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Recent Concepts in Myocardial Regeneration

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ABSTRACT

Functional restoration of the damaged heart presents a challenge as the available treatment options do not help in reduction of scar size after myocardial infarction or significant improvement of an impaired cardiac pumping ability in Heart Failure (HF). Nowadays, Stem Cell technology is rapidly gaining popularity as a way to improve the prognosis of patients with coronary artery disease and HF. Ideally, transplanted cells would mimic the lost myocytes morphologically and functionally. Therefore, we at AIIMS undertook following studies to evaluate the safety and efficacy of Stem Cell (SC) injection in Acute Myocardial Infarction (AMI) and Dilated Cardiomyopathy (DCM). A) To Study the Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection. B) Intracoronary stem cell implantation in patients with dilated cardiomyopathy. (B.1) pilot study- 6 months (B.2) final long-term (3-year) follow-up. C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with AMI - MI3 trial.

Keywords: Acute Myocardial Infarction, Dilated Cardiomyopathy, Heart Failure, Stem Cells.

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INTRODUCTION

Cardiovascular diseases (CVDs) are one of the world's leading causes of mortality. Cardiomyocytes are considered to be terminally differentiated and cardiac injury causes permanent myocardial loss resulting in cardiac dysfunction. There is loss of myocardial tissue during Myocardial Infarction (MI) which results in scar formation, progressive remodelling of the left ventricle (LV), and development of ischemic cardiomyopathy (ICM).

Myocardial infarction (MI) secondary to coronary artery disease is a leading cause of morbidity and death throughout the world. Although reperfusion therapies have provided dramatic advances in the treatment of acute MI, a substantial fraction of patients is not able to undergo successful reperfusion promptly. In patients having large MIs, loss of more than a billion cardiomyocytes can occur, overwhelming the hearts intrinsic reparative capacity. Without further intervention, the damaged myocardium is replaced by fibrous non-contractile tissue (scar), and the resulting left ventricular (LV) dysfunction can initiate a spiral of adverse remodeling progressing to end-stage heart failure.

The current conventional therapeutic strategies ameliorate the symptoms of heart failure but at the same time they fail to reconstitute the dead myocardium with functional new cardiomyocytes and vessels, ultimately failing to show any major improvement. Heart transplantation is an effective means of treating patients with heart failure. But the vast majority of patients are restricted by the age, the donor, surgical complications, medical costs, and so forth. In the search to improve the outcomes in patients with CVDs, a new approach has gained momentum during last few years which is repair of CVDs with Stem Cells. Cellular cardiomyoplasty is a new potential therapeutic approach that uses exogenous cells to repair regions of damaged myocardium. There are number of sources of Stem cells which have been investigated for cardiac therapy **(Table 1).**

The first cell source studied in detail in both animal models and humans was autologous skeletal myoblasts. Although myoblasts formed stable grafts in the heart, they failed to differentiate into cardiomyocytes and were unable to improve myocardial function. Autologous bone marrow mononuclear cells, which poses a broader differentiation potential than myoblasts, have also been tested in animal models and clinical trials. A range of rodent post-MI studies provided clear evidence of functional benefit resulting from transplantation of bone marrow–derived mononuclear cells to the infarcted myocardium; however, the mechanisms of benefit have been debated. Despite questions about underlying mechanisms, bone marrow mononuclear cells are being broadly tested in early stage clinical trials with results ranging from a small benefit on cardiac functional parameters to no significant effect (1,2). Patient-specific cardiac stem cells

Table 1. Cell Sources Investigated For Cardiac Therapy

Autologous	Allogeneic
Skeletal myoblasts	Fetal cardiomyocytes
Bone marrow-derived cells	Embryonic stem cells and derivatives
c-kit^{+}lin^{-}	
Bone marrow mononuclear cells	
Endothelial progenitor cells	Mesenchymal stem cells*+
CD34+	
CD133+	
Cardiac stem/progenitor cells	Parthenogenetic stem cells and derivatives
Side population	
C-kit+	
Cardiosphere derived	
Epicardial progenitors	
Spermatogonial stem cells and derivatives	
Induced pluripotent stem cells and derivatives*	

*can be both autologous and allogeneic.
+can be derived from multiple tissues, including bone marrow and adipose.

isolated from the adult heart hold promise (3,4). Experiments in animal models and more recently in early stage clinical trials have shown encouraging results testing autologous cardiac stem cells and cardiosphere-derived cells (5,6). However, scalability, senescence and dysfunction secondary to the underlying pathology are major potential limitations for cardiac stem cells (7,8). Alternatively, the use of mesenchymal stem cells (MSCs) has been investigated. Improved heart function following the transplantation of mesenchymal stem cells (MSCs) has been reported in animal models of acute MI as well as in clinical studies on patients with heart failure (9). Various favourable characteristics, such as multilineage differentiation potential, ability to evade the host immune system, immunomodulatory capacities and ease of proliferation *in vitro*, make MSCs particularly attractive for cell therapy (10). It has been well established that MSC infusion improves the function of infarcted myocardium (11). MSCs can be derived from a variety of tissues, including bone marrow and adipose. These cells can be extensively expanded in culture and exhibit apparent immune privilege.

MSCs transplanted post-MI animal hearts have shown benefits, which seem to be primarily paracrine in nature (12,13). MSCs have also been tested in early phase clinical trials, including MSCs treated with a cardiogenic cocktail, and show signs of functional benefits (14,15). **Table 2** highlights the different cell sources used for Cellular Cardiomyoplasty and their limitations.

