Safety Analysis and Improved Cardiac Function Following Local Autologous Transplantation of CD133+ Enriched Bone Marrow Cells After Myocardial Infarction

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Abstract: The CD133⁺ bone marrow cell (BMC) population includes primitive multipotent stem cells which induce neoangiogenesis. Studies suggested transplantation of these cells to infarcted myocardium can have a favorable impact on tissue perfusion and contractile performance. We assessed the feasibility, safety and functional outcomes of autologous CD133⁺ BMC transplantation during coronary artery bypass grafting (CABG) in patients with recent myocardial infarction. In a prospective, nonrandomized, open-label study, 27 patients with recent myocardial infarction underwent CABG and intramyocardial injection of autologous bone marrow-derived CD133⁺ cells (18 patients, BMC group) or CABG alone (9 patients, control group). At 6 months after CABG, the Wall Motion Score Index (WMSI) was significantly reduced for akinetic/dyskinetic segments treated with CD133⁺ cells compared with the control group (P<0.006). Likewise, comparison between baseline and follow up results of dobutamine stress echocardiography and myocardial perfusion scintigraphy showed improvement of myocardial viability and local perfusion of the infarcted zone of the BMC group compared with the control group. No complications related to CD133⁺ cell transplantation were noted, either procedurally or during postoperative at a mean of 14 months follow up. In patients with recent myocardial infarction, transplantation of CD133⁺ cells to the peri-infarct zone during CABG surgery is feasible and safe, with no evidence of early or late adverse events. Moreover, these cells might restore tissue viability and improve perfusion of the infarcted myocardium, suggesting that they may induce myogenesis as well as angiogenesis.

Key Words: Myocardial infarction, stem cells, transplantation, bone marrow, adult stem cell.

INTRODUCTION

Congestive heart failure (CHF) secondary to ventricular remodeling following infarction continues to be a major medical problem world-wide. The high morbidity of CHF and the shortage of donor hearts for transplantation along with problems associated with immunosuppression complications and functional failure of the transplanted organs demand a search for new approaches to prevent heart failure after a myocardial infarction (MI) (Davani et al., 2005; Hassink et al., 2003).

Recently, many investigators have used a variety of stem and progenitor cell populations to regenerate damaged human myocardia including bone marrow-derived mononuclear cells, hematopoietic stem cells, mesenchymal stem cells, circulating blood-derived progenitor cells, skeletal myoblasts, and endothelial progenitor cells. Embryonic stem cells (ESCs) may an alternative for damaged myocardia. Kolossov et al. (2006) in a comprehensive study demonstrated that highly purified ESC–derived cardio-myocytes are the most suitable candidates for cellular cardiomyo-plasty, as these cells enhance, in contrast to BMCs, the contractile function of the lesioned myocardium without tumor formation. It is, however, still unclear and controversial which is the most promising cell source. These cells have been delivered by intracoronary injection, direct myocardial injection, or mobilization from the periphery by administration of granulocyte colony-stimulating factor (for review see Wollert et al., 2003). It has been suggested that these cells might contributed to cardiac repair by transdifferentiation into cardiac myocytes, angiogenesis, and/or inhibiting apoptosis (Hassink et al., 2003; Wollert et al., 2005; Mann et al., 2005; Siminiak et al., 2003). Thus, cell transplantation has emerged as a new strategy for the therapy of a large number of patients with MI. Each potentially therapeutic cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings (Wollert et al., 2005).

