Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: A prospective randomized study

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Background: Autologous adult stem cell transplantation has been touted as the latest tool in regenerative medical therapy. Its potential for use in cardiovascular disease has only recently been recognized. A randomized study was conducted with a novel epicardial technique to deploy stem cells as an adjuvant to conventional revascularization therapy in patients with congestive heart failure.

Methods: After institutional review board and government approval, adult autologous stem cell transplantation ($CD34^+$) was performed in patients with ischemic cardiomyopathy and an ejection fraction of less than 35% who were scheduled for primary off-pump coronary artery bypass grafting. Preoperatively, the patients underwent echocardiography, stress thallium imaging single photon emission computed tomography, and cardiac catheterization to identify ischemic regions of the heart and to guide in the selection of stem cell injection sites. The patients were prospectively randomized before the operative therapy was performed. Patient follow-up was 1, 3, and 6 months with echocardiography, single photon emission computed tomography, and angiography.

Results: There were 20 patients enrolled in the study. Ten patients had successful subepicardial transplantation of autologous stem cells into ischemic myocardium. The other 10 patients, the control group, only had off-pump coronary artery bypass grafting. There were 8 male and 2 female subjects in each group. The median number of grafts performed was 1 in both groups. On angiographic follow-up, all grafts were patent at 6 months. The ejection fractions of the off-pump coronary artery bypass grafting group versus the off-pump coronary artery bypass grafting plus stem cell transplantation group were as follows: preoperative, 30.7% $\pm$ 2.5% versus 29.4% $\pm$ 3.6%; 1 month, 36.4% $\pm$ 2.6% versus 42.1% $\pm$ 3.5%; 3 months, 36.5% $\pm$ 3.0% versus 45.5% $\pm$ 2.2%; and 6 months, 37.2% $\pm$ 3.4% versus 46.1% $\pm$ 1.9% ($P < .001$). There were no perioperative arrhythmias or neurologic or ischemic myocardial events in either group.

Conclusions: Autologous stem cell transplantation led to significant improvement in cardiac function in patients undergoing off-pump coronary artery bypass grafting for ischemic cardiomyopathy. Further investigation is required to quantify the optimal timing and specific cellular effects of the therapy.

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Congestive heart failure (CHF) is a complex clinical syndrome that results from myocardial dysfunction that impairs the heart's ability to circulate blood at a rate sufficient to maintain the metabolic needs of peripheral tissues and various organs. Heart failure is a relatively common clinical disorder estimated to affect more than 5 million patients in the United States. About 400,000 new patients are diagnosed with CHF each year. Morbidity and mortality rates are high; annually, approximately 900,000 patients require hospitalization for CHF, and

Abbreviations and Acronyms
CHF = congestive heart failure
GCSF = granulocyte colony-stimulating factor
NYHA = New York Heart Association
OPCAB = off-pump coronary artery bypass grafting
PBS = phosphate-buffered saline
SPECT = single photon emission computed tomography

up to 200,000 patients die from this condition. The average annual mortality rate is 40% to 50% in patients with severe (New York Heart Association [NYHA] class IV) heart failure. In the United States CHF treatment is estimated to cost more than 25 billion dollars for 2004.[1]

The initial stages of heart failure are managed with medical therapy, and end-stage heart failure is managed with surgical procedures in addition to medical therapy. Some of the proven surgical procedures include myocardial revascularization, ventricular assist devices, and heart transplantation.[2]

Although surgical and catheter-based revascularization of ischemic myocardium can treat angina, reduce the risk of myocardial infarction, and improve function of viable myocardium, the viability of severely ischemic myocardium, necrotic myocardium, or both cannot be restored.

The major process to reverse the left ventricular remodeling would be the enhancement of regeneration of cardiac myocytes, as well as the stimulation of neovascularization within the affected area of the myocardium.[3] Thus the aim of cardiac cellular transplantation is to repopulate the ailing myocardium with cells that could restore contractility and blood supply. This can be achieved by introducing progenitor cells that are capable of differentiating into cardiac myocytes or that promote neovascularization. It has been well established that adult bone marrow is a rich reservoir of tissue-specific stem and progenitor cells. Several studies have shown that bone marrow–derived cells functionally contribute to neoangiogenesis during wound healing and limb ischemia,[4-14] postmyocardial infarction,[15-19] endothelialization of vascular grafts,[20-23] atherosclerosis,[24] retinal and lymphoid organ neovascularization,[25-27] and vascularization during neonatal growth.[28]

In human subjects whole autologous bone marrow mononuclear cells have been delivered by means of arterial and venous catheters into the coronary vessels feeding the infarcted and ischemic tissue,[29-32] by means of transendocardial injections,[33] by means of guided electrochemical mapping directly into infarcted myocardium,[34] or by means of direct epicardial injections.[35] In another study the efficacy of the ex vivo expanded peripheral blood mononuclear cells was compared with bone marrow–derived endothelial progenitor cells in restoring revascularization after acute myocardial infarction.[36] In all of these studies, there was improved blood flow and left ventricular function, suggesting that infusion of autologous progenitor cells appears to be feasible and safe and might confer short-term therapeutic benefit.

