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## Translational Findings from Cardiovascular Stem Cell Research

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### Abstract

The possibility of using stem cells to regenerate damaged myocardium has been actively investigated since the late 1990s. Consistent with the traditional view that the heart is a “post-mitotic” organ that possesses minimal capacity for self-repair, much of the preclinical and clinical work has focused exclusively on introducing stem cells into the heart, with the hope of differentiation of these cells into functioning cardiomyocytes. This approach is ongoing and retains promise but to date has yielded inconsistent successes. More recently, it has become widely appreciated that the heart possesses endogenous repair mechanisms that, if adequately stimulated, might regenerate damaged cardiac tissue from in situ cardiac stem cells. Accordingly, much recent work has focused on engaging and enhancing endogenous cardiac repair mechanisms. This article reviews the literature on stem-cell based myocardial regeneration, placing emphasis on the mutually enriching interaction between basic and clinical research.

### Keywords

regeneration; stem cell; infarction; myocardium

### Introduction

Congestive heart failure (CHF) is the consequence of myocardial cell death and subsequent cardiac remodeling. Despite noteworthy therapeutic advances during the past half-century, CHF continues to be a major cause of morbidity and mortality and is the leading cause of hospitalization among people aged 65 years and older (Roger et al., 2012). Systematic exploration of regenerating lost myocardium in CHF began in the late 1990s. This article reviews progress in this area, emphasizing mutually beneficial interactions between basic and clinical research.

### The preclinical and clinical studies of different exogenous stem cells

#### Skeletal myoblasts

The era of active research on cardiac regeneration may be dated to initial attempts to employ skeletal myoblasts for cardiac repair reasoning that sufficient plasticity might exist between precursors of related cell lineages, in this case muscle (Koh, Klug, Soonpaa, & Field, 1993).

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A noteworthy attempt was reported in the 1998 publication by Taylor et al., describing the transplantation of autologous skeletal myoblasts into myocardial scar in a rodent model of acute MI. The transplanted skeletal myoblasts created islands of skeletal and cardiac cells and improved myocardial contractility in the infarcted area (Taylor et al., 1998). In 2001, Menasche et al. translated these observations to patients by injecting skeletal myoblasts into scarred regions of the heart during coronary artery bypass surgery (CABG) and subsequently showed improved contractility and viability in the grafted scar on echocardiography and positron emission tomography 5 months after cell delivery (Menasche et al., 2001).

**Safety of skeletal myoblast injections**—Several key safety concerns emerged early in the translational development of skeletal myoblast therapy. Early work on skeletal myoblasts resulted in high incidence of sustained ventricular tachycardia, suggesting that cell injections could be pro-arrhythmic (Menasche, et al., 2001). In a subsequent large randomized, placebo-controlled trial, injection of skeletal myoblasts in patients with ischemic cardiomyopathy during CABG resulted in reverse left ventricular (LV) remodeling and no improvement in left ventricular ejection fraction with increased risk of arrhythmia in the early post-operative period (Menasche et al., 2008). There are ongoing preclinical investigations on a subpopulation of skeletal myoblasts called myoendothelial cells, which have a great propensity to differentiate into cardiomyocytes and endothelial cells compared to the undifferentiated pool of myoblasts. Although there is great promise for higher efficacy in cardiac repair using myoendothelial cells, the risk of arrhythmia will not be eliminated due to their inability to express connexin-43 and form gap junctions with existing cardiac myocytes (Menasche, 2008). Cardiac transplantation of myoblasts overexpressing connexin43 has been shown to improve the electrical coupling of skeletal myoblasts and cardiomyocytes, and in some studies eliminated pro-arrhythmogenic effect of skeletal myoblasts in small animal models of myocardial infarction (Fernandes et al., 2009).

