



## Concise Review: Mesenchymal Stem Cells in Cardiovascular Regeneration: Emerging Research Directions and Clinical Applications

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**Key Words.** Mesenchymal stem cells • Clinical applications • Somatic cell therapy • Stem cell transplantation • Cardiovascular regeneration

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Received December 9, 2016; accepted for publication July 18, 2017; first published August 24, 2017.

<http://dx.doi.org/10.1002/sctm.16-0484>

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### ABSTRACT

Experimental and early clinical data suggest that, due to several unique properties, mesenchymal stem cells (MSCs) may be more effective than other cell types for diseases that are difficult to treat or untreatable. Owing to their ease of isolation and culture as well as their secretory and immunomodulatory abilities, MSCs are the most promising option in the field of cell-based therapies. Although MSCs from various sources share several common characteristics, they also exhibit several important differences. These variations may reflect, in part, specific regional properties of the niches from which the cells originate. Moreover, morphological and functional features of MSCs are susceptible to variations across isolation protocols and cell culture conditions. These observations suggest that careful preparation of manufacturing protocols will be necessary for the most efficient use of MSCs in future clinical trials. A typical human myocardial infarct involves the loss of approximately 1 billion cardiomyocytes and 2–3 billion other (mostly endothelial) myocardial cells, leading (despite maximized medical therapy) to a significant negative impact on the length and quality of life. Despite more than a decade of intensive research, search for the “best” (safe and maximally effective) cell type to drive myocardial regeneration continues. In this review, we summarize information about the most important features of MSCs and recent discoveries in the field of MSCs research, and describe current data from preclinical and early clinical studies on the use of MSCs in cardiovascular regeneration. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:1859–1867

### SIGNIFICANCE STATEMENT

This concise review discusses present and future applications of mesenchymal stem cells (MSC) in therapy of cardiovascular disorders. It summarizes both preclinical and clinical trials conducted in this area with strong emphasis on mechanisms of MSCs action. Its main impact lies in comprehensive summary of ongoing and finished studies.

### INTRODUCTION

Cardiovascular diseases (CVDs) are the number one cause of death worldwide [1]. CVDs affect not only elderly people but also middle-aged people at the peak of their working and social capacities; hence, CVDs are an enormous medical and economic problem in society.

Recent decades have witnessed tremendous progress in pharmacological and endovascular therapies, as well as in surgical techniques, and today, a great amount of effort is being directed toward cardiac disease prevention [1]. Nevertheless, CVDs remain a chronic and progressive burden in a significant proportion of patients, leading to heart failure that requires heart transplantation or permanent left ventricular support [1].

Among the experimental therapies of the future, artificial (mechanical) heart replacement [2] and cardiac regenerative approaches

(including biological hearts) remain the most promising. Today, stem cells are a major focus in regenerative therapeutic strategies.

Discovered in 1970 [3], mesenchymal stem cells (MSCs) possess several specific features that make them important candidates for future regenerative cardiac therapies. Today, MSCs are defined by the International Society for Cellular Therapy as self-renewing, multipotent cells that exhibit plastic adherence under standard culture conditions and express CD73 and CD90 but not CD45, CD34, CD14, CD11b, CD79 $\alpha$ , CD19, or HLA-DR surface markers, with in vitro multilineage differentiation capacity [4]. MSCs are also known as mesenchymal stromal stem cells, multipotent adult progenitor cells, medicinal signaling cells, and mesenchymal progenitor cells (MPCs); however, MPCs are also occasionally classified as a separate population of cells [5].

Among the sources of MSCs, bone marrow [3] and adipose tissue [6] have been the most commonly studied to date. However, MSCs are also found in umbilical cord blood [7], dental pulp [8], synovial fluid [9], amniotic fluid [10], and urine [11]. Umbilical cord Wharton's jelly (WJ)-derived MSCs have recently been gaining significant attention, owing to some of their unique properties and their feasibility of use as an "unlimited" off-the-shelf source of regenerative cells [12, 13].

Although MSCs from various sources share several characteristics, they also exhibit several differences. These variations in MSCs populations may reflect particular regional properties of the niches from which they originate. MSCs features are also susceptible to variations in cell culture conditions and isolation protocols [14–16].

