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Cell therapy for heart failure: A comprehensive overview of experimental and clinical studies, current challenges, and future directions

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Abstract

Despite significant therapeutic advances, the prognosis of patients with heart failure (HF) remains poor and current therapeutic approaches are palliative in the sense that they do not address the underlying problem – the loss of cardiac tissue. Stem cell-based therapies have the potential to fundamentally transform the treatment of HF by achieving what would have been unthinkable only a few years ago – myocardial regeneration. For the first time since cardiac transplantation, a therapy is being developed to eliminate the underlying cause of HF, not just to achieve damage control. Since the initial report of cell therapy (skeletal myoblasts) in HF in 1998, research has proceeded at lightning speed and numerous preclinical and clinical studies have been performed that support the ability of various stem cell populations to improve cardiac function and reduce infarct size in both ischemic and nonischemic cardiomyopathy. Nevertheless, we are still at the dawn of this therapeutic revolution. Many important issues (e.g., mechanism(s) of action of stem cells, long-term engraftment, optimal cell type(s), dose, route, and frequency of cell administration) remain to be resolved, and no cell therapy has been conclusively shown to be effective. The purpose of this article is to critically review the large body of work carried out with respect to the use of stem/progenitor cells in HF, both at the experimental and clinical level, and to discuss current controversies, unresolved issues, challenges, and future directions. The review focuses specifically on chronic HF; other settings (e.g., acute myocardial infarction, refractory angina) are not discussed.

Keywords

Stem cells; myocardial infarction; congestive heart failure; myocardial regeneration

Introduction

Heart failure (HF) is a common, lethal, disabling, and expensive disorder. Its prevalence in industrialized nations has reached epidemic proportions and continues to rise. Despite significant therapeutic advances, the prognosis for patients who are admitted to the hospital with HF remains poor, with a 5-year mortality of nearly 50% – worse than that for patients with breast or colon cancer [1]. In the United States, HF affects nearly 6 million persons, kills over 300,000 people per year, and is directly responsible for more than $40 billion in healthcare expenditures [2].

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None.

Although current therapeutic approaches to HF improve symptoms and prolong life, they are palliative in the sense that they do not address the fundamental problem – the loss of cardiac tissue. It is for this reason that stem cells have sparked intense interest. Stem cell-based therapies have the potential to dramatically transform the treatment and prognosis of HF by achieving what would have been unthinkable only a few years ago – myocardial regeneration. For the first time since cardiac transplantation, the goal is not damage control, but damage elimination – i.e., removal of the underlying cause of HF. It is the curative potential of this new therapy that explains why translational efforts have proceeded at lightning speed (Fig. 1). The first study of bone marrow cells in experimental myocardial infarction (MI) was published in 2001 [3]; within a year, this therapy had been applied in patients [4]. In the setting of HF, it took only three years from the first use of stem cells (skeletal myoblasts) in an animal model [5] to the first use of these cells in humans [6]. Few ideas in medicine have been translated from the experimental laboratory to the clinical arena faster than the use of stem cells in heart disease.

Over the past 15 years, numerous preclinical and clinical studies have been performed that support the ability of various stem cell populations to improve cardiac function and attenuate adverse left ventricular (LV) remodeling in both ischemic and nonischemic cardiomyopathy. Despite this rapid progress, however, many fundamental issues remain to be resolved and, to date, no cell therapy has been conclusively shown to be effective in patients with HF. The purpose of this article is to critically review the large body of work carried out with respect to the use of stem/progenitor cells in HF, both at the experimental and clinical level, and to discuss current controversies, unresolved issues, challenges, and future directions. This review focuses specifically on chronic HF; studies of stem cells in acute MI, refractory angina, and other conditions not relevant to chronic HF are not discussed.

Stem cell types investigated heretofore in HF

Stem cells are undifferentiated, self-renewing cells that possess a multi-lineage differentiation potential. As illustrated in Fig. 2, various types of stem cells have been considered for the treatment of HF. The preclinical and clinical studies that have assessed the utility of stem cells in chronic HF are summarized in Tables 1 and 2, respectively.

i) Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent cells harvested from the inner cell mass of preimplantation-stage blastocysts [7]. When cultured as 3-dimensional cystic aggregates (embryoid bodies), both mouse and human (h) ESCs have the capacity to differentiate into cells of all three germ layers, namely, ectoderm, endoderm, and mesoderm (including cardiomyocytes) [8, 9]. hESC-derived cardiomyocytes, which can be isolated from embryoid bodies by either mechanical dissection or enzymatic methods [10], exhibit adult cardiomyocyte morphology with properly organized sarcomeric proteins, and express cardiac-specific transcription factors such as Nkx2.5, GATA-4, and MEF2C [11]. Also, they display spontaneous beating activity with characteristic atrial, ventricular, and nodal action potentials [12, 13]. The strong cardiogenic potential of ESCs and the availability of hESC-derived cardiomyocytes have motivated research into their effects in HF. In the only study of these cells conducted in a large animal model to date, Menard *et al.* [14] reported that cardiac-committed mouse ESCs, transplanted into infarcted sheep myocardium, differentiated into cardiomyocytes and improved LV function. Similarly, using hESC-derived cardiomyocytes, Caspi *et al.* [15] and Cai *et al.* [16] reported formation of stable cardiomyocyte grafts, attenuation of LV remodeling, and improvement in LV systolic function in rat models of old MI (although in the latter study [16] they caused formation of teratomas).

Despite the well-documented capacity of ESCs for cardiac differentiation, both ethical and biological concerns have prevented their use as a treatment modality in patients. Specifically, because of their pluripotency and allogeneic nature, adoptive transfer of ESCs is plagued by teratoma formation [7,17] and graft rejection [17], two formidable problems that essentially preclude the clinical use of these cells. In contemporary clinical research, the margin of tolerance for such catastrophic effects as tumor formation is zero, and no matter how much the probability of tumors is reduced by various ESC manipulations [18-20], it is unlikely that it will be completely eliminated. One teratoma would be sufficient to halt clinical investigation of ESCs for years. On the other hand, the recent emergence of induced pluripotent stem cells (iPSCs), which have pluripotency comparable to ESCs, has provided an alternative that obviates one of the two major problems inherent in ESC-based therapies – graft rejection.

For ESCs, the chasm between promises made and results delivered has been striking. Since the late 1990s [7], these cells have been enthusiastically heralded as a major breakthrough in medicine that will usher in unprecedented opportunities for the treatment of human disease. [21-25] Despite these claims, however, no clinical trial of ESCs in cardiovascular disease has been conducted or even initiated, nor, to the best of our knowledge, is any such trial even being planned. During the same time frame, adult stem cells have been used safely in thousands of patients, with results that were sufficiently encouraging to warrant phase II and phase III trials. Clearly, the expectations raised by the advocates of ECSs have not been met. This sobering realization, coupled with the problems of tumorigenesis and rejection, makes it unlikely that enthusiasm for the therapeutic use of ESCs will continue unabated. The most reasonable interpretation of current knowledge is that ESC-based therapies have no future in terms of clinical application, at least in the next few years, and will probably become obsolete – a thing of the past, which will be remembered as an unfulfilled promise.

ii) Induced pluripotent stem cells

In 2006, Takahashi and Yamanaka [26] produced a population of iPSCs by transducing mouse adult fibroblasts with defined transcription factors (OCT3/4, Sox2, c-Myc, and Klf4) (the “Yamanaka factors”). These iPSCs express ESC surface markers and exhibit morphology and growth properties similar to those of ESCs [26]. It was subsequently demonstrated that the cardiogenic potential of iPSCs is very similar to that of ESCs, and that iPSC-derived cardiomyocytes possess functional properties typical of cardiac cells, such as spontaneous beating, contractility, and ion channel expression [27]. However, to date, no study has specifically assessed the therapeutic potential of iPSCs in animal models of HF.

Although iPSCs hold great promise for cardiac regeneration, the transcription factors used to generate these cells (c-Myc, Oct4, and Klf4) are known oncogenes that can produce teratomas. Newer methods that involve transient expression of the reprogramming factors may obviate this problem [28, 29], but the pluripotent nature of these cells may still promote tumorigenesis [30]. Other problems include the low efficiency of iPSC generation and the variability from one cell line to another [31]. Given the rapidly evolving technology in this field, it is possible that these technical hurdles will soon be overcome and iPSC-based approaches will prove to be helpful for the therapy of HF; at present, however, iPSCs are not ready for clinical application.

iii) Skeletal myoblasts

Skeletal myoblasts are derived from satellite cells, a skeletal muscle progenitor cell population present under the basal membrane of myofibers. With muscle injury, these satellite cells undergo proliferation and promote regeneration by differentiating into myotubes and new muscle fibers [32, 33]. Because of their ease of procurement from muscle

biopsies, rapidity of expansion *in vitro*, and resistance to hypoxic and ischemic conditions [34], skeletal myoblasts were the first cells to be tested both in preclinical [5] and clinical [6] studies of HF. However, myoblasts transplanted in injured hearts have been found to form skeletal (striated) muscle fibers rather than cardiac muscle [35].

The ability of skeletal myoblasts to promote cardiac repair has been evaluated in small [36, 37] and large [38-42] animals models of HF. Both after intramyocardial and intracoronary administration, these cells have been shown to differentiate into myotubes and form viable skeletal muscle-like grafts in the scarred myocardium, which was associated with attenuation of adverse ventricular remodeling, decreased interstitial fibrosis, and improvement of cardiac performance [36, 43, 44]. The reduction in fibrosis has been ascribed to correction of the imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) [45]. The ability of skeletal myoblasts to improve cardiac function has also been shown in nonischemic cardiomyopathy (induced by doxorubicin and δ-sarcoglycan gene mutation in rats [46] and CHF147 Syrian hamsters, respectively [47]); in both studies, intramyocardial injection of myoblasts improved LV function and decreased interstitial fibrosis. In the latter study, the benefits were ascribed to extracellular matrix (ECM) remodeling and activation of cardiac stem cells secondary to the secretion of growth factors [47].

These encouraging results from animal studies were quickly translated into clinical trials in HF. The first human transplantation of myoblasts was performed by Menasche *et al*. in patients with severe ischemic HF [6, 48] (Fig. 1). In this phase I study, injection of 871 million cells into a scarred LV region at the time of coronary artery bypass grafting (CABG) was associated with a significant improvement in New York Heart Association (NYHA) functional class and LV function. These observations, however, were difficult to interpret because of the confounding effects of concomitant surgical revascularization and lack of a suitable control group. Furthermore, 4 of 10 patients experienced ventricular tachycardia, warranting the use of implantable cardioverter-defibrillators (ICDs). This electrical instability has been ascribed to the lack of electromechanical coupling, due to the failure of differentiated myotubes to express key gap junction proteins such as N-cadherin and connexin-43 [49].

After this trial, several small, nonrandomized studies showed augmented LV function [48, 50-59], improved LV remodeling [50, 52, 60], and histological evidence of myoblast survival in the myocardium [61] following intramyocardial injection in patients with ischemic cardiomyopathy. Based on the promising results of these studies, Menasche *et al*. conducted MAGIC, a phase II randomized, placebo-controlled, double-blind trial that examined the effects of intramyocardial injection of skeletal myoblasts (at two doses: 400 or 800 millions) plus CABG vs. CABG alone (controls) in 97 patients with severe LV dysfunction (LV ejection fraction [EF] between 15-35%). There were no significant differences in cardiac function and occurrence of malignant arrhythmias between patients receiving myoblasts and controls at the end of 6 months; however, in a substudy, it was found that patients treated with 800 million cells had attenuation of LV remodeling and a decrease in LV volumes [62].

