ONLINE SUPPLEMENT

SUPPLEMENTAL METHODS:

Study Design and Patient Selection

Details of the study design and justification have been previously reported.[1] Breifly, eight patients with a diagnosis of ischemic cardiomyopathy were enrolled between October 2008 and June 2009 to document the feasibility and procedural safety of intramyocardial injections of autologous bone marrow progenitor cells via the Helical Infusion Catheter (BioCardia, San Carlos, CA) after remote MI. Data was collected over one year after cell transplantation to assess safety, clinical outcomes, and conduct detailed cardiac structure and function phenotyping using cardiac magnetic resonance imaging (CMR). Patients were eligible if they had chronic ischemic left ventricular dysfunction (LVEF 20-50%) secondary to a previous MI and were on maximal medical therapy for heart failure. Patients were documented to have chronic myocardial scars by CMR, and in all cases were fully revascularized in the infarct related territory by documented cardiac catheterization prior to enrollment. Computed tomography (CT) of the chest, abdomen, and pelvis was obtained and patients excluded if an occult malignancy was identified. Written informed consent was obtained from all patients on this University of Miami Institutional Review Board (IRB) approved protocol.

Harvest, Processing, and Delivery of Bone Marrow Cells

All patients underwent a bone marrow aspiration (BMA) from the iliac crest. Bone marrow aspirate was immediately transported to the University of Miami Good Manufacturing Practice cell lab. Bone marrow mononuclear cells were isolated using Ficoll density gradient centrifugation. The cells were then washed and prepared for infusion. Cells were suspended in 2.5ml of phosphate buffered saline for the first 4 patients and 5ml for the subsequent 4 patients. Patients who were assigned to the bone marrow mononuclear cell arm (n=4) were injected approximately 4-hours following BMA. In the patients allocated to the bone marrow mesenchymal stem cell arm (n=4), the MSCs were isolated from the bone marrow mononuclear cells based on plastic adherence and expanded in culture. After approximately 4-5 weeks in culture, sufficient quantities of MSCs were available and injected into the patients.

The bone marrow cells were delivered to the myocardium by transendocardial injections using the BioCardia Helical Infusion Catheter during cardiac catheterization. Right anterior oblique (RAO) and left anterior oblique (LAO) ventriculograms were obtained with biplane angiography. End-diastolic endocardial border contours were traced over the ventriculograms. Using the previously acquired CMR delayed hyperenhancement images and the wall motion abnormalities on the ventriculograms, an akinetic infarct zone (IZ) and hypokinetic border zone (BZ) were marked. The suspension of bone marrow progenitor cells was divided into 10 syringes of 0.25ml for the first 4 patients and 0.5ml aliquots for the next 4 patients. The ventriculogram tracings were used to guide the Helical Infusion Catheter to the IZ and BZ, where the corkscrew tip of the catheter was inserted. A small injection of iodinated contrast was injected via the side port of the catheter to confirm engagement with the myocardium. The syringes containing the cells were injected followed by a dwell time to prevent washout of cells. This was repeated in 10 areas of the IZ and BZ.

Safety Assessment

The primary outcome of the study was to assess the safety of transendocardial administration of bone marrow mononuclear and mesenchymal stem cells as determined by the incidence of treatment emergent serious adverse events (TE-SAE) defined as the composite of death, non-fatal myocardial infarction (MI) stroke, hospitalization for worsening heart failure, cardiac perforation, pericardial tamponade, ventricular arrhythmias lasting > 15 seconds or with hemodynamic compromise, or atrial fibrillation one-month post-catheterization. Secondary outcomes assessed clinical, laboratory and CMR endpoints, as discussed below. All patients were admitted to the hospital following catheterization for a 72-hour observation period. Peri-procedural safety monitoring included echocardiograms to evaluate for pericardial effusion, Holter ECG monitoring, and cardiac enzymes. Pulmonary function testing was performed at baseline and at 12 months. A 12-month whole body CT was compared to the pre-injection CT to assess for ectopic tissue growth.

