

## Direct Myocardial Implantation of Human Fetal Stem Cells in Heart Failure Patients: Long-term Results

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### ABSTRACT

**Background:** End-stage heart failure (HF) is refractory to current standard medical therapy, and the number of donor hearts is insufficient to meet the demand for transplantation. Recent studies suggest autologous stem cell therapy may regenerate cardiomyocytes, stimulate neovascularization, and improve cardiac function and clinical status. Although human fetal-derived stem cells (HFDSCs) have been studied for the treatment of a variety of conditions, no clinical studies have been reported to date on their use in treating HF. We sought to determine the efficacy and safety of HFDSC treatment in HF patients.

**Methods and Results:** Direct myocardial transplantation of HFDSCs by open-chest surgical procedure was performed in 10 patients with HF due to nonischemic, nonchagasic dilated cardiomyopathy. Before and after the procedure, and with no changes in their preoperative doses of medications (digoxin, furosemide, spironolactone, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers), patients were assessed for New York Heart Association (NYHA) class, performance in the exercise tolerance test (ETT), ejection fraction (EF), left ventricular end-diastolic dimension (LVEDD) via transthoracic echocardiography, performance in the 6-minute walk test, and performance in the Minnesota congestive HF test. All 10 patients survived the operation. One patient had a stroke 3 days after the procedure, and although she later recovered, she was unable to perform the follow-up tests. Another male patient experienced pericardial effusion 3 weeks after the procedure. Although it resolved spontaneously, the patient abandoned his control tests and died 5 months after the procedure. An autopsy of the myocardium suggested that new young cells were present in the cardiomyocyte mix. At 40 months, the mean ( $\pm$ SD) NYHA class decreased from  $3.4 \pm 0.5$  to  $1.33 \pm 0.5$  ( $P = .001$ ); the mean EF increased 31%, from  $26.6\% \pm 4\%$  to  $34.8\% \pm 7.2\%$  ( $P = .005$ ); and the mean ETT increased

291.3%, from 4.25 minutes to 16.63 minutes (128.9% increase in metabolic equivalents, from  $2.46$  to  $5.63$ ) ( $P < .0001$ ); the mean LVEDD decreased 15%, from  $6.85 \pm 0.6$  cm to  $5.80 \pm 0.58$  cm ( $P < .001$ ); mean performance in the 6-minute walk test increased by 43.2%, from  $251 \pm 113.1$  seconds to  $360 \pm 0$  seconds ( $P = .01$ ); the mean distance increased 64.4%, from  $284.4 \pm 144.9$  m to  $468.2 \pm 89.8$  m ( $P = .004$ ); and the mean result in the Minnesota test decreased from  $71 \pm 27.3$  to  $6 \pm 5.9$  ( $P < .001$ ).

**Conclusion:** Although these initial findings suggest direct myocardial implantation of HFDSCs is feasible and improves cardiac function in HF patients at 40 months, more clinical research is required to confirm these observations.

### INTRODUCTION

Heart failure (HF) is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood. It results from coronary artery disease in approximately two thirds of patients; the remaining patients have nonischemic cardiomyopathy, the cause of which may be known (eg, hypertension, valvular disease, or myocarditis) or unknown (eg, idiopathic dilated cardiomyopathy) [Hunt 2001]. A relatively common disorder, HF is estimated to affect nearly 5 million people in the United States, with approximately 550,000 new patients each year. Morbidity and mortality rates are high: Each year in the United States, HF causes approximately 970,000 hospitalizations and 53,000 deaths and is a likely contributing factor in 265,000 deaths [ASA/AHA 2005]. The 1-year mortality rate for patients in New York Heart Association (NYHA) classes III to IV is nearly 40% [Jones 2004]. The cost of medical treatment for HF was projected to be \$27.9 billion in the United States in 2005 [ASA/AHA 2005].

Cardiac transplantation is currently the only established surgical treatment for refractory end-stage HF (stage D), but it is available to fewer than 2500 patients in the United States each year. Other standard treatments for HF are limited to measures that only slow its progression or manage its symptoms; such treatments include various pharmacologic therapies and surgical interventions [Hunt 2001].

