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RESEARCH ARTICLE

A pilot clinical trial of cell therapy in heart failure with preserved ejection fraction

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Aims We investigated the effects of $CD34^+$ cell therapy in patients with heart failure with preserved ejection fraction (HFpEF).

Methods and results In a prospective pilot study, we enrolled 30 patients with HFpEF. In Phase 1, patients were treated with medical therapy for 6 months. Thereafter, all patients underwent $CD34^+$ cell transplantation. Using electroanatomical mapping, we measured local mechanical diastolic delay and myocardial viability to guide the targeting of cell injections. Patients were followed for 6 months after cell transplantation (Phase 2), and the primary endpoint was the difference in change in E/e′ between Phase 1 and Phase 2. In Phase 1, the decrease in E/e′ was significantly less pronounced than in Phase 2 (-0.33 ± 1.72 vs. -3.77 ± 2.66, $p = 0.001$). During Phase 1, there was no significant change in global systolic strain (GLS; from $-12.5 \pm 2.4\%$ to $-12.8 \pm 2.6\%$, $p = 0.77$), N-terminal pro-B-type natriuretic peptide (NT-proBNP; from 1463 ± 1247 pg/ml to 1298 ± 931 pg/ml, $p = 0.31$), or 6-min walk test (6MWT; from 391 ± 75 m to 402 ± 93 m, $p = 0.42$). In Phase 2, an improvement was noted in NT-proBNP (from 1298 ± 931 pg/ml to 887 ± 809 pg/ml, $p = 0.02$) and 6MWT (from 402 ± 93 m to 438 ± 72 m, $p = 0.02$). Although GLS did not change significantly in Phase 2 (from $-12.8 \pm 2.6\%$ to $-13.8 \pm 2.7\%$, $p = 0.36$), we found improved local systolic strain at cell injection sites ($-3.4 \pm 6.8\%$, $p = 0.005$).

Conclusions In this non-randomized trial, transendocardial $CD34^+$ cell therapy in HFpEF was associated with an improvement in E/e′, NT-proBNP, exercise capacity, and local myocardial strain at the cell injection sites.
Clinical Trial Registration: ClinicalTrials.gov NCT02923609.

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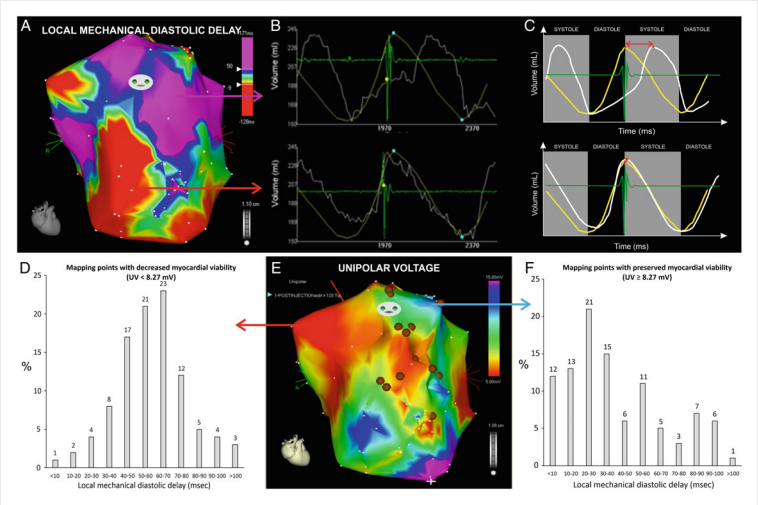
Graphical Abstract



Targeting cell therapy in heart failure with preserved ejection fraction based on local mechanical diastolic delay. NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Keywords Heart failure • Heart failure with preserved ejection fraction • Diastolic function • Cell therapy

Introduction

Heart failure with preserved ejection fraction (HFpEF) represents the most common cause of hospitalization for heart failure, now surpassing heart failure with reduced ejection fraction (HFrEF).[1] Despite this increase in incidence, few effective treatments of HFpEF are available except for the recently noted beneficial effects of sodium–glucose cotransporter 2 inhibitor therapy with empagliflozin.[2] While the HFpEF syndrome is heterogeneous, there is increasing evidence that HFpEF results from a systemic proinflammatory state, microvascular endothelial inflammation and impairment in endothelial-cardiomyocyte nitric oxide signalling, which lead to diastolic dysfunction via altered cardiomyocyte function and extracellular matrix.[3] This hypothesis was confirmed by autopsy data demonstrating that patients with HFpEF have lower coronary microvascular density and more severe fibrosis than control subjects regardless of the severity of epicardial coronary disease.[4]

Microvascular rarefaction represents an imbalance between vessel destruction and regeneration and can be caused either by destruction of microvasculature or insufficient angiogenesis. Formation of new microvasculature in multiple ischaemic tissues, including ischaemic myocardium, appears to be dependent on $CD34^+$ cells, which include hematopoietic stem cell lines as well as endothelial progenitor cells.[5] Although the exact underlying mechanisms remain to be better defined, they are thought to involve direct incorporation into the newly developing vasculature and the production and secretion of angiogenic cytokines. In patients with HFpEF, the levels of circulating $CD34^+$ cells are decreased, with $CD34^+$ numbers inversely correlating with the degree of diastolic impairment.[6] This suggests that impaired $CD34^+$-related vasculogenesis and angiogenesis in HFpEF may represent an interesting target for potential therapeutic interventions.

Our group has previously demonstrated that disease progression in HFrEF can be partially reversed by application of autologous $CD34^+$ cells.[7] This therapeutic approach was also associated with improvement in regional myocardial perfusion[8] and diastolic parameters.[9] Based on these findings our hypothesis is that $CD34^+$ cell therapy may improve diastolic characteristics associated with HFpEF. Therefore, the aim of this pilot study was to investigate the safety and efficacy of $CD34^+$ cell therapy in patients with symptomatic HFpEF by the use of novel delivery technique based on the measurement of local mechanical diastolic delay.

