Effects of Intracoronary CD34+ Stem Cell Transplantation in Nonischemic Dilated Cardiomyopathy Patients

5-Year Follow-Up

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Rationale: CD34+ transplantation in dilated cardiomyopathy was associated with short-term improvement in left ventricular ejection fraction and exercise tolerance.

Objective: We investigated long-term effects of intracoronary CD34+ cell transplantation in dilated cardiomyopathy and the relationship between intramyocardial cell homing and clinical response.

Methods and Results: Of 110 dilated cardiomyopathy patients, 55 were randomized to receive CD34+ stem cell transplantation (SC group) and 55 received no cell therapy (controls). In the SC group, CD34+ cells were mobilized by granulocyte colony-stimulating factor and collected via apheresis. Patients underwent myocardial scintigraphy and cells were injected in the artery supplying segments with the greatest perfusion defect. At baseline, 2 groups did not differ in age, sex, left ventricular ejection fraction, or N-terminal B-type natriuretic peptide levels. At 5 years, stem cell therapy was associated with increased left ventricular ejection fraction (from 24.3±6.5% to 30.0±5.1%; P=0.02), increased 6-minute walk distance (from 344±90 m to 477±130 m; P<0.001), and decreased N-terminal B-type natriuretic peptide (from 2322±1234 pg/mL to 1011±893 pg/mL; P<0.01). Left ventricular ejection fraction improvement was more significant in patients with higher myocardial homing of injected cells. During follow-up, 27 (25%) patients died and 9 (8%) underwent heart transplantation. Of the 27 deaths, 13 were attributed to pump failure and 14 were attributed to sudden cardiac death. Total mortality was lower in the SC group (14%) than in controls (35%; P=0.01). The same was true of pump failure (5% vs 18%; P=0.03), but not of sudden cardiac death (9% vs 16%; P=0.39).

Conclusions: Intracoronary stem cell transplantation may be associated with improved ventricular function, exercise tolerance, and long-term survival in patients with dilated cardiomyopathy. Higher intramyocardial homing is associated with better stem cell therapy response. (Circ Res. 2013;112:165-173.)

Key Words: bone marrow cells ■ CD34+ cells ■ dilated cardiomyopathy ■ granulocyte colony-stimulating factor mobilization ■ heart failure

The pioneering administration of intracoronary bone marrow cells (BMCs) opened the field of clinical stem cell therapy >10 years ago. Since then, several studies have investigated the role of BMC therapy in various clinical settings, primarily focusing on patients with acute myocardial infarction.1 Despite the promising short-term results, clinical trials have not consistently shown benefits of intracoronary BMC therapy. Some studies such as the Clinical Benefit and Long-term Outcome after Intracoronary Autologous Bone Marrow Cell Transplantation in Patients with Acute Myocardial Infarction (BALANCE) trial,2 the study by Cao et al,3 and the study by Assmus et al4 have shown long-term benefits of BMC therapy in the setting of acute myocardial infarction. The end points in these studies were a change in left ventricular ejection fraction (LVEF) or combined end points of myocardial infarction or readmission. By contrast, the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI)5 and the Bone Marrow transfer to Enhance ST-elevation Infarct Regeneration (BOOST)6 trials failed to show long-term benefits of autologous BMC therapy. Although the reasons for the differences in long-term outcomes of BMC-treated patients with ischemic heart disease...
remain largely unclear, they may be partially explained by the different degrees of functional exhaustion of BMCs in patients after myocardial infarction.\(^7\) Patients with dilated cardiomyopathy (DCM) also have impairment in circulating BMCs and endothelial progenitor cells.\(^8,9\) In patients with DCM, lower number of circulating BMCs have been associated with worse functional class and increased neurohormonal activation.\(^9\) However, compared with patients with ischemic cardiomyopathy, patients with DCM have higher numbers of circulating progenitor cells with better functional capacity,\(^10\) which could represent a potential advantage for BMC-based therapy.

