Chapter 7

Mesenchymal Stromal Cells as a Therapeutic Intervention

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Additional information is available at the end of the chapter

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Abstract

Mesenchymal stem cells, also known as mesenchymal stromal cells (MSCs), are a safe and promising biologic therapeutic for inducing tissue repair and regeneration in a broad array of chronic diseases. The mechanisms underlying the beneficial effects of MSCs include immunomodulation, reduction in inflammation and fibrosis, and stimulation of neovascularization and endogenous regeneration. Accumulating evidence from a multitude of clinical trials support the notion that both autologous and allogeneic MSCs are not only safe but also possess the capacity for repair of diverse organ systems and amelioration of multiple chronic disease processes. However, there are many questions regarding the underlying mechanisms of action, the most efficacious cell characteristics, tissue source, dose/concentration, route of delivery, and timing of administration, interactions with concurrent therapies, sustainability of effect, donor and patient characteristics, and adverse effects, including infections and malignancy, that remain to be resolved. Answering these questions will require well-designed and rigorously conducted multicenter clinical trials with well-established and defined clinical endpoints and appropriately defined patient populations, number of patients, and duration of follow-up. This chapter will review the current state of knowledge in the use of MSCs as a therapeutic strategy for organ structural and functional repair in chronic diseases.

Keywords: cell transplantation, mesenchymal stem cells, regenerative medicine

1. Introduction

Mesenchymal stem cells (a.k.a. mesenchymal stromal cells, MSCs) hold enormous promise as a durable, sustainable, and novel cell-based biologic therapeutic for a diverse range of clinical applications aimed at preventing or reversing organ injury and promoting tissue regeneration.

Substantial data have accumulated regarding the safety of administering both autologous and allogeneic MSCs to patients with a broad array of diseases. In addition, it is increasingly clear that MSCs exert anti-fibrotic, pro-angiogenic, regenerative, and immunomodulatory effects, and therefore, offering therapeutic potential in a wide range of presently untreatable conditions. The growing evidence supporting the use of MSCs as therapeutic strategy includes their relative ease of isolation and expansion in culture, multilineage differentiation capacity, immunomodulatory, anti-inflammatory, anti-microbial, and regenerative effects, homing and migratory capacity to injury sites, safety profile in allogeneic transplantation, and few ethical considerations [1, 2]. The use of large animal models in preclinical studies has been instrumental in deciphering the underlying mechanisms of action of MSC therapy [3]. Moreover, substantial human phenotypic data has demonstrated that MSC therapy is safe [4–10] and holds the potential for repair and regeneration of diverse organ systems and amelioration of multiple chronic illnesses for which there is currently no cure [4, 6, 7, 9–24]. There are currently various MSC sources under investigation in preclinical and clinical studies, namely bone marrow, adipose tissue, umbilical cord blood, umbilical cord, and amniotic membranes/placenta (**Figure 1**). Multiple mechanisms of action underlie successful MSC therapy, including MSC engraftment and differentiation, and more importantly, the secretion of bioactive paracrine molecules that inhibit apoptosis, fibrosis, and inflammation and promote neovascularization/neo-angiogenesis and endogenous stem cell recruitment, proliferation, and differentiation [25–27] (**Figure 2**). In particular, cell-cell interactions between MSCs

