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Comparison of Allogeneic vs Autologous Bone Marrow–Derived Mesenchymal Stem Cells Delivered by Transendocardial Injection in Patients With Ischemic Cardiomyopathy

The POSEIDON Randomized Trial

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RECENT CLINICAL TRIALS SUGGEST that bone marrow–derived cell preparations, including mononuclear cells^{1–5} and mesenchymal stem cells (MSCs),^{6–8} can ameliorate left ventricular (LV) re-

Context Mesenchymal stem cells (MSCs) are under evaluation as a therapy for ischemic cardiomyopathy (ICM). Both autologous and allogeneic MSC therapies are possible; however, their safety and efficacy have not been compared.

Objective To test whether allogeneic MSCs are as safe and effective as autologous MSCs in patients with left ventricular (LV) dysfunction due to ICM.

Design, Setting, and Patients A phase 1/2 randomized comparison (POSEIDON study) in a US tertiary-care referral hospital of allogeneic and autologous MSCs in 30 patients with LV dysfunction due to ICM between April 2, 2010, and September 14, 2011, with 13-month follow-up.

Intervention Twenty million, 100 million, or 200 million cells (5 patients in each cell type per dose level) were delivered by transendocardial stem cell injection into 10 LV sites.

Main Outcome Measures Thirty-day postcatheterization incidence of pre-defined treatment-emergent serious adverse events (SAEs). Efficacy assessments included 6-minute walk test, exercise peak $\dot{V}O_2$, Minnesota Living with Heart Failure Questionnaire (MLHFQ), New York Heart Association class, LV volumes, ejection fraction (EF), early enhancement defect (EED; infarct size), and sphericity index.

Results Within 30 days, 1 patient in each group (treatment-emergent SAE rate, 6.7%) was hospitalized for heart failure, less than the prespecified stopping event rate of 25%. The 1-year incidence of SAEs was 33.3% (n=5) in the allogeneic group and 53.3% (n=8) in the autologous group (P=.46). At 1 year, there were no ventricular arrhythmia SAEs observed among allogeneic recipients compared with 4 patients (26.7%) in the autologous group (P=.10). Relative to baseline, autologous but not allogeneic MSC therapy was associated with an improvement in the 6-minute walk test and the MLHFQ score, but neither improved exercise $\dot{V}O_2$ max. Allogeneic and autologous MSCs reduced mean EED by –33.21% (95% CI, –43.61% to –22.81%; P<.001) and sphericity index but did not increase EF. Allogeneic MSCs reduced LV end-diastolic volumes. Low-dose concentration MSCs (20 million cells) produced greatest reductions in LV volumes and increased EF. Allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions.

Conclusions In this early-stage study of patients with ICM, transendocardial injection of allogeneic and autologous MSCs without a placebo control were both associated with low rates of treatment-emergent SAEs, including immunologic reactions. In aggregate, MSC injection favorably affected patient functional capacity, quality of life, and ventricular remodeling.

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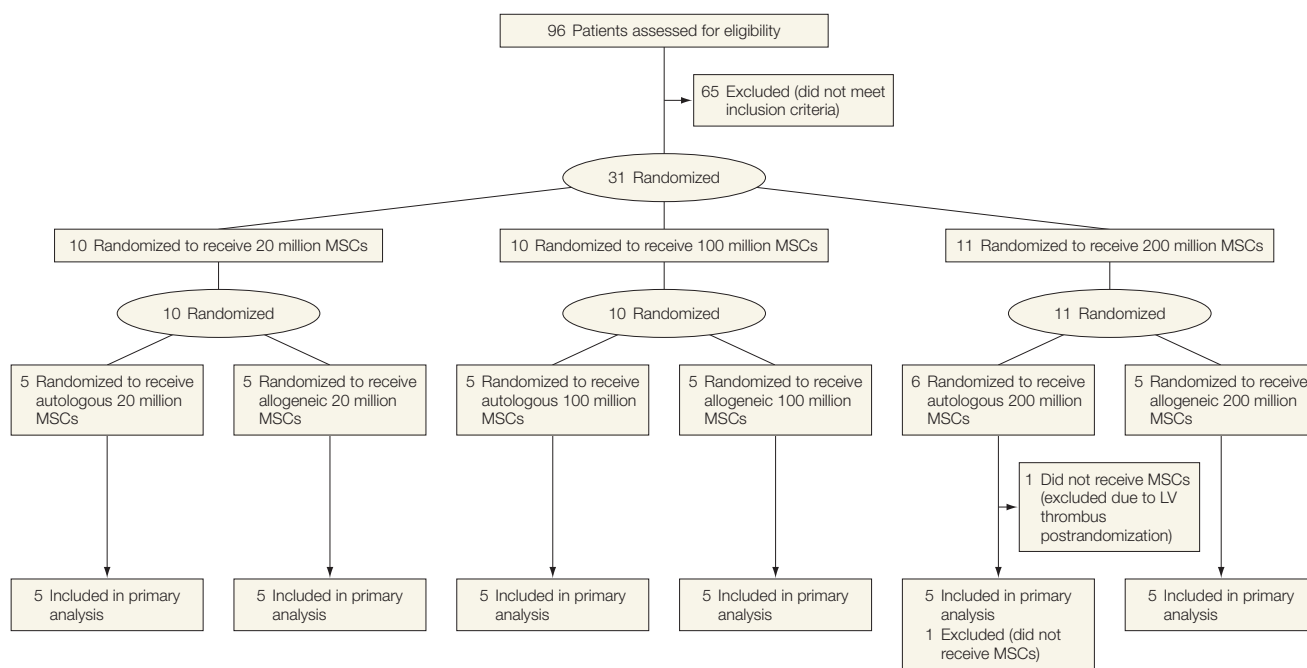
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modeling in patients with acute^{1,3,8} and chronic^{2,4,6,9} ischemic cardiomyopathy (ICM). An important issue in this new field is whether a certain cellular

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Figure 1. Study Flow Diagram

MSCs indicates mesenchymal stem cells; LV, left ventricular.

constituent of bone marrow—the MSCs—can be used as an allograft. The immunoprivileged and immunosuppressive properties¹⁰ of MSCs result from the absence of major histocompatibility class II antigens¹¹ and the secretion of T helper type 2 cytokines,¹² respectively. Recent experimental findings, however, have called into question the long-term persistence of MSCs injected into myocardium, suggesting that allogeneic MSCs may be cleared to a greater extent than autologous cell preparations,¹³ possibly via formation of alloreactive antibodies.

