ONLINE FIRST

Effect of Transendocardial Delivery of Autologous Bone Marrow Mononuclear Cells on Functional Capacity, Left Ventricular Function, and Perfusion in Chronic Heart Failure

The FOCUS-CCTRN Trial

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for the Cardiovascular Cell Therapy Research Network (CCTRN)

Context Previous studies using autologous bone marrow mononuclear cells (BMCs) in patients with ischemic cardiomyopathy have demonstrated safety and suggested efficacy.

Objective To determine if administration of BMCs through transendocardial injections improves myocardial perfusion, reduces left ventricular end-systolic volume (LVESV), or enhances maximal oxygen consumption in patients with coronary artery disease or LV dysfunction, and limiting heart failure or angina.

Design, Setting, and Patients A phase 2 randomized double-blind, placebo-controlled trial of symptomatic patients (New York Heart Association classification II-III or Canadian Cardiovascular Society classification II-IV) with a left ventricular ejection fraction of 45% or less, a perfusion defect by single-photon emission tomography (SPECT), and coronary artery disease not amenable to revascularization who were receiving maximal medical therapy at 5 National Heart, Lung, and Blood Institute–sponsored Cardiovascular Cell Therapy Research Network (CCTRN) sites between April 29, 2009, and April 18, 2011.

Intervention Bone marrow aspiration (isolation of BMCs using a standardized automated system performed locally) and transendocardial injection of 100 million BMCs or placebo (ratio of 2 for BMC group to 1 for placebo group).

Main Outcome Measures Co-primary end points assessed at 6 months: changes in LVESV assessed by echocardiography, maximal oxygen consumption, and reversibility on SPECT. Phenotypic and functional analyses of the cell product were performed by the CCTRN biorepository core laboratory.

Results Of 153 patients who provided consent, a total of 92 (82 men; average age: 63 years) were randomized (n=61 in BMC group and n=31 in placebo group). Changes in LVESV index (–0.9 mL/m^2 [95% CI, –6.1 to 4.3]; P=.73), maximal oxygen consumption (1.0 [95% CI, –0.42 to 2.34]; P=.17), and reversible defect (–1.2 [95% CI, –12.50 to 10.12]; P=.84) were not statistically significant. There were no differences found in any of the secondary outcomes, including percent myocardial defect, total defect size, fixed defect size, regional wall motion, and clinical improvement.

Conclusion Among patients with chronic ischemic heart failure, transendocardial injection of autologous BMCs compared with placebo did not improve LVESV, maximal oxygen consumption, or reversibility on SPECT.

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CELL THERAPY HAS EMERGED AS an innovative approach for treating patients with advanced ischemic heart disease, including those with refractory angina and/or heart failure. Early clinical studies have been performed primarily using autologous stem/progenitor cells.[1-13] In patients with ischemic heart

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disease and heart failure, treatment with autologous bone marrow mononuclear cells (BMCs) has demonstrated safety and has suggested efficacy.[10,14-17]

None of the clinical trials performed to date, however, have been powered to evaluate specific efficacy measures. In addition, autologous cell therapy trials have usually enrolled older patients with acute or chronic left ventricular (LV) dysfunction without evaluating the effect of these parameters or cell function on clinical outcome. These factors are important because ischemic heart failure has the potential to limit the beneficial influences of cellular effects.[6,18]

The present study was undertaken by the National Heart, Lung, and Blood Institute–sponsored Cardiovascular Cell Therapy Research Network (CCTRN).[19] It builds on work from a pilot study in Brazil,[10] which in turn led to the first phase 1 randomized trial of autologous BMC therapy for heart failure in the United States (FOCUS-HF) approved by the US Food and Drug Administration.[16] These initial studies showed that transendocardial delivery of BMCs was feasible and appeared safe in patients with chronic heart failure due to multivessel coronary artery disease.[10,16] Although these preliminary studies also evaluated LV function, perfusion, and functional capacity, a definitive assessment of efficacy was not possible due to the small number of patients. Thus, the present trial was designed as a larger study to investigate the effects of transendocardial-delivered BMCs in patients with chronic ischemic heart disease and LV dysfunction with heart failure and/or angina.[20]

METHODS

A phase 2 randomized double-blind, placebo-controlled trial, FOCUS-CCTRN (First Mononuclear Cells injected in the United States conducted by the CCTRN) was designed to evaluate the safety and efficacy of BMCs in patients with chronic ischemic heart disease and LV dysfunction who have no other revascularization options. The primary objective was to determine whether transendocardial administration of 100×10^6 total BMCs improves measures of LV performance and perfusion at 6 months compared with baseline levels.

Briefly, we enrolled patients aged 18 years or older with clinically stable coronary artery disease, LV ejection fraction (LVEF) of 45% or less, limiting angina (Canadian Cardiovascular Society [CCS] class II-IV) and/or congestive heart failure (New York Heart Association [NYHA] class II-III), a perfusion defect by single-photon emission computed tomography (SPECT), and no revascularization options while receiving guideline-based medical therapy.[20]

The study was conducted at the 5 CCTRN centers; the organizational structure and oversight of the CCTRN have been described.[20] The protocol was reviewed and approved by the local institutional review boards at each center. All patients provided written informed consent.

