

Safety and efficacy of bone marrow-derived autologous CD133⁺ stem cell therapy

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1. ABSTRACT

The Phase I clinical study was designed to assess the safety and feasibility of a dose escalating intracoronary infusion of autologous bone marrow (BM)-derived CD133⁺ stem cell therapy to the patients with chronic total occlusion (CTO) and ischemia. Nine patients were received CD133⁺ cells into epicardial vessels supplying collateral flow to areas of viable ischemic myocardium in the distribution of the CTO. There were no major adverse cardiac events (MACE), revascularization, re-admission to the hospital secondary to angina, or acute myocardial infarction (AMI) for the 24-month period following cellular infusion. In addition, there were no periprocedural infusion-related complications including malignant arrhythmias, loss of normal coronary blood flow or acute neurologic events. Cardiac enzymes were negative in all patients. There was an improvement in the degree of ischemic myocardium, which was accompanied by a trend towards reduction in anginal symptoms. Intracoronary infusion of autologous CD133⁺ marrow-derived cells is safe and feasible. Cellular therapy with CD133⁺ cells to reduce anginal symptoms and to improve ischemia in patients with CTO awaits clinical investigation in Phase II/III trials.

2. INTRODUCTION

Therapies for coronary ischemia and subsequent myocardial necrosis include acute revascularization, coronary artery bypass grafting (CABG) and pharmacologic interventions targeted at reducing ischemia and ultimately preserving myocardium and prolonging life. Morbidity and mortality related to myocardial necrosis regardless of etiology has been reduced dramatically with the institution of these therapies. Others and we reported that hematopoietic stem cells have the promise in reducing total ischemia via neovascularization in various animal models (1-4). Researchers and clinicians are interested to develop improved therapeutic regime for the treatment of ischemia and ultimately facilitate improvement of symptoms for the betterment of patient's life. Specifically, patients with chronic total occlusion (CTO) provide a therapeutic challenge as they experience stable chronic angina, which is difficult to control and or ameliorate (5). Percutaneous coronary intervention (PCI) to CTO account for approximately 12-16% of all interventions in the United States (6). The Occluded Artery Trial recently revealed that PCI does not reduce MACE and may in fact provoke myocardial ischemia with increase reinfarction rates in clinically stable patients with persistent total occlusion (7).

Many of these patients have areas of viable, yet jeopardized areas of myocardium. Development of collateral growth is an important compensatory mechanism with respect to CTO. Collateralization may provide adequate augmentation of blood flow to ischemic tissue protecting patients with CTO from angina. However, collateralization may not provide this protection and further augmentation of collateral growth may be necessary to generate reduction or cessation of symptoms.

Hematopoietic stem cells, which express CD34 and CD133 surface markers have been shown in models of acute and chronic ischemia to augment blood flow and prevent myocardial necrosis (4, 8-16). Postulated mechanisms for this process include paracrine effects, which may provide substrate for promoting myocardial healing and/or angiogenesis (17, 18). Recently, a number of clinical trials have attempted to determine the role of stem cells in promoting an *in situ* reparative milieu (19-29). The studies utilized autologous cellular products, primarily whole mononuclear cell preparations. In addition, different delivery techniques were attempted and a unified cell dose was not used. We postulate that a selected cell population may have a therapeutic advantage over whole cell preparations because it provides a pure potent stem cell fraction, which were reportedly having the potential for neovascularization and differentiation ultimately resulted to reduction of ischemia. Moreover, all of the current studies have illustrated safety with single dose applications without an attempt at titration with respect to safety. Therefore, we aimed to determine if infusion with increasing cell dose of autologous CD133⁺ selected stem cells was safe and feasible in patients with CTO.