To date, few trials have investigated the effects of intracoronary BMC therapy in patients with DCM. In the Transplantation of Progenitor Cells and Functional Regeneration Enhancement Pilot Trial in Patients With Nonischemic Dilated Cardiomyopathy (TOPCARE-DCM) trial, such therapy resulted in significant improvement in LVEF, regional hypokinesia, and N-terminal brain natriuretic peptide (NT-proBNP) at 1 year.\(^11\) In accordance with these findings, the Autologous Bone Marrow Cells in Dilated Cardiomyopathy (ABCD) trial demonstrated an improvement in ejection fraction and quality of life during a mean follow-up of 4 years.\(^12\) Previously, in a pilot randomized study, we have found that intracoronary BMC transplantation was associated with improvement in ventricular remodeling and exercise tolerance in DCM patients.\(^13\) Based on these preliminary data, the aim of the present study was to evaluate long-term effects (5 years) of intracoronary BMC transplantation in patients with DCM. In an exploratory analysis, we also sought to investigate the relationship between intramyocardial homing and response to stem cell therapy.

**Methods**

**Patient Population**

The study design consists of an open-label randomized study conducted at the Advanced Heart Failure and Transplantation Center at University Medical Center Ljubljana in collaboration with the Methodist DeBakey Heart Center and Stanford University. All patients were randomized between January 10, 2005 and May 15, 2006, and followed-up for 5 years.

Patients with heart failure were referred to Advanced Heart Failure and Transplantation Center at University Medical Center Ljubljana to be considered for inclusion in the study. Inclusion criteria consisted of the following: age 18 to 65 years old, diagnosis of nonischemic DCM according to European Society of Cardiology position statement,\(^14\) optimal medical management for at least 6 months, marked ventricular systolic dysfunction (LVEF <30%), and New York Heart Association functional class III for at least 3 months before referral. Patients with acute multiorgan failure or history of hematologic neoplasms were not included. Informed consent was obtained for all patients before participation in the study, and the study protocol was approved by the National Medical Ethics Committee. The trial was registered according to the Slovenian Drug Law and with clinicaltrials.gov (NCT01350310).

**Study Design**

In phase 1 of the study, all patients received granulocyte colony-stimulating factor (G-CSF) therapy (5 mg/kg; 5 days) to assess bone marrow reactivity and potential effects of G-CSF on cardiac function (Figure 1). An independent investigator blinded to the clinical data performed and analyzed echocardiograms at baseline and 1 month after G-CSF therapy. Patients in whom G-CSF therapy was associated
with a transient increase in absolute neutrophil count by at least 50% and no change in cardiac function at 1 month (defined as no improvement in LVEF [<5%] and no decrease in left ventricular end-diastolic dimension [LVEDD]) were enrolled in phase 2.

In phase 2, patients were randomly allocated in a 1:1 ratio to receive intracoronary transplantation of autologous CD34+ stem cells (SC group) or no intracoronary infusion (control group). At the time of enrollment, and at yearly intervals thereafter, we performed detailed clinical evaluation, echocardiography, 6-minute walk test, and measured plasma levels of NT-proBNP. To better define the potential role of inflammatory response, we also measured plasma inflammatory markers (tumor necrosis factor [TNF]-α and interleukin [IL]-6) at the time of CD34+ stem cell injection.

Echocardiography and 6-Minute Walk Test
The echocardiogram data were recorded and analyzed by an independent echocardiographer who was blinded both to randomization and timing of the recordings. LVEF was estimated using the Simpson biplane method and LVEDD was measured in the parasternal long-axis view by a side-by-side comparison. Both the LVEF and the LVEDD were averaged over 5 cycles. Similarly, the 6-minute walk test was performed by a blinded observer according to the consensus of the European Society of Cardiology.15

NT-proBNP, TNF-α, and IL-6 Measurement
Blood was collected into an EDTA-coated tube containing aprotinin, immediately placed on ice for up to 4 hours, and then centrifuged at 4500 rpm for 15 minutes at 0°C. The serum was extracted and stored at −80°C until NT-proBNP assay was performed. All NT-proBNP assays were performed at a central independent laboratory blinded to the clinical data using a commercially available kit (Roche Diagnostics). TNF-α was determined using high-sensitivity human TNF-α immunoassay (Quantikine HS; R&D Systems) and IL-6 was determined using human IL-6 immunoassay (Quantikine HS; R&D Systems).

