Evolving Technology

Intramyocardial delivery of CD133$^+$ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: Safety and efficacy studies

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Supplemental material is available online.

Objectives: Cell therapy may offer novel therapeutic options for chronic ischemic heart disease. In a clinical trial, we first assessed the feasibility and safety of intramyocardial CD133$^+$ bone marrow cell injection together with coronary artery bypass grafting (CABG). We then tested the hypothesis that CABG plus CD133$^+$ cell injection would result in better contractile function than CABG alone.

Methods: Fifteen patients took part in the safety study, followed by 40 patients who underwent either CABG with cell therapy or CABG alone. Bone marrow was harvested from the iliac crest one day before surgery, and purified CD133$^+$ progenitor cells were injected in the infarct border zone during the CABG operation. LV function was measured by echocardiography and myocardial perfusion by SPECT.

Results: In the safety study, no procedure-related complications were observed for up to 3 years. LV injection fraction (LVEF) increased from 39.0% ± 8.7% preoperatively to 50.2% ± 8.5% at 6 months and 47.9% ± 6.0% at 18 months ($F = 6.03$, $P = .012$). In the efficacy study, LCEF rose form 37.4% ± 8.4% to 47.1% ± 8.3% at 6 months in the group with CABG and cell therapy ($F = 24.16$, $P < .0001$) but only from 37.9% ± 10.3% to 41.3% ± 9.1% in the CABG-only group ($F = 7.72$, $P = .012$). LVEF was significantly higher at 6 months in the group with CABG and cell therapy than in the CABG-only group ($P = .03$). Similarly, perfusion of the infarcted myocardium improved more in patients treated with CABG and cell therapy than in those treated with CABG alone.

Conclusion: Intramyocardial delivery of purified bone marrow stem cells together with CABG surgery is safe and provides beneficial effects, though it remains to be seen whether thewe effects produce a lasting clinical advantage.

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Cellular therapy for ischemic heart disease has attracted tremendous attention, especially since initial experimental studies have suggested that somatic stem cells can regenerate both blood vessels and cardiomyocytes after myocardial infarction. Recently, the capacity of bone marrow–derived stem cells to form new heart muscle cells has been questioned, but clinical pilot trials have been initiated nevertheless.[E1,E2] In the setting of acute myocardial infarction, several

Abbreviations and Acronyms
CABG = coronary artery bypass grafting
ECG = electrocardiography
LV = left ventricle
LVEF = LV ejection fraction
MRI = magnetic resonance imaging
PBS = phosphate-buffered saline solution

studies have shown a functional benefit of intracoronary infusion of bone marrow cells relative to the standard treatment alone,[E3-E5] but patients with chronic ischemic heart disease and impaired heart function may require a different approach. Our group therefore developed a protocol for injection of purified $CD133^+$ bone marrow stem cells directly into the diseased myocardium at the time of coronary artery bypass grafting (CABG). Because of the encouraging results in the first 6 patients,[E6] we completed a dose-escalation safety trial and then conducted an efficacy study to compare the outcome with that of standard CABG. Results of both trials are presented here.

Patients and Methods

The study was approved by the institutional review board and ethics committee at Rostock University (including the safety and the efficacy trial as well as all subsequent protocol modifications). Inclusion criteria were as follows: (1) history of myocardial infarction at least 14 days previously, (2) indication for bypass surgery on coronary arteries other than the infarcted vessel, and (3) distinct area of akinetic left ventricular (LV) myocardium corresponding with the infarct localization. Exclusion criteria were as follows: (1) debilitating chronic disease (eg, 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 before the onset of the phase II trial. First, patients needing concomitant mitral valve repair for regurgitation were included. Second, in the absence of a distinctly akinetic area of LV myocardium, a globally reduced LV ejection fraction (LVEF) was accepted. Once the presence of the main inclusion criteria was determined, enrollment was discussed with the patient and informed consent was obtained. Then the patient was referred for myocardial perfusion scintigraphy, Holter electrocardiography (ECG), and, lately, cardiac magnetic resonance imaging (MRI). In the efficacy study, patient allocation to the CABG alone or CABG plus cellular treatment group was performed as described in Appendix E1. Preoperative patient characteristics were similar between the groups and are given in Table E1. Enrollment for the safety trial began in August 2001 and was completed in February 2003. The efficacy study began in May 2003 and was terminated in February 2005. The recruitment history according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines, is given in Figure 1.

Cell Preparation

One day before CABG, with local anesthesia, bone marrow was aspirated from the iliac crest with preheparinized syringes. The marrow was brought to the hematology clean room lab, and $CD133^+$ stem/progenitor cells were isolated by magnetic separation with ferrite-conjugated antibody (Miltenyi CliniMacs System; Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometry–based quality control measurements were performed at various steps throughout the procedure. Details are given in Appendix E2.

Surgical Procedure

All patients were operated on with cardiopulmonary bypass and cardioplegic arrest. The left thoracic artery was used in most but not all cases (depending on the presence of an anterior vessel that could be grafted), and saphenous vein grafts or radial artery grafts were harvested. When there was mitral regurgitation grade III or higher according to transesophageal echocardiography on the operating table, the mitral valve was repaired by ring annuloplasty. All coronary arteries with relevant stenoses and sufficient diameter were grafted, including, if possible, the previously infarcted vessel. Once the graft–coronary artery anastomoses had been completed, the infarcted area was visualized, and 10 injections of 0.2 mL of cell suspension were made into the infarct border zone if 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 LV 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 CABG alone patients, no sham injection was performed. After their stay in the intensive care unit and the intermediate care unit, patients recovered on the surgical ward for at least 12 days or were transferred to the referring cardiology unit earlier. Standard postoperative medication included aspirin (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.

Outcomes and Follow-up

In the safety study, the primary outcome was freedom from death from cardiac disease or major cardiac event at 12 months. Secondary outcomes were ventricular arrhythmia and any class III or class IV event according to a modified Centers for Disease Control and Prevention classification. In the efficacy trial, the following null hypothesis was formulated: At 6 postoperative months, there would be no difference in average LVEF between CABG alone and CABG with cell injection. Secondary outcome parameters were myocardial perfusion in the infarcted area and the same safety parameters as in the safety study. Before referral to a cardiac rehabilitation program at approximately 2 postoperative weeks, Holter ECG, transthoracic echocardiography, and myocardial perfusion scintigraphy were performed. The next Holter ECG and echocardiogram were recorded at the end of the rehabilitation program in the respective institution; however, the echocardiography data were not used for quantitative analysis in the study. Echocardiography and myocardial perfusion scintigraphy were repeated in our institution at 6