One potential advantage of allogeneic cells is the possibility of their use as an “off-the-shelf” therapeutic agent, avoiding the need for bone marrow aspiration and tissue culture delays before treatment. It is also hypothesized that the function of autologous MSCs could be impaired in patients with comorbidities or advanced age.^{14,15} We performed a randomized dose-finding comparison study of allogeneic vs autologous MSCs delivered by transen-

docardial stem cell injection (TESI) in patients with ICM.

METHODS

Study Design and Enrollment

The POSEIDON study protocol entitled “A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction” was approved under the US Food and Drug Administration Investigational New Drug 13568. The primary goal was to demonstrate the safety of allogeneic MSCs administered by TESI in patients with chronic LV dysfunction secondary to myocardial infarction (MI). The secondary goals were (1) to compare the long-term safety of allogeneic MSCs with autologous MSCs and (2) to demonstrate the long-term efficacy of allogeneic MSCs with autologous

MSCs administered by TESI in these patients.

Patient enrollment took place at the University of Miami Miller School of Medicine, Miami, Florida, and The Johns Hopkins University School of Medicine, Baltimore, Maryland, between April 2, 2010, and September 14, 2011. All patients provided written informed consent on the University of Miami Institutional Review Board–approved protocol. The National, Heart, Lung, and Blood Institute Gene and Cell Therapy Data and Safety Monitoring Board was responsible for safety oversight and recommended study continuation after each review period.

Randomization occurred centrally using an electronic data entry system. The dose of cells in each group was serially escalated from 20 million to 100 million to 200 million cells. Cells were delivered to the myocardium by TESI during retrograde left heart catheterization using the Biocardia Helical Infusion Catheter.^{16,17} Thirty-one patients were randomized to either allo-

geneic or autologous MSCs in a 1:1 ratio using random permuted blocks to each of the 3 increasing dose levels of 10 patients each (FIGURE 1).

Patient Population

Eligible patients had chronic ischemic LV dysfunction secondary to MI as documented by confirmed coronary artery disease with a corresponding area of myocardial akinesis, dyskinesis, or severe hypokinesis. Additional criteria included age 21 to 90 years, LV ejection fraction (EF) of less than 50% within 6 months before screening and not during or recently after an ischemic event, and eligibility for cardiac catheterization within 5 to 10 weeks of screening as determined by the study team. Patients were excluded for a non-cardiac condition limiting life expectancy to less than 1 year, glomerular filtration rate of less than 50 mL/min/1.73 m², serious radiographic contrast allergy, clinical requirement for coronary revascularization, a life-threatening arrhythmia in the absence of an implanted defibrillator, or a malignancy within 5 years of screening.

Study Procedures and Timeline

Baseline studies included chemistry and hematology laboratories, echocardiography, and computed tomographic (CT) scans of the heart, chest, abdomen, and pelvis. Patients randomized to the autologous MSC group underwent bone marrow aspiration from the iliac crest 4 to 6 weeks before cardiac catheterization; autologous MSCs were prepared in culture from the marrow aspirate.⁶ Allogeneic MSCs were derived from bone marrow aspirates of healthy donors.

Mesenchymal stem cells were delivered to 10 sites in an infarcted myocardial territory as previously described.^{16,17} Following TESI, patients were hospitalized for a minimum of 4 days and were observed 2 weeks after catheterization, monthly thereafter for 6 months for all safety and efficacy assessments, at 12 months for selected efficacy and safety assessments, and at 13 months for follow-up CT scans of the heart, chest, ab-

domen, and pelvis. One patient did not undergo the 13-month CT scan because of renal dysfunction and 2 patients were not included in the 13-month CT scan analysis because of intervening heart transplantation (n=1) or LV assist device implantation (n=1).

Study End Points

The primary end point was the incidence, within 1 month after TESI, of any treatment-emergent serious adverse events (SAEs), defined as the composite of death, nonfatal MI, stroke, hospitalization for worsening heart failure, cardiac perforation, pericardial tamponade, or sustained ventricular arrhythmias (>15 seconds or causing hemodynamic compromise). Additional safety assessments included monitoring for adverse events (AEs), SAEs, and major adverse cardiac events (MACEs); serial troponin and creatine kinase MB; CT scans of the chest, abdomen, and pelvis to identify ectopic tissue formation; and 48-hour ambulatory electrocardiography, hematology, chemistry, urinalysis, spirometry, and serial echocardiography.

In addition to cardiac imaging studies, efficacy assessments included exercise peak oxygen consumption per unit time ($\dot{V}O_2$), a 6-minute walk test, New York Heart Association (NYHA) class (class I, no limitation; class II, slight limitation of physical activity; class III, marked limitation of physical activity; and class IV, unable to perform any physical activity without symptoms), and the Minnesota Living with Heart Failure Questionnaire (MLHFQ).¹⁸⁻²⁰ The 21-item MLHFQ²⁰ is an instrument used to assess the effect of heart failure on a patient's quality of life. The response scale of each item ranges from 0 (none) to 5 (very much). The total score is the sum of the responses to the 21 items, ranging from 0 (for no effect) to 105 (for strong effect of heart failure on daily living). This instrument has been validated as effective and efficient.¹⁹ A score increase of more than 10% between consecutive assessments has been shown to identify high-risk patients within the next 12 months.¹⁸

Cardiac CT Scanning

Contrast-enhanced CT scan was performed at screening and at 13-month follow-up using 128-slice (Siemens AS+, Siemens Medical Solutions) or 356-slice (Toshiba) scanners. Cardiac CT scans provided global LV function and volumes (iNtuition software version 4.4.7.47; TeraRecon Inc), sphericity index,²¹⁻²³ and early enhancement defect (EED) of the myocardium, as described previously^{24,25} and in the eMethods (available at <http://www.jama.com>). Early enhancement defect is a correlate of myocardial blood flow and is imaged immediately after injection. We used EED as a measure of myocardial infarct scar size; in patients with chronic infarction and without signs of myocardial ischemia, the EED closely approximates scar. Left ventricular sphericity index was calculated by $LDEDV/(\pi LVLd^3/6)$, according to Lamas et al.²³

Immunologic Monitoring

Serum samples were collected preinfusion and at 24 hours, and 1 and 6 months postinfusion. Participant anti-HLA allogeneic-antibody sensitization was assessed as described previously²⁶ and in the eMethods.