The above data suggest that bone marrow or mobilized peripheral blood progenitor cells play a role in the revascularization of the ischemic myocardium. Most of these studies, however, are small series or case reports, are poorly controlled, and are not randomized. There is lot of variability in the factors and conditions to validate the true benefits of cellular therapy. Therefore the goal of this study was to perform a prospective randomized study of autologous stem cell therapy in patients with ischemic CHF requiring revascularization.

Methods

After hospital institutional review board and Argentina Ministry of Health approval, patients with documented ischemic heart failure were screened to evaluate suitability for the study protocol. All patients underwent preoperative electrocardiography, 2-dimensional stress echocardiography, single photon emission computed tomography (SPECT), chest roentgenography, and standard hematologic laboratory tests for general anesthesia and cardiac surgery. Inclusion criteria were as follows: ischemic heart failure with an ejection fraction of 35% or less on 2 imaging studies (echocardiography and multiplanar cardiac catheterization) and NYHA heart failure functional class III or IV. All patients had prior percutaneous coronary interventions and had optimal medical management of their heart failure. All patients screened for participation in this study were from the Benetti Foundation in Argentina, where the standard of care involves hybrid revascularization procedures and where anterior vessels are bypass grafted and posterior vessels are stented. This might not be the case in other countries. Patients were excluded if they had a current or prior malignancy, any hematologic disorder, renal failure requiring dialysis, left ventricular aneurysm, prior cardiac surgery, valvular disease requiring surgery, preoperative steroid therapy, or were within 6 days of an acute coronary event.

On the day of the operation, the patients were randomized to off-pump coronary artery bypass grafting (OPCAB) or OPCAB plus stem cell therapy by using a nonparticipant in the study to pick a red ball (OPCAB plus stem cell therapy group) or blue ball (OPCAB-only group). After this was determined, the patients were given a general anesthetic, and monitoring lines were placed. Patients in the OPCAB-only group had a standard sternotomy and OPCAB performed with both apical suction and pressure stabilization of the heart (Guidant Corp). Patients in the stem cell therapy group were placed prone, and bone marrow was harvested from the iliac bone in a sterile fashion after achievement of general anesthesia. To minimize the anesthetic time, we designed a special multiholed harvest needle with a 60-mL syringe. It was introduced into the iliac bone between both posterior iliac spines at both sides. Using this technique, we were able to harvest 500 to 600 mL of bone marrow with a minimal number of puncture sites. At least 250 mL of bone marrow must be harvested to continue with the protocol. The harvested bone marrow was placed in a blood bag

TABLE 1. Studies used to evaluate patients

	Preoperative	Postoperative, 1 wk	1 mo	3 mo	6 mo
ECG	X	X	X	X	X
CXR	X	X	X	X	X
SPECT	X		X	X	X
CATH	X				X
ECHO	X		X	X	X

ECG, Electrocardiogram; *CXR*, chest roentgenogram; *SPECT*, single photon emission computed tomography; *CATH*, cardiac catheterization; *ECHO*, echocardiography.

with 10,000 U of heparin sulfate and 400 μm of lysine acetylsalicylate to avoid platelet clumping. The bone marrow was filtered on a 500-μm filter followed by a 200-μm filter. The resulting solution was mixed with hydroethylstarch 6%. The supernatant was centrifuged at 400*g* for 15 minutes. The cellular pellet was resuspended in phosphate-buffered saline (PBS). The cell solution was mixed 3:1 with a solution of 155 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$ and 0.1 mmol/L EDTA and set for 5 minutes at room temperature. The solution was then centrifuged at 400*g* for 10 minutes. The pellet was washed with PBS and resuspended. The cell suspension was placed over Ficoll Paque (1.077 density) 4:1 and centrifuged at 400*g* for 30 minutes. The upper layer was aspirated, leaving the mononuclear cell layer at the interphase. The interphase cells were transferred to a new conical tube with PBS and centrifuged at 300*g* for 10 minutes. The supernatant was completely removed, and the cell pellet was resuspended in PBS. Cells counts were performed, and magnetic labeling with Isolex 300i was performed as per standard protocol for peripheral blood progenitor cell products to obtain an enriched product of at least 70% $CD34^+$ cells. The resulting cell solution was resuspended in 30 mL of the patient's own plasma and 10,000 U of heparin sulfate.