Table 2. Cellular Cardiomyoplasty: Cell Sources and Limitations

Cell Source	Ethical Problems	Acquisition Concerns	Rejection	Oncogenicity
Fetal cardiomyocytes	Yes	Yes	Yes	No
Adult cardiomyocytes	No	Yes	No	Yes
Skeletal Myoblast	No	Yes	No	No
Embryonic Stem Cell	Yes	Yes	No	Yes
Marrow Stromal Cell	No	No	No	No

Clinical Trial Using MSCs:

A team from Rigshospitalet University Hospital Copenhagen (Copenhagen, Denmark) at the American College of Cardiology's 63rd Annual Scientific Session has suggested that heart failure patients may benefit from a new treatment in which stem cells derived from bone marrow are injected into the heart.

This study is the largest placebo-controlled, double-blind randomized trial to use mesenchymal stromal cells injected directly into the heart muscle to treat patients with chronic ischemic heart failure. A total of 59 patients with chronic ischemic heart disease and heart failure were included in the study. A small amount of bone marrow was extracted from each patient and the mesenchymal stromal cells were then isolated and induced to self-replicate. Patients were then given an injection containing either a saline placebo or their own cultured mesenchymal stromal cells directly into the heart muscle via a catheter inserted in the groin; a procedure requiring only local anesthesia. After 6 months, treated patients showed an 8.2-ml decrease in end systolic volume, the study's primary end point and a key measure of the heart's pumping ability. An increase in end systolic volume of 6 ml was observed in patients in the placebo group.

These results support previous findings from smaller studies that demonstrated reduced scar tissue in the heart in patients treated with stem cells. Researchers will now continue to monitor these patients in order to evaluate the long-term outcomes. A larger, Phase III clinical trial is now required in order to progress towards the acceptance of this treatment for widespread use in patients with ischemic heart failure (*Regen. Med.* (2014) 9(3), 255–257).

Embryonic Stem Cells & CVDs :

Embryonic stem cells (ESCs) provide another allogeneic cell source investigated for post-MI therapy and tested in animal models. ESCs have undoubted potency to generate all cell types present in the heart. ESCs and their derivatives have shown functional benefit in various animal MI models. Nevertheless, concerns about immune rejection, safety and the embryonic source of these cells have delayed clinical applications. In addition, other pluripotent stem cell sources, including spermatogonial stem cells and parthenogenetic stem cells, have been

suggested as potential cell sources for cardiac repair, but little data exist at this time on these cell sources and particularly with regard to human cells.

The recent discovery of induced pluripotent stem cells (iPSCs) and other advances in reprogramming technologies, such as induced cardiomyocytes, have provided more potential avenues for cardiac repair. Because iPSCs are produced by reprogramming somatic cells, such as dermal fibroblasts, they can provide autologous cells for patients, reducing the risk of immune rejection. Another promising feature of iPSCs is that they can be extensively expanded for the production of large quantities of potentially any cell type desired to repair the myocardium. Initial studies have begun to examine the use of iPSCs and their derivatives for cellular therapy to treat a variety of diseases using animal models (16).

A number of pre clinical studies have been done in MI model using iPSCs and their derivatives (**Table 3**).

Stem cells are the origin cells of various mature cells. They have the potential of self-renewal and differentiation. Either immediately after isolation or after expansion *in vitro*, stem cells are transplanted into a specific region of the heart, and ultimately replace and repair the myocardial necrosis or pathological cells; then the aim of curing heart failure can be achieved and it has brought a bright prospect for the treatment of heart failure. Many clinical trials using the stem cell transplantation for acute and chronic heart failure have been carried out. The interest is based on the assumption that left ventricular dysfunction is largely due to the loss of a critical number of cardiomyocytes and

Table 3: Preclinical Studies of Post –MI Cell Therapy Using iPSCs and Derivatives

References	Cell Source For Reprogramming	Reprogramming Method	Cell Types Transplanted	Delivery Method	Animal Model	Duration Of Study	Summary Of Results
Nelson et al	MEFs	Lentiviral-human (KOSM)	iPSCs	Intramyocardial injection	Mouse	4wk	↑Ventricular function↓ pathological remodeling. engraftment and differentiation into CMs SMs, ECs. No teratomas in immunocompetent mice. Teratomas in immunodeficient mice
Singla et al	Mouse H9c2 cardiomyoblasts	Plasmid-mouse (KOSM)	iPSCs	Intramyocardial injection	Mouse	2wk	↑Ventricular function↓ apoptosis, No teratomas in immunocompetent mice
Templin et al	Human cord blood	Lentiviral-human (NOLS)	iPSCs	Intramyocardial injection	Pig	12-15 wk	iPSCs coinjected with human MSC survived and differentiated into endothelial cells. iPSCs injected alone failed to survive. Pigs were immunosuppressed

that it may be partly reversed by implanting new contractile cells into the post infarction scars or regions of wall thinning.

Recently, the results of a few small trials were reported that suggest a beneficial role of stem cell therapy in nonischemic dilated cardiomyopathy (17-19). Kalil et al. (17) showed intramyocardial transplantation of bone marrow stem cells in dilated cardiomyopathy patients with improvement in functional class without improvement in left ventricular function. Wang et al. (18) also found similar results after intracoronary infusion of autologous mesenchymal stem cells. Kaparthi et al. (19) showed improvement in functional status and left ventricular function after intracoronary autologous bone marrow mononuclear cells infusion. Resident CMPCs by contrast have only relatively recently been identified, but are already generating excitement because they appear to differentiate into bona fide cardiomyocytes *in vitro* with high efficiency. This is exceptional for any adult stem cell source studied to date.

While experimental studies and early phase clinical trials tend to support the concept that cell therapy may enhance cardiac repair,several key issues still need to be addressed before introduction into routine clinical practice. These include (1) the optimal type of donor cells in relation to the clinical profile, (2) the mechanism by which cell engraftment improves cardiac function, (3) optimization of cell survival, (4) development of less invasive cell delivery techniques, and (5) the relevance to non-ischaemic heart failure.