Statistical Analysis

The study was designed to estimate the confidence interval of treatment-emergent SAE at 30-day post-TESI in both the allogeneic and autologous MSC groups. The underlying rate of 30-day treatment-emergent SAE was assumed to be 25% across all 3-dose levels. In this setting, the exact binomial 95% CI is 0.04 to 0.48. Rates of treatment-emergent SAEs, AEs, and SAEs were compared using Fisher exact test at 30 days and 12 months. For continuous measures, normality of data was tested using the Shapiro-Wilk test, and differences between groups used *t* test or nonparametric tests, as appropriate. Normally distributed efficacy parameters were evaluated with a repeated measures analysis of variance model using the entire data set, including between-group comparisons as well as time and group $\times$ time interaction

terms. Bonferroni correction was applied post hoc. A 2-sided $P < .05$ was considered statistically significant. Analy-

ses were conducted using SAS version 9.2 (SAS Institute Inc) and conformed to the prespecified goals of the trial. Addi-

tional methods are shown in the eMethods.

RESULTS

Patient Population

The study population was predominantly male and white race (TABLE 1). Most patients had mild to moderate (NYHA classes II and III) heart failure symptoms and impaired 6-minute walk test and MLHFQ scores. Thirty-one patients were randomized to either allogeneic MSCs or autologous MSCs and to each of the 3 increasing dose levels of 10 patients each (Figure 1). One enrolled patient in the third dose level (200 million MSCs) was excluded according to a protocol-defined contingency (heart failure exacerbation with development of left intraventricular thrombus, which precluded catheterization for cell delivery). As a result, 30 patients received the study injection (5 patients in each cell type per dose level combination).

Safety

In 1 patient, contamination of cell culture required repeat bone marrow aspiration and expansion of autologous MSCs. In all patients, TESI was technically successful.

The primary end point of the study was the 30-day event rate of predefined treatment-emergent SAEs. One patient in each group had a treatment-emergent SAE (hospitalization for heart failure) within 30 days (TABLE 2). This event rate did not approach the prespecified stopping guidelines. There were 6 AEs (0.4 per patient) in the allogeneic group vs 17 AEs (1.13 per patient) in the autologous group. Three patients (20%) in the allogeneic group and 9 (60%) in the autologous group experienced AEs (Fisher exact test, $P = .06$). Reactions suggestive of an acute immunogenic reaction, such as fever, urticaria, hemolysis, hypotension, liver dysfunction, and/or thrombocytopenia, did not occur in any patient. Accordingly, the study met its primary safety event end point documenting the safety of TESI using allogeneic MSCs in patients with ICM.

Prespecified procedural stopping criteria included hemodynamic compro-

Table 1. Patient Characteristics by Type of Mesenchymal Stem Cell Injection^a

Characteristics	Mesenchymal Stem Cell Type	
	Allogeneic (n = 15)	Autologous (n = 15)
Age, mean (SD), y	62.8 (10.5)	63.7 (9.3)
Male sex	13 (86.7)	13 (86.7)
Hispanic or Latino ethnicity	3 (20.0)	3 (20.0)
White race	13 (86.7)	15 (100.0)
Ejection fraction assessed by CT scan, mean (SD), %	27.1 (9.6)	29.0 (8.8)
History of coronary revascularization	13 (86.7)	13 (86.7)
History of atrial or ventricular arrhythmia	13 (86.7)	11 (73.3)
History of hypertension	13 (86.7)	13 (86.7)
New York Heart Association class		
I	2 (13.3)	2 (13.3)
II	9 (60.0)	9 (60.0)
III	4 (26.7)	4 (26.7)
Device		
AICD	11 (73.3)	9 (60.0)
Biventricular pacemaker	3 (20.0)	6 (40.0)
No device	1 (6.7)	0
History of congestive heart failure	8 (53.3)	12 (80.0)
Time since last MI, median (range), y	9.0 (0.2-27.1)	12.8 (2.4-31.8)
Infarct location		
Anterior, inferior, lateral	5 (33.3)	9 (60.0)
Anterior, inferior	5 (33.3)	4 (26.7)
Anterior	3 (20.0)	1 (6.7)
Anterior, lateral	0	1 (6.7)
Inferior	1 (6.7)	0
Inferior, lateral	1 (6.7)	0
History of valvular heart disease	4 (26.7)	3 (20.0)
History of smoking	7 (46.7)	12 (80.0)
Current smoker	0	2 (13.3)
History of diabetes	4 (26.7)	4 (26.7)
Peak $\dot{V}O_2$, mean (SD), mL/kg/min	16.2 (4.6)	17.2 (5.1)
6-Minute walk test, mean (SD), m	391.2 (85.9)	369.2 (103.7)
% Predicted FEV ₁ , mean (SD)	84.4 (18.9)	78.6 (17.6)
MLHFQ, mean (SD)	38.9 (31.7)	43.6 (31.0)
Cardiac CT parameters, mean (SD)		
LV ejection fraction, %	27.7 (9.3)	26.3 (9.3)
End-diastolic volume, mL	268.7 (88.1)	294.6 (79.5)
End-systolic volume, mL	198.5 (81.2)	220.6 (75.3)
Stroke volume, mL	70.2 (24.0)	74.0 (24.1)
End-diastolic myocardial volume, mL	201.0 (49.5)	218.9 (53.9)
End-diastolic myocardial mass, g	210.9 (51.5)	233.8 (58.6)
End-diastolic diameter, mm	64.7 (6.9)	69.3 (6.6)
End-systolic diameter, mm	55.8 (7.8)	58.3 (11.0)
Sphericity index	0.47 (0.10)	0.51 (0.11)
MI size (early enhancement defect), g	19.6 (9.2)	23.1 (15.5)
Scar as % of LV mass, %	9.3 (3.8)	10.1 (5.9)

Abbreviations: AICD, automatic implantable cardioverter-defibrillator; CT, computed tomography; FEV₁, forced expiratory volume in first second of expiration; LV, left ventricular; MI, myocardial infarction; MLHFQ, Minnesota Living with Heart Failure Questionnaire; $\dot{V}O_2$, oxygen consumption per unit time.