### **Bone-marrow derived stem cells**

Testing a new hypothesis that greater plasticity could be brought to bear for cardiac regeneration, Orlic et al. reported myocardial and vascular regeneration after myocardial injury resulting from injection of bone marrow derived c-kit+, lin- stem cells (Orlic et al., 2001). The subsequent preclinical and translational studies using bone-marrow derived stem cells ignited research on myocardial regeneration and cardiac cell therapy (Menasche, 2011; Orlic, et al., 2001; Rota et al., 2005), leading to multiple clinical trials of whole bone marrow or its constituents. Meta-analyses examining the clinical trials of autologous bone marrow cells demonstrated safety and temporary improvement in left ventricular ejection fraction (Abdel-Latif et al., 2007; Menasche, 2011). These clinical trials by and large are small, heterogeneous in design with respect to cell type, dosage, timing, route of administration and have varied outcomes.

**Lessons learned from clinical trials of bone marrow stem cells**—The lessons learned from this heterogeneous group of clinical trials, as well as from subsequent mechanistic studies in basic science laboratories, influenced the design of subsequent clinical trials. In an earlier study of intracoronary autologous bone-marrow cell transfer after myocardial infarction, there was modest, but temporary improvement in left ventricular ejection fraction (LVEF) at 6 months (Wollert et al., 2004), which did not persist at 18-month follow up (Meyer et al., 2006). In the landmark REPAIR-AMI (Reinfusion of Enriched Progenitor Cell and Infarct Remodeling) trial, intracoronary infusion of bone marrow cells within 3-7 days of myocardial infarction (MI) resulted in a modest improvement in left ventricular ejection fraction (LVEF) (Schachinger et al., 2006), which persisted in a 2-year follow up (Assmus et al., 2010a). A more recent study examining the

effect of intracoronary infusion of bone marrow cells 2-3 weeks post-MI failed to show a similar improvement in LVEF (Traverse et al., 2011).

Taken together, the findings of these studies suggest that the timing of stem-cell transplantation following an acute myocardial injury may be a crucial determinant of the regenerative efficacy. Specifically, it appears that early transplantation, within a few days following acute myocardial infarction (AMI), is necessary for benefit. An important concept has emerged from these early trials, that clinical benefits might derive out of proportion to improvements in cardiac performance, and in this regard it is noteworthy that a two-year follow up in the REPAIR-AMI trial showed improved mortality regardless of whether and to what degree the ejection fraction improved (Assmus et al., 2010b). Accordingly, it is now important to start considering the inclusion of clinical endpoints in trial design of cell-based therapies for heart disease.

In the above studies, the working hypothesis was that benefits attained from the transplantation of exogenous stem cells were due to engraftment and differentiation of implanted cells into cardiomyocytes and possibly blood vessels. More recent data have shown that the benefits of cell therapy can arise from more elaborate, multi-factorial mechanisms. Animal studies have demonstrated that transplanted exogenous stem cells release paracrine factors that reduce apoptosis of native cardiomyocytes and improve LV remodeling (Burdon, Paul, Noiseux, Prakash, & Shum-Tim, 2011). These studies also raise the possibility that a time-dependent paracrine influence on the remodeling process may explain why early but not late transplantation is effective, post myocardial infarction.

The results of the earlier clinical trials and the recent mechanistic discoveries influenced the design of two large randomized, placebo-controlled trials. The TIME trial is an ongoing double-blind trial of 120 patients that is evaluating the safety and efficacy of administering bone marrow mononuclear cells at 3 vs. 7 days following AMI (Traverse et al., 2009). The European Society of Cardiology for Stem Cells and Cardiac Repair is planning to conduct a large 3000-patient trial to evaluate all-cause mortality in patients who receive bone-marrow derived mononuclear cells following AMI. This will be the first study to evaluate, as a primary outcome measure, the effect of stem cells on an outcome other than LVEF.

### Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are an emerging and highly promising therapeutic option. MSCs, which are widely distributed in tissues, can be easily isolated and expanded from bone marrow aspirates. As they lack MHC cell-surface markers, MSCs do not induce an immune response, and may therefore facilitate allogeneic transplantation of these cells. As a practical matter, it is convenient to use allogeneic cells because they can be harvested from a pool of individuals for transplant into multiple patients. As a result, MSCs have emerged as a viable candidate for stem cell transplantation in cardiac diseases (Williams & Hare, 2011). As demonstrated in preclinical and clinical studies, MSCs can differentiate into cardiomyocytes, endothelial cells, and vascular muscle cells (Amado et al., 2005; Quevedo et al., 2009; Toma, Pittenger, Cahill, Byrne, & Kessler, 2002). MSCs also have cytoprotective effects via paracrine factors, although the efficacy of injected cells exceeds those of the MSC conditioned medium containing the paracrine factors (Hatzistergos et al., 2010b). The history of MSCs in cardiac regeneration provides a robust example of translation from preclinical mechanistic studies into clinical trials. The translational influence is particularly evident regarding routes of administration of cells including intravenous, intracoronary, and transendocardial cell delivery methods.

**Lessons learned from different routes of administration of MSCs**—Intravenous injection of MSCs following AMI in animal models demonstrated that only 0.4% of the

MSCs were subsequently identified within the myocardium (Toma, et al., 2002). Furthermore, the majority of MSCs were found sequestered in the lungs (Halkos et al., 2008). This is not surprising, given that the lung is the first capillary bed encountered following intravenous (IV) administration. Beyond merely diverting cells from the cardiac targets, the pulmonary accumulation of pluripotent cells raised concerns that cell growths of unpredictable lineages might occur in the lung. Although a subsequent clinical trial of IV MSCs following AMI showed an excellent safety profile associated with 6 EF unit improvement in LVEF at 3 months (Hare et al., 2009) (Figure 1), persistent concerns about pulmonary sequestration led to a greater emphasis on intracoronary and transendocardial routes of cell administration.

A potential advantage of intracoronary administration of MSCs is that it delivers a higher concentration of stem cells to the heart than the intravenous route. Also, the conventional over-the-wire balloon angioplasty catheters can be used for injection of the cells (Wang et al., 2009). In a swine model of AMI, intracoronary delivery of MSCs resulted in decreased scar size and improved EF (Qi et al., 2008). Similarly, an earlier clinical trial of 69 patients receiving intracoronary MSCs following AMI resulted in an improvement in LVEF (Chen et al., 2004).

In the transendocardial approach, cells are injected across the endocardial wall directly into ventricular muscle tissue via a needle-tipped catheter (Sherman, Martens, Viles-Gonzalez, & Siminiak, 2006). Using a canine model of AMI, Perin et al. compared the retention rates of the delivered stem cells via the transendocardial vs. intracoronary routes. They found that transendocardial delivery resulted in greater cardiac retention of delivered cells and a more robust functional recovery (Perin et al., 2008). Transendocardial delivery of MSCs has also been studied in swine models of both AMI and chronic ischemic cardiomyopathy (Amado, et al., 2005; Quevedo, et al., 2009). In these studies, the approach was found to be safe and effective, with evidence of engraftment and differentiation of the MSCs into cardiomyocytes, vascular smooth muscle cells and endothelial cells. Also, scar size was reduced, while there was an improvement in blood vessel density, tissue perfusion and EF (Amado, et al., 2005; Hatzistergos et al., 2010a; Quevedo, et al., 2009).

Based on these positive findings, transendocardial MSC delivery was studied in clinical trials. Transendocardial injection of MSCs in 8 patients with ischemic cardiomyopathy resulted in improved contractility in the region of the injections. At 12 months, there was a reduction of end-diastolic and end-systolic volumes. Importantly, there was no increase in the risk of arrhythmias during 12 months of follow up (Williams et al., 2011). The promising findings of this study have led to several larger trials of endomyocardial delivery of MSCs and bone marrow cells in patients with ischemic (Trachtenberg et al., 2011), and non-ischemic cardiomyopathy (clinicaltrials.gov NCT01392625).

The issue of immunogenicity of MSCs is being examined in the Percutaneous StEm Cell Injection Delivery Effects On Neomyogenesis (POSEIDON-DCM) trials, (clinicaltrials.gov NCT01087996, and NCT01392625). In these randomized clinical trial, the safety and efficacy of transendocardial injection of allogeneic and autologous MSCs on LV function recovery will be compared head to head. Two parallel trials will test this hypothesis first in patients with ischemic cardiomyopathy and then in patients with non-ischemic dilated cardiomyopathy.

