Effects of Intracoronary Cd34+ Stem Cell Transplantation in Non-Ischemic Dilated Cardiomyopathy Patients: 5-Year Follow Up

Bojan Vrtovec1,5, Gregor Poglajen1, Luka Lezaic2, Matjaz Sever3, Dragoslav Domanovic4, Peter Cernele1, Aljaz Socan2, Sonja Schrepfer5,6, Guillermo Torre-Amione7, François Haddad5,6, and Joseph C. Wu5,6

1Advanced Heart Failure and Transplantation Center, UMC Ljubljana, Slovenia; 2Department of Nuclear Medicine, UMC Ljubljana, Slovenia, 3Department of Hematology, UMC Ljubljana, Slovenia; 4National Blood Transfusion Institute, Ljubljana, Slovenia; 5Department of Medicine, Division of Cardiology, Stanford University School of Medicine, Stanford, CA, USA; 6Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA USA; and 7Methodist DeBakey Heart Center, Houston, TX, USA.

Running title: Stem Cell Therapy in Dilated Cardiomyopathy

Subject codes:
[23] Catheter-based coronary and valvular interventions: other

Address for correspondence:
Dr. Bojan Vrtovec
Advanced Heart Failure and Transplantation Center
Department of Cardiology
Ljubljana University Medical Center
Zaloska 7
Ljubljana, MC SI-1000
Slovenia
Tel: (+3861)522-2844
Fax: (+3861)522-2828
bvrtovec@stanford.edu

In September 2012, the average time from submission to first decision for all original research papers submitted to Circulation Research was 11.5 days.

DOI: 10.1161/CIRCRESAHA.112.276519
ABSTRACT

**Rationale:** CD34+ transplantation in dilated cardiomyopathy (DCM) was associated with short-term improvement in LVEF and exercise tolerance.

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

**Methods and Results:** Of 110 DCM patients, 55 were randomized to receive CD34+ cell transplantation (SC group), and 55 received no cell therapy (controls). In SC group, CD34+ cells were mobilized by G-CSF 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, gender, LVEF, or NT-proBNP levels. At 5 years, stem cell therapy was associated with increased LVEF (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 NT-proBNP (from 2322±1234 pg/mL to 1011±893 pg/mL; P<0.01). LVEF 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 to sudden cardiac death. Total mortality was lower in SC group (14%) than 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 DCM. Higher intramyocardial homing is associated with better stem cell therapy response.

**Keywords:**
Bone marrow cells, G-CSF mobilization, CD34+ cells, dilated cardiomyopathy, heart failure

**Non-standard Abbreviations:**
DCM dilated cardiomyopathy
BMC bone marrow cell
LVEF left ventricular ejection fraction
LVEDD left ventricular end-diastolic dimension
IL-6 interleukin 6
NT-proBNP N-terminal B-type natriuretic peptide
TNF–alpha tumor necrosis factor alpha