3. METHODS

3.1. Patient selection

A total of nine patients were enrolled between January and June 2006 and followed for a period of 24 months after the date of the procedure. Patients underwent screening for enrollment within 30 days of therapy. This Phase I, single center study enrolled patients of at least 18 years of age who experienced class II-IV angina (Canadian Cardiovascular Society classification). Identification by nuclear imaging of at least one region of chronically ischemic or viable (hibernating) myocardium formerly perfused by a non-revascularizable totally occluded coronary artery was required for inclusion. In addition, well-established collateral vessels of at least 1.5-mm in luminal diameter to the viable myocardium at the time of diagnostic coronary angiography must have been present to be included in the trial. Patients included had also a left ventricular (LV) ejection fraction of greater than 45%, as measured by echocardiography. Patients with coronary lesions amenable to PCI including brachytherapy, or where CABG was indicated was excluded. Any contraindication for cardiac catheterization, PCI and BM aspiration as per institutional guidelines, patients with an AMI within the previous 3 months and/or New York Heart Association (NYHA) class III or IV congestive heart failure were also excluded. Patients with baseline electrocardiogram (ECG) abnormalities that would hinder interpretation of baseline

ECG un-interpretable for ischemia (e.g., left bundle branch block, LV hypertrophy with strain pattern, Wolff-Parkinson-White syndrome) were excluded. Hematologic abnormalities including a documented bleeding diathesis, anemia with a hemoglobin concentration of < 8 mg/dl, a platelet count < 100,000 and known malignancy involving the hematopoietic or lymphoid system excluded entry into this study. Moreover, the presence of severe co-morbidities including renal and hepatic failure was additionally excluded. Informed consent was obtained from those patients that fulfilled these criteria.

3.2. Study design and parameters of safety

The study design and protocol was approved by the institutional review board of Case Western Reserve University and University Hospitals of Cleveland. Informed consent was obtained from all participants. The primary endpoint of the study was to assess the safety and feasibility of a dose-escalating injection of autologous BM derived CD133⁺ hematopoietic stem cells in chronic ischemic patients with a staged twenty-four months follow up. Secondary endpoints included reduction in the area of ischemic myocardium, improvement in LV function and myocardial viability and reduction of symptoms. Pre-enrollment procedures included coronary angiogram, two-dimensional echocardiogram, pharmacologic stress test with nuclear imaging, ECG and 24 hour Holter monitor, laboratory studies and completion of a Seattle Angina Questionnaire. After the infusion, all patients were monitored for 16-24 hours on telemetry and had vital signs per routine until discharge. Cardiac enzymes and basic metabolic panels were collected at 8, 16, and 24 hours following the procedure. An ECG was completed at 24 hours post-injection or at the time of discharge. Staged follow-up began immediately following infusion and subsequently at 7, 14, 30, 90, 180 days, 12 months, 18 months and 24 months. At each visit the following assessments were performed: (1) Focused cardiovascular history and angina questionnaire, (2) Limited physical exam, (3) Review of medication list, (4) ECG, (5) Evaluation of MACE as defined as death (cardiac and non-cardiac), PCI or CABG, readmission to the hospital secondary to angina and AMI. Laboratory and other testing including cardiac enzymes, basic metabolic panel, complete blood count and coagulation parameters (7, 30, 90, 180 days, 12 months, 18 months and 24 months), 24 hour Holter monitoring (7 and 30 days) and temperature log (at every visit) were carried out. At the 1, 6, 12 and 18-month follow-ups, all of the above items were completed in addition to an echocardiogram.

3.3. Cell preparation and delivery

Cellular preparation was carried out on the morning of the day of infusion. BM aspiration was performed in a sterile fashion. The posterior iliac crest was prepped with betadine and a 25-gauge needle introduced with 1% lidocaine to provide local anesthesia. A 4-inch, 11-gauge Jamsheedi biopsy needle was introduced into the marrow cavity and 150-200 ml of BM was removed. Following isolation of BM, mononuclear cells were isolated by density gradient separation using Ficoll-Paque solution. Selection of CD133⁺ cells was achieved by initial

magnetic labeling of CD133⁺ cells with CD133 microbeads (Miltenyi, Bergisch Gladbach, Germany) and subsequent separation of these cells from CD133⁻ cells in the same marrow aliquot by passing all cells through a positive selection column, which is placed in a magnetic field. The magnetically-labeled CD133⁺ cells were retained in the column while the unlabeled CD133⁻ cells pass through. After removal of the column from the magnetic field, the magnetically retained CD133⁺ cells were eluted as the positively selected fraction. The final cell harvest was centrifuged prior to final formulation and three washes in buffering solution were performed. The final product was brought up to five ml in buffering solution at a concentration corresponding to the appropriate dose.

