Concise Review: Rational Use of Mesenchymal Stem Cells in the Treatment of Ischemic Heart Disease

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Key Words. Cardiac • Mesenchymal stem cells

ABSTRACT

The capacity of stem and progenitor cells to stimulate cardiac regeneration has been studied for almost 20 years, with very promising preclinical data and mixed clinical results. Several cell types have been studied, identified by their cell surface markers, differentiation capacity and their secreted growth factors. Bone marrow derived mesenchymal stem cells (MSCs) have been found to have potent regenerative capacity, through multiple mechanisms, including mesoderm lineage differentiation, immunomodulation, and paracrine stimulation. MSCs also secrete exosomes and microvesicles, which themselves contain potent angiogenic cytokines or mRNA molecules with effects on their local milieu. This concise review summarizes the mechanisms of MSC-based cardiac regeneration and highlighting results from molecular and preclinical studies. We also discuss clinical trial results to date, and ongoing studies. Furthermore, we discuss novel approaches for the enhancement of MSC based cardiac regeneration, such as genetic modification.

SIGNIFICANCE STATEMENT

This concise review summarizes results from experiments using a specific type of stem cells, called mesenchymal stem cells, which have shown a capacity to repair and regenerate the heart following injury. This article summarizes the mechanisms by which these cells act, and discusses ongoing research in how to improve their effect.

INTRODUCTION

Cardiovascular diseases remain one of the leading causes of death worldwide. Myocardial infarction (MI) from atherosclerotic plaque rupture remains the most common cause, frequently leading to the development of heart failure (HF) [1, 2]. In industrialized countries, the prevalence of HF is high, affecting 1%–3% of total population, representing one of health care’s most expensive diagnoses [3].

As a result of a pathological stimulus, the left ventricle undergoes a robust plasticity response known as pathological remodeling [4]. This process refers to the change in cardiomyocyte biology and cardiac structure post insult, and is the culmination of a series of transcriptional, signaling, structural, electrophysiological, and functional events occurring within the cardiomyocyte, along with a range of events which occur in fibroblasts, vascular smooth muscle cells, endothelial cells, and leukocytes [5]. While these changes are aimed at stabilizing the heart in the short term, the long-term consequence is an inexorable progression to pump failure and death. Current therapy involves beta blockade, angiotensin-converting enzyme inhibition, aldosterone blockade [6], and biventricular pacing strategies [7, 8]. These strategies primarily work by reducing pathological left ventricle (LV) remodeling via inhibition of “neuro-hormonal activity,” which include sympathetic and renin-angiotensin-aldosterone activation. Despite medical therapy, the mortality and morbidity from HF secondary to MI remains unacceptably high. For example, recent data in Ontario, Canada, demonstrates that the 1-year mortality for a diagnosis of congestive heart failure (CHF), regardless of the etiology is approximately 25% [9].

Given the limited capacity for self-renewal, the concept of cell-based strategies to “regrow” lost cardiomyocytes or to promote endogenous repair became popular in the late 1990s. Since then, the field of regenerative medicine has dramatically expanded, with a growing body of the literature to support the safety and efficacy of this approach. However, there lacks definitive clinical data to move this field into mainstream medical practice. This review will focus upon a well-studied and safe stem cell subpopulation known as mesenchymal stem cells (MSCs). We will further focus upon the use of MSCs as a therapeutic...
strategy to reverse deleterious LV remodeling, and outline current and future clinical trials using this regenerative approach.

MSC DIFFERENTIATION

MSCs are a subset of bone marrow cells that can be isolated from other bone marrow derived mononuclear cells (BM-MNCs) by their rapid adherence to plastic tissue culture dishes. Following culture, the remaining cells typically express markers CD29 (integrin β-1), CD44 (hCAM), CD90 (th-y-1), CD105, and CD117 (c-kit) and are negative for the hematopoietic and vascular markers CD34, CD45, and CD11b [10, 11].