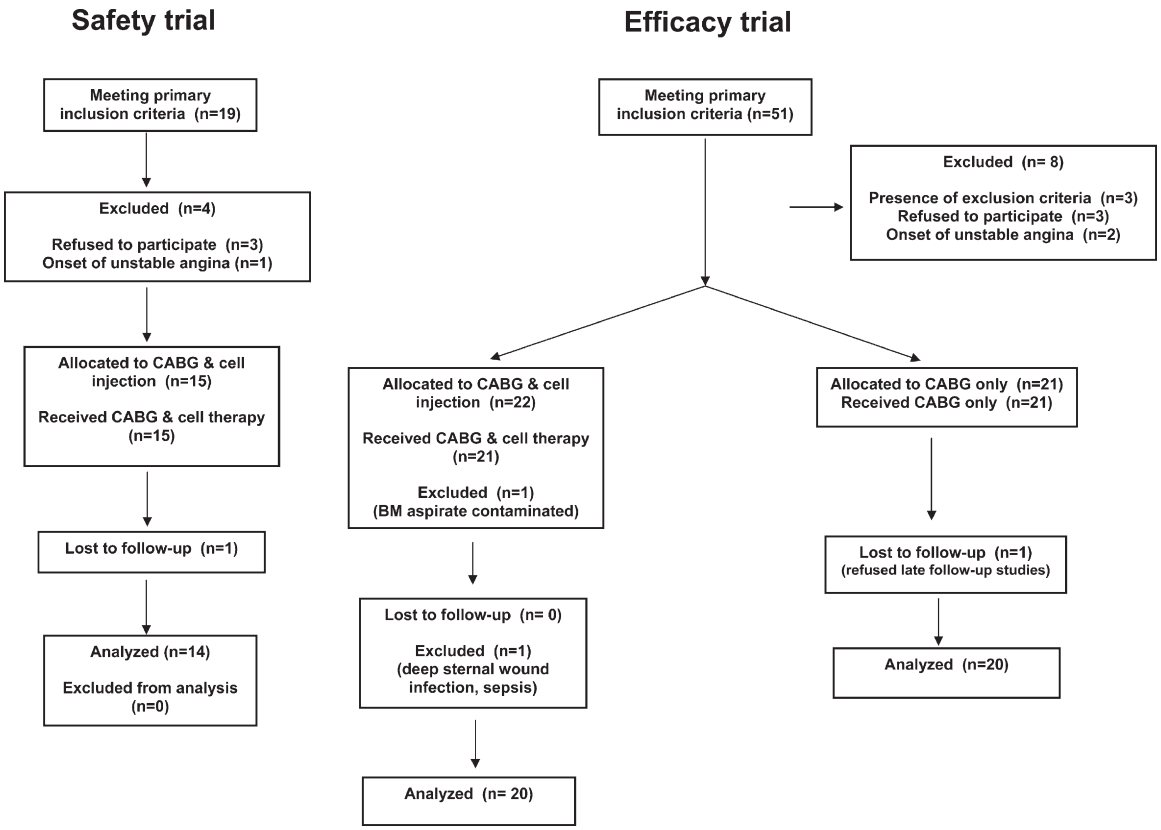

Figure 1. Consolidated Standards of Reporting Trials flowchart of trial history. *CABG*, coronary artery bypass grafting.

postoperative months in the efficacy trial and also after 18 months in the safety trial.

Echocardiography

Cardiac transthoracic ultrasonographic studies were performed for measurement of global LV contractility and dimensions. The method is described in detail in Appendix E3. The studies were carried out by two experienced echocardiographers (A.D., C.N.) who were blinded to the presence and location of the cell injection. Measurements obtained by the two independent echocardiographers were consistent. In a separate set of patients, the echocardiographic data were validated with cardiac MRI, and a close correlation of the LVEF measurements was found (Figure E1).

Myocardial Perfusion Scans

For myocardial-perfusion, single-photon emission computed tomography at rest, 100 MBq thallous chloride Tl 201 was intravenously injected, and scans were done 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.

Statistical Analysis

Continuous data are presented as mean ± SD. For variables not normally distributed, medians and ranges are presented (cell isolation data, infarct time, myocardial perfusion data). To compare preoperative patient characteristics between the groups, the Student t test was used for continuous data and the χ^2 test was used for categorical data. Comparisons of changes in functional data, including LVEF, LV end-systolic volume, LV end-diastolic volume, LV end-systolic diameter, and LV end-diastolic diameter with time, were done with repeated-measures analysis of variance with the Greenhouse–Geisser F test to evaluate treatment and time effects.[E7] Variables not conforming to a normal distribution (myocardial perfusion data) were compared with the Mann–Whitney U test. Agreement between echocardiography-based and cardiac MRI determinations of LVEF was determined by the Bland-Altman method, and the mean difference was used to assess bias and 95% confidence intervals (2 SD).[E8] On the basis of the results of the safety trial, the efficacy trial required (version 6.0, nQuery Advisor; Statistical Solutions, Saugus, Mass) 20 patients in each group to attain 80% power for detecting a relative difference of 10% in average LVEF between the groups, assuming an 8% SD (effect size of 1.25, $\alpha = .05$, $\beta = 0.2$). Statistical analysis was performed with the SPSS software package (version 14.0; SPSS Inc, Chicago, Ill).

Results

Patient- and procedure-related baseline data are given in Table E1, and no significant differences were found between the two groups. Overall, 80% of the patients were male, and the mean age at surgery was 63 ± 5 years. The interval between documented myocardial infarction and surgery ranged from 2 weeks to 3.2 years, with a median of 7.9 weeks. All patients had moderate to severe symptoms of coronary artery disease with reduced exercise capacity and chest pain, and the indication for surgery was triple- or double-vessel disease with or without left main coronary artery stenosis in all but 1 case. In that case, the primary indication for surgery was mitral valve incompetence after extensive transmural anterior myocardial infarction; the infarcted vessel had been revascularized by percutaneous transluminal coronary angioplasty and stent placement 5 years previously. During surgery, 3.5 ± 0.7 bypass–coronary artery anastomoses per patient were constructed, and the cell injection was performed as described previously. During the postoperative intensive care unit stay, most patients received low-dose inotropic support until the first postoperative day, but none had symptoms of low cardiac output requiring high-dose inotrope infusion or intra-aortic balloon counterpulsation. Two patients per group showed elevated creatine kinase levels on postoperative day 1, but without evidence of acute transmural infarction on ECG. These patients were not excluded from further analysis.

Bone Marrow Cell Preparation

Between 91 and 265 mL (median 156 mL) of bone marrow was harvested by aspiration from the iliac crest. The median percentage of $CD34^+$ cells in all bone marrow aspirates was 0.8% (range 0.26%-1.44%), corresponding to a median absolute number of 2.95×10^7 $CD34^+$ cells (range 3.85×10^6-1.03×10^8). After cell selection with AC133/1(CD133) monoclonal antibody, the median number of $CD133^{selected}/CD34^+$ cells was 5.80×10^6 (range 1.08×10^6-8.35×10^7), with a median purity of 75.8% (range 53.1%-89.6%). In only 1 patient (aged 40 years) was the final cell dose higher than 10×10^6. Median recovery of $CD34^+$ cells was 18.3%. Viability of the cell product, as measured by propidum iodide exclusion, ranged between 77% and 99% (median 94%). For details, see Table E2.

Safety Trial Results

All 15 patients included in the dose-escalation safety trial tolerated the operation well and were extubated during the first postoperative night. One patient underwent a rethoracotomy for bleeding from the internal thoracic artery on the day of surgery. Two patients had symptoms of respiratory tract infection that were treated with antibiotics, and another 2 patients had transient pleural effusions. Otherwise, in-hospital convalescence was uneventful, and all patients were referred to a cardiac rehabilitation program during the third postoperative week. Follow-up time currently ranges between 30 and 50 months, encompassing a total of 625 patient-months. No relevant ventricular arrhythmia was recorded at any point by online telemetric monitoring or Holter ECG, and the reported exercise tolerance improved in all patients. A 75-year-old patient was unavailable for follow-up 9 months after surgery. He had cerebrovascular disease with a history of multiple transient cerebral ischemic events but without gross neurologic deficits at the time of operation. Subsequent investigation revealed that he had died of a stroke. No autopsy was performed. All other patients were alive and well at the time of preparation of the paper. By the end of the rehabilitation process, all patients but 1 were in New York Heart Association functional class I. Recatheterization was not performed, but there was no evidence of new regional contractile dysfunction indicating relevant graft dysfunction. The echocardiographic data of the safety trial patients are depicted in Figure 2. Overall, average LVEF increased significantly, from 39.0% ± 8.7% preoperatively to 50.2% ± 8.5% at 6 months and 47.9% ± 6.0% at 18 months (Greenhouse–Geisser $F = 6.03$, $P = .012$, repeated-measures analysis of variance). LV end-systolic volume declined significantly, from 92.3 ± 35 mL preoperatively to 65.4 ± 20 mL at discharge ($P = .004$), 66.2 ± 24 mL at 6 months ($P = .008$) and 65.8 ± 11 mL at 18 months ($P = .013$). LV end-diastolic volume decreased, from 144 ± 37 mL preoperatively to 121 ± 23 mL at 6 months and 127 ± 18 mL at 18 months, but this did not represent a significant change with time ($F = 2.07$, $P = .18$).