Cellular aliquots were subjected to flow cytometry to evaluate the expression of cellular markers prior to and after cell selection. Cellular markers evaluated included phycoerythrin (PE) conjugated CD133 and fluorescein isothiocyanate-conjugated CD45. Other lineage markers to determine other contaminating cells included PE conjugated CD3, CD13, CD19 (Invitrogen, Carlsbad, California), CD33, CD41, CD61, CD71, and CD56 (Becton Dickinson, Franklin Lakes, New Jersey). These surface phenotype analyses served to innumerate and characterize the purity of CD133⁺ cells and included identification and quantification of any contaminating cells. Sterility was assessed by gram stain and assays for mycoplasma and endotoxin as well as bacterial and fungal cultures. The minimum acceptable total number of infused CD133⁺ cells was 1×10^6 (range $0.5 - 1.5 \times 10^6$ cells), 2×10^6 (range $1.5 - 2.5 \times 10^6$ cells) and 3×10^6 (range $2.5 - 3.5 \times 10^6$ cells). A minimum purity and viability of $\geq 70\%$ CD45⁺ CD133⁺ cells was deemed acceptable for infusion. Mononuclear cells were assessed for colony-forming units per manufacturer protocol (StemCell Technologies, Vancouver, British Columbia). The cells were transported to the catheterization laboratory at ambient temperature for infusion within 8 hours after final formulation.

An intracoronary perfusion over the wire dilation catheter (Maverick, Boston Scientific, Natick, Massachusetts) was utilized for cellular infusion. The epicardial coronary artery supplying collateral vessels to the viable myocardium was identified via angiography. Equipment was passed through an introducer sheath placed in a peripheral vessel for the index PCI. The target parent vessel was cannulated with an infusion catheter through a guide catheter that was placed in the ostium of the appropriate coronary artery in the sinus of Valsalva. Patients received a cell dose of CD133⁺ cells by manual injection over five minutes followed by a flush as indicated by the respective cohort (e.g., 1×10^6 , 2×10^6 , or 3×10^6). The infusion was performed by an independent operator without knowledge of the dosing strategy. At the end of the treatment, a selected coronary angiogram of the vessel was performed to assess Thrombolysis in Myocardial Infarction (TIMI) flow and to evaluate the integrity of the vessel wall.

3.4. Evaluation of safety and feasibility

A data safety and monitoring board was established and comprised of independent experts in

relevant biomedical fields and managed through the independent assistance of the Harvard Clinical Research Institute. The board reviewed the protocol prior to study initiation and thereafter periodically monitored progress, serious adverse events, data, outcomes, toxicity, safety and other confidential data from this trial.

3.5. Pharmacologic stress test and ^{99m}Tc-sestamibi assessments

At baseline, 1, 6, 12 and 18-month follow-up a pharmacologic stress test was done on each patient. Reduction in the area of ischemia was evaluated by ^{99m}Tc-sestamibi imaging coupled with the pharmacologic study. A standard scintillation camera was used to assess viable myocardium under ischemia. Planar views were captured at rest and stress with identification of a reversible defect corresponding to the appropriate vascular territory. Ischemic burden was quantified on the four (short axis, vertical long axis, horizontal long axis and transaxial) tomographic projections. A straight anterior projection was used to determine LV function following radiotracer injection.

3.6. Angina assessment

The Seattle Angina Questionnaire was given to patients at 7, 30, 90, 180 days, 12 months and 24 months. The questionnaire consisted of eleven questions designed to measure cardiovascular clinical outcomes. It assessed physical limitation, anginal stability and frequency, treatment satisfaction and disease perception as a measure of the impact of coronary artery disease on a patient's quality of life.

3.7. Statistics

A paired Student's *t* test was used to evaluate the p-value. A p-value less than 0.05 is considered statistically significant.