## Endogenous cardiac repair and the paradigm shift

### Cardiac Stem Cells

In early 2000, the concept of endogenous cardiac repair via resident cardiac stem cells (CSCs) was introduced, and challenged the old belief that the heart is a post-mitotic organ (Anversa & Nadal-Ginard, 2002; Hierlihy, Seale, Lobe, Rudnicki, & Megeney, 2002). Resident CSCs are self-renewing, clonogenic and multipotent. They can differentiate into cardiomyocytes, smooth muscle and vascular endothelial cells (Linke et al., 2005; Oh et al., 2003). Several laboratories have identified CSCs expressing different stem cell antigens including c-kit, Sca-1, MDR1, and CD31 (Linke, et al., 2005; Messina et al., 2004b; Oh, et al., 2003; Pfister et al., 2005). The different phenotypes of CSCs are believed to represent different stages of maturation of the resident cardiac stem cells (Laflamme & Murry, 2011). Accumulating evidence supports the concept that the dominant cell in the hierarchy of cardiac precursors expresses the c-kit receptor (Ferreira-Martins et al., 2012). Clusters of CSCs form structural and functional units called “stem cell niches” that are connected to fibroblasts and cardiomyocytes via junctional and adhesion proteins (Urbanek et al., 2006).

The paradigm shift in cardiac biology and the recent advances in our understanding of cardiomyocyte homeostasis have generated exciting opportunities for cardiac regeneration. There is growing interest in either using isolated cardiac stem cells as a line of therapy or developing strategies to potentiate the existing endogenous myocardial repair mechanisms (Linke, et al., 2005). Pre-clinical studies have shown that CSCs have the potential to reduce the infarct size and improve LV remodeling in animal models of myocardial injury (Johnston et al., 2009; Rota et al., 2008).

The robust preclinical data with CSCs has already been translated into clinical trials. The phase I SCIPIO trial (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy) demonstrated the safety and efficacy of intracoronary delivery of autologous c-kit+, lin-cardiac stem cells in patients with ischemic cardiomyopathy undergoing CABG (Bolli et al., 2011) (Figure 2). In this trial the c-kit+ cells were amplified from right atrial appendage tissue, were shown to have telomere lengths that were 7.5 kbp, and had minimal evidence of lineage commitment as shown by expression of c-kit in 75-98%, and expression of NKX2.5, GATA4, and Mef2c in only 0.1% of the cells. These findings suggested that the amplified cells retained the features of primitive stem cells, yet to undergo lineage commitment towards adult cellular constituents of the heart, and as such represent a population of true cardiac stem cells.

Another approach to cell-based therapy employing a cell product amplified from myocardial tissue entails the culturing of cardiospheres generated from endomyocardial biopsies (Messina et al., 2004a). Cardiospheres are clusters of undifferentiated, clonogenic stem cells, and were studied in a porcine model of acute MI, and were shown to reduce infarct size, and improve LV hemodynamics (Johnston, et al., 2009). In a recent phase I trial, CARDIOSphere-Derived aUTologous stem CELls to reverse ventricUlar dySfunction (CADUCEUS), the safety of intracoronary infusion of cardiosphere derived cells, grown from endomyocardial biopsies, was examined in patients following myocardial infarction (Makkar et al., 2012). This approach led to a reduction in scar tissue, and augmentation of viable tissue. In a somewhat paradoxical observation, this trial failed to detect an increase in ejection fraction. Future larger studies will be required to compare the effects of cardiospheres vs other cell approaches in populations of patients with well-defined cardiac disorders.