Preclinical studies have shown cell therapy to regenerate myocardial cells in the injured or necrotic myocardium,

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stimulate angiogenesis, and improve both systolic and diastolic ventricular function [Taylor 1998; Kocher 2001; Orlic 2001a, 2001b; Tomita 2002; Fujii 2003; Pouly 2004; Zhang 2004]. In patients with myocardial infarction, autologous stem cell therapy has been shown to stimulate angiogenesis, repair local cardiac tissue, and improve cardiac function [Kang 2004; Schachinger 2004]. In patients with ischemic HF, such therapy has been demonstrated to improve ejection fraction (EF), heart-pumping action, quality of life, NYHA class, and exercise capacity [Perin 2004]. In ischemia, bone marrow-derived stem cell therapy has been shown to be safe in terms of arrhythmias and/or other adverse events [Patel 2005].

Stem cells can be derived from 3 main sources: adult tissues (eg, bone marrow), blastocysts (embryonic stem cells), and fetal tissue from terminated ectopic pregnancies, elective abortions, or spontaneous miscarriages. Most cell therapy administered to HF patients to date has been bone marrow-derived adult autologous stem cells. Human fetal-derived stem cells (HFDSCs) are thought to be more pluripotent than adult stem cells; ie, the former can develop into a wider range of specialized cells [O'Donoghue 2004]. Although HFDSCs have been used to treat a variety of conditions, including blood and immune system disorders [Touraine 1999], spinal cord injuries [Tsybalyuk 2003], stroke [Zorin 2003], other neurologic and eye disorders [Salogub 2003], and diabetes [Grischenko 2003], there have been no reports of the use of HFDSCs in HF therapy.

Given the promising findings to date of autologous stem cell therapy in HF patients, the possibly greater differentiating potential of HFDSCs and their successful application in treating a variety of other disorders, we designed this trial to investigate the safety and efficacy of HFDSC implantation for the treatment of idiopathic cardiomyopathy.

## METHODS

This investigation was an open-label, single-arm, prospective clinical study performed at Luis Vernaza Hospital, Guayaquil, Ecuador. The study was approved by the Ethics Committee of the hospital, and prospective participants were fully informed about the potential risks of the surgical procedure and HFDSC transplantation. Informed consent was obtained from all patients.

### Patient Population

All patients were assessed at baseline with respect to biochemistry profile, complete blood count, coagulation profile, electrocardiogram, chest radiograph, transthoracic echocardiogram with a Vivid 7 device (GE Healthcare, Piscataway, NJ, USA), cardiac catheterization with coronary angiography to exclude ischemic heart disease, a 6-minute walk test over a 30-m flat surface, an exercise tolerance test (ETT) according to a modified Naughton protocol, NYHA classification, and a Minnesota congestive HF test.

Patients participating in the study met the following inclusion criteria: American Heart Association diagnostic criteria for dilated nonischemic, nonchagasic cardiomyopathy [Hunt 2001]; an EF  $\leq$ 35% by transthoracic echocardiography; a

NYHA functional class of III or IV; bilirubin, creatinine, blood urea nitrogen, serum glucose, glutamic-oxaloacetic transaminase (aspartate aminotransferase), and glutamic-pyruvic transaminase levels  $<$ 2.5 times normal values; and a symptomatic condition despite optimal drug therapy for HF.

Exclusion criteria included the following: valvular heart disease requiring surgical treatment; other concurrent life-threatening disease, infectious disease, blood disease, diagnosis of epilepsy, or positivity in human immunodeficiency virus or Venereal Disease Research Laboratory testing; intolerance or hypersensitivity to biological substances; participation in another clinical trial; a history of drug or alcohol abuse, psychiatric disturbances, or suicide attempts in the previous 2 years; renal failure needing dialysis; a white blood cell count  $<$ 5000/ $\mu$ L or  $>$ 12,000/ $\mu$ L, a hematocrit  $<$ 30%, or pulmonary thromboembolism within the previous 6 months; mechanical ventilation support within the previous 10 days; and morbid obesity.