4. Results

4.1. Baseline characteristics

Table 1 illustrates the patient demographics including clinical variables. The majority of patients were white males with an average age of 65 years. They experienced primarily class I and II anginal symptoms and had preserved LV ejection fractions. Clinical histories were similar and consistent with respect to the study population. All patients were discharged on aspirin and a beta-blocker. ACE inhibitor and statins were used widely.

4.2. Procedural and cellular characteristics

Parent vessel anatomy was consistent with population studies of CTO with the RCA being the most prevalent vessel occluded (Table 2). Marrow aspirations were carried out without complication. Aspirated volumes were adequate and similar among patients. Mononuclear cell preparations and subsequent selection of CD133⁺ cells were variable in number ranging from $8-48 \times 10^6$ and yielded substantially more cells than required for infusion. Purity fell within the range of acceptable as defined in the protocol for all patients but was also highly variable (77-

Table 1. Baseline characteristics

	n	%
Demographic Variables		
Age, yrs	65.2 ± 8.1	
Gender		
Male	7	
Female	2	
Race		
White	9	
Black	0	
Clinical History		
Diabetes	5	
Hypertension	8	
Smoking	5	
Dyslipidemia	8	
CAD	5	
CVA or TIA	0	
Ejection fraction		57.8 ± 6.44
Myocardial infarction	6	
PCI	5	
CHF	0	
Prior CABG	5	
Angina		
Class 1 or 2	6	
Class 3 or 4	3	
Sestamibi Grade		
Enrollment		
Mild	7	
Moderate	2	
6 months		
Absent	6	
Mild	3	
Cell Therapy		
Target vessel		
LAD	1	
RCA	6	
LCX	2	
CD133 purity		85.0 ± 7.37
Cell viability		84.1 ± 10.0
Colony-forming units/well	0.87 ± 0.81	
Discharge medications		
Aspirin (%)	9	100
Clopidogrel (%)	3	33
ACE inhibitor (%)	6	67
Beta-blocker (%)	9	100
Statin (%)	8	89

Values are expressed as mean ± SD. CAD = coronary artery disease; CVA = cerebral vascular accident; TIA = transient ischemic attack; PCI = percutaneous coronary intervention; CHF = congestive heart failure; CABG = coronary artery bypass grafting; ACE = angiotensin-converting enzyme; LAD = left anterior descending artery; LCX = left circumflex; RCA = right coronary artery.

Table 2. Patient infusion data

Patient	Age	Marrow volume (ml)	Number of selected CD133 ⁺ (10 ⁶)	CD133 ⁺ purity (%)	Target vessel	CD133 ⁺ infused
1	65	200	23.2	97.0	LCX	1 x 10 ⁶
2	76	200	48.5	77.0	RCA	1 x 10 ⁶
3	76	185	11.6	77.3	RCA	1 x 10 ⁶
4	65	170	8.0	80.8	RCA	2 x 10 ⁶
5	71	200	11.8	91.0	OM2	2 x 10 ⁶
6	61	200	30.2	94.5	RCA	2 x 10 ⁶
7	63	200	19.5	78.0	LAD	3 x 10 ⁶
8	51	200	13.2	88.3	RCA	3 x 10 ⁶
9	59	200	17.8	80.8	RCA	3 x 10 ⁶
Summary	65.2 ± 7.67	195 ± 10.0	20.4 ± 11.8	85.0 ± 7.37		

Values are expressed as mean ± SD.

97%). *In vitro* analysis of the whole cell preparations from all patients produced few and in some cases no colony-forming units. Analysis of the CD133⁺ selected cells as a percent of CD45⁺ cells (hematopoietic cells) is expressed in Table 3. All hematopoietic lineages were represented in the contaminating population, the largest percentage of which

was myeloid precursors and megakaryocytes. Each of the three dosing tiers was tolerated and then triggered an additional dose escalation (doubling of cell number). Cells were held, transported and infused to the target vessel with timely success. There was a lack of infusion related morbidity including acute obstruction or dissection and no