After OPCAB, the preselected sites of myocardial dyskinesis and akinesis, but not the infarcted regions, were injected with the stem cell preparation by using a 22-gauge needle apparatus. The injections were in the peri-infarcted, viable but dykinetic, or akinetic areas. There were no injections into the scar itself. The needle apparatus does not have an end hole like most needles but only has side holes. This reduces the amount of leakage that would be generated during a standard needle with a distal end hole. The injection placement was based on prior echocardiography and SPECT viewing to determine ventricular wall thickness, preventing direct introduction of cells into the ventricle. The cell preparation was injected in 1.0-mL aliquots as the needle was withdrawn from the myocardium over a 2-second period. The injections were spaced up to 1 cm apart and spaced to avoid coronary vessels. The injections were 3 to 5 mm in depth on the basis of echocardiographic findings of wall thickness. No direct intracoronary injections were performed. Once this was completed, the chest was closed in standard fashion after good hemostasis and placement of drainage tubes. The patients were extubated in the operating room and taken to the intensive care unit for monitoring. The patients were transferred to the telemetry floor with continuous monitoring and, when appropriate, discharged home. The patients were monitored-evaluated postoperatively as seen in Table 1.

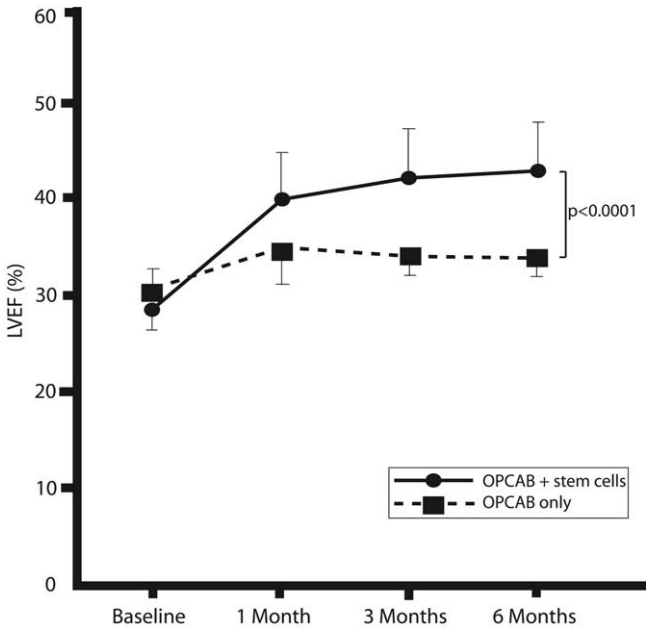

Figure 1. Effects of stem cell transplantation on LVEF recovery. *LVEF*, Left ventricular ejection fraction; *OPCAB*, off-pump coronary artery bypass grafting.

Both groups were followed in the same manner. Statistics were performed by using a 2-way repeated measures analysis of variance, and this resulted in the ability to compare the increase in left ventricular ejection fraction over time in the stem cell group with the increase in left ventricular ejection fraction over time in the control subjects (Statistica software, STAT Soft; see Tables E1-E4 and Figure E1). The Student paired *t* test for means was used to compare the NYHA functional class preoperatively and 6 months postoperatively for each group (Statistica software, STAT Soft; see Tables E1-E4 and Figure 1). The study was blinded for the patients and reviewers of the imaging studies (cardiologists).

Results

There were 48 patients screened for the study. Twenty patients were enrolled and randomized into the study. The demographics of the OPCAB versus OPCAB plus stem cell therapy groups were as follows: male/female sex, 8:2 versus 8:2; mean age, 63.6 versus 64.8 years; and prior myocardial infarction, 7 versus 9 patients. All 20 patients enrolled in the study had successful completion of the treatment in their respective study arms. Ten patients underwent successful OPCAB, with grafting of the left internal thoracic artery to the left anterior descending artery in the control group. In the OPCAB plus stem cell therapy group, 10 patients underwent successful OPCAB from the left internal thoracic artery to the left anterior descending artery, and 1 patient also underwent a saphenous vein graft to the circumflex artery. The median amount of bone marrow harvested was

