
Stem cell	41.9% ± 8.8%	49.5% ± 6.7%	49.4% ± 7.6%	52.5% ± 8.8%
Treated beyond 12 wk after myocardial infarction				
Control	43.6% ± 6.5%	44.6% ± 7.4%	42.0% ± 8.2%	42.2% ± 18.7%
Stem cell	43.5% ± 7.3%	53.5% ± 3.4%	50.3% ± 7.1%	44.0% ± 13.8%