Myocardial perfusion in the area of interest was assessed by thallium single-photon emission computed tomographic scans. The activity in the area at risk—expressed as the ratio of postoperative to preoperative activity—demonstrated improved perfusion in the previously nonperfused or hypoperfused infarction zone in 13 patients. The median perfusion ratio after CABG with $CD133^+$ cell injection increased by 15% to 1.15 at 2 weeks ($P < .01$, Wilcoxon signed rank test) and remained stable with a ratio of 1.14 at 6 and 18 months (Figure E2). Figure 3 depicts representative perfusion scans from a patient who received 5×10^6 $CD133^{selected}/CD34^+$ cells in the border zone of a posterior transmural myocardial infarction, where no bypass graft could be placed. At the time of discharge, there was no relevant improvement, but perfusion of the ischemic tissue had virtually returned to normal 6 months later. This secondary gain in tissue blood supply may have been attributable to the cell injection.

Efficacy Trial Results

A total of 43 patients were assigned to undergo either CABG with cell injection or CABG alone. In 1 patient of

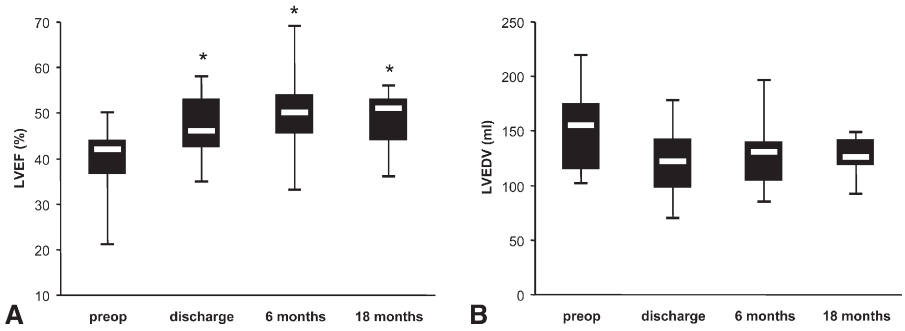

Figure 2. Echocardiographic data of patients included in safety trial (n = 15). A, Relative to preoperative *(preop)* data, left ventricular ejection fraction *(LVEF)* increased significantly in response to coronary artery bypass grafting with CD133^{+} cell injection ($F = 6.03$, $P = .012$, repeated-measures analysis of variance). Left ventricular ejection fraction values were significantly higher than preoperative values at discharge and at 6 and 18 months (all $P < .05$). B, Trend toward sustained reduction in left ventricular end-diastolic volume ($P = .06$, preoperative vs discharge). *Box plots* indicate 25th and 75th percentiles *(solid box),* median *(white bar),* and minimum and maximum values of each data set *(whiskers).* Asterisk indicates $P < .05$ versus preoperative data.

the CABG with cell injection group, the primary bone marrow aspirate tested positive for bacterial contamination on microscopy; the final cell product, although sterile, was not delivered. Another patient, who had a long history of diabetes, had deep sternal wound infection in the second postoperative week and required repeated wound revision

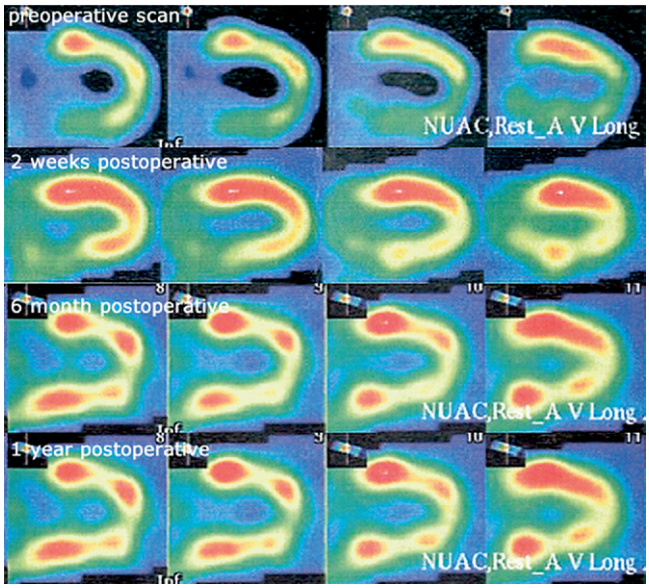

Figure 3. Representative single-photon emission computed tomographic scans of patient in safety trial who underwent bypass grafting to left anterior descending coronary artery and its branches, as well as injection of 5×10^6 CD133^{selected}/CD34^{+} cells in posterior infarct area. At discharge *(second panel),* tracer activity in infarct area was still diminished, but at 6 months *(third panel),* perfusion had virtually returned to normal.

and open wound healing. He was hospitalized for 4 months and refused further follow-up examinations. In the CABG alone group, 1 patient was retrospectively excluded because she refused any follow-up examinations after discharge. All but 1 patient who remained in the study had an uneventful postoperative course. The only early postoperative complication was a low cardiac output syndrome with acute renal failure in 1 patient, requiring medium-dose catecholamine treatment and temporary hemofiltration. This patient recovered completely and was transferred to the ward on postoperative day 6. During the follow-up period, no major adverse events (death, myocardial infarction, or cardiac reintervention) were reported, and all patients were alive and well at most recent follow-up. There was no difference in New York Heart Association functional class between groups.

The echocardiographic data on LV function are summarized in Table E3, and the data relevant for the primary outcome parameter, LVEF at 6 months, are depicted in Figure 4. The average LVEF rose, from 37.4% ± 8.4% to 47.1% ± 8.3% at 6 months in patients undergoing CABG with cell injection ($P < .0001$) and from 37.9% ± 10.3% to 41.3% ± 9.1% in patients undergoing CABG alone ($P = .012$). As required by the study protocol, direct comparison of the primary outcome parameter (average LVEF at 6 months) between the two treatment groups achieved significance ($P = .03$), with the 95% confidence interval for the mean difference in LVEF between 3% and 11%. Within the range of probability defined by the statistical power, the null hypothesis is therefore rejected, indicating that CABG with cell injection results in significantly better LVEF than does CABG alone. The average changes in LVEF were +9.7% ± 8.8% in the CABG with cell injection group and +3.4%

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± 5.5% in the CABG alone group ($P = .02$). The mean difference between groups in the change in LVEF from preoperative baseline to 6 postoperative months was 6.3% (95% confidence interval for difference 3%-11%). Figure 5 shows that this difference developed late after CABG, during the interval between the time of discharge and 6-month follow-up.

As determined with single-photon emission computed tomographic imaging, myocardial perfusion in the area of interest at 6 months had improved in 4 CABG alone patients, versus 11 patients who were treated with CABG with cell injection ($P < .05$, Fisher exact test). The distribution of the ratio of colorimetric quantification of tracer activity in the area of interest was determined relative to the preoperative value. Improvement in perfusion at 6 months was greater in the CABG with cell injection group (median ratio 1.02, interquartile range 0.95-1.11) than in the CABG alone group (median ratio 0.95, interquartile range 0.91-1.03; Figure E3).