INTRODUCTION

The pioneering administration of intracoronary bone marrow cells (BMCs) opened the field of clinical stem cell therapy more than 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 BALANCE trial (2), the study of Cao et al. (3), and the study of Assmus et al. (4) 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 ASTAMI (5) and the 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 to 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, very few trials have investigated the effects of intracoronary BMC therapy in patients with DCM. In the TOPCARE-DCM trial, such therapy resulted in significant improvement in left ventricular ejection fraction, regional hypokinesia, and N-terminal brain natriuretic peptide (NT-proBNP) at 1 year (11). In accordance with these findings, the 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 is 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 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 18-65 years old, diagnosis of non-ischemic dilated cardiomyopathy 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 (NYHA) functional class III for at least 3 months before referral. Patients with acute multi-organ failure or history of hematologic neoplasms were not included. Informed consent was obtained in 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 http://clinicaltrials.gov (Number 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 for 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) 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)-alpha 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 for randomization and timing of the recordings. LVEF was estimated using the Simpson’s biplane method and left ventricular end-diastolic dimension (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-alpha 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, Mannheim, Germany). TNF-alpha was determined using high-sensitivity human TNF-a immunoassay (Quantikine HS, R&D Systems, Minneapolis, MN) 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 b.i.d.). On the fifth day, 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, IL). The magnetic cell separator Isolex 300i (Nexell Therapeutics Inc, Irvine, CA) 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, Oslo, Norway). 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 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 one of the major coronary arteries (LAD, LCX, or RCA) supplying segments of reduced tracer accumulation on scintigraphy. After full heparinization, a microcatheter (Progreat Microcatheter System, Terumo, Leuven, Belgium) was positioned in a mid-portion of the target coronary artery and the cells resuspended in saline were injected intracoronary. Each patient received 10 injections (10 mL each). To avoid trauma of 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 oxyme (HMPAO). Cell solution was centrifuged, supernatant solution was removed, and sedimented stem cells were incubated with a solution of 99mTc-labeled HMPAO. 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 was 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 that the median value of the general activity level.
Follow-up and end points.
All patients were followed over a period of 5 years. The primary endpoints included changes in LVEF and LVEDD. Secondary endpoints 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 (ICDs), sudden cardiac death was defined as either a witnessed cardiac arrest or death within 1 hour after the onset of acute symptoms, or an unexpected death in a patient known to have been well within the previous 24 hours (16). In patients with ICDs, sudden cardiac death was defined as appropriate ICD shock. Pump failure death was defined as a death resulting from multi-organ 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.

Statistical analysis.
The minimal sample size for the study was calculated using a pre-specified 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’s test for continuous variables. Comparisons of categoric variables were made by use of a chi-square test. Univariable and multivariable stepwise Cox proportional hazard regression analysis were performed to identify independent correlates of 5-year mortality. The probability value for entering was set at 0.3 and for staying in the model was set at 0.05. The Kaplan-Meier method was used to analyze and compare survival in the stem cell group and controls. A value of 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 rise after G-CSF stimulation. The remaining 110 patients were randomly allocated into SC group (n=55) and control group (n=55) (Figure 1). At baseline, the 2 groups did not differ with regards to age, gender, DCM etiology, 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 millions. Average stem cell viability was 91.3%. Viability of labeled and unlabeled stem cells was 89.9% and 92.3%, respectively, and did not differ significantly (P=0.24). The area of reduced tracer uptake and contractile dysfunction was variable between patients. In 25 patients, cells were injected in the LAD, in 11 patients in the LCX, and in 19 patients in the RCA. 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 non-sustained VTs 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 to 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-poBNP.

Exercise capacity in the SC group increased significantly within the first year and remained stable throughout the follow-up period, leading to a significant inter-group difference at the end of the study. In parallel, we found a significant decrease in NT-proBNP levels in SC group at 1 year, 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 two 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 post injection was 7.1 ± 1.5 %. At delayed imaging (18 hours post injection), 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 LAD is shown in Figure 4.

Although cell engraftment was documented in all patients, there was significant inter-patient variability. Patients with good (≥ 50th percentile; n=22 patients) and poor (<50th percentile; n=21 patients) homing did not differ with regards to LVEF, LVEDD, NTpro-BNP levels, target coronary artery, liver, kidney function or plasma levels of TNF-alpha. However, we found decreased myocardial homing in older patients and in patients with higher levels of interleukin-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 non-ischemic dilated cardiomyopathy (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 controls.

Several factors may contribute to the beneficial effects of stem cell therapy on cardiac function. In pre-clinical 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 anti-inflammatory effects (18). Other studies have also shown that BMC administration can attenuate the effects of circulating autoantibodies that may be involved in the pathogenesis of DCM (19); this is probably mediated by tolerization of autoreactive T and B cells.