As, there was no clinical data on the role of stem cell therapy in nonischemic dilated cardiomyopathy; therefore we undertook a pilot study of intracoronary stem cell implantation in patients with dilated cardiomyopathy. From a cohort of 44 patients, 24 were randomly allocated to the stem cell therapy arm and 20 to the control arm. There was a significant improvement ($p < 0.001$) in NYHA (New York Heart Association) functional class in the treatment arm, with 16 patients (62%) improving by at least 1 functional class, as compared to only 2 patients (10%) improving in the control arm.

In continuation with the previous study, next study was again in DCM patients (n=85) with a follow up of 3 years. The EF improved in the treatment arm by 5.9% with a reduction in end-systolic volumes and no change in end-diastolic volumes. There was no significant improvement in the EF in the control patients.

MATERIALS & METHODS

A) Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection:

Patients with Coronary artery disease (CAD) undergoing CABG and with reduced LV function with an area of nonviable myocardium were included. 86% had a documented previous MI, 43%

had hypertension, 36% were chronic smokers and 14% had diabetes.

Materials & Methods:

35-40 ml of bone marrow was aspirated from patients giving mean cell count of 15±22 million/ml. Number of injections per patient ranged from 25 to 48 with amount of 0.25cc/sq cm. All patients underwent serial echocardiograms and stress thallium 20-segment analysis.
Mean follow up was14.5±3 months.

B) Role of intracoronary implantation of stem cells in dilated cardiomyopathy (DCM):

B.1) Short term study: In this study 44 patients were included, who had dilated cardiomyopathy with an ejection fraction (EF) of ≤35%, where New York Heart Association (NYHA) functional class II or more symptomatic for more than 6 months, had normal coronary arteries, and had no other comorbidities such as chronic renal or liver failure or any malignancy. Out of which, 24 were randomly allocated to the stem cell therapy arm and 20 to the control arm.
Mean follow-up period was 6 months.

B.2) Long Term Study: This includes the final long-term (3-year follow-up) results of the trial. The study included patients between 15 and 70 years of age with idiopathic dilated cardiomyopathy with normal coronary arteries, an ejection fraction (EF) of <40%, and no other severe comorbidities (e.g., chronic renal failure).

This was an open-label, randomized trial in which 85 patients were enrolled. The end points of the study were 1) change in New York Heart Association (NYHA) functional class, 2) change in quality of life as per the Kansas City Cardiomyopathy Questionnaire (KCCQ), 3) change in left ventricular function (Vivid 7TM, Wipro GE Healthcare, offline analysis using Simpson's method), and 4) mortality. An endomyocardial biopsy was performed in 8 patients.

The mean follow-up period was 28 ± 9 months.

Materials & Methods:

50-60 ml bone marrow was aspirated from the iliac crest of the patients. Mononuclear cells were separated from the bone marrow using Ficoll density gradient separation. The mononuclear cells constituted 89 ±2% of the cells, were 28 ±16 million /ml, and CD 34+ cells and were 1.6 million/ml. The viability of these cells was 99 ±1%. The patients then underwent right heart catheterization and endomyocardial biopsy from the right side of the interventricular septum. The coronary sinus was then engaged using a Swan-Ganz catheter (Arrow International, Reading, Pennsylvania) that was passed up the coronary sinus, and the balloon was inflated. This was done so that the coronary circulation was slowed and the stem cells would get more time to transmigrate into the myocardium. Once the coronary sinus catheter was inflated, the stem cells were slowly injected into

the coronary arteries by hooking the arteries with a Judkins catheter. Two-thirds of the mononuclear cell concentrate was injected into the left coronary artery and one-third was injected into the right coronary artery. The coronary sinus balloon was kept inflated for 3 min during the intracoronary injection.

The patients were kept under monitoring for 24 h with electrocardiographic monitoring and serial cardiac enzymes. Follow-up was done at 1 week, 1 month, and then every 3 months for 1 year. At 3 months, Holter monitoring, an echocardiogram, and an endomyocardial biopsy were repeated. An echocardiogram was also repeated at 1 year. Left ventricular function assessment was performed offline by the modified Simpson method by 2 observers blinded to the underlying treatment. All patients were on the maximum tolerated doses of angiotensin-converting enzyme inhibitors and beta-blockers. Diuretic doses (including frusemide and torsemide, and spironolactone) were adjusted to ensure the absence of pedal edema. The end points of the study were: 1) change in NYHA functional class, 2) a change in left ventricular function, 3) mortality, and 4) endomyocardial biopsy and histopathologic evaluation.

Continuous variables were compared by a Wilcoxon 2-sample test (for within-group differences) and the Mann-Whitney U test (between-group differences). Differences in mortality and change in functional class between the 2 groups were compared by the Fisher exact test. A value of $p < 0.05$ was considered statistically significant. All analyses were performed with SPSS for Windows.

C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with Acute Myocardial Infarction - MI3 trial (Mononuclear infusion in Myocardial infarction, Multicentric-Trial, India):

In this study, 250 patients with AMI were included. They were given Intracoronary infusion of either autologous bone marrow derived mononuclear cells (MNC) or standard of care medical therapy.

Materials & Methods:

The study was a randomized multicentric phase III trial to evaluate the efficacy of stem cell in improvement of LV function in patients with AMI. Patients of AMI following left anterior descending (LAD)artery occlusion from five premier centres namely, Army Hospital (Research and Referral), New Delhi; Military Hospital, Cardio Thoracic Centre (MHCTC), Pune; Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGI), Lucknow; Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh; Christian Medical College (CMC), Vellore and All India Institute of Medical Sciences (AIIMS), New Delhi were included in the study w.e.f 07 July 2007 to 08 July 2010.