To date, few human studies have been designed to investigate safety and effectiveness of CD133⁺ cells during coronary artery bypass grafting (CABG) after MI (Bartunek et al., 2005; Stamm et al., 2003; 2004; Agbulut et al., 2004; Ghodsizad et al., 2004; Shmelkov et al., 2005). CD133, a prominin 5 transmembrane glycoprotein 1, is a marker for more primitive multipotent stem and endothelial progenitor
cells and is of particular interest in studies directed at therapeutic angiogenesis, as these cells have been shown to differentiate into endothelial cells (Bartunek et al., 2005; Shmelkov et al., 2005; Quirici et al., 2001). Importantly, another rationale for using purified CD133+ cells is to avoid injection of a large number of leukocytes and their progenitors, which have limited plasticity and the presence of which, in large numbers, may give rise to an unwanted inflammatory response at the site of a graft (Haider et al., 2005). However, there is limited information on the direct inflammatory response at the site of a graft (Haider et al., 2005).

The study was initiated in June 2004 and recruited 29 patients with recent myocardial infarction who were candidates for coronary artery bypass grafting (CABG). Patients were eligible for inclusion into the study if they had: a history of transmural infarction less than 3 months prior to admission for surgery, the presence of at least 4 non viable segments in LAD territory as determined by angiography, myocardial thickness of akinetic area more than 3 mm, the absence of severe concomitant disease such as renal and hepatic disease, and the absence of ventricular aneurysm. Patients were excluded if one of the following criteria was met: old infarction (>3 months), severe left ventricular dysfunction (ejection fraction <25%), malignant arrhythmias, abnormal liver function tests or renal insufficiency.

The study was conducted with the approval of the institutional review board of the Royan Institute and Tehran Heart Center Hospital, Tehran University of Medical Sciences, Iran. Patients were briefed in detail about the treatment and diagnostic procedures, and informed consent was obtained from all of them. Patients were enrolled sequentially, 20 patients assigned to both the CABG and stem cell transplantation (BMCC group) and 9 patients to CABG alone (control group). Before surgery, Dobutamine Stress Echocardiography (DSE), myocardial perfusion scintigraphy, 24 hours holter monitoring, standard hematologic laboratory tests for cardiac surgery and evaluation of cardiac enzymes for assessment of myocardial ischemia were performed on all patients. Left ventricular function was assessed by analysis of LV echocardiography and myocardial perfusion scan.

**MATERIALS AND METHODS**

**ECHOCARDIOGRAPHY STUDIES**

Global and regional contractility studies were performed with two-dimensional echocardiography (2D echo) (Vivide 7 dimension, VingMed, GE) using a sonor 5500 ultrasound system (Philips). For data analysis, the left ventricle was divided into 16 segments according to the recommendations of American Society of Echocardiography. The wall motion was scored as 1, normal; 2, hypokinetic; 3, akinetic; 4, dyskinetic and 5, aneurysm for each segment. The Wall Motion Score Index (WMSI) was computed for akinetic and dyskinetic segments as the sum of their scores divided by the number of segments evaluated. A left ventricle WMSI was calculated at baseline and peak dobutamine dosage for akinetic/dyskinetic segments. The accuracy of DSE to predict recovery of resting segmental function was calculated for low dose (5 μg/kg/min) and for full protocol dobutamine infusion (10 to 15 μg/kg/min) as in cases of stunning, dobutamine stress echocardiography is an excellent means for detecting hibernation myocardium. A segment was defined as viable when during low dose dobutamine, wall motion appeared basally dyskinetic; and became normal or near-normal during peak dose of dobutamine. Absence of viability in the akinetic/dyskinetic area was assumed if no change in segmental contractility after administration of 5, 10, and 15 μg/kg per min of dobutamine with no increase in the heart rate >10 beats/min has been observed. The echocardiographic data were analyzed by 2 independent, blinded, experienced observers.

**MYOCARDIAL PERFUSION SCINTIGRAPHY**

Because of the clinical status of the selected patients, myocardial perfusion Single Photon Emission Computed Tomography (SPECT) (ADAC Double head vertex) was applied only at rest. At rest images were obtained after trinitroglycerin (TNG) ingestion and intravenous injection of 20 mCi of (99m) Tc-MIBI (methoxyisobutyl isonitrile). All data were collected using an Adac dual head gamma camera and low energy-all purpose collimator, and applying 180° SPECT. Short axis, horizontal long axis and vertical long axis views were obtained from the data. The medial parts of all axis views were evaluated. All the segmental views were scored visually. The study was blinded for the reviewers of imaging studies. Uptake below 50% of maximum uptake was indicative of non viability.