^aData are presented as No. (%) unless otherwise specified. New York Heart Association class I indicates no limitation; class II, slight limitation of physical activity; and class III, marked limitation of physical activity. Current smoker indicates patient actively smoking at time of enrollment.

mise, ischemia, or ventricular arrhythmias; these did not occur in any patients. No patients had a significant pericardial effusion postprocedure.

Creatine kinase MB and serum troponin I were monitored every 12 hours for 2 days postinjection (Table 2). Myocardial biomarkers increased, peaking within 12 hours and returning to baseline in all patients by 36 hours (mean creatine kinase MB from 1.5 ng/mL preinjection to 3.9 ng/mL, upper limit of normal 0.0-6.0 ng/mL; mean troponin I from 0.1 ng/mL preinjection to 0.9 ng/mL, upper limit of normal 0.04-0.07 ng/mL).

Long-term AEs

Over the duration of the study, there were no deaths. One patient who received 100 million allogeneic MSCs underwent orthotopic heart transplantation and 1 patient who received 20 million autologous MSCs had LV assist device implantation. Over 12 months, there were 24 AEs in 10 patients (66.7%) treated with allogeneic MSCs and 38 AEs in 11 patients (73.3%) treated with autologous MSCs ($P > .99$) (eTable 1). No differences were observed in the 1-year incidence of heart failure or MACE. The 1-year incidence of at least 1 SAE was found in 5 patients (33.3%) in the allogeneic MSC group and in 8 patients (53.3%) in the autologous MSC group ($P = .46$).

Arrhythmias

At 12 months, no patients (allogeneic MSC group) and 2 patients (autologous MSC group) experienced ventricular tachycardia lasting longer than 15 beats ($P = .48$). During the study duration, 1 patient who was treated with allogeneic MSCs experienced a ventricular tachycardia AE compared with 5 patients (33.3%) in the autologous MSC group ($P = .08$). No ventricular arrhythmia SAEs were observed among allogeneic MSC recipients compared with 4 patients (26.7%) in the autologous group ($P = .10$) (eTable 1).

Rehospitalization and MACEs

Rehospitalization during 12 months occurred in 5 patients (33.3%) in the

allogeneic MSC group and 6 patients (40.0%) in the autologous MSC group ($P > .99$), at a median 70 days and 69 days, respectively ($P = .54$). Rehospi-

talization for worsening heart failure occurred in 2 patients (13.3%) in the allogeneic group and 4 patients (26.7%) in the autologous group

Table 2. Safety Summary by Cell Type Injection Within 30 Days of Transendocardial Stem Cell Injection^a

	Mesenchymal Stem Cell Type	
	Allogeneic (n = 15)	Autologous (n = 15)
Total No. of AEs	6	17
No. of AEs per patient, mean (median [range])	0.40 (0 [0-3])	1.13 (1 [0-4])
Total No. of SAEs	1	3
No. of SAEs per patient, mean (median [range])	0.07 (0 [0-1])	0.20 (0 [0-2])
Primary safety end point	1 (6.7)	1 (6.7)
Patients with ≥ 1 AEs	3 (20.0)	9 (60.0)
Cardiac disorders	1 (6.7)	5 (33.3)
Atrial fibrillation	1 (6.7)	0
Heart failure	0	1 (6.7)
Palpitations	0	1 (6.7)
Ventricular tachycardia	0	3 (20.0)
Gastrointestinal disorders	0	1 (6.7)
General disorders and administration site conditions	0	2 (13.3)
Infections and infestations	1 (6.7)	1 (6.7)
Metabolism and nutrition disorders	2 (13.3)	0
Musculoskeletal and connective tissue disorders	0	1 (6.7)
Nervous system disorders	0	3 (20.0)
Renal and urinary disorders	0	1 (6.7)
Respiratory, thoracic, and mediastinal disorders	1 (6.7)	0
Skin and subcutaneous tissue disorders	0	1 (6.7)
Vascular disorders	1 (6.7)	1 (6.7)
Patients with ≥ 1 SAEs	1 (6.7)	3 (20.0)
Cardiac disorders	0	1 (6.7)
Heart failure	0	1 (6.7)
Nervous system disorders	0	1 (6.7)
Renal and urinary disorders	0	1 (6.7)
Respiratory, thoracic, and mediastinal disorders	1 (6.7)	0
Major adverse cardiac event	1 (6.7)	1 (6.7)
Deaths	0	0
Ectopic tissue formation	0	0
Creatine kinase MB, mean (95% CI), ng/mL		
Baseline	1.3 (1.1-1.6)	1.7 (1.2-2.2)
12 h	3.0 (2.2-3.7)	4.8 (3.0-6.5)
24 h	1.6 (1.2-2.1)	2.3 (1.6-3.0)
36 h	1.2 (0.9-1.5)	1.7 (1.1-2.2)
48 h	1.1 (0.8-1.4)	1.2 (0.8-1.7)
Serum troponin I, mean (95% CI), ng/mL		
Baseline	0.0 (0.0-0.1)	0.1 (0.0-0.1)
12 h	0.8 (0.5-1.1)	0.9 (0.4-1.5)
24 h	0.4 (0.2-0.6)	0.7 (0.2-1.2)
36 h	0.3 (0.1-0.5)	0.4 (0.1-0.6)
48 h	0.2 (0.1-0.4)	0.3 (0.1-0.5)

Abbreviations: AEs, adverse events; SAEs, serious adverse events.

^aData are presented as No. (%) unless otherwise specified. Major adverse cardiac event is defined as the composite incidence of death, hospitalization for worsening heart failure, or nonfatal recurrent myocardial infarction. SAEs and AEs are categorized according to MedDRA by System Organ Class. Cardiac disorders are further classified according to the MedDRA preferred term. No statistically significant difference was noted for any parameter.

($P = .65$) during 12 months. One patient who received 200 million allogeneic MSCs had an MI and was hospitalized on day 339 following TESI. The 12-month incidence of MACEs occurred in 3 patients (20.0%) in the allogeneic group and 4 patients (26.7%) in the autologous group ($P > .99$).

Ectopic Tissue Formation

All patients underwent 13-month CT scans of chest, abdomen, and pelvis; no ectopic tissue formation was detected.