Patients aged 20-65 years

presenting with first acute ST elevation anterior wall myocardial infarction (AMI) who underwent coronary angiography (CAG) between 1 - 3 weeks were included in the study if they fulfilled the following (a) Killip Class I - III at admission; (b) Proximal and/or mid LAD artery involvement on CAG and (c) LVEF of 20-50% by multigated acquisition scan (MUGA).

Patients with multi-vessel coronary artery disease (CAD), pulmonary edema, Killip class IV, advanced renal or hepatic dysfunction, associated mechanical complications like ventricular septal rupture, previous history of angioplasty or significant circumflex and right coronary artery (RCA) involvement, LVEF < 20% by echocardiography (ECHO), percutaneous coronary intervention (PCI) done within 2 hrs of AMI, and pregnant women were excluded from the study.

RESULTS

A) Role of Stem Cells in ischemic cardiomyopathy by direct intra myocardial injection:

All the patients underwent serial echocardiograms ,ECG and stress thallium 20 segment analysis. There was a significant improvement in New York Heart Association class from a baseline of 2.9±0.7 to 1.25±0.6 (P<.001). Echocardiographic LV ejection fraction analysis revealed evidence of improvement to 41% ± 9%. A mean 3.7±2.6 segments showed improvement, 10.0±1.6 segments showed no change and 2.3±2.6 segments showed worsening. Overall, 56.1% of infarcted areas injected with stem cells improved (**Fig. 1**).

On stress thallium 20 segment analysis at last follow-up, the number of scarred segments reduced from 5.4±2.7 to 4.6±2.6, and the number of normal segments increased from 6.2±3.9 to 8.2±4.1 (**Fig. 2**).

On stress thallium evaluation of injected infarcted segments (n=7±2), 1.8±1.9 segments showed improvement, the other remained same and none worsened.

Bone-marrow derived stem cell transplantation during Coronary artery bypass grafting (CABG) is feasible and safe and the BM obtained from sternum at the time of CABG provides an adequate number of stem cells.

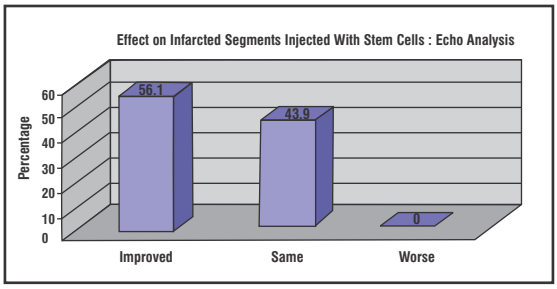


Fig. 1: Effect on infarcted segments injected with stem cells: echocardiography analysis

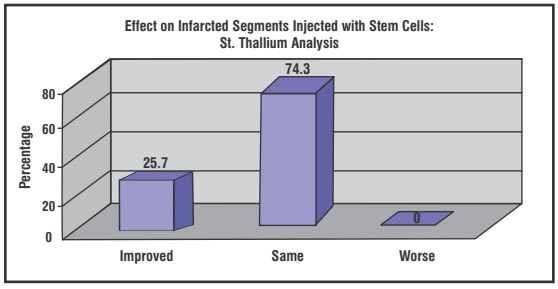


Fig. 2: Effect on infarcted segments injected with stem cells: stress thallium analysis

B) Role of intracoronary implantation of stem cells in dilated cardiomyopathy (DCM):

B.1) Short term Follow-up (6 months): 24 patients underwent intracoronary stem cell injection with coronary sinus blockage. Four patients died during the 6-month follow-up. Overall EF showed a small but significant improvement of 5.4%. There was a decrease in end-systolic volumes, but no change in end-diastolic volumes. Endomyocardial biopsy done at 3 months showed no significant change in the number of myocytes or capillaries, but the ratio of capillaries to myocytes showed an insignificant increase. There were soft data to suggest cell proliferation (binucleate cells and Ki 67 positivity).

B.2) Long term Follow-up (up to 3 years): Two patients in the treatment arm were lost to follow-up, and another 2 patients underwent biventricular pacing. Among the remaining 41 patients, 10 (24.4%) patients died within 3 years. There were 12 NYHA functional class IV patients, and of them, 6 died during the follow-up period and 5 patients showed improvement (1 patient to class I, 1 patient to class III, and the remaining 3 patients to class II). Mortality was not significantly different between the treatment and control arms. The EF improved in the treatment arm by 5.9% with a reduction in end-systolic volumes and no change in end-diastolic volumes. Both NYHA functional class III and IV groups in the treatment arm showed improvement, although the effect on the NYHA functional class III patients (EF: 23.6±10.6.% to 30.1±11%) was greater than that on the NYHA functional class IV patients (EF: 20.1 ±9% to 24 ±13.8%). There was no significant improvement in the EF in the control patients **(Table 4)**. There was a significant improvement in quality of life as assessed by KCCQ and functional status on long-term follow-up in the treatment group **(Table 4)**.

In summary, the clinical follow-up results of a first-in-man pilot study of stem cell therapy in patients with dilated cardiomyopathy at the completion of 3 years of follow-up demonstrate that the benefit is sustained and without any long-term side effects. Although the effect was less in patients with more severely damaged myocardium. This study establishes the long-term safety and long-term efficacy of this therapy in dilated cardiomyopathy.