Properties of MSCs derived from bone marrow (BM-MSCs), adipose tissue (AT-MSCs) and WJ (WJ-MSCs) vary in different culture conditions and during differentiation [15–17]. For instance, WJ-MSCs express the highest proliferative potential independently of cell culture conditions [18, 19]. AT-MSCs and BM-MSCs, but not WJ-MSCs, cultured in the presence of serum produce high amounts of extracellular matrix components. Only AT-MSCs are able to produce collagen (I, II, and III). Regardless of cell culture conditions, BM-MSCs preserve high proangiogenic features [15–17]. Other studies have shown that BM-MSCs are the most immunosuppressive cells. These observations suggest that the properties of MSCs strongly depend on cell source and culture conditions [18, 20, 21] and might suggest the most efficient use of various MSCs types in future clinical trials.

This review is intended to present concise information on recent discoveries and the clinical use of MSCs in the field of cardiovascular research.

## ROLES OF MSCs IN CARDIOVASCULAR THERAPY

### Direct Differentiation: Not the Primary Mechanism of MSCs' Action

The heart is a pump built of an extracellular matrix skeleton populated with cells, approximately 30% of which are cardiomyocytes and 70% of which are endothelial cells [22]. MSCs have the potential to differentiate into several cell types, including cardiomyocytes [23, 24]. MSC-like cells can be found in perivascular adventitial niches [25]; moreover, these cells can be differentiated epigenetically *in vitro* into cardiovascular precursors [26]. Some authors have suggested even broader differentiation potential of MSCs both *in vitro*—into neural and glial cells, skeletal myocytes, hepatocytes, and endothelial cells [27]—and *in vivo*, because new cardiomyocytes [28], vascular smooth muscle cells, and endothelial cells have been found at sites of MSCs injections [29]. These studies and other research [30] have shown that MSCs contribute to neovascularization via large and small vessel formation regardless of new muscle generation. Although MSCs are able to differentiate into different cell types, including cardiomyocytes and endothelial cells, this is probably not their primary mechanism of action in cardiovascular regeneration [29].

### Cardiac Retention versus Engraftment of MSCs

Effective delivery and enhanced retention of regenerative cells are fundamental to produce a meaningful therapeutic effect [31–35] because if the cells do not reach the target zone in the first place,

they have no chance to exert any effect. A further fundamental issue is long-term engraftment of the therapeutic cells. The latter may be, to some extent, evaluated in animal models [36] but not yet systematically in humans due to technical and safety limitations and label-specific limitations such as any potential toxic effect on the cell [37] and/or excretion of the label from the therapeutic cell (that is, in most cases, time-dependent) and may provide a false signal of the cell presence (cell vs. label presence) [38].

Engraftment rate of MSCs appears to be rather low [28]. This phenomenon contradicts many preclinical and clinical observations in which robust beneficial effects of MSCs transplantation, such as a decrease in fibrosis, the stimulation of angiogenesis, and the restoration of contractile function, have been observed. Using an improved delivery technique, our group has recently achieved a high and reproducible retention rate ( $\approx 30\%$ ) of  $^{99}\text{Tc}$ -labeled WJ-MSCs in the peri-infarct zone in humans after recent myocardial infarction [39].

Among the key MSCs mechanisms of action, paracrine secretion [40–43] and cell–cell interactions [44–46] appear to be most important. With these mechanisms, repeated administration of the therapeutic cells may be far more relevant to the therapeutic effect than the focus on long-term engraftment.

### Secretion of Diverse Compounds is a Unique Feature of MSCs

MSCs secrete various cytokines, including hematopoietic cell proliferation and differentiation signals such as interleukin-6, *fms*-like tyrosine kinase 3 ligand, a granulocyte and macrophage colony-stimulating factors [47, 48]. They are able to induce cardioprotection via inhibition of cardiomyocyte apoptosis around the area of administration through secretion of anti-apoptotic and angiogenic factors, such as secreted frizzled-related protein 2, which modulates the Wnt signaling pathway [41], and vascular endothelial growth factor (VEGF), which stimulates angiogenesis [40]. The secretion of proangiogenic molecules is crucial for neovascularization in infarcted hearts, because MSCs lacking VEGF are less effective [40].