Peripheral Blood BMC Mobilization, Collection, and Viability Assessment
Peripheral blood BMCs were mobilized by daily subcutaneous injections of G-CSF (5 mg/kg BID twice daily). On day 5, a full blood count and peripheral blood CD34+ cell count were performed. Peripheral blood stem cells were then collected with the Amicus cell separator (Baxter Healthcare). The magnetic cell separator Isolux 300i (Nexell Therapeutics) was used for the immunomagnetic-positive selection of CD34+ cells. In the closed system, the collected cells were washed to remove the platelets, sensitized with mouse monoclonal anti-CD34 antibodies, and then incubated with immunomagnetic beads coated with polyclonal sheep anti-mouse antibodies (Dynabeads; Dynal AS). The bead/CD34+ cell rosettes were separated in the magnetic field from other cells and CD34+ cells were released from the Dynabeads using an octapeptide with an affinity for anti-CD34 antibodies. After immunomagnetic selection, cells were assessed for viability using methylene blue and reassessed for viability 2 hours thereafter, before intracoronary injection.

Target Area Selection and Intracoronary Delivery
Before cell transplantation, patients underwent myocardial perfusion scintigraphy with 99mTc-sestamibi and nitrate augmentation (Figure 2). Tracer uptake in myocardium was quantified using a 20-segment model and normalized to maximum uptake in the heart muscle. Target areas were defined as viable segments of reduced tracer accumulation and contractile dysfunction. Target coronary artery was defined as 1 of the major coronary arteries (left anterior descending, left circumflex, or right coronary artery) supplying segments of reduced tracer accumulation on scintigraphy. After full heparinization, a microcatheter (Progreat Microcatheter System; Terumo) was positioned in a mid-portion of the target coronary artery and the cells were resuspended in saline were injected intracoronary. Each patient received 10 injections (10 mL each). To avoid trauma of

Figure 2. Changes in clinical parameters in stem cell-treated patients (SC group) and controls. At 1 year, left ventricular ejection fraction (LVEF) was significantly increased in the SC group compared with controls, which persisted up to year 3 and was still significantly higher at the end of the study (A). By contrast, we observed no statistical difference for left ventricular end-diastolic dimension (LVEDD) at any time point (B). In the SC group, exercise capacity increased significantly within the first year and remained stable (C), and N-terminal B-type natriuretic peptide (NT-proBNP) levels were significantly decreased (D). 6MWD indicates 6-minute walk test distance.
the target vessel, we performed no balloon inflations at the time of the procedure.

Assessment of Myocardial Homing

Before intracoronary injection, a predefined volume (20%) of cell solution was labeled with 99mTc-hexamethylpropylene-amine oxime. Cell solution was centrifuged, supernatant solution was removed, and sedimented stem cells were incubated with a solution of 99mTc-labeled hexamethylpropylene-amine oxime. After an incubation period of 10 minutes, the cells were resuspended and again centrifuged. The average measured activity of cell preparation was 150 MBq. Two hours after intracoronary delivery of the cells, cell imaging was undertaken to assess myocardial engraftment and distribution. Planar anterior and posterior projections and tomographic imaging of cardiac region were performed on a dual-head gamma camera. After 18 hours, imaging was repeated to detect potential cell migration. Good homing was predefined as measured activity value greater than the median value of the general activity level.

Follow-up and End Points

All patients were followed-up over a period of 5 years. The primary end points included changes in LVEF and LVEDD. Secondary end points included changes in exercise capacity and NT-proBNP levels. In an exploratory analysis, we also compared cardiac mortality, which included sudden cardiac death and death secondary to pump failure. In patients without implanted cardioverter–defibrillators, sudden cardiac death was defined as appropriate implanted cardioverter–defibrillator shock. Pump failure death was defined as a death resulting from multiorgan failure caused by heart failure progression. Heart transplantation was performed according to the standard Eurotransplant protocol, which requires each patient to be confirmed by 3 independent auditors.

