Molecular Therapy
Review

Mesenchymal Stem Cell-Based Therapy for Cardiovascular Disease: Progress and Challenges

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Administration of mesenchymal stem cells (MSCs) to diseased hearts improves cardiac function and reduces scar size. These effects occur via the stimulation of endogenous repair mechanisms, including regulation of immune responses, tissue perfusion, inhibition of fibrosis, and proliferation of resident cardiac cells, although rare events of transdifferentiation into cardiomyocytes and vascular components are also described in animal models. While these improvements demonstrate the potential of stem cell therapy, the goal of full cardiac recovery has yet to be realized in either preclinical or clinical studies. To reach this goal, novel cell-based therapeutic approaches are needed. Ongoing studies include cell combinations, incorporation of MSCs into biomaterials, or pre-conditioning or genetic manipulation of MSCs to boost their release of paracrine factors, such as exosomes, growth factors, microRNAs, etc. All of these approaches can augment therapeutic efficacy. Further study of the optimal route of administration, the correct dose, the best cell population(s), and timing for treatment are parameters that still need to be addressed in order to achieve the goal of complete cardiac regeneration. Despite significant progress, many challenges remain.

The last two decades have witnessed the development of novel regenerative medicine approaches for cardiovascular diseases. However, the ultimate goal of complete repair of the heart in response to damage has yet to be realized. Among the biggest hurdles to this goal is the inability of the adult heart to promote sufficient *de novo* cardiomyogenesis to replace cells lost to disease due to its (1) intractable genetic and epigenetic state, (2) limited cellular plasticity, and (3) proclivity to succumb to pro-inflammatory and pro-fibrotic immune pathways. To this end, gene and stem cell therapies are among the most promising regenerative approaches, and several designs are currently being tested, both alone and in combination.

Some of the most promising gene transfer therapies are designed to up-[1–6] or downregulate[7,8] the expression of cardiac, vascular, or immune system genes, which are abnormally expressed in the pathologic heart. Other approaches ectopically express oncogenes to force proliferation of adult cardiomyocytes.[9] More recently, the transfer of lineage reprogramming gene cocktails into myocardial scars for converting non-cardiomyocytes into beating cardiomyocyte-like cells has been gaining support.[10] Finally, recent advances in the development of high-precision genome-engineering tools, such as the CRISPR/Cas system,[11] have introduced the possibility of using gene therapy to permanently edit and correct disease-causing mutations in the genome of adult cardiomyocytes.[12,13] However, despite the great promise, most preclinical and clinical studies have illustrated important limitations in the translation of gene therapy toward the clinical setting. For example, recent gene transfer clinical trials in patients with heart disease did not succeed in reaching the anticipated levels of efficacy seen in preclinical animal models.[14–16] Similarly, a major caveat in using gene transfer for lineage reprogramming or cell proliferation-based cardiac therapies is the high risk of such interventions introducing ectopic cardiomyocytic or neoplastic formation if the recipient cell(s) and the activity of the transferred gene(s) are not well controlled. Finally, preclinical proof-of-concept experiments highlight important limitations in the applicability of the CRISPR/Cas system for cardiac regenerative medicine, such as the need for *in situ* genome editing of billions of cardiomyocytes at the single-cell level, without disrupting their function or introducing unwanted off-target mutations,[12] as well as the genetic complexity of heart diseases, which are generally of polygenic or unknown genetic origin.

Cardiac cell-based therapies aim to overcome the limitations of gene therapy via the adoptive transfer of healthy cells, rather than isolated genes. Cells are thought to operate either directly, by replacing the unhealthy cells in the damaged tissue, or indirectly, via the secretion of molecules and microvesicles that stimulate endogenous mechanisms of immune regulation and cardiac regeneration. The cell grafts are derived from adult tissues, such as bone marrow,[17,18] skeletal muscle,[19] and the heart itself[20,21] or from pluripotent stem cells[22,23], and they may be autologous[17] or allogeneic[18,24] in origin. However, as with gene therapy, cell therapy faces many challenges toward clinical translation. For example, contrary to the original hypothesis that

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Table 1. Comparison of Different Regenerative Approaches for Cardiovascular Disease

	MSCs	CSCs	iPSCs	Gene Therapy
Accessibility	readily accessible in large quantities from bone marrow[55] and adipose tissue,[61] also from placenta, umbilical cord, etc.;[25] ideal for clinical use as allogeneic (MHC-mismatched), off-the-shelf cellular therapy due to immunoprivileged phenotype[25]	requires access to adult cardiac tissue;[20,21] most likely clinical use as autologous cell therapy; allogeneic use may be possible;[20,21,41,48,164,165] the on-going CAREMI trial is testing the safety, feasibility, and efficacy of allogeneic CSCs in patients with acute myocardial infarction;[165] unclear if immunosuppression necessary	requires reprogramming of somatic cells followed by directed differentiation into MSCs, CPCs, coronary vascular cells or CMs;[35,166] development of patient-specific, autologous cells clinically challenging due to manufacturing (costs and time);[167] most likely clinical use as allogeneic (MHC matched) therapy[24]	multiple techniques (e.g., TALENs, CRISPR/Cas); require development of live viral vectors for delivery in patient[168]
Clinical and preclinical efficacy	reduction of fibrosis in scarred tissues;[151] improved LVEF and endothelial function[42,60]	reduction in infarct size; improved LVEF[20]	improved LVEF[24,169]	improved LVEF[90,170,171]
Safety	no evidence for tumorigenic or arrhythmogenic risk after extensive preclinical and clinical testing both as autologous or allogeneic therapy[17,18,42,60]	no evidence for tumorigenic or arrhythmogenic risk after preclinical and clinical testing both as autologous or allogeneic (CDC) therapy[20,21,41,48]	no clinical data; preclinical studies indicate increased risk for arrhythmogenesis[24,172] and possibly tumor formation;[173–175] high risk for immune rejection if histocompatibility mismatch	minimal tumorigenic, immunogenic, or arrhythmogenic risk from current clinical studies;[176,177] risk for mosaic gene disruption and introduction of novel off-target mutations following CRISPR/Cas gene therapy;[12,13] safety of direct cardiac cell reprogramming or cell proliferation strategies currently undetermined
Mechanism of action	anti-inflammatory and antifibrotic effects; stimulate endogenous repair by paracrine signaling (growth factors and microvesicles);[32,87,88] limited capacity for direct tissue replacement[78–80]	stimulate endogenous repair by paracrine signaling (growth factors and microvesicles); limited capacity for direct tissue replacement[41,48,178]	unclear; evidence for direct remuscularization[24,172] or no evidence of remuscularization;[112] evidence for paracrine effects[37,112,169]	modulates gene expression