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TABLE 2. Ejection fractions for the OPCAB-only group

OPCAB patient no.	Age (y)	NYHA class preoperatively	NYHA class post operatively	Preoperative EF (%)	EF 1 mo (%)	EF 3 mo (%)	EF 6 mo (%)	LVEDV preoperatively (mL)	LVEDV 6 mo (mL)
1	71	IV	III	30	35	35	35	164	159
2	64	III	II	34	42	41	43	107	105
3	57	III	III	32	36	35	35	118	116
4	66	III	III	31	37	37	38	139	135
5	62	III	II	29	34	34	36	156	150
6	60	IV	II	33	40	43	44	132	121
7	55	III	II	34	37	37	37	126	125
8	69	IV	IV	29	35	34	35	148	145
9	68	IV	III	26	33	33	33	175	164
10	64	III	III	29	35	35	36	171	169
Mean ± SD	63.6 ± 4.9	3.4	2.7	30.7 ± 2.5	36.4 ± 2.6	36.4 ± 3.0	37.2 ± 3.4	144 ± 23	139 ± 22

OPCAB, Off-pump coronary artery bypass grafting; *NYHA*, New York Heart Association functional class; *EF*, ejection fraction; *LVEDV*, left ventricular end-diastolic volume; *SD*, standard deviation.

550 mL, and a median of 22×10^6 $CD34^+$ cells was found in the final specimen. The ejection fractions for preoperative, 1-month, 3-month, and 6-month analyses are found in Table 2 for the OPCAB-only group and Table 3 for the OPCAB plus stem cell therapy group; Table 4 compares the means of both groups. Also found in Tables 2 and 3 are the ages and preoperative and postoperative (6-month) NYHA functional classes of all the patients. The OPCAB-only patients did have a decrease from 3.4 to 2.7 ($P = .001$). However, there was even a larger decrease in the OPCAB plus stem cell therapy group from 3.5 to 0.7 ($P = 5.9 \times 10^{-9}$). There was no statistical difference in the preoperative ejection fractions between the 2 groups: 30.7% ± 2.5% in the OPCAB group versus 29.4% ± 3.6% in the OPCAB plus stem cell therapy group ($P = .381$). There was, however, a significant difference in ejection fractions at 1, 3, and 6 months for the OPCAB-only group versus the OPCAB plus stem cell therapy group: 36.4% ± 2.6 versus 42.1% ± 3.5, 36.4% ± 3.0% versus 45.5% ± 2.2%, and 37.2% ± 3.4% versus 46% ± 1.9%, respectively ($P < .001$, Figure 1). There was one patient in the OPCAB plus stem cell therapy group who had a hematoma at the bone marrow harvest site. There were no other adverse events in either group (ie, neurologic, hematologic, vascular, death, or infection events). No patients had any postoperative arrhythmias.

Discussion

Our study is one of the first prospective randomized approaches to cellular therapy for ischemic heart failure. The inclusion criteria were broad to help enrollment into the study. Exclusion criteria, however, were kept very strict to decrease the risk to these patients with very few,

TABLE 3. Ejection fractions for the OPCAB plus stem cell therapy group

SC patient no.	Age (y)	NYHA class preoperatively	NYHA class postoperatively	Preoperative EF (%)	EF 1 mo (%)	EF 3 mo (%)	EF 6 mo (%)	LVEDV preoperatively (mL)	LVEDV 6 mo (mL)
1	56	IV	I	26	39	44	44	184	152
2	76	III	0	34	48	50	50	111	95
3	72	IV	II	32	43	46	47	115	97
4	65	IV	II	27	41	45	46	168	141
5	57	III	0	29	43	47	46	145	136
6	61	III	0	31	46	47	47	133	110
7	66	IV	I	23	39	45	45	171	133
8	61	IV	I	26	37	43	44	176	159
9	60	III	0	32	39	42	45	119	98
10	74	III	0	34	46	46	46	109	93
Mean ± SD	64.8 ± 3.9	3.5	0.7	29.4 ± 3.6	42.1 ± 3.5	45.5 ± 2.2	46 ± 1.9	143 ± 29	121 ± 26

OPCAB, Off-pump coronary artery bypass grafting; *SC*, stem cell; *NYHA*, New York Heart Association functional class; *EF*, ejection fraction; *LVEDV*, left ventricular end-diastolic volume; *SD*, standard deviation.