Importantly, beyond cytokine production, MSCs secrete metalloproteinases that reorganize the extracellular matrix in scar tissue [49]. Reverse remodeling of scar tissue and antifibrotic effects in necrotic myocardial tissue are required for the regeneration and functional restoration of infarcted hearts. Moreover, MSCs also directly stimulate the proliferation and differentiation of endogenous cardiac stem cells (CSCs) [50], thus contributing to muscle regeneration.

Interestingly, soluble cytokines and remodeling factors are not the only agents secreted by MSCs. Exosomes are small extracellular vesicles that may contain microRNAs and induce biological effects, even at distant locations. MSCs have been shown to secrete exosomes that decrease infarct size in a mouse model of myocardial ischemia/reperfusion injury [43].

### Immunomodulation: A Key Attribute of MSC Regenerative Potential

Both innate and adaptive immunity coordinate distinct and mutually nonexclusive events governing cardiac repair. Elimination of the cellular debris, compensatory growth of the remaining cardiac tissue, activation of resident or circulating precursor cells, quantitative and qualitative modifications of the vascular network,

formation of a fibrotic scar and the inflammatory response guide the regenerative process following cardiac damage [51].

The most remarkable feature of MSCs is their moderate HLA class I expression and their lack of HLA class II expression, thus resulting in their immunoprivilege [4]. In many clinical trials MSCs have been found not to trigger immunologic reactions for as long as 12 months post-transplantation [52]. In contrast, they are known to have immunosuppressive properties, for example, by promoting monocyte maturation toward anti-inflammatory type M2 macrophages and producing soluble mediators such as transforming growth factor- $\beta$ 1, hepatocyte growth factor, prostaglandin E2, indoleamine 2,3-dioxygenase, heme oxygenase-1, soluble HLA-G5, and anti-inflammatory interleukin 10 [53]. MSCs also arrest B cell and dendritic maturation, downregulate the activating receptors of natural killer cells, suppress proliferation of both T helper cells and cytotoxic T cells, and inhibit T cell production of pro-inflammatory cytokines [54]. Owing to their immunomodulatory properties, MSCs are used to treat graft-versus-host disease [55] and may resolve inflammation in infarcted hearts.

### Direct MSC Communication with Target Cells

MSCs also interact with other cells directly through cell–cell contacts involving gap junctions [46] and tunneling nanotubes. For instance, MSCs are able to transfer mitochondria through nanotubes [45], thus achieving cardioprotection via respiratory chain salvage in myocytes.

Through direct and indirect communication with cells at injured sites, MSCs recruit other stem cells to facilitate regeneration of injured tissue. One example of such interactions is the SDF-1 $\alpha$ /CXCR4 axis, which regulates homing of hematopoietic stem cells to the injured myocardium [56]. Moreover, cardiomyocytes can reenter the cell cycle after treatment with some cytokines secreted by MSCs (e.g., TGF $\beta$ ). These observations suggest that MSCs can trigger the repair of injured tissue. These intrinsic features of MSCs make them ideal candidates for regenerative cardiac therapy. Figure 1 summarizes biological mechanisms of MSCs action.

### PRECLINICAL CARDIOVASCULAR STUDIES INVOLVING MSCs

Most of the aforementioned cellular mechanisms through which MSCs act in CVDs were originally identified in animal studies. The potential of MSCs to differentiate into cardiomyocytes and engraft into the myocardium has been shown in pioneering experiments in mice [28], which have revealed expression of desmin,  $\beta$ -myosin heavy chain,  $\alpha$ -actin, cardiac troponin T, and phospholamban, as well as sarcomeric organization of the contractile proteins, in the left ventricles of mice injected with human BM-MSCs. That and another animal study [57] have shown that the beneficial effects after MSCs injection exceed those attributable to simple differentiation and engraftment of MSCs. Owing to their immunosuppressive properties, MSCs have been found to ameliorate conditions related to non-ischemic cardiac disorders by resolving inflammation and improving cardiac function via paracrine actions in a rat model of acute myocarditis [58].