transplanted cells would directly remuscularize and regenerate the damaged myocardium, most of the adult cell types show limited cardiomyocyte differentiation capacity, while their long-term engraftment is minimal regardless of histocompatibility, due to immune clearance from the host myocardium.[25] Similarly, strategies with bona fide cardiomyogenic cells, such as pluripotent stem cell-derived cardiac precursors and cardiomyocytes,[26] or reprogrammed cells[27] suffer from poor engraftment, regardless of histocompatibility, and, more importantly, they may become a source of neoplasia[28] or arrhythmogenesis[24,29] until cleared from the host myocardium by the immune system. Nonetheless, the current consensus is that, despite the lack of evidence for durable, direct tissue replacement, numerous cell therapy regimens for cardiac dysfunction exert unequivocal beneficial effects, in part via incompletely understood paracrine mechanisms, which stimulate endogenous repair[30–32] by, among other mechanisms, stimulating endogenous cardiac precursors and cardiomyocyte proliferation,[32–36] secreting proangiogenic and prosurvival molecules and microvesicles,[37–40] and modulating the immune system[41–44] (Table 1).

Combinatorial approaches may prove to be the most efficacious. Combining cell and gene therapy offers the advantage that cells can be genetically engineered *ex vivo* prior to transplantation, offering a safer alternative and more precise control of gene expression compared to gene therapy alone.[27,38,45–47] Another such approach involves the *ex vivo* engineering of cell grafts with mixtures of physiologically relevant cell types, such as cardiomyocytes; cardiac precursors; and vascular, neuronal, immune, and mesenchymal cell types,[35,41,48–54] which may offer a more comprehensive regenerative strategy compared to each cell type alone, given that both cardiomyogenic and non-cardiomyogenic cells are necessary for heart function and regeneration.

Here we focus on the role of one particular cell type, the mesenchymal stem cell (MSC), in cell-based therapies for heart disease. We review the progress and challenges of MSC-based therapy in cardiac regeneration, emphasizing recent works and controversies, and we discuss future perspectives for MSCs in cardiovascular regeneration.

Characteristics of MSCs

MSCs are stromal cells found in most fully developed organs, including bone marrow, adipose tissue, placenta, umbilical cord, heart, and peripheral blood.[25,54–57] They are multipotent stem cells, likely derived from diverse embryonic lineages, that self-renew and differentiate into several cell types, both mesoderm and neuroectoderm derived.[58] Currently, the precise definition of MSCs remains unclear; but, to be classified as MSCs, cells must meet the following minimal characterization criteria, as defined by the International Society for Cellular Therapy: express a certain set of cluster of differentiation (CD) markers (CD105, CD73, and CD90); lack expression

of hematopoietic lineage CD markers (CD45, CD34, CD14, CD19, and HLA-DR); be plastic adherent when maintained under standard culture conditions; and exhibit the ability to form into colony-forming unit fibroblasts (CFU-Fs) and differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*.[59]

MSCs are considered an attractive option for cell-based therapy because of their ease of isolation and secretion of bioactive molecules and microvesicles that activate tissue repair processes and modulate immune and inflammatory cells.[25] Immune rejection is an important concern with allogeneic (allo) cell-based therapy, but the lack of cell surface histocompatibility complex (HLA) class II molecules and T cell costimulatory molecules and their paracrine-mediated immunomodulatory activity[25,42] provide MSCs an immunoprivileged status that abrogates the need for pharmacological immunosuppression and evades their destruction by the host immune system. Hence, MSCs are safely used as allografts and are well tolerated in cardiomyopathy patients, similar to autologous (auto) MSCs.[60]

Most studies have examined MSCs derived from bone marrow (BM) and adipose tissue (AT). MSCs from these tissues are easily isolated in large quantities, retain their immunomodulatory characteristics,[55] and can produce high amounts of extracellular matrix components. However, these MSCs are not identical. Adipose tissue-derived MSCs (AT-MSCs) produce collagen (I, II, and III), while bone marrow-derived MSCs (BM-MSCs) exhibit high proangiogenic activity[61] and have a greater immunosuppressive effect compared to AT-MSCs.[62–64] BM-MSCs are the most competent cell type for the repression of induced T cell proliferation, and, via a paracrine mechanism, they showed a high anti-proliferative effect on phytohemagglutinin-primed T cells.[64]

Other potentially important sources of MSCs include the umbilical cord matrix, amniotic fluid, peripheral blood, and the heart itself, and each appears to have characteristic properties. For example, while MSCs isolated from the umbilical cord matrix (UCM-MSCs) have similar effects on blocking T cell activation as BM- and AT-MSCs, they have no apparent effect on B cells, unlike BM- and AT-MSCs, both of which inhibit B cell activation.[65] Amniotic fluid-derived MSCs (AF-MSCs) have multipotent differentiation capability,[66,67] and, compared to BM-MSCs, they may also express the core pluripotency transcription factor OCT4.[68,69] Similarly, a type of circulating MSC present in the peripheral blood[70] may also express pluripotency markers, such as SSEA-4, Oct-3/4, and TRA-1-81.[71] However, the role of these markers remains unclear, since these circulating cells lack pluripotent differentiation capacity.[72]