**PREPARATION OF CD133+ CELLS**

One day before CABG surgery, bone marrow was aspirated from the iliac crest of BMC group patients in a standard fashion after achievement of sedation. The harvested bone marrow was placed in sterile tubes containing 1500 U/50ml of heparin sulfate to avoid platelet clumping. The procedure of stem cell isolation was performed in a clean room (FS 209 E & ISO 14644). To reduce the volume of red blood cells, hydroxy ethyl starch was used.

Mononuclear cells were separated by Ficoll-Hypaque (Lymphodex, inno TRAin, H9L6114) and then these cells were diluted in cliniMACS buffer. The bone marrow LDMNCs were incubated for 45 min at 4 °C with the AC133/1 monoclonal antibody (mAb) directly labeled to microbeads (MACS, Miltenyi Biotec GmbH, 172-01, Bergisch Gladbach, Germany), washed with cliniMACS buffer and placed on a column in the miniMACS cell separator (Miltenyi Biotec). The labeled cells were separated using a high-gradient magnetic field, and eluted from the column after their removal from the magnet. The positive fraction was then placed on a new column and the magnetic separation step repeated. At the end of the separation, the cells were counted and assessed for viability using Trypan Blue dye exclusion; their purity was determined using a FACSCalibur flow cytometer (Becton Dickinson, San José, CA, USA). CD133+ cells enriched were stored at 4 °C in 2% human serum albumin (Human Albumin 20%, USP, Bayer,
683-20) in a sterile tube until intramyocardial injection on
the next day.

PROCEDURE OF STEM CELL TRANSPLANTATION
AND CABG

All patients were operated on by a cardiac surgery team
according to our standardized surgical protocol. During
CABG, after all distal anastomosis of grafts and immediately
before removal of aortic cross clamp, the infarcted area was
visualized and palpated. Then 10 samples of 0.2 ml of stem
cell suspensions each (total 2 ml) were injected along the
border zone of the infarcted area using a 22-gauge needle.
After the final injection, the aortic cross clamp was removed
and the operation was completed as usual.

FOLLOW UP

Patients were transferred to intensive care unit (ICU) and
extubated after 5.5±1.8 hours. All patients were monitored
by electrocardiography in the ICU and the routine blood tests
were performed. We aimed to discharge all patients from the
ICU within 18–24 h, and to discharge them from the hospital
on the 5th or 6th postoperative day. 2D echocardiography
was performed one week after operation and, thereafter,
every 3 months. Myocardial perfusion scintigraphy and DSE
were repeated at 6 months after the operation. The patients
were followed for 12–18 months except 2 of 20 cases who
did not come back for echo and scan studies. The latter were
excluded from the analysis.

STATISTICAL ANALYSIS

All analyses were performed by operators blinded to all
clinical and other functional data using SAS system version
9.1 (SAS Institute Inc., Cary, NC). All measurements are
expressed as mean± the standard error of the mean (SEM).
Paired t test and t-test were used as appropriate. A P-value of
less than 0.05 was considered to indicate a significant differ-
ence.

RESULTS

Bone marrow aspiration volume, the number of isolated
mononuclear cells (MNCs), and the viability of the isolated
cells are summarized in Table 1. The mean number of in-
jected stem cells and viability were 1.89×10^6 ±0.03 and
84.80±8.06, respectively. The enrichment of CD133^+ cells,
measured only in 8 samples because of the technical difficul-
ties, ranged from 41.6% to 85.7% (75±9.2%) with a total
number from 0.2 to 5.1×10^6 CD133^+ cells per sample (Fig.
1).