Methods

Patient population

This study consists of a pilot prospective sequential study design conducted at the Advanced Heart Failure and Transplantation Center at University Medical Center Ljubljana between January 2016 and March 2020 in collaboration with the Stanford Cardiovascular Institute at the Stanford University School of Medicine.

Patient inclusion criteria consisted of the following: age 18 to 70 years, symptomatic heart failure with New York Heart Association

class III symptoms in the last 3 months, preserved left ventricular systolic function on echocardiography (left ventricular ejection fraction [LVEF] >50%), a septal E/e′ >15, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels >300 pg/ml. Patients with acute multiorgan system failure, a history of haematologic neoplasms, amyloidosis, hypertrophic cardiomyopathy, obstructive coronary artery disease (evidence of at least one lesion >70% on coronary angiography) or presence of permanent atrial fibrillation were not included. Informed consent was obtained in all patients prior to participation in the study and the study protocol was approved by the National Ethics Committee. The trial was registered in ClinicalTrials.gov (NCT02923609) and with National Agency for Medicinal Products and Medical Devices.

Study design

The study was designed in a prospective sequential fashion (*Figure 1*) with a run-in period without intervention. In Phase 1 of the study, all patients were treated with optimization of medical therapy for 6 months. Thereafter, in Phase 2 of the study, participants received transendocardial CD34$^+$ cell therapy. Follow-up period of Phase 2 lasted for 6 months. At the time of enrolment (6 months before cell therapy), at the time of cell therapy, and 6 months thereafter, we performed detailed history and clinical evaluation (including measurement of body mass index, blood pressure and electrocardiogram), laboratory assays, echocardiography, 6-min walk test (6MWT), and measured plasma levels of NT-proBNP. All patients completed the follow-up at all time points, except for two patients who were not able to perform 6MWT at 6-month follow-up due to orthopaedic problems.

Peripheral blood stem cell mobilization and collection

Patients underwent stem cell mobilization and collection as described previously.[7] In short, peripheral blood stem cells were mobilized by daily subcutaneous injections of granulocyte-colony stimulating factor (G-CSF; 10 μg/kg, divided bid) for 5 days; CD34$^+$ cells were then collected from the peripheral blood via apheresis using a cell separator (Amicus, Fenwal, Inc). A positive selection of CD34$^+$ cells from the apheresis product was performed after overnight storing at 4°C using a cell separation system (CliniMACS Plus device, Miltenyi Biotec). The leukapheresis product was transferred to a 600 ml transfer bag and diluted with a PBS/EDTA 1 0.5% HSA buffer with twice the volume of the leukapheresis product. Platelet-rich plasma was removed using centrifugation. The cell suspension was incubated with 3 ml of human IgG (Octagam, Octapharma) and with 7.5 ml of CD34 reagent (CliniMACS, Miltenyi Biotec) for 30 min at room temperature for specific labelling. The cell product was positively selected for CD34$^+$ cells on the cell separation system (CliniMACS Plus device, Miltenyi Biotec) and ultimately 6 ml of CD34$^+$ cell suspension was obtained. A tubing set (CliniMACS, Miltenyi Biotec) was used for the selection. The CD34$^+$ cells in the final product were enumerated using flow cytometry (FACSCalibur, Becton Dickinson) and analysed with the accompanying software (Cell Quest Pro, Becton Dickinson). All the samples were analysed using the three-color single platform variant of the ISHAGE guidelines with the addition of the viability dye 7-aminoactinomycin D. The average viability of injected CD34$^+$ cells was 99.1 ± 0.3%. Of the recovered CD34$^+$ cells, 80 million were used for transendocardial injections.

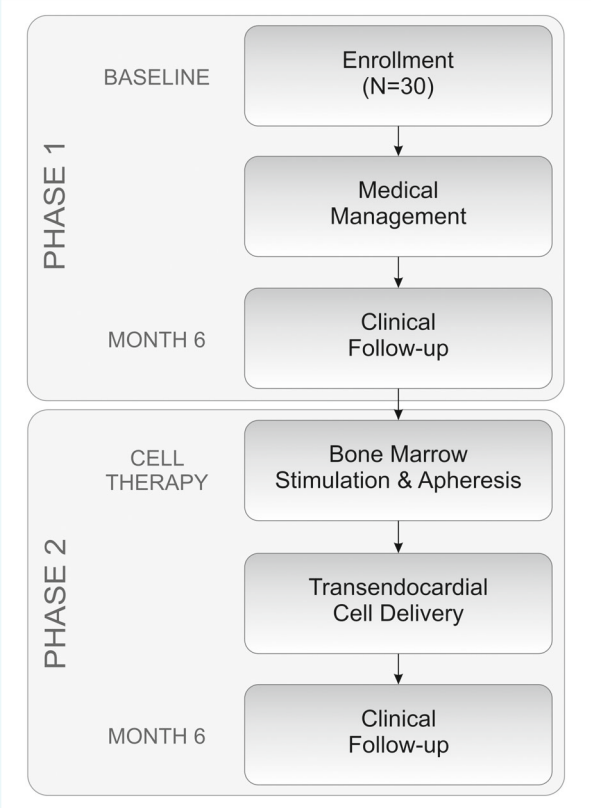


Figure 1 Flow-chart of the study design. After enrolment all patients were treated with optimization of medical therapy for 6 months (Phase 1). Thereafter, all patients received transendocardial CD34$^+$ cell therapy, and were followed for another 6 months (Phase 2). At the time of enrolment (6 months before cell therapy), at the time of cell therapy, and 6 months thereafter we performed detailed clinical evaluation, laboratory assays, echocardiography, 6-min walk test, and measured plasma levels of N-terminal pro-B-type natriuretic peptide.