CMR Analysis

CMR was used to analyze secondary endpoints of structural and functional effects of bone marrow progenitor cell injections on chronic ischemic heart failure. Cine CMR was used to determine global cardiac function[2] and tagged cine myocardial imaging[3] was used to assess regional LV function. Delayed myocardial enhancement with intravenous gadolinium was used to quantify infarct size.[4] All patients underwent CMR at baseline, 3 months, 6 months, and 1 year after stem cell transplantation; one patient refused his 3 month and 1 year CMR

CMR was performed on a 1.5 T scanner (Signa HDx, GE Healthcare, Waukesha, WI) using an 8-channel body coil with ECG gating and breath-hold acquisitions. Steady-state free precession (SSFP) cine images in short axis planes (8mm slices with 2mm gap, FOV 35-40cm, matrix 256x200, TR/TE 3.7ms/1.6ms) were obtained; fast gradient echo tagged short axis images with grid pattern (8mm slices with 2mm gap, matrix 256x128, TR/TE 6.8ms/3.2ms); and short axis and multiple long axis views of delayed myocardial enhancement imaging acquired 10 minutes following intravenous gadolinium (0.2mmol/kg; Magnevist, Bayer Healthcare, Wayne, NJ) (8mm slices with 2mm gap, matrix 192x160, TR/TE 4.4ms/1.3ms). In patients with ICD devices, fast gradient echo cine images (matrix 192x128, TR/TE 5.1ms/2.8ms) were substituted for the SSFP cine images to limit artifacts. Patients enrolled in this study that had an implantable cardioverter defibrillator (ICD) were imaged in the magnet based on a previously reported protocol.[5,6] One patient refused his 3 and 12 month CMR scan

CMR analytical software Segment (Medviso AB; Lund, Sweden) was used to calculate LV mass, end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) from short axis cine images. Scar volume and scar size as a percentage of LV mass were calculated from delayed enhancement (DE). At end-diastole and end-systole, epicardial and endocardial contours were drawn in sub-centimeter continguous slices covering the apex to mitral valve plane to obtain left ventricle EDV, ESV, and LV mass. Global left ventricle EF was calculated as [(EDV-ESV)/EDV] x 100%. Infarct scar size was determined from the short axis DE images covering the apex to the mitral valve annulus; tissue an intensity signal > 2 standard deviations above reference normal myocardium was identified as scar and calculated as absolute scar volume and scar as a percentage of LV mass. Because of artifact from the ICD, three patients did not have interpretable delayed enhancement scans and were excluded from the DE scar analysis.

Regional function was measured by tagged CMR images using HARP software (Diagnosoft; Cary, NC). Three contiguous short axis tagged images encompassing the scar were selected for analysis. User defined epicardial and endocardial contours were drawn to create a 24-segment mesh for each slice, and eulerian circumferential strain (Ecc) for each segment at each time point of the cardiac cycle was measured. Using the RV insertion as a reference point, corresponding delayed enhancement and tagged images were segmented into infarct zone (IZ), border zone (BZ), remote zone (RZ). The hyperenhanced zone that was the target for intramyocardial stem cell injections was identified as the IZ and both lateral neighboring 15-degree segments defined as the BZ. A 45-degree segment away from the IZ without enhancing myocardium was marked as RZ. The peak Ecc for each zone was calculated by averaging the peak Ecc (more negative is greater contractility) from each individual segments of the specified zone. The same slices and zones were used between all time points. One patient had poor tracking of tagged images due to ICD artifacts and was excluded from this analysis.

Statistical Analysis

Endpoints following stem cell injection were analyzed with a repeated-measures analysis of variance (ANOVA) with terms for time. Least squares mean and standard errors were estimated along with p-value from the F-statistic using PROC MIXED (SAS version 9.2, Cary, NC) assuming compound symmetry covariance structure. All values are presented as means ± standard error of mean (SEM) unless otherwise noted. Safety data were summarized with the use of descriptive statistics. Linear correlation analyses were applied to the difference in peak Ecc to the changes in EDV and ESV. All tests were 2-sided and a p-value less than 0.05 was considered statistically significant.

SUPPLEMENTAL RESULTS:

Patient Population and Safety Outcomes

Eight patients were enrolled in this study with baseline characteristics summarized in the Online Table I. The mean age was 57.2±13.3 years, all were male, and all patients had suffered a remote MI (68.8±21.4 months, range 4 months to 11 years). All patients tolerated the bone marrow aspiration without complication and all cell products were manufactured to the protocol defined total dose. Six patients received all 10 injections, of which one had clumping of BM-MSCs and received 50 million cells in the required total volume. Due to losses in transfer of final cell product, one patient received 8 injections (160 million cells) and one patient received 9 injections (150 million cells).