The demographic, clinical, and angiographic characteris-
tics of the study population are shown in Table 2. There were
no significant differences in any of the baseline parameters
between patients receiving BMCs and control group. Al-
though only 4 female patients were included in the study,
separate analysis did not disclose any trend toward a poten-
tial differential response of female versus male patients. The
duration of post infarction time was same in both groups;

Table 1. Results of BM Aspiration, the Number of Cells, and the CD133^+ Cell Viability Of Enrolled Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>BM Volume (ml)</th>
<th>No. MNC × 10^6</th>
<th>No. CD133^+ × 10^6</th>
<th>Viability of CD133^+ (%)</th>
<th>Total Viable CD133^+ × 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>12</td>
<td>0.2</td>
<td>97</td>
<td>0.194</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>57</td>
<td>2</td>
<td>80</td>
<td>1.600</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>17</td>
<td>0.24</td>
<td>65</td>
<td>0.156</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>96</td>
<td>2</td>
<td>90</td>
<td>1.800</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>98</td>
<td>0.8</td>
<td>81</td>
<td>0.648</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>166</td>
<td>3</td>
<td>73</td>
<td>2.190</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>81</td>
<td>1</td>
<td>93</td>
<td>0.930</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>169</td>
<td>3</td>
<td>87</td>
<td>2.610</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>76</td>
<td>4</td>
<td>78</td>
<td>3.120</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>120</td>
<td>1.8</td>
<td>85</td>
<td>1.530</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>112</td>
<td>1.5</td>
<td>88</td>
<td>1.320</td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>312</td>
<td>1.8</td>
<td>85</td>
<td>1.530</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>200</td>
<td>2</td>
<td>80</td>
<td>1.600</td>
</tr>
<tr>
<td>14</td>
<td>45</td>
<td>100</td>
<td>1.5</td>
<td>95</td>
<td>1.425</td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td>165</td>
<td>5.1</td>
<td>80</td>
<td>4.080</td>
</tr>
<tr>
<td>16</td>
<td>45</td>
<td>75</td>
<td>1.7</td>
<td>90</td>
<td>1.530</td>
</tr>
<tr>
<td>17</td>
<td>45</td>
<td>193</td>
<td>0.5</td>
<td>90</td>
<td>0.450</td>
</tr>
<tr>
<td>18</td>
<td>47</td>
<td>65</td>
<td>2</td>
<td>90</td>
<td>1.800</td>
</tr>
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</table>
ranging 55 to 88 days (55-86 days for BMC group and 59-88 days for control subjects). Duration of cardiac injury and number of viable segments in LAD territory, and the correlation with improvement in treated subjects compared to control subjects was summarized in Table 2 and 3.

DSE and SPECT were performed at 6 months of follow-up for each patient. During follow-up two patients of BMC group were excluded, as explained above. Table 3 summarizes the echocardiography parameters. There was a significant increase in global LVEF (calculated by 2D echocardiography) from baseline to 6 months of follow-up in treated subjects compared to control subjects. LVEF was significantly higher in treated subjects compared to control subjects at 6 months of follow-up (48.6%±2.3% vs. 50.8%±1.56%, p<0.05).

Table 2. Baseline Characteristics of Studied Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>BMC Group</th>
<th>Control Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>48.63±2.31</td>
<td>50.86±1.56</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, M/F (%)</td>
<td>91.6</td>
<td>57.1</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of cardiac injury (day)</td>
<td>74.1±2.20</td>
<td>74.6±3.03</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary angiography LAD/LCX/RCA as affected vessel</td>
<td>18/16/11</td>
<td>9/9/6</td>
<td>NS</td>
</tr>
<tr>
<td>Risk Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>53.8</td>
<td>57</td>
<td>NS</td>
</tr>
<tr>
<td>Positive family history, %</td>
<td>53.8</td>
<td>42.8</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>76.9</td>
<td>57</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>53.8</td>
<td>57.1</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>38.4</td>
<td>42.8</td>
<td>NS</td>
</tr>
<tr>
<td>Classification of CCS</td>
<td>2.62±0.8</td>
<td>3.2±0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Classification of NYHA</td>
<td>1.85±0.69</td>
<td>1.8±0.45</td>
<td>NS</td>
</tr>
</tbody>
</table>