Electroanatomical mapping and transendocardial cell delivery

Electroanatomical mapping was performed using the Biosense NOGA system (Biosense Webster, Diamond Bar, CA, USA).[7] For each patient, colour-coded unipolar voltage and their corresponding 'bull's-eye' maps, consisting of at least 150 sampling points were generated. Points were acquired when the catheter tip was stable on the endocardium (defined as simultaneous stability of local activation time, location stability, loop stability, and cycle length stability). A map of the left ventricle was reconstructed with all endocardial regions adequately represented. In accordance with previous studies in heart failure,[9,10] viable myocardium was defined as unipolar voltage (UV) ≥8.27 mV and non-viable myocardium was defined as areas with UV <8.27 mV. Local diastolic function was assessed by a novel algorithm based on the measurement of local mechanical diastolic delay (*Figure 2*). At each sampling point, local mechanical diastolic delay was measured as timing difference between end-diastolic time (based on left ventricular volume) and maximum local diastolic time (based on local wall movement of the mapping point). To better define the target areas for cell

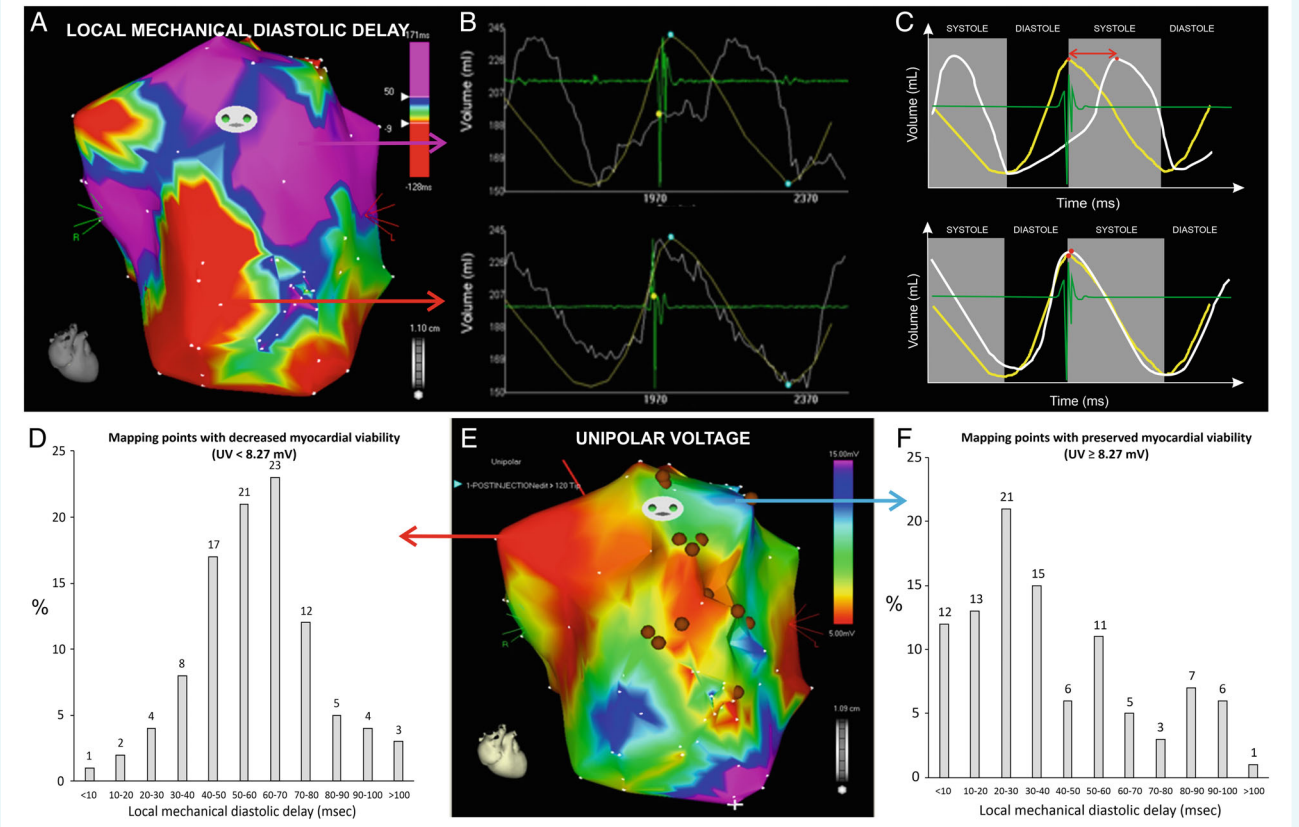


Figure 2 Measurement of local mechanical diastolic delay and myocardial viability. (*A*) Exemplary electroanatomical mapping of the left ventricle with measurement of local mechanical diastolic delay. (*B*) Examples of mapping points with (top) and without (bottom) local mechanical diastolic delay. (*C*) Schematic presentation of the local mechanical diastolic delay measurements from panel B. Prior to stem cell application, we performed electroanatomical mapping of the left ventricle consisting of at least 150 mapping points. At each point we measured local movement of left ventricular wall (white lines in panels *B* and *C*). Local mechanical diastolic delay (red arrows in panel *C*) was defined as timing difference between maximum local diastolic time and end-diastolic time based on left ventricular volume (yellow lines in panels *B* and *C*). In panel (*A*), blue and purple areas represent regions with increased local mechanical diastolic delay; red, yellow and green areas represent the regions without significant local mechanical diastolic delay. Green lines in panels (*B*) and (*C*) represent local action potential. (*E*) Exemplary unipolar voltage map: red and yellow areas represent regions with decreased viability; green, blue and purple areas represent regions with preserved viability. (*D*) Distribution of local mechanical diastolic delay in mapping points with decreased viability. (*F*) Distribution local mechanical diastolic delay in points with preserved viability. Overall, we found a significantly increased local mechanical diastolic delay in regions with decreased viability. Stem cell injections (brown dots in panel *E*) were performed targeting the areas with increased local mechanical diastolic delay and preserved myocardial viability.

injections, we analysed the data from our previous study investigating the correlates of local diastolic properties in patients with dilated cardiomyopathy.[9] In these patients, increased left ventricular filling pressure (E/e′ >15) correlated with a prolongation of local diastolic delay by a mean of 50 ms. Thus, in the present study, local mechanical diastolic delay was considered increased if the time delay between end-diastolic time and local diastolic time was at least 50 ms. All patients had areas of local diastolic delay of >50 ms and thus were eligible for treatment.