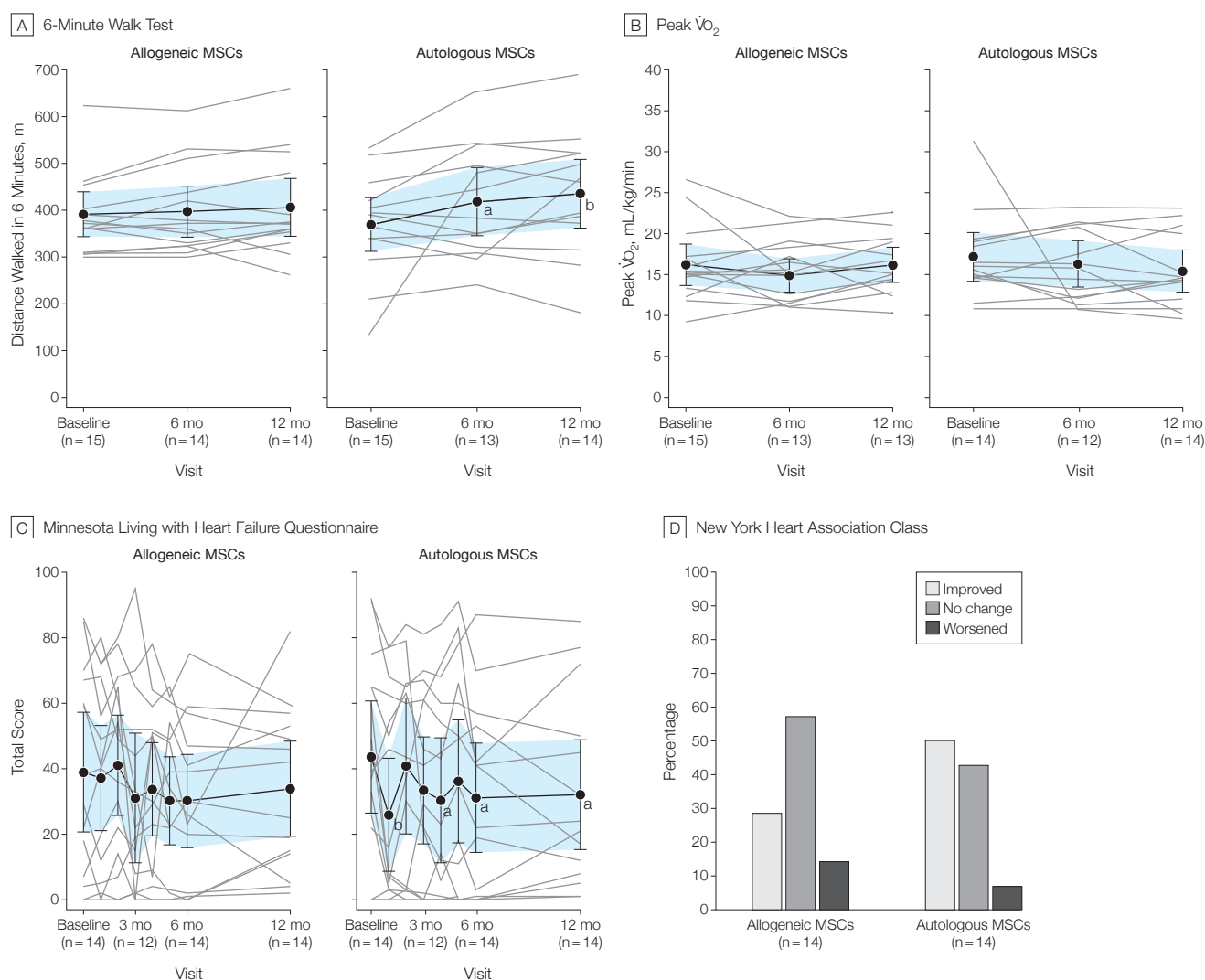
Functional Status, Quality of Life, and Pulmonary Function

Patient functional status and quality of life were monitored serially using

6-minute walk test, peak $\dot{V}O_2$, MLHFQ, and NYHA class. Several of these parameters suggested clinical improvement; the effects were similar between the allogeneic and autologous groups.

The changes in the 6-minute walk test, MLHFQ, and NYHA classification all suggested functional improvement after TESI (FIGURE 2). The dis-

Figure 2. Functional Outcomes of the Effect of Allogeneic and Autologous Mesenchymal Stem Cell (MSC) Transendocardial Stem Cell Injection on Patient Functional Capacity and Quality of Life



A, Autologous but not allogeneic MSC therapy was associated with an improvement in the 6-minute walk test. When considering both groups combined, patients exhibited increased 6-minute walk test distance in both groups ($P = .003$ for analysis of variance with repeated measures; $P = .004$ vs baseline for all patients; and $P = .21$ for cell type $\times$ time interaction). B, Patients exhibited no change in peak exercise oxygen consumption per unit time ($\dot{V}O_2$ max). C, Minnesota Living with Heart Failure Questionnaire (MLHFQ) score improved in the autologous but not the allogeneic group. When considering both groups combined, MLHFQ scores improved ($P < .001$ for analysis of variance with repeated measures; and $P = .54$ for cell type $\times$ time interaction). D, Change in New York Heart Association classification from 12 months to baseline is shown as the proportion of patients who either improved, did not change, or worsened. Individual patient values for the 6-minute walk test, peak $\dot{V}O_2$, and total score of the MLHFQ are shown across time as gray lines with the corresponding cell type mean and 95% CIs indicated by bars and shaded across time. Within-group P values are noted as ^a $P < .05$ and ^b $P < .01$.

tance walked in 6 minutes significantly increased in the autologous group by 48.0 m (95% CI, 8.3-87.7 m) at 6 months ($P=.02$) and 65.8 m (95% CI, 27.2-104.5 m) at 12 months ($P=.001$). However, in the allogeneic group, the increase of 10.7 m (95% CI, -28.0 to 49.3 m) at 6 months and 19.7 m (95% CI, -19.0 to 58.3 m) at 12 months was not significant ($P=.58$ and $P=.31$, respectively). When combined, both groups increased the 6-minute walk test at 6 and 12 months ($P=.009$) and did not differ between the 2 cell types ($P=.87$). Mean increase in distance walked at 6 months was 31.0 m (95% CI, 0.8-61.2 m; $P=.04$) and at 12 months was 43.5 m (95% CI, 9.1-77.0 m; $P=.003$). Neither peak $\dot{V}O_2$ nor forced expiratory volume in the first second of expiration (FEV_1) exhibited changes. At 12 months among both groups, the mean change from baseline in peak $\dot{V}O_2$ was -0.6 mL/kg/min (95% CI, -2.7 to 1.4 mL/kg/min; $P=.39$) and pulmonary function (FEV_1 ; % predicted) was -3.1 (95% CI, -7.8 to 1.7; $P=.33$).

