Intramyocardial Angiogenic Cell Precursor Injection for Cardiomyopathy
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INTRODUCTION
Stem cell transplantation is an emerging therapeutic approach to various diseases, which has been investigated extensively. Heart failure due to cardiomyopathy is one of the diseases in which stem cells might play an important role in the future. The aim of transplantation is to replace damaged cells and establish new blood vessels to restore contractility and blood supply to the heart. This can be achieved by introducing progenitor cells capable of differentiating into cardiomyocytes or promoting neovascularization. Animal studies have been conducted widely, but clinical data are limited. Results depend on the type of cell used, e.g., skeletal myoblast, bone marrow stem cell, circulating progenitor cell. Whether these cells can transdifferentiate into cardiomyocytes is controversial, particularly in terms of generating contractile function. The results also depend on delivery methods: intravenous, intracoronary, or direct intramyocardial injection (either transendocardial or transepicardial). The advantages of direct intramyocardial cell injection are that it is simple and achieves maximal cell delivery; the drawbacks are its invasiveness compared to the transcatheter approach, and the potential for arrhythmia. Microinvasive technology has a potential benefit in minimizing the trauma that can occur with the conventional approach. The objective of this study was to assess the safety, efficacy and short-term results of thoracoscopic direct injection of angiogenic cell precursors into patients with endstage cardiomyopathy. Cells were obtained from the patient’s own blood, avoiding immunological concerns. The number of cells prior to injection was $29.1 \pm 18.9 \times 10^6$. Forty-one patients with cardiomyopathy (mean age, $58.5 \pm 14.3$ years) underwent stem cell injection; 21 had dilated cardiomyopathy and 20 had ischemic cardiomyopathy. Overall ejection fraction improved significantly by $4.8\% \pm 7.5\%$ at $149 \pm 98$ days postoperatively. It increased from $25.9\% \pm 8.6\%$ to $28.7\% \pm 9.8\%$ in dilated cardiomyopathy, and from $26.6\% \pm 5.8\%$ to $33.6\% \pm 7.8\%$ in ischemic cardiomyopathy. New York Heart Association functional class was significantly better at 2 months in both groups. It was concluded that thoracoscopic intramyocardial angiogenic cell precursor injection is feasible and safe in patients with cardiomyopathy. The early results are good, and phase II trials are in progress.

ABSTRACT
Stem cell therapy for heart failure is a rapidly progressing field. The objective of this study was to assess the safety, and short-term results of thoracoscopic direct injection of angiogenic cell precursors into patients with endstage cardiomyopathy. Cells were obtained from the patient’s own blood, avoiding immunological concerns. The number of cells prior to injection was $29.1 \pm 18.9 \times 10^6$. Forty-one patients with cardiomyopathy (mean age, $58.5 \pm 14.3$ years) underwent stem cell injection; 21 had dilated cardiomyopathy and 20 had ischemic cardiomyopathy. Overall ejection fraction improved significantly by $4.8\% \pm 7.5\%$ at $149 \pm 98$ days postoperatively. It increased from $25.9\% \pm 8.6\%$ to $28.7\% \pm 9.8\%$ in dilated cardiomyopathy, and from $26.6\% \pm 5.8\%$ to $33.6\% \pm 7.8\%$ in ischemic cardiomyopathy. New York Heart Association functional class was significantly better at 2 months in both groups. It was concluded that thoracoscopic intramyocardial angiogenic cell precursor injection is feasible and safe in patients with cardiomyopathy. The early results are good, and phase II trials are in progress.

ORIGINAL CONTRIBUTION
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ABSTRACT
Stem cell therapy for heart failure is a rapidly progressing field. The objective of this study was to assess the safety, and short-term results of thoracoscopic direct injection of angiogenic cell precursors into patients with endstage cardiomyopathy. Cells were obtained from the patient’s own blood, avoiding immunological concerns. The number of cells prior to injection was $29.1 \pm 18.9 \times 10^6$. Forty-one patients with cardiomyopathy (mean age, $58.5 \pm 14.3$ years) underwent stem cell injection; 21 had dilated cardiomyopathy and 20 had ischemic cardiomyopathy. Overall ejection fraction improved significantly by $4.8\% \pm 7.5\%$ at $149 \pm 98$ days postoperatively. It increased from $25.9\% \pm 8.6\%$ to $28.7\% \pm 9.8\%$ in dilated cardiomyopathy, and from $26.6\% \pm 5.8\%$ to $33.6\% \pm 7.8\%$ in ischemic cardiomyopathy. New York Heart Association functional class was significantly better at 2 months in both groups. It was concluded that thoracoscopic intramyocardial angiogenic cell precursor injection is feasible and safe in patients with cardiomyopathy. The early results are good, and phase II trials are in progress.