Transendocardial delivery of cell suspension was then performed with MyoStar® (Biosense Webster) injection catheter targeting the areas with increased local diastolic delay and preserved myocardial viability. After acquiring a stable mapping point with the tip of the catheter perpendicular to the endocardial surface, the endocardial surface was punctured and the needle was advanced into the myocardium, and transendocardial injections were performed. Each patient received 20 injections of stem cell suspension (0.3 ml each).

Echocardiography, 6-min walk test and NT-proBNP measurements

Echocardiography was performed in accordance with the American Society of Echocardiography/European Association of Cardiovascular Imaging expert consensus[11] using the Vivid E9 system (GE Healthcare, Horten, Norway). The echocardiographic data were de-identified and analysed offline (EchoPAC, GE Healthcare, Horten, Norway) at the end of the follow-up period of Phase 2 by a single investigator, blinded to the timings of the recordings. The primary endpoint parameter (E/e′) was also re-measured by a second blinded independent

reviewer, and intraclass correlation coefficient estimate and its 95% confidence interval were calculated based on a single-rating, consistency, two-way random-effects model. This yielded a value of 0.92 (0.88–0.95), suggesting good reliability of the performed echocardiographic measurements. We obtained left ventricular diameters from two-dimensional long parasternal view and calculated ventricular volumes and LVEF by the biplane method. The E/e′ ratio was calculated from early transmitral velocity divided by peak left ventricular relaxation velocity. Diastolic dysfunction was graded according based on E/A ratio and left atrial pressure (LAP) estimation: grade 1 was defined as E/A ratio ≤0.8 with a peak E velocity ≤0.5 m/s and LAP normal or low; grade 2 was defined as E/A ratio between 0.8 and 2 in addition to elevated LAP; and grade 3 was present when E/A ratio was ≥2 and LAP was increased. LAP was considered increased in the presence of at least two of the following criteria: E/e′ >14, tricuspid regurgitation velocity >2.8 m/s, or left atrial volume index >34 ml/m^2. Left ventricular longitudinal strains were analysed by speckle tracking echocardiography from apical four-chamber, two-chamber, and long-axis views. Endocardial borders were traced manually at the end-diastolic frame. The software tracked speckles along the endocardial border throughout the cardiac cycle. Peak longitudinal strains were computed automatically to generate regional data from each of the 17 segments and then averaged to calculate global longitudinal strain.

In all patients, 6MWT was performed by a blinded observer according to the standard protocol.[12] All NT-proBNP assays were performed at a central independent laboratory, blinded to the patient's clinical data using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

Study endpoints

The primary endpoint was defined as the difference in change in E/e′ assessed by echocardiography between Phase 1 and Phase 2. The secondary endpoints included changes in parameters of diastolic function, global systolic strain, NT-proBNP levels, and exercise capacity. The safety endpoint included serious adverse events (SAEs), defined as any serious event that may result in persistent or significant disability or incapacity and included death, heart transplantation, sustained ventricular arrhythmia (ventricular tachycardia or ventricular fibrillation), and heart failure exacerbation requiring hospitalization. In an exploratory analysis, we followed the patients for SAEs for another year after the completion of Phase 2 of the trial. A data and safety monitoring committee was responsible for monitoring SAEs during the study and would report to the study principal investigator.

Statistical methods

Based on our previous findings on transendocardial injection on diastolic function in non-ischaemic heart failure[9] and the results of cell therapy on diastolic function in ischaemic heart failure,[13] the sample size calculation for this study was based on the 90% probability that the study will detect a treatment difference with a 5% two-sided significance level, if the true difference in E/e′ between time of cell therapy and 6 months thereafter is 1.8 (assumed standard deviation [SD]: 6.7). Continuous variables were expressed as mean ± SD and categorical variables were expressed as number and percentage. Continuous variables were explored for normal distribution with the Kolmogorov–Smirnov test. Differences between groups were analysed using a *t*-test for continuous variables with correction for unequal variance when appropriate, and with chi-square or Fisher exact test when appropriate. Correlation analysis between E/e′, NT-proBNP, exercise capacity, UV, and local mechanical diastolic delay was performed with Pearson correlation coefficient. Statistical significance was assumed for *p*-values of <0.05. All statistical analyses were performed with SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The included 30 patients represent a cohort of consecutive consenting patients. We screened 46 patients; 12 were not included due to the presence of permanent atrial fibrillation, three due to E/e′ <15, and one because of LVEF <50%. Patient characteristics at baseline, at the time of cell injection (end of Phase 1), and 6 months thereafter (end of Phase 2) are presented in *Table 1*. The patient population represents a cohort with significant heart failure symptoms and high comorbidity with the majority having diabetes mellitus, systemic hypertension and dyslipidaemia. On inclusion of the study, the majority of patients were treated with diuretics and renin–angiotensin inhibitors; about half of patients received beta-blockers and aldosterone antagonists. No patients were treated with sodium–glucose cotransporter 2 inhibitors. We found no significant differences in body mass index, blood pressure, heart rate, sodium, and parameters of kidney and liver function between the pre-specified time points. In Phase 1, we found an increased dosing of perindopril and a trend of increased dosing of furosemide. In Phase 2, no significant changes in drug dosing were found.

Myocardial viability and local mechanical diastolic delay

Using electroanatomical mapping prior the cell transplantation we generated a total of 4820 mapping points in 30 patients. Local viability of myocardial segments, measured by UV, is presented in *Table 2*. UV was significantly higher in the myocardial segments receiving cell injections, particularly in the anterior region. The mean local mechanical diastolic delay was 47 ± 33 ms, and the coefficient of variation was 0.70. The distribution of local mechanical diastolic delay in points with preserved myocardial viability (UV ≥8.27 mV) and decreased myocardial viability (UV <8.27 mV) is presented in *Figure 2*. We found a significant inverse correlation between UV and local mechanical diastolic delay ($r = -0.18$, $p = 0.001$). On average, cell injections were performed in 5.1 of nine electroanatomical segments (56%), which corresponded to 321 of 510 echocardiographic segments (63%).