$CD105^+/CD90^+/CD45^-$ MSC/MSC-like cells have been isolated from adult human,[54] mouse,[56,57] and swine[57] hearts. The human adult resident cardiac MSCs express cardiac lineage markers such as GATA-4 and smooth muscle actin. Compared to BM-MSCs, cardiac MSCs express comparable levels of major histocompatibility complex (MHC) classes I and II, but they exhibit slow growth *in vitro*.[54] Importantly, they also express the costimulatory molecule CD40. Therefore, it is unclear if they exhibit the same immunoprivileged characteristic as BM-MSCs.[54,73] Murine cardiac mesenchymal cells (CMCs) can be separated into two groups based on the time for plastic adherence.[57] The rapidly and slowly adhering CMCs were administered intramyocardially 3 days post-myocardial infarction (MI); 30 days later, the slowly adhering CMCs exhibited greater therapeutic efficacy than the rapidly adhering cells. Despite the therapeutic potential of these and other cardiac-derived stem cells, isolating these cells requires a cardiac biopsy, a further limitation.

MSCs derived from induced pluripotent stem cells (iPSC-MSCs) exhibit a similar phenotype as BM-MSCs; they differentiate into multiple cell types, exhibit tissue repair potential in ischemic disease,[74,75] and display important anti-inflammatory actions.[76] iPSC-MSCs exhibit enhanced proliferative capacity compared to BM-MSCs, and they can be expanded for up to 40 passages while maintaining a normal diploid karyotype and a stable gene expression and surface antigen profile.[74] With these inherent advantages, iPSC-MSCs represent a potential alternative cell source for tissue repair in ischemic disease. However, prior to clinical applications, systematic *in vivo* preclinical studies must be conducted to assess safety and tumor formation.[75,77]

MSC Therapy: Mechanism of Action in Cardiac Regeneration

Under the proper conditions, MSCs are capable of transdifferentiating into functional cardiomyocytes *in vivo* and *in vitro*.[78–80] For example, transplantation of human MSCs, expressing a β-galactosidase reporter gene, into immunodeficient mice with experimental myocardial damage resulted in β-galactosidase$^+$ cardiomyocytes within the host myocardium, suggestive of cardiomyocyte differentiation of the xenografted MSCs.[81] Similarly, sex-mismatched cell therapy experiments, in porcine models of acute[32] and chronic MI,[82] provide evidence that engrafted male porcine MSCs differentiated into cardiomyocytes within the female host myocardium, as ascertained by co-localization of the Y chromosome with cardiac transcription factors GATA-4, Nkx2.5, and the structural cardiac protein α-sarcomeric actin. Importantly, differentiated myocytes showed evidence of coupling to host myocardium via gap junctions composed of connexin-43.[82] More recently, Cre-loxP-based genetic fate-mapping studies in mice delineated a pool of adult resident cardiac MSC-like epicardial cells, which could be culture expanded, and they retained full capacity to differentiate into cardiomyocytes *in vitro* and *in vivo*.[56] However, the majority of these studies demonstrate that the level of direct MSC contribution to cardiomyocyte replacement is low and, therefore, unlikely to represent a therapeutically meaningful MSC mechanism of action.[17,32,60,83,84]

Many studies indicate that beneficial effects of MSC therapy can occur through paracrine signaling. The MSC secretome modulates several key cell processes that contribute to cardiovascular protection and/or repair under different pathological conditions.[32,33,82,85–90] While MSCs from different sources share a substantial degree of similarity, there are variations in marker expression profile and secretomes.[55,91,92] For example, AT-MSCs secrete higher amounts of

angiogenic and anti-apoptotic growth factors, such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), as well as interleukin-6 (IL-6) *in vitro*, whereas BM-MSCs secrete higher amounts of the cell migration-related chemokine stromal cell-derived factor (SDF)-1α.[92] UCM- (Wharton's jelly) MSCs secrete higher levels of immune-signaling molecules and neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), compared to BM-MSCs, suggesting a greater beneficial role for Wharton jelly-MSCs in neurodegenerative diseases.[93] Similarly, MSCs derived from embryonic stem cells (ESC-MSCs), which, like iPSC-MSCs, appear to represent an alternative source for stem cell therapy, may also better support neurogenic-related processes compared with BM-MSCs, which enhance angiogenesis *in vitro*[94,95] and in the damaged myocardium.[85,96,97] Furthermore, a recent clinical study by Premer et al.[86] shows that allogeneic MSC therapy improves endothelial function in patients with heart disease. Allogeneic MSCs secrete higher levels of nitric oxide than autologous MSCs, and patients receiving allogeneic MSCs had reduced levels of circulating VEGF compared to autologous MSCs.[86] These differences in the MSC secretome suggest the potential use of differentially sourced MSCs to address specific medical conditions.[98]

MSCs promote endogenous cardiomyocyte regeneration. For example, MSC therapy stimulates survival and proliferation of adult cardiomyocytes via Akt-mediated pathways.[32,87,88] In addition, SDF-1 secretion by MSCs increases the migration, proliferation, and cardiomyocyte differentiation of endogenous c-kit$^+$ cardiac stem and/or progenitors.[32,33,89] Notably, a recent study suggested that, despite its beneficial chemotactic effects, antagonism of SDF-1 in mobilized cardiac precursor cells enhanced their differentiation into cardiomyocytes, highlighting a potentially important mechanism of cardiomyogenesis involving transient up- and downregulation of the SDF-1/CXCR-4-signaling pathway. Based on these observations, it is intriguing to speculate that persistent activation of SDF-1 with gene therapy may be less preferable than transient, cell-based approaches for the treatment of heart failure.[90]