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Angiogenic Cells for Cardiomyopathy

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same time. Multipotent progenitor cells rich in CD45, CD31Bright, CD34+CD45-Dim, and CD34Bright cells were isolated from peripheral blood. Cells at a concentration of 1.5–3.0×10^6·mL^-1 were cultured with vascular endothelial growth factor (R&D Systems, Minneapolis, MN, USA) and 5 IU·mL^-1 heparin (Kamada, Beit-Kama, Israel). The process of cell expansion took 5 days. The resulting ACPs expressed CD34, CD133, KDR, Tie-2, CD144, von Willebrand factor, CD31 Bright, concomitant binding of ulex-lectin, and uptake of acetylated low-density lipoprotein, secreted interleukin-8, vascular endothelial growth factor, angiogenin and formed tube-like structures in vitro. The number and viability of cells were checked and passed the quality control before use.

In the DCM group, cells were injected into the entire LV area. In the ICM group, cells were injected into nonviable myocardium and hypokinetic areas determined by MRI or echocardiogram and sestamibi myocardial perfusion scan. Cardiac MRI was conducted using 3.0 Tesla MR Scanner (Achieva 3.0T systems with Philips Quasar Dual gradients, Philips Medical Systems, The Netherlands). An initial first-pass myocardial perfusion MRI examination was performed using 0.1 mmol gadolinium chelate contrast material per kilogram of body weight, followed by a second 0.1 mmol·kg^-1 dose of the agent. This protocol yielded a cumulative 0.2 mmol·kg^-1 dose of gadolinium chelate contrast material that was administered as part of the myocardial viability assessment. No imaging was conducted during the second contrast material injection. The time between injections was the time required for

| Table 1. Characteristics of Dilated Cardiomyopathy (DCM) and Ischemic Cardiomyopathy (ICM) Groups |
|---------------------------------|-----------------|-----------------|
| Variable                        | DCM (n = 21)    | ICM (n = 20)    |
| Age (years)                     | 54.86 ± 16.73   | 62.35 ± 10.38   |
| Males                           | 16 (76.2%)      | 19 (95.0%)      |
| Mean NYHA class                 | 2.88 ± 0.83     | 3.00 ± 0.68     |
| Diabetes                        | 6 (28.6%)       | 10 (50.0%)      |
| Hypertension                    | 6 (28.6%)       | 15 (75.0%)      |
| Renal failure                   | 3 (14.3%)       | 4 (20.0%)       |
| COPD                            | 0               | 4 (20.0%)       |
| Pulmonary hypertension          | 4 (19.0%)       | 5 (25.0%)       |
| Cerebrovascular accident        | 1 (4.8%)        | 4 (20.0%)       |
| Mitral regurgitation Mild       | 3 (14.3%)       | 0               |
| Moderate                        | 6 (28.6%)       | 3 (15.0%)       |
| Severe                          | 3 (14.3%)       | 7 (35.0%)       |
| Preoperative LVEF               | 24.97% ± 8.74%  | 26.09% ± 6.70%  |
| BNP (pg·mL^-1)                  | 2,988.41 ± 2,760.46 | 5,208.05 ± 5,938.85 |
| No. of ACPs (×10^6)             | 27.05 ± 20.74   | 31.33 ± 16.99   |
| Cell viability                  | 94.68% ± 4.35%  | 94.44% ± 3.10%  |

ACPs = angiogenic cell precursors, BNP = B-type natriuretic peptide, COPD = chronic obstructive pulmonary disease, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association.
myocardial perfusion MRI (1–2 min). The dose of contrast agent was the same as that used in 1.5 T MRI. In fact, the dose of contrast agent should be reduced by approximately half (0.5 mmol·kg⁻¹, total accumulation = 0.1 mmol·kg⁻¹), but this protocol did not work with our equipment. The delayed contrast enhancement study was initiated 15–20 min after the 2nd injection of gadolinium chelate. Wall motion at rest was assessed visually and described as normal, hypokinetic, akinetic or dyskinetic. For analysis of perfusion, the uptake pattern of contrast medium by myocardial tissue was assessed. Distinct uptake of contrast medium during the capillary phase was described as normal perfusion. The absence of uptake or clearly diminished uptake was described as a perfusion defect. Delayed hyperenhancement was classified as absent or present. The transmural extent of delayed enhancement was classified as 1%–25%, 26%–50%, 51%–75% or 76%–100%. The total volume of infarcted (hyperenhancing) tissue was calculated as follows. For each slice, hyperenhancing regions were contoured manually, and the area of hyperenhancing tissue was calculated. The volume was calculated by adding the areas and multiplying the resulting value by the slice thickness. The total mass of infarcted tissue was calculated by multiplying the total volume by the myocardial density (1.05). The number of infarcted segments per patient was counted and reported as percentage of total LV mass.