Table 3. CD133⁺ contamination characteristics

Patient	CD3	CD3neg/CD56 ⁺	CD13 ⁺ /SSC high	CD14	CD19 immature	CD19 mature	CD61	CD71
1	2.8	0.06	4.7	0.68	4.5	1.9	4.6	0.21
2	7.7	0.43	7.7	0.59	1.5	0.26	0.5	0.78
3	1.3	0.01	3.8	0.23	0.55	0.17	2.9	0.58
4	1.4	0.17	6.3	0.49	2.6	0.47	22.8	0.28
5	0.95	0.05	11.1	0.61	5.4	0.2	0.13	2.2
6	--	--	--	--	--	--	3.1	0.15
7	0.57	0.05	15.1	0.09	1.2	0.02	1.3	1.7
8	2.6	0.03	6.2	0.36	3.7	0.88	2.0	1.5
9	10.9	0.15	5.2	0.99	3.2	1.9	9.9	0.3
Summary	3.53 ± 3.50	0.12 ± 0.13	7.51 ± 3.55	0.51 ± 0.26	2.83 ± 1.58	0.73 ± 0.72	5.25 ± 6.79	0.86 ± 0.71

Absolute values are expressed as percent. Summary values are expressed as mean ± SD

Table 4. Clinical safety markers

Procedural complications *	0
Creatinine kinase (U/l) †	
Baseline	80.5 ± 39.4
6-8 h after cell therapy	180.1 ± 78.9
12-16 h after cell therapy	162.9 ± 58.8
20-24 after cell therapy	145.1 ± 58.6
Creatinine kinase -MB (ng/ml) ‡	
Baseline	1.11 ± 0.57
6-8 h after cell therapy	1.33 ± 0.67
12-16 h after cell therapy	1.33 ± 1.05
20-24 after cell therapy	1.22 ± 1.03

Values are expressed as mean ± SD. *Reduction in TIMI flow, dissection, aneurysm and thrombosis. † upper normal limit = 232. ‡ upper normal limit = 7

Table 5. 12-Month change in seattle angina questionnaire score

Scale of the Seattle Angina Questionnaire	Baseline Mean Value	12-Month Mean Value	Mean Difference	
Physical limitation		71.35	76.79	5.43
Angina stability		50.00	65.63	15.63
Angina frequency		77.50	83.75	6.25
Treatment satisfaction	85.94	91.41	5.47	
Disease perception		53.13	67.71	14.58

change in TIMI flow of the target vessel (Table 4). Figure 1 is a representative angiogram and illustrates collateral vessel directed infusion of cells. Cardiac biomarkers remained within normal limits for all patients at time points up to 24 hours following infusion. There were no detected arrhythmias or ECG abnormalities during the post-procedural period. Moreover, patients experienced no symptoms consistent with angina following infusion.

4.3. Outcomes

In our single center study of nine patients the isolation and infusion of autologous CD133⁺ cells was feasible. No in-hospital events were recorded including death, AMI and ventricular arrhythmias. No patients were lost to follow-up and all clinical and ischemic functional assessments were completed. The primary outcome of safety over the course of the study was met. No MACE was incurred during the twenty-four months follow up period. There were no arrhythmias detected by 24 hour Holter monitoring and no laboratory abnormalities attributable to infusion. With respect to secondary outcomes, seven of the nine patients had a reduction in the area of reference ischemia (Figure 2). There was suggestion of an increase in anginal stability and quality of life (Table 5). However, there appeared to be no change in treatment satisfaction, anginal frequency or improvement in physical limitation as assessed by the Seattle Angina Questionnaire.

5. DISCUSSION

In this phase I pilot study we demonstrated that coronary infusion of autologous BM derived CD133⁺ hematopoietic stem cells is feasible and safe in patients with CTO. This safety was achieved with a step-wise dose escalation approach.

Recent clinical trials in humans have illustrated reasonable safety and feasibility. However, there remain additional questions regarding efficacy, timing of delivery, cell potential and dosing. As with other pharmaceutical agents dosing kinetics and toxicity are critical. This interplay has not been addressed in previous studies with cellular products. The largest trials to date have generated varying results with respect to efficacy and those that have shown benefit reflect nominal responses (20, 24, 25). In addition, these studies employed large numbers of heterogeneous cell populations. Heterogeneity of cell populations prohibits analysis of potency because the functionally effective cell is not specifically quantified. Additionally, there are no studies to date addressing the question of cell dose and the relationship with clinical safety.