Other investigators have used catheter-based intramyocardial injection of skeletal myoblasts in ischemic HF [51, 53, 55, 58, 59]. A small (10 patients) phase I study of percutaneous trans-coronary-venous myoblast transplantation (the POZNAN trial) [55] reported an improvement in NYHA class and LVEF at 6 months of follow-up. Other studies in small patient cohorts by Biagini *et al*. [58] and Dib *et al*. (CAuSMIC trial) [59] reported improved NYHA functional class and increased LVEF at 1 year after therapy, but in the former study [58], the improvement in LV function was noted only during dobutamine infusion. A double-blind, randomized, placebo-controlled, multicenter study of transcatheter intramyocardial

administration of myoblasts in HF (the MARVEL trial), designed to enroll 330 patients, was terminated prematurely because of financial constraints; the preliminary results in 23 patients showed improvement in 6-min walk distance at 3 and 6 months, but also an increase in the occurrence of sustained ventricular tachycardia in 7 of 15 patients [63].

The long-term effects of intramyocardial myoblast injection in patients with ischemic cardiomyopathy have been evaluated in four trials [54, 56, 57, 64] (including a follow-up of the first Menasche study[56]). Although in three of these trials [54, 56, 57, 64] cardiac function improved, myoblasts were transplanted during surgical revascularization (CABG) or LVAD placement, which, as pointed out above, complicates the interpretation of the outcome. In the fourth study [64], in which myoblasts were delivered percutaneously by transendocardial injection, there was no beneficial effect on global or regional LV function at 4-year follow-up. These findings are consistent with the results of the SEISMIC trial, a recent phase IIa, randomized, open-label trial of percutaneous intramyocardial transplantation of myoblasts in HF patients [65]. In this study, myoblast therapy was not associated with any improvement in LVEF at 6-month follow-up, although there was an improvement in 6-min walk distance [65].

In summary, most of the smaller, nonrandomized clinical trials of skeletal myoblasts have yielded encouraging results, but the largest study to date (the MAGIC trial) failed to corroborate these findings. It must also be noted that many of these trials were performed in conjunction with CABG or LVAD procedures, making it difficult to separate the effects of myoblasts from those of revascularization. Because of the negative results of MAGIC, the risk of arrhythmias, and the availability of other cell types, interest in skeletal myoblasts has waned, and it seems unlikely that these cells will play a role in cell therapy of HF.

iv) Bone marrow-derived stem cells

The bone marrow harbors different types of hematopoietic and nonhematopoietic stem cell populations that have the potential to differentiate into diverse phenotypes (Fig. 2). Due to the relatively greater concentration of stem cells in the bone marrow and the ease of procurement of these cells, most of the preclinical and clinical studies in HF have utilized bone marrow-derived stem cells (Fig. 1, Tables 1 and 2).

a) Unfractionated bone marrow mononuclear cells—Bone marrow mononuclear cells (BMMNCs) are a heterogeneous population composed of mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), and more committed cell lineages. As BMMNCs can be easily procured using density gradient centrifugation and as these cells do not require extensive culture techniques, they have been used by many investigators in animal models of acute MI [3, 66, 67]. Relatively fewer studies have been performed in the setting of chronic HF, and the results are conflicting. In sheep [68] and pig [69] models of post-infarction HF, BMMNCs (injected directly into the scar tissue) produced no improvement in LV function (although one study reported increased angiogenesis and reduction in infarct size [69]). In contrast to these findings, studies in dogs (post-infarction HF) [70] and rats (cryoinjury-induced HF) [71] have reported improvement in myocardial function, reduction in plasma N-terminal pro brain natriuretic peptide (NT-proBNP) levels, and induction of angiogenesis.

Conflicting results have also been obtained in patients with HF. Perin *et al.* [72, 73] were the first to evaluate the safety and efficacy of autologous BMMNCs, injected transendocardially with a NOGA Myostar catheter, in patients with chronic ischemic HF (Fig. 1). At 2 and 4 months after therapy, there was a significant improvement in LVEF and a reduction in end-systolic volume in cell-treated patients [72]. During longer follow-up (6 and 12 months), these patients exhibited not only improved cardiac performance, but also an increase in myocardial perfusion and exercise capacity compared with controls [73, 74]. Directionally

concordant observations were made by other investigators, who reported that intramyocardial injection of BMMNCs (performed during surgery [75] or percutaneously via a NOGA device [76]) was associated with a decrease in HF symptoms and an improvement in LV function in patients with severe ischemic LV dysfunction. In contrast, trials using in-scar injections of BMMNCs in patients with ischemic HF failed to show improved LV function [77, 78]. The reasons for these differences are not obvious; one possibility is the site of cell delivery, as in the study by Perin *et al*. [72, 73] cells were injected into the peri-infarct viable myocardium rather than into the scar itself.

In addition to the intramyocardial route, numerous studies have examined the effect of intracoronary infusion of BMMNCs in patients with HF, again with mixed results. A number of trials have reported an improvement in various parameters of LV function and anatomy [79-81]. In the TOPCARE-CHD study, Assmus *et al*. [82] compared the effects of intracoronary infusion of $22\pm11\times10^6$ circulating EPCs or $205\pm110\times10^6$ BMMNCs on global LV function in 75 patients with chronic ischemic cardiomyopathy. At 3 months after therapy, LVEF improved significantly in patients receiving BMMNCs (+3.7±4.0 absolute EF units) but not in those receiving circulating EPCs (+0.4±3.0 absolute EF units) [82]. This difference in response may be due to the functional impairment of circulating EPCs in chronic HF patients [83], which limits their recruitment into the scar tissue, or it may reflect the contribution of cell types other than circulating EPCs. In the TOPCARE–CHD registry, Assmus *et al*. [84] enrolled 121 patients with ischemic HF and reported a significant reduction of both NT-proBNP and NT-proANP serum levels and a reduction in mortality at 3 months after intracoronary infusion of BMMNCs. However, other trials have failed to confirm the beneficial effects of intracoronary delivery of BMMNCs in HF [78, 85]. For example, when BMMNCs were given (intramyocardially or intracoronarily) during CABG surgery [78], there was no improvement in regional or global LV function and no reduction in scar size.

BMMNCs have also been studied in the setting of nonischemic cardiomyopathy [86, 87]. In TOPCARE-DCM [87], intracoronary infusion of $259\pm135\times10^6$ BMMNCs in 33 patients with DCM was associated with an improvement in regional contractile and microvascular function and a decrease in NT-proBNP serum levels, suggesting a beneficial effect on LV remodeling. Interestingly, the increase of regional contractile function was directly proportional to the functionality of the infused cells as measured by their colony-forming capacity [87].

In summary, studies of BMMNC administration in patients with chronic ischemic HF have yielded inconsistent results; all of these trials, however, have been small. Larger, phase II trials are needed to achieve definitive conclusions.

b) Mesenchymal stem cells—MSCs, also known as bone marrow stromal cells, are a subset of nonhematopoietic cells that are multipotent and plastic-adherent under culture conditions. MSCs can differentiate into chondrocytes, adipocytes, osteoblasts, and skeletal muscle cells, and have also been reported to differentiate into cardiomyocytes [88, 89] and endothelial cells [90], although this cardiogenic potential remains controversial [91]. MSCs typically express CD105, CD73, CD90, and STRO-1 but lack hematopoietic markers (CD45, CD34 and CD14/CD11b) [92].

The results of MSC administration in animal models of chronic HF have been encouraging. Direct epicardial injection of allogeneic MSCs in a dog model of ischemic HF induced by ameroid constriction resulted in differentiation of MSCs into smooth muscle cells and endothelial cells, increased vascularity, and improved myocardial function [93]. Similarly, autologous MSCs, injected directly into a myocardial infarct scar, have been reported to attenuate LV remodeling and reduce infarct size in a swine model of ischemic

cardiomyopathy [94]. These data provided the groundwork for an ongoing randomized, double-blind, placebo-controlled study of autologous MSCs in patients with chronic ischemic LV dysfunction undergoing CABG (PROMETHEUS; NCT00587990) (Table 3). In rat models of both ischemic [95-97] and nonischemic [98] cardiomyopathy, intramyocardial injection of MSCs has been shown to improve cardiac function [95-98], increase angiogenesis [95, 98], and reduce myocardial fibrosis [96, 98]. To date, the only clinical study that has examined the effects of MSCs in patients with HF is the POSEIDON trial by Hare *et al*. [99], which compared three doses of autologous or allogeneic MSCs (20, 100 and 200 × 10^6 cells) in patients with ischemic cardiomyopathy and demonstrated that all doses favorably affected patient functional capacity, quality of life, and ventricular remodeling (Table 2).

c) Hematopoietic stem cells and endothelial progenitor cells—HSCs reside in the bone marrow and differentiate into cells of both myeloid and lymphoid lineages. EPCs, on the other hand, are mobilized into peripheral blood in response to ischemic injury and promote neovascularization by differentiating into endothelial cells (reendothelialization) [100, 101]. CD34 is a typical surface marker of both HSCs and EPCs [102]. Thus, CD34+ cells are found in the bone marrow and in the peripheral blood and have the potential to give rise to all blood cell types as well as endothelial cells (<1% of nucleated cells in the blood are CD34+).

Autologous CD34+ cell transplantation has been performed in patients with both ischemic [103] and nonischemic [104, 105] cardiomyopathy (Fig. 1). In the former setting, injection of CD34+ cells into the peri-infarct, viable LV regions during off-pump CABG surgery produced a greater improvement in contractile function than did CABG alone [103]. Also, a small pilot study evaluating the safety and feasibility of intracoronary CD133+ or CD133-, CD34+ cell therapy in patients with old anterior MI reported a sustained improvement in regional perfusion and LV remodeling with both cell types [106]. In the setting of nonischemic cardiomyopathy, a study by Vrtovec *et al*. concluded that intracoronary infusion of CD34+ cells led to an increase in LVEF and 6-min walk distance and a decrease in NT-proBNP levels [104]. Importantly, these beneficial effects were sustained during long-term follow-up [105]. Another surface marker of HSCs and EPCs is CD133 (AC133) [107]. Stamm *et al*. [108] examined the effects of CD133+ cells, given by intramyocardial injection during CABG, in patients with ischemic HF. At 6 months after treatment, LVEF and perfusion of the infarcted myocardium increased to a greater extent in patients who received CABG and CD133+ therapy than in those who received CABG alone.

Recently, Perin *et al*. [109] investigated a novel population of hematopoietic cells, referred to as aldehyde dehydrogenase-bright ($ALDH^{br}$) cells, in 20 patients with ischemic HF (10 control and 10 treated). $ALDH^{br}$ cells, which have been isolated from human bone marrow and peripheral blood, express CD34, CD117, CD105, CD133, and CD166 and include primitive CD34+/CD38- cells [110]. Transendocardial delivery of $ALDH^{br}$ cells produced a significant decrease in LV end-systolic volume at 6 months and a trend toward improved maximal oxygen consumption [109].

In summary, the initial experience with CD34+ and CD133+ cells in HF (both of ischemic and nonischemic origin) is encouraging but limited by the small size of the trials. As is the case for other cells, larger studies will be necessary to evaluate the role of these cell types in the treatment of HF.

v) Adipose-derived MSCs

Adipose tissue contains a pool of multipotent stem cells, designated as adipose-derived MSCs that are able to replicate as undifferentiated cells, to develop as mature adipocytes,

and to differentiate into other cell types along the mesenchymal lineage. Reports that adipose-derived MSCs can differentiate into cardiomyocytes [111] and endothelial cells [112] have motivated studies in animal models of HF. Using a cell sheet technology, Miyahara *et al*. [113] reported that transplantation of monolayered MSCs into scarred myocardium reversed wall thinning in the scar area and improved cardiac function. In another study [114], the effects of transplanting undifferentiated or cardiac pre-differentiated adipose-derived MSCs were compared with those of BMMNCs in a rat model of chronic MI. One month after transplantation, adipose-derived MSCs induced an improvement in LVEF, an increase in angiogenesis, and a decrease in fibrosis that were significantly greater than those effected by adipose-derived cardiomyogenic cells or BMMNCs [114]. Additionally, intramyocardial injection of adipose stem cells at 1 week after coronary occlusion has been reported to mitigate the deterioration in cardiac contractile function and enhance angiogenesis in infarcted rat hearts [115].