The percutaneous injection of allogeneic MSCs into infarcted swine hearts has been found to result in long-term engraftment, improvement in the ejection fraction, decreased scar tissue formation, and benefits to general cardiac function. Moreover, this procedure has been found to be safe and to produce immunoprivilege effects in transplanted cells, because they are not rejected

by allogeneic recipients [59]. The beneficial effects of MSCs are not restricted to animal models of acute and/or subacute myocardial infarction. Promising results have also been observed in a chronic model of ischemic heart disease in dogs in which MSCs have been found to be able to differentiate into smooth muscle cells and endothelial cells, thus causing increased vascularity and improving cardiac function [60]. Autologous MSCs have also been safely delivered into a chronic model of ischemia–reperfusion-induced cardiomyopathy in pigs, thus resulting in structural and functional reverse remodeling [61].

Large-animal models such as pigs are best for bridging the gap between basic research and clinical application because their size, anatomy and physiology are similar to those of humans. These models aid in not only selecting the optimal number of transplanted cells and time of transplantation but also establishing the best method (transendocardial vs. intracoronary vs. intravenous) for delivering and imaging transplanted MSCs [62]. Although results in preclinical studies are very promising, showing improvement in a wide range of cardiac functions—increased ejection fraction, decrease in scar tissue, reversed remodeling, improved contractility, augmented heart perfusion, and increased blood vessel density [28–30, 40, 43, 53, 58, 61, 63, 64]—the long-term assessment of the safety and efficacy of MSCs is still needed.

### TRANSLATION OF MSC REGENERATIVE POTENTIAL INTO CARDIOVASCULAR CLINICAL TRIALS

Fundamental considerations in the clinical applications of cellular therapies to stimulate myocardial repair and regeneration are uncompromised safety and maximized clinical efficacy. A typical human myocardial infarct involves the loss of approximately 1 billion cardiomyocytes and 2–3 billion other (mostly endothelial) myocardial cells [65], leading (despite maximized medical therapy) to a significant negative impact on the length and quality of life [1]. On a laboratory level, maximization of clinical safety involves evaluation of chromosomal stability [66]. Maximization of the cell potential for regenerative capacity involves cell type identification or choice, potential cell pretreatment, and the delivery method to ensure high uptake in the target zone.

### Effect of MSCs Transplantation in Acute Myocardial Infarction

In trials focused on the application of MSCs in acute/subacute myocardial infarction, BM-MSCs have commonly been used (Table 1). In one pioneering study, the short-term (6 months) safety of intravenous injections of allogeneic MSCs has been analyzed. No arrhythmogenicity or tumorigenicity was observed, and global symptom scores and ejection fractions tended to improve versus the effects in the placebo group [67]. In another study, BM-MSCs have been found to be safe for small group of patients with acute myocardial infarction during a 5-year follow-up [68].

In addition to BM-MSCs, AT-MSCs have also been tested for efficacy in myocardial regeneration (Table 1). In the APOLLO trial, application of AT-MSCs resulted in improved cardiac function, elevated perfusion, and a decrease in the extent of scar tissue [69]. On the basis of the results of this study, an ongoing phase III ADVANCE (NCT01216995) trial was launched. MPCs are also being tested for their safety, feasibility and efficacy in the treatment of acute ST-elevation myocardial infarction after their intracoronary administration in the AMICI trial (NCT01781390).

WJ is also a promising source of MSCs for clinical application in treating acute myocardial infarction. WJ-MSCs have been shown to be safe and beneficial in two independent studies [13, 70], and had also positive effects on infarct size and left ventricular contractility [59].

In a study using a different design—CADUCEUS—the application of cardiospheres (mixture of autologous MSCs with CSCs) has been performed. This study has found a moderate decrease in scar tissue and increased viable heart mass and contractility in the treatment group; however, there was no change in ejection fraction [71]. One-year follow-up showed that the safety and therapeutic effects of the intervention were maintained [72].

### Efficiency of MSC Transplantation Is Highest in Chronic Ischemic Cardiomyopathy

Chronic ischemic cardiomyopathy is another cardiovascular disorder in which MSCs are being intensively evaluated and are thought to be highly efficient (Table 1). In the POSEIDON trial, allogeneic and autologous transendocardial applications of BM-MSCs have been compared. Both types of cells delivered similar effects—improvement in ejection fraction and a decrease in scar size within 1 year after intervention [73].