The data of the analysis at 6 months are presented. Four patients died between 1 and 3 months in the treatment arm. Three died of progressive heart

Table 4: Evaluation at Follow up of Cell Therapy

	Treatment Arm (n = 41)		Control Arm (n = 40)	
	Baseline	3 Yrs	Baseline	3 Yrs
Clinical data				
Age, yrs	45 ±15		49 ± 9	
Sex, male	33		35	
NYHA functional class for dyspnea				
I	0	4 (9)	0	9
II	0	22 (54)	14 (35)	10 (25)
III	29 (71)	6 (15)	12 (30)	18 (45)
IV	12 (29)	9 (22)	14 (35)	12 (30)
Mortality		12 (24.4)		14 (30)
KCCQ				
Functional status score	51.19 ± 19.90	67.02 ± 21.8*	51.52 ± 18.12	52.74 ± 18.8†
Clinical summary score	59.81 ± 20.27	75.22 ± 18.31*	59.95 ± 18.44	61.17 ± 19†
Echocardiography data				
End-systolic volume, ml	137.3 ± 62.6	120 ± 52*	145.7 ± 74.7	147.8 ± 79.9†
End-diastolic volume, ml	176.7 ± 76.40	166.5 ± 65.5	184.9 ± 94.6	187.7 ± 98.8
Ejection fraction, %	22.5 ± 8.3	28.4 ± 11.8*	20.8 ± 9.3	21.2 ± 9.2†
Treatment at 3 yrs				
ACE inhibitor or ARB (ramipril equivalent), % taking drug		41 (100)		40 (100)
Dose, mg		7.5 ±1.2		7.4 ±1.7
Beta-blocker (carvedilol equivalent), taking drug		29 (70)		29 (72)
Dose, mg		12.5 ± 5		12.1 ± 3

Values are mean ± SD or n (%) of patients. Continuous variables were compared using a Wilcoxon 2-sample test (for within-group differences) and the Mann-Whitney U test (between-group differences). Differences in mortality were compared by the Fisher exact test. All analyses were performed with SPSS for Windows (version 10.0.1, 1999, SPSS Inc., Chicago, Illinois). *p < 0.05 between baseline and 3 years for treatment group. †p < 0.05 between treatment arm and control arm.
ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; KCCQ = Kansas City Cardiomyopathy Questionnaire; NYHA = New York Heart Association.

failure and 1 experienced sudden cardiac death. The mortality was not significantly different (p value not significant) between the treatment and control (2 patients died) arms. There was a significant improvement (p < 0.001) in NYHA functional class in the treatment arm, with 16 patients (62%) improving by at least 1 functional class, as compared to only 2 patients (10%) improving in the control arm. The EF improved by 5.4% (20 ±7.4% to 25 ±12%, p ± 0.05) (**Fig. 3**) with a reduction in end-systolic volumes (144 ±85 ml to 116 ±68 ml, p± 0.05) and no change in end-diastolic volumes.

None of the patients who were functional class IV and had recently required inotropic infusions showed any improvement. There was no significant improvement in the EF in the control arm (baseline EF:16 ±5.4% to final EF: 16 ±4.7%). Endomyocardial biopsy was performed at 3 months. Histopathology revealed no evidence of persisting stem cells, no evidence of any new immature myocytes, and also no evidence of any inflammation, infarction or neovascularization.

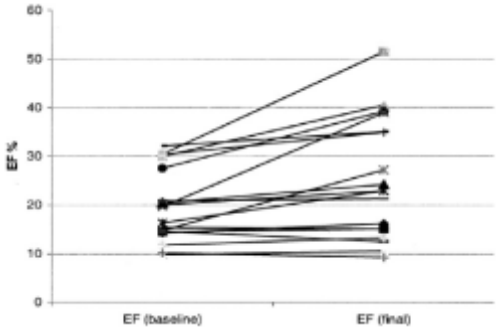


Fig. 3: Change in ejection fraction (EF) after 6 months of follow-up

Two patients had scattered binucleate cells and evidence of cell proliferation (Ki 67). (**Fig. 4**) Morphometric analysis showed no change in the absolute number of myocytes (457 ±210 cells/mm2 [before] and 340 ±150 cells/mm2 [after]). There was also no change in the number of capillary endothelial cells (stained by CD34 class II, 696 ±206 cells/mm2 [before] and 569 ±150 cells/mm2 [after]). The ratio of capillaries to myocytes showed an increase, but it was not significant (1.7 ± 0.7 to 1.9 ±0.8). There was no significant difference between the mortality in the treatment (16.7%) and control (10%) arms. In the control group, 2 patients died. There was no significant change in functional class or left ventricular function in this group. In summary, 24 patients underwent intracoronary stem cell injection with coronary sinus blockage. Four patients died during the 6-month follow-up. Overall EF showed a small but significant improvement of 5.4% (**Fig. 3**). There was a decrease in end-systolic volumes, but no change in end-diastolic volumes.

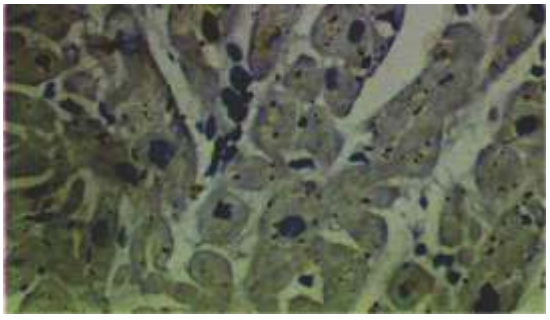
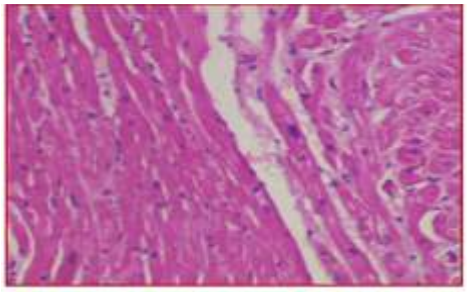
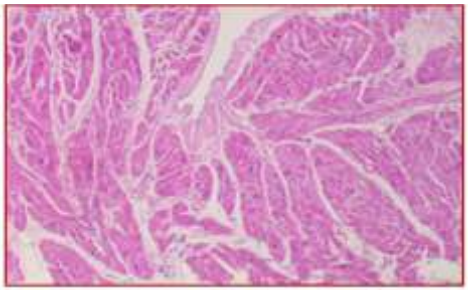