In the clinical arena, no full report of adipose-derived MSCs in HF is available yet. The preliminary results of the PRECISE trial by Perin *et al*. [116] in 27 patients indicate that administration of adipose-derived cells resulted in stabilization of infarct size and improvement in maximal oxygen consumption.

vi) Cardiac stem cells

One of the most dramatic developments in the history of cardiac biology has been the recent recognition that the adult heart undergoes a continuous turnover of its cellular components (including myocytes) [117]. This process is thought to be underlain by a population of resident stem cells that possess the capacity to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells [117] (Fig. 2). The discovery that the heart is a self-renewing organ has not only refuted the long-held doctrine that the myocardium is a postmitotic tissue (composed of cells that have withdrawn from the cell cycle and are terminally differentiated), but has also opened exciting therapeutic avenues.

a) c-kit+ Cardiac stem cells—In 2003, Beltrami *et al*. described a population of cells isolated from the adult rat heart that expressed the tyrosine kinase receptor c-kit (a marker of stemness) but lacked any markers of hematopoietic lineage [118]. These c-kit+ cardiac stem cells (CSCs) were shown to be self-renewing, clonogenic, and multipotent, exhibiting the ability to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells both *in vitro* and *in vivo* [118-120]. Four years later, a similar population of c-kit+ CSCs was identified in the adult human heart [120]. Injection of human CSCs into infarcted rodent myocardium resulted in improvement of LV function and structure and formation of a chimeric heart that contained human myocardium composed of myocytes and coronary vessels [120].

In the past decade, the ability of human and rodent CSCs to alleviate LV dysfunction and remodeling and promote regeneration has been repeatedly demonstrated by several laboratories in various preclinical animal models of acute MI [119, 121-124]. Evidence that ischemic cardiomyopathy is associated with loss of functionally competent CSCs [125] has ignited interest in investigating the effects of CSCs in the setting of chronic HF as well. Intramyocardial injection of c-kit+ CSCs at the borders of an infarct 20 days after a permanent coronary occlusion in rats was reported to result in replacement of ~42% of the scar with new myocardium, attenuation of LV dilation, and preservation of LV function [126]. However, in contemporary medicine, most infarcts are reperfused. Furthermore, from a practical standpoint, the technique most conducive to widespread use of CSCs in patients with HF would be intracoronary delivery. To address these issues, Tang *et al*. [127] investigated whether administration of CSCs is effective in regenerating cardiac tissue and

alleviating postinfarction LV remodeling and dysfunction when these cells are infused intracoronarily in the setting of an old MI produced by a temporary coronary occlusion followed by reperfusion. One month after coronary occlusion/reperfusion, rats received an intracoronary infusion of vehicle or EGFP-labeled CSCs. Thirty-five days later, CSC-treated rats exhibited more viable myocardium in the risk region, less fibrosis in the noninfarcted region, and improved LV function [127]. However, the number of EGFP+ cells expressing markers of cardiogenic commitment was too small to account for the augmentation of LV function (EGFP+ cells accounted for only 2.6±1.1% of the region at risk and 1.1±0.4% in the noninfarcted region). These observations suggest that an important mechanism whereby CSCs produced their salutary effects was the secretion of cytokines/growth factors that exerted paracrine actions on endogenous cells, particularly endogenous CSCs, which in turn proliferated and differentiated into adult cardiac cells. In support of this hypothesis was the finding that the pool of endogenous CSCs expanded to a greater degree in CSC-treated than in control rats [127].

The efficacy of CSCs in chronic ischemic cardiomyopathy [126, 127] was surprising, as a scar would seem to be a very hostile environment to the homing and survival of transplanted cells, and the signals (adhesion molecules and growth factors) that attract and activate CSCs soon after ischemia-reperfusion would be expected to have largely abated once the healing process is complete. To verify these rat findings [126, 127] in a large, clinically-relevant species, a similar study was performed in pigs that underwent a 90-min coronary occlusion followed by reperfusion [128]. At the time of occlusion, the right atrial appendage was harvested for isolation and expansion of c-kit+ CSCs; 3 months after MI, 1 million autologous CSCs were infused into the infarct-related artery using a balloon catheter. Similar to the results obtained in rats, one month later the pigs treated with CSCs exhibited an increase in LVEF and systolic thickening fraction in the infarcted LV wall, as well as a decrease in LV end-diastolic pressure (LVEDP) and an increase in LV dP/dt_{max} [128]. The encouraging results of these studies of intracoronary CSC infusion in the setting of an old MI [127, 128] laid the groundwork for SCIPIO, the first clinical trial of CSCs (Fig. 1).

SCIPIO was a phase I, randomized, open-label trial of autologous CSCs for the treatment of ischemic HF. The target population consisted of patients with LVEF ≤40% who underwent CABG. Approximately 4 months after CABG, 1 million autologous CSCs (isolated and expanded from myocardial tissue harvested during surgery) were administered by intracoronary infusion; controls were not given any treatment. Although the two-year follow-up has not been completed, the interim results are very encouraging [129, 130]. In 20 CSC-treated patients, LVEF (measured by 3-D echo) increased from 29.0 ± 1.7% before CSC infusion to 36.0 ± 2.5% at 4 months after infusion. By contrast, in 13 control subjects, LVEF did not change. The salubrious effects of CSCs persisted and, if anything, became even more pronounced at 1 year (LVEF: +8.1% vs. baseline, n=17) and 2 years (LVEF: +12.9%, n=8) [131]. In nine CSC-treated patients in which MRI could be performed, there was a profound reduction in infarct size at 4 months (from 34.9±2.3 to 21.6±2.7 g [-38.1%]) and even more at 1 year (from 33.9±3.0 to 18.7±3.6 g [-44.8%]) [129]. These salubrious effects were associated with a significant improvement in the NYHA functional class and in the quality of life (measured by the Minnesota Living with Heart Failure Questionnaire).

Aside from the setting of ischemic cardiomyopathy, CSCs have also been found to exert salutary effects in a rat model of anthracycline-induced cardiomyopathy [132].

In summary, several studies have documented the ability of CSCs to promote regeneration and alleviate LV dysfunction and remodeling in various preclinical models of post-MI cardiomyopathy. The results of the first clinical trial (SCIPIO) are consistent with this preclinical work and suggest that intracoronary infusion of autologous CSCs results in a

substantial and sustained improvement in LV systolic function, in a reduction in infarct size, and in clinical improvement in patients with ischemic HF. These promising observations warrant larger, phase II studies. It is important to note that although in SCIPIO CSCs were isolated from the right atrial appendage, it is now possible to isolate and expand these cells from endomyocardial biopsy specimens [133], which makes the use of autologous CSCs potentially applicable to most patients with HF.

b) Cardiospheres and cardiosphere-derived cells—Cardiospheres were first described by Messina *et al*. [134] in 2004. Using subcultures of atrial or ventricular human biopsy samples and murine hearts, these authors isolated a population of cells that grew as self-adherent clusters and could differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells. Messina *et al*. termed these clusters "cardiospheres" [134]. Three years later, Smith *et al*. [135] presented a method in which cardiospheres obtained from percutaneous endomyocardial biopsy specimens were plated to yield cardiosphere-derived cells (CDCs). These CDCs were reported to differentiate into electrically stable cardiomyocytes *in vitro* and, when injected into a murine infarct model, to promote cardiac regeneration and improved cardiac function [135]. In 2009, Johnston *et al*. reported that intracoronary delivery of human CDCs in pigs with old MI resulted in cardiac regeneration, reduction in "relative" infarct size, attenuation of adverse LV remodeling, and improvement in cardiac function [136].

Phenotypically, cardiospheres and CDCs are a heterogeneous mixture of many different cell types, including cells that express endothelial (KDR [human]/ flk-1 [mouse], CD31), stem cell (CD34, c-kit, Sca-1), and mesenchymal (CD105, CD90) antigenic markers [134] (Fig. 2). Which of these cells type(s) is responsible for the observed effects on cardiac function and remodeling is unknown. In CADUCEUS, 98% of CDCs infused were positive for CD105, suggesting a mesenchymal nature [137]. In a recent study by the same group [138], the safety and efficacy of direct intramyocardial injection of CDCs and cardiospheres were compared in a porcine model of post-MI HF; although CDCs and cardiospheres had equivalent effects on LVEF, the latter were superior in improving hemodynamics and regional function and in mitigating ventricular remodeling. The enhanced potency of cardiospheres for myocardial repair has been attributed to enhanced "stemness" and cell-matrix interactions [139].

This preclinical work was translated by Makkar *et al*. [137] into a phase I, randomized trial (CADUCEUS) in patients with a recent MI and LVEF≤45% but ≥25%. At 1.5-3 months after MI, 17 patients received an intracoronary infusion of escalating doses of autologous CDCs (12.5, 17.3, or 25 million cells), which were produced from an endomyocardial biopsy. (However, the amount of tissue used to produce CDCs was reported to be 276 mg [SD 177, range 93–891 mg] [137], which is all but impossible to obtain with endomyocardial biopsies.) Eight control patients received standard care. In two patients, CDCs were found to be aneuploid (trisomy 8) and had to be discarded. At 12 months of follow-up, CDC-treated patients exhibited a 42% reduction in scar size (from 24% to 12% of the left ventricle), concomitant with an increase in viable tissue and regional systolic wall thickening in the infarcted region. However, CDC therapy failed to increase LVEF, reduce LV volumes, and improve NYHA functional class or quality of life as assessed with the MLHFQ [137]. Although the increase in non-gadolinium enhanced tissue in CDC-treated patients was claimed to be proof of cardiac regeneration [137], it could also be accounted for by other changes unrelated to regeneration, such as hypertrophy, decreased interstitial space, reduced vascular permeability, and/or improved perfusion [140-144].

In summary, CDCs are a mixture of different cell types (predominantly expressing mesenchymal markers) that have been reported to promote regeneration and alleviate post-MI dysfunction and remodeling in various preclinical models [135, 136, 138, 145, 146]. The

clinical effects of CDCs are unclear. The MRI data reported in CADUCEUS are consistent with regeneration (but they do not prove it); however, evidence that CDCs have beneficial effects on global LV function and clinical status is still lacking. Given the heterogeneous nature of this cell preparation, it will be difficult to identify which component(s) accounts for the salubrious effects. As is the case of c-kit+ CSCs, larger Phase II studies are needed to evaluate the therapeutic potential of CDCs.

c) Other cardiac progenitor cells

Sca-1+ cardiac stem cells: The existence of Sca-1+ progenitors in the adult mouse heart was reported by Oh *et al.* [147]. These cells expressed CD31 and cardiogenic transcription factors (GATA-4, MEF2C, and MEF-1) but lacked blood lineage markers, c-kit, Flt-1, Flk-1, vascular endothelial cadherin, von Willebrand factor, and hematopoietic stem cell markers (CD45 and CD34) [147]. *In vitro*, Sca-1+ cells have the ability to express cardiac structural genes and differentiate into beating cardiomyocytes upon treatment with 5-azacytidine [147] and oxytocin [148] Transplantation of Sca-1+ cells into the peri-infarct and infarct zones in a murine model of MI resulted in endothelial and cardiomyogenic differentiation of these cells with attenuation of LV remodeling [149]. However, the effects of these cells in the setting of chronic HF remain to be determined; further, the lack of a human homologue of Sca-1 makes translation difficult.

Side population cells: The so-called side population (SP) cells are characterized by their ability to exclude the Hoechst 33342 dye via the ATP binding transporters Bcrp1/Abcg2 and MDR1 [150]. First identified in murine bone marrow as HSCs [151], SP cells were subsequently isolated by Martin *et al.* [152] from adult as well as embryonic mouse hearts and characterized as CD31-, Sca-1^{high}, c-kit^{low}, CD34^{low}, and CD45^{low}. Although cardiac SP cells have been reported to differentiate into mature cardiomyocytes, endothelial cells, and smooth muscle cells and to regenerate cryoinjured myocardium [153], their ability to induce cardiac repair has not been tested.