TABLE 4. Comparison of OPCAB-only versus OPCAB plus stem cell therapy group ejection fractions

	OPCAB	OPCAB + stem cell therapy
N	10	10
Mean EF (%)		
Preoperative	30.7 ± 2.5	29.4 ± 3.6
1 mo	36.4 ± 2.6	42.1 ± 3.5*
3 mo	36.5 ± 3.0	45.5 ± 2.2*
6 mo	37.2 ± 3.4	46.1 ± 1.9*

OPCAB, Off-pump coronary artery bypass grafting; *EF*, ejection fraction. *$P < .001$.

if no other, options for treatment. Patients with ischemic disease have the potential benefit of cellular therapy from both angiogenesis and myogenesis. Even though not all patients had clinical myocardial infarctions, all patients did have evidence of severely ischemic nonrevascularizable regions. The 10 patients in both groups had optimal medical therapy for their heart failure before they were referred for the study. This information aided in the selection process, showing which patients really have no other treatment options.

Our results demonstrate that all the patients in the OPCAB plus stem cell therapy group had significantly improved ejection fractions over those in the OPCAB-only group. There was, however, also a slight improvement in the OPCAB-only group when compared with baseline values. This might be due to the recruiting of hibernating myocardium as a result of coronary revascularization. The most dramatic ejection fraction improvement effect was within 1 month of therapy, and this improvement was maintained over the 6-month follow-up period. The patients improved both on imaging studies and clinically.

A factor that might be important and might play a role in stem cell function is the type of harvesting: direct bone marrow aspirate or peripheral after stimulation. We evaluated this concept before designing the trial. Peripheral bone marrow harvesting requires that preoperative granulocyte colony-stimulating factor (GCSF) be given to the patients for 4 days before harvesting. This methodology, however, might not be practical when planning surgical intervention because it requires multiple procedures for the patient at separate settings. Our rationale was based on performing a cellular therapy where the patient would have all procedures completed at one setting thus reducing the potential risk of complications from the GCSF therapy, the risk of cell contamination, the risk of multiple anesthesia, and the risk to the quality of the cells harvested making this procedure a safer therapy. Our volume of bone marrow harvested might appear high at 500 to 600 mL; however, because we do not stimulate with GCSF or culture ex vivo, this is the amount we found necessary to have adequate numbers of viable cells. Recently, Kang and colleagues[38] found that peripherally harvested stem cells stimulated with GCSF increased microinfarcts in the myocardium and did not find this to occur with their previous work with direct bone marrow. They believed that stimulated cells might be larger than $CD34^+$ cells directly filtered from pure bone marrow. Until further studies are performed to validate one technique over the other, both will continue to be used at the preference of the investigators.

Direct injection into the heart might have been a confounding factor as a result of the site of injury causing local inflammatory angiogenesis.[39] In this study 28 to 30 injections per patient were performed, and this was similar to our pilot study. Our prior safety and efficacy study, presented at the International Society for Heart and Lung Transplantation 2004, compared a group of 6 patients with OPCAB plus serum injection versus OPCAB plus stem cells. The patients with OPCAB plus stem cells had significant improvement in their ejection fractions over a 6-month period. On the basis of these findings, the ethics board recommended that we not continue with serum injections in the current study. Also, there is always the issue of stem cells with OPCAB. There are always going to be some overlapping regions of circulation. Therefore this must always be considered, but the control group reduces some of the confounding issues.

Another factor that might contribute to stem cell function is whether the patient was started on a cardiopulmonary bypass machine before or during the therapy. The inflammatory process associated with the heart-lung machine results in the release of a number of cytokines and other substances. This process has not been fully evaluated with stem cells, and therefore we decided not to introduce it into the trial. There might be no effect on stem cell function with a cardiopulmonary bypass machine. However, that is a question for another study.

The role of performing OPCAB might also have an effect on stem cell function and the clinical findings. Randomization was performed to decrease this confounding variable. In our other trial, we are currently enrolling patients with nonrevascularizable CHF and performing stem cell therapy with minimally invasive techniques to decrease the risks of surgical intervention in these very ill patients.

The imaging techniques used in this early study, echocardiography, multiplanar cardiac catheterization, and SPECT, are simple ways that most centers could attempt to replicate and validate our findings. In our more recent trials, we are using gated diffusion magnetic resonance imaging. Even though this imaging modality is very important in the evaluation of patients with heart failure, it is limited because it is very expensive and because of the fact that it cannot be used in patients with implantable antiarrhythmic devices.