In our study no procedural complications were encountered and there were no subsequent post-procedural arrhythmias, ischemia or immediate acceleration of angina in any patients. Each dose was tolerated and triggered an

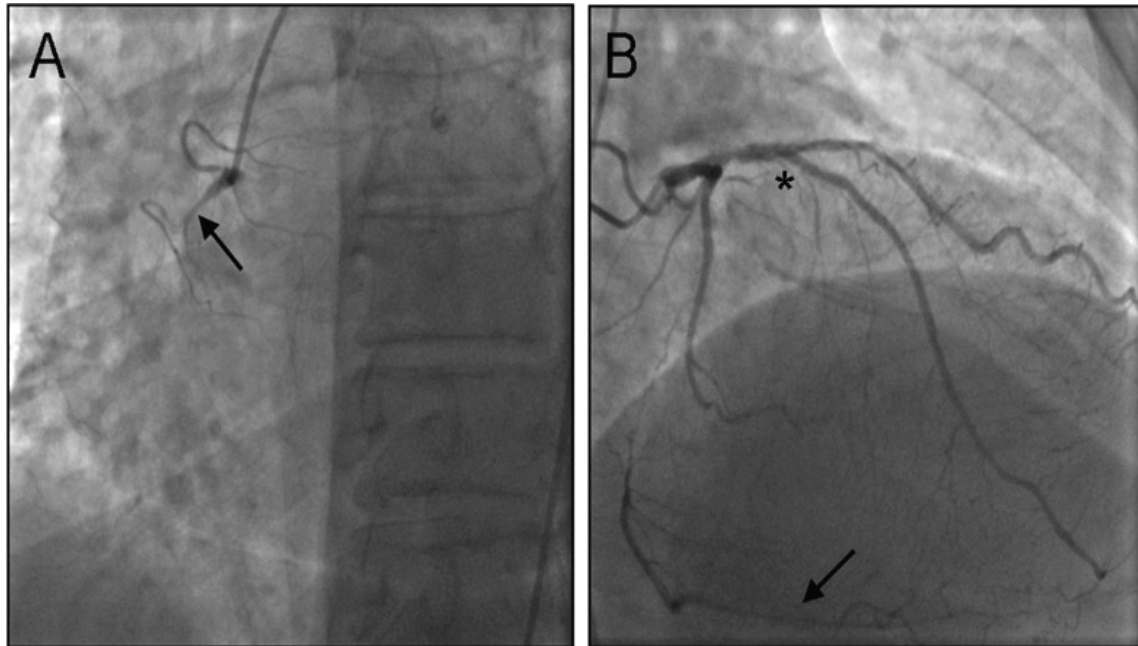


Figure 1. (A) Representative coronary angiograms illustrating a chronic total occlusion (CTO) of the right coronary artery (RCA) and (B*) target left anterior descending artery (LAD) during directed cellular infusion. Arrowheads indicate the regions of occlusion.

additional two increases in cell dose without acute clinical consequences. There were no patients lost to follow up and all patients completed the components of the clinical follow up protocol. During the twenty-four months monitoring period following cell infusion there were no documented arrhythmias or MACE.

There were no logistical issues related to harvesting and selecting adequate numbers of a relatively pure fraction of hematopoietic stem cells. Isolation of distinct populations of stem cells is possible in most large clinical centers with appropriate core facilities as this is done regularly for bone marrow transplantation. Large volume bone marrow aspirates generated significantly more CD133⁺ cells than required for infusion and cell preparations were free of infectious agents after processing. The wide range of cell numbers retrieved may represent the health of the patient's bone marrow. Although adequate numbers of cells were selected, there was a range of purity of these cells. The variability in purity reflects small numbers of contaminating precursor cells and may be related to antibody specificity. However, because this fraction represents a minute portion of the total numbers of cells infused we don't believe they are significantly involved in mediating toxicity or the potential for efficacy. Further investigation of this cell fraction is ongoing to determine the exact role of these cells and their functional capacity.