Islet-1+ cells: During cardiogenesis, Isl-1+ cells give rise to cardiac muscle, the conduction system, and endothelial and smooth muscle cells in the heart compartments [154]. Laugwitz *et al.* [155] proposed that Isl-1+ cells represent endogenous cardiac progenitors that display conversion to a mature cardiac phenotype, with intact calcium dynamics and action potentials [155]; however, the ability of these cells to repair injured myocardium *in vivo* has never been demonstrated. Importantly, these cells do not exist in the postnatal ventricular myocardium, either under normal conditions or after MI, making it unlikely that they serve as cardiac progenitors or will have any clinical application [156].

Potential mechanisms of actions of stem cells in HF

Taken together, the studies reviewed above (Tables 1 and 2) suggest that at least some types of cell therapy are likely to improve cardiac function in chronic HF. What remains largely unknown, however, is the mechanism(s) responsible for these beneficial effects. Below we discuss briefly the various hypotheses that have been proposed (Fig. 3).

(Trans)differentiation of transplanted cells into cardiac cells

Although this may seem the most obvious explanation for the salubrious effects of stem cells, the evidence obtained thus far does not support (trans)differentiation of transplanted cells as the only, or even the major, mechanism of action. As mentioned above, Reinecke *et al.* [157] found that transplanted skeletal myoblasts differentiate into skeletal muscle fibers and do not express cardiac-specific genes. Transdifferentiation of bone marrow cells into cardiac myocytes remains highly controversial, with studies both supporting [3, 71, 158] and

refuting [66, 67] this concept. Others have suggested fusion of bone marrow cells with resident cardiomyocytes as the responsible mechanism [159, 160], but this has also been refuted [161, 162]. Similarly, transdifferentiation of human peripheral blood CD34+ cells into cardiomyocytes and vascular smooth muscle cells remains controversial [163, 164]. Although the therapeutic benefits of MSCs have been ascribed to differentiation towards cardiac and vascular lineages [88, 89, 98, 165], most studies have not supported this concept, suggesting instead that the major actions of MSCs are paracrine [166-168].

A similar uncertainty applies to cardiac-derived cells. As discussed above, CSCs are multipotent, being able to differentiate into myocytes, endothelial cells, and vascular smooth muscle cells *in vitro* [118]. When transplanted in injured hearts, CSCs give rise to vascular cells and to cells that express myocyte-specific proteins (although these cells are usually small and do not resemble adult myocytes) [121, 124, 126-128]. In some studies, particularly in models of acute MI, the magnitude of this regenerative process has been found to be substantial [118, 119, 169, 170]. However, in a rat [127] and pig [128] model of chronic post-MI HF, differentiation of transplanted CSCs into myocytes or myocyte-like cells was quantitatively insufficient to account for the improvement in LV function. In the case of CDCs, differentiation into cardiac cells has been reported to be either a minor mechanism of action [171] or non-existent [172, 173].

In summary, differentiation of transplanted cells along the cardiac lineage may occur. However, the key issue is the magnitude of this phenomenon vis-à-vis the improvement in function. In most of the studies reported to date, the functional benefits appear to be disproportionate to the relatively small number of new cardiac cells formed by differentiation of transplanted cells; consequently, the former cannot be accounted for solely by the latter. Other mechanisms must be at work.

Formation of new blood vessels from transplanted cells

Differentiation of transplanted cells into new blood vessels has been reported with various cells (e.g., MSCs [93], adipose-derived cells [174, 175], CD34+ cells [176, 177] and CSCs [118, 178]). Experimentally, this phenomenon may be important in models of chronic coronary occlusion, which can be associated with the presence of ischemic but viable myocardium [118, 119, 169, 170], but not in models in which the artery that supplies the infarcted/scarred myocardium is patent [127, 128]. Clinically, formation of new vessels may contribute to improved cardiac performance in some patients with ischemic heart disease, but it is difficult to envision how it could do so in the setting of nonischemic cardiomyopathy or in patients with ischemic heart disease who do not have flow-limiting coronary lesions (e.g., revascularized patients).

Paracrine mechanisms

The inability to explain the salutary effects of transplanted stem cells on the basis of their differentiation has led to the “paracrine hypothesis” [167], that is, the concept that transplanted cells induce myocardial repair by releasing signals (cytokines, chemokines, growth factors, possibly exosomes or microparticles) into the surrounding tissue, which in turn promote a number of restorative processes including activation of endogenous CSCs, neovascularization, inhibition of apoptosis, inhibition of hypertrophy, and favorable alterations of the ECM. Collectively, these actions result in enhanced LV function, improved perfusion, and myocardial repair [167].

i. Activation of endogenous CSCs. In the aforementioned study by Tang *et al*. [127] in a rat model of chronic HF, infusion of exogenous CSCs was found to promote proliferation of endogenous CSCs in both the infarcted and noninfarcted regions, suggesting that activation of the endogenous pool of CSCs via paracrine

mechanisms was a major mechanism of benefit. It is known that CSCs secrete growth factors (such as hepatocyte growth factor [HGF] and insulin growth factor-1 [IGF-1]) that stimulate other CSCs to migrate through the myocardial interstitium, proliferate, and differentiate into myocytes and vascular structures. [126, 168]. Activation of endogenous CSCs has also been suggested to be an important mechanism underlying the beneficial effects of other cell types, including MSCs [168].

ii. Induction of neovascularization. Many stem cells can induce neovascularization by secreting chemokines (stromal cell-derived factor-1 [SDF-1]) [70, 179, 180] and proangiogenic factors (vascular endothelial growth factor [VEGF]), basic fibroblast growth factor [FGF], HGF, IGF-1, tissue growth factor-β [TGF-β], and angiopoietin-1) [45, 98, 181, 182]. EPCs recruited to the ischemic area can also secrete the endothelial and inducible isoforms of nitric oxide synthase (eNOS and iNOS) and promote proliferation of endothelial cells [183]. The resulting neovascularization may improve blood supply to the viable cells that remain in the infarcted region and thus improve cardiac function in settings of chronic coronary occlusion; as mentioned above, however, this mechanism would not account for improved function in experimental models of reperfused infarction, where no residual ischemia is present, or in patients without persistent ischemia.

iii. Inhibition of apoptosis. A number of studies suggest that paracrine factors (such as IGF-1) released by stem cells following transplantation inhibit cardiomyocyte death by apoptosis (e.g., [98]). *In vitro* and *in vivo* data in models of acute MI suggest that Akt overexpressing MSCs decrease cardiomyocyte apoptosis [167, 182]. Combined transplantation of skeletal myoblasts and AC133+ cells was also reported to improve cardiac function by reducing myocardial apoptosis [44].

iv. Inhibition of hypertrophy. Administration of stem cells in models of HF is associated with a reduction in the hypertrophic response of surviving myocytes [36, 71, 95, 126, 127]. It remains uncertain, however, whether this is a primary action of transplanted cells or it is secondary to improved cardiac performance.

v. Remodeling of the extracellular matrix. Stem cells can modulate various constituents of the ECM, thereby limiting infarct expansion, LV remodeling, and myocardial fibrosis. Skeletal myoblasts have been reported to preserve matrix collagen architecture [36], to reduce fibrosis in the peri-infarct and infarct-remote regions, [37] and to modulate MMP-2 and TIMP-4 levels [45], suggesting a favorable effect on the ECM metabolism. The importance of ECM alterations in CSC-dependent repair is underscored by the findings of Rota *et al*. [126], who reported that CSCs increased MMP-2, MMP-9, and MMP-14 levels and decreased TIMP-4 levels in a rat model of post-MI HF.

Cell fusion

In 2004, spontaneous cell fusion was proposed as an alternative mechanism by which transplanted bone marrow cells produce apparent regeneration of various adult tissues [66, 67, 160]. This concept was based on work by Alvarez-Dolado *et al*., who used a method based on Cre-Lox recombination for detecting cell fusion events of bone marrow cells with cardiomyocytes [159]. Subsequent studies [161, 162], however, concluded that c-kit+ bone marrow cells differentiated into myocytes and coronary vessels independent of cell fusion. The use of Cre-Lox recombination as an appropriate model to study cell fusion has been challenged, as the unmodified Cre-recombinase in the progenitor cells can cross the membrane of the recipient cell [184], thus mimicking cell fusion. The notion that cells fusion

is an important mechanism underlying the salubrious effects of stem cells has lost support in recent years.

Current challenges, unresolved issues, and future directions

Taken together, the preclinical and clinical work performed to date suggests that administration of stem cells has considerable potential to improve cardiac function and regenerate viable myocardium in HF. Despite these encouraging results, however, no cell type has been conclusively demonstrated to be effective in alleviating HF in patients. It is clear that in order to unleash the full potential of cell-based therapies and proceed toward clinical translation, a number of major unresolved issues will have to be resolved; for example, what are the optimal cell type(s), the optimal cell dose, the optimal route of cell administration, and the optimal frequency of treatment? These questions can be answered only by conducting careful preclinical and clinical studies.

Unfortunately, the current environment does not support studies that compare cells, doses, routes of administration, and frequency of treatment. At the preclinical level, this type of work is likely to receive low priority scores by peer review groups because it is, by definition, descriptive and lacks mechanistic insights and conceptual novelty. In the clinical arena, comparisons of different cell types or doses are expensive and time-consuming. It is hoped that sponsors and funding agencies will recognize that this type of research is indispensable to translate cell base-therapies to humans and will identify it as a priority for funding.

i) Cell type

It is unknown which, among the many different types of stem/progenitor cells that have been studied to date (Tables 1 and 2), is most effective in a given pathophysiological setting. Despite the obvious importance of this question, very few studies have directly compared different cell types with respect to the outcomes of therapy [45, 70, 97, 185]. Such studies are difficult because they require that the dose-response relationships for each cell type be defined and compared (as simply comparing one dose of cells would be inadequate). This has not been done heretofore. For example, the claim that CDCs are “superior” to CSCs is untenable because it is predicated upon the use of one dose of cells [185]. Similarly, the few studies that have compared different cell types [45, 70, 97] have not evaluated the dose-response relationships for each cell type.

A related and unresolved issue is whether combinations of different cell types may be more efficacious than a single cell type. Theoretical considerations, as well as preclinical studies of BMMNCs, skeletal myoblasts [44, 186, 187], MSCs, and CSCs [188], suggest that the former approach may offer advantages, as the actions of different cells may be complementary or even synergistic [188].

ii) Cell dose

It is evident from Tables 1 and 2 that the doses of cells used to treat chronic HF have varied enormously. Although it seems obvious that the effects of cell-based therapies will depend on the number of cells administered, the nature of this relationship is still unknown for most cell types. In the clinical realm, only two studies have addressed the dose-dependency of the effects of stem cells in HF. In the MAGIC trial [62], a higher dose (8×10^6) of skeletal myoblasts was more effective in decreasing LV volumes and reversing LV remodeling than a low dose (4×10^6), although neither dose improved LV function. In the POSEIDON trial, Hare *et al.* [99] compared three doses of autologous or allogeneic MSCs (20, 100, and 200 × 10^6 cells) in patients with ischemic cardiomyopathy and demonstrated that all doses

favorably affected patient functional capacity, quality of life, and ventricular remodeling, although 200×10^6 was (unexpectedly) less effective than 20×10^6. These results differ from those obtained by these investigators in a swine model of ischemic cardiomyopathy, in which both a high dose (200×10^6 cells) and a low dose (20×10^6 cells) of MSCs increased regional function, but only the high dose effected reverse remodeling [94]. To address this important issue, an ongoing phase II dose-escalation study (REVASCOR) is assessing the feasibility and safety of transendocardial delivery of three doses of allogeneic mesenchymal precursor cells (25, 75, 150×10^6 cells) in patients with HF (NCT00721045) (Table 3). Similar studies of the dose-response relationship are needed for other cell types.

iii) Route of administration

As is the case for the optimal cell type and dose, the most effective technique to deliver cells to the heart is still unknown. The major routes used to date are direct injection into the LV wall (transendocardially or transepicardially) and intracoronary infusion. Transepicardial injection is performed during cardiac surgery [54, 62]; this method offers direct visualization of the scarred regions but is limited by the requirement for surgery. With transendocardial injection, cells can be delivered directly into the LV wall by using an injection catheter advanced across the aortic valve and positioned against the endocardial surface. The advantages of this technique over intracoronary infusion are that: i) electromechanical mapping of the endocardial surface with a NOGA system can be used to trace viable, ischemic, and scarred myocardium, thereby enabling targeted injection of cells into the scar or into the border zone, and ii) cells can be delivered to a scarred region even if the coronary artery supplying it is totally occluded. Because of these advantages, transendocardial injection has been used extensively in the clinical arena [51, 53, 58, 59, 63-65, 72-74, 76]. However, intramyocardial injections may disrupt tissue architecture and create cell clumps that lack adequate blood supply, resulting in cell death. Further, the distribution of cells within the infarcted region is usually inhomogeneous [124, 189].