Our small yet compelling prospective randomized study for the cellular treatment of CHF demonstrates early benefits. A number of questions as to the type of cells, method of harvesting and implantation, and further mechanisms of action will still need to be answered.[40-42] None of the previous studies have adequately addressed the differential commitment of stem cells to either cardiomyocytes or angiogenesis. This might in part be dependent on the reversibility of extracellular matrix changes in the myocardium caused by CHF. Future studies will need to be performed in which the pathology can be more closely examined. We are planning similar studies in patients who will be unloaded by left ventricular assist devices as a bridge to cardiac transplantation. Similar work has been performed with skeletal myoblasts by Pagani and colleagues,[43] who demonstrated viable skeletal myocytes that were injected into the scar. However, there was no electromechanical connection, as demonstrated by connexin 43 expression. The patient with a ventricular assist device is a great model for future cellular therapy trials. This will provide a unique perspective on the specific cellular transformations that occur in ischemic cardiomyopathy because the native heart will be excised before transplantation. This study will help form a foundation in surgical cellular therapy as we continue to expand our clinical trials.[37]

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Discussion

Dr Robert C. Robbins *(Stanford, Calif)*. So all the grafts were done to the anterior wall, and the injections were done to all other areas of the heart?

Dr Patel. Yes, sir.

Dr Robbins. And although you do mention in the article a potential limitation that you are using this in a model of reperfusion, how do you know that the improvement was not just from coronary myocardial revascularization?

Dr Patel. That was the reason for the standard group, which also received coronary bypass on the basis of the analysis of their SPECT and their echocardiogram to make sure that of those 37 patients screened, the 20 who were chosen to be in the study had very similar infarct areas. Therefore there are some confounding variables when you revascularize, and that is why we have started to do a minimally invasive technique in which there is no revascularization for patients who have idiopathic or similar to transmyocardial laser revascularization–type patients. Therefore there are some confounding variables when you do this.

Dr Robbins. There are no control groups. Somebody could stand up and argue that putting needles in the heart made them better because you did not have a control group, but I do not buy that.

The one thing that I will ask you to explain is that you show this beautiful connexin 43 expression from septal biopsy specimens that I am assuming were done from the jugular vein or an endomyocardial biopsy.

Dr Patel. It is from a left femoral artery retrograde.

Dr Robbins. But it is a right ventricular septal biopsy. How did the stem cells that you are injecting not in the septum cause this beautiful.

Dr Patel. There were injections into the septum, as I described in the article, but these were actually left side of the heart catheterizations. Therefore that is why I said the septum that is biopsied is from the left side of the heart, where the injections are.

Dr Robbins. So epicardially you injected cells into the septum. How was that done?

Dr Patel. With transesophageal echocardiography.

Dr Michael Mann *(San Francisco, Calif)*. I will try to condense the question to save time. I wonder whether you could say a few words about the preclinical animal model that you used as the basis for these studies because I did not catch that, and this leads into the real part of my question, which has to do with mechanism.

Nearly every one of the dozens if not hundreds of studies that have looked at this phenomenon have pretty much demonstrated that there is no functional coupling of whatever cells survive this transplantation process with the rest of the heart, and therefore I am wondering what your hypothesized mechanism is for this dramatic improvement on the basis of the possible survival of a small percentage of your cells, which, granted, have not been clearly characterized.

And also within the context of mechanism, clearly the patients who underwent the bone marrow process were not blinded, and as we have seen from the transmyocardial laser revascularization studies and other studies, there is a huge placebo effect that plays into myocardial remodeling after revascularization procedures. How do you factor that into your analysis?

Dr Patel. To answer your last question first, in our safety and efficacy study, which I presented this past week at the International Society for Heart and Lung Transplantation, these patients actually had not only the injections but also the bone marrow to serve as a control subject for the basis of this study.

In terms of mechanism, when you look at some of these other studies, we are not saying that these cells alone survive. There might be a mechanism of other recruitment of not only paracrine activity but actually a systemic response that might recruit cells, and those are further animal studies that are ongoing. Our initial animal model was a pig-based model that had left anterior descending coronary artery ligation over time and had injection of CD34-CD45 cells, which showed some improvement, and this led to further analysis and then clinical trials in South America.

Our next trial, which is going to be performed here in the United States, is actually looking at all of these theories, with a little more in-depth analysis in patients with ventricular assist devices so that we could harvest these hearts and get a more specific potential mechanism and characterize the exact type of cells that are left when these changes of just functional or clinical improvement occur.

Dr Thoralf Sundt *(Rochester, Minn)*. Are you saying that these patients were blinded to their treatment group?

Dr Patel. Yes.

Dr Sundt. Therefore the patients who did not have stem cells injected still had a bone marrow aspirate?