Subgroup Analysis

By univariate analysis within each group and also within the combined cohort of all cell-treated patients (safety trial and efficacy trial), there was no correlation between the functional effect of the operation (defined as change in LVEF) and patient age, cell dose, interval between infarct and surgery, or any other variable except one preprocedural or periprocedural variable. The only relevant association indicated a moderate inverse correlation between preoperative LVEF and the gain in LVEF after CABG with cellular treatment (Pearson $r = -0.56$, $P < .001$, n = 35). That is, a lower LVEF before the operation was associated with a larger increase in LVEF after CABG with cell injection (Figure 6, *A*). Indeed, when all patients who underwent CABG with cellular treatment are grouped according to preoperative LVEF (< 35% vs ≥35%), the notion that patients with a poorer LVEF benefit more is further supported. Patients with a preoperative LVEF less than 35% showed a mean increase of 15.3% (95% confidence interval 10.8%-20.4%), significantly greater than the change in LVEF in patients with preoperative LVEF of at least 35% (increase of 7.8%, 95% confidence interval 4.1%-11.5%, $F = 5.87$, $P = .02$, 2-way analysis of variance; Figure 6, *B*).

Discussion

We evaluated the effects of intramyocardial delivery of $CD133^+$ bone marrow cells in chronically ischemic hearts of patients undergoing CABG. In the initial safety trial, no cell injection-related complications were observed during up to 4 years follow-up. LV function improved; however, the safety trial did not allow differentiation between the effects of cell injection and CABG. In the subsequent efficacy trial, 40 patients were stratified to undergo CABG with cell injection or CABG alone, and we found that global LV systolic function at 6 months was moderately but significantly better in cell-treated patients. It therefore appears that concomitant injection of $CD133^+$ bone marrow cells yields a functional benefit in addition to CABG.

Adult stem or progenitor cells derived from blood or bone marrow are readily available for clinical use, although experimental evidence regarding their true myocardial regeneration capacity remains inconclusive[E1,E9,E10] Nevertheless, numerous small and large animal studies have provided evidence of functional benefits of bone marrow–derived stem cells in ischemic myocardium, even in the absence of quantitatively relevant cardiomyocyte differentiation.[E2,E11] Clinically available $CD34^+$ and $CD133^+$ bone marrow stem cells have proved especially effective for improving blood supply to ischemic tissue.[E10] $CD133^+$ cells readily assume an endothelial cell phenotype in vitro[E12] and have been shown to improve myocardial function in rats.[E7] Our own preclinical evaluation in mice showed that human $CD133^+$ bone marrow cells increase blood vessel count and reduce cardiomyocytes apoptosis in the infarct border zone.[E8] Other possible mechanisms include beneficial effects on extracellular matrix composition.[E13] In this context, recent research has identified the hibernating myocardium, which is to some degree nearly always present in the chronic infarct border zone, as a particularly responsive target of experimental and clinical cardiac cellular therapy.[E14,E15] On the basis of the existing preclinical evidence, we and others have come to the decision that clinical pilot trials are justified and in fact needed. In 2001, we initiated a phase I analogous safety trial with incremental escalation of the cell dose. We chose to inject bone marrow cells enriched for CD133 to avoid potential proinflammatory side effects of unmodified mononuclear cell preparations on direct delivery to the myocardium. Furthermore, we deemed it important to work with a well characterized, distinct cell population. Data from the first patients have been reported before,[E6,E16] and the encouraging results prompted us to complete the safety trial and proceed with an efficacy study. Even though the observed difference in LVEF at 6 months is modest, we believe it still serves to provide proof of principle, namely that direct intramyocardial injection of purified bone marrow stem/progenitor cells does have beneficial effects on chronically ischemic human hearts. This notion has recently been corroborated by other investigators. Erbs and colleagues[E14] showed functional improvement after intracoronary injection of peripheral blood-derived progenitor cells in patients with chronic ischemia, and Patel and associates[E17] reported on a trial similar to ours. In the latter study, $CD34^+$ bone marrow cells were implanted at the time of off-pump CABG and induced a significantly greater improvement of contractile function than did CABG alone.

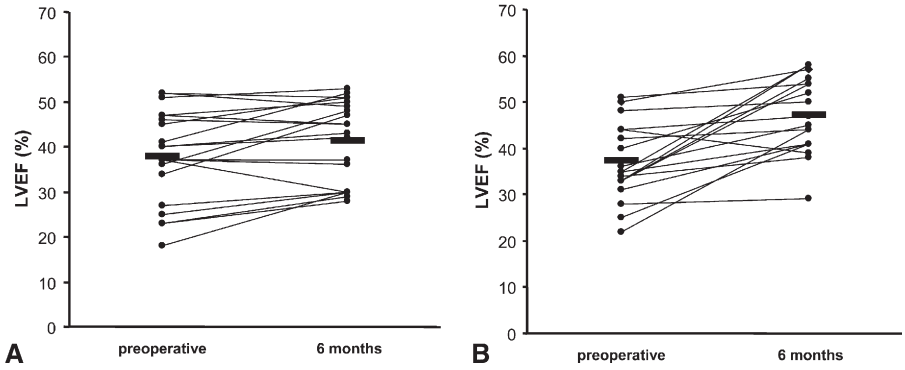

Figure 4. Left ventricular ejection fraction *(LVEF)* before surgery *(preoperative)* and at 6 months' follow-up *(6 months)* for individual patients. A, In patients undergoing coronary artery bypass grafting alone (n = 20), average LVEF *(horizontal bar)* rose from 37.9% ± 10.3% to 41.3% ± 9.1% ($F = 7.72$, $P = .012$, repeated-measures analysis of variance). B, In patients who underwent coronary artery bypass grafting with $CD133^+$ bone marrow cell injection (n = 20), average left ventricular ejection fraction rose from 37.4% ± 8% to 47.1% ± 7% ($F = 14.84$, $P < .0001$, repeated-measures analysis of variance). Analysis of variance revealed significantly greater increase with coronary artery bypass grafting with $CD133^+$ cell injection relative to coronary artery bypass grafting alone ($P = .02$).

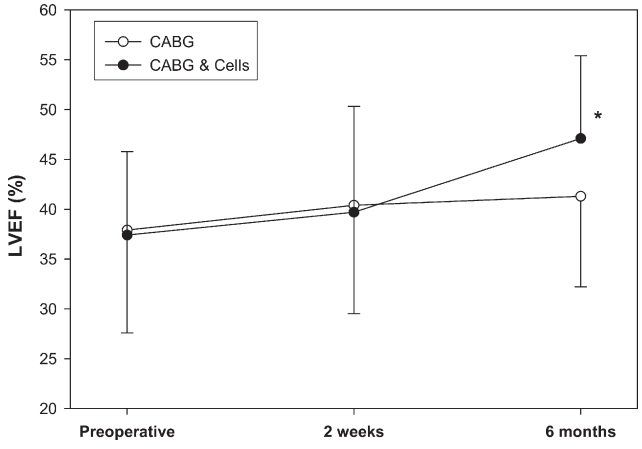

Figure 5. Mean left ventricular ejection fraction *(LVEF)* for coronary artery bypass grafting *(CABG)* and coronary artery bypass grafting with cellular therapy *(CABG & cells)* groups, indicating significant improvement for both groups between preoperative baseline and 6 months but with much larger improvement in patients treated with coronary artery bypass grafting with cells than with those treated with coronary artery bypass grafting alone (9.7% ± 8.8% vs 3.4% ± 5.5%, respectively). There was no significant mean difference in left ventricular ejection fraction between 2 weeks and 6 months in coronary artery bypass grafting alone group ($P = .52$), whereas significant increase was seen in coronary artery bypass grafting with cellular therapy group ($F = 14.84$, $P < .001$). *Error bars* represent SD. Asterisk denotes significant group difference at 6 months (mean difference 6.3%, 95% confidence interval 3%-11%, $P < .01$).