The mean (SD) MLHFQ score at baseline was 43.6 (8.0) in the autologous group and 38.9 (8.5) in the allogeneic group. In the autologous group, MLHFQ score significantly decreased at 6 months (-14.0; 95% CI, -23.6 to -4.3; $P=.005$) and at 12 months (-13.0; 95% CI, -22.6 to -3.3; $P=.009$). The reductions in the allogeneic group were not statistically significant at 6 months (-11.2; 95% CI, -32.1 to 9.7; $P=.29$) or at 12 months (-10.2; 95% CI, -31.1 to 10.7; $P=.34$). When both groups were combined, in a repeated measures model, significant improvements in the MLHFQ score occurred at all assessment periods relative to baseline ($P=.009$), but there were no statistically significant differences between the cell types ($P=.84$). Among both groups, mean reduction in MLHFQ score at 6 months was 10.1 (95% CI, -18.1 to -2.1; $P=.004$) and at 12 months was 7.6 (95% CI, -15.2 to 0.0; $P=.02$).

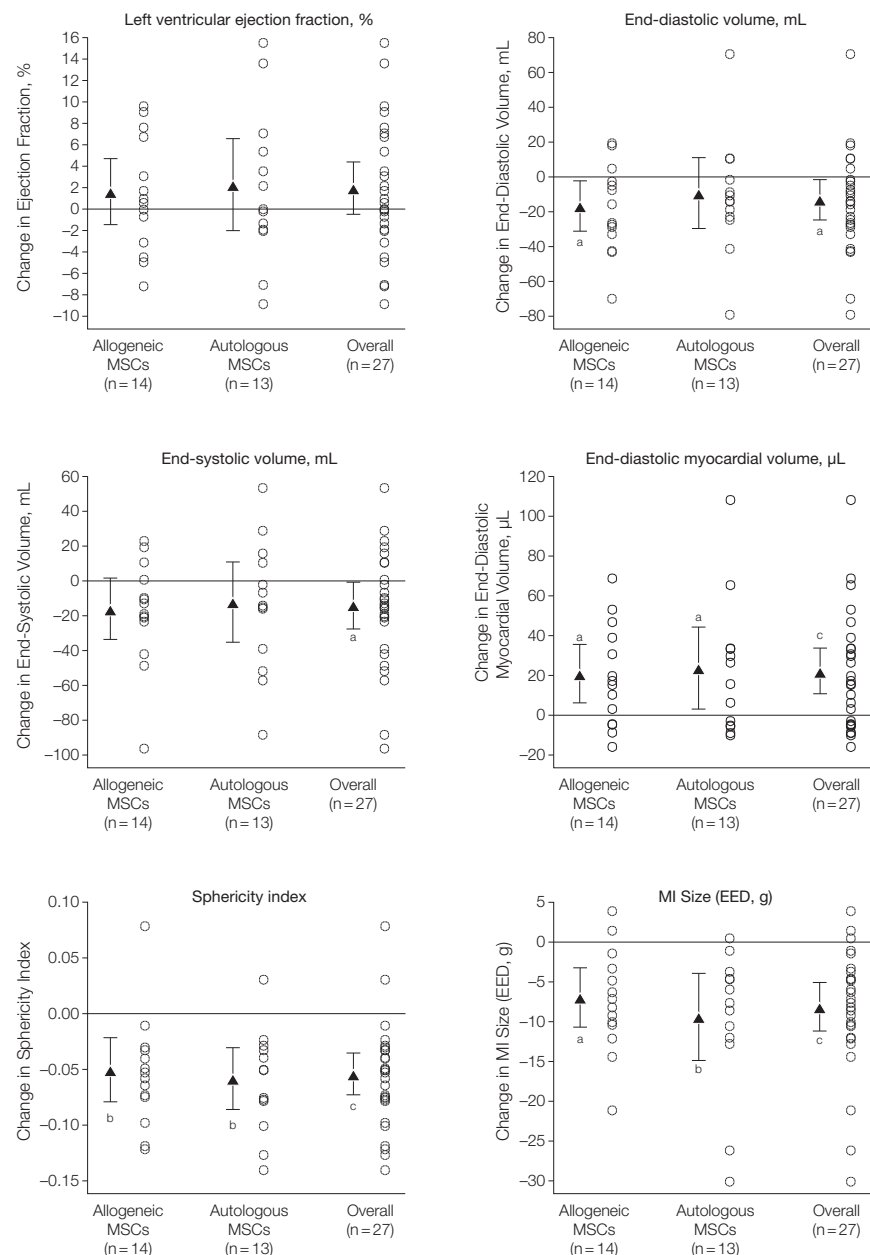
The NYHA class improved (allogeneic, 28.6%; autologous, 50.0%) or did not change (allogeneic, 57.1%; autolo-

gous 42.9%) at 12 months. Two patients in the allogeneic MSC group and 1 patient in the autologous MSC group had worsened NYHA class at 12 months ($P=.55$).