MSCs also exert beneficial effects on extracellular matrix remodeling. For example, MSCs modulate the phenotype of cardiac fibroblasts and their ability to degrade extracellular matrix, and, consequently, they promote the reduction of fibrosis in scarred tissues.[99] They also exert a local antifibrotic effect following transplantation. Exposure of MSCs to type I collagen present in fibrotic tissue promotes dysregulation of myocyte regeneration and repair, and it leads to a downregulation of growth and inflammatory gene expression and a resultant decrease in MSC-induced myoblast proliferation.[100,101] The secretion of unique paracrine compounds, such as HGF, amplifies cardioprotection after MSC transplantation into the injured myocardium.[102]

Perhaps the most potent cardioprotective effects of MSC administration are elicited through immunomodulatory and anti-inflammatory actions. MSCs attenuate the cytotoxic activity of splenic lymphocytes, inhibit nuclear factor κB (NF-κB), reduce tumor necrosis factor alpha (TNF-α) and IL-6 protein expression, and stimulate IL-10 expression in the peri-infarct myocardium.[25,61,103] TNF-α is an important activator of inflammation and TNF-α levels are increased in heart disease.[104] In the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON)-dilated cardiomyopathy (DCM) clinical trial (ClinicalTrials.gov: NCT01392625), patients with non-ischemic dilated cardiomyopathy (NIDCM) had elevated levels of TNF-α at baseline, but these levels were significantly reduced 6 months following the administration of either allo- or auto-BM-MSCs, although allo-MSCs exhibited superior efficacy. This reduction correlated with improvements in cardiac function.[42]

As mentioned above, the lack of HLA class II surface markers and their immunomodulatory properties provide MSCs with the ability to exert their effects without being recognized by the host immune system. Paradoxically, pretreating MSCs with the IL-6 family member cardiotrophin-1, prior to intramyocardial injection into infarcted mice, improved engraftment and infarct size reduction.[105] This result suggests that the host immune system itself helps to maintain MSC immunoprotection. This interplay was further illustrated by ixmyelocel-T cells, a mixed population of bone marrow mononuclear cells enriched for CD90$^+$ MSCs (50-fold) and CD45$^+$CD14$^+$ M2 macrophages (~200-fold). This mixture of cells improved angiogenesis and endothelial protection in a rat hindlimb ischemia model.[44] Open-label phase IIA trials of ixmyelocel-T (ClinicalTrials.gov: NCT00765518 and NCT01020968) demonstrated that intramyocardial delivery of this cell combination improves clinical, functional, symptomatic, and quality-of-life outcomes in patients with heart failure due to ischemic dilated cardiomyopathy.[106] A phase IIB clinical trial (ClinicalTrials.gov: NCT01670981) that has completed recruitment is designed to test the efficacy, safety, and tolerability of ixmyelocel-T compared to placebo (vehicle control) when administered via transendocardial, catheter-based injections to patients with end-stage heart failure due to ischemic dilated cardiomyopathy. Results up to 12 months post-injection suggest that the treatment significantly reduces clinical cardiac events, thereby improving patient outcomes.[49,107]

Several modes of intercellular communications play a role in MSC mechanism of action. In addition to the release of paracrine factors, MSCs actually form tunneling nanotubes with adjacent cells capable of transferring organelles, such as mitochondria, and they also exchange microvesicles, macromolecular complexes, and exosomes[40,108,109] between cells. The regulation of intercellular mitochondrial transport from MSCs to other cells has been attributed to an intrinsic expression of MIRO1, a mitochondrial Rho GTPase1, in the MSC.[110] Moreover, the efficiency of this transfer is enhanced by the formation of tunneling nanotubes (TNTs) via activation of the TNT-α/NF-κB-signaling pathway. iPSC-MSCs overexpress MIRO1 and also have greater sensitivity to the pro-inflammatory cytokine TNF-α compared to BM-MSCs, boosting mitochondrial transfer potency.[111] Simultaneously, the vesicles, macromolecular complexes, and exosomes release a wide range of functional proteins, mRNAs, and microRNAs (miRNAs) capable of protecting cardiac

tissue from ischemic injury by promoting blood vessel formation and enhancing cell proliferation, thereby reducing infarct size and preserving cardiac systolic and diastolic performance.[40,108,109,112]

Extracellular vesicles (EVs), released by a variety of cells, are thought to exert a wide variety of effects on target tissues. These small EVs, also known as exosomes, are 30–100 nm in diameter, and they contain large quantities of bioactive compounds, such as functional proteins, mRNAs, and miRNAs.[108] EVs released by MSCs exposed to hypoxia and/or starvation produce positive effects on neoangiogenesis and functional recovery in the infarcted heart. MSCs release a large quantity of EVs upon exposure to hypoxic conditions *in vitro*. These EVs appear to protect the cells from undergoing apoptosis.[109] In an acute MI rat model, the intramyocardial injection of MSC-EVs markedly enhanced blood flow recovery, reduced infarct size, and preserved cardiac systolic and diastolic performance.[109] MSC-derived exosomes (MSC-Exos) boost the effect of cardiac stem cells (CSCs) in an MI model. Zhang et al.[40] pre-treated CSCs with MSC-Exos *in vitro* and observed an incremental increase in the proliferation, migration, and angiogenic potency of the CSCs. This *in vivo* study showed that the treatment of CSCs with MSC-Exos enhanced engraftment and capillary density, reduced cardiac fibrosis, and improved cardiac outcome in a rat acute MI model.[40] A recent novel study by Mayourian et al.[113] utilized a mathematical model associated with a three-dimensional human engineered cardiac tissue (hECT) to elucidate the paracrine signaling and heterocellular coupling (connexins forming gap junctional channels between different cell types) of MSCs on human cardiac contractility and arrhythmogenicity. Their results confirmed that MSC-Exos are taken up by the cardiomyocytes and fibroblasts that constitute hECT and this paracrine signaling is responsible for significantly increasing the contractility of the hECT, also producing an anti-arrhythmic effect.[113]