Under general anesthesia with one-lung ventilation, the patient was placed in the right lateral decubitus position. A small incision was made in the left chest at the 5th intercostal space on the posterior axillary line. The thoracoscope, connected to a video camera, was placed through this incision. The chest cavity was examined, and the 2 other access ports were introduced at the 3rd and 7th intercostal spaces on the posterior axillary line (Figure 1). A thoracoscope with 30-degree and 0-degree lenses was used to allow the surgeon to open the pericardium longitudinally anterior and posterior to the phrenic nerve. This approach was accomplished with ease when the left pleural and pericardial cavity had never been invaded before. Time must be spent to free the pericardium from the heart without injury to the phrenic nerve. Damage to the phrenic nerve could be detrimental in this group of very seriously ill patients. The thoracoscopic instruments and appropriately placed pericardial traction stitches assisted in reaching all regions of the LV wall. Cell injection was carried out using a 23-gauge butterfly needle with a home-made guard (Figure 2). The thickness of the LV wall, which varied according to the severity and extent of infarction and was predetermined by MRI, helped decide the depth of injection. The needle was
RESULTS
Because of preexisting poor LV function and high comorbidity, all patients required inotropic support during intensive care unit stay. The plasma creatine kinase-MB level was 3.95 ± 1.21 ng·mL⁻¹, which may confirm the absence of significant cell damage during the injection. There were no major adverse cardiac events, such as neurological deficit, excessive postoperative bleeding, new renal insufficiency or pulmonary failure, and no malignant arrhythmias. All patients tolerated cardiac rehabilitation very well. The 6-min walk tests showed an improvement of nearly 126 meters in walking capacity (from 343.3 to 469.4 meters, \( n = 9 \), at 90 days). At a mean of 180 days after the injection, NYHA functional class improved from 2.69 ± 0.79 preoperatively to 1.63 ± 0.81 in the DCM group (\( n = 16 \), \( p < 0.05 \)), and from 3.0 ± 0.52 to 1.94 ± 0.85 in the ICM group (\( n = 16 \), \( p < 0.05 \)). The overall EF in both groups improved by 4.8 ± 7.5 percentage points (from 26.2% ± 7.2% to 31.1% ± 9.0%) at 149 ± 98 days postoperatively (\( n = 29 \), \( p < 0.05 \)). Ejection fraction improved by 2.8 ± 9.1 percentage points (from 25.9% ± 8.6% to 28.7% ± 9.8%) at 135 ± 88 days in the DCM group (\( n = 15 \), \( p = 0.3 \)), and 7.1 ± 4.6 percentage points (from 26.6% ± 5.8% to 33.6% ± 7.7%) at 164 ± 109 days in the ICM group (\( n = 14 \), \( p < 0.05 \)). In the ICM group, the number of follow-up MRI scans was insufficient for analysis; however, there was a trend towards a decrease in percentage of infarction (Figure 3).

DISCUSSION
The newly discovered blood-borne progenitor cells have not undergone substantial trials in humans. This cell type has only just been separated and cultivated in amounts believed to be enough to repair damage in various organs, particularly the heart. Angiogenic cell precursors are characterized by features similar to endothelial progenitor cells with regard to bone marrow development, migration into the circulation and function. However, as ACPs induce blood vessel formation by a variety of means, and may differentiate into both blood vessel-forming cells and heart muscle cells. The term ACP is preferred over endothelial progenitor cell. Angiogenic cell precursors secrete tissue survival and regeneration factors and cytokines, which also facilitate the mobilization of additional progenitor cells and important elements involved in the healing process in the ischemic area. This potent combination of beneficial effects makes VesCell a powerful treatment for severe cardiac disorders.