Randomization was computer-generated and used variable block sizes of 6 or 9, randomly selected and stratified by center. All randomized patients underwent baseline testing, bone marrow harvesting, and automated cell processing that was performed locally.[20] Patients were randomized in a 2:1 ratio to receive either BMCs or a placebo (cell-free) preparation. The cell-containing or cell-free preparation was delivered to viable myocardial regions identified during electromechanical mapping of the LV endocardial surface (NOGA, Biologics Delivery Systems, Cordis Corporation). All caregivers and patients were masked to treatment. At 6 months, all baseline testing was repeated in an identical fashion.

Baseline assessments have been described.[20] Demographic and clinical variables were determined by interview and documented from each patient's medical record. Race and ethnicity were recorded as self-described by the patients.

Cell harvesting and processing procedures for all CCTRN protocols have been reported.[21,22] Rigorous automated methods for local cell processing were implemented to ensure quality and uniformity of cell preparation. Briefly, approximately 80 to 100 mL of bone marrow was aspirated from the iliac crest using standard techniques. The aspirate was processed with a closed, automated cell processing system[21] (Sepax, Biosafe SA). Composition of CD34 and CD133 cells was determined by flow cytometry. After the cells passed stipulated lot release criteria, including viability (>70%) and sterility, randomization was performed by the data coordinating center. Treatment assignment was masked to all but 1 designated cell processing team member at each center not involved in patient care. The target dose was 100×10^6 total BMCs. The BMC final product was suspended in normal saline containing 5% human serum albumin and adjusted to a concentration of 100×10^6 cells in 3 mL distributed into three 1-mL syringes. The placebo group received a cell-free suspension in the same volume.

Within 12 hours of aspiration, BMCs or placebo were delivered in 15 separate injections (0.2 mL each) to LV endocardial regions identified as viable (unipolar voltage ≥6.9 mV) by electromechanical mapping as described elsewhere.[20] A 2-dimensional echocardiogram was performed immediately after the injection procedure and on the next day before hospital discharge. Serial measurements of creatine kinase, creatine kinase MB, and troponin also were obtained. All patients remained in the hospital overnight and were then discharged with instructions for guideline-recommended therapy. Patients were examined for safety and efficacy at 6 months. All events deemed to be potential major adverse clinical events were assessed by 2 independent cardiologists not affiliated with any clinical site and masked to treatment assignment. Follow-up for safety continues (up to 12 months postintervention) with annual telephone calls at 2, 3, 4, and 5 years postintervention.

The CCTRN established a cell biorepository core laboratory to advance the understanding of the relationship between cell product characteristics (composition or phenotype and func-

tion) and clinical outcomes.[23] The remaining processed cells (unused cells in the BMC group and all cells from patients in the placebo group) were shipped to the biorepository core laboratory with patient consent.

Echocardiographic measurements were performed by an echocardiography core laboratory according to published guidelines[24] and included LV end-systolic volume (LVESV), LV end-diastolic volume, regional wall motion, and LVEF. Myocardial contrast was used to enhance endocardial definition. These measurements were computed using the biplane rule methods of Simpson as described by Maret et al.[25] The LV volume data were normalized for body surface area and indexed data are presented.

SPECT or adenosine myocardial perfusion tests were performed to identify changes in ischemic (reversible) defects from rest and after adenosine infusion over 4 minutes (or if contraindicated, with a regadenoson bolus) using standardized protocols. To enhance viability detection on resting images, sublingual nitroglycerin was administered 15 minutes before injection of technetium Tc 99m sestamibi for the resting image. Changes in fixed perfusion defects by SPECT also were measured.

Maximal oxygen consumption was assessed by using the Naughton treadmill protocol. Blood flow improvement was examined by magnetic resonance imaging (MRI) in patients without MRI contraindications.

Clinical improvement by CCS classification, NYHA classification, and change in antianginal medications was explored. Serum brain-type natriuretic peptide (BNP) levels were collected in patients with congestive heart failure, and changes in the levels were assessed at 6 months. Major adverse clinical events were assessed and defined as new myocardial infarction, rehospitalization for percutaneous coronary intervention in treated coronary artery territories, death, and rehospitalization for non–myocardial infarction acute coronary syndrome or congestive heart failure.

The 3 prespecified primary end points of change (6-month follow-up minus baseline) in LVESV, maximal oxygen consumption, and defect size on SPECT were compared between the BMC group and the control group.

For each co-primary end point, a sample size was computed based on estimates of the effect size and the standard deviation of the difference from the prior data.[20] Type I error was apportioned at the .05 level to be conducted at 80% power with 10% of patients anticipated as lost to follow-up. All testing was 2-sided. The study was designed to detect a mean difference between the 2 groups of 27 mL for LVESV, 5 mL/kg/min for maximal oxygen consumption, and 10 absolute percentage points for reversible ischemia. To ensure adequate power for each of the 3 end points, the sample size was computed for each one, and the maximum sample size was selected.[20] This produced a sample size of 86 patients, which was administratively increased to 92 patients (31 in the placebo group and 61 in the BMC group). No type I error adjustment for multiple comparisons was incorporated because this was a phase 2 study.

All statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc).[26] Descriptive statistics for baseline characteristics were generated for demographic variables, medical history, physical examination, laboratory data, and clinical events. χ^2 Statistics and *t* tests were used to evaluate the differences between the 2 study groups. General linear modeling techniques assessed the effects of treatment on the continuous primary and secondary outcomes of the study. Both unadjusted and baseline covariate-adjusted treatment effects were computed. Dichotomous secondary end points (ie, clinical improvement at 6 months, change in CCS anginal score and NYHA class, decrease in weekly need for antianginal medication [nitrates]) were analyzed using χ^2 and Fisher exact tests. The time-to-event end points (ie, major adverse clinical events) could not be reliably assessed due to the paucity of events.

Prespecified subgroup analyses for hypothesis generation examined the effects of treatment stratified by age, sex, race, diabetes, serum BNP levels in patients with heart failure, preexisting comorbidity, number of endothelial colony forming cells, and baseline LVEF. Two-sided significance testing was used; *P* values of less than .05 were deemed statistically significant.

RESULTS

Screening commenced in March 2009 and 153 patients provided consent. Between April 29, 2009, and April 18, 2011, 92 patients were randomized (n=61 in BMC group and n=31 in placebo group). Of the 273 patients screened, most were excluded due to not having evidence of reversibility (prior to protocol amendment during the final third period of enrollment) or having an LVEF greater than 45% (**FIGURE 1**).[27]

Briefly, this was an older, white male population (**TABLE 1**). Most (76%) patients had an implantable cardioverter-defibrillator. No statistically significant differences were seen in baseline characteristics between the BMC and placebo groups, except for greater ranolazine use in the BMC group (**TABLE 2**), which was consistent with more patients with CCS class II to IV angina in the BMC group. The mean (SD) LVEF on the qualifying echocardiogram was 32.4% (9.2%) in the BMC group and 30.2% (7.8%) in the placebo group.

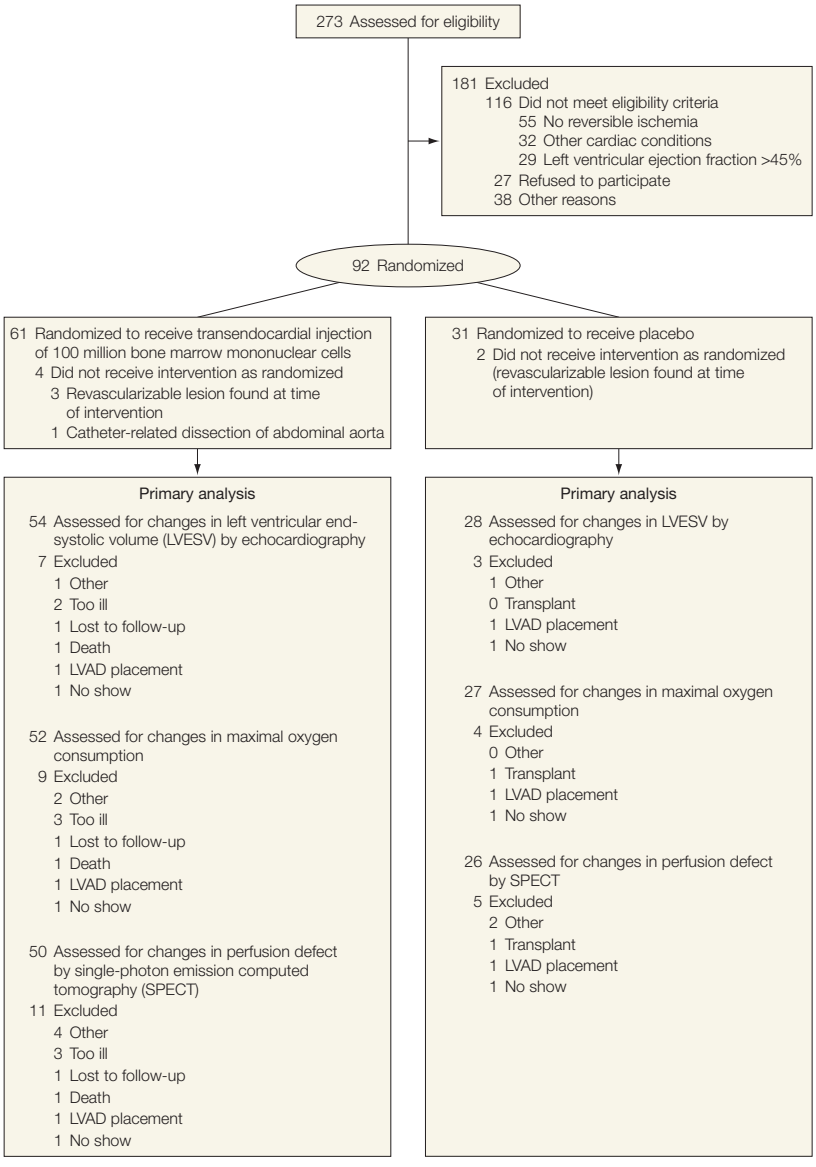
All randomized patients had their bone marrow processed with Ficoll using the automated Sepax device.[21] The mean (SD) volume of bone marrow harvested was 93.7 (8.3) mL. The mean (SD) time from aspiration to product injection was 8.9 (1.2) hours in the BMC group and 8.6 (2.2) hours in the placebo group.