Using growth-factor rich selective media, MSCs have been shown to be able to differentiate into multiple mesoderm lineages and differentiated cell types, including osteoblasts [12], adipocytes [13], skeletal muscle myocytes/myotubes [14], pancreatic islet cells [15], and cardiomyocytes [16, 17]. If delivered in vivo, they have been shown to engraft and transdifferentiate into cardiomyocytes, repairing the infarcted myocardium [18, 19]. Further studies challenged these findings, as very limited engraftment was found, although there was still benefit on overall myocardial function in small animal models [20, 21]. In pigs, 2 weeks following coronary injection, only 2% of cells were found in the heart, and there was no evidence of cardiomyocyte differentiation [22]. Overall, animal studies have shown that MSCs can improve cardiac function, but likely not exclusively through replacement of injured contractile cardiomyocytes. Figure 1 summarizes the mechanisms listed below.

Figure 1. Mechanisms of MSC-mediated cardiac regeneration. The initial reported mechanisms of MSCs’ impact on cardiac regeneration were via replacement of necrotic contractile myocardium with differentiated cardiomyocytes (CMs; left side of figure). The relative contribution of this mechanism is likely quite small, with greater contribution from paracrine mechanisms, whether from secreted paracrine signals or encapsulated signals in microvesicles or endosomes (right side of figure). Together, these processes lead to improved cardiomyocyte survival, reduced inflammation, and preserved myocardial function. Abbreviations: CM, conditioned medium; MSC, mesenchymal stem cell; SMC, smooth muscle cell.

PARACRINE EFFECT

MSCs also secrete multiple cytokines and growth factors, together termed their “secretome,” which contribute to their paracrine therapeutic effect. These factors are released in soluble form, or in exosomes and in extracellular vesicles (EVs), and can be sampled by collecting the medium in which the cells are cultured, so-called “conditioned medium” (CM) [23]. Over 30 systematic proteomic studies on MSC CM have been conducted, reporting a multitude of growth factors that could have potent paracrine effects. These include hepatocyte growth factor (HGF) [20], interleukin-1 (IL1) and -6 (IL6) [24], stem-cell derived factor-1 (SDF-1) [25], and several others [23]. Within the EVs or exosomes, several mRNAs have been found, such as miR221 and miR-19a, which are involved with suppressing apoptosis or stimulating Akt.
Several groups have shown benefit of MSC-derived growth factors and CM for cardiac repair and regeneration. In a rat model of acute MI, CM from cultured MSCs were able to preserve myocardial contractile capacity, inhibit apoptosis of cardiomyocytes, and allow the formation of new vessels in damaged tissues [28]. This study also showed upregulation of vascular endothelial growth factor (VEGF) and IL-1ß in CM from MSCs cultured under hypoxic conditions, suggesting that hypoxia might stimulate production of these vasculoprotective and anti-apoptotic cytokines. With MSCs engineered to overexpress Akt, CM from hypoxia-treated cells was able to prevent in vitro apoptosis of rat cardiomyocytes, and in vivo lead to reduced infarct size and preserved LV contractility [29, 30].

The paracrine factors secreted by MSCs likely exert a pleiotropic effect on the myocardium, with improved local angiogenesis, cardiac stem-cell stimulation, and reduced cardiomyocyte death. There is also evidence of reduced fibroblast activation and cell-mediated immune response, with corresponding reduction in myocardial fibrosis.

Various preclinical studies have been shown to enhance the ability of MSCs to secrete soluble angiogenic markers, such as VEGF and Placental growth factor (PLGF) [20]. MSCs transduced with GATA-4, a GATA zinc finger transcription factor family member, showed increased production of insulin-like growth factor-1 (IGF-1) and VEGF [26]. Injection of the cells into a rat model of MI increased peri-infarct neovessel formation and reduced overall infarct size [31]. In a swine model of MI, human MSC CM was injected intravenously and lead to increased capillary density and preserved cardiac function [32]. By echocardiography, animals who received a CM product showed increased peri-infarct neovessel formation and reduced overall infarct size [31]. In a porcine model of myocardial I/R was able to limit infarct size and improve systolic function via reduction of TGF-ß signaling and apoptosis [49]. Fractionation analyses then revealed that cardioprotection was mediated by components with a size between 100 and 220 nm, suggesting the presence of large particles rather than secreted cytokines. The same group then showed that highly purified exosomes isolated from CM of the same MSCs had a radius of 55–65 nm and induced significant cardioprotection when injected in a murine MI model [50]. Interestingly, this effect was only produced by intact, not lysed, exosomes [51].