Administration of BMCs could also 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 (20). There has also been growing evidence of defective vascularization and impaired vasculogenesis in patients with DCM (21). Although the exact underlying mechanisms remain to be defined, they appear to be related to impaired survival of endothelial cells due to increased expression of VE-cadherin/beta-catenin (22). Myocardial ischemia in patients with DCM could also 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 non-homogeneous 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 a recent pre-clinical animal study whereby early cell engraftment by positron emission tomography (PET) reporter gene imaging was found to predict late cardiac functional recovery (23). Homing is a complex process depending on interplay between cytokines, cytokine receptors, adhesion molecules, and intracellular signaling cascades. When compared to ischemic heart disease, patients with DCM display down-regulation of myocardial homing factors (10). 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 (24). 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 (12) or in the target coronary artery (11). Since CD34+ cells contain more endothelial lineage-determined cells than CD1333 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 to other studies in DCM, which also found an improvement in LVEF in the range of 4-6% (11,12). 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 may primarily be limited to the early period after the procedure (4,6). If further validated, 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 (11,12). 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 (25); this could represent the underlying mechanisms of improvement in ventricular function in our study. It could also explain why BMC transplantation may be beneficial in DCM without directly leading to novel myocyte generation.
beneficial effects on ventricular function were also reflected by improvement in NT-BNP levels by more than 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 (11), these results suggest the long-term beneficial effects of CD34+ stem cell therapy in DCM patients.

In our exploratory analysis, we have also found a significantly lower mortality rate in patients receiving stem cell therapy as compared to the controls, 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 (13). In contrast to some other more undifferentiated cell types, BMCs have been proven several times not to possess an arrhythmogenic potential (1), 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 dilated cardiomyopathy (e.g., 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 double-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 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 all patients (Phase 1) and randomized only those in whom G-CSF had no effects on LVEF and LVEDD (Phase 2). Of 131 patients entering Phase 1, we excluded only 2 patients (1.5%) because of significant improvement of cardiac function. In accordance with other studies (26), this suggests that the effects of G-CSF stimulation on cardiac function in dilated cardiomyopathy 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 BMCs (27) and 111In-oxine imaging of circulating progenitor cells (28) have not shown significant cellular toxicity by the radiotracers. Finally, we recognize that patients with dilated cardiomyopathy are a heterogeneous patient population and dynamic changes in ventricular function may be multi-factorial.

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 develop methods to further improve stem cell homing and survival.
SOURCES OF FUNDING
Ministry of Health, Republic of Slovenia: Tertiary Care Scientific Grants (# 20110130 and 20100368).
Stanford Cardiovascular Institute Seed Grants (JCW, FH).

DISCLOSURES
None.

REFERENCES


DOI: 10.1161/CIRCRESAHA.112.276519
FIGURE LEGENDS

**Figure 1.** Flow chart of the study design summarizing the two phases of the study. Phase 1 represents the G-CSF Stimulation Phase. Phase 2 represents the BMC transplantation randomization phase.

**Figure 2.** Changes in clinical parameters in stem cell treated patients (SC Group) and controls. At 1 year, LVEF was significantly increased in the SC group compared to controls, which persisted up to the third year and was still significant higher at the end of the study (Panel A). By contrast, we observed no statistical difference for LVEDD at any time point (Panel B). In the SC group, exercise capacity increased significantly within the first year and remained stable (Panel C), and NT-proBNP levels were significant decreased (Panel D). 6MWD, 6-minute walk test distance.

**Figure 3.** Survival and causes of death in stem cell treated patients (SC Group) and controls. Five-year survival as evaluated by Kaplan-Meier analysis was 2.3 times higher in the SC group than in controls (right), primarily due to differences in pump failure mortality (left).

**Figure 4.** Homing of injected CD34+ cells. Example of homing analysis 2 hours after cell injection in the LAD. 99mTc-HMPAO tracer accumulation is evident in the anterior and anteroseptal areas.