Of the 92 patients randomized, 5 patients were identified as having a lesion suitable for percutaneous revascularization (although no patient had such a lesion identified on the qualifying angiogram). Per protocol, these 5 patients underwent revascularization rather than receive the study product.

A sixth patient experienced a limited retrograde catheter-related dissection of the abdominal aorta that precluded study product delivery.

Electromechanical map–guided injection of cells or placebo was conducted per protocol in the remaining 86 patients. Mean viability of the cell product (Trypan blue exclusion) was 98.6% (TABLE 3). In the BMC group, all but 6 patients received the targeted total dose of 100×10^6 nucleated cells, which contained an average of 2.6% of CD34 cells and 1.2% of CD133 cells. Five of these patients had harvests that contained less than 100 million cells (99.9, 99.6, 99, 80, and 61 million cells). The other patient experienced recurrent ventricular tachycardia with hypotension after each injection and received only a small volume of cell product (approximately 13 million cells).

Figure 1. Flow Diagram of Patients in FOCUS-CCTRN Trial



FOCUS-CCTRN indicates First Mononuclear Cells injected in the United States conducted by the Cardiovascular Cell Therapy Research Network.

A total of 54 patients in the BMC group and 28 patients in the placebo group had paired LVESV data at baseline and at 6 months (FIGURE 2). The mean (SD) LVESV index (LVESVI) at baseline was 57.9 (26.1) mL/m^2 in the BMC group and 65.0 (19.8) mL/m^2 in the placebo group. At 6 months, the mean (SD) LVESVI was 57.0 (25.5) mL/m^2 in the BMC group and 65.0 (23.3) mL/m^2 in the placebo group. The difference in the change in LVESVI between the BMC group and the placebo group was not statistically significant (–0.9 mL/m^2 [95% CI, –6.1 to 4.3]; P=.73).

A total of 52 patients in the BMC group and 27 patients in the placebo group had paired maximal oxygen consumption data at baseline and at 6 months (Figure 2). The mean (SD) baseline maximal oxygen consumption was 14.6 (3.8) mL/kg/min in the BMC group and 15.3 (4.6) mL/kg/min in the placebo group. At 6 months, the mean (SD) maximal oxygen consumption was 15.0 (4.5) mL/kg/min in the BMC group and 14.7 (5.1) mL/kg/min in the placebo group. The difference in the change in maximal oxygen consumption between the BMC group and placebo group was not statistically significant (1.0 [95% CI, –0.42 to 2.34]; P=.17).

A total of 52 patients in the BMC group and 25 patients in the placebo group had paired SPECT evaluations at baseline and at 6 months (Figure 2). The mean (SD) percent reversible defect during the baseline period was 25.1% (27.8%) in the BMC group and 11.8% (20.4%) in the placebo group. At 6 months, the mean (SD) percent reversible defect was 21.3% (26.6%) in the BMC group and 9.2% (9.1%) in the placebo group. The difference in the change for percent reversible defect between the BMC group and placebo group was not statistically significant (–1.2 [95% CI, –12.50 to 10.12]; P=.84).

There were no significant differences in the change between the 2 groups over time for percent total myocardial defect (–0.9 [95% CI, –5.0 to 3.3]), total defect size (–1.6 [95% CI, –5.1 to 1.9]), or fixed defect size (–0.7 [95% CI, –4.8 to 3.4]).

The small number of patients without contraindications for MRI (n=17) precluded performing an informative analysis on the MRI data. There were no significant differences in the change between the 2 groups in regional wall motion (–0.1 [95% CI, –0.30 to 0.14]; *P*=.47) and LV end-diastolic volume index (2.5 [95% CI, –4.4 to 9.3]; *P*=.48).

Forty percent of patients in the BMC group and 47% of patients in the placebo group were NYHA class III at baseline. The decrease over time in the percentage of patients in the BMC group who were NYHA class III was statistically significant (40% vs 20%; for difference: 95% CI, 3% to 37%; *P*=.02); there was no significant difference in the analogous change for the placebo group. However, when the between-group analysis was applied, this finding was not statistically significant. Similarly, there were no significant differences in the change in CCS class (difference in the percent change: 0.18 [95% CI, –0.07 to 0.43]; *P*=.49), serum BNP levels (regular BNP: –40.3 pg/mL [95% CI, –120.2 to 39.7]; *P*=.32 and probrain-type natriuretic peptide: 150.2 pg/mL [95% CI, –1215.2 to 1515.6]; *P*=.82), or decrease in the need for antianginal medication between the 2 groups at 6 months (2 in BMC group and 0 in placebo group; difference in the percent change: 0.04 [95% CI, –0.01 to 0.09]; *P*=.28).

Subgroup analyses examined the effects of demographics, comorbidities (age, sex, diabetes mellitus, hypertension, angina, and hyperlipidemia), and cell surface markers (CD34 and CD133) on end point measures. There were no significant differences. Additional outcomes were examined for exploratory purposes.