In the TAC-HFT trial, the effects of BM-MSCs and bone marrow mononuclear cells (BMMNCs) have been compared. Neither cell type triggered serious adverse effects; however, BM-MSCs, but not BMMNCs, caused a decrease in infarct size and improvements in contractility and overall quality of life; however, no changes in ejection fraction have been observed [74].

BM-MSCs' beneficial effects in treating chronic ischemic cardiomyopathy are clear, but the effects tend to be limited and localized to the injection site. In the PROMETHEUS study, patients undergoing coronary artery bypass grafting received autologous MSCs. An 18-month follow-up showed improved contraction and perfusion and decreased scar tissue size in injected segments. However, the small number of participants and the lack of a placebo group restricts the degree to which these results can be generalized [75].

The effects of MSCs transplantation may be limited not only by the site of injection but also by the number of transplanted cells. Most of the aforementioned trials used dose-escalation approaches (ranging from  $12.5 \times 10^6$  to  $11 \times 10^8$ ), whereas the ongoing TRIDENT trial—a phase II clinical trial (NCT02013674)—intends to establish the optimal number of transendocardially transplanted allogeneic MSCs, which should at least correspond to the number of cells lost during myocardial infarction while still being a number that is possible to culture and inject.

Bone marrow is not the only source of MSCs that has been tested for treating chronic ischemic cardiomyopathy. AT-MSCs also yield improvements in total left ventricular mass, heart contractility and perfusion in no-option patients with chronic ischemic cardiomyopathy, as shown by the PRECISE study [76]. An ongoing phase II trial (CONCERT-CHF) is testing the safety and efficacy of transendocardial injections of autologous MSCs together with c-kit-positive CSCs in patients with chronic heart failure.

A slightly different approach involves pretreatment of MSCs with cytokines before transplantation. In the C-CURE study, MSCs were preconditioned with a cardiogenic cytokine cocktail before application. Increase in the ejection fraction, end-systolic volume, 6-minute walking distance and general quality of life were observed, with no systemic toxicity or adverse effects within 2 years [77]. In this approach, MSCs with an increased commitment to a cardiopoietic lineage are believed to be more promising than

unstimulated MSCs. The C-CURE results inspired the multinational CHART-1 trial, conducted in 39 hospitals. A recent update from this study has demonstrated the safety of cardiogenic conditioned BM-MSCs from patients 39 weeks after transplantation [78].

A similar approach has been used in the MyStromalCell study, in which patients received VEGF-stimulated AT-MSCs [79]. In that study, prior to transplantation, AT-MSCs were stimulated to differentiate toward an endothelial lineage by culturing for 7 days in VEGF- $A_{165}$ -stimulation medium.

Interestingly, there are no current trials making direct comparisons of the effects of MSCs from different sources (e.g., AT-MSCs vs. BM-MSCs) in the treatment of any cardiac disorder. Similarly, no studies have compared cell delivery methods in this manner. This lack of information complicates making assumptions about optimal cell sources or delivery methods.

Studies to date have generally provided optimistic observations concerning the application of MSCs in the treatment of cardiovascular disorders (acute or chronic).

In several models, MSCs have been shown to decrease scar tissue size, increase perfusion and contractility of the injured heart, induce neovasculogenesis and antifibrotic effects in damaged cardiac tissue, and generally improve quality of life. However, there is still a need for large, comprehensive, randomized controlled multicenter studies comparing crucial features of MSCs application in CVDs (e.g., source and number of cells, culture conditions, time, and method of application). Several such studies are in progress, thus warranting cautious optimism with regard to the clinical application MSCs in the near future. Table 1 presents selected clinical trials involving MSCs in cardiovascular disorders.

### ENHANCING THE EFFICIENCY OF MSC THERAPY: FUTURE GOALS

Despite the promising results of clinical studies involving MSCs, constant efforts to enhance MSC performance are being made, primarily because effects observed in preclinical studies are stronger than those in clinical trials. To achieve the best clinical results, optimal conditions for transplantation must be established. These conditions involve duration of the disease (acute or chronic disorder); the dose of cells applied; the overall patient condition, sex and age of the patient; and the age of the cell donor in cases of allogeneic transplants.