Intracoronary delivery involves the infusion of cells into a coronary artery, usually during a brief coronary occlusion produced by inflating a balloon at the tip of the catheter. The rationale for stopping flow is to prevent the rapid “washout” of the cells and to facilitate their extravasation into the interstitium. Compared with transendocardial injection, intracoronary delivery offers several advantages: i) it results in a much more uniform distribution of cells within the infarcted region [124], ii) it does not require specialized training or the purchase of specialized equipment, and iii) it is technically easier, and therefore more practical for widespread utilization in clinical practice. The widespread distribution of cells within the infused vascular bed has also the theoretical advantage of enabling them to “decide where to go” in response to local cues. However, intracoronary delivery has also certain disadvantages vs. transendocardial injection: i) the immediate retention of cells is lower [190, 191] (e.g., 2.6±0.3% after intracoronary infusion compared with 11±3% after intramyocardial injection [192]), presumably because of rapid wash out of cells, ii) microvascular occlusion can occur when large cells, such as MSCs (10-20 μm) [193, 194], skeletal myoblasts (~20 μm) [195], and CDCs (~21 μm) [134, 136, 139] are infused (this problem is not encountered when smaller cells, such as CSCs and BMMNCs, are used), and iii) delivery of cells to a myocardial region supplied by an occluded artery is not possible.

To date, relatively few studies have compared different routes of cell delivery [37, 41, 78, 124, 191, 194, 196-198], with discrepant results. None of them has used a range of doses, which, as discussed above, is necessary to achieve valid conclusions. Comparisons of the intracoronary and transendocardial delivery routes in large animal models using a range of doses of cells are needed to resolve this issue.

iv) Frequency of administration

There is no *a priori* reason to posit that the effects of a single cell administration cannot be improved by a repeated administration. Most stem cells can be frozen, stored, and re-used at a later time. Consequently, it seems rather curious that almost every study performed heretofore has used a single injection of cells to determine whether this therapy is efficacious in HF. This would be tantamount to determining the effect of an antibiotic on an infectious disease by giving only one dose. The lack of studies evaluating repeated cell injections is all the more perplexing when one considers that there is evidence suggesting a dose-dependent response relationship between number of cells injected and functional benefit [62, 94], as discussed above. The effects of stem cells in HF patients should not be labeled as "negative", "modest", or "small" on the basis of the results obtained with a single treatment; in our opinion, the effects of repeated administrations of stem cells need to be compared with those of a single administration, lest a cell therapy may be inappropriately dismissed as ineffective.

The few available data do support the concept that repeated injections of cells are more efficacious than a single injection. In animal models of old MI, repeated injections of skeletal myoblasts were more effective than single injections in increasing LVEF [42, 199] and vasculogenesis and in decreasing fibrosis [42]. Clearly, further studies are necessary to determine the relationship between the number/frequency of cells administered and their effects on cardiac function.

While it is appreciated that the issues discussed above (items i-iv) are not conceptually challenging, it is our opinion that they have enormous practical importance and need to be addressed. It is unlikely that optimal clinical application of cell therapy will be achieved until we have an answer to these questions.

v) Cell retention, survival, long-term engraftment, and lineage commitment

Stem cell studies have consistently shown very low rates of long-term cell engraftment: regardless of cell type, dose, and mode of delivery, more than 90% of injected cells disappear in the first few days and <2% can still be found 4 weeks after transplantation [200, 201]. This massive cell loss is the result of two sequentially distinct events. During or immediately after delivery, there is significant loss due to failure of cells to extravasate (intracoronary infusion) or leakage through transepicardial/transendocardial puncture holes coupled with removal through the venous system (intramyocardial injection). For example, in the acute phase of MI, only ~10% of CSCs [201] and <10% of MSCs [202] were found in the myocardium 24 hours after intramyocardial injection in mice and only 2–5% of BMMNCs a few hours after intracoronary infusion in humans [203]. In a porcine model of cardiopulmonary bypass, only 10% of epicardially injected microspheres approximating the size of MSCs were retained within the sites of injection after 30 min [204]. Then, during the first weeks after transplantation, most of the cells that were initially retained die because of ischemia caused by poor vascularization of the injected region, inflammation with attendant oxidative stress and release of cytotoxic cytokines, immune destruction of allogeneic cells, and apoptosis following disengagement of anchorage-dependent cells from their extracellular matrix (anoikis).

Clearly, the massive loss of transplanted cells is a major unresolved problem that limits the efficacy of any type of cell therapy. Improving cell homing, survival, and engraftment in the hostile ischemic environment is therefore important for optimizing therapeutic benefits. Several strategies are currently under investigation, including pretreatment of the target tissue, ex vivo pretreatment of cells (genetic modifications[205]; physical or pharmacologic preconditioning), and implantation of cells included in scaffolds made of biocompatible

matrix. Pretreatment of the host tissue has been accomplished with ultrasound-mediated destruction of microbubbles in the coronary circulation (which improves recruitment of BMMNCs and MSCs, probably by creating capillary pores [206, 207]) and extracorporeal shock wave treatment (which has shown benefit in patients with ischemic heart failure receiving intracoronary BMMNCs in the CELLWAVE trial [208]). With regard to ex vivo pretreatment of stem cells, many promising strategies have emerged. One is the overexpression of antiapoptotic genes, such as heme oxygenase 1, Bcl-2, Akt, or Pim-1, which has been shown to increase the survival and function of MSCs [202, 209, 210] and CSCs [122] including their capacity to secrete paracrine mediators [209]. Augmenting either the expression of SDF-1 in the myocardium or that of its receptor, CXCR4, on stem cells increases cell recruitment 205, 211, [212]. Preconditioning EPCs with antibodies, HMGB-1, or small molecules increases their neovascularization capacity by activating β2 integrins [213, 214]. Similarly, preconditioning human EPCs and BMMNCs with the endothelial nitric oxide synthase transcription enhancer AVE9488 improves their migratory and neovascularization potential [215]. Many studies have found that preconditioning MSCs and EPCs with simulated ischemia upregulates prosurvival, angiogenic, and migratory proteins, such as HIF-1α, Akt-1, Bcl-2, Ang-1, VEGF, as well as the receptors CXCR4 and c-Met, and imparts beneficial effects [212, 216, 217]. Preconditioning human CSCs with the HO-1 inducer CoPP significantly enhances their resistance to apoptosis [218].

The importance of promoting the lineage commitment of transplanted cells is illustrated by the recently reported C-CURE (Cardiopoietic stem Cell therapy in heart failure) trial, in which lineage specification of MSCs was achieved by exposing them to a cardiogenic cocktail regimen that triggered expression and nuclear translocation of cardiac transcription factors; in this study, administration of autologous bone marrow-derived mesenchymal cardiopoietic cells was found to effect favorable LV remodeling and improve cardiac function in patients with ischemic HF [219].

Embedding cells in natural (e.g. matrigel, collagen, fibrin, alginate) or synthetic (e.g. peptide nanofibers) biomaterials is another means of enhancing stem cell function. Biomaterials promote cell engraftment, retention, and differentiation because of their low viscosity and their similarity to myocardial extracellular matrix, which preserves cell-to-matrix signals [220]. The two main approaches in cardiac tissue engineering are *in vitro* engineering, which consists of seeding cells on pre-formed porous scaffolds that are cultivated *in vitro* and then applied on the epicardial surface, and *in vivo* engineering, in which a mixture of biomaterials and cells is injected and the formation of a biocomplex occurs *in situ* [221, 222]. Conceptually, biomaterials could be designed to release growth factors in a controlled manner that promotes survival and engraftment of cells, and also guides cell phenotype decisions [221, 222].

In summary, improving cell survival and engraftment is crucial to the progress of cell therapy and thus should be a high priority area for research. The strategies summarized above (pretreatment of target tissue, pretreatment of cells, embedding cells in a matrix) are not mutually exclusive and may have additive or even synergistic effects.

Ongoing clinical trials

At the time of this writing, ClinicalTrials.gov lists ten clinical trials that are testing the safety and efficacy of stem cells in HF patients (Table 3).

To evaluate the effects of intramyocardial injection of BMMNCs and MSCs in patients with ischemic cardiomyopathy, three phase I/II randomized, double-blind, placebo-controlled trials are being conducted at the University of Miami. The primary end-point of Prospective

Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) is to test the safety of intramyocardial injection of autologous human MSCs in patients with chronic MI undergoing CABG. The Transendocardial Autologous Cells (hMSCs or hBMCs) in Ischemic Heart Failure Trial (TAC-HFT) is directly comparing hMSCs and hBMMNCs in a prospective manner. The recently published preliminary data from the phase I pilot study of TAC-HFT suggest that transendocardial injection of autologous bone marrow progenitor cells (hMSCs or hBMMNCs) improves regional contractility in a myocardial scar and reverse LV remodeling [223, 224]. Owing to the absence of major histocompatibility complex class II, MSCs are immunoprivileged and suppress T-cell proliferation. These cells are being evaluated in the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in Dilated Cardiomyopathy (POSEIDON-DCM), which is comparing allogeneic MSCs with autologous MSCs in patients with nonischemic dilated cardiomyopathy. In the early-stage study of patients with ischemic cardiomyopathy, POSEIDON demonstrated that transendocardial injection of allogeneic and autologous MSCs favorably affected patient functional capacity, quality of life, and ventricular remodeling [99].

Cardio3 BioSciences is currently recruiting patients in its phase III trial (CHART-1) to examine autologous bone marrow-derived mesenchymal cardiopoietic cells (C3BS-CQR-1) in patients with chronic HF. In this study, the investigators are using a unique ‘cardiopoietic cocktail’ of growth factors (transforming growth factor-β1, bone morphogenetic protein-4, activin A, retinoic acid, insulin-like growth factor-1, fibroblast growth factor-2, alpha-thrombin, and interleukin-6), which has been reported to engage MSCs to differentiate into cardiac stem cells [225]. Using a patient-specific multicellular therapy expanded from a small sample of a patient’s own bone marrow, Aastrom Biosciences is using Ixmyelocel-T (primarily CD90+ MSCs, CD14+ monocytes and alternatively activated macrophages) to evaluate the efficacy, safety and tolerability of transendocardial injection in subjects with HF due to ischemic dilated cardiomyopathy. The NOGA-DCM (Safety and Efficacy Study of Intramyocardial Stem Cell Therapy in Patients with Dilated Cardiomyopathy) study is using CD34+ cells in HF patients. This study is being conducted by Dr. Vrtovec’s group, who has recently demonstrated that intracoronary stem cell transplantation is associated with improved ventricular function, exercise tolerance, and long-term survival (up to 5-years) in patients with dilated cardiomyopathy [105]. NOGA-DCM is designed to directly compare the effects of intracoronary and intramyocardial stem cell delivery in non-ischemic dilated cardiomyopathy at 1-year follow-up. Aside from these studies using bone marrow-derived cells, ALLSTAR (Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration), sponsored by Capricor Inc., is a phase I/II study that tests the safety and efficacy of intracoronary delivery of allogeneic CDCs in patients with an anterior MI and HF.