A recent study showed that murine MSCs released exosomes enriched with miR-22, which were internalized by cocultured cardiomyocytes. MiR-22 prevented CM apoptosis via interaction with methyl CpG binding protein 2 (Mecp2) [52]. Another group showed that MSCs transduced with GATA-4 produced exosomes with high levels of several miRNAs, among them miR-21 and miR-19a [27]. These miRNAs reduced apoptosis of ischemic cardiomyocytes via inhibition of p53-upregulated modulator of apoptosis, a subclass of the Bcl-2 protein family [53], and inhibition of Phosphatase and tensin homolog (PTEN) with resultant activation

**EVs and Exosomes**

The use of EVs and exosomes, without the cells themselves, is a growing practice for regenerative therapy. EVs have a size between 100 nm and 1 μm and derive from the detachment of cytoplasmic protrusions. EVs from MSCs express CD13, CD29, CD44, CD73, and CD105, similar to MSCs themselves [40–42]. Exosomes have a size ranging between 30 and 100 nm and originate from fusion of endosomes with the plasma membrane, which are released by exocytosis. Both contain nucleic acids, coding mRNA and noncoding RNA. Coding mRNAs present in EVs include transcripts related to control of transcription, cell proliferation, and immune regulation [42, 43]. Among the noncoding RNAs contained in released MSC-EVs, there are selected patterns of miRNAs [44, 45], which can be transferred to target cells and downregulate miRNA translation and protein expression [46, 47].

Recent studies suggest that the therapeutic effect of MSCs is in large part due to secreted EVs and exosomes [48]. In particular, the CM of human embryonic stem cell-derived MSCs injected in a porcine model of myocardial I/R was able to limit infarct size and improve systolic function via reduction of TGF-ß signaling and apoptosis [49]. Fractionation analyses then revealed that cardioprotection was mediated by components with a size between 100 and 220 nm, suggesting the presence of large particles rather than secreted cytokines. The same group then showed that highly purified exosomes isolated from CM of the same MSCs had a radius of 55–65 nm and induced significant cardioprotection when injected in a murine MI model [50]. Interestingly, this effect was only produced by intact, not lysed, exosomes [51].

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of Akt and extracellular signal-regulated kinase (ERK) pathways [27].

**CLINICAL TRIALS OF MSCS FOR ISCHEMIC HEART DISEASE**

While most cell therapy trials for ischemic heart disease (IHD) have concentrated on BM-MNCs, isolated MSCs have also been used in trials of acute and chronic IHD. Table 1 summarizes these studies.

Chen et al. [53] randomized 69 patients post acute MI and injected $48\times10^6$ to $60\times10^6$MSCs into the infarct related coronary artery 10 days following reperfusion and stenting. At 3 and 6 months follow-up, they found a significant difference in the improvement of left ventricular ejection fraction (LVEF) in the MSC group compared to placebo (17% vs. 5%), in addition to reduced infarct size [66]. In 2009, Hare et al. [55] published a dose escalation study of allogeneic MSCs ($0.5/1.6/5$ per kg) in patients post-percutaneous coronary intervention (PCI) for acute MI. They found an increase in LVEF at 3, 6, and 12 months in the MSC group compared to placebo. At 12 months post injection, the improvement was of 5.2% versus 1.8% by cardiac magnetic resonance imaging (MRI), with the greatest benefit being in patients with anterior MI. Lee et al. [58] randomized 69 patients post MI to receive $7.2\times10^6$ autologous MSCs or placebo and found a similar result at 6 months, with an improvement in LVEF of 5.2% versus 1.6% at 6 months (using SPECT).