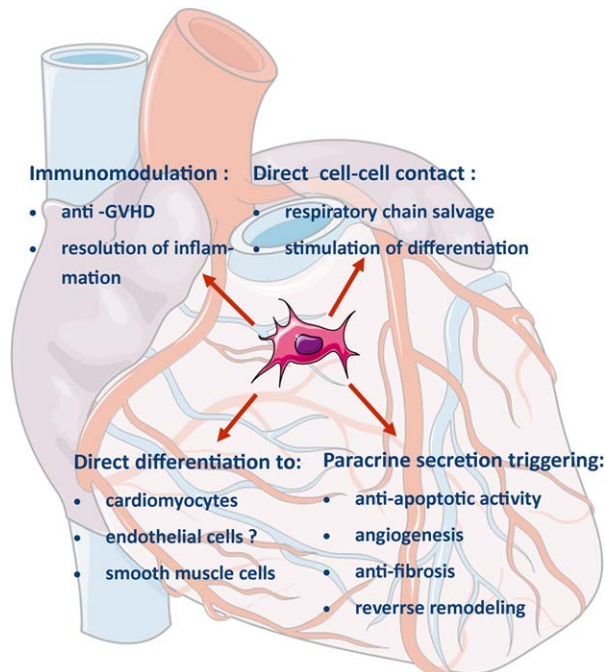
The method of cell delivery (intracoronary vs. transendocardial vs. intravenous) is also being debated [37]. On the basis of the conclusions of cardiovascular clinical trials, the transendocardial application of  $20\text{--}100 \times 10^6$  MSCs in treating chronic ischemic cardiomyopathy may deliver the best results. However, there is a lack of comprehensive studies discussing these issues and showing a reliable efficacy of MSCs transplantation that exceeds the efficacy of standard procedures alone. Ultimately, combined therapies may prove most viable. An interesting concept is to test MSCs as an adjunctive therapy in patients receiving left ventricular assist devices [80].

There is also a lack of data showing the optimal source of MSCs for transplantation. As shown in basic science studies, MSCs can differ across sources in their regenerative potential, that is, in their level of secreted trophic factors or propensity toward different lineages. However, there are many discrepancies among published data regarding the properties of BM-, AT-, and WJ-MSCs. Therefore, comprehensive studies are needed to obtain consistent results. Such studies may also improve cell preparation methods for specific clinical trials.