Table 1. Baseline Patient Characteristics

|                | All (n=110) | SC Group (n=55) | Control Group (n=55) | P
|----------------|-------------|-----------------|----------------------|---
| Age, y         | 54±9        | 53±8            | 55±7                 | 0.64 |
| Male           | 89 (81)     | 45 (82)         | 44 (80)              | 0.81 |
| DCM pathogenesis |            |                 |                      |     |
| Cardiomyopathy with history of viral infection | 78 (71) | 38 (69) | 40 (73) | 0.43 |
| Familial       | 15 (14)     | 7 (13)          | 8 (15)               |     |
| Idiopathic     | 17 (15)     | 10 (18)         | 7 (13)               |     |
| LVEF, %        | 25.2±4.2    | 24.3±6.5        | 25.7±4.1             | 0.40 |
| LVEDD, cm      | 7.0±0.8     | 6.9±1.0         | 7.0±0.7              | 0.83 |
| Creatinine, mg/dL | 1.45±0.62 | 1.42±0.42       | 1.49±0.47            | 0.40 |
| Sodium, mmol/L | 138±7       | 138±4           | 136±9                | 0.52 |
| NT-proBNP, pg/mL | 2390±1974 | 2322±1234       | 2431±1995            | 0.56 |
| Therapy        |             |                 |                      |     |
| Loop diuretics | 101 (92)    | 51 (93)         | 50 (91)              | 0.73 |
| Digoxin        | 20 (18)     | 9 (16)          | 11 (20)              | 0.62 |
| Spironolactone | 77 (70)     | 41 (75)         | 36 (65)              | 0.30 |
| RAAS inhibitors | 105 (95)   | 51 (93)         | 54 (98)              | 0.17 |
| β-blockers     | 89 (81)     | 43 (79)         | 46 (84)              | 0.47 |
| CRT            | 22 (20)     | 13 (24)         | 9 (16)               | 0.34 |
| ICD            | 19 (17)     | 10 (18)         | 9 (16)               | 0.80 |

Statistical Analysis

The minimal sample size for the study was calculated using a prespecified power of 90% and P value of 0.05. Continuous variables were expressed as mean±SD. Differences between survivors and patients who died and the effects of cell homing on LVEF were analyzed by means of 1-factor ANOVA followed by Tukey test for continuous variables. Comparisons of categoric variables were made by use of a χ² test. Univariable and multivariable stepwise Cox proportional hazard regression analyses were performed to identify independent correlates of 5-year mortality. The P value for entering was set at 0.3, and for staying in the model it was set at 0.05. The Kaplan-Meier method was used to analyze and compare survival in the stem cell group and controls. P<0.05 was considered significant.

Results

Patient Characteristics

Of 131 patients entering phase 1, we excluded 2 patients because of significant improvement of cardiac function at 1 month and 19 patients because of inadequate neutrophil increase after G-CSF stimulation. The remaining 110 patients were randomly allocated into the SC group (n=55) or control group (n=55) (Figure 1). At baseline, the 2 groups did not differ with regard to age, sex, DCM pathogenesis, LVEF, LVEDD, plasma sodium, creatinine, NT-proBNP, or medical/device management (Table 1).

Stem Cell Delivery

The average number of intracoronary injected CD34+ stem cells was 113±26 million. Average stem cell viability was 91.3%. Viability rates of labeled and unlabeled stem cells were 89.9% and 92.3%, respectively, and did not differ significantly (P=0.24). The area of reduced tracer uptake and contractile
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dysfunction was variable between patients. In 25 patients, cells were injected in the left anterior descending, in 11 patients cells were injected in the left circumflex, and in 19 patients cells were injected in the right coronary artery. No cases of distal coronary artery occlusion, acute cardiac dysfunction, or significant troponin leak occurred. Average plasma troponin I levels were 0.09 ± 0.01 ng/mL at baseline, 0.11 ± 0.02 ng/mL 6 hours after the procedure, and 0.08 ± 0.01 ng/mL 12 hours after the procedure. In 2 cases, patients experienced nonsustained ventricular tachycardias during the procedure.