Cardiac Remodeling

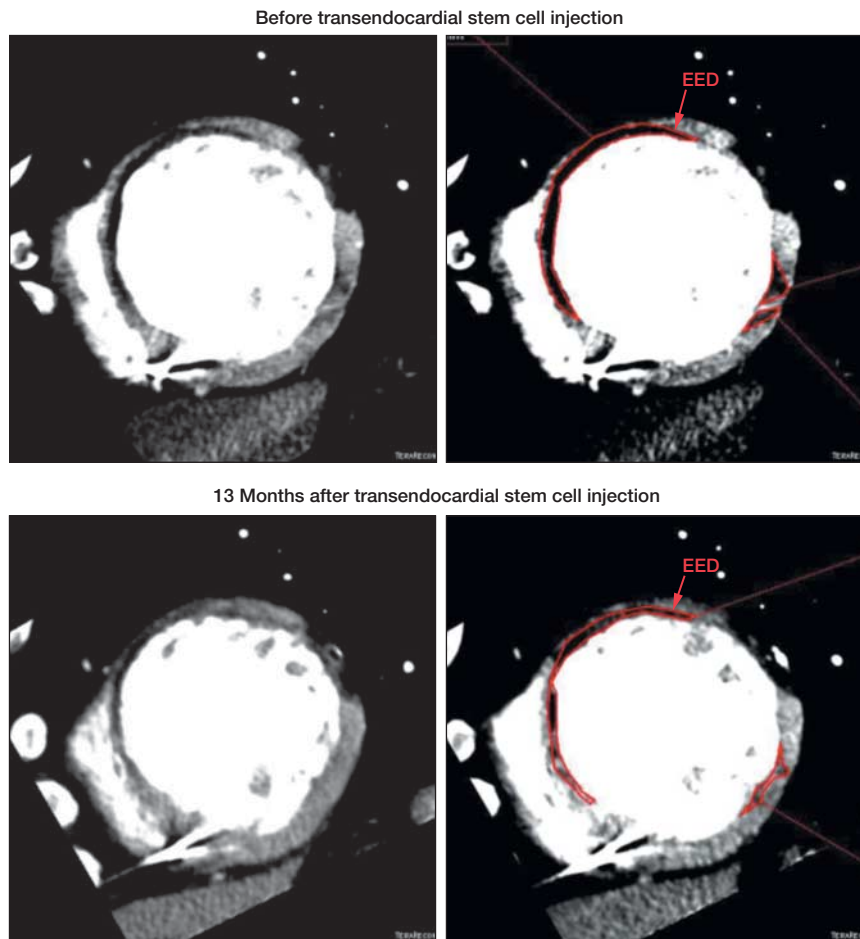
Patients receiving MSCs experienced reverse LV remodeling as assessed by CT scan^{27,28} (Table 1, FIGURE 3, and eTable 2), primarily by a dramatic reduction

Figure 3. Computed Tomography (CT) Parameters Change From Baseline



MSCs indicates mesenchymal stem cells; EED, early enhancement effect. Mean changes from baseline to 13 months are noted by triangles and depict change in cardiac phenotype assessed by cardiac CT scan. Error bars indicate 95% CIs. Individual patient changes from baseline are shown as circles. Shown are changes in cardiac structural and functional parameters from baseline to 13-month follow-up in allogeneic, autologous, and combined patient groups. Within-group P values are noted as ^a $P<.05$, ^b $P<.01$, and ^c $P<.001$.

Figure 4. Improvement in Early Enhancement Defect (EED) After Transendocardial Allogeneic Stem Cell Injection



Upper panel, baseline early-phase short-axis multidetector computed tomography images in a representative patient with chronic myocardial infarction (EED total, 40.34 g). Lower panel, 13 months after transendocardial stem cell injection of allogeneic mesenchymal stem cells (100 million cells). There was a decrease of 34.68% in EED (26.35 g). The reduction of the myocardial defects was accompanied by decrease of end-diastolic volume from 433.68 mL to 365.81 mL, decrease of end-systolic volume from 371.83 mL to 277.38 mL, increase of ejection fraction from 14.26% to 24.17%, and improvement of sphericity index from 0.60 to 0.51. See Interactive of the patient's 3D multidetector tomography scan reconstructions showing the EED before and after transendocardial stem cell injection.

in EED,^{24,25} a measure of infarct size (Figure 3, FIGURE 4, eFigure 1, and Interactive), which was 22.10 g (95% CI, 16.94-27.27 g) at baseline and 13.98 g (95% CI, 10.99-16.97 g) at 13 months ($P < .001$). Allogeneic and autologous MSCs reduced mean EED by 33.21% (95% CI, 43.61%-22.81%; $P < .001$). Similar reduction was observed in the allogeneic (-31.61%; 95% CI, -49.24 to -13.99; $P < .001$) and in the autologous (-34.93%; 95% CI, -48.18 to -21.68; $P < .001$) groups ($P = .75$). Re-

garding the effect of baseline EED on the change in EED, patients whose baseline EED was below the median (18.3 g) had a -4.5-g change in EED at 13 months compared with a -11.5-g change in EED in patients whose baseline EED was above the median ($P = .01$). This suggests that a larger infarct at baseline resulted in a larger reduction in infarct size. Reverse remodeling was also evident by a reduction in the LV sphericity index (Figure 3).^{21,22}

Left ventricular volumes in individual groups revealed a statistically significant reduction in LV end-diastolic volumes only in the allogeneic group (Figure 3, eTable 2). When combined, LV systolic and diastolic volumes decreased in both groups, and EF increased by 1.96 (95% CI, -0.47 to 4.39; $P = .11$) (Figure 3, eFigure 1, and Interactive), although the increase in EF was not statistically significant. The reduction in end-systolic volume and end-diastolic volume did not differ between groups ($P = .77$ and $P = .53$, respectively). The EED at baseline and baseline EF were not significantly associated with the change in EF. Left ventricular mass increased in all patients (eTable 2), potentially consistent with myocardial regeneration. Improvement in these parameters did not differ between groups.

There was an inverse dose response to cell therapy with regard to EF, LV end-systolic volume, and EED improvements (eFigure 2). For these parameters, the effect of 20 million cells was significantly more than that of 200 million cells. This dose effect could not be attributed to differences in baseline EF or EED, because the mean EFs at baseline (20 million, 26.4%; 100 million, 24.9%; 200 million, 29.4%) and baseline EED (20 million, 16.2 g; 100 million, 26.3 g; 200 million, 24.06 g) did not significantly differ by dose level.

Immunologic Responses

More than 30% of patients tested showed sensitization to HLA antigens (8 of 27) at baseline. A majority of the sensitized patients (7 of 8 [87.5%]) demonstrated sensitization at all time points with minimal variation of antibody levels following infusion, indicating preexisting allogeneic antibody before infusion without subsequent allogeneic antibody development after exposure to allogeneic MSCs.

Two patients in the allogeneic group showed sensitization only at the 6-month time point. Of these, 1 patient developed low-level HLA class I antibodies to HLA antigen specificities not expressed by the donor MSC.