Parameters of diastolic function

The individual changes in E/e′ in Phase 1 and Phase 2 are presented in *Figure 3* and parameters of diastolic function are presented in *Table 3*. In Phase 2, the decrease in mean E/e′ was more pronounced than in Phase 1. Similarly, in Phase 2, we found more pronounced improvement in grades of diastolic dysfunction, deceleration time, and maximal tricuspid regurgitation velocity. E-wave velocity and E/A ratio remained relatively unchanged throughout the study period.

Table 1 Patient characteristics

	Baseline ($n = 30$)	End of Phase 1 ($n = 30$)	End of Phase 2 ($n = 30$)	p-value
Age, years	62 ± 10			
Male sex	23 (77)			
Duration of heart failure, months	27.4 ± 5.5			
Hypertension	27 (90)			
Diabetes mellitus	23 (77)			
Dyslipidaemia	25 (83)			
Non-obstructive CAD	15 (50)			
Paroxysmal atrial fibrillation	9 (30)			
BMI, kg/m^2	28.8 ± 4.1	28.2 ± 4.3	27.9 ± 3.9	NS
Systolic blood pressure, mmHg	142 ± 42	138 ± 38	137 ± 40	NS
Diastolic blood pressure, mmHg	95 ± 13	87 ± 11	86 ± 13	NS
Mean blood pressure, mmHg	109 ± 20	103 ± 14	103 ± 16	NS
Heart rate, bpm	72 ± 15	69 ± 13	70 ± 14	NS
Serum creatinine, mg/dl	1.05 ± 0.40	1.09 ± 0.32	1.04 ± 0.31	NS
Total bilirubin, μmol/L	14 ± 7	16 ± 2	16 ± 2	NS
ALT, μkat/L	1.3 ± 0.9	1.4 ± 0.9	1.3 ± 5.1	NS
AST, μkat/L	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.3	NS
Total cholesterol, mmol/L	5.9 ± 3.2	5.6 ± 2.8	5.5 ± 3.0	NS
Sodium, mmol/L	141 ± 3	140 ± 3	140 ± 4	NS
QRS duration, msec	108 ± 21	112 ± 17	110 ± 18	NS
Drug therapy				
Loop diuretics	27 (90)	30 (100)	30 (100)	NS
ACEI/ARB	25 (83)	26 (87)	26 (87)	NS
MRA	17 (56)	22 (73)	22 (73)	NS
Beta-blockers	15 (50)	16 (53)	16 (53)	NS
Dosing of medications				
Furosemide, mg	46 ± 29	53 ± 28	55 ± 29	0.08[a]
Perindopril, mg	5.3 ± 1.9	6.4 ± 2.0	6.4 ± 2.0	0.02[a]
Ramipril, mg	6.3 ± 2.5	7.5 ± 2.9	7.5 ± 2.9	NS
Telmisartan, mg	50 ± 20	60 ± 23	60 ± 23	NS
Spironolactone, mg	22.3 ± 5.3	22.9 ± 4.8	22.9 ± 4.8	NS
Bisoprolol, mg	5.5 ± 2.2	5.8 ± 1.8	5.9 ± 2.0	NS

Values are presented as mean ± standard deviation, or n (%).
ACEI, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase, BMI, body mass index; CAD, coronary artery disease; MRA, mineralocorticoid receptor antagonist.
[a]Baseline versus end of Phase 1.

Parameters of systolic function

We found no significant changes in LVEF in either Phase 1 (from 58.7 ± 7.3% to 57.6 ± 6.1%, $p = 0.78$) or Phase 2 (from 57.6 ± 6.1% to 62.2 ± 8.8%, $p = 0.22$). Similarly, changes in global longitudinal strain did not reach statistical significance in any of the 2 phases (Phase 1: from −12.5 ± 2.4% to −12.8 ± 2.6%, $p = 0.77$; Phase 2: from −12.8 ± 2.6% to 13.8 ± 2.7%, $p = 0.36$; *Figure 4A*). However, we found a significant improvement in local systolic strain at the sites of cell injections (−3.4 ± 6.8%, $p = 0.005$); the changes were particularly pronounced when the cells were injected in the anterior wall of the left ventricle (*Table 2*).

NT-proBNP levels and exercise capacity

Individual changes in NT-proBNP levels and exercise capacity are presented in *Figure 4B,C*. During Phase 1, there was no change in NT-proBNP levels (from 1463 ± 1247 pg/ml to 1298 ± 931 pg/ml, $p = 0.31$) or 6MWT (from 391 ± 75 m to 402 ± 93 m, $p = 0.42$). In Phase 2, an improvement was noted in NT-proBNP (from 1298 ± 931 pg/ml to 887 ± 809 pg/mL, $p = 0.02$) and 6MWT (from 402 ± 93 m to 438 ± 72 m, $p = 0.02$). We also performed the correlation analysis between the changes in E/e′ and NT-proBNP and E/e′ and 6MWT in Phase 2 and found a significant correlation between changes in E/e′ and NT-proBNP ($r = 0.37$, $p = 0.04$), and a trend of inverse correlation between E/e′ and exercise capacity ($r = −0.34$, $p = 0.06$).

Adverse events

Data on SAEs are presented in *Table 4*. The numbers of SAEs did not differ between Phase 1 and Phase 2; there was a trend of lower rates of heart failure hospitalizations in Phase 2, but it did not reach statistical significance. During the exploratory 1-year

Table 2 Local viability (unipolar voltage) and changes in local strain of myocardial segments receiving transendocardial CD34⁺ cell injections and the segments without cell injections

	Injected segments ($n = 321$)	Non-injected segments ($n = 189$)	p-value
Unipolar voltage, mV			
Anterior	15.2 ± 5.1	6.2 ± 5.2	0.001
Lateral	10.3 ± 4.1	6.7 ± 3.8	0.002
Posterior	10.1 ± 3.8	6.8 ± 5.1	0.003
Septal	9.1 ± 4.1	5.3 ± 3.2	0.003
Changes in local strain			
Anterior	−4.3 ± 4.2	+0.7 ± 3.5	0.001
Lateral	−3.3 ± 6.2	+1.4 ± 4.1	0.001
Posterior	−3.5 ± 7.2	+2.0 ± 3.2	0.001
Septal	−2.1 ± 5.3	+2.2 ± 3.1	0.002

Values are presented as mean ± standard deviation.