**Lessons learned from the paradigm shift**—The recent paradigm shift and discovery of CSCs has expanded our understanding of the mechanism of action of other types of stem

cells. For example, we have demonstrated that MSCs can mobilize clusters of c-kit<sup>+</sup> CSCs into the infarct zone (Figure 3), and form structures resembling stem cell niches (Hatzistergos, et al., 2010a; Urbanek, et al., 2006). The mechanism of activation of endogenous cardiac stem cells are not well known, but paracrine factors (Mohsin, Siddiqi, Collins, & Sussman, 2011a), and cell-cell interaction between exogenous stem cells and stem cell niches (Williams & Hare, 2011) are two suggested mechanisms. Identification of the paracrine factors involved in stimulation of CSCs can help optimize the effect of cell therapy by preconditioning stem cells or using genetic engineering to enhance their efficacy in activating endogenous regeneration (Mohsin, Siddiqi, Collins, & Sussman, 2011b).

### Pluripotent stem cells

Embryonic stem cells (ESCs) are derived from the inner cell mass of mammalian blastocysts, while induced pluripotent stem (iPS) cells are generated from somatic cells such as fibroblasts using retrovirus-mediated transduction of four transcription factors. iPS cells were first described by Takahashi and Yamanaka in 2006 in a mouse animal model (Takahashi & Yamanaka, 2006). The following year, human iPS cells were isolated from human fibroblasts (Takahashi et al., 2007). The morphology and gene expression of human iPS cells are similar to human ESCs. The two main challenges with iPS cells are: 1) retrovirus-mediated transduction imposes an increased risk of tumor formation due to reactivation of the integrated transgenes, and 2) there is poor efficacy of iPS differentiation into cardiomyocytes (Yoshida & Yamanaka, 2011). There are ongoing efforts to improve the method of iPS cell generation using adenoviruses and plasmid vectors for transduction of transcription factors while reducing the risk of tumor formation (Yoshida & Yamanaka, 2011). The focus is also shifted to directed differentiation of the iPS cells using signaling molecules to improve the efficacy of cardiomyocyte differentiation (Narazaki et al., 2008).

In a rodent model of MI, intramyocardial delivery of human iPS cells demonstrated in-situ generation of cardiomyocytes, smooth muscle cells and endothelial cells, and improvement of LV contractility (Nelson et al., 2009). However, the translation of this field into clinical studies will depend on improvements in the safety profile of the iPS cells and higher efficacy of generating these cells.

### Future translational findings

In the last decade, the efforts in the field of stem cell therapy in cardiovascular disease have resulted in generating a wealth of data on efficacy and safety of different types of stem cells. The key element in moving the field forward has been the constant mutually reinforcing interactions between basic and clinical/translational research approaches. Although some of the more rigorous clinical trials have failed to reproduce earlier positive results from smaller trials, the findings in larger clinical trials highlighted possible mechanistic questions that should be answered by more focused experiments in the basic science laboratories.

Ongoing efforts are needed to optimize the cell delivery techniques, cell preparations and cell survival. The discovery of CSCs and their role in endogenous repair of the myocardium has opened a new chapter in studying the cellular constituents and pathways involved in myocardial repair.