### Table 1. Summary of key clinical trials of MSC therapy for ischemic heart disease

| Study | n    | Cell source | Cell dose ($\times10^6$) | Design | Delivery | Key findings |
|-------|------|-------------|--------------------------|--------|----------|-------------|
| Acute myocardial infarction |
| Chen et al. [53] | 69   | Autologous BM | 4800–6000 | RPCT   | IC       | Improved LVEF, perfusion and wall motion |
| Katritsis et al. [54] | 22   | Autologous BM | 1–2 | Open   | IC       | Improved wall motion and perfusion |
| Hare et al. [55] | 53   | Allogeneic BM (Provacel) | 0.5/1.6/5 per kg | RPCT   | IV       | Safety; improved LVEF and remodeling |
| Houtgraaf et al. [56] | 14   | Autologous BM | 20 | RPCT   | IC       | Improvement in perfusion and myocardial scar |
| Gao et al. [57] | 41   | Autologous BM | 3.1 | RPCT   | IC       | No difference in viability, perfusion or LVEF |
| SEED-MSC [58] | 80   | Autologous BM | $72\pm9$ | Open   | IC       | Improved LVEF |
| Chronic ischemic heart disease |
| Chen et al. [53] | 22   | Autologous BM | 5 | Open   | IC       | Increased LVEF and improved symptoms |
| Mohyeddin-Bonab et al. [59] | 8    | Autologous BM | 5.6 | Open   | IC/IM    | Improved LVEF, reduced infarct size |
| Friis et al. [60] | 31   | Autologous BM | 22 | Open   | IM       | Improved LVEF and exercise capacity |
| POSEIDON [61] | 30   | Allogeneic/autologous BM | 20/100/200 | Randomized open | IM       | Safe |
| Mathiasen et al. [62] | 60   | Autologous BM | 83 | RPCT   | IM       | Improved LVEF and muscle mass |
| Perin et al. [63] | 60   | Allogeneic BM | 25/75/150 | RPCT   | IM       | Safety, feasible |
| Qayyum et al. (2017) | 60   | Autologous adipose tissue | $72\pm45$ | RPCT   | IM       | No difference in exercise capacity |
| TAC-HFT [64] | 65   | Autologous BM | 40 | RPCT   | IM       | Improved exercise tolerance and reduced infarct size. |
| PROMETHEUS [65] | 9    | Autologous BM | 20–40 | RPCT   | IM       | Increased LVEF and decreased scar. |

Abbreviations: BM, bone marrow; IC, intracoronary; IM, intramyocardial; IV, intravenous; LVEF, left ventricular ejection fraction; MSC, mesenchymal stem cell; RPCT, randomized placebo-controlled trial.

**References**

1. [Chen et al. (2009)](#)
2. [Katritsis et al. (2009)](#)
3. [Hare et al. (2009)](#)
4. [Houtgraaf et al. (2009)](#)
5. [Gao et al. (2009)](#)
6. [Lee et al. (2009)](#)
7. [Chen et al. (2010)](#)
8. [Mohyeddin-Bonab et al. (2010)](#)
9. [Friis et al. (2010)](#)
10. [POSEIDON (2010)](#)
11. [Mathiasen et al. (2010)](#)
12. [Perin et al. (2010)](#)
13. [Qayyum et al. (2010)](#)
14. [TAC-HFT (2010)](#)
15. [PROMETHEUS (2010)](#)
predominantly intramyocardial. Friis et al. [60] conducted a safety study and enrolled 31 patients with stable, moderate-severe angina with no further revascularization options. MSCs (mean: $21.5 \times 10^6$, range $3–62 \times 10^6$) were delivered by intramyocardial injection, and all recipients were followed for 6 months. There were no ventricular arrhythmias or other major adverse cardiac events (MACE) associated with the cells. SPECT analysis showed no difference in the perfusion score, and cardiac MRI showed improvement in LVEF from 55.9% to 57.9% ($p < .001$). Clinically there was an improvement in exercise capacity and Canadian Cardiovascular Society (CCS) class of angina, although these results were not placebo-controlled. In 2015, Mathiasen et al. published results of the MSC-HF trial, a randomized controlled trial for patients with symptomatic ischemic cardiomyopathy (LVEF < 45%) [62]. Sixty patients were enrolled and randomized in a 2:1 fashion, and MSC recipients received a mean of $8.3 \times 10^7$ autologous cells via intramyocardial injection. At 6 months of follow-up, LV end-systolic volume (LVESV) was significantly reduced in the MSC group compared to placebo ($213.0$ ml; $p = .001$). Compared with placebo, there were also significant improvements in LVEF of 6.2% ($p < .0001$), stroke volume of 18.4 ml ($p < .0001$), and myocardial mass of 5.7 g ($p = .001$).