Patients who were initially included but who were non-compliant with the protocol (tests or treatments), were lost to follow-up, or developed an unrelated new illness were excluded from the study.

For each patient, preoperative medications (digoxin, furosemide, spironolactone, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers) were maintained throughout the study and the follow-up.

### Stem Cells

HFDSCs were provided by the Institute for Regenerative Medicine, Barbados, and were processed and prepared by the Institute for Problems of Cryobiology and Cryomedicine (IPCC) (Kharkov, Ukraine). The IPCC obtains HFDSCs from fetuses of 5 to 12 weeks' gestation from legally consenting, noncompensated donors who have undergone terminated ectopic pregnancies, elective abortions, or spontaneous miscarriages. The HFDSCs are prepared from harvested fetal liver tissue under sterile conditions and undergo polymerase chain reaction testing for human immunodeficiency virus, hepatitis B and C, mycoplasma, toxoplasmosis, cytomegalovirus, herpes simplex viruses I and II, rubella, and *Treponema pallidum*; HFDSCs also undergo culture tests for bacterial and fungal contamination. Cell preparations are stored in cryopreservatives at  $-196^{\circ}\text{C}$  in liquid nitrogen. The percentage of viable cells was 60% according to the IPCC certification.

The IPCC shipped HFDSCs in minishipper containers in a cryopreserved state ( $-150^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$ ) to Luis Vernaza Hospital for this study, and they were maintained in this state until use. Just before the procedure, HFDSCs were thawed to room temperature. In 9 patients, the cells were diluted in 80 mL of saline solution at  $37^{\circ}\text{C}$ ; in 1 patient who underwent the procedure via a minithoracotomy approach, the cells were diluted in 15 mL. Each patient received 60 to  $80 \times 10^6$  HFDSCs, according to the information issued by the provider.

### Anesthesia and Surgical Technique

Patients were anesthetized with 0.50  $\mu\text{g}/\text{kg}$  fentanyl as a premedication, with 2 mg/kg thiopental as induction, with

## Improvement in Patient Characteristics from Baseline at 40 Months following Human Fetal-Derived Stem Cell Transplantation\*

Variable	Result			P
	Before Transplantation	40 Months Postoperatively	Change	
NYHA class	3.4 ± 0.5	1.33 ± 0.5		.001
Ejection fraction, %	26.6% ± 4.0%	34.8% ± 7.2%	31%↑	.005
ETT, min	4.25	16.63	291.3%↑	<.0001
ETT, METS	2.46	5.63	128.9%↑	
LVEDD, cm	6.85 ± 0.6	5.80 ± 0.58	15%↓	<.001
6-MWT, s	251 ± 113.1	360 ± 0	43.2%↑	.01
Mean distance, m	284.4 ± 144.9	468.2 ± 89.8	64.4%↑	.004
Minnesota CHF test	71 ± 27.3	6.0 ± 5.9		<.001

\*Data are presented as the mean ± SD where indicated. NYHA indicates New York Heart Association; ETT, exercise tolerance test; METS, metabolic equivalents; LVEDD, left ventricular end-diastolic dimension; 6-MWT, 6-minute walk test; CHF, congestive heart failure.

1 mg/kg atracurium for relaxation, and with 0.025 µg/kg per minute of remifentanyl and 0.5% to 1.5% sevofurane for maintenance during the procedure. Nine patients underwent a midline sternotomy, and 1 patient had a left anterior minithoracotomy in the fifth intercostal space. Prior to the injections, 80 marks (1 cm apart) were made with a blue methylene marker on the anterolateral, posterolateral, and diaphragmatic left ventricular walls and on the anterolateral right ventricular wall, with care taken to avoid coronary blood vessels.