The baseline LVEF was available in 54 patients in the BMC group and in 28 patients in the placebo group. The mean (SD) baseline LVEF was 34.7% (8.8%) in the BMC group and 32.3% (8.6%) in the placebo group. At 6 months, the mean (SD) LVEF change was an increase of 1.4% (5.2%) in the BMC group and a decrease of –1.3% (5.1%) in the placebo group. This difference was significant (2.7 [95% CI, 0.3 to 5.1]; *P*=.03). Findings for stroke volume were similar, with a mean (SD) increase of 2.7 (12.9) mL in the BMC group and a decrease of –5.8 (15.2) mL in the placebo group; this difference was significant (8.4 [95% CI, 2.1 to 14.8]; *P*=.01).

In an exploratory analysis, BMC therapy was associated with an improvement in maximal oxygen consumption for patients with number of endothelial colony-forming cells greater than the median value of 80 (change: 2.5 [95% CI, 0.16 to 4.88]). However the interaction test for this assessment was not significant (interaction effect size: 2.61 [95% CI, –0.30 to 5.51]; *P*=.08).

A regression analysis showed that higher CD34 cell or CD133 cell counts were associated with greater absolute unit increase in LVEF. The range of CD34 was 0.5% to 6.9% (SD, 1.2%). Assuming that differences of 1.96 for SD or 2.4% are more likely due to biological variability, the effect of differences in CD34 cell level beyond that expected due to natural variability was examined, using a 3% level to be conservative. Every 3% higher level of CD34 cells was associated with on average a 3.0% greater absolute unit increase in LVEF in a multiple variable model that included age and treatment as predictor variables (3.06 [95% CI, 0.14-5.98]; *P*=.04). An analogous computation for CD133 cells (range, 0.1%-3.6%; SD=0.62) revealed that every 3% higher level of CD133 cells was associated with on average a 5.9% greater absolute unit increase in LVEF (5.94% [95% CI, 0.35%-7.57%]; *P*=.04).

Table 1. Patient Baseline Characteristics

	BMC Group (n = 61)	Placebo Group (n = 31)	*P* Value
Mean (SD)			
Age, y	63.95 (10.90)	62.32 (8.25)	.47
Height, cm	174.50 (8.79)	177.42 (9.60)	.15
Weight, kg	91.53 (22.00)	99.99 (24.23)	.10
Body mass index[a]	30.10 (6.14)	31.80 (6.60)	.23
Blood pressure, mm Hg			
Systolic	120.59 (19.69)	122.13 (15.78)	.71
Diastolic	70.95 (11.18)	74.77 (10.35)	.12
Heart rate, beats/min			
Mean (SD)	67.90 (10.45)	72.61 (13.60)	.07
Median (range)	65.00 (51.00-100.00)	70.00 (49.00-107.00)	
No. (%)			
Female sex	8 (13.11)	2 (6.45)	.49
White race	58 (95.08)	30 (96.77)	>.99
Hispanic ethnicity	3 (4.92)	1 (3.23)	>.99
New York Heart Association classification			
I	6 (9.84)	2 (6.45)	.59
II	32 (52.46)	14 (45.16)	
III	23 (37.70)	15 (48.39)	
IV	0	0	
Canadian Cardiovascular Society classification	(n = 54)	(n = 25)	
I	13 (24.07)	10 (40.00)	.45
II	24 (44.44)	10 (40.00)	
III	16 (29.63)	5 (20.00)	
IV	1 (1.85)	0	
Qualifying LVEF by echocardiography, mean (SD), %	(n = 60) 32.43 (9.23)	(n = 31) 30.19 (7.76)	.25
Aspiration to injection time, h	(n = 58)	(n = 29)	
Mean (SD)	8.95 (1.18)	8.56 (2.22)	.28
Median (range)	9.01 (6.52-11.40)	8.98 (0.22-11.40)	

Abbreviations: BMC, bone marrow mononuclear cell; LVEF, left ventricular ejection fraction.
[a] Calculated as weight in kilograms divided by height in meters squared.

The patients were divided based on median age of the population (≤62 years and >62 years). No statistically significant effect of therapy was seen for the primary end points (LVESV, maximal oxygen consumption, and percent reversibility on SPECT) for age. No significant differences were seen on the secondary end points or cell product variables in the subgroup analysis, except for those described below.