**Table 1.** Selected clinical trials involving MSCs in cardiovascular disorders

Disorder	Trial acronym and/or number	Phase	Type of trial	Cells applied	Amount of cells	Time from onset	Delivery method	Results	Reference
Myocardial infarction	NCT00114452	I	randomized, double-blind, placebo-controlled, dose escalation	alio BM-MSCs	0.5 × 10 <sup>6</sup> /kg, 1.6 × 10 <sup>6</sup> /kg, 5.0 × 10 <sup>6</sup> /kg	1–10 days	intravenous	no arrhythmogenicity, no tumorigenicity	[67]
Myocardial infarction	NCT00877903	II	randomized, double-blind, placebo-controlled	alio BM-MSCs	undisclosed	<7 days	intravenous	↓ hypertrophy ↓, arrhythmia, left ventricle reverse remodeling	-
Myocardial infarction	NTR1553	I	nonrandomized, controlled	auto BM-MSCs	>10 × 10 <sup>6</sup>	<1 month	intramyocardial	no adverse effects	[68]
Myocardial infarction	APOLLO, NCT00442806	I/IIa	randomized, double-blind, placebo-controlled	auto AT-MSCs	average 17.4 ± 4.1 × 10 <sup>6</sup>	<24 hours	intracoronary	no adverse effects ↓, scar tissue ↑, perfusion	[69]
Myocardial infarction	-	pilot	first-in-man	alio WJ-MSCs	30 × 10 <sup>6</sup>	5–7 days	transcoronary	no adverse effects	[13]
Myocardial infarction	NCT01291329	II	randomized, double-blind, placebo-controlled	alio WJ-MSCs	6 × 10 <sup>6</sup>	5–7 days	intracoronary	↑ ejection fraction ↓ heart perfusion	[70]
Myocardial infarction	CADUCEUS, NCT00893360	I	prospective, randomized, controlled	auto MSCs + CSCs	12.5, 25 × 10 <sup>6</sup>	1, 5–3 months	intracoronary	↓ scar tissue ↑ contractility	[71]
Chronic ischemic cardiomyopathy	POSEIDON, NCT01087996	I/II	randomized comparison, dose escalation	alio vs auto BM-MSCs	20, 100, 200 × 10 <sup>6</sup>	not applicable	transendocardial	↑ ejection fraction ↓ scar tissue	[73]
Chronic ischemic cardiomyopathy	TAC-HFT, NCT00768066	I/II	randomized, blinded, placebo-controlled	auto BM-MSCs vs auto BMMNCs	100, 200 × 10 <sup>6</sup>	not applicable	transendocardial	no adverse effects ↓ infarct size	[74]
Chronic ischemic cardiomyopathy	PROMETHEUS, NCT00587990	I/II	randomized, blinded, placebo-controlled	auto BM-MSCs	2 × 10 <sup>7</sup> , 2 × 10 <sup>8</sup>	not applicable	intramyocardial	local ↑ contraction ↓ scar tissue	[75]
Chronic ischemic cardiomyopathy	PRECISE, NCT00426868	II	randomized, placebo-controlled, double-blinded	auto AT-MSCs	0.4 × 10 <sup>6</sup> /kg, 0.8 × 10 <sup>6</sup> /kg, 1.2 × 10 <sup>6</sup> /kg	not applicable	transendocardial	↑ left ventricular mass ↑ contractility ↑ perfusion	[76]
Chronic ischemic cardiomyopathy	C-CURE, NCT00810238	II/III	randomized, single-blinded,	preconditioned auto BM-MSCs	6–11 × 10 <sup>8</sup>	not applicable	endoventricular	↑ ejection fraction	[77]
Ischemic heart failure	CHART-1, NCT01768702	III	prospective, multicentre, randomized, controlled, double-blinded	auto BM-MSCs	>24 × 10 <sup>6</sup>	not applicable	endomyocardially with a retention-enhanced catheter	no adverse effects	[78]
Chronic ischemic cardiomyopathy, refractory angina	MyStromal Cell, NCT01449032	II	randomized, double-blind, placebo-controlled	VEGF-stimulated alio AT-MSCs	undisclosed	not applicable	intramyocardial	unpublished	[79]

Abbreviations: AT-MSCs, MSCs derived from adipose tissue; BMMNCs, bone marrow mononuclear cells; BM-MSCs, MSCs derived from bone marrow; WJ-MSCs, Wharton's jelly-derived MSCs.



**Figure 1.** Mesenchymal stem cells mechanisms of action in cardiovascular diseases. Abbreviation: GVHD, graft-versus-host disease. Source: Servier Medical Art, modified (<https://creativecommons.org/licenses/by/3.0/legalcode>).

The absence of differences between the effect of autologous and allogeneic BM-MSCs used in clinical studies for the treatment of ischemic cardiomyopathy has previously been reported [73]. However, allogeneic cells have advantages over autologous cells in that they can be prepared, expanded and characterized more quickly as off-the-shelf-products that are ready to be applied when needed. Our [13] and another group [70] have suggested the use of an innovative source of allogeneic MSCs, WJ, for treating cardiac disorders. In the CIRCULATE study, WJ-MSCs will be isolated from umbilical cords and characterized on the basis of their molecular features and their ability to treat cardiac disorders both in *in vivo* models and in a clinical trial. This approach may address the unmet needs regarding the clinical application of MSCs, which include but are not restricted to the poor availability of abundant autologous cells in the short time period after heart failure. It has been estimated that myocardial infarction is associated with loss of approximately  $10^9$  cardiac myocytes [65]. Thus, that is the order of magnitude of cells necessary for transplantation within days after a cardiac incident. An off-the-shelf approach appears to be more feasible than autologous cell expansion to meet this need.