No patient experienced a TE-SAE as previously defined. Perforation, MI, and sustained ventricular arrhythmias are potential complications of intramyocardial injection and were not observed in this study. All patients tolerated the stem cell injections with only transient PVCs, similar to guidewire induced PVCs during catheterization. One patient experienced a non-sustained VT on day 4 post-injection, which resulted in placement of an ICD. There were 2 other patients that received prophylactic ICD as recommended by their primary cardiologist and not due to study related complications. Echocardiography showed one case of a trivial pericardial effusion (less than 0.5cm) at 48-hours post injection that resolved without intervention. Post-procedure and monthly Holter-ECG recordings showed all patients (n=8) to

be in normal sinus rhythm and no cases of ventricular fibrillation were documented (n=0). All patients had episodes of asymptomatic, non-sustained ventricular tachycardia within one-month of injection (n=8, range 3-12 beats). As shown in Online Table II, intramyocardial injection caused a small but significant increase in Troponin-I and CPK-MB at 12 hours which, began resolving by 24 hours. These increases in enzymes are likely the result of needle manipulation of myocardium and did not produce clinical symptoms or ST changes on ECG similar to previously documented animal models.[7] Whole body CT showed no evidence of new ectopic tissue formation in any patient at 12 months. Pulmonary function tests (PFTs) demonstrated no change in forced expiratory volume in 1 second (FEV1) between baseline and 12 months [2.92±0.25 vs. 2.94±0.22 (81.4 vs. 90.1% of predicted value), p=NS]. Our safety data at one year shows a consistent profile with other clinical trials using intramyocardial stem cell injections of autologous bone marrow cells with the Helical Infusion Catheter.[8]

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Online Table I. Patient characteristics

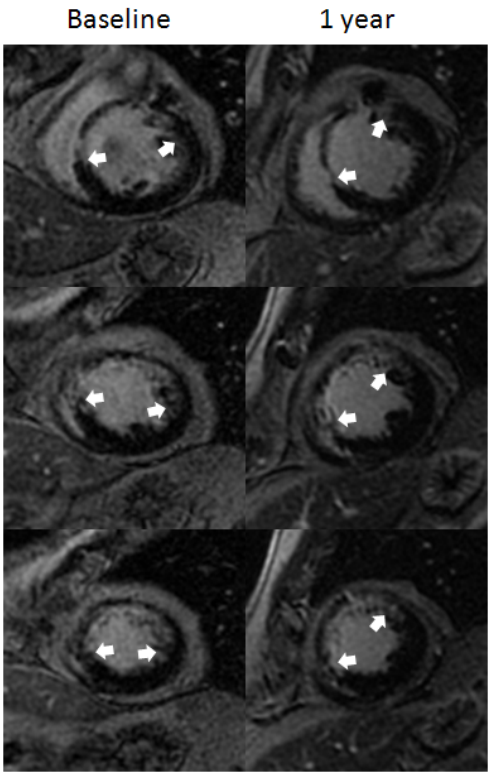
Pt	Age (yrs)	Gender	NYHA Class	HR	BP	History of smoking	DM	Beta blocker	ACE-I or ARB	Previous PCI/CABG*	Age of infarct at injection	Infarct target wall	Bone Marrow Cells (number)
1	65	M	1	51	121/51	N	N	Y	Y	CABG	8 years	Inferior	MNC (100 million)
2	35	M	1	65	108/63	Y	N	Y	Y	PCI	4 months	Anterior/ Septum	MSC (100 million)
3	78	M	1	53	141/78	N	N	Y	Y	PCI	9 years	Lateral	MSC (100 million)
4	51	M	2	69	108/74	Y	N	Y	Y	PCI	6 months	Septum	MNC (100 million)
5	65	M	1	60	147/68	Y	N	Y	Y	PCI	11 years	Anterior/ Septum	MNC (200 million)
6	45	M	1	71	119/73	N	Y	Y	N	PCI	11 years	Anterior/ Septum	MSC (200 million)
7	61	M	3	73	159/85	Y	N	Y	Y	PCI	4 years	Inferior	MSC (200 million)
8	54	M	2	63	101/71	Y	N	Y	Y	PCI	5 months	Anterior	MNC (200 million)

Pt=patient, Age=Age at Injection, M=Male, Y=yes, N=no, NYHA=New York Heart Association Class, HR=heart rate, BP=blood pressure, DM=Diabetes Mellitus, Beta Blocker=On a beta blocker pre-injection, ACE-I=On angiotensin converting enzyme inhibitor or angiontensin receptor blocker (ARB) pre-injection, PCI=percutaneous coronary intervention, CABG=coronary artery bypass grafting, MNC=mononuclear cells, MSC=mesenchymal stem cells. *As per study protocol, all patients underwent coronary revascularization a minimum of 3 months prior to stem cell injection.