Left Ventricular Function and Dimensions
Time-related changes in LVEF and LVEDD are presented in Figure 2. At 1 year, there was an increase in LVEF in the SC group but not in controls, which led to a significant intergroup difference. The improvement of LVEF in the SC group persisted up to the third year; after that, it progressively declined. However, when compared with the controls, LVEF at the end of the study still remained significantly higher. Although there was a trend toward a decrease in LVEDD in the SC group at year 1, there was no statistical difference between the groups at any time point.

Exercise Capacity and NT-proBNP
Exercise capacity in the SC group increased significantly within the first year and remained stable throughout the follow-up period, leading to a significant intergroup difference at the end of the study. In parallel, we found a significant decrease in NT-proBNP levels in the SC group at year 1, which persisted up to 5 years (Figure 2).

Patient Outcome
During follow-up, 27 (25%) patients died and 9 (8%) underwent heart transplantation. Of the 27 deaths, 13 were attributed to pump failure and 14 were attributed to sudden cardiac death. Total mortality was lower in patients receiving SC therapy (8/55; 14%) than in controls (19/55; 35%) (P=0.01). The same was true of the pump failure (3/55 [5%] vs 10/55 [18%]; P=0.03), but not of the sudden cardiac death group (5/55 [9%] vs 9/55 [16%]; P=0.39). Heart transplantation numbers did not differ between the 2 groups (4/55 [7%] vs 5/55 [9%]; P=0.73). Five-year survival as evaluated by Kaplan-Meier analysis was 2.3-times higher in the SC group than in controls (P=0.015) (Figure 3).

Univariable and Multivariable Predictors of Outcome
The results of the univariable and multivariable Cox proportional hazards regression analysis of survival are presented in Table 2. In a model that included baseline LVEF, LVEDD, NT-proBNP levels, and age, stem cell therapy was the only independent correlate of outcome at 5 years.

Homing and Its Relationship With Clinical Response
Using cell labeling, we quantified cell engraftment in patients from the SC group (n=43 patients). Average early cell engraftment 2 hours postinjection was 7.1 ± 1.5%. At delayed imaging (18 hours postinjection), retention of cells in the myocardium decreased to 5.3 ± 1.3% (P<0.001). We found no significant difference in cell retention rates between different target areas at 2 and 18 hours after the procedure. Representative cell engraftment 2 hours after the injection in left anterior descending is shown in Figure 4.

Table 2. Univariable and Multivariable Predictors of 5-Year Survival

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Univariable Hazard Ratio</th>
<th>95% CI</th>
<th>Multivariable Hazard Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cell therapy</td>
<td>3.4</td>
<td>1.05–5.77</td>
<td>0.04</td>
<td>3.4</td>
<td>1.05–5.77</td>
</tr>
<tr>
<td>NT-proBNP &lt;1000 pg/mL</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LVEF &gt;20%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age &lt;60 y</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

CI indicates confidence interval; LVEF, left ventricular ejection fraction; and NT-proBNP, N-terminal brain natriuretic peptide.
to LVEF, LVEDD, NT-proBNP levels, target coronary artery, liver function, kidney function, or plasma levels of TNF-α. However, we found decreased myocardial homing in older patients and in patients with higher levels of IL-6 (Table 3). Patients with good homing displayed a significant increase in LVEF at 3 and 12 months after the procedure, in contrast to those with poor homing who did not significantly increase their LVEF at any time point (Figure 5).