Previous trials have utilized a cell source, which potentially may have reduced potential as a number of studies suggest that stem cell dysfunction

may be inherent to patients with chronic disease (30-32). Functional quantification was carried out in this study utilizing a colony-forming assay with cells selected from each patient. None of the patient samples yielded significant numbers of colony-forming units suggesting a possible diminution of function.

The patients enrolled in this study were required to have an LV ejection fraction greater than 45% and thus represent a cohort without significant impairment of myocardial function. Additionally, six of nine patients had symptoms that did not dramatically impact their function (i.e. class I and II angina). There was an overall trend towards reduction in ischemic myocardium.

5.1. Limitations

Although a number of products have been utilized with varying success it remains unclear as to the efficacy of stem cells and the potential role in myocardial ischemia whether acute or chronic. Many of the patients in this trial may have received benefit from the infusion however the study was not powered to answer this provocative question. Overall myocardial function was initially good with established collateralization in this mostly functional population and thus represents a population difficult to detect functional or symptomatic improvement.

6. CONCLUSION

Cellular therapy for myocardial ischemia and subsequent functional deficiency has been shown to be beneficial in preclinical studies. Furthermore, application of

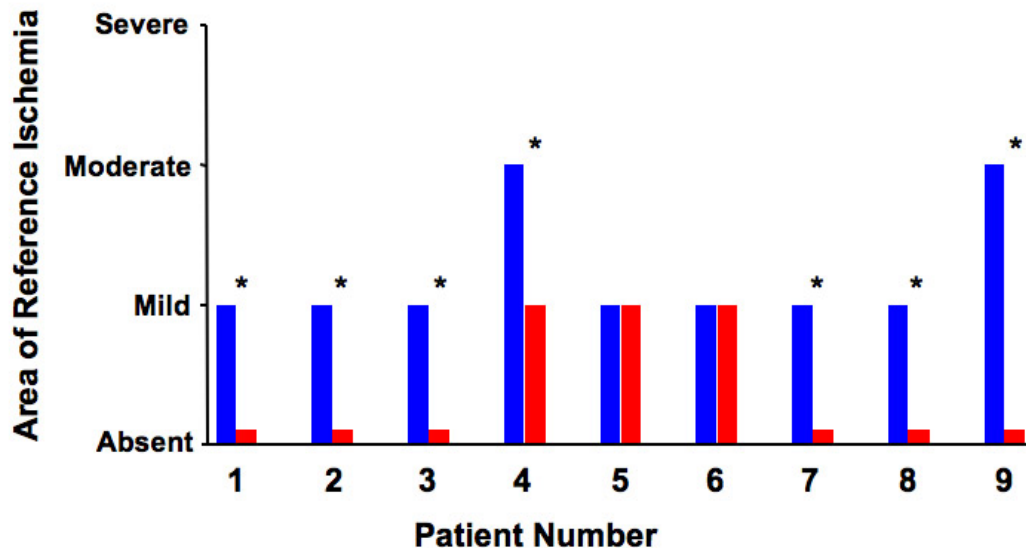


Figure 2. Comparison of the area of reference ischemia, measured by ^{99m}Tc-sestamibi. Columns in the left (blue) and in the right (red) represent the degree of ischemia at the time of enrollment and at 12 months, respectively for each patient. (*) indicates statistical significance (p<0.001).

cellular therapy *in vivo* can enhance repair via angiogenesis and possibly regenerate myocardium. Although improvement has been achieved in animal studies it remains unclear as to whether this form of therapy is effective in humans. There is a paucity of therapeutic options for patients with CTO and perhaps augmentation of the compensatory response with stem cells may be effective. Our study provides a novel approach of collateral vessel targeting for stem cell delivery in patients with CTO and appears to be safe. We believe this study paves the way for future trials powered to answer questions of efficacy of a selected hematopoietic stem cell in this difficult to treat population of patients.

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