Fig. 4: Presence of Ki67 (Proliferation seen in one patient)

Endomyocardial biopsy done at 3 months showed no significant change in the number of myocytes or capillaries, but the ratio of capillaries to myocytes showed an insignificant increase. There were soft data to suggest cell proliferation (binucleate cells and Ki 67 positivity) (Fig. 4). Also, Hematoxylin Eosin Staining showed that there was no

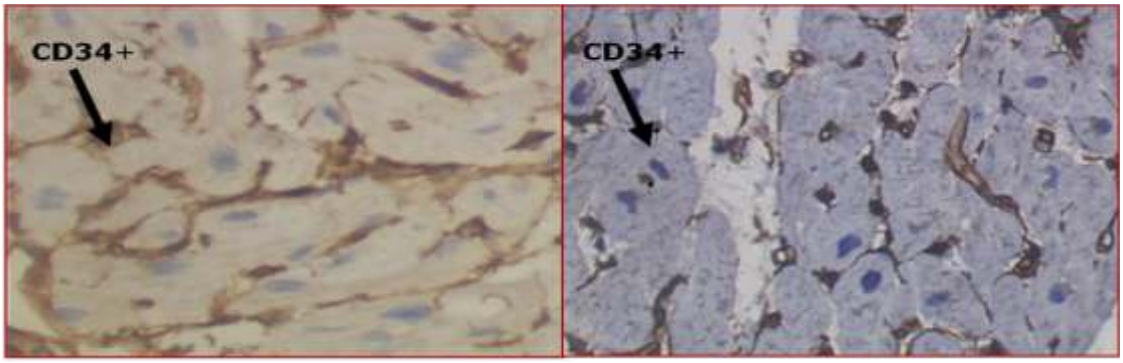


- Pre
- Hypertrophy of fibers with nuclear enlargement
- Mild interstitial fibrosis



- Post
- No abnormal vasculature
- No inflammation
- No primitive cells

Fig. 5: Hematoxylin Eosin Staining of the Cardiac biopsy tissue of the patient



- MYOCYTE : CD34+ Ratio
  - Pre stem cell 1 : 0.8
  - Post stem cell 3 wks 1 : 1.5
    6 mths 1 : 1.4

Fig. 6: Presence of Cd34+ Stem cells after Stem Cell Transplantation

inflammation and no abnormal vasculature formation occurred supporting the safety of stem cell injections (**Fig. 5**). The ratio of myocyte: CD34 cells was slightly increased at 3 months, however this was insignificant (**Fig. 6**).

C) Efficacy of Stem Cell in improvement of Left Ventricular Function in patients with Acute Myocardial Infarction - MI3 trial (Mononuclear infusion in Myocardial infarction, Multicentric-Trial, India):

Between 07 July 2007 and 08 July 2010, 621 patients were screened to assess their eligibility for participating in the trial. Two hundred and fifty patients were randomly assigned, in 1:1 ratio, either to a non Stem Cell Treatment (SCT) arm (n: 125) that received standard of care medical therapy or to a SCT arm (n: 125) that received intracoronary infusion of MNCs in addition to standard of care medical therapy. In the SCT arm of the 125 patients, a total of 114 patients received the stem cells. While in the non SCT arm, all 125 patients received standard of care medical therapy as per current guidelines after PCI. The final cohort followed up for six months included, 109 patients in SCT arm and 117 in non SCT arm (**Fig. 7**).

RESULTS:

Since only 71 patients received the predefined cell dose, a stratified analysis of this group of patients was done with a nested cohort matched for age and sex. The baseline LVEF was similar in both groups (34.22 ±7.03% in SCT vs. 35.75 ± 4.1% in non SCT group). The difference in LVEF observed at the end of six months was approximately 3 per cent (7.03 vs. 4.1%) with a possible benefit in the SCT group (**Fig. 8A**); however, this was not significant. Stratified analysis comparing 38 trial deviates with a nested cohort from