**Discussion**

This is the first randomized study to date investigating the long-term effects of intracoronary administration of G-CSF-mobilized CD34+ stem cells in patients with nonischemic DCM. During the 5-year follow-up period, cell therapy was associated with a significant improvement in cardiac function and exercise capacity and a significant decrease in NT-proBNP levels. In an exploratory analysis, we also found that total mortality rates were lower in patients randomized to the SC therapy group than in controls.

Several factors may contribute to the beneficial effects of stem cell therapy on cardiac function. In preclinical models, it has been shown that BMC administration can improve cardiac function through paracrine effects. These factors can attenuate apoptosis of endogenous cardiomyocytes and endothelial cells,17 promote angiogenesis, activate resident cardiac stem cells, or induce antiinflammatory effects.18 Other studies also have shown that BMC administration can attenuate the effects of circulating autoantibodies that may be involved in the pathogenesis of DCM19; this is probably mediated by tolerization of autoreactive T cells and B cells.

**Table 3. Characteristics of Patients With Good and Poor Myocardial Cell Homing**

<table>
<thead>
<tr>
<th></th>
<th>Good Homing (≥50th Percentile; n=22)</th>
<th>Poor Homing (&lt;50th Percentile; n=21)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48 ± 8</td>
<td>54 ± 7</td>
<td>0.017</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>24 ± 7</td>
<td>25 ± 8</td>
<td>0.62</td>
</tr>
<tr>
<td>LVEDD, cm</td>
<td>6.8 ± 0.8</td>
<td>7.1 ± 1.1</td>
<td>0.37</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>2454 ± 2088</td>
<td>2121 ± 3489</td>
<td>0.75</td>
</tr>
<tr>
<td>Target vessel, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD:</td>
<td>LAD: 44</td>
<td>LAD: 46</td>
<td>0.78</td>
</tr>
<tr>
<td>RCA:</td>
<td>RCA: 30</td>
<td>RCA: 36</td>
<td></td>
</tr>
<tr>
<td>LCX:</td>
<td>LCX: 26</td>
<td>LCX: 18</td>
<td></td>
</tr>
<tr>
<td>Number of injected cells (×10⁶)</td>
<td>119 ± 57</td>
<td>102 ± 34</td>
<td>0.34</td>
</tr>
<tr>
<td>Bilirubin, µmol/L</td>
<td>18 ± 10</td>
<td>23 ± 18</td>
<td>0.43</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.10 ± 0.25</td>
<td>1.09 ± 0.28</td>
<td>0.89</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>3.3 ± 1.9</td>
<td>11.5 ± 9.9</td>
<td>0.009</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>4.2 ± 2.9</td>
<td>5.5 ± 2.1</td>
<td>0.33</td>
</tr>
</tbody>
</table>

IL-6, interleukin-6; LAD, left anterior descending; LCX, left circumflex; LVEDD, left ventricular end-diastolic dimension; LVEF indicates left ventricular ejection fraction; NT-proBNP, N-terminal brain natriuretic peptide; RCA, right coronary artery; and TNF-α, tumor necrosis factor-α.

All values, except for P values, represent mean±SD.
Administration of BMCs also could lead to improvement in vasculogenesis and angiogenesis. Studies in animal models suggest that implantation of BMCs improves angiogenesis, arteriogenesis, and tissue perfusion, as well as left ventricular function. There also has been growing evidence of defective vascularization and impaired vasculogenesis in patients with DCM. Although the exact underlying mechanisms remain to be defined, they appear to be related to impaired survival of endothelial cells attributable to increased expression of vascular endothelial-cadherin/β-catenin. Myocardial ischemia in patients with DCM also could account for disease progression. Based on similar mechanisms, delivery of CD34+ stem cells could improve tissue perfusion and left ventricular function in patients with DCM. In accordance with this hypothesis, we found that DCM patients exhibit nonhomogeneous tissue perfusion on nuclear imaging. The heterogeneity of perfusion defects was the basis for target area selection for stem cell administration.