We administered 80 injections of 1 mL each in the marked areas. The injections were made 3 mm deep with a 25-gauge needle and a catheter. Only 15 injections were made in the anterolateral wall in the patient who underwent the minithoracotomy (S.B., female, 48 years). During the procedure, patients were monitored for arterial pressure, central venous pressure, urine output, electrocardiogram, oxygen saturation, and end-tidal carbon dioxide concentration in the expired air. Infusions of potassium (20 mEq/hour) and magnesium (1 g/hour) were started before the operations and maintained up to the time of chest closure. All patients were extubated in the operating theater.

At 40 months after the procedure, each patient underwent reassessment for NYHA classification, an ETT, the EF, the left ventricular end-diastolic dimension (LVEDD), and performance in a 6-minute walking test. The Minnesota congestive HF test was performed before the operation and at 40 months.

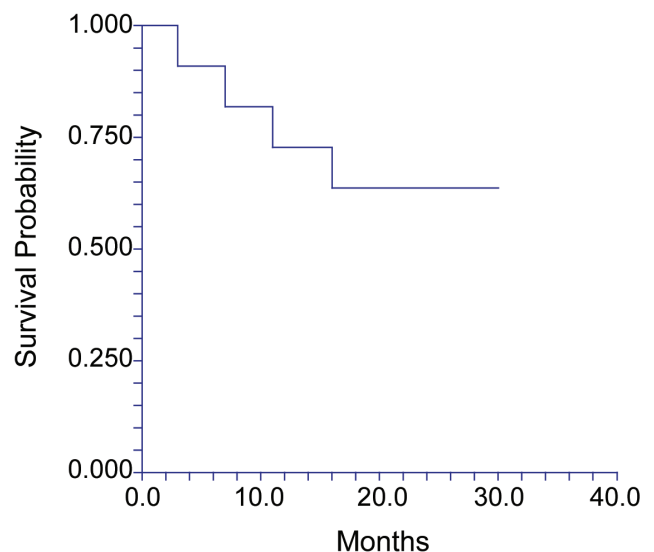
### Statistical Analysis

Mean values for parameters measured just before and after the procedure were compared with paired Student *t* tests (Primer program; Primer-E, Ivybridge, UK).

## RESULTS

Six female and 4 male patients (age range, 47–77 years) met the inclusion criteria and participated in the study.

There was no operative or perioperative mortality. One male patient (U.J., 69 years) experienced a single transient intraoperative ventricular fibrillation during the procedure but before receiving injections; the ventricular fibrillation was terminated by electrical cardioversion. One man (M.J., 66 years) and 1 woman (V.M., 77 years) required temporary pacemakers postoperatively because of severe bradycardia (<40 bpm), for 24 hours and 48 hours, respectively. The former patient received dobutamine for 24 hours. He also had a mild pericardial effusion at 3 weeks, which resolved spontaneously. He was later excluded from the trial for noncompliance (he abandoned his controls), and he ultimately died at 5 months. The heart autopsy showed nests of cardiomyocytes among the fibrotic tissue, but it was not possible to determine whether they were new growing myocardium or remaining native fibers. Immunohistochemistry analyses of the heart revealed the expression of



Kaplan-Meier plot of the probability of survival to 40 months.

cell markers CD34, CD117, smooth muscle actin, vimentin, and myogenin, as well as increased neovascularization in adjacent fibrotic tissue. Electron microscopy analysis did not clarify whether the new fibers were self-regenerating heart cells or were due to the implants. The results of 2 polymerase chain reaction tests for chimerism were negative.

One female diabetic patient (Q.A., 52 years) had a right hemiparesis 3 days after implantation due to ischemic stroke that was confirmed by computed tomography scanning. Although the patient was alive and recovering as of this writing, she was excluded from the protocol because she was unable to perform the follow-up tests of the study. She died 18 months after the procedure from diabetes complications.

Another female patient (B.M.) was hospitalized 3 times (at 2, 4, and 7 months postoperatively) because of abdominal pain caused by severe gastroenteritis that led to transient decompensation. She therefore required inotropic support with dobutamine for 48, 48, and 72 hours, respectively. Although no improvement in her EF was noted at 210 days postoperatively, there was a significant improvement in her ETT performance, the LVEDD, and her performance in the 6-minute walk test. At the 210-day follow-up, no other complications were observed. The patient died suddenly at 8 months postoperatively.