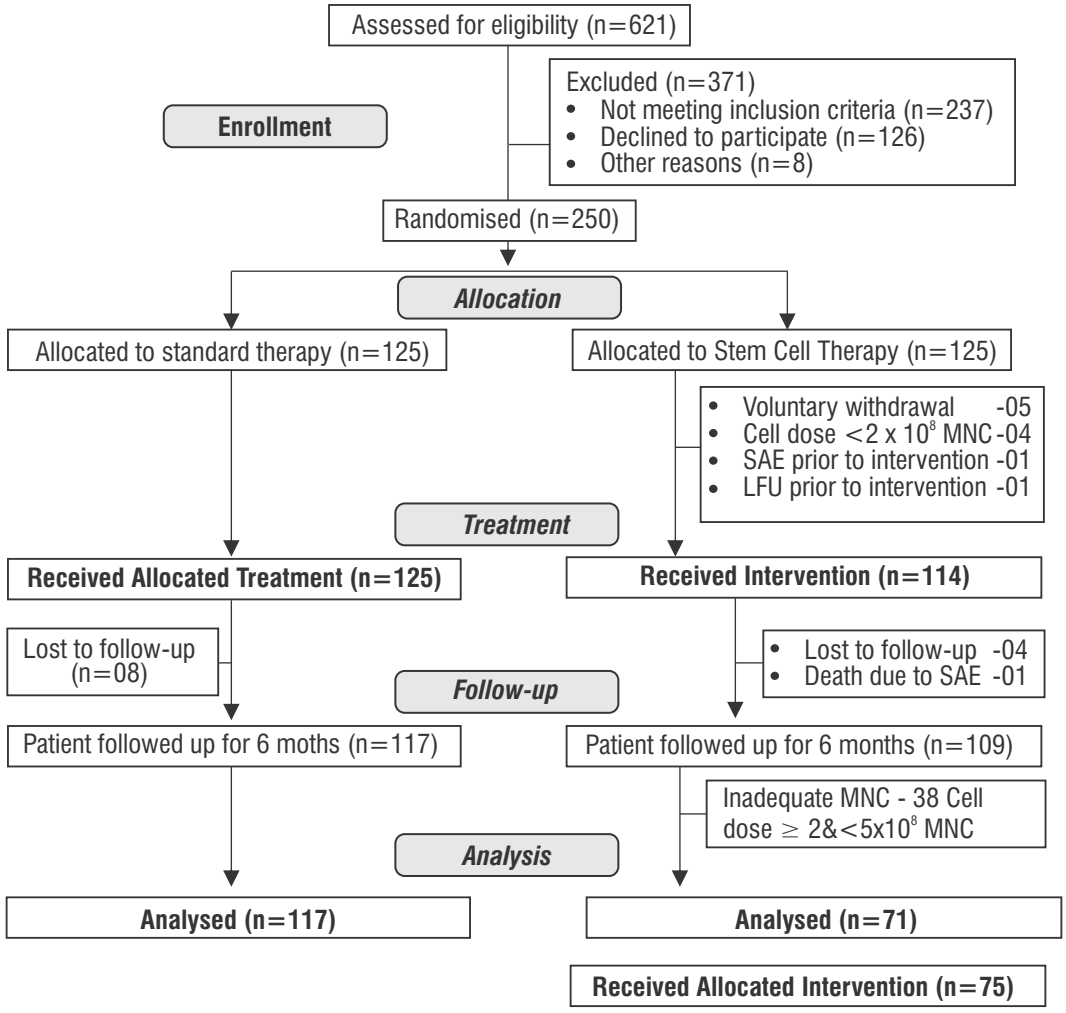


Figure 7.Consort diagram depicting patient enrolment and follow up

the non SCT group showed no significant improvement between the two groups (**Fig. 8B**). It was observed that the baseline LVEF did not differ significantly between the two groups. At six months, LVEF showed an increase in both groups. The mean change in LVEF from baseline to six months being 5.17 ± 8.90 per cent in non SCT group and 4.82 ± 10.32 per cent in SCT group. The median change in LVEF from baseline to six months was 4% in non SCT and 3.5 % in SCT group. However, the difference was not significant. It was found that the cell dose showed a positive impact when infused in the intended dose of ≥ 5 X 10^8 (n=71) when compared with a subset of trial deviates (n=38) who did not receive the predefined cell dose, namely ≥ 2 or <5 x 10^8 cells (**Fig. 8C**). Also , there was no difference noted in the group infused stem cells prior to or beyond 10 days (upto 21

days) of onset of AMI. There was no impact of age and baseline LVEF noted on the primary outcome (**Fig. 8D**).

Adverse effects (AEs) and serious adverse events (SAE) recorded during six months follow up were equally distributed in both the groups with no significant difference.

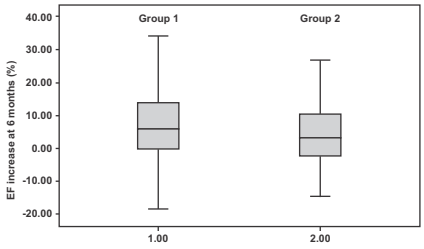


Fig. 8A. Stratified analysis of SCT group with nested cohort: Effect on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, SCT (n=71) and Group 2 Nested cohort from non SCT group (n=71). Actual increase in EF at 6 months between SCT group (7.03 ± 10.33 %, Median 6, IQR 0-14) and nested cohort from non SCT arm (4.1 ± 9.1%, Median 3.01, -2.15-10.45) was not significantly different.

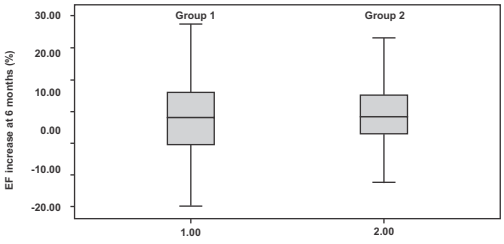


Fig. 8B. Stratified analysis of Trial deviates with nested cohort: Effect on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1,Trial deviates (n=38) and Group 2, Nested cohort from non SCT arm (n=38). Actual increase in EF at 6 months between trial deviates group (2.75 ± 9.6%, Median 3.25, IQR -3.91-9.49) and nested cohort from non SCT arm (4.37 ± 8.87% Median 3.5, -0.75-8.86) was not significantly different.

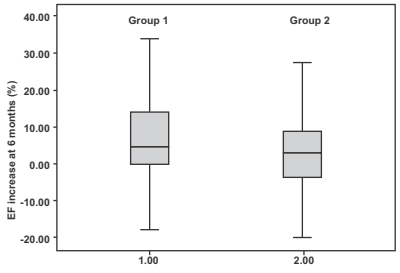


Fig. 8C. Impact of cell dose administered on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, SCT (n=71) and Group 2, Trial deviates (n=38). Actual increase in EF at 6 months between SCT group (7.03 ± 10.33%, Median 6, IQR 0-14) and Trial deviates (2.75 ± 9.6%, Median 3.25, IQR -3.91-9.49) was significant (P<0.05).