Another poorly understood modulator is the effect of aging on MSC function. This understanding is important so as to optimize the use of autologous cells in the elderly. MSC function and therapeutic efficacy appear to decrease with donor age. Older individuals are typically afflicted by cardiovascular and other diseases. Disease and the cumulative exposure to other stressors, including reactive oxygen species, telomere attrition, and a decline in the fidelity of DNA repair and protein turnover systems, compromises the self-renewal potential and differentiation capacity of stem cells.[114,115] This age-dependent decline in stem cell number and function, including multilineage differentiation, homing, immune modulation, and wound healing,[116] can shift the secretome profile of human MSCs toward the expression of senescence-associated factors.[117] MSCs from patients with atherosclerosis secrete higher levels of cytokines, including interferon (IFN)-γ, IL12p70, IL-13, IL-2, and IL-4, key factors of the senescence-associated secretome.[118] These changes suggest that it is of primary importance to monitor and prevent the appearance of a senescent phenotype in *ex vivo* expanded human MSCs (hMSCs).[119] Not only does MSC senescence *in vitro* correlate with diminished function, but it may also explain why MSCs from older donors are less effective for *in vitro* immunopotency assays.[120] A possible candidate to reverse MSC age-related senescence is SIRT3. SIRT3 expression is reduced upon MSC expansion. Increasing SIRT3 expression reverses this decline and improves MSC longevity and differentiation capacity.[120]

Does the age of the recipient limit the response to cell therapy?[83,121,122] In the Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT) and POSEIDON trial, older patients with chronic ischemic cardiomyopathy (ICM) were administered allogeneic or autologous MSCs via a transendocardial route. These trials demonstrate that older patients ($\geq$60 years old) respond similarly to younger patients (<60 years old) with respect to reduction in scar size, improvement in 6-min walk distance test (6MWD), and the Minnesota living with heart failure questionnaire (MLHFQ) total score.[83] This result suggests that recipient age does not reduce the effects of MSC therapy in patients with ICM.

MSCs for Cardiac Repair

Preclinical Studies

The cardiac regenerative capacity of MSCs has been explored in several small[81,123–125] and large animal[32,126–134] models of heart disease, including acute and chronic ischemic cardiomyopathies. Autologous,[127,129] allogeneic,[32,82,128] or xenogeneic[135,136] MSCs have been studied, using different routes of administration, including intracoronary,[129,131–134,137] transendocardial,[32,128,135] systemic,[124] and intravenous.[43,125,132] Most of these approaches support that MSC therapy, regardless of source, is effective at reducing scar size and improving left ventricular ejection fraction (LVEF). Specifically, a meta-analysis of 52 preclinical animal studies of cell therapy for ischemic heart disease[138] suggested that MSC therapy is safe and associated with moderate (~7.5%) but significant improvements in LVEF. However, the effect of cell therapy on LVEF decreased slightly after 8-week follow-up. Animals treated with BM-MSCs also exhibited a better outcome compared to those treated with mononuclear cells.[138] While BM-MSCs have been most frequently utilized as a treatment of diseased hearts in a large animal model,[32,41,48,139,140] other sources, such as obtained from adipose tissue[132–134] and umbilical cord blood,[131] have also produced significant improvement in LV function, perfusion, and remodeling in a large animal model of MI.

The inability of a single cell type to completely reverse the effects of MI prompted the examination of cell combination therapy (CCT; Figure 1). In swine models of MI, BM-MSCs and CSCs from xenogeneic (human),[140] autologous,[48] or allogeneic[41] sources were co-injected into the border zone. In all three studies, CCT has greater therapeutic efficacy than a single cell type, augmenting cardiac regeneration and LV functional recovery without adverse immunologic reaction.[41,48,140] The success of the combinatorial approach formed the basis for the CONCERT-HF (Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure; ClinicalTrials.gov: NCT02501811) clinical trial. CONCERT-HF is a double-blind, placebo-controlled trial in which patients receive 150×10^6 autologous MSCs and/or 5×10^6