This patient subsequently underwent uneventful cardiac transplantation with negative cross-match results. The other sensitized patient showed low-level donor-specific HLA class I antibodies to the HLA B57, A24, and A26 antigens as well as antibody to A25, a known cross-reactive specificity. Flow cytometric cross-match with serum from the second patient to fresh donor T cells showed a weak positive reaction, indicating low titer, *de novo* allogeneic sensitization with class I donor antigens.

COMMENT

The POSEIDON study addresses the major issue of the use of allogeneic MSCs as a cell-based therapeutic. Mesenchymal stem cells are both immunoprivileged and immunosuppressive, thus bearing the potential to be used as an allograft.^{8,11,29-33} To address potential advantages and disadvantages of allogeneic MSCs, we performed a randomized comparison of allografting vs autologous therapy in patients with ICM. Both allogeneic and autologous cells were safe, with both cell types demonstrating potential regenerative bioactivity in patients with ICM, particularly by reducing infarct size and improving ventricular remodeling measured by sphericity index. The majority of patients receiving allografted cells did not mount increased panel-reactive antibodies in response to the therapy. Together, our findings strongly support the ongoing development of allogeneic MSC therapy in patients with a variety of chronic conditions.^{8,15,29,30,34}

To date, limited clinical investigations have been performed with MSCs. We previously conducted a study of intravenous delivery of allogeneic MSCs for patients with acute MI.⁸ More recently, we obtained proof of concept data in patients with ICM that TESI resulted in reverse LV remodeling.⁶ The present study results further substantiate that MSC therapy delivered by TESI produces reverse remodeling of the ventricle as evidenced by improved sphericity index and reduced diastolic and systolic LV volumes, although only allogeneic MSCs re-

duced LV end-diastolic volumes. These changes are driven by decreases in MI size observed in both treatment groups.

Our study shows for the first time, to our knowledge, evidence of clinical improvement (patients receiving cell therapy had improved NYHA classification, 6-minute walk test, and MLHFQ scores; these effects were preferentially observed in the autologous group; however, when groups were combined, the effects were statistically significant without a detectable difference between groups). These findings suggest that cell therapy with MSCs improves functional status and quality of life in patients with advanced ICM. Although these findings will require further substantiation in future larger placebo-controlled studies, the concordance of findings among these efficacy and cardiac remodeling parameters is encouraging.

Two other recent early-stage investigations reported cell therapy for patients with ICM: the SCIPIO³⁵ and CADUCEUS³⁶ trials used cardiac c-kit + stem cells and cardiospheres, respectively. The SCIPIO trial showed very dramatic increases in EF in response to intracoronary infusion of c-kit + cardiac stem cells. Patients in the CADUCEUS trial had decreases in infarct size without significant changes in EF or LV volumes. The degree of infarct size reduction reported herein is similar to that observed in patients receiving intracoronary cardiospheres in the CADUCEUS trial,³⁶ but we found that infarct size reduction was also accompanied by reverse remodeling of the ventricle as evidenced by reductions in LV systolic and diastolic dimensions and improvement in sphericity index (Figure 3 and eFigure 1).

We used multidetector CT scanning to evaluate heart function. Computed tomography has some advantages over magnetic resonance imaging, which is also a highly valuable modality. Although cardiac magnetic resonance imaging can be safely performed in many patients with cardiac rhythm management devices,³⁷ image artifact from those devices can ob-

scure the interpretation of cardiac structure and function. Computed tomographic scanning allowed us to obtain structure and function measures regardless of the presence of cardiac defibrillators or pacemakers. We chose to quantify EED rather than delayed enhancement as a correlate of infarct size because of (1) better contrast between abnormal and normal myocardium; (2) automatic implantable cardioverter-defibrillator lead artifacts exhibited substantial degradation of delayed enhancement images; and (3) the margins of thin chronically infarcted myocardial tissue were often difficult to identify on the delayed images.

An intriguing finding of our study is the potential for an inverse dose response. With 20 million cells, increases in EF were evident and reductions in systolic volume were substantially greater in the patients who received 20 million vs 200 million cells (eFigure 2). The increase in EF and decrease in EED in the 20 million cells group are remarkable for their large effect sizes, which are potentially meaningful and will require substantiation in larger trials. Preclinical investigations are consistent with the inverse dose response; cell retention, survival, performance, or all 3 may be impaired if certain dosing thresholds are exceeded.^{38,39} The inverse dose response may reflect the concentration of cells and not total cell number, because the volume of injectate was constant over the different doses. These findings highlight the importance of dose finding studies before undertaking large clinical trials.

Several mechanisms of action underlying the ability of MSCs to produce reverse remodeling in chronically scarred ventricles can be inferred from preclinical studies.^{40,41} Mesenchymal stem cells engraft and persist for several months in myocardium when delivered by TESI^{31-33,42} and exert a range of effects, including reducing tissue fibrosis, differentiating into small and large blood vessels (vasculogenesis), participating in myogenesis, and releasing paracrine factors.^{31,42,43} Mesenchymal stem cells produce myogenesis not only by direct myocyte differ-

entiation, but also by stimulating endogenous cardiac stem cells to proliferate, undergo lineage commitment, and form transient amplifying cells.^{32,44}

Our pilot study is limited primarily by the lack of a placebo group and its open-label design. Accordingly, the key findings reported herein will require further substantiation in larger phase 2 studies. Because of the small size of this study, some of the important effects did not reach statistical significance when individual group were considered alone. Notably, MHLFQ and 6-minute walk test were not significantly improved in the allogeneic group, whereas changes in LV end-diastolic volumes were not improved in the autologous group. The inverse dose response suggesting that 20 million cells is better than higher cell doses or concentrations is worth noting. Future studies are planned to further investigate this important observation. Another limitation of the study is that we were unable to conduct covariate adjustment with some important clinical characteristics of patients because of the limited sample size. The importance of baseline characteristics in the study design should be considered in future larger phase 2 studies.

In conclusion, we addressed the issue of allogeneic vs autologous MSC therapy by randomizing patients to receive either cell type by TESI without a control group. Our findings in this phase 1/2 study reveal a satisfactory safety profile and document for the first time that alloimmune reactions in patients receiving allogeneic MSCs for ischemic LV dysfunction were low (3.7%). We also observed that those patients treated with both allogeneic and autologous MSCs had improvement in structural and functional measures over time. These data support future investigation of these MSCs within double-blind, randomized, placebo-controlled trials in ICM.

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