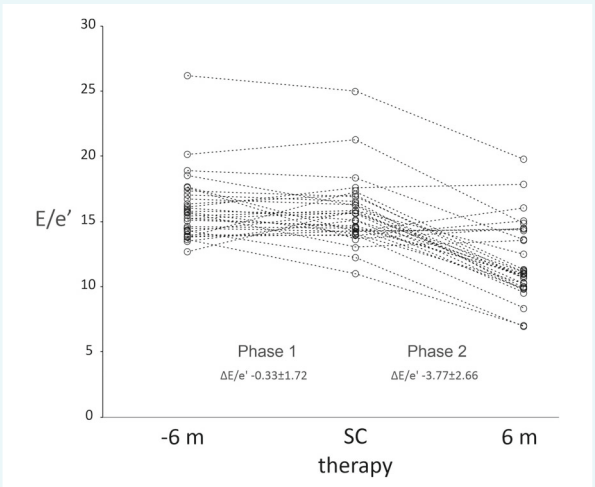


Figure 3 Changes in E/e′. The chart displays individual changes in E/e′ between baseline (6 months before cell therapy), the time of cell therapy, and 6 months thereafter. The decrease in E/e′ was significantly more pronounced in Phase 2 than in Phase 1 ($p = 0.001$). SC, stem cell.

follow-up after the completion of the trial, 28/30 (93%) patients survived, one patient died due to myocardial infarction, and one patient died of non-cardiac causes; hospitalization for heart failure occurred in four patients (13%).

Discussion

This is the first clinical study to date investigating the effects of cell therapy in patients with HFpEF using a novel approach based on local measurement of mechanical diastolic delay. Our data show

Table 3 Parameters of diastolic function

	Baseline ($n = 30$)	End of Phase 1 ($n = 30$)	End of Phase 2 ($n = 30$)	p-value
E-wave velocity, cm/s	98 ± 11	96 ± 13	95 ± 18	NS
Septal E/e′ velocity ratio	18.0 ± 3.5	17.4 ± 3.0	11.9 ± 2.6	0.001*
Lateral E/e′ velocity ratio	14.0 ± 2.4	13.9 ± 2.4	11.8 ± 4.0	0.001*
Mean E/e′ velocity ratio	16.0 ± 2.6	15.7 ± 2.6	11.9 ± 3.0	0.001*
E/A velocity ratio	1.83 ± 0.55	1.84 ± 0.70	1.83 ± 0.64	NS
DT, ms	156 ± 31	159 ± 38	182 ± 38	0.02*
TR Vmax, m/s	2.94 ± 0.16	2.95 ± 0.1	2.72 ± 0.22	0.01*
Diastolic dysfunction				
Grade 1	0 (0)	1 (3)	14 (47)	0.01*
Grade 2	19 (63)	20 (67)	11 (37)	
Grade 3	11 (37)	9 (30)	5 (17)	

Values are presented as mean ± standard deviation, or n (%).
DT, deceleration time; TR Vmax, maximal tricuspid regurgitation velocity.
*$p < 0.05$ (end of Phase 2 vs. end of Phase 1).

that transendocardial CD34⁺ cell transplantation in HFpEF patients is feasible and safe, and associated with favourable signal based on E/e′, NT-proBNP, and 6MWT distance. Although global systolic function of the left ventricle did not change significantly after cell therapy, we found a significant improvement in local systolic function at cell injection sites.

Currently, the effects of medical management in patients of HFpEF are limited, mainly focusing on treatment of heart failure symptoms and comorbidities as large clinical trials investigating the effects of candesartan,[14] irbesartan,[15] perindopril,[16] spironolactone,[17] and more recently, sacubitril/valsartan[18] in patients with HFpEF all failed to reach their primary endpoints. However, recent data demonstrate that empagliflozin therapy reduces the combined risk of cardiovascular death or hospitalization for heart failure in HFpEF patients, regardless of the presence or absence of diabetes.[2] Although the underlying mechanisms for these findings are not clearly defined, they may be related to the empagliflozin-induced reduction in inflammation-dependent endothelial dysfunction resulting in improved cardiomyocyte contractility.[19]

In our study, no patients were receiving empagliflozin, and the optimization of other medical therapy was not associated with improvement in left ventricular systolic and diastolic function, NT-proBNP levels, or exercise capacity. In contrast, 6 months after cell therapy, we found a significant improvement in E/e′, NT-proBNP, exercise capacity, deceleration time, and maximal tricuspid regurgitation velocity. This is in line with the results of the previous trials demonstrating beneficial effects of cell therapy on diastolic parameters and exercise capacity in patients with ischaemic heart failure,[13] non-ischaemic dilated cardiomyopathy[9] and acute myocardial infarction.[20] To date, no clinical trials evaluating the effects of cell therapy in HFpEF have been published. However, in a pre-clinical rat model of HFpEF, intracoronary application of cardiosphere-derived cells was associated with improvements in left ventricular relaxation and filling pressures.[21] In this animal study, cell therapy normalized arteriolar density and significantly increased capillary density at 4 weeks after application. Similarly, in an aged rat model, systemic delivery of adipose-derived cells