LAD: left anterior descending coronary artery; LCX: left circumflex coronary artery; RCA: right coronary artery; CCS: Canadian Clinical Score; NYHA: New York Heart Association.
CD133+ Cell Transplantation in AMI

In both groups (Fig. 2). However, improvements in global LV EF did not differ between patients receiving CD133+ cells compared with control patients. Moreover, LV end-systolic volume and end-diastolic volume were slightly smaller at 6 months follow up in both groups (Table 3).
Detailed analysis of regional wall motion revealed an improvement in the infarct zone from baseline to follow up in both BMC and control group. The akinetic/dyskinetic WMSI of BMC group was 2.38±0.13 at baseline versus 1.53±0.17 at follow up (P<0.001) while there was no significant change in WMSI at baseline and follow up in the control group (Table 3, Fig. 2). The most prominent improvement in the infarct zone in form of changing in WMSI in the BMC group was observed in compared with the control group as well (P<0.006).

Importantly, patients receiving a higher number of viable CD133+ cells (≥1×10⁶, N= 14, 2.31±0.29) showed significantly more improvement in WMSI (P<0.001) compared to patients receiving a lower number of viable CD133+ cells (<1×10⁶, N= 4, 0.43±0.13). The mean NYHA class (New York Heart Association) was reduced from 1.85±0.69 at baseline to 1.00±0.20 in follow up, in patients receiving BMCs (P<0.04). Illustrations of myocardial perfusion scans at baseline and 6 months after surgery of a patient transplanted with CD133+ cells are shown in Fig. (3). Comparison of the two scans indicates improvement of some segments in the form of an increase in MIBI uptake as shown by arrows in B.

![Illustrations of myocardial perfusion scan](image)

**Fig. (3).** Illustrations of myocardial perfusion scan in a patient with transplanted CD133+ cells at baseline (A) and 6 months after surgery (B). Arrows indicate infarct tissue before and after surgery. Improved viability and perfusion can be seen as showed by arrows in B.

No ventricular arrhythmias were detected after treatment with bypass surgery and CD133+ cell transplantation during the hospitalization period or during the 12~18 months of postoperative follow up (the mean follow up of 14 months). None of the patients showed evidence of myocardial ischemia on postoperative ECG recording or complications related to CD133+ cell transplantation. Also there was no mortality during hospitalization or the outpatient follow up period.

**DISCUSSION**

Stem cells hold great promise for tissue repair and regenerative medicine. Recent studies have shown that cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes, and cigarette smoking are inversely correlated with cell number transplantation and function (Yao et al., 2006). For the effect decrease of these factors on our results we compared the baseline parameters between patients receiving BMCs and control group that there were no significant differences in any of the factors between two groups. In this prospective study, we demonstrated a significant improvement of regional wall motion of akinetic/dyskinetic segments and perfusion of infarcted areas in patients receiving local transplantation of autologous CD133+ cells in conjunction with CABG surgery, compared with a control group. Our results showed although global EF was improved in BMC patients, it was not significant in comparison with the control group. Similar results of increased LVEF and improved tissue perfusion and improvement in cardiac function after 12 months follow up following CD133+ cell transplantation have been reported by Stamm et al. (2004). No evidence of adverse effects such as ventricular arrhythmia or neoplasia was detected in their study (Stamm et al., 2003; 2004). Newly, subsequent results in phase-2 of Stamm’s study of 40 patients randomized to undergo CABG and CD133+ cell injection or CABG alone was presented in 86th Annual Meeting AATS in Philadelphia (Steinhoff et al., 2006). Comparing results both studies showed that the mean LVEF and perfusion were significantly improved in the cell-treated group at 6 months. Regression analysis indicated that the patients with preoperative LVEF <30% benefit more from CABG and cell therapy than those with only moderately impaired LVEF (Steinhoff et al., 2006). Since we excluded patients with severe LV dysfunction (EF <25%) from our study, so direct comparisons with aforementioned study cannot be made. In addition, researchers using CD133+ cells infused into the human infarct-related artery illustrated that global LVEF, regional wall motion, and tissue perfusion increased in the cell transfer group (Bartunek et al., 2005). More recently, Klein et al. (2007) applied intramyocardial implantation of CD133+ stem cells without CABG in ten patients with end-stage chronic ischemic cardiomyopathy (EF <22%) and found CD133+ cell transplantation alone improved cardiac function.