A number of reports on other clinical studies have described similar advantageous effects of bone marrow mononuclear cells injected in the infarct-related coronary artery of patients early after acute infarction[E3,E5,E18]; however, other trials have shown little if any clinical effect.[E19] Other than differences in cell type ($CD133^+$ vs bone marrow mononuclear cells), delivery route (intramyocardial vs intracoronary), and concomitant procedures (percutaneous transluminal coronary angioplasty vs CABG), the most relevant distinction of our approach is patient selection. With an interval between myocardial infarction and cellular treatment of several months or years, acute ischemia and subsequent local inflammatory infiltration have abated, and myocardial remodeling processes, including scar formation, are most likely completed. The cellular mechanisms required to beneficially influence myocardial function may be completely different from those occurring in the face of cellular therapy in acutely ischemic hearts. In that respect, our patient cohort is not homogenous. In some cases, the interval between infarct and cellular treatment was a few weeks; in others, several years. It therefore seems likely that the amount of hibernating myocardium varies greatly among individual patients, and this confounding factor could contribute to the heterogeneity of the functional treatment response. In future studies, we plan to localize and quantify areas of hibernating myocardium before cellular treatment and use this information for patient selection or retrospective correlation with functional outcome data.

In any event, it should be noted that both cellular therapy targets (acutely and chronically ischemic myocardium) are

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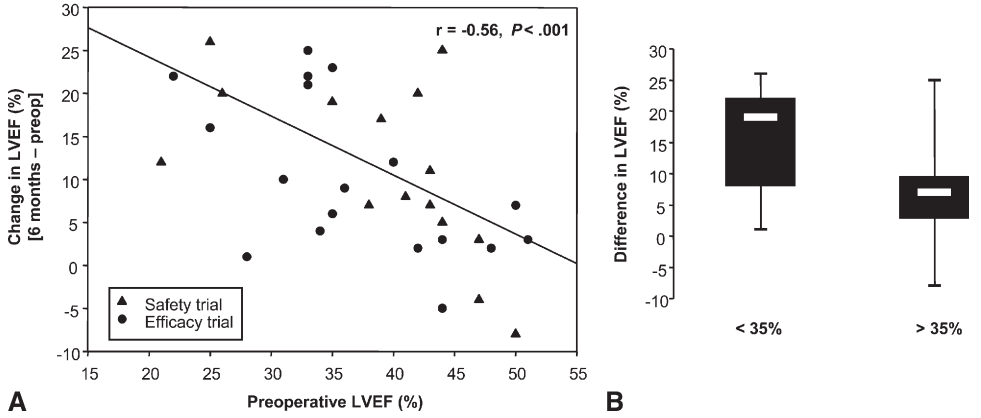

Figure 6. Association between preoperative left ventricular ejection fraction *(LVEF)* and gain in left ventricular ejection fraction at 6-month follow-up. A, Linear regression indicated trend toward inverse correlation between preoperative left ventricular ejection fraction and postoperative change in left ventricular ejection fraction. B, All cell-treated patients (safety and efficacy trials combined) grouped according to preoperative left ventricular ejection fraction (<35% vs >35%). Patients with preoperative left ventricular ejection fraction less than 35% had increase in LVEF by average of 15.4%, whereas patients with baseline LVEF greater than 35% had increase by only 7.8% ($F = 5.87$, $P = .02$, 2-way analysis of variance).

not mutually exclusive or competitive. Even if treatment of acute myocardial infarction can be further optimized by rapid cellular therapy, there will always be a substantial number of patients who are first seen with heart function already impaired because of silent ischemic events or failure of emergency treatment.

Limitations

Our study has a number of limitations, which should be taken into account when interpreting the results. We did not have cardiac MRI, the current clinical criterion standard for analysis of global and regional LV contractility, available during the first years of the trial, and echocardiographic LVEF measurements are expected to be less accurate. The greater variability of the LVEF data should be reflected in both the cellular treatment group and the CABG alone group, however, rendering it unlikely that a systematic error favors either cohort. Moreover, we validated our echocardiography protocol by direct comparison with cardiac MRI–derived LVEF data and found a close correlation between the techniques. It is also clear that there is a variable degree of interplay between myocardial revascularization by CABG and the effects of the cellular treatment. This cannot be completely avoided in combination therapy studies, even if one strictly avoids grafting the previously infarcted vessel. Another limitation is the heterogeneity of the preoperative LV contractility, with LVEF ranging between 18% and 48%. During the course of the trial, we had the impression that patients with very poor preoperative LV function benefited more from CABG with cell injection than did those with better preservation of LV function. In fact, our data clearly indicate this to be the case. We will therefore aim at treating only patients with LVEF less than 35% in future trials. As discussed previously, we do not know the amount of hibernating myocardium in our patients, which could help explain the variability of the functional response to cellular treatment. This is illustrated by the finding that most of the functional improvement occurred during the early postoperative period in the phase 1 study arm, whereas the gain in LVEF developed rather late in the safety trial. Finally, our study was underpowered in terms of detecting differences in LV volumes, which needs to be addressed in large-scale trials.

Given that autologous bone marrow stem cells can indeed improve the function of chronically ischemic myocardium in addition to the beneficial effects of traditional revascularization procedures, we believe that there is room for substantial further improvement. The cell number we used is rather small (only 1 of our patients received 80×10^6 $CD133^+$ cells; all others received between 1.2 and 10×10^6 cells), and the overnight storage of the cell product may have impaired its biologic activity. In a recent study by Asahara and coworkers,[E20] there was a clear dose-response relationship of human $CD34^+$ cells in rats, but it is not clear how this translates into the clinical setting. Other cell types with a greater likelihood for true cardiomyocyte differentiation (mesenchymal stem cell–derived cells) might ultimately prove more efficient. Strategies to precondition cells before implantation by pharmacologic, genetic, or physical means are also currently under evaluation. For the time being, however, clinicians have to resort to clinically avail-

able cell products. On that basis, we believe the approach that we have chosen to be effective.

Discussion

Richard D. Weisel *(Toronto, Canada).* I greatly appreciate the excellent presentation, and I thank Drs Steinhoff and Stamm for sending me the manuscript in advance.

This study is important for cardiac surgeons because it echoes the information I presented last year suggesting that surgeons not only need to bypass coronary arteries, fix ventricles, and repair valves but also need to change the response of the heart to our interventions. Surgeons should introduce biologic interventions whenever they perform mechanical interventions. Your study demonstrates that biologic interventions can have profound effects of the response of the heart to our mechanical interventions. Unfortunately, you have not identified the mechanisms responsible for the benefit, and we therefore may have difficulty integrating this approach into our surgical practice.

When we originally implanted cells into the heart, we thought we were producing new heart cells. Subsequent studies have determined that none of the cells implanted into the heart transdifferentiated into new heart cells. The mechanism responsible for the improved function seen after the implantation of a variety of cell types has thus not been elucidated. How does cellular transplantation work? We have previously demonstrated that cellular transplantation induces angiogenesis and matrix remodeling, as well as recruiting endogenous stem cells from the heart and the bone marrow to the heart. If these are the mechanisms responsible for the improved function, then perhaps we need to augment those effects with any surgical interventions on the heart. So my first question for you is as follows: What is the mechanism responsible for the functional benefit, and should you augment your cells with genes or proteins to increase the benefit of cellular transplantation? Do you believe that your cells will transdifferentiate into cardiomyocytes?