Moreover, because cardiovascular disorders mainly affect elderly people with comorbidities (e.g., diabetes), it is safe to assume that their autologous cells would also suffer “comorbidities,” thereby diminishing the long-term therapeutic effects of transplanted autologous cells. This risk is overcome via the application of “healthy, young” allogeneic cells.

Sophisticated methods for increasing MSCs efficacy involve (a) cell transplantation in combination with pharmacotherapy [81]; (b) MSCs genetic modification (e.g., increasing engraftment potential [82]), which may be effective but also hazardous and nonphysiological; (c) MSCs preconditioning (e.g., with VEGF, insulin-like growth factor 1, bone morphogenetic protein 2 or basic fibroblast growth factor) [64, 79, 83] to induce their differentiation or

increase their paracrine properties; and d) application of MSCs on scaffolds [84] or in microcapsules [85] to increase their retention. Future studies are expected to reveal which of these approaches provide the greatest treatment efficacy.

Despite numerous clinical studies showing beneficial effects of MSCs in treating cardiovascular disorders, some authors have called into question the nature of MSCs, and have even suggested that MSCs and fibroblasts cannot be distinguished on the basis of morphology, cell-surface markers, differentiation potential or immunologic properties [86–88]. This highlights the importance of defining MSCs properly and may reflect the trap of inaccurate nomenclature, because no stem-cell nature would be expected in fibroblasts.

Interestingly, despite of all abovementioned concerns, the level of improvement in left ventricular ejection fraction observed in cell therapy trials is comparable to the levels observed with the use of the most effective pharmacological treatments [39]. One common criticism of cellular therapies to stimulate myocardial repair and regeneration involves their seemingly small effect on myocardial contractility, typically evaluated as the left ventricular ejection fraction (LVEF). It needs to be noted that typically reported improvements in LVEF in patients with heart failure by  $\approx 2\%$ – $4\%$  [89] are not different from the typical effect of widely recognized pharmacological therapies (e.g., beta-blockers +2.9% [90], angiotensin receptor blockade +1.3% [91], aldosterone inhibition +2.0% [92] or cardiac resynchronization therapy +2.7% [93]). It is expected that improvements in cell therapy including the use of unlimited cell sources, reproducible cell harvest, preparation protocols and standardized delivery methods taking advantage of the latest technology will translate into advancing beyond the magnitude of the effect of contemporary pharmacotherapy.

## CONCLUSION

A number of unique features of MSCs discussed above make them unique and promising therapeutic agents, in the field of stem cell research. Rather than being *typical stem cells* that differentiate into effector cells, which directly trigger the regeneration of damaged tissues (similar to construction workers at a construction site), they act as *governing cells* that secrete mediators and/or directly interact with other cells and subsequently stimulate or recruit those cells to perform regenerative actions (similarly to construction site supervisors). To conclusively demonstrate these effects, additional well-designed randomized multicenter studies are needed before MSCs treatment can become a therapy of choice for the fundamental health problem worldwide, CVDs. Allogeneic MSCs are particularly interesting as therapeutic agents because they are not only free of fundamental biologic limitations of autologous cells [94] but also can be used as “off-the-shelf” therapeutic agents [13].

In a mutual relationship to clinical trials, important issues that need to be addressed at the pre-clinical and early clinical stage of MSCs applications involve (a) reduction or elimination of cell antigenicity to reduce or eliminate rejection [54], (b) continued development of improved delivery techniques to enhance myocardial retention and engraftment [62], and (c) cell engineering and /or preconditioning [95] to enhance regenerative capacities and enhance survival. Recent study in subacute myocardial infarction in humans indicates an unprecedented high-grade (systematically 30%–35%) myocardial uptake of transcoronary-administered naturally low-immunogenic WJ-MSCs [39]. This exceeds by  $\approx 5$ -fold the myocardial uptake of other cell types (such as unselected or selected bone-marrow hematopoietic or mesenchymal cells) [96]

in subacute human myocardial infarction, indicating an important clinical research direction [97].

#### ACKNOWLEDGMENT

This work was supported by research grant (Stratemed2/265761/10/NCBR/2015) from the National Center for Research and Development.

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#### AUTHOR CONTRIBUTIONS

M.M., M.S., B.B., and P.M.: manuscript writing, final approval of the manuscript.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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