**Figure 5.** Changes in left ventricular ejection fraction in patients with good and poor homing. Patients with good homing (defined as ≥ 50 percentile, upper panel) displayed an increase in LVEF, while patients with poor homing (defined as < 50 percentile, lower panel) did not.
Table 1. Baseline Patient Characteristics

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

All values, except for P values, represent either mean ± standard deviation or number of patients (%). DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic dimension; NT-proBNP, NT-proB-type natriuretic peptide; RAAS, renin-angiotensin-aldosterone; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator.
Table 2. Univariable and Multivariable Predictors of 5-Year Survival

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$ Hazard</td>
<td>95% CI</td>
<td>$P$ Hazard</td>
</tr>
<tr>
<td>Stem cell therapy</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>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LVEF &gt;20%</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age &lt;60 years</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal brain natriuretic peptide.
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</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>44</td>
<td>46</td>
<td>0.78</td>
</tr>
<tr>
<td>RCA:</td>
<td>30</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>LCX:</td>
<td>26</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Number of injected</td>
<td>119 ± 57</td>
<td>102 ± 34</td>
<td>0.34</td>
</tr>
<tr>
<td>cells (x10^6)</td>
<td></td>
<td></td>
<td></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-alpha, pg/ml</td>
<td>4.2 ± 2.9</td>
<td>5.5 ± 2.1</td>
<td>0.33</td>
</tr>
</tbody>
</table>

All values, except for P values, represent mean ± standard deviation. LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; IL-6, interleukin 6; TNF-alpha, tumor-necrosis factor alpha.
Novelty and Significance

What Is Known?

- Intracoronary transplantation of bone-marrow cells has been intensely studied in clinical trials of ischemic heart disease for more than 10 years.

- Some reports indicate that when compared to patients with ischemic cardiomyopathy, patients with non-ischemic dilated cardiomyopathy have higher numbers of circulating progenitor cells with better functional capacity.

- Bone-marrow transplantation in non-ischemic 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 non-ischemic 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 non-ischemic 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 non-ischemic cardiomyopathy patients, emphasize the important role of imaging to track cell fate, and may serve as a background for larger multi-center stem cell trials in this patient population.
Figure 1.

Enrollment

Phase 1: GCSF stimulation (n=131)

Excluded Phase 1 of the Study
LVEF improvement (n=2)
Inadequate BMC mobilization (n=19)

Phase 2: Randomization (n=110)

Stem Cell Therapy Group (n=55)

Control Group (n=55)

Scintigraphy guided BMC transplantation

5 year follow-up
Figure 2.
Figure 3.

![Bar graph showing patient number and Event Free Survival over time for Stem Cell Group and Controls.](image-url)

- **Bar Graph:**
  - X-axis: Causes of death/transplantation (Pump Failure Death, Sudden Cardiac Death, Heart Transplantation)
  - Y-axis: Patient Number
  - Legend:
    - Stem Cell Group
    - Controls
  - Statistics:
    - p = 0.03
    - p = 0.39
    - p = 0.73

- **Survival Graph:**
  - X-axis: Time (months)
  - Y-axis: Event Free Survival (%)
  - Legend:
    - Stem Cell Group
    - Controls
  - Statistics:
    - p = 0.015

DOI: 10.1161/CIRCRESAHA.112.276519
Figure 4.
Figure 5.

GOOD HOMING

Baseline 3 months 1 year 5 years

p=0.05  p=0.02  p=0.08

Baseline 3 months 1 year 5 years

p=0.42  p=0.60  p=0.54

LVEF (%)
Effects of Intracoronary Cd34+ Stem Cell Transplantation in Non-Ischemic Dilated Cardiomyopathy Patients: 5-Year Follow Up
Bojan Vrtovec, Gregor Poglajen, Luka Lezaic, Matjaz Sever, Dragoslav Domanovic, Peter Cernelec, Aljaz Socan, Sonja Schrepfer, Guillermo Torre-Amione, Francois Haddad and Joseph C. Wu

Circ Res. published online October 12, 2012;
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/early/2012/10/12/CIRCRESAHA.112.276519

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/