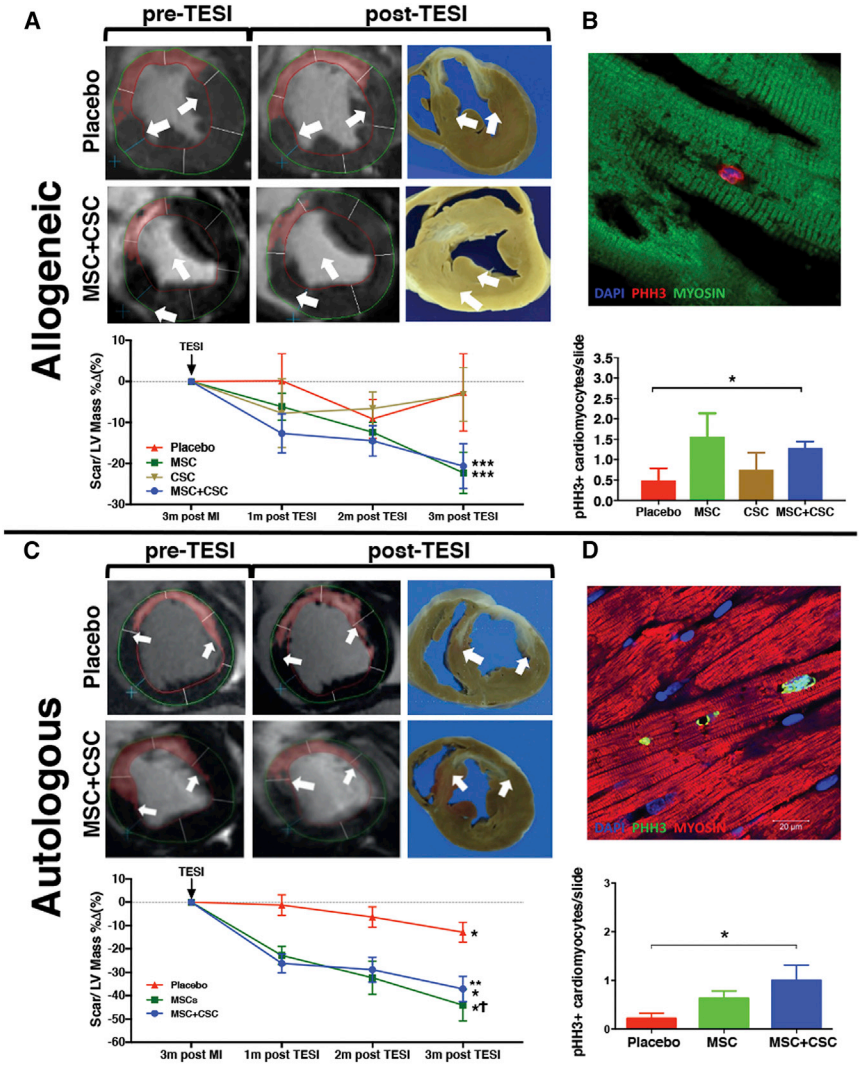

Figure 1. Cell Combination Therapy (MSC + CSC) Reduces Scar Size and Promotes Mitosis of Endogenous Cells in a Swine Chronic Myocardial Infarction Model

Delayed enhancement cardiac magnetic resonance short-axis representative images showing and quantifying the chronologic change of scar size in placebo and combination cell treatment groups pre- and post-transendocardial stem cell injection (TESI), from both allogeneic (A) and autologous (C) studies in swine MI. Graphs show that change in scar mass, as a percentage of left ventricular (LV) mass, decreased significantly following TESI of allogenic and autologous cell combination and mesenchymal stem cell therapies compared to placebo or cardiac stem cell therapy alone. *$p < 0.05$ within group, **$p < 0.001$ MSC + CSC versus placebo at all time points post-TESI, ***$p < 0.0001$ MSC + CSC or MSC versus placebo at all time points post-TESI, †$p < 0.05$ MSC versus placebo at all time points post-TESI. MSC, mesenchymal stem cell; CSC, ckit$^+$ cardiac stem cell. (B and D) Representative confocal microscopy images showing mitosis of cardiomyocytes in response to allogeneic (B) or autologous (D) combination stem cell treatment, using the mitosis-specific marker phospho-histone H3 (PHH3). Graphs represent PHH3 cardiomyocytes/slide in the remote (allogeneic) and border (autologous) zones. *$p < 0.05$ MSC + CSC versus placebo. Adapted from Natsumeda et al.[41] (allogeneic cell study) and Karantalis et al.[48] (autologous cell study).

autologous CSCs. MSCs have also been tested in combination with ESCs and produced significant functional benefit in murine studies. The transplantation of hMSCs into an immunocompetent rat model of MI created a suppressive local microenvironment that mitigated the expected rejection of the co-injected hESCs, thus favorably affecting cell engraftment and functional recovery.[50] These and other studies,[141–144] demonstrating that CCT is a more effective treatment for MI than a single cell type, represent just one example of the next generation of approaches designed to optimize cell-based therapy.

Another promising MSC-based strategy is combining cells with biomaterials designed to improve retention. One of the first attempts consisted of a patch comprising a fibrin matrix seeded with autologous MSCs labeled with β-galactosidase in a porcine MI model.[127] This approach significantly increased LV systolic wall thickness in the infarct zone, and transplanted cells differentiated into cells with myocyte-like characteristics. A robust increase of neovascularization, as indicated by von Willebrand factor-positive angioblasts and capillaries in transplanted hearts, was also observed.[127] This approach has since seen significant improvements. In small animals, a variety of studies show that biomaterials comprising collagen[145,146] or natural extracellular matrix (ECM) scaffold, containing decellularized bovine pericardial tissue scaffold[147] and decellularized human myocardium[148] and combined with a cellular component (MSCs), restores mechanical function to the heart.[145–148] Likewise, studies in large animals are also being performed associating MSCs with a polymeric cardiac patch material containing poly(glycerol sebacate) (PGS), fibrinogen, and VEGF scaffold[149] or natural ECM scaffolds with decellularized porcine myocardium[150] and urinary bladder matrix.[151] The ideal properties of these biomaterials include, but are not limited to, high porosity, high surface area-to-cell volume ratio, microenvironment similar to natural ECM, good mechanical properties, biodegradability, and biocompatibility.[152] Despite the tremendous advances in engineered cardiac tissue, the complete restoration of myocardial function remains elusive, and work needs to be done in order to potentiate the biological activity of these biomaterials to offer biophysical support to the damaged heart.