Dr Patel. They had an aspirate that was just frozen.

Dr Sundt. You said that these folks also had stents placed?

Dr Patel. Yes, sir.

Dr Sundt. And was it the same number of stents in everybody?

Dr Patel. It was the same number of stents before this intervention.

Dr Sundt. The third thing is, this is fundamentally a regional therapy, and yet the end point you are looking at is global, global ejection fraction and functional class and so on. Do you have any regional data? For example, do you have echocardiographic data region by region that shows improvement?

Dr Patel. We actually do have analysis of all 16 segments, which was a little long for both this article and the article that one of the cardiologists is going to be presenting soon. But they actually show, region by region on the basis of what your preoperative dysfunction is, improvement segment by segment.

Dr Sundt. Each segment or only the segments that you injected?

Dr Patel. The segments that are injected.

Dr John G. Byrne *(Boston, Mass)*. A follow-up on Thor's question. In stenting, not all stenting is created equal, and therefore obviously that is a huge confounding variable for global function. If someone got 3 stents to the circumflex system and none to the right, how do you control for that?

Dr Patel. These were all posterior stents, and that is why there was almost twice the number of patients screened for this study as opposed to actually enrolled in it. All these are confounding factors that we tried to control, which is a little harder to do when you are working with human subjects as opposed to a straight animal model, but all stents were delivered to a posterior circulation. That is why in the article you see there was one patient who received a left anterior descending coronary artery graft and a circumflex graft, but all posterior circulation was treated with drug-eluting stents.

Dr Robert C. Robbins. I am going to go back to this connexin 43 stuff. I am going to give you a pass on that and say that you did do that. Therefore are you telling us then that this increase in connexin 43 expression was from the stem cells magically turning into new cardiac myocytes?

Dr Patel. No, and I am glad you asked that. I was hoping you would. In all the connexin studies that have been shown before, all the persons who are doing myoblast therapy have failed to demonstrate any connexin; even though they show global improvement, there is no actual integration of these new muscles. Our theory of mechanism is not that these cells alone become muscle, but at the level, because these are ischemic patients, could actually cause angiogenesis recruiting muscle that we are not currently getting blood flow or oxygen to, along with the potential of muscle. Therefore everyone hopes that this is just a muscle answer.

We do not believe this is a muscle-only answer. We believe this is a combined effect, not only at a paracrine level but also there is some systemic mechanism of recruitment, and those are the cells and markers that we are currently looking at, at what else is going on regionally other than these stem cells. Just to believe that these stem cells alone turn magically into blood vessels or muscles would not be safe or even reasonable to expect as the only answer.

There is something else systemically happening in terms of recruitment of other factors, and we are looking at cytokine levels and other growth factors because we have shown in the animal model that something like insulin-like growth factor or transforming growth factor β have changes in the animal model in terms of the thickness of the muscle that we are seeing in these patients at the animal level.

Dr Carmelo A. Milano *(Durham, NC)*. There are a number of multicenter skeletal myoblast transplant protocols. Several of them have required automatic implantable cardioverter defibrillators to be present in the patients before the experiment because of the observation of ventricular arrhythmias, and you stated that that has not been seen. Do you have an understanding of why marrow stem cells might not be arrhythmogenic, whereas we have these concerns with skeletal myoblasts?

Dr Patel. We initially had similar concerns until we looked at a number of different trials and our own data of more than 47 patients now. The persons who are doing the largest myoblast studies who we have spoken to believe that it is not only the total number of cells but also the size of cells and the injections that are disrupting the membranes that are causing this arrhythmia potential.

If you look at all the autologous stem cell literature, the only trial that showed a significant amount of arrhythmia created was by

Dr Stamm in Germany, where patients were receiving both revascularization on pump and stem cells at the same time; there were 5 of his patients who ended up with significant arrhythmias. We believe that being on the pump actually had some contributing factor to this inflammatory response, not only to the disruption of the systemic response but also actually at the cellular level, and that was another reason why we decided not to use any perfusion or a heart-lung machine during the stem cell implantation to cut down the inflammatory response. To summarize, there would be both a mechanical disruption, the number of cells, size of cells, and the inflammatory response.

We are still very lucky and fortunate that we have not seen these arrhythmias, but we have 5 other centers working with us who have not seen this phenomenon either. There is no specific answer that we could tell you yet as to why they are not having this. But in our OPCAB data alone—forget about stem cells—our arrhythmia rate of just postoperative atrial fibrillation is less than 5% on the basis of what the anesthesiologists give the patients of magnesium infusions.