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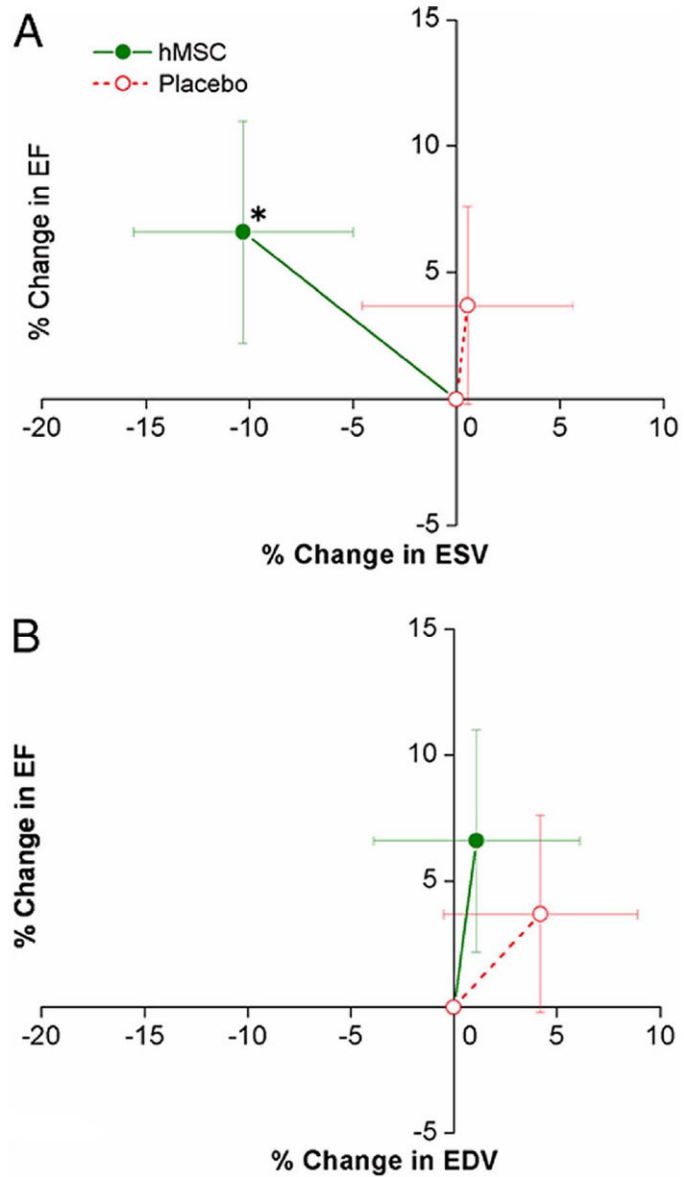
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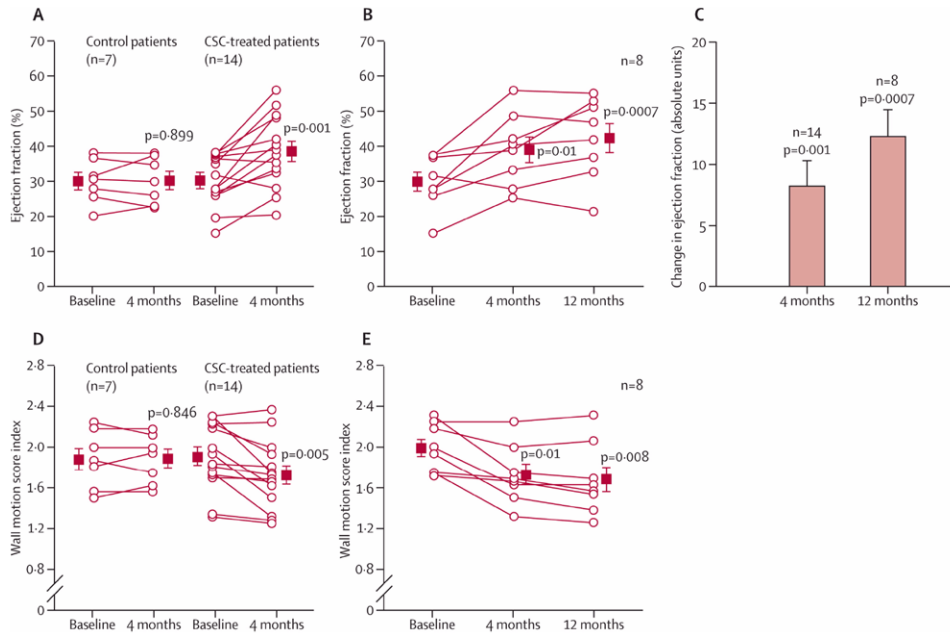