Patients who failed to respond to medical and surgical treatment were included in this study. Most were in NYHA class II and III and waiting to become transplant candidates, so much sicker than those undergoing coronary artery bypass and valvular procedures. Almost half (DCM + ICM) had a moderate degree of mitral
and tricuspid regurgitation, pulmonary hypertension and multiple comorbidities. The improvements in NYHA functional class, 6-min walk test and LVEF are comparable to those of intracoronary or transendocardial autologous bone-marrow cell or intracoronary endothelial progenitor cell injection in both acute and chronic myocardial ischemia models. Left ventricular ejection fraction increased 6.7 percentage points after intracoronary injection of autologous bone-marrow cells and successful percutaneous coronary intervention for acute ST-segment elevation myocardial infarction in the BOOST trial. Schachinger and colleagues found no difference in the increase of LVEF (5–8 percentage points) with circulating progenitor cells (endothelial progenitor cells) or bone marrow-derived progenitor cells. Similar results were found in chronic myocardial ischemia: LVEF increased by 9.2% at 1 week and 10.5% at 3 months after intracoronary transplantation of autologous bone marrow-derived mononuclear cells. Left ventricular ejection fraction remained improved at 6 months and at 1 year after cell injection. Wang and colleagues reported no significant change in LV function or LV end-diastolic diameter after intracoronary injection of autologous mesenchymal stem cells in DCM; however, 6-min walk distance was significantly improved at 6 months, as in our study.

The proposed mechanisms of action of these cells are angiogenesis, transdifferentiation, cytokine release/paracrine action and homing signal. The actual mechanisms are not quite clear at this point. Surgical trauma itself has a potential effect of angiogenesis. The mechanism may be similar to that of transmyocardial laser revascularization, believed to be stimulation of neoangiogenesis via growth factors. It might also be the combination of growth factors enhancing the results of the treatment; however, experimental results are conflicting, and long-term clinical results of laser revascularization are unsatisfactory. In DCM, the mechanism of action is unclear.

The few complications in this study did not differ from the trans-catheter approach in which death, acute myocardial infarction or stent thrombosis were found after intracoronary autologous bone-marrow cell injection. The same complications after intracoronary endothelial progenitor cell injection were reported in the TOPCARE-AMI trial, at rates of 0%, 3.3% and 3.3%, respectively. During 18-month’s follow-up in the BOOST randomized controlled trial, there was 3.3% recurrence of myocardial infarction and 17% of patients required target-vessel revascularization. There was no documented ventricular tachycardia or syncope in our study or in previously reported series. Echocardiography revealed no evidence of intramyocardial tumor formation or calcification, and no cancer was diagnosed during follow-up. Perin and colleagues reported trans-myocardial injection of bone marrow mononuclear cells via the NOGA Myostar catheter in chronic myocardial ischemia. There was 1 (7%) death at 14 weeks, presumed to be a sudden cardiac death, and no other major periprocedural complication such as sustained arrhythmias, nor did any significant arrhythmias occur while the patients were hospitalized.

This study was carried out to determine the feasibility of direct injection of progenitor cells into the myocardium. The safety of this procedure has never been documented, and our findings confirm that it is safe. We also investigated whether thoracoscopic and microinvasive intercostal approaches were feasible. Both endoscopic and microinvasive approaches allowed surgeons to reach all LV wall areas with minimal difficulty, even in redo cases, and the injection could be performed with ease. Further development would be to identify the type of cell with the greatest ease of harvesting and expansion. The dose-response relationship needs to be evaluated, and patient selection is also important. More basic science is required to explain the results and clarify patient selection. Large prospective randomized studies are needed to establish the role of stem cells in the treatment of heart failure.

The limitations of this study were due to its nature as a safety study, and thus the efficacy of intramyocardial angiogenic precursor cell injection was unclear. It was not randomized, and we did not have a control group with medical treatment alone. Left ventricular ejection fraction was followed up by either cardiac MRI or echocardiogram; therefore, there is bias in the evaluation of LV function. Follow-up was incomplete as the patients were mostly from overseas. Also, cardiologists were not blinded to the previous results of LV function tests. However, the NYHA class and quality-of-life score could be obtained directly from the patient.

It was concluded that thoracoscopic intramyocardial autologous ACP transplantation is feasible and safe in all cases of DCM and in both first-time and redo ICM patients. The early results are good; long-term results are pending. Many unanswered questions remain before expanding usage, such as what is the most effective type of stem cell, the underlying mechanism, optimal dosage, delivery route and long-term safety and efficacy? Randomized, double-blind, placebo-controlled trials are needed to confirm the benefit of this type of cell transplantation.

REFERENCES


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