Meta-analyses of trials using bone marrow derived progenitor and stem cells, with a total sample size of 2,602, albeit not

| Study | n | Cell source | Condition | Design | Delivery | ClinicalTrials ID |
|-------|---|-------------|-----------|--------|----------|-------------------|
| RELIEF | 135 | Autologous BM | Acute MI | Phase III | IC | NCT01652209 |
| CIRCULATE | 105 | Allogeneic BM | Acute MI | Phase II/III | IC | NCT03404063 |
| HUC-HEART | 79 | Autologous/allogeneic BM | Pre-CABG | Phase I/II | IM | NCT02323477 |
| Kumar et al. | 20 | Allogeneic BM | Acute MI | Phase I/II | IV | NCT00883727 |
| Perin et al. | 25 | Allogeneic BM | Acute MI | Phase I/II | IM | NCT00555828 |
| Skerrett et al. | 220 | Allogeneic BM (PROCHYMAL) | Acute MI | Phase II | IV | NCT00877903 |
| Musialek et al. | 115 | Allogeneic BM (Cardiocell) | Acute MI | Phase I/III | IC | NCT03404063 |
| AMICI | 105 | Allogeneic BM | Acute MI | Phase II | IC | NCT01781390 |
| ESTIMATION | 50 | Autologous BM | Postacute MI | Phase III | IM | NCT01394432 |
| Jerome et al. | NYD | Autologous BM | Ischemic CM (LVAD) | Phase I | IM | NCT02460770 |
| MESAMI2 | 90 | Autologous BM | Chronic ischemic CM | Phase II | IM | NCT02462330 |
| Dai et al. | 45 | Autologous BM | Chronic ischemic CM | Phase I/II | Collagen scaffold | NCT02635464 |
| CONCERT-HF | 144 | Autologous BM | Ischemic CM | Phase II | IM | NCT02501811 |
| Antonitsis et al. | 30 | Allogeneic BM | Ischemic CM needing CABG | Phase I | IM | NCT01753440 |
| Antonitsis et al. | 5 | Allogeneic BM | Ischemic CM with LVAD | Phase I | IM | NCT01759212 |
| Kastrup et al. | 10 | Allogeneic adipose tissue | Ischemic CM | Phase I | IM | NCT02387723 |
| Kastrup et al. | 81 | Allogeneic adipose tissue | Ischemic CM | Phase II | IM | NCT03092284 |
| SCIENCE | 138 | Allogeneic adipose tissue | Ischemic CM | Phase II | IM | NCT02673164 |
| UCMSC-Heart | 40 | Allogeneic UC | Ischemic CM | Phase I/II | IC | NCT02439541 |
| TRIDENT | 40 | Allogeneic BM | Ischemic CM | Phase II | IM | NCT02013674 |
| DREAM HF-1 | 600 | Allogeneic BM (rexlemestrocel-L) | Ischemic CM | Phase III | IM | NCT02032004 |
| SEESUPIHD | 64 | Allogeneic UC | Ischemic CM | Phase I/II | IC | NCT02666391 |
| TRAABPIHD | 200 | Autologous BM | Ischemic CM | Phase I/II | NYD | NCT02504437 |
| Maskon et al. | 80 | Autologous BM | Ischemic dilated CM | Phase II | IC | NCT01720888 |
| Harjula et al. | 60 | Autologous BM | Ischemic CM needing CABG | Phase II | IM | NCT00418418 |
| TAC-HFT-II | 55 | Autologous BM ± CSC | Ischemic CM | Phase I/II | IM | NCT02503280 |
| TEAM-AMI | 124 | Autologous BM | Ischemic CM | Phase II | IC | NCT03047772 |
| Hu et al. | 30 | Umbilical cord | Idiopathic dilated CM | Phase I | IM | NCT01219452 |
| Olson et al. | 45 | Allogeneic BM | Anthracycline-mediated CM | Phase I | IV | NCT02408432 |
| Fernandez-Avilez et al. | 70 | Autologous BM | Idiopathic dilated CM | Phase I/II | IM | NCT01957826 |
| Bartolucci et al. | 30 | Allogeneic UC | Dilated CM | Phase I/II | IV | NCT01739777 |