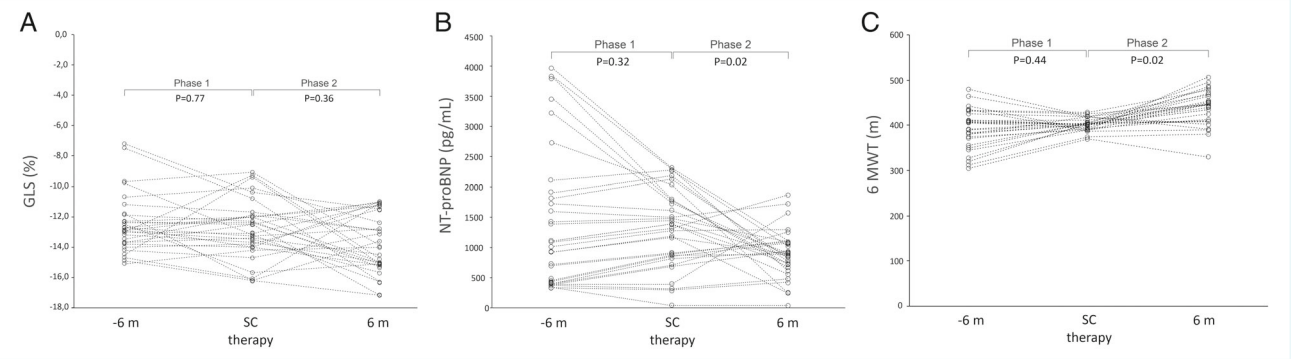


Figure 4 Changes in global and local longitudinal strain, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and exercise capacity. In Phase 1, we found no change in global longitudinal strain (GLS); although GLS showed a trend of decrease in Phase 2, the changes did not reach statistical significance (*A*). Levels of NT-proBNP remained stable throughout Phase 1 but decreased significantly in Phase 2 (*B*). In Phase 1, 6-min walk test (6MWT) distance did not change; however, we found a significant improvement in 6MWT distance in Phase 2 (*C*). SC, stem cell.

Table 4 Serious adverse events

	Phase 1 ($n = 30$)	Phase 2 ($n = 30$)	*p*-value
Death	0 (0)	0 (0)	1.00
Heart transplantation	0 (0)	0 (0)	1.00
Sustained ventricular arrhythmia	1 (3)	2 (7)	0.56
Hospitalization for heart failure	5 (17)	1 (3)	0.09

Values are presented as *n* (%). Sustained ventricular arrhythmia was defined as ventricular tachycardia or ventricular fibrillation requiring cardioversion, and hospitalization for heart failure was defined as exacerbation of heart failure symptoms and signs requiring hospitalization, with concomitant increase in N-terminal pro-B-type natriuretic peptide levels.

enhanced coronary microvascular perfusion and improved diastolic function.[22] Thus, it appears that although the underlying mechanisms of the effects of cell therapy in HFpEF are incompletely understood, they may partly result from changes in microvasculature and perfusion of the myocardium.

Recent clinical evidence from a large cohort of patients with chronic heart failure suggests that the number of circulating endothelial progenitor cells expressing CD34 epitopes is significantly decreased in both patients with HFrEF and HFpEF.[23] In their study, lower numbers of $CD34^+$ cells were a strong, independent predictor of mortality in HFpEF population. In addition, decreased numbers of $CD34^+$ cells have also been associated with more advanced symptoms and worse diastolic parameters in HFpEF; this relationship was independent from patient age.[6] Based on these data, one might speculate that an increase in peripheral and myocardial numbers of $CD34^+$ cells could lead to improved diastolic function and beneficial clinical effects in heart failure. In accordance with this hypothesis, the effects of cell therapy on left ventricular filling pressures in patients with ischaemic heart failure have been shown to directly correlate with the numbers of injected $CD34^+$ cells.[23] In our trial, the number of $CD34^+$ cells used for transendocardial delivery was very high (80 million), which may have contributed to the magnitude of observed clinical effects.

To maximize the potential benefits of cell therapy in our study, we opted to use a novel targeting strategy aiming to apply the cell suspension to the sites of preserved myocardial viability and increased local mechanical diastolic delay. Using electroanatomical mapping, we found a significant inverse correlation between local mechanical diastolic delay and myocardial viability, which may offer additional insights into pathophysiology of HFpEF. Our results also demonstrate that, in HFpEF patients, electroanatomical mapping and transendocardial injections of $CD34^+$ cells are safe, with the incidence of adverse events being low and comparable to those occurring in patients with HFrEF.[24] Using this approach, we were able to demonstrate a significant improvement of local systolic strain at cell injection sites 6 months after therapy, with the changes particularly pronounced in the myocardial areas with higher unipolar voltage. This is in accordance with our previous findings in patients with dilated cardiomyopathy[25] and the results of the studies with ischaemic heart failure, where the application of cell therapy in the regions with high unipolar voltage resulted in improved local systolic strain and a decrease in left ventricular filling pressures.[26,27] Since the majority of effects after transendocardial cell therapy appears to occur at the cell injection sites,[28] the use of advanced imaging algorithms to guide transendocardial cell delivery could further improve the efficacy of this therapeutic approach.

Study limitations

The results of our study are subject to several limitations. For instance, our patient population only included patients with HFpEF without permanent atrial fibrillation, the patients were relatively young and predominantly male, so the generalizability of the effects of $CD34^+$ cell therapy should be interpreted with caution. Since this is a pilot trial of cell therapy in HFpEF, the study design did not include a placebo arm, and thus the results should be viewed as hypothesis-generating. Although local diastolic delay may

be heart rate-dependent, the heart rate of patients during electroanatomical mapping did not change significantly, and thus the heart rate-dependent parameters were comparable in segments with and without local mechanical diastolic delay. In an attempt to minimize the potential effects of medical management, all patients were followed in our heart failure outpatient clinic for 6 months before inclusion, which allowed for adequate optimization of medical therapy (Phase 1). After cell therapy (Phase 2), no additional changes in heart failure therapy were performed. Our study protocol did not include any daily life activity measurements or questionnaires, and due to open-label design, the results of the 6MWT may be less reliable. However, we did find a stable trend of a decrease in heart failure hospitalization rates after cell therapy. Finally, we recognize that our sample size was small, which makes the study underpowered to be definitive.

Conclusions

In HFpEF patients, local mechanical diastolic delay appears to correlate with myocardial viability. In this pilot trial of cell therapy in HFpEF, we found an improvement in E/e′, NT-proBNP levels, exercise capacity, and some parameters of diastolic function at 6 months after transendocardial $CD34^+$ cell application. Although encouraging, the results of this study should be viewed as preliminary; further larger studies are warranted to validate these signals, better define the underlying mechanisms and to investigate whether such approach can be safely and effectively used in a broader population of HFpEF patients.