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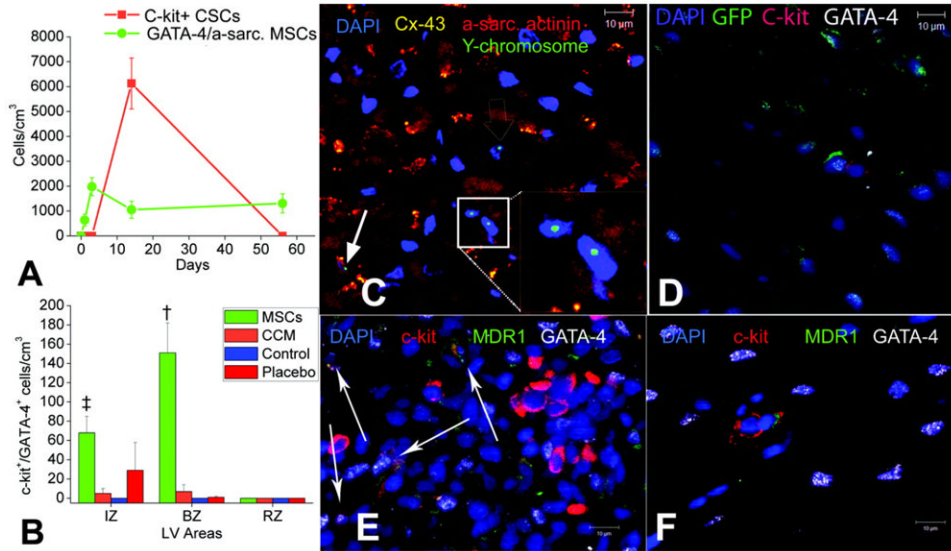


**Figure 1. Impact of human MSC treatment on prevention of LV remodeling following myocardial infarction, with permission from Hare et al., JACC 2009; 54(24): 2277-2286** Changes in left ventricular (LV) ejection fraction (EF) are plotted against the changes in LV end-systolic volume (ESV) (**A**) and end-diastolic volume (EDV) (**B**) during follow-up. Human mesenchymal stem cell (hMSC) patients (n = 21 at 3 months, n = 18 at 6 and 12 months) exhibit evidence of attenuated remodeling with no increase in LV EDV and a decline in LV ESV, whereas placebo patients (n = 13 at 3 months, n = 11 at 6 and 12 months) demonstrate evidence of LV chamber enlargement consistent with progressive ventricular remodeling. \*p = 0.005 versus baseline.



**Figure 2. Echocardiographic analysis of CSC-treated patients and controls in SCIPIO trial, with permission from Bolli et al., *Lancet* 2011; 378(9806): 1847-1857**

(A) Left ventricular ejection fraction (measured by use of three-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. (B) Ejection fraction at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. (C) Change in ejection fraction from baseline at 4 months and 12 months in CSC-treated patients. (D) Wall motion score index at 4 months after baseline in control and CSC-treated patients. (E) Wall motion score index at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. Boxes represent the mean values and (error bars represent SE). P values are reported for difference between baseline and 4 months and between baseline and 12 months. CSC=cardiac stem cell. As shown, CSC-treated patients had improvement in the regional wall motion score index, and overall ejection fraction at 4 and 12 months following treatment.



**Figure 3. MSCs stimulate endogenous CSCs, with permission from Hatzistergos et al. *Circulation Research* 2010;107:913-922**

(A) Graph depicting the contribution of cardiomyocyte precursors following exogenous administration of MSCs (green line) and endogenous CSCs (orange line) during cardiac repair after MI. At 2 weeks, MSCs activate endogenous expansion of c-kit<sup>+</sup> CSCs (orange line) (B) Two weeks following TEI, the number of C-kit<sup>+</sup> cells co-expressing GATA-4 is greater in MSCs versus non-MSCs treated hearts. (C and D) The 2-week-old chimeric myocardium contains mature cardiomyocytes (open arrow), immature MSCs (inset), and cardiac precursors of MSCs origin (arrow), coupled to host myocardium by connexin-43 gap junctions. Interestingly, endogenous c-kit<sup>+</sup> CSCs are found in close proximity to MSCs (D). (E) Cluster of c-kit<sup>+</sup> CSCs in an MSC-treated heart; numerous CSCs are committed to cardiac lineage documented by GATA-4 and MDR-1 co-expression (arrows). (F) Few isolated c-kit<sup>+</sup> cells were found in non-MSC-treated animals.