Conclusions

When considering the current status of cell-based therapies for HF, it is important to keep a historical perspective. We are still at the dawn of the era of regenerative medicine. Only 15 years ago, suggesting that it was possible to regenerate dead myocardium would have been considered science fiction. Notwithstanding the many mechanistic, pathophysiological, and practical issues that remain unresolved, it is important to remember that tremendous progress has been made in a relatively short time. Many promising candidates for cell therapy have been identified, both in experimental animals and in humans, and several studies are ongoing in patients with chronic HF (Fig. 1, Tables 1-3). Never has an idea been translated from preclinical models to humans so quickly. Importantly, cell therapy appears to be safe – to date, no adverse effect of stem/progenitor cells has been reported.

It is true that the precise mechanism of action of stem cells remains unclear, and their efficacy in HF has not been proven. But wouldn't it be surprising if a conclusive answer to these complex questions had been achieved in just a decade? How long did it take for reperfusion therapy to become a routine part of the management of acute MI? And do we understand the mechanism of action of all therapies that we use daily? We must not succumb to irrational impatience or premature nihilism. When a novel therapy comes along, the clinical trials conducted in the first few years are generally small and inconclusive. This has indeed been the case for stem cells in HF; nevertheless, the results are encouraging, and the therapy appears safe. What is important now is: i) to resolve issues concerning optimal cell type, dosage, and route and timing of administration, and ii) to proceed with rigorous, large-scale, rationally-designed, randomized clinical trials. With this approach, we believe that cell-based therapies are likely to become a clinical reality that may revolutionize the management of HF.

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Nonstandard Abbreviations and Acronyms

ALDH^{br}	aldehyde dehydrogenase-bright
BMMNCs	Bone marrow mononuclear cells
CABG	coronary artery bypass grafting
CDCs	cardiosphere-derived cells
CSCs	cardiac stem cells
ECM	extracellular matrix
EPCs	endothelial progenitor cells
ESCs	embryonic stem cells
FGF	fibroblast growth factor
HF	Heart failure
HGF	hepatocyte growth factor
HSCs	hematopoietic stem cells
ICD	implantable cardioverter-defibrillators
IGF-1	insulin growth factor-1
IPSCs	induced pluripotent stem cells
LV	left ventricular

LVEDP	LV end-diastolic pressure
MMPs	matrix metalloproteinases
MSCs	mesenchymal stem cells
NYHA	New York Heart Association
NT-proBNP	N-terminal pro brain natriuretic peptide
SDF-1	stromal cell-derived factor-1
TIMPs	tissue inhibitors of matrix metalloproteinases
TGF-β	tissue growth factor-β
VEGF	vascular endothelial growth factor

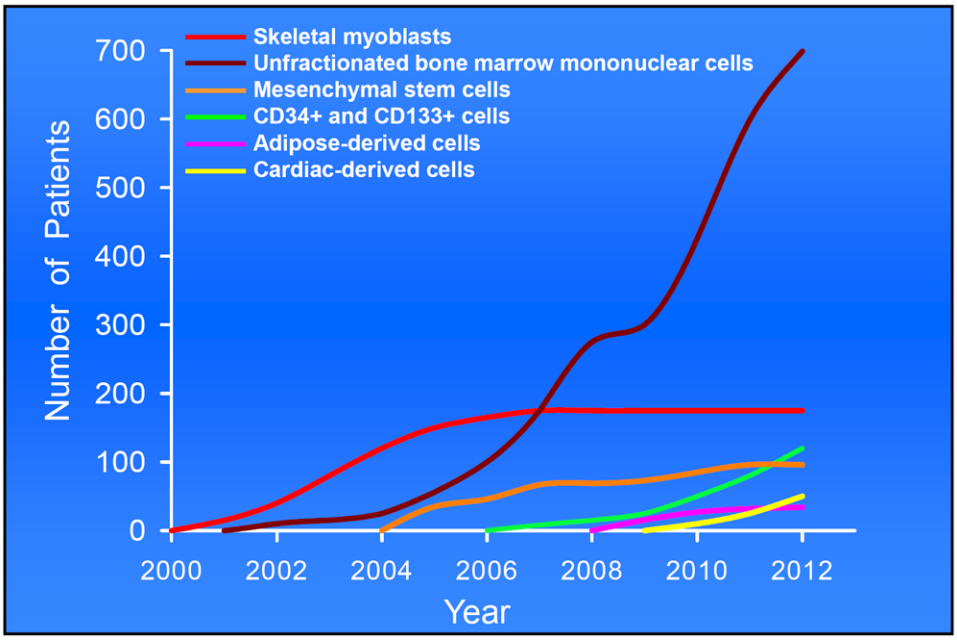

Figure 1. Use of various types of stem cell therapies in patients with cardiovascular disease
Illustrated is the number of patients treated with six major types of cells from 2000 (when the first cell therapy for heart disease was performed) to 2012.

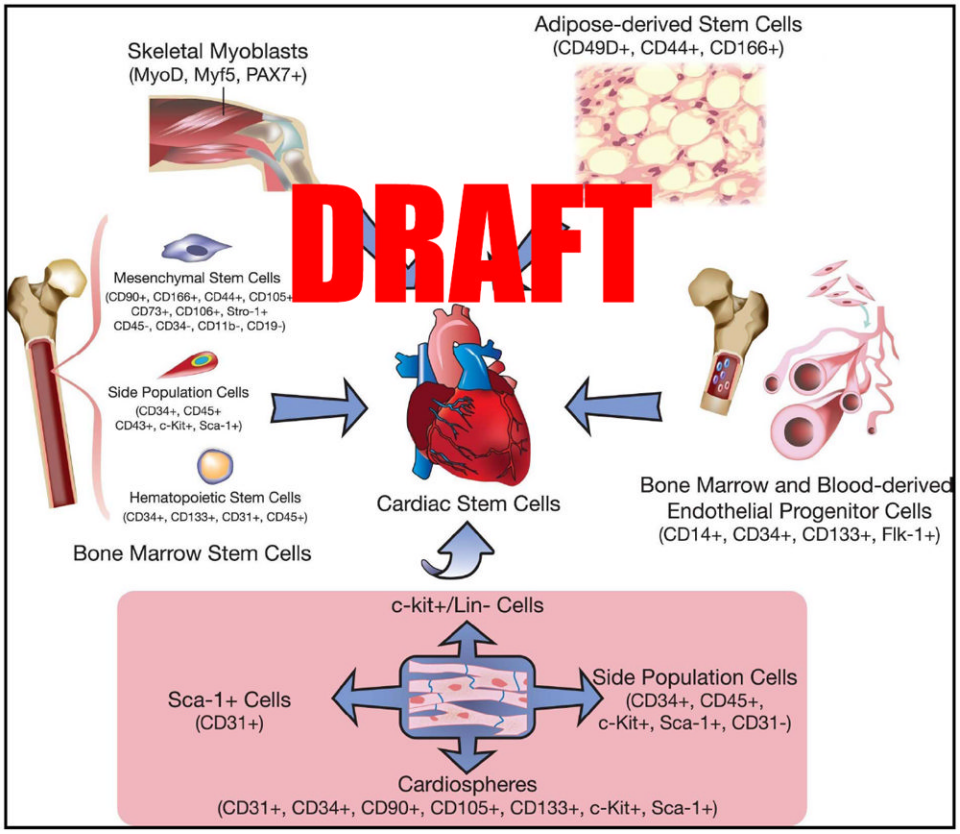

Figure 2. Sources of stem cells used for cardiac repair

Bone marrow-derived stem cells include a broad range of cells, from mesenchymal stem cells to endothelial progenitor cells, hematopoietic stem cells, and unfractionated mononuclear cells. (Illustration Credit: Ben Smith)

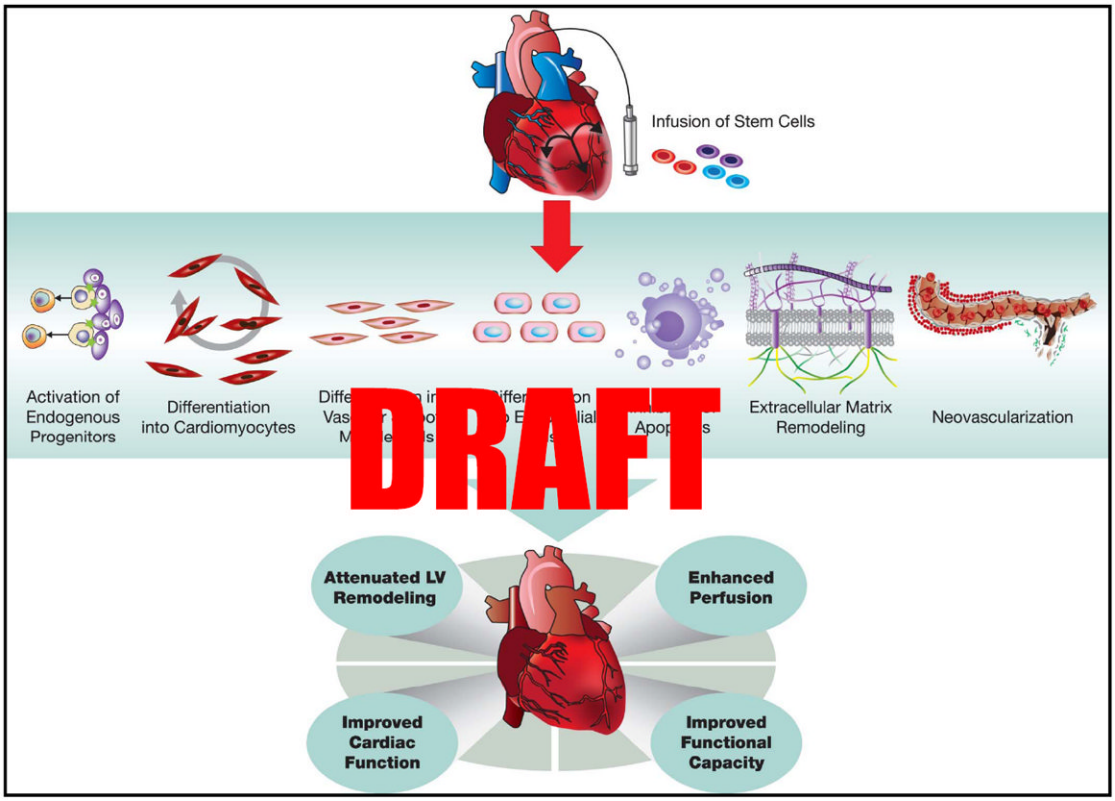

Figure 3. Potential mechanisms of action of stem cells

Implantation of stem cells in the injured heart initiates myocardial repair via several direct and indirect mechanisms: activation of endogenous precursors, differentiation into cardiac and vascular cells, promotion of neovascularization, favorable modulation of the extracellular matrix, and inhibition of apoptosis. Together these events reduce adverse cardiac remodeling and hypertrophy, increase perfusion, and improve cardiac function, leading to improvement in clinical status. (Illustration Credit: Ben Smith)