Table 2. Baseline Patient Medical History, Medication Use at Time of Randomization, and Laboratory Evaluations

	No. (%) of Patients[a]		
	BMC Group (n = 61)	Placebo Group (n = 31)	*P* Value
Medical history			
Diabetes	21 (34.43)	16 (51.61)	.12
Hypertension	49 (80.33)	24 (77.42)	.79
Hyperlipidemia	57 (93.44)	29 (93.55)	>.99
Angina	21 (34.43)	12 (38.71)	.82
Former or current smoking	46 (75.41)	20 (64.52)	.33
Prior myocardial infarction	**(n = 57)** 53 (92.98)	**(n = 31)** 29 (93.55)	>.99
Prior revascularization	51 (83.61)	26 (83.87)	>.99
Prior coronary artery bypass graft surgery	47 (77.05)	25 (80.65)	.79
No. of operations			
1	33 (70.21)	21 (84.00)	.39
2	13 (27.66)	4 (16.00)	
3	1 (2.13)	0	
Congestive heart failure			
Yes	36 (59.02)	20 (64.52)	.66
Prior hospitalization	14 (22.95)	9 (29.03)	.61
Asymptomatic carotid disease	11 (18.03)	3 (9.68)	.37
History of stroke or transient ischemic attack	8 (13.11)	1 (3.23)	.26
Valvular heart disease	18 (29.51)	8 (25.81)	.81
Peripheral vascular disease	13 (21.31)	3 (9.68)	.25
History of arrhythmia	**(n = 56)** 29 (51.79)	**(n = 28)** 14 (50.00)	>.99
Cardiac pacemaker	42 (68.85)	23 (74.19)	.64
Implantable cardioverter-defibrillator	3 (4.92)	2 (6.45)	>.99
Dual chamber pacing	17 (18.5)	10 (10.9)	.81
Medication use at time of randomization			
ACE inhibitor or ARB	37 (60.66)	22 (70.97)	.37
Aldosterone inhibitor	9 (14.75)	8 (25.81)	.26
Aspirin/P2 Y12	53 (86.89)	29 (93.55)	.49
β-Blockers	57 (93.44)	30 (96.77)	.66
Warfarin	10 (16.39)	4 (12.90)	.77
Digitalis	4 (6.56)	4 (12.90)	.44
Diuretics	41 (67.21)	23 (74.19)	.63
Nitrates	39 (63.93)	18 (58.06)	.65
Statins	44 (72.13)	21 (67.74)	.81
Ranolazine	21 (34.43)	3 (9.68)	.01
Laboratory evaluations, median (range)			
Hemoglobin, g/dL	14.0 (10.0-16.9)	14.3 (12.4-16.6)	.21
High-sensitivity C-reactive protein, mg/L	**(n = 54)** 1.4 (0.1-37.0)	**(n = 29)** 1.1 (0-86.4)	.60
Glomerular filtration rate, mL/min/1.73 m^2	**(n = 58)** 71.2 (29.6-155.4)	**(n = 29)** 70.1 (30.5-107.3)	.96
Brain-type natriuretic peptide, pg/mL	**(n = 46)** 132.0 (16.0-545.0)	**(n = 23)** 105.0 (26.0-140.0)	.68
Probrain natriuretic peptide, pg/mL	**(n = 15)** 833.0 (50.0-9793.0)	**(n = 8)** 828.0 (103.0-5778.0)	.95

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMC, bone marrow mononuclear cell.
SI conversion factors: To convert C-reactive protein to nmol/L, multiply by 9.524; hemoglobin to g/L, multiply by 10.0; natriuretic peptide to ng/L, multiply by 1.0.
[a]Unless otherwise indicated.

When LVEF was assessed, patients aged 62 years or younger showed a statistically significant effect of therapy. Patients in the BMC group demonstrated a mean (SD) increase in LVEF of 3.1% (5.2%) from baseline to 6 months, whereas patients in the placebo group showed a decrease of –1.6% (6.6%). The difference in the change between groups was significant (4.7% [95% CI, 1.0% to 8.4%]; *P*=.02).

There were no in-hospital events (other than the dissection noted earlier). One patient died 29 days after BMC delivery due to pump failure, which was deemed unlikely to be associated with cell therapy. Another patient had a myocardial infarction 61 days after BMC delivery; the infarction did not occur in the targeted injection area, and the patient was discharged from the hospital 4 days later. There were no rehospitalizations in either group for percutaneous coronary intervention prior to the 6-month visit. Eight patients (3 in the BMC group and 5 in the placebo group) were rehospitalized for congestive heart failure, with 1 additional patient in the BMC group rehospitalized for acute coronary syndrome during this same time frame. One patient in the placebo group underwent heart transplantation, and 2 other patients (1 in each group) had LV assist device placements before the 6-month visit.

COMMENT

The CCTRN was developed by the National Heart, Lung, and Blood Institute to advance cell therapy for patients with cardiovascular diseases by using a collaborative network approach to facilitate larger studies with wide applicability. The FOCUS-CCTRN trial is the first, to our knowledge, adequately powered study of cell therapy in patients with chronic ischemic heart disease and LV dysfunction (LVEF ≤45%) to be completed in the United

States. We found no significant differences in a priori selected primary end points between patients treated with BMCs and placebo in this first-in-man study that administered 100 million cells via transendocardial injection. The protocol randomized 92 patients from a cohort of 153 patients who provided consent, demonstrating the efficiency and expertise of network recruiting as well as the perceived need by the community with heart failure for therapy to address this disease.