Recent animal experiments using cell transplantation techniques showed that human umbilical cord blood-derived CD133+ progenitor cells can prevent scar thinning, attenuate systolic dilatation, and improve LV function in rat model of MI (Leor et al., 2006). Also, transplantation of bone marrow-derived CD133+ progenitors into the infarcted myocardium 10 days after coronary artery ligation in rats promoted an increase in LVEF and improved cardiac function (Agbulut et al., 2004).

One of the most arguable subjects in our study is the beneficial effect of CABG that may have influenced. Naturally it is not possible to distinguish between the effect of the CABG operation and the CD133+ cell injection because both effects overlap in the infarct border zone. Although surgical and interventional revascularization of ischemic myocardium improves the function of viable myocardium, the viability of
necrotic myocardium cannot be restored (Stamm et al., 2003). To increase the specificity of the determination of the potential beneficial effect on cardiac function of CD133+ cell transplantation, we examined the improvement in the WMSI of akinetic/dyskinetic (as nonviable) and not for hypokinetic segments. Although the efficacy of cell transplantation may be difficult to evaluate and ascertain if CABG is performed simultaneously (Wollert et al., 2005), studies have shown that akinetic/dyskinetic segments often demonstrated perfusion but not contractile reserve after revascularization by using CABG and generally did not recover function, while most hypokinetic segments improved and manifested both contractile reserve and perfusion following the surgical technique (deFilippi et al., 1995). Based on our results, we did not find any correlation between the days’ number of transmural infarction (the duration of cardiac injury) and viable segments number in LAD territory with the improvement of treated subjects compared with the control subjects. We considered duration of cardiac injury duration less than 3 months that this period has not this effect on akinetic/dyskinetic segments, because the time of cardiac injury was very near from 55-88 days. This is not surprising that hypokinetic segments could be reducible for duration of MI can affect on this process but akinetic/dyskinetic segments could be influenced improbly by this time (deFilippi et al., 1995). The improvement WMSI and increase in MIBI uptake observed in the area where cells had been transplanted suggest that viable tissue was present in the area of the infarct. Thus we hypothesize that the overall improvement can not solely be due to the revascularization and is mainly mediated by CD133+ cells since no viable tissue could be detected on dobutamine echo and myocardial perfusion scan of the area of the infarct preoperatively. The concomitant increase in MIBI uptake and myocardial contractility raises the intriguing possibility of recovery of the contractile elements. Thus, the effect of CD133+ cell therapy might be both the improvement of neovascularization and the replacement of necrotic myocytes by transdifferentiating or fusion of these cells (Haider et al., 2005; Mathur et al., 2004).