Online Table II. Cardiac enzymes following transendocardial bone marrow injections

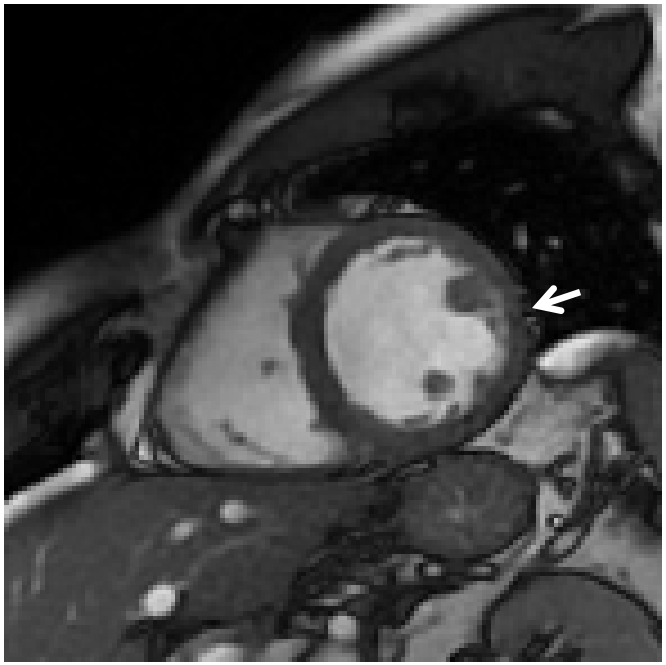
	Time after stem cell injection					
Cardiac Enzyme	**Baseline**	**12 hr**	**24 hr**	**36 hr**	**48 hr**	**p value**
Troponin-I (ng/mL)	0.008±0.01	0.85±0.2	0.44±0.2	0.36±0.2	0.32±0.2	0.003
CPK-MB (ng/mL)	3.10±0.8	4.01±1.1	1.88±0.5	1.31±0.3	1.33±0.4	0.002

All values are means ± SEM.

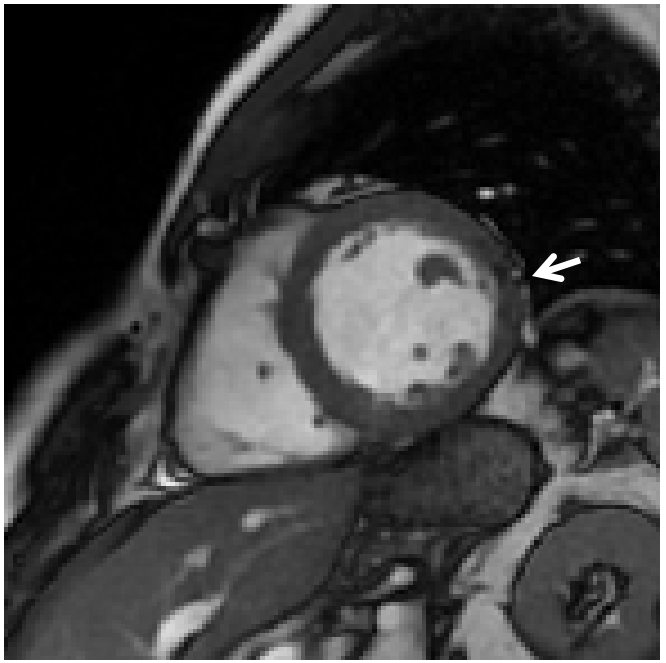


Online Figure I. Example changes in an anterior wall infarct one year after transendocardial bone marrow stem cell injections. Sequential gadolinium delayed-enhancement cardiac MRI short-axis images from base (top) to apex (bottom) of an anterior/septal infarct (between white arrows) at baseline (left) and at one year (right). The EDV decreased from 225.2 to 196.3mL and the ESV decreased from 143.9 to 124.8mL from baseline to 1 year, respectively. A 25% reduction in scar size as a percentage of LV mass was evident at 1 year.

A.



B.



Online Figure II. Short-axis cine cardiac MRI at (A) baseline and (B) 1 year post-injection of bone marrow progenitor cells. This patient had a chronic lateral wall infarct (white arrow) that was treated and showed improved regional contractility at 1 year.