Abbreviations: BM, bone marrow; CABG, coronary artery bypass grafting; CM, cardiomyopathy; CSC, cardiac stem cells; IC, intracoronary; IM, intramyocardial; IV, intravenous; LVAD, left ventricular assist device; MI, myocardial infarction; MSC, mesenchymal stem cell; NYD, not yet determined; UC, umbilical cord.
focused exclusively on MSCs, have shown the limitation of trials to date [67, 68]. All have been relatively under powered studies and have used diverse protocols. The exact cell type and number of cells have been quite variable, as exemplified in the MSC trials using a 10,000-fold difference in the amount of delivered cells. Not unexpectedly, one meta-analysis showed that cell number was an independent predictor of outcome on LV function, with trials using greater than $5 \times 10^6$ cells having more efficacy than those using less [68]. Regardless, the data for BM-derived cells overall show a small but significant benefit in LVEF (+1.92%), reduction in infarct size (−2.25%), and LVE SV (−6.37 ml) compared with standard therapy [68]. Furthermore, a 2014 meta-analysis comparing various selected stem cell populations performed an analysis of MSC efficacy for acute MI specifically, and found that MSCs lead to an overall benefit in LVEF of 4.41% compared to placebo control, an effect that was statistically significant ($p = .01$) [67].

There are many other limitations to the clinical trials conducted to date, including differences in the timing of cell delivery, delivery method (intramyocardial, intracoronary, or intravenous delivery), follow up, and cell processing. The trials were mostly locally driven translational studies in the absence of standard procedures across the trials. Unfortunately, the heterogeneity of the trials has reduced the impact of their data, especially in the meta-analyses, ultimately creating more confusion than clarity.

Currently, there are over 25 trials of MSC delivery for cardiac regeneration registered with clinicaltrials.gov, including for acute MI and ischemic cardiomyopathy. There are also trials using MSCs for nonischemic conditions, such as anthracycline-mediated cardiomyopathy. Many are phase Ia/b, with LV function or MACE as primary outcomes. There are still no larger scale efficacy trials, likely due to regulatory, fiscal, and institutional limitations. Table 2 summarizes the ongoing trials.

## Future Directions

Generating reliable and effective cell-based therapy for IHD requires optimization of the product, delivery method, and recipient selection. Many preclinical studies have shown benefit of cell modification to enhance their survival, proliferative capacity, and secretion of paracrine factors. These include genetic manipulation, in vitro preconditioning (with hypoxia or with pharmaceutical agents, for example), or pretreatment with growth factors or other cytokines [69]. Gene delivery of Akt [29] or haem-oxygenase 1 (HO-1) [70] in MSCs prior to transplantation have shown benefit in cell survival, with resulting improvement in rat myocardial function postdelivery. Similarly, transfection of MSCs with anti-apoptotic genes such as bcl-2 [71], bcl-xL [72], connexin43 [73], and survivin [74] have been found to improved MSC survival in vivo, and result in moderate improvement of LVEF in rats.

## Conclusion

While there are many preclinical approaches that have shown promise, there is a great need for larger scale clinical trials showing efficacy. MSC-based cell therapy, either using the cells themselves or their derived products, offers promise, and may provide more convincing data compared to a more heterogeneous cell population such as BM-MNCs. Investment into this field is imperative to the development of feasible treatments, and requires engagements from both the public and private sector. Without a manufactured product per se, there are limitations to the generation of a marketable product, but regardless, from a therapeutic point of view, harnessing stem cell biology may hold great promise.

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## Author Contributions

M.R.W. contributed as the lead author on this review. A.A. was a contributing author. K.A.C. was the senior author. No other authors or writers were involved with this manuscript.

## Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest for any of the authors.
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