Determining the route(s) of administration that maximizes the therapeutic benefits of MSCs has been difficult. Despite the numerous

preclinical and clinical studies, there is no overall consensus as to the preferable route. However, a recent meta-analysis suggests that, for acute MI, the transendocardial route produces superior reduction in scar size and improvement in LVEF in both swine and human studies.[153] Further studies directly comparing the route of injection are needed to confirm this conclusion and to determine the most effective route of injection in other cardiovascular diseases in order to optimize the design of new clinical studies.[154]

Clinical Trials

The encouraging outcomes of preclinical studies using MSCs as a treatment for diseased myocardium have set the scene for worldwide clinical trials, many of which have used BM-MSCs.[17,42,60,106,155,156] In the POSEIDON study, administration of allo- and auto-hMSCs to patients with ICM was equally safe, and there was no increase in serious adverse events or immunologic reactions with allo-hMSC therapy.[60] Similarly, safety and clinical efficacy was revealed following allo-MSC or auto-MSC transplantation in patients with NIDCM.[42] In both studies, allo-MSCs, unlike auto-MSCs, improved endothelial function.[86] In the recent TRIDENT (Transendocardial Stem Cell Injection Delivery Effects on Neomyogenesis Study) study,[155] no evidence of immune reaction was seen in ICM patients who received either 20 or 100 million allogeneic BM-MSCs. Only one patient had a calculated panel reactive antibody (cPRA) response incidence following cell transplantation, corroborating previous hMSC allograft studies,[42,60] but the patient did not experience immunologic rejection. Treatment with human BM-MSCs also reduced the serum levels of TNF-α,[155] a marker of heart disease progression.[157] Functionally, both doses reduced infarct scar size, but only the group that received 100 million hBM-MSCs demonstrated improved LVEF, suggesting that the higher dose provides a greater benefit to cardiac function.[155] Similar to the TRIDENT study, allo mesenchymal precursor cells (MPCs), delivered by transendocardial injection to patients with heart failure, improved cardiac structure and function without clinically symptomatic immune responses. Three different cell doses were tested (25, 75, and 150 million). The highest dose produced a reduction in major adverse cardiac events and provided some beneficial effects on left ventricular remodeling.[158]

In the MSC-HF (Autologous Mesenchymal Stromal Cell Therapy in Heart Failure) trial (Clinicaltrials.gov: NCT00644410), patients with severe ICM were administered a mean dose of 77.5 + 67.9 $\times$ 10^6 auto-BM-MSCs, depending on their MSC proliferation. Cell treatment improved numerous parameters of cardiac function, but only left ventricular end systolic volume (LVESV) exhibited a dose-dependent effect. These patients had significant reductions in hospitalization for angina, suggesting that autologous MSC transplantation can be beneficial for severe heart failure treatment.[156]

In CHART-1 (Safety and Efficacy of Autologous Cardiopoietic Cells for Treatment of Ischemic Heart Failure) (Clinicaltrials.gov: NCT01768702), the therapeutic potential of BM-MSCs exposed to a cardiopoietic treatment (cardiopoietic stem cells) was studied. Similar to the MSC-HF study, the number of both intramyocardial injections (<16 injections, 16–19 injections, and >20 injections) and cells injected ($\leq$600 million autologous cardiopoietic stem cells) was dependent on *ex vivo* proliferation. The major findings in this study suggest that fewer injections ($\leq$20, directly correlated with a lower dose received per patient) improve indices of remodeling and function better than higher doses of cells, consistent with the idea that there is an upper limit to cell dosing.[159]

Comparable to the positive cardiac effect promoted by the transplantation of BM-MSCs, AT-MSCs have shown a potential benefit in heart failure. Ventricular function, myocardial perfusion, and exercise capacity were preserved in the PRECISE (A Randomized Clinical Trial of Adipose-Derived Stem & Regenerative Cells In the Treatment of Patients With Non Revacularizable Ischemic Myocardium) clinical trial, the first randomized, placebo-controlled, double-blind trial to examine the safety and feasibility of transendocardial injections of autologous adipose-derived regenerative cells (ADRCs) into patients with ICM.[160] In another study, the efficacy and safety of cryopreserved allogeneic AT-MSCs (called CSCC-ASC; Allogeneic Adipose Tissue-Derived Stromal/Stem Cell Therapy in Patients With Ischemic Heart Disease and Heart Failure - a Safety Study) from healthy donors delivered by intramyocardial injection into 10 patients with ischemic heart disease and ischemic heart failure (IHF) was tested. Approximately 15 injections of 0.3 mL CSCC-ASC (total of 100 million cells) were delivered via transendocardial stem cell injection into viable myocardium into the border zone of infarcted tissue. As a primary endpoint result (major immunologic reaction of allogeneic CSCC-ASC therapy), four of ten patients developed donor-specific *de novo* HLA class I antibodies, and only two patients had donor-specific antibodies at baseline.[161] The study is currently in phase II (ClinicalTrials.gov: NCT02387723) to assess the safety and efficacy of CSCC-ASC in a larger placebo-controlled trial in IHF patients.[162] Another approach is to pre-treat autologous AT-MSCs prior to administration into ICM patients. In the MyStromalCell trial (Mesenchymal Stromal Cell Therapy in Patients With Chronic Myocardial Ischemia; ClinicalTrials.gov: NCT01449032), AT-MSCs were pre-treated with VEGF-A_{165}. Patients receiving these cells significantly improved their exercise capacity whereas the placebo group did not.[163] This small example of the many recent clinical trials illustrates the variety of approaches and sources of MSCs being studied as a therapeutic approach to heart disease.

Future Perspectives

The first stage of cardiac regenerative medicine with MSCs has been successfully completed with a number of high-quality, independently conducted clinical studies supporting its safety and efficacy in patients with heart disease, while highlighting important limitations that need to be addressed as the field continues to move forward.