I also had some concerns with your study. You had difficulty with the randomization. You were unable to complete the study according to your original trial design because of the unavailability of the room to perform the bone marrow biopsy. I am therefore concerned that you may have biased the randomization.

In your article, you report a significant difference between the two groups in end-systolic volume. I was concerned that the control group, the CABG alone group, had larger hearts before the operation and that this could not be improved with any type of therapy. Only 6 of your control patients had improvement postoperatively and 14 did not, which is not what we would anticipate after CABG. In addition, you did not use the Canadian laser in your control group. The Canadian laser is the insertion of a needle into the heart, which previous investigators have demonstrated to increase angiogenesis. The needle injection itself may have improved the functional outcome, and this procedure was only used in the cell transplant group, not the control group. I suggest that you use needle injection in your proposed phase III trial.

Finally, I was concerned about the randomization, because only 2 additional patients showed an improvement in LVEF in the treatment versus the control groups. The LVEF increased in 8 treated patients and 6 control patients. This difference was small but statistically significant. The difficulty you had with randomization thus could have influenced the outcomes.

In summary, I think that this is an important study, and it certainly will advance the field. Cardiac surgeons should go on to the next phase to develop a new treatment to restore cardiac function in our patients undergoing CABG. We should establish the mechanisms responsible for the improved function, however, but then augment those effects by adding genes or proteins to the cells implanted into the infarcted myocardium. Biologic interventions may be as important as mechanical treatments to restore our patients to full activity.

Dr. Steinhoff. Thank you, Dr. Weisel. The introduction of such a method has several phases, and this first phase I and II study of CD133+ intramyocardial stem cell transplantation is testing safety, and biological effects. Of course, the last is a difficult option with the diagnostic methods we have available. To unravel the underlying mechanisms there has to be a high correspondence between experimental models and clinical studies. I think it is difficult, at present, to exactly understand the sequence of cellular reactions that lead to cardiac regenerative processes.

We just had the basic science lecture about apoptosis, and apoptosis is also probably an important feature of exchange of cells necessary for tissue regeneration. So I think the addition of anti-apoptotic substances such as genes may be important; we have done research in anti-apoptotic gene transfer with stem cells and found a higher therapeutic effect in experimental models. Or proteins may be added. There are a number of candidate proteins that can improve stem cell function in the heart, which may lead the next clinical introductory phase. However, we have to learn step-by-step how stem cells can be used in cardiac therapy, what therapeutic effects they have, whether they are safe, whether or not they have side effects, how we can apply them, and in what disease condition.

In our study, we tried to find such a clinical therapeutic window treating chronic ischemia with intramyocardial injection of autologous CD133+ stem cells as an adjunct to a conventional CABG procedure. Of course, we are well aware of the weak points of our data. As you mentioned, we do not have a sham needle injection in the control group. We also had to overcome logistic problems in the prospective randomization of patients considering the bone marrow stem cell harvest and cell isolation methods. As compared to controls, however, we have seen in 35 patients treated with stem cells a consistent improvement in cardiac function—as great as 27% and with a mean of 10%—and I think that is really impressive. The lack of side effects is giving us confidence to go to the next clinical phase III study and to extend the experience. There are, of course, continuing improvements in isolation methods and conditioning of cells.

With respect to your mention of the control group, I agree that sham needle injection would be needed, and a next controlled study should include that. Also, a double-blinded study, as here it is only a single-blinded study, will be necessary to give the hard data needed for clinical introduction. Of course, this will take some time, but I am positive that we have good prospects for cardiac stem cell therapy in chronic ischemic heart disease.

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Appendix E1: Patient Stratification

For the efficacy 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 put in the CABG alone group. Availability of the clean room was beyond the control of the investigators, resulting in the following allocation sequence: 01000011001110010001 01010100101011110111, where *0* represents CABG alone and *1* represents CABG with cell injection.

Appendix E2

Cell Preparation

Handling of the bone marrow after aspiration took place in a good manufacturing practice unit providing a particle-reduced environment of European good manufacturing practice guidelines level A in level B. Before further preparation of the bone marrow, cell samples were drawn for measurement of stem cell number and viability and for proof of sterility. After density centrifugation, cells were transferred into a Cobe 2991 cell processor (Gambro BCT, Lakewood, Colo).[E21] The cells were washed twice with phosphate-buffered saline solution (PBS) containing 5% human serum albumin (HSA). After washing, the cell suspension was concentrated to a volume of 85 mL. Then the AC133 reagent (Miltenyi Biotec) was added to the cell processing bag for 30 minutes of incubation. The cell suspension was washed again twice with PBS with HSA and adjusted to a final volume of 150 mL. Samples were drawn again for quantification of stem cell number and viability to monitor the performance of the cell labeling procedure. $CD133^+$ cells were isolated with a CliniMACS Magnetic Cell Separation device (Miltenyi Biotec). The cell product was processed according to the standard program for $CD133^+$ cell selection with maximum cell numbers of 6×10^{10} leukocytes and 6×10^8 $CD133^+$ target cells. The CliniMACS column yielded

a purified $CD133^+$ cell product suspended in approximately 70 mL PBS with HSA.[E22] Samples were drawn from the transplant and the waste fraction for measurement of cell numbers, purity, and viability. The sample for proof of sterility was drawn from the waste fraction. After calculation of the number of viable stem cells, the $CD133^+$-enriched cell suspension was centrifuged for 10 minutes at 200*g,* resuspended in PBS with HSA, and then adjusted to a cell concentration according to the Fibonacci dose escalation scheme used in the safety study protocol. The cells were aliquoted into 2-mL vials. In the efficacy trial, all purified cells were concentrated to a final volume of 2 mL.

Flow Cytometry

Samples were drawn from the unmanipulated bone marrow, after incubation with anti-CD133 antibody (before CliniMACS column), from the purified $CD133^+$ cell product, and from the waste fraction of the CliniMACS system. To avoid competitive binding between fluorochrome-conjugated AC133/1 monoclonal antibody (CD133) and the ferrite-conjugated AC133/1 antibody used for CliniMACS cell selection, stem cell enumeration was done with a CD34 (clone 8G12) monoclonal antibody ($CD133^{selected}/CD34^+$ cells). The clone AC133/2 was not available at the time of the safety trial. Later, fluorochrome-conjugated AC133/2 monoclonal antibody was used in addition for determination the stem cell number in the phase II trial. Cell counting was done according to the Interdisziplinäre Gruppe für Labor und Durchflusszytometrie and International Society of Hematotherapy and Graft Engineering protocol.[E23,E24] In all samples derived from the selection procedure, cell viability was also measured with propidium iodide staining and flow cytometry.

Cell Products

The results of bone marrow cell preparation are summarized in Table E2. Because the preparation of the transplant of the first patient in the safety study was done without density centrifugation, the previously described analyses were performed without this patient (that cell preparation resulted in a final transplant dose of only 1.18×10^5 $CD133^{selected}/CD34^+$ cells with a purity of 3.5% and a recovery of only 2%). By the end of the safety study, a second CD133 antibody (clone AC133/2), not interfering with the AC133/1 used for cell selection, became available for diagnostic use. In 9 cell preparations of the efficacy trial, the stem cell number was calculated on the basis of both $CD133^+$ and $CD34^+$ cells. Comparison of the cell counts showed a median number of 6.75×10^6 $CD133^{selected}/CD34^+$, compared with 7.2×10^6 $CD133^{selected}/CD133^+$ cells in the final cell product. Median purities were 77% in the calculation with $CD133^{selected}/CD34^+$ cells and 80% when $CD133^{selected}/CD133^+$ cells were used for calculation. These results indicate that CD34 and CD133 measurements are equally valid for monitoring the efficacy of the cell selection procedure.