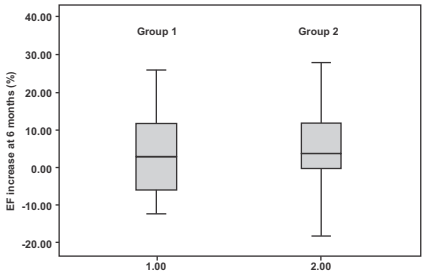


Fig. 8D. Impact of timing of infusion in SCT arm on primary outcome. Box and whisker plot showing primary outcome at 6 months in 2 groups. Group 1, early (infusion given in < 10 days, n=21) and Group 2, late (infusion given between day 10 to day 21, n=50). : Actual increase in EF at 6 months between the early group (6 ± 10.45 %, Median 7.5, IQR 0.49-14) and late group (7.47 ± 10.97, Median 4, IQR -2 - 14) was not significant.

DISCUSSION

Cell-based therapy for ischemic cardiomyopathy and heart failure has emerged as a highly promising therapeutic approach that will expand the benefits obtained by current pharmacologic and revascularization approaches by directly reversing scar formation and promoting myocardial regeneration. The next stage of development for the clinical use of cell therapy should focus on investigating novel formulations, particularly the best cell type(s) and/or cell combinations to use and understand the mechanisms by which various stem cells interact with host cells and/or each other and elicit their regenerative effect. In summary, the clinical follow-up results of a first-in-man pilot study of stem cell therapy in patients with dilated cardiomyopathy at the completion of 3 years of follow-up demonstrate that the benefit is sustained and without any long-term side effects.

This study establishes the long-term safety and long-term efficacy of this therapy in dilated cardiomyopathy. This is the first study of stem cell therapy in dilated nonischemic cardiomyopathy. Over a 6-month period, there was a small albeit significant improvement in ventricular function. Previous clinical studies have also shown a small degree of change in ventricular function of a similar magnitude (20, 21). Laboratory experiments in nonischemic dilated cardiomyopathy (22) have previously suggested that benefit from stem cell therapy in this group comes mainly from a decrease in fibrosis and an increase in vascularity, but no evidence has been found supporting transdifferentiation of stem cells to myocytes. Our data also suggest that the benefit of stem cell therapy could be a paracrine effect with changes in vascularity, perhaps stimulation of cell proliferation, or by some still-unexplored mechanism. We did not find any evidence of transdifferentiation. In this study, we wish to highlight a number of issues. It is the first study to show the benefit of stem cells in nonischemic dilated cardiomyopathy, and the first study that uses coronary sinus occlusion to increase cell contact time. It is also the first study in which we have endomyocardial biopsies performed after stem cell therapy.

It provides a stimulus for exploring the benefits of stem cell therapy in nonischemic dilated cardiomyopathy. A double-blind study is being planned to further explore the benefit seen in this preliminary study. The small magnitude of benefit could perhaps be because all patients in this study were in very late stages of their cardiomyopathy, and we probably need to consider stem cell therapy at a much earlier stage. Endomyocardial biopsy performed for the first time in stem cell therapy, shows no evidence of transdifferentiation of stem cells to myocytes but provides soft data pointing to a possible paracrine effect.

Similarly, the results of MI3 trial demonstrates that autologous MNCs can be safely administered in patients with AMI and the dose of stem cells has important role in determining its efficiency in regeneration (23).

Although there has been significant progress in the clinical translation of cell therapy over the past decade, uncertainties remain regarding the most efficacious cell type, source and quantity, as well as route and timing of delivery (24). Collectively, these issues highlight the need for further investigation of the mechanisms underlying stem cell survival, plasticity, and function.

Future Prospects :

Stem cell treatment is gaining momentum in treatment of CVDs but still a lot needs to be explored so that its use can be translated into better clinical outcomes. Therefore, pharmacologic and genetic strategies are being developed in an effort to improve stem cell survival, homing, and engraftment (25, 26, 27-32). Different strategies are being explored to utilize the combination of different stem cells or of cell and gene therapy (25, 26, 27-32). In addition, the discovery of microRNAs as regulators of cardiovascular biology and stem cell differentiation have made them attractive targets to optimize cell-based therapies. One of the major challenges of cell-based therapy is the survival of cells after delivery into the recipient tissue microenvironment. Ischemia creates a hostile microenvironment due to locally expressed pro-inflammatory and pro-apoptotic cytokines inducing cell death. Various approaches to inhibit local inflammation and promote cell survival and tissue regeneration are being investigated, including preconditioning, by *in vitro* incubation of stem cells with pro- survival factors, or transfection of stem cells with pro-survival or antiapoptotic genes prior to cell delivery (25, 26, 27-32).

New methodologies are being developed for direct differentiation of fibroblasts into beating cardiomyocytes. Three transcription factors (TFs), GATA4, Mef2c, and Tbx5, are essential for cardiac myocyte differentiation for direct reprogramming of heart fibroblasts to cardiomyocyte formation *in vitro* (33). Recently, it was shown that these TFs induce cardiomyocyte formation not only *in vitro* but also *in vivo* (34).

Use of Stem Cells has shown beneficial effects in hearts with post myocardial infarction LV remodelling (MI) and has emerged as a highly promising therapeutic approach. The next stage of development for the clinical use of cell therapy should focus on applying a prefabricated bioartificial cardiac tissue (35-36), a "cardiac muscle patch", over the surface of a myocardial infarct and investigating novel formulations, particularly the best cell type(s) and/or cell combinations to use and elucidation of the mechanisms by which various stem cells interact with host cells and/or each other and elicit their regenerative effects.

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