The female patient who underwent her procedure via minithoracotomy died at 12 months from HF caused by a mitral insufficiency. She had refused a mitral valve operation.

The patients who provided 40 months of follow-up data demonstrated improvements both clinically and in imaging studies. With regard to the imaging studies, we noted an increased wall thickness, both eccentric and concentric. Patients improved in association with increased contractility in these regions. Compared with baseline assessments, we noted other improvements: The mean ( $\pm$ SD) NYHA class decreased from  $3.4 \pm 0.5$  to  $1.33 \pm 0.5$  ( $P = .001$ ); the mean EF increased 31%, from  $26.6\% \pm 4.0\%$  to  $34.8\% \pm 7.2\%$  ( $P = .005$ ); performance in the ETT increased 291.3%, from 4.25 minutes to 16.63 minutes (128.9% in metabolic equivalents, 2.45 to 5.63) ( $P < .0001$ ); the mean LVEDD decreased 15%, from  $6.85 \pm 0.6$  cm to  $5.80 \pm 0.58$  cm ( $P < .001$ ); mean performance in the 6-minute walk test increased 43.2%, from  $251 \pm 113.1$  seconds to  $360 \pm 0$  seconds ( $P = .01$ ); the mean distance increased 64.4%, from  $284.4 \pm 144.9$  m to  $468.2 \pm 89.8$  m ( $P = .004$ ); and the mean result in the Minnesota congestive HF test decreased from  $71 \pm 27.3$  to  $6 \pm 5.9$  ( $P < .001$ ) (Table). The Kaplan-Meier probability of survival at 40 months was 66% (Figure).

## DISCUSSION

Current treatment options for refractory end-stage HF are limited in effectiveness, and no current treatment can totally repair ischemic or necrotic myocardial tissue. Studies have suggested that autologous stem cell therapy can be beneficial in improving cardiac function in patients with HF due to coronary diseases [Patel 2005]. The preliminary findings from this study constitute the first report of the application of HFDSCT therapy in HF patients. We found statistically

significant improvement in left ventricular function (EF and LVEDD), NYHA class, and performance in the ETT, the 6-minute walk test, and the Minnesota congestive HF test at 40 months after direct implantation of myocardial cells. All patients were maintained on their preoperative medications and dosages throughout the study.

It is worth noting that no new recurrent or permanent arrhythmias were seen after implantation in this surgical series of patients. As previously reviewed [Patel 2005], studies have found arrhythmias in patients with HF or myocardial infarction who were given skeletal myoblast cell therapy, but this complication appears to be less of a problem with autologous adult stem cells.

The improvement seen in the present cohort suggests that HFDSCTs have some therapeutic effect. The mechanism of action, as seen via echocardiographic imaging, suggests, however preliminarily, that the increased wall thickness and contractility in the regions described might be due to an increase in the number of cardiomyocytes. More studies are needed to validate this hypothesis and to evaluate for the presence of DNA from the donor.

Whether the expression of these cell markers and the electron microscopy analyses show that these phenomena are constant or just appeared in a patient who did not get as good an outcome as the rest of the cohort remains a question that also requires further investigation to be answered.

We recognize that the relatively small number of patients may represent a significant limitation of this study. These initial findings suggest, however, that HFDSCT transplantation improves cardiac function in HF patients at 40 months. No rejection reactions or malignancy has been seen as of this writing. We believe that the sustained effect of HFDSCT therapy indicates that it offers another possibility for treating patients with advanced HF and represents a new approach that could be used before other major surgical treatments, including heart transplantation, by having them available "on the shelf," thereby avoiding the time-consuming procedures of autologous bone marrow harvesting and processing. Irrespective of the improvement seen in this trial, it is still premature to determine accurately the mechanism of action, indications, and doses; therefore, more research is needed.

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As a potential conflict of interest, F.B. and L.G. disclose that they were consultants for this study. The other investigators have no conflicts of interest.

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