One of the most important parameters limiting the success of cell therapy is the low number of stem cells retained in the myocardium. In our clinical study, patients with poor cell homing did not significantly improve left ventricular function at any time point. Our data are also consistent with those of a recent preclinical animal study in which early cell engraftment by positron emission tomography reporter gene imaging was found to predict late cardiac functional recovery. Homing is a complex process depending on interplay between cytokines, cytokine receptors, adhesion molecules, and intracellular signaling cascades. When compared with ischemic heart disease, patients with DCM display downregulation of myocardial homing factors. In our patient cohort, decreased myocardial homing was associated with high levels of IL-6, an inflammatory marker. In addition to its effects on myocardial apoptosis and decreased cardiac contractility, IL-6 plays an important role in regulating cell survival, growth, and differentiation in various cell types, including CD34+ cells. In CD34+ cell culture, the addition of IL-6 to early-acting cytokines was associated with decreased long-term repopulating capacity of the cells despite the increased cellular expansion. Based on a similar mechanism, increased IL-6 levels could probably suppress myocardial homing of CD34+ cell in patients with DCM.

To date, stem cell trials in DCM have been using unfractioned BMCs, with only low numbers of CD34+ cells. The cells were injected intracoronary with balloon inflations either in coronary sinus or in the target coronary artery. Because CD34+ cells contain more endothelial lineage-determined cells than CD133+ or unfractioned BMCs, we decided to use a protocol based on peripheral CD34+ mobilization, which led to significantly increased numbers of injected CD34+ cells. Although we did not perform any balloon inflations during the procedure, the retention rates in our study are in the range of those reported in the previous studies, possibly questioning the role of balloon inflation as a useful strategy to improve myocardial homing.

In the present study, CD34+ stem cell therapy was associated with an increase in LVEF at 5 years by a mean of 5.7%. This is comparable with other studies in DCM, which also found an improvement in LVEF in the range of 4% to 6%. In terms of timing, LVEF improvement appears to occur early after CD34+ stem cell transplantation (within the first year), and may slowly decrease in the long-term follow-up (after the third year). Other studies investigating the long-term effects of BMC transplantation also suggest that the beneficial effects of intracoronary BMC transplantation primarily may be limited to the early period after the procedure. If further validated, then future trials could consider multiple administrations of stem cell therapy in patients with decreased systolic function.

As previous studies have shown, BMC transplantation was not associated with a significant change in left ventricular size. This may suggest that BMCs may improve myocardial function to a greater extent than structural remodeling. In preclinical models, improvement in myocyte function was primarily associated with improved tissue perfusion; this could represent the underlying mechanisms of improvement in ventricular function in our study. It also could explain why BMC transplantation may be beneficial in DCM without directly leading to novel myocyte generation. The beneficial effects on ventricular function also were reflected by improvement in NT-BNP levels by >50% and by improvement in exercise tolerance. The time course of these changes correlated with changes in LVEF, with the majority of the improvement occurring within the first year. Taken together, and with the findings of TOPCARE-DCM trial, these results suggest the long-term beneficial effects of CD34+ stem cell therapy in DCM patients.

In our exploratory analysis, we also have found a significantly lower mortality rate in patients receiving stem cell therapy as compared with the controls, with the difference being largely a consequence of reduced death rates from pump failure. This suggests that improvement in left ventricular
function after stem cell therapy also translates into long-term clinical benefits. The positive effect of stem cell therapy on mortality was evident primarily within the first year, which strongly correlates with the time course of other clinical parameters in this study. We found no effect of stem cell therapy on sudden cardiac death rates, but the study was underpowered for this effect. A previous study from our group also showed that stem cell therapy did not significantly affect parameters of ventricular repolarization. In contrast to some other more undifferentiated cell types, BMCs have been proven several times not to possess an arrhythmogenic potential, a finding consistent with the results of our study.