Table 1

Animal studies of stem cell therapy in heart failure

Study	Host	Type of heart failure	Time of cell therapy	Dose and route of administration	Follow-up period after cell therapy	Outcomes
SKELETAL MYOBLASTS						
Suzuki *et al.*[46]	Lewis rat	Doxorubicin-induced cardiomyopathy	4 weeks after last doxorubicin dose	1×10^6 cells, intracoronary	4 weeks	↓ Mortality Improved hemodynamic parameters
Ghostine *et al.*[38]	Sheep	Embolization using absorbable hemostatic gauze	14 days after MI	50,000 cells, Intramyocardial	12 months	↑ LVEF ↓ LVEDV Improved global wall motion score
Pouly *et al.*[47]	CHF147 Syrian hamster	δ-sarcoglycan deficiency-induced dilated cardiomyopathy	------	5×10^6 cells, Intramyocardial	4 weeks	↑ FAC ↓ Fibrosis
Chachques *et al.*[39]	Sheep	Permanent coronary occlusion	3 weeks after MI	70×10^6 cells, intramyocardial	3 months	↑ LVEF ↓ LV remodeling
He *et al.*[40]	Dog	Coronary microembolization	After hemodynamic confirmation of establishment of heart failure	270 to 830×10^6 cells, Intramyocardial	10 weeks	↑ LVEF ↓ LV remodeling Improved hemodynamic parameters
Gavira *et al.*[41]	Gottingen mini-pig	Vascular embolization in the intermediate branch of first or second marginal artery	8 weeks after MI	$407.55 \pm 115\times10^6$, intramyocardial or intracoronary	3 months	↑ LVEF ↓ Fibrosis ↑ Vasculogenesis
Farahmand *et al.*[36]	Lewis rat	Permanent coronary occlusion	either 5 days after MI or 30 days after MI	5×10^6 cells, Intramyocardial	30 days	↑ LVFS ↓ LV remodeling Improved hemodynamic parameters Attenuated matrix remodeling
Fukushima *et al.*[37]	Sprague Dawley rat	Permanent coronary occlusion	3 weeks after MI	5×10^6 cells, intramyocardial or intracoronary	84 days	↑ LVEF

Study	Host	Type of heart failure	Time of cell therapy	Dose and route of administration	Follow-up period after cell therapy	Outcomes
						Improved physical activity ↔ Mortality
BONE MARROW MONONUCLEAR CELLS						
Tomita *et al.*[71]	Sprague Dawley rat	Cryosurgery	3 weeks after surgery	1×10^6 cells, Intramyocardial	3 weeks	Improved hemodynamic parameters ↓ LV remodeling ↑ Angiogenesis Cardiac differentiation +
Bel *et al.*[68]	Sheep	Ligation of circumflex artery	3 weeks after MI	422×10^6 cells, Intramyocardial	2 months	↔ LVEF ↔ LV remodeling No differentiation into endothelial cells or cardiomyocytes
Waksman *et al.*[69]	Pig	Permanent coronary occlusion	4 weeks after MI	24×10^6 cells, Intramyocardial	4 weeks	↔ Global wall motion score ↓ Infarct size ↑ Angiogenesis
BONE MARROW AND ADIPOSE-DERIVED MESENCHYMAL CELLS						
Nagaya *et al.* (Bone-marrow MSCs) [98]	Lewis rat	Myosin-induced autoimmune myocarditis	5 weeks after immunization	5×10^6 cells, Intramyocardial	4 weeks	Improved hemodynamic parameters ↑ Angiogenesis Cardiomyocyte differentiation + ↓ Fibrosis
Silva *et al.* (Bone-marrow MSCs) [93]	Dog	Ameroid-induced chronic coronary occlusion	30 days after MI	100×10^6 cells, Intramyocardial	30 days	↑ LVEF Neovascularization +
Miyahara *et al.* (Adipose- derived MSCs) [113]	Sprague Dawley rat	Permanent coronary occlusion	4 weeks after MI	$5\text{-}8\times10^5$ cells as monolayered grafts into myocardium	4 weeks	↓ Mortality Improved hemodynamic parameters Cardiac regeneration +
Liu *et al.* (Bone-marrow MSCs) [95]	Sprague Dawley rat	Permanent coronary occlusion	4 weeks after MI	1×10^6 cells, Intramyocardial	4 weeks	↓ Infarct size ↓ LV remodeling

Study	Host	Type of heart failure	Time of cell therapy	Dose and route of administration	Follow-up period after cell therapy	Outcomes
						↑ LVEF ↓ Fibrosis Cardiac differentiation + ↑ Angiogenesis
Mazo *et al.* (Adipose-derived MSCs) [114]	Sprague Dawley rat	Permanent coronary occlusion	5 weeks after MI	1×10^6 cells, Intramyocardial	3 months	↑ LVEF Improved tissue metabolism ↓ Infarct size ↓ Fibrosis Neovascularization +
Li *et al.* (Bone-marrow MSCs) [96]	Wistar rat	Isoproterenol-induced heart failure	4 weeks after isoproterenol injection	3×10^6 cells, Intramyocardial	4 weeks	↑ LVEF ↓ Fibrosis
Schuleri *et al.* (Bone-marrow MSCs) [94]	Gottingen pig	Ischemia/reperfusion injury	12 weeks after MI	20×10^6 to 200×10^6 cells, Intramyocardial	24 weeks	<u>High dose:</u> ↑ LVEF ↓ Infarct size <u>Both high and low dose:</u> ↑ regional contractility and myocardial blood flow
Mazo *et al.* (Bone-marrow MSCs) [97]	Sprague Dawley rat	Permanent coronary occlusion	4 weeks after MI	1×10^6 cells, Intramyocardial	4 wk	↑ LVEF ↓ Fibrosis ↑ Angiogenesis
CARDIAC STEM CELLS						
Rota *et al.* (c-kit+ cells) [126]	Fischer 344 rat	Permanent coronary occlusion	20 days after MI	40,000 cells, Intramyocardial	2 weeks	↑ LVEF Attenuated matrix remodeling ↓ Fibrosis Cardiac regeneration + Neovascularization + Improved hemodynamic parameters

Study	Host	Type of heart failure	Time of cell therapy	Dose and route of administration	Follow-up period after cell therapy	Outcomes
						↓ LV remodeling
Johnston *et al*. (CDCs) [136]	Mini-pig	Permanent coronary occlusion	4 weeks after MI	10×10^6 cells, intracoronary	8 weeks	↓ Infarct size Improved hemodynamic parameters ↔ LVEDV ↓ LV remodeling Cardiac regeneration +
Tang *et al*. (c-kit+ cells) [127]	Fischer 344 rat	Ischemia/reperfusion injury	30 days after MI	40,000 cells, intracoronary	35 days	↑ LVEF Improved hemodynamic parameters Attenuated matrix remodeling ↓ Fibrosis ↓ LV remodeling Cardiac regeneration +
Lee *et al*. (Cardiospheres) [138]	Mini-pig	Permanent coronary occlusion	4 weeks after MI	1×10^6 cells, intracoronary	8 weeks	↑ LVEF ↓ LV remodeling
Bolli *et al*. (c-kit+ cells) [128]	Pig	Ischemia/reperfusion injury	90 days after MI	500,000 cells, intracoronary	31 days	↑ LVEF Improved hemodynamic parameters ↓ Fibrosis ↓ LV remodeling Cardiac regeneration + Angiogenesis +

CDC, cardiosphere-derived cell; FAC, fractional area change; LV, left ventricular; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVFS, LV fractional shortening; MI, myocardial infarction; MSC, mesenchymal stem cell. ↑, increased; ↓, decreased; ↔, no change.

Table 2

Clinical trials of stem cell therapy in heart failure

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
SKELETAL MYOBLASTS								
Menasche *et al*.[48]	Non-randomized, uncontolled study	Cell treatment =10 No controls	Intramyocardial injection during CABG	871×10^6 cells	Echocardiography	10.9 months	↑ LVEF ↑ regional wall motion ↓ NYHA class	Ventricular arrhythmias in 4/10 patients, 2 deaths
Smits *et al*.[51]	Non-randomized, uncontrolled pilot study	Cell treatment =5 No controls	Intramyocardial (Transendocardial)	$196\pm105\times10^6$ cells	MRI, LV angiography, nuclear radiography, echocardiography	3 to 6 months	↑ wall thickening ↑ LVEF ↑ regional wall motion at 3 mth but not at 6 mth	Ventricular arrhythmias in 1/5 patients
Herreros *et al*.[50]	Non-randomized, uncontolled study	Cell treatment =12 No controls	Intramyocardial injection during CABG	221×10^6	Echocardiography, PET scan	3 months	↑ LVEF ↑ myocardial contractility and tissue viability ↑ regional wall motion	No major complications reported
Siminiak *et al*.[52]	Non-randomized, uncontolled study	Cell treatment =10 No controls	Intramyocardial injection during CABG	4×10^5 cells	Echocardiography	12 months	↑ contractility ↑ LVEF ↑ regional wall motion	Ventricular arrhythmias in 4/10 patients, 1 death
Ince *et al*.[53]	Non-randomized, case-controlled study	Cell treatment =6 Controls =6	Intramyocardial (Transendocardial)	$210\pm150\times 10^6$ cells	Echocardiography	12 months	↑ LVEF ↑ walking distance ↓ NYHA class	2 patients developed early ventricular arrhythmias, which was not sustained
Siminiak *et al*. **[POZNAN]** [55]	Non-randomized, uncontolled study	Cell treatment =10 No controls	Percutaneous transcoronary-venous	100×10^6 cells	Echocardiography	6 months	↓ NYHA class ↑ LVEF	No major complications reported
Dib *et al*.[54]	Non-randomized, uncontolled study	Cell treatment =30 No controls	Intramyocardial injection during CABG (24 patients) and LVAD (6 patients)	CABG group: 10, 30, 100, 300×10^6 cells cells; LVAD group: 300×10^6 cells	Echocardiography, PET scan	24 months	↑ LVEF ↑ regional wall motion ↑ viability ↓ LVESV and LVEDV	CABG group: Ventricular arrhythmias in 4/24 patients, 1death and 1 MI; LVAD group: Ventricular

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
							↓ NYHA class	arrhythmias in 2/6 patients, 3 deaths
Biagini *et al*.[58]	Non-randomized, uncontolled study	Cell treatment =10 No controls	Intramyocardial (Transendocardial)	15×10^6 cells	Echocardiography	12 months	↑ LVEF ↓ LVESV ↓ NYHA class	No major complications reported
Hagege *et al*.[56]	Cohort study	Cell treatment =9 No controls	Intramyocardial injection during CABG	62 to $1{,}100\times10^6$ (871×10^6) cells	Echocardiography	18-58 (49.4) months	↑ LVEF ↓ NYHA class	Ventricular arrhythmias in 5/9 patients
Gavira *et al*.[57]	Non-randomized, contolled study	Cell treatment =12 Controls =14	Intramyocardial injection during CABG	50×10^6 cells	Echocardiography, PET scan	12 months	↑ LVEF ↑perfusion and viability ↑ regional contractility	No major complications reported
Veltman *et al*.[64]	Non-randomized, contolled study	Cell treatment =14 Controls =28	Intramyocardial (Transendocardial)	3 to 50×10^6 cells	Echocardiography	4 yrs	↔ LVEF ↔ myocardial performance index	Ventricular arrhythmias in 7 cell treated patients, 3 and 11 deaths in cell treated and control groups, respectively.
Menasche *et al*. **[MAGIC]** [62]	Randomized, placebo-controlled, double-blind study	Cell treatment =97 (low dose: 33 patients, high dose: 34 patients) Controls =30	Intramyocardial injection during CABG	Low dose: 400×10^6 High dose: 800×10^6 cells	Echocardiography	6 months	↔ LVEF ↔ regional wall motion ↓ LVESV and LVEDV in high dose group	Low dose: 4 patients with ventricular arrhythmias and 5 deaths High dose: 5 patients with ventricular arrhythmias and 4 deaths
Dib *et al*. **[CAUSMIC]** [59]	Randomized, placebo-controlled, double-blind study	Cell treatment =12 Controls =11	Intramyocardial (Transendocardial)	3 patients/dose group, receiving 30, 100, 300 or 600 $\times10^6$ cells	Echocardiography	12 months	↓ NYHA class ↓ LV dimension ↑ LVEF ↑ regional wall motion ↑ viability	Ventricular arrhythmias in 6/12 patients
Duckers *et al*. **[SEISMIC]** [65]	Prospective, randomized, open-label study	Cell treatment =26	Intramyocardial (Transendocardial)	150 to 800×10^6 cells	MUGA scan	6 months	↔ LVEF ↑ 6MWD ↓ NYHA class	Ventricular arrhythmias in