Appendix E3

Echocardiography

Cardiac transthoracic ultrasonographic studies were performed with a Philips SONOS 7500 echocardiography system (Philips Nederland) equipped with a 4-MHz vector array transducer and an ATL HDI 5000 echocardiography system equipped with a vector array adult cardiac transducer (ATL Ultrasound, Bothell, Wash). Patients were positioned supine left lateral, with the head slightly elevated when the echocardiograms were performed. The standard approach included the parasternal long- and short-axis views and the apical 3-, 4-, and 5-chamber views. In addition to 2-dimensional images and loops, we also acquired color flow mappings, pulsed and continuous wave Doppler images to assess the function of the heart valves. Two-dimensional techniques were used to provide visual assessment of LV systolic function, both regional and global. LVEF was calculated with the Simpson method, which divides the LV cavity into multiple slices of known thickness and diameter by taking multiple short-axis views at different levels along the LV long axis and then calculates the volume of each slice (area $\times$ thickness). Images and loops were recorded on VHS videotape or on magneto-optical disk storage devices for later analysis. The studies were carried out by two experienced echocardiographers (A.D., C.N.) who were blinded to the presence and location of the cell injection. Measurements obtained by the two independent echocardiographers were consistent.

Validation of Echocardiography by Cardiac MRI

Methods. To determine validity and reproducibility of echocardiography-based LVEF determination, a separate set of patients ($n = 13$) with similarly impaired LV function who were not included in the study were examined with cardiac MRI, and echocardiography was performed by the same investigators using the same protocol described previously. Cardiac MRI was done with ECG-gated sequences in a 1.5-T scanner (Avanto; Siemens AG, Munich, Germany). To determine LVEF, LV end-diastolic and end-systolic volumes were determined for calculation of LVEF with breath-hold gradient echo sequences (Cine-True Fast Imaging With Steady Precession). Sequence parameters were as follows: TR 40.05 ms, TE 1.3 ms, flip angle 80° to 65°, matrix 192 $\times$ 156, slice thickness 8 mm, and field of view 34 to 40 cm. The LV was covered by a continuous stack of short-axis slices. An end-diastolic, end-expiratory 4-chamber view served as a reference to plan the short-axis slices. Image analysis was done blinded, without knowledge of the echocardiographic data, with Argus software (Siemens).

Results. In 13 separate patients with ischemic heart disease and impaired LV contractility (average LVEF 31% $\pm$ 5%), LVEF was measured by both echocardiography ($LVEF_{echo}$) and cardiac MRI ($LVEF_{MRI}$). There was a significant positive linear correlation between $LVEF_{echo}$ and $LVEF_{MRI}$ ($r^2 = 0.84$, $P < .001$), described by the equation $y = 0.97x + 3.7$. Bland-Altman analysis indicated a mean difference (bias) between MRI and echocardiography of 2.8%, with an SD (precision) of 2.1% (95% confidence interval of difference -1.5% to 7.1%; Figure E1). Cardiac MRI measurements of LVEF on average are 2.8% higher than those obtained by echocardiography. The bias was constant across the range of LVEF, as indicated by a slope not different from 0 ($P = .16$).

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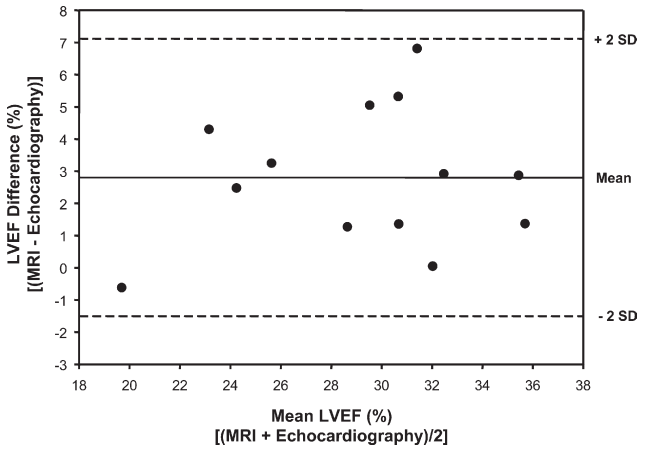

Figure E1. Bland–Altman plot showing relationship between average left ventricular ejection fraction *(LVEF)* versus left ventricular ejection fraction difference between magnetic resonance imaging *(MRI)* and echocardiography and revealing in general that echocardiography provides slight linear underestimation of left ventricular ejection fraction relative to cardiac magnetic resonance imaging. Mean difference is 2.8% *(solid line),* and variability of difference is represented as 95% confidence interval or limits of agreement (±2 SD, *dashed lines*). Bland–Altman plot revealed significant underestimation of left ventricular ejection fraction with echocardiography relative to magnetic resonance imaging ($P < .01$, paired *t* test), although average difference was constant through range of left ventricular ejection fraction (slope not significantly different from 0, $P = .16$).

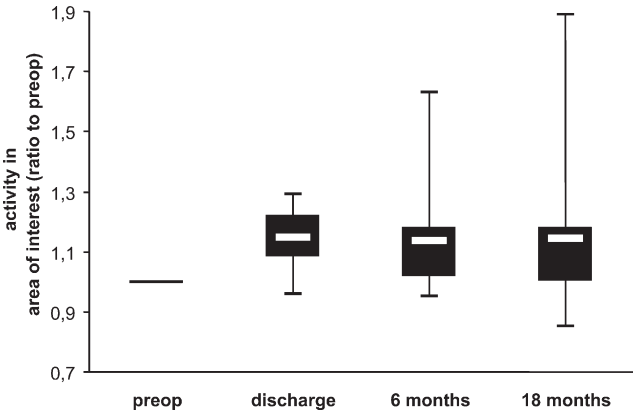

Figure E2. Myocardial perfusion in ischemic myocardium of patients included in safety study. Activity in cell-treated area of interest was quantified by computerized colorimetric analysis and expressed asl ratio with respective preoperative *(preop)* value. Overall, there was sustained improvement of perfusion in target area ($P < .01$, Wilcoxon signed rank test). *Box plots* indicate 25th and 75th percentiles *(solid box),* median *(white bar),* and minimum and maximum values of each data set *(whiskers).*

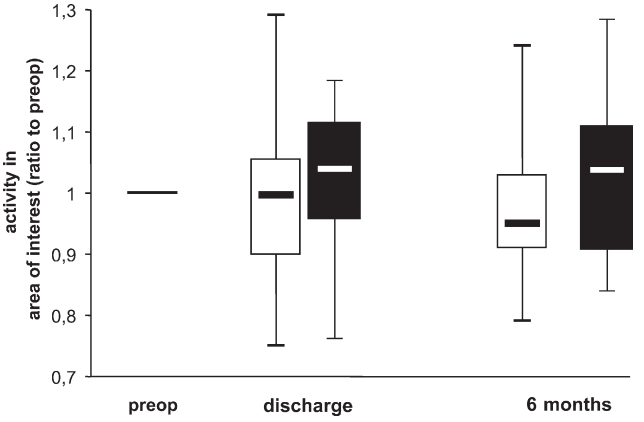

Figure E3. Myocardial perfusion in ischemic myocardium of patients included in efficacy study before operation *(preop),* at discharge, and at 6 months' follow-up. Activity in cell-treated area of interest was quantified by computerized colorimetric analysis and expressed as ratio with respective preoperative value. *Solid box* represents patients undergoing coronary artery bypass grafting with $CD133^+$ cell injection; *open box* represents patients undergoing coronary artery bypass grafting alone. *Box plots* indicate 25th and 75th percentiles *(box), median (bar),* and minimum and maximum values of each data set *(whiskers).*