First, although the original idea of cell-based regenerative medicine strategies envisioned the development of autologous cell grafts for direct tissue replacement, the majority of preclinical and clinical trials, regardless of whether the cells are of somatic or PSC origin, indicates that the success of the field will likely require the development

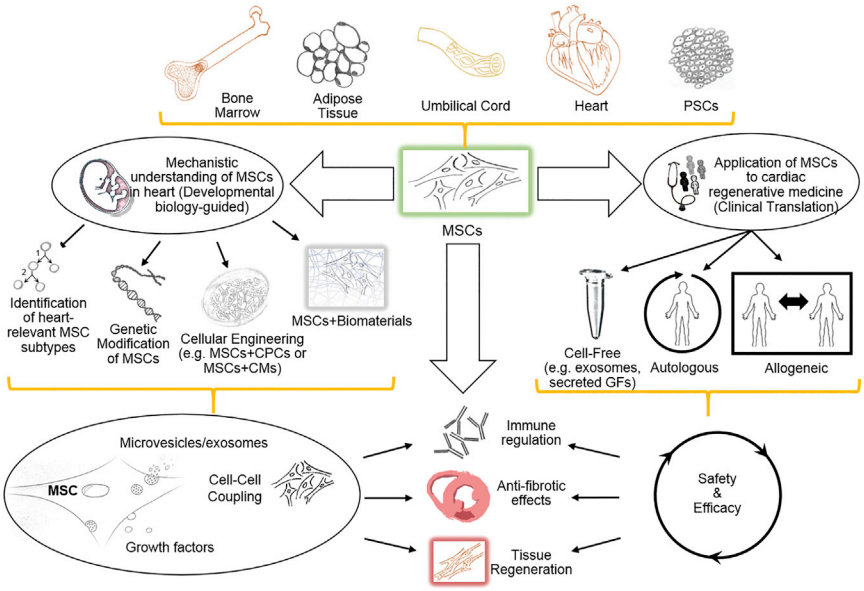

Figure 2. MSCs Are a Particularly Attractive Cellular Platform for Cell-Based Cardiac Regenerative Medicine

MSCs can be isolated from virtually all tissues, including bone marrow, adipose tissue, umbilical cord, and the heart itself, or they can be generated from pluripotent stem cells. Ongoing research, examining the relationship of MSCs to heart development and repair, will guide the development of more refined strategies, such as isolating bona fide cardiac MSC lineages, generating genetically engineered MSCs with better defined growth factor and exosome secretomes, and optimizing combination strategies of MSCs with cardiac progenitor cells and/or biomaterials more relevant to cardiac organogenesis. Translation of this research should address the most important clinical concerns, including feasibility, safety, and efficacy of utilizing MSCs as the basis for cell-based and novel cell-free, autologous and/or allogeneic regenerative medicine approaches. These approaches are designed to safely and effectively augment MSC repair of the damaged heart by delivering microvesicles/exosomes and growth factors and by cell-cell coupling that together activate anti-inflammatory and antifibrotic pathways and stimulate the proliferation of endogenous cardiac precursors, cardiomyocytes, and coronary vascular cells.

of allogeneic, off-the-shelf products with the capacity to promote endogenous mechanisms of regeneration. To this end, MSCs from young or perinatal healthy tissues, or derived from PSCs, are likely to continue dominating the field, since, compared to other somatic and PSC-based cell types, they can be easily manufactured under a relatively low regulatory and financial burden, and they can be readily available either as autologous or allogeneic grafts without compromising safety and efficacy.[17,42,60]

Second, there is an increasing need for improving our understanding of the biology and role of the different types of MSCs, in order to refine their manufacturing and maximize their capacity to promote repair. It will be particularly important to compare the different types of MSCs from different tissue sources (e.g., bone marrow, adipose, perinatal, and cranial and/or dental tissues) in terms of immunologic, antifibrotic, and cardiac regenerative properties. What are the normal functions of each cell type within the host tissue? Does the sex of the donor affect cell function? To what extent are these cell types affected by aging and/or disease?

To this end, focusing on developmental biology-guided basic research approaches could facilitate the identification of MSC lineages that are particularly relevant to cardiac development, growth, and/or regeneration, as well as provide key mechanistic insights regarding the molecular pathways underlying their self-renewal and therapeutic properties. For example, preconditioning and genetic manipulation can reprogram MSCs to secrete appropriate stimulatory or inhibitory molecules, and they can be combined with the above approaches. These approaches can also be directed toward increasing survival and engraftment of MSCs post-administration or for the production of therapeutic exosomes and/or EVs (Figure 2).

While continuing to improve MSCs, the next (interrelated) direction is to optimize cell administration. The most effective route of cell administration must be determined. The transendocardial route (transendocardial stem cell injection [TESI]) appears to be the most efficacious compared to delivering cells through the circulation.[153] However, cell-laced bioengineered patches are also effective, albeit requiring more invasive surgical procedures. Unique delivery systems may be optimal for distinct types of heart disease.

A final direction is to continue exploring the synergy of combining MSCs with another cell type that complements the properties of MSCs (e.g., CSCs[41,48,140,141] or macrophages[49]) to improve cardiac function more than with MSCs alone. Combinations of more than two cell types (sub-populations) and/or combining cell and pharmaceutical approaches are also potential approaches. Over the past ~20 years, stem cell therapy for heart disease has progressed from being virtually non-existent to being a feasible, safe, and encouraging clinical alternative. The challenge is to maintain this momentum by continuously optimizing approaches so as achieve complete cardiac repair.

CONFLICTS OF INTEREST

K.E.H. reports having a patent for cardiac cell-based therapy and holding equity in Vestion Inc. J.M.H. reports having a patent for cardiac cell-based therapy and holding equity in Vestion Inc. J.M.H. maintains a professional relationship with Vestion Inc. as a consultant and member of the Board of Directors and Scientific Advisory Board. Vestion Inc. did not play a role in the design, conduct, or funding of the study. J.M.H. is the Chief Scientific Officer, a compensated consultant, and advisory board member for Longeveron and holds equity in Longeveron. J.M.H. is also the co-inventor of intellectual

property licensed to Longeveron. Longeveron did not play a role in the design, conduct, or funding of the study. L.B. and W.B. have no conflicts of interest.

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