Primary end points used in previous cell therapy trials of heart failure have been arbitrarily chosen due to lack of sufficient historical data in stem/progenitor cell trials. In these previous studies of patients with ischemic heart disease, both LVEF (by echocardiography) and myocardial ischemia (by SPECT) were used to measure outcomes of interest and suggested improvement.[5,28] However, these trials enrolled patients with mostly preserved LVEF (ranging from 48% to 56%). The recently published FOCUS-HF trial is one of the first reported studies of autologous BMC therapy in patients with ischemic heart failure and low LVEF.[14] That phase 1 trial also demonstrated a lack of improvement in the measures that were selected for the current study (LVESV, maximal oxygen consumption, and percent reversibility on SPECT); however, at the time the CCTRN-FOCUS was designed, the results of FOCUS-HF were unknown.

Power calculations for the primary end points selected for the current study assumed ambitious improvements after BMC injections in maximal oxygen consumption of 5 mL/kg/min, in LVESV of 22 mL, and in reversibility on SPECT of 10% based on results from a pilot study from Brazil.[10] Since then, exercise training in patients with heart failure and low LVEF (HF-ACTION [A Controlled Trial Investigating Outcomes of Exercise Training]) resulted in only a 0.6 mL/kg/min improvement in maximal oxygen consumption using the same protocol used in FOCUS-CCTRN.[29] Defining end points in this field continues to be a major challenge.

Table 3. Bone Marrow Mononuclear Cell Product Characteristics

	Mean (SD)		
	BMC Group (n = 61)	Placebo Group (n = 31)	*P* Value
Total nucleated cells/product, $\times 10^6$	99.03 (5.58)	100.03 (0.18)	.32
% Viability/product by Trypan blue exclusion	98.56 (1.11)	98.70 (0.89)	.52
% of CD34 cells/product[a]	**(n = 57)** 2.71 (1.19)	**(n = 30)** 2.60 (0.93)	.67
% of CD133 cells/product[a]	**(n = 57)** 1.21 (0.62)	**(n = 30)** 1.14 (0.48)	.59
Colony-forming units-Hill/product[a]	**(n = 55)** 109.41 (206.29)	**(n = 30)** 151.33 (244.20)	.40
Endothelial colony-forming cells/product[a]	**(n = 49)** 131.84 (164.62)	**(n = 28)** 156.44 (240.12)	.60

Abbreviation: BMC, bone marrow mononuclear cell.
[a]Four patients either declined to participate or had insufficient product for the biorepository.

In the present phase 2 study, exploratory analyses revealed that LVEF improved in the BMC group compared with the placebo group by 2.7%. This difference is in keeping with results from a previous meta-analysis of BMC therapy in patients with chronic ischemic heart disease and in smaller, individual trials that evaluated BMC therapy in similar patients.[1,30-32] The modest improvement in LVEF in our study is consistent and may be more meaningful in light of the larger number of patients enrolled.

To examine this finding further, we assessed LVEF with respect to the bone marrow characteristics of the patient. Improvement in LVEF correlated with the percentage of CD34 and CD133 cells in BMC samples. This correlation was based on a central biorepository assessment of cell surface markers present in the cell product. Evaluating inherent variability in the cell product may provide mechanistic insight into the relationship between cell characteristics and both patient baseline characteristics and clinical outcomes.

Losordo et al[33] recently found a reduction in angina and increased exercise duration with the delivery of autologous CD34 cells in patients with refractory angina who had normal LVEF (≥55%) overall. Because baseline LVEF in patients in the BMC group (32.4%) in our study represented patients with significant LV dysfunction, a meaningful comparison of the results of the 2 studies is difficult. However, both CD133 and CD34 cell populations have been shown to give rise to endothelial and vascular progenitor cells and to secrete chemokines and cytokines capable of recruiting cells and promoting cell survival.[34-37] These findings support a model in which CD34 and CD133 cells might improve myocardial oxygenation and LV function in areas of ischemia and/or hibernating myocardium; however, further study is needed.

In the FOCUS-HF trial,[16] maximal oxygen consumption improved in the younger patients and was correlated with cell function in an exploratory analysis. In the present study, maximal oxygen consumption improvement was correlated with endothelial cell function, which warrants further investigation. Additional analyses of cell function will be forthcoming from the CCTRN biorepository and may provide further meaningful correlations with outcome measures.