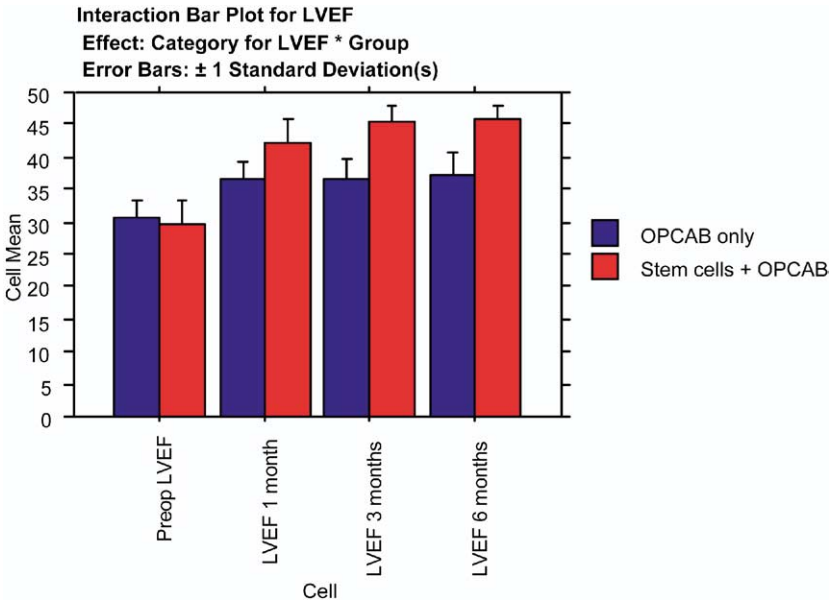

Figure E1. Analysis of variance *(ANOVA)* for ejection fraction *(EF)* over time. *LVEF*, Left ventricular ejection fraction; *OPCAB*, off-pump coronary artery bypass grafting.

ET

TABLE E1. Paired *t* test for NYHA functional class for OPCAB-only group

t Test: paired 2-sample test for means		
	Variable 1	**Variable 2**
Mean	3.4	2.7
Variance	0.266667	0.455556
Observations	10	10
Pearson correlation	0.382546	
Hypothesized mean difference	0	
df	9	
t Stat	3.279649	
P value (T ≤t) 1-tailed	.004767	
t Critical 1-tailed	1.833113	
P value (T ≤t) 2-tailed	.009535	
t Critical 2-tailed	2.262157	

NYHA, New York Heart Association; *OPCAB*, off-pump coronary artery bypass grafting.

TABLE E2. Paired *t* test for NYHA functional class for OPCAB plus stem cell therapy group

t Test: Paired 2-sample for means		
	Variable 1	**Variable 2**
Mean	3.5	0.7
Variance	0.277778	0.677778
Observations	10	10
Pearson correlation	0.896258	
Hypothesized mean difference	0	
df	9	
t Stat	21	
P value (T ≤t) 1-tailed	2.95E-09	
t Critical 1-tailed	1.833113	
P value (T ≤t) 2-tailed	5.9E-09	
t Critical 2-tailed	2.262157	

NYHA, New York Heart Association; *OPCAB*, off-pump coronary artery bypass grafting.

TABLE E3. Paired *t* test for EDV for OPCAB-only group

t Test: Paired 2-sample for means		
	Variable 1	**Variable 2**
Mean	143.6	138.9
Variance	531.8222	478.1
Observations	10	10
Pearson correlation	0.988186	
Hypothesized mean difference	0	
df	9	
t Stat	4.068624	
P value (T ≤t) 1-tailed	.001403	
t Critical 1-tailed	1.833114	
P value (T ≤t) 2-tailed	.002805	
t Critical 2-tailed	2.262159	

EDV, End-diastolic volume; *OPCAB*, off-pump coronary artery bypass grafting.

TABLE E4. Paired *t* test for EDV for OPCAB plus stem cell therapy group

t Test: paired 2-sample test for means		
	Variable 1	**Variable 2**
Mean	143.1	121.4
Variance	869.2111	650.9333
Observations	10	10
Pearson correlation	0.961422	
Hypothesized mean difference	0	
df	9	
t Stat	7.988482	
P value (T ≤t) 1-tailed	1.12E-05	
t Critical 1-tailed	1.833114	
P value (T ≤t) 2-tailed	2.24E-05	
t Critical 2-tailed	2.262159	

EDV, End-diastolic volume; *OPCAB*, off-pump coronary artery bypass grafting.