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
		Controls =14						12/26 patients, 1death
Povsic *et al*.[63]	Randomized, double-blind, controlled study	Cell treatment =15 Controls =8	Intramyocardial (Transendocardial)	Low dose: 400×10^6 High dose: 800×10^6 cells	Doubutamine stress echocardiography, MUGA scan	6 months	↑ 6MWD	Ventricular arrhythmias in 7/15 cell-treated patients
BONE-MARROW MONONUCLEAR CELLS								
Perin *et al*.[72]	Prospective, nonrandomized, open-label study	Cell treatment =14 Controls =7	Intramyocardial (Transendocardial)	$25.6\pm6.3\times 10^6$ cells	Echocardiography, SPECT	2 and 4 months	2 months: ↓ NYHA class, ↓ CCSAS, ↑ LVEF, ↓ LVESV and LVEDV 4 months: ↑ LVEF, ↓ LVESV and LVEDV	1 sudden cardiac death in cell treated group
Perin *et al*.[73]	Prospective, nonrandomized, open-label study	Cell treatment =11 Controls =9	Intramyocardial (Transendocardial)	$25.6\pm6.3\times 10^6$ cells	Echocardiography, SPECT	6 and 12 months	↑ exercise capacity ↑ perfusion ↔ LVEF	No major complications reported
Galinanes *et al*.[75]	Non-randomized, uncontolled study	Cell treatment =14 No Controls	Intramyocardial injection during CABG	CD34+ $(31.5\pm3.5\times10^6)$ and CD117+ $(0.61\pm0.1\times10^6)$ cells	Doubutamine stress echocardiography	6 weeks and 10 months	↑ LVEF Improved wall motion score	No major complications reported
Blatt *et al*.[79]	Non-randomized, uncontolled study	Cell treatment =6 No Controls	Intracoronary	16.7×10^6 cells	Doubutamine stress echocardiography	4 months	↑ LVEF ↓ NYHA class Improved wall motion score	No major complications reported
Assmus *et al*. **(TOPCARECHD)** [82]	Randomized, controlled study	Cell treatment =52 (28 patients BMCs, 24 patients circulating progenitor cells) Controls =23	Intracoronary	BMCs: $205\pm110\times10^6$ Circulating progenitor cells: $22\pm11\times10^6$	Echocardiography, SPECT, MRI	3 months	↑ LVEF (BMCs only) ↓ NYHA class (BMCs only)	1 episode of ventricular arrhythmia and 5 deaths in circulating progenitor cell group
Hendrikx *et al*.[77]	Randomized, controlled trial	Cell treatment =10 Controls =10	Intramyocardial injection during CABG	$60\pm31\times10^6$ cells	MRI	4 months	↔ LVEF ↑ systolic thickening ↓ NYHA class and LVESV	No major complications reported

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
Gao *et al*.[80]	Non-randomized, contolled study	Cell treatment =14 Controls =14	Intracoronary	28 to 32 $\times 10^6$ cells	Echocardiography	3 months	↑ LVEF ↓ LVESV	No major complications reported
Seth *et al*.[86]	Pilot study	Cell treatment =24 Controls =120	Intracoronary	~120 $\times 10^6$ cells	Echocardiography	3 months	↑ LVEF ↓ LVESV ↓ NYHA class ↓ NYHA class	No major complications reported
Beeres *et al*.[76]	Non-randomized, uncontolled study	Cell treatment =15 No Controls	Intramyocardial (Transendocardial)	94±14×10^6 cells	SPECT	3 months	↑ LVEF ↓ NYHA class ↑ perfusion ↑ regional wall motion	1 death due to heart failure
Yao *et al*.[85]	Randomized, placebo-controlled trial	Cell treatment =24 Controls =23	Intracoronary	12×10^6 cells	Echocardiography, MRI, SPECT	6 months	↔ LVEF ↔ LVEDV and LVESV ↔ perfusion ↔ infarct size	No major complications reported
Ang *et al*.[78]	Randomized, controlled, single-blinded trial	Cell treatment =42 (21 intramyocardial, 21 intracoronary) Controls =23	Intramyocardial injection during CABG or Intracoronary	Intramyocardial: 84±56×10^6 BMCs and 142±166× 10^3 CD34+/CD 177+ cells Intracoronary: 115±73×10^6 BMCs and 245±254×10^3 CD34+/CD 177+ cells	Echocardiography, MRI	6 months	↔ LVEF ↔ LVEDV and LVESV ↔ infarct wall motion ↔ infarct size	No major complications reported
Diederichsen *et al*.[81]	Non-randomized, uncontolled study	Cell treatment =32 No Controls	Repeated intracoronary	1st infusion: 647±382×10^6 cells 2nd infusion: 889±361×10^6 cells	Echocardiography	12 months	↔ LVEF Improved LV filling	No major complications reported
Perin *et al*. **(FOCUS-HF)** [74]	Randomized, double-blinded, controlled study	Cell treatment =20 Controls =10	Intramyocardial (Transendocardial)	30×10^6 cells	Echocardiography, SPECT	6 months	↔ LVEF ↓ CCSAS ↑ perfusion	No major complications reported

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
MESENCHYMAL STEM CELLS								
Hare *et al*. **(POSEIDON)** [99]	Randomized pilot Study	Cell treatment =31 No controls	Intramyocardial (Transendocardial)	Three different doses: 20, 100, 200×10^6	Computed tomography	12 months	↔ LVEF Improved physical performance ↓ LVEDV	1 patient in each group was hospitalized for HF
BONE MARROW PROGENITOR CELLS								
Patel *et al*.[103]	Randomized, controlled study	Cell treatment =10 Controls =10	Intramyocardial injection during CABG	22×10^6 cells	Echocardiography, SPECT	6 months	↑ LVEF	No major complications reported
Manginas *et al*.[106]	Pilot, controlled study	Cell treatment =12 Controls =12	Intracoronary	CD133+: $16.9\pm4.9\times10^6$ cells CD133-/CD34+: $8\pm4\times10^6$ cells	Echocardiography	28±8.7 months	↑ LVEF ↓ LV remodeling ↓ LVESV and LVEDV ↑ perfusion	1 patient developed restenosis at the cell delivery site
Stamm *et al*.[108]	Nonrandomized, controlled study	Cell treatment =20 Controls =20	Intramyocardial injection during CABG	5.8×10^6 cells	Echocardiography, SPECT	6 months	↑ LVEF ↑ perfusion	No major complications reported
Fischer-Rasokat *et al*.[87]	Pilot study	Cell treatment =33 No controls	Intracoronary	$259\pm135\times10^6$ cells	MRI, LV angiography	3 months, 12 months	↑ LVEF Improved regional wall motion	No major complications reported
Vrtovec *et al*.[104]	Randomized, controlled study	Cell treatment =28 Controls =27	Intracoronary	$123\pm23\times10^6$ cells	Echocardiography	12 months	↑ LVEF ↑ 6MWD	5 patients died of cardiac causes and 5 patients underwent heart transplantation
Vrtovec *et al*.[105]	Randomized, controlled study	Cell treatment =55 Controls =55	Intracoronary	$123\pm23\times10^6$ cells	Echocardiography	5 years	↑ LVEF ↑ 6MWD	27 patients died of cardiac causes and 9 patients underwent heart transplantation
Perin *et al*.[109]	Randomized, controlled, double-blind study	Cell treatment =10 Controls =10	Intramyocardial (Transendocardial)	$2.37\pm1.31\times10^6$ cells	Echocardiography, SPECT	6 months	↓ LVEDV Improved maximal oxygen consumption	No major complications reported
CARDIAC STEM CELLS								

Study / Name of the trial	Study design	Number of patients	Delivery method	Cell Dose	End-point evaluation method	Follow-up period	Outcomes	Side effects in cell-treated patients
Bolli *et al*. **(SCIPIO)** [129]	Open label, randomized, controlled study	Cell treatment =16 Controls =7	Intracoronary	1×10^6 cells	Echocardiography, MRI	4 and 12 months	↑ LVEF ↓ infarct size ↓ NYHA class	No major complications reported
Makkar *et al*. **(CADUCEUS)** [137]	Randomized, controlled study	Cell treatment =17 Controls =8	Intracoronary	$12.5\text{-}25\times10^6$ cells	MRI	6 and 12 months	↔ LVEF ↔ LV volumes ↓ scar mass	4 cell-treated patients had serious adverse events

BMC, bone marrow cell; CABG, coronary artery bypass grafting; CCSAS, Canadian Cardiovascular Society Angina Score; LV, left ventricular; LVAD, LV assist device; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LVEF, LV ejection fraction; MRI, magnetic resonance imaging; MUGA, Multi gated acquisition scan; MWD, minute walk distance, NYHA, New York Heart Association; PET, positron emission tomography; SPECT, single photon emission computed tomography. ↑, increased; ↓, decreased; ↔, no change.

Table 3

Ongoing clinical trials of stem cell therapy in heart failure registered at clinicaltrials.gov (April 2013)

Trial Design Phase and title	Cell type	Status	Design	Estimated patient enrollment	Delivery Method	Reference
Phase I/II; Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery **(PROMETHEUS)**	Autologous MSCs	Active, not recruiting	Randomized, double-blinded, placebo-controlled	45	Intramyocardial	NCT 00587990
Phase I/II; The Transendocardial Autologous Cells (Hmsc or Hbmc) in Ischemic Heart Failure Trial **(TAC-HFT)**	Autologous hMSC or hBMC	Recruiting	Randomized, double-blinded, placebo-controlled	67	Intramyocardial (Transendocardial)	NCT 00768066
Phase I/II; The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in Dilated Cardiomyopathy **(POSEIDON-DCM)**	Autologous MSCs Allogenic MSCs	Recruiting	Randomized, open-label, pilot study	36	Intramyocardial (Transendocardial)	NCT 01392625
Phase I/II; Autologous Mesenchymal Stromal Cell Therapy in Heart Failure	Mesenchymal stromal cells	Recruiting	Randomized controlled	60	Intramyocardial	NCT 00644410
Phase II; A Phase II Dose-escalation Study to Assess the Feasibility and Safety of Transendocardial Delivery of Three Different Doses of Allogeneic Mesenchymal Precursor Cells (MPCs)in Subjects With Heart Failure **(REVASCOR)**	Mesenchymal Precursor Cells	Active, not recruiting	Dose-escalation study	60	Intramyocardial (Transendocardial)	NCT 00721045
Phase II; Safety and Efficacy Study of Intramyocardial Stem Cell Therapy in Patients With Dilated Cardiomyopathy **(NOGA-DCM)**	Autologous BM-HSCs (CD34+ cells)	Recruiting	Randomized, double-blinded, placebo-controlled	60	Intramyocardial	NCT 01350310
Phase I; Cardiac Stem cell Infusion in Patients with Ischemic Cardiomyopathy **(SCIPIO)**	c-kit+ Cardiac Progenitor Cells	Active, not recruiting	Randomized, open-label	33	Intracoronary	NCT 00474461
Phase I/II; Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration **(ALLSTAR)**	Cardiosphere-Derived Cells (CDCs)	Recruiting	Randomized, double-blind, placebo-controlled	274	Intracoronary	NCT 01458405
Phase III; Safety and Efficacy of Autologous Cardiopoietic Cells for Treatment of Ischemic Heart Failure **(CHART-1)**	Bone Marrow-derived Mesenchymal Cardiopoietic Cells (C3BS-CQR-1)	Recruiting	Randomized, double-blind, placebo-controlled	240	Intramyocardial	NCT 01768702
Phase II; An Efficacy, Safety and Tolerability Study of Ixmyelocel-T Administered Via Transendocardial Catheter-based Injections to Subjects With Heart Failure Due to Ischemic Dilated Cardiomyopathy **(ixCELL DCM)**	Bone marrow-derived cells, including primarily CD90+ MSCs, CD14+ monocytes and alternatively activated macrophages	Recruiting	Randomized, double-blind, placebo-controlled	108	Intramyocardial (Transendocardial)	NCT 01670981

BMC, bone marrow cell; BMMNCC, bone marrow mononuclear cell; CSC, cardiac stem cell; MSC, mesenchymal stem cell.