Study Limitations
The results of our study are subject to several limitations. Although our patient population included patients with DCM (eg, viral, familial, or idiopathic), no biopsies were performed to exclude secondary cardiomyopathies. Our sample size was small, but the groups were well-matched at baseline. Because of our pilot study design, the study was not placebo-controlled or blinded. To minimize this potential bias, echocardiographic and exercise capacity evaluation were performed by independent observers blinded to the patient grouping. To minimize patient trauma and obtain a purified solution of CD34+ cells, our protocol included bone marrow stimulation with G-CSF. To exclude potential direct effects of G-CSF on CD34+ cells, our protocol included bone marrow stimulation to minimize patient trauma and to obtain a purified solution of CD34+ cells, our protocol included bone marrow stimulation with G-CSF. To exclude potential direct effects of G-CSF on left ventricular function, we performed G-CSF stimulation in accordance with other studies.26 This suggests that the effects of G-GSF stimulation on cardiac function in DCM may not be significant. Although we found no effect of cell labeling on viability assessed by methylene blue staining, we have not measured cell proliferation and migration parameters to verify that the nuclear tracer had no effect on the cells. However, previous studies using 18F-FDG imaging of BMCs32 and 111In-oxine imaging of circulating progenitor cells33 have shown significant cellular toxicity by the radiotracers. Finally, we recognize that patients with DCM are a heterogeneous patient population and dynamic changes in ventricular function may be multifactorial.

Conclusions
Intracoronary transplantation of autologous CD34+ cells appears to be a safe treatment modality in patients with DCM. Our results suggest long-term improvement in cardiac function and exercise tolerance, and a decrease in NT-proBNP. This may translate into improved outcome of this patient population. Most of the benefits of the therapy were observed within the first year, which may serve as a background for potential repeated stem cell transplantation in selected patients. Finally, we have shown that better homing can be associated with better response to stem cell therapy. Further studies are needed to define the underlying mechanisms of stem cell therapy response and to develop methods to further improve stem cell homing and survival.

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Disclosures
None.

References


15. Olsson LG, Svedberg K, Clark AL, Wite KK, Cleland JG. Six minute corridor walk test as an outcome measure for the assessment of treatment
27. Vrtovec et al. Stem Cell Therapy in Dilated Cardiomyopathy

**Novelty and Significance**

**What Is Known?**

- Intracoronary transplantation of bone marrow cells has been intensely studied in clinical trials of ischemic heart disease for >10 years.
- Some reports indicate that when compared with patients with ischemic cardiomyopathy, patients with nonischemic dilated cardiomyopathy have higher numbers of circulating progenitor cells with better functional capacity.
- Bone marrow transplantation in nonischemic dilated cardiomyopathy has been associated with short-term improvement in left ventricular function and exercise tolerance.

**What New Information Does This Article Contribute?**

- In an open-label, randomized study, intracoronary transplantation of bone marrow cells is associated with long-term improvement in cardiac function and exercise tolerance in patients with nonischemic dilated cardiomyopathy.
- Improvement in functional capacity may translate into improved outcome of this patient population.
- Better intramyocardial cell homing is associated with better response to CD34+ stem cell therapy.

The long-term effects of bone marrow cell therapy in patients with nonischemic dilated cardiomyopathy have not been completely characterized. In the first randomized prospective study to date, we investigated the effects of intracoronary administration of mobilized CD34+ stem cells in this patient cohort. During the 5-year follow-up period, cell therapy was associated with a significant improvement in cardiac function and exercise capacity. In an exploratory analysis, we also found that total mortality rates were lower in patients randomized to the stem cell therapy. Higher intramyocardial cell homing was associated with better stem cell therapy response. The findings of this study demonstrate that stem cell therapy appears to be beneficial in nonischemic cardiomyopathy patients, emphasize the important role of imaging to track cell fate, and may serve as a background for larger multicenter stem cell trials in this patient population.
Effects of Intracoronary CD34+ Stem Cell Transplantation in Nonischemic Dilated Cardiomyopathy Patients: 5-Year Follow-Up
Bojan Vrtovec, Gregor Poglajen, Luka Lezaic, Matjaz Sever, Dragošlav Domanovic, Peter Cernelc, Aljaz Socan, Sonja Schrepfer, Guillermo Torre-Amione, François Haddad and Joseph C. Wu

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