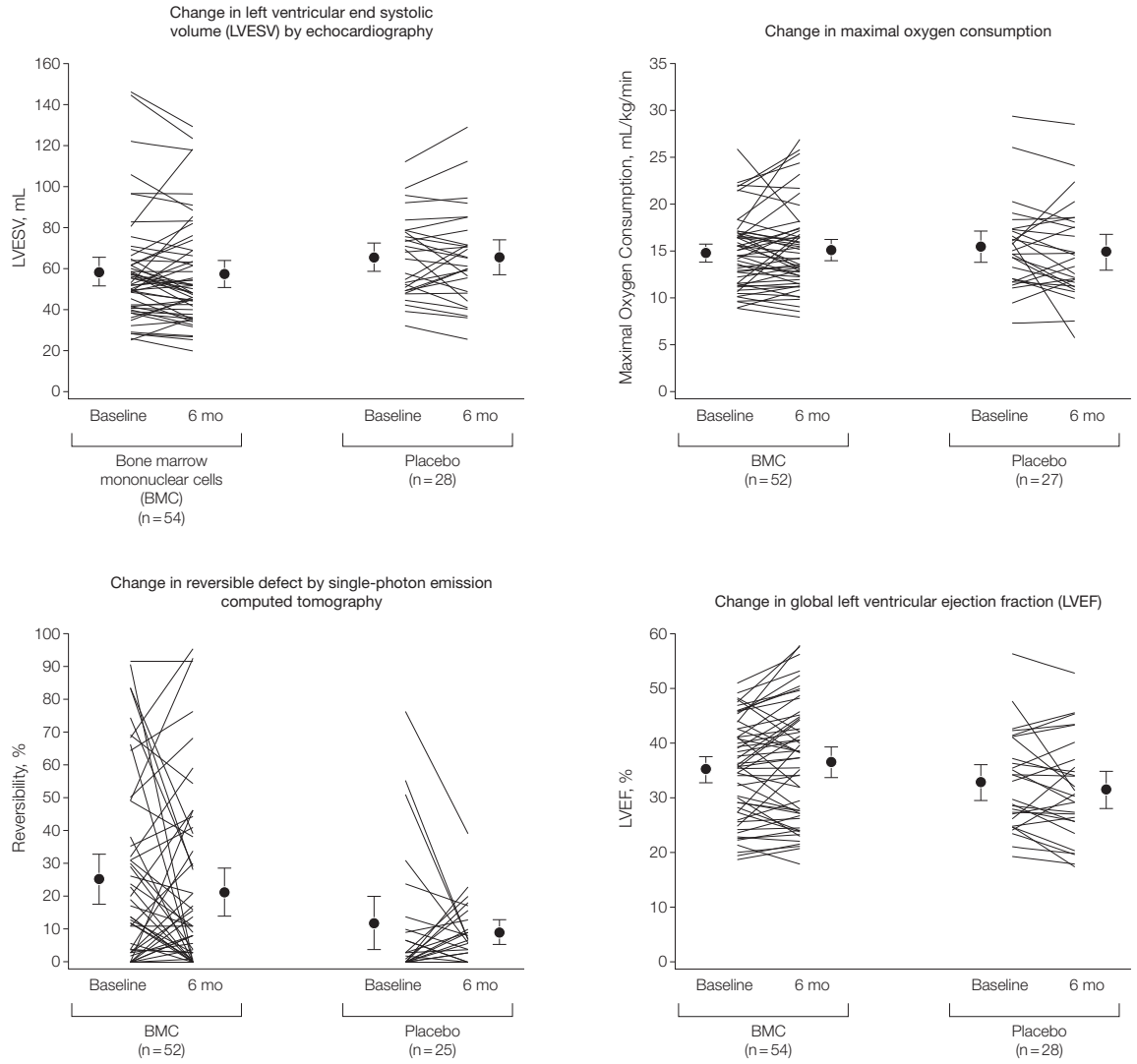
Establishment of the biorepository core laboratory by the CCTRN marks an important step forward in understanding the role of cell function in cardiac cell therapy. By providing mechanistic insight, cell phenotypic and functional studies will help to define meaningful end points for future studies and will aid in selecting patients most likely to receive maximal benefits from autologous therapy.

Although this study enrolled more patients than previous heart failure trials in patients with ischemic heart disease and low LVEF, the sample size was still relatively small. The sample size chosen required large improvements in selected end points to show a significant treatment effect. This choice occurred principally due to the paucity of data available for these evaluations. A large percentage of the patients in our study had contraindications for MRI, thus precluding a meaningful evaluation of the MRI data.

SPECT sestamibi often underestimates myocardial viability and reversibility in patients with multivessel coronary artery disease compared with SPECT thallium or positron emission tomography.[38-41] After approximately 20 months of enrollment in the present study, investigators noted a discrepancy between the amount of reversibility present on baseline SPECT and the subsequent finding of viable myocardium by electromechanical mapping as well as the presence of angina.

Even with minimal reversibility (eg, 1%) on baseline SPECT sestamibi, a significant amount of myocardial viability was noted on electromechanical mapping. For this reason and to facilitate enrollment because many patients were being excluded due to the SPECT reversibility criterion, in November 2010 the protocol was amended during the final third period of enrollment to include patients with any perfusion defects (ie, fixed or reversible). This may have skewed the population to patients with a lesser degree of myocardial viability, limiting the areas suitable for cell injection. In addition, the study size precludes any determina-

Figure 2. Changes in Major Outcomes Over Time by Therapy



Solid circles indicate mean values at baseline and 6 months. Error bars indicate 95% confidence intervals.

tion of the effect of therapy on the occurrence of community-wide accepted clinical outcomes (eg, total mortality), which must be addressed in larger forthcoming studies. Although there was a difference in LVEF in an exploratory analysis, its clinical significance is conditional.

CONCLUSIONS

In the largest study to date of autologous BMC therapy in patients with chronic ischemic heart disease and LV dysfunction, we found no effect of BMC therapy on prespecified end points. Further exploratory analysis showed a significant improvement in LVEF associated with treatment. Our findings provide evidence for further studies to determine the relationship between the composition and function of bone marrow product and clinical end points. Understanding these relationships will improve the design and interpretation of future studies of cardiac cell therapy.

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