Importantly, very recent reports have shown that adult mouse bone marrow contains cells with the ability to form cardiomyocytes (Eisenberg et al., 2006). Although a study (Leor et al., 2006) was not able to confirm or refute the possible aforementioned mechanism(s), they suggested that the benefit is likely a result of factors secreted by the human CD133+ derived cells or another type of interaction with the healing of the infarct rather than a direct mechanical or angiogenic contribution. This suggests that cells within injured tissue express trophic and growth factors adjusted to the needs of the tissue. The idea that progenitor cells may protect the myocardium without directly participating in myocardial repair is supported by several recent works in cardiac models (Agbulut et al., 2004; Balsam et al., 2004). These cells may work through prevention of scar thinning by autologous myofibroblast accumulation. By thickening the scar, wall stress is reduced (Laplace law) and the degree of outward motion of the infarct that occurs during systole (paradoxical systolic bulging) is reduced and leading to a lower end-systolic volume (Muller-Ehmsen et al., 2002; Yao et al., 2003). Also Nygren et al. (2004) showed bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. To find the mechanism of improvement it is important to trace the transplanted cells. Recently, Ruhrparwar et al. (2006) presented a clinically applicable technique using advanced ultra high-field 7-Tesla (7T) magnetic resonance imaging (MRI) of nanoparticle-labeled transplanted human bone marrow CD133+ cells in porcine ischemic hearts to track the fate of the transplanted cells.

The accurate selection of the delivery route of cells to infarcted myocardium, due to uncertainties of safety, has remained controversial (Wollert et al., 2005; Bartunek et al., 2005; Ott et al., 2005). A recent clinical trial reported an increased incidence of coronary events after intracoronary injection of CD133+ bone marrow progenitor cells (Bartunek et al., 2005). On the other hand, the goal of any cell delivery strategy is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area, preventing homing of transplanted cells into other organs (Wollert et al., 2005; Strauer et al., 2003). Therefore, to achieve a maximum concentration at the site of ischemic injury and due to these safety concerns (Wollert et al., 2005; Stamm et al., 2003; Stamm et al., 2004; Ott et al., 2005), we directly injected CD133+ cells into infarcted zone during CABG. Furthermore, the main advantage of the surgical procedure is injection under visualization, which allows anatomic identification of the target area and even distribution of the injections (Strauer et al., 2003). Another factor that may influence the results is the number of cells injected into infarcted myocardium. Functional recovery is likely dependent on the number of engrafted cells (Bartunek et al., 2005). The mean number of injected cells in our patients was approximately 2×10^6 cells while injection of various numbers (1.5-19×10^6) of CD133+ cells were reported in related studies (Ghodsizad et al., 2004, Ghodsizad et al., 2006, Klein et al., 2007). Based on the comparison of our results of patients receiving higher than 1×10^6 CD133+ cells to those receiving a lower number of cells, we can conclude the number of injected cells may correlate with cardiac improvement. However this conclusion must be considered with caution because of the small number of cases compared. Dose-response studies with more patients are required to determine the appropriate cell number to inject. The mean purity of injected stem cells was 75±9.2% in our study. Although we were unable to obtain pure CD133+ cell populations, the enriched CD133+ cells contained minimal contamination of natural killer cells, B cells, or monocytes. The other limitations of this study are the small number of patients (20 patients received CD133+ cells and 9 patients no cells) enrolled and the study design (non-randomized), which limits any conclusions about efficacy. Indeed, at the beginning of the study, our intention was patients receiving CD133+ cells alone then we added 9 patients as control group to increase the quality of study. So, we did not randomize the patients and were entered “sequentially” in our study such as Perin’s report (Perin et al., 2003). Because of ethics committee concerns, patients in the control group were not enrolled concurrently with treated patients and did not receive a placebo injection. It should also be taken into consideration that this was not a blinded study and those patients who received CD133+ cells were aware of the treat-

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ment and may have experienced subjective benefits that could be placebo related.

Taken together our results suggest that cell transplantation with bypass surgery is associated with improvement in cardiac function, increased tissue viability, perfusion, regional wall motion, and lack of side effects suggesting that this is a promising therapy for patients with infarcted myocardium. Further clinical research is warranted by these promising results.

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