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References

1. Nair N. Epidemiology and pathogenesis of heart failure with preserved ejection fraction. *Rev Cardiovasc Med*. 2020;**21**:531–40.
2. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, et al.; EMPEROR-Preserved Trial Investigators. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med*. 2021;**385**:1451–61.
3. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;**62**:263–71.
4. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. *Circulation*. 2015;**131**:550–9.
5. Sietsema WK, Kawamoto A, Takagi H, Losordo DW. Autologous $CD34^+$ cell therapy for ischemic tissue repair. *Circ J*. 2019;**83**:1422–30.
6. Chiang CH, Huang PH, Leu HB, Hsu CY, Wang KF, Chen JW, et al. Decreased circulating endothelial progenitor cell levels in patients with heart failure with preserved ejection fraction. *Cardiology*. 2013;**126**:191–201.
7. Vrtovec B, Poglajen G, Lezaic L, Sever M, Socan A, Domanovic D, et al. Comparison of transendocardial and intracoronary $CD34^+$ cell transplantation in patients with nonischemic dilated cardiomyopathy. *Circulation*. 2013;**128**:S42–9.
8. Lezaic L, Socan A, Poglajen G, Peitl PK, Sever M, Cukjati M, et al. Intracoronary transplantation of $CD34^+$ cells is associated with improved myocardial perfusion in patients with nonischemic dilated cardiomyopathy. *J Card Fail*. 2015;**21**:145–52.
9. Bervar M, Kozelj M, Poglajen G, Sever M, Zemljic G, Frljak S, et al. Effects of transendocardial $CD34^+$ cell transplantation on diastolic parameters in patients with nonischemic dilated cardiomyopathy. *Stem Cells Transl Med*. 2017;**6**:1515–21.
10. Hutchinson MD, Gerstenfeld EP, Desjardins B, Bala R, Riley MP, Garcia FC, et al. Endocardial unipolar voltage mapping to detect epicardial ventricular tachycardia substrate in patients with nonischemic left ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol*. 2011;**4**:49–55.
11. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2016;**17**:1321–60.
12. Olsson LG, Swedberg K, Clark AL, Witte KK, Cleland JG. Six minute corridor walk test as an outcome measure for the assessment of treatment in randomized, blinded intervention trials of chronic heart failure: a systematic review. *Eur Heart J*. 2005;**26**:778–93.
13. Diederichsen AC, Møller JE, Thayssen P, Videbaek L, Saekmose SG, Barington T, et al. Changes in left ventricular filling patterns after repeated injection of autologous bone marrow cells in heart failure patients. *Scand Cardiovasc J*. 2010;**44**:139–45.
14. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, et al.; CHARM Investigators and Committees. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved trial. *Lancet*. 2003;**362**:777–81.
15. Massie BM, Carson PE, McMurray JJ, Komajda M, McKelvie R, Zile MR, et al.; I-PRESERVE Investigators. Irbesartan in patients with heart failure and preserved ejection fraction. *N Engl J Med*. 2008;**359**:2456–67.
16. Cleland JG, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J; PEP-CHF Investigators. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. *Eur Heart J*. 2006;**27**:2338–45.
17. Pitt B, Pfeffer MA, Assmann SF, Boineau R, Anand IS, Claggett B, et al.; TOPCAT Investigators. Spironolactone for heart failure with preserved ejection fraction. *N Engl J Med*. 2014;**370**:1383–92.
18. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, et al.; PARAGON-HF Investigators and Committees. Angiotensin-neprilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med*. 2019;**381**:1609–20.
19. Pabel S, Hamdani N, Singh J, Sossalla S. Potential mechanisms of SGLT2 inhibitors for the treatment of heart failure with preserved ejection fraction. *Front Physiol*. 2021;**5**:752370.
20. Jiang M, Mao J, He B. The effect of bone marrow-derived cells on diastolic function and exercise capacity in patients after acute myocardial infarction. *Stem Cell Res*. 2012;**9**:49–57.
21. Gallet R, de Couto G, Simsolo E, Valle J, Sun B, Liu W, et al. Cardiosphere-derived cells reverse heart failure with preserved ejection fraction (HFpEF) in rats by decreasing fibrosis and inflammation. *JACC Basic Transl Sci*. 2016;**1**:14–28.
22. Kelm NQ, Beare JE, Yuan F, George M, Shofner CM, Keller BB, et al. Adipose-derived cells improve left ventricular diastolic function and increase microvascular perfusion in advanced age. *PLoS One*. 2018;**13**:e0202934.
23. Samman Tahhan A, Hammadah M, Sandesara PB, Hayek SS, Kalogeropoulos AP, Alkhoder A, et al. Progenitor cells and clinical outcomes in patients with heart failure. *Circ Heart Fail*. 2017;**10**:e004106.
24. Vrtovec B, Poglajen G, Sever M, Zemljic G, Frljak S, Cerar A, et al. Effects of repetitive transendocardial $CD34^+$ cell transplantation in patients with nonischemic dilated cardiomyopathy. *Circ Res*. 2018;**123**:389–96.
25. Zemljic G, Poglajen G, Sever M, Cukjati M, Frljak S, Androcec V, et al. Electroanatomic properties of the myocardium predict response to CD34+ cell therapy in patients with ischemic and nonischemic heart failure. *J Card Fail*. 2017;**23**:153–60.
26. Schneider C, Jaquet K, Geidel S, Rau T, Malisius R, Boczor S, et al. Transplantation of bone marrow-derived stem cells improves myocardial diastolic function: strain rate imaging in a model of hibernating myocardium. *J Am Soc Echocardiogr*. 2009;**22**:1180–9.
27. Merino A, Gaya A, Calvo J, Rotger R, Nuñez J. Intracoronary injection of haematopoietic precursor cells regenerates the borders, but not the core, of old myocardial scars. *ESC Heart Fail*. 2020;**7**:2962–71.
28. Suncion VY, Ghersin E, Fishman JE, Zambrano JP, Karantalis V, Mandel N, et al. Does transendocardial injection of mesenchymal stem cells improve myocardial function locally or globally? An analysis from the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) randomized trial. *Circ Res*. 2014;**114**:1292–301.