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TABLE E1. Patient- and procedure-related baseline data

Variable	Safety trial (n = 15)	Efficacy trial		
		CABG with cell injection (n = 20)	CABG only (n = 20)	*P* value
Age (y, mean ± SD)	65.2 ± 9.1	62 ± 10.2	63.5 ± 8.4	.61
Sex (male/female)	15:0	15/5	16/4	.99
Infarct time (wk, median and range)	7 (2.5-12)	9 (2-1200)	7.5 (2-830)	.91
NYHA class (No.)				.74
II	2 (13%)	1 (5%)	2 (10%)	
II-III	9 (60%)	16 (80%)	14 (70%)	
III	4 (27%)	3 (15%)	4 (20%)	
Coronary artery stenoses (No.)				
LMCA	5 (33%)	9 (45%)	7 (35%)	.75
LAD	14 (93%)	18 (90%)	20 (100%)	.49
CX	13 (87%)	14 (70%)	17 (85%)	.45
RCA	15 (100%)	17 (85%)	18 (90%)	.99
Target area (No.)				
Anterior	3	7	7	.93
Posterior	8	10	11	
Apex	4	3	2	
Holter (No.)				
Relevant VES	0	0	0	
Atrial fibrillation	0	0	0	
Intraoperative				
No. of bypasses (mean ± SD)	3.7 ± 0.8	3.3 ± 1.3	3.6 ± 0.8	.38
LITA use (No.)	14	15	20	.03
Mitral valve plasty (No.)	0	1	1	
Cell dose ($\times 10^6$ cells)	0.6-5	7.5 (1.2-80)	NA	
No. of injections	10	10	NA	
Postoperative				
Need for inotropes	10 (67%)	11 (55%)	12 (60%)	.99
Mechanical ventilation (h, mean ± SD)	6.8 ± 2	5.6 ± 3	6 ± 2	.62
Postoperative creatine kinase (IU, median and range)	435 (155-3500)	485 (181-3700)	507 (195-1166)	.07
Postoperative creatine kinase isoenzyme MB (IU, median and range)	26 (11-84)	24 (15-421)	28 (12-43)	.24
VES (% of all QRS)	0.004% ± 0.009%	0.009% ± 0.02%	0.02% ± 0.04%	.39
C-reactive protein (mg/L, median and range)	36 (11-75)	41 (12-136)	26 (3-113)	.16
Hospital stay (d, mean ± SD)	14 ± 2	14 ± 3	12 ± 2	.18

Preoperative, perioperative, and postoperative baseline data of patients enrolled in the safety study and in the efficacy trial (grouped by treatment). There were no relevant differences between groups. *CABG,* Coronary artery bypass grafting; *NYHA,* New York Heart Association; *LMCA,* left main coronary artery; *LAD,* left anterior descending coronary artery; *CX,* circumflex coronary artery; *RCA,* right coronary artery; *VES,* ventricular extrasystoles; *LITA,* left internal thoracic artery; *NA,* not applicable.

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TABLE E2. Results of bone marrow cell preparation

	BM volume (mL)	% $CD34^+$ in BM	Total $CD34^+$ in BM	% $CD34^+$ after AC133/1 selection	% $AC133/2^+$ after cell selection	Absolute $CD34^+$ after cell selection	Recovery (%)	Total $CD133^+$ after cell selection	Viability (%)
All	156	0.8%	2.9×10^7	75.8%	NA	5.8×10^6	18.3%	NA	93.4%
Safety trial	139	0.9%	3.4×10^7	80.3%	NA	5.02×10^6	19.1%	NA	90.6%
Efficacy trial	163	0.7%	2.8×10^7	73.2%	NA	5.96×10^6	18.3%	NA	94.3%
Procedures with AC133/2 count	145	0.7%	2.5×10^7	77.3%	79.8	6.75×10^6	25.6%	7.19×10^6	93.5%

Results of the bone marrow cell preparation. Data are given as median. *BM,* Bone marrow; *% $CD34^+$ in BM,* percentage of $CD34^+$ cells in fresh bone marrow relative to all $CD45^+$ cells; *Total $CD34^+$ in BM,* total number of $CD34^+$ cells in fresh bone marrow; *% $CD34^+$ after AC133/1 selection,* percentage of $CD34^+$-labeled cells in cell product after CliniMACS isolation of $CD133^+$ cells; *% $AC133/2^+$ after cell selection,* percentage of AC133/monoclonal antibody-positive labeled cells in CliniMACS cell product; *Absolute $CD34^+$ after cell selection,* total number of $CD34^+$-labeled cells after $CD133^+$ selection; *Recovery (%),* median recovery of $CD34^+$ cells; *Viability (%),* percentage of viable cells as assessed by propidium iodide staining; *NA,* Not applicable.

TABLE E3. Echocardiographic data

		Efficacy trial	
Variable	**Safety trial (n = 15)**	**CABG with cell injection (n = 20)**	**CABG only (n = 20)**	***P* value**
Left ventricular ejection fraction (%)				
Preoperative	39.0 ± 8.7	37.4 ± 8.4	37.9 ± 10.3	.86
Discharge	46.9 ± 6.8*	39.7 ± 10.6	40.4 ± 10.9	.55
6 mo	50.2 ± 8.5*	47.1 ± 8.3*	41.3 ± 9.1*	.03†
18 mo	47.9 ± 6.0*	NA	NA	
Left ventricular end-diastolic volume (mL)				
Preoperative	147.9 ± 38	153.9 ± 28	153.7 ± 35	.98
Discharge	122.7 ± 32	145.9 ± 37	157.9 ± 35	.34
6 mo	126.5 ± 29	142.8 ± 42	149.3 ± 35	.62
18 mo	127.2 ± 18	NA	NA	
Left ventricular end-systolic volume (mL)				
Preoperative	92.3 ± 35	96.2 ± 20	94 ± 33	.81
Discharge	65.4 ± 20	89.1 ± 33	92.2 ± 32	.78
6 mo	66.2 ± 24	77.1 ± 31	88.5 ± 29	.31
18 mo	65.8 ± 11	NA	NA	
Left ventricular end-systolic diameter (mm)				
Preoperative	39.5 ± 7.2	42.5 ± 6.2	48.1 ± 7.7	.03†
Discharge	36.4 ± 7.7	42.3 ± 7.7	46.3 ± 10.8	.31
6 mo	41.3 ± 8.8	40.5 ± 7.0	48.3 ± 9.0	.02†
18 mo	39.2 ± 6.2	NA	NA	
Left ventricular end-diastolic diameter (mm)				
Preoperative	54.7 ± 3.9	57.1 ± 5.4	58.9 ± 6.3	.35
Discharge	51.1 ± 5.3	53.1 ± 9.6	56.4 ± 9.2	.89
6 mo	52.8 ± 3.6	54.5 ± 6.9	57.0 ± 5.1	.21
18 mo	52.4 ± 3.1	NA	NA	

Echocardiographic data of patients enrolled in safety study and in efficacy trial (grouped by treatment). *P* values refer to comparison of coronary artery bypass grafting alone versus coronary artery bypass grafting with cell injection by 2-way repeated measures analysis of variance. Data are given as mean ± SD. *CABG,* Coronary artery bypass grafting; *NA,* not applicable. *Significant difference compared with preoperative data; †Statistically significant.