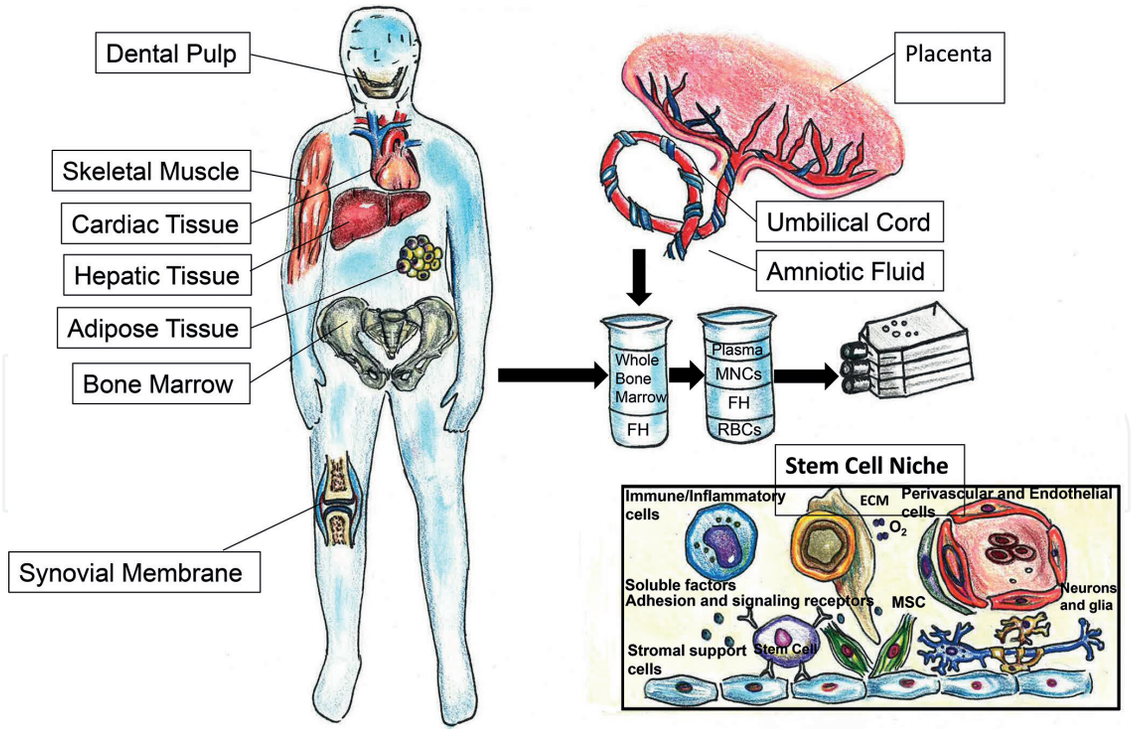

Figure 1. Mesenchymal stem cell tissue sources, ex vivo expansion, and role in stem cell niche. Initially identified in bone marrow, MSCs can be isolated from various tissues in the body. To isolate MSCs from a bone marrow biopsy, first the mononuclear cells are isolated from red blood cells by Ficoll density centrifugation, and subsequently, the MSCs are separated from the mononuclear cells by plastic adherence in culture. Inset: the constituents of a stem cell niche are depicted in this schematic. ECM extracellular matrix. *Adapted from Wagers AJ et al., Cell Stem Cell, 2012.*

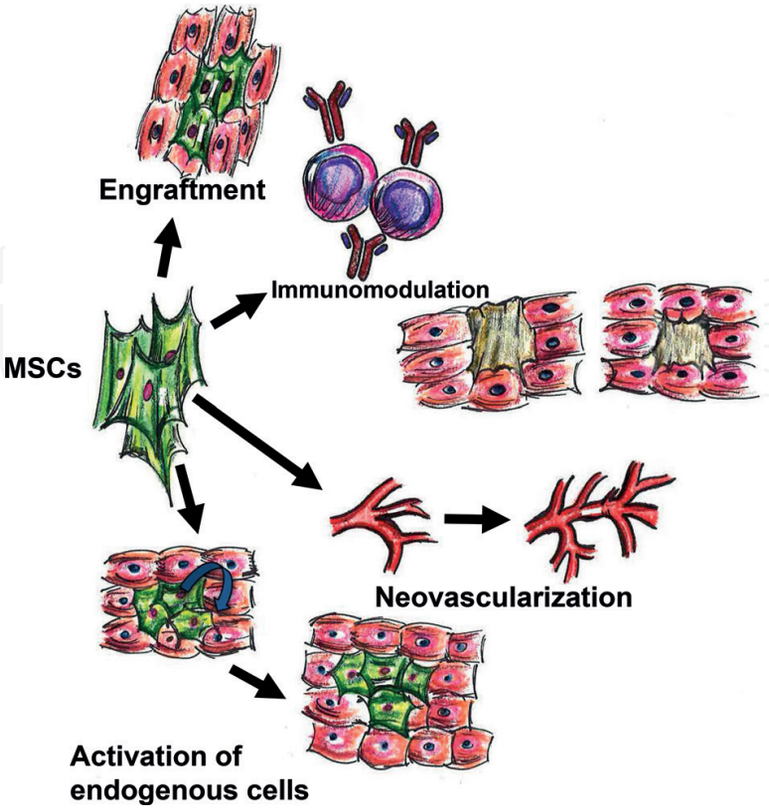

Figure 2. Mechanism of action of mesenchymal stem cell therapy.

and endogenous host cells within stem cell niches provide structural support and produce the soluble signals that regulate stem cell function in tissues[1, 28–30] (**Figure 1** inset). An in-depth molecular understanding of how MSCs produce the therapeutic benefits demonstrated in numerous clinical trials is critical for the development and design of new clinical trials as well as for the development of newer generations of MSC products that have greater efficacy and sustainability. This chapter will review the current state of knowledge in the use of MSCs as a therapeutic strategy for organ structural and functional repair.

2. Biology of mesenchymal stem cells

MSCs are non-hematopoietic stem cells with multilineage potential that originate from the mesodermal germ layer. The pioneering studies conducted by Friedenstein et al. provided the first evidence that these fibroblast-like cells, described as spindle-shaped and clonogenic in culture conditions could be isolated from bone marrow via their inherent adherence to plastic in culture [31, 32]. MSCs are an integral part of the stromal microenvironment and support hematopoietic stem cells and regulate hematopoiesis, although they comprise only ~0.01–0.001% of the total nucleated cells in the bone marrow [33, 34]. Moreover, MSCs have been isolated from virtually every tissue type, including adipose tissue, liver, lung, skeletal and heart muscle, synovial membrane, amniotic fluid, placenta, umbilical cord blood, and dental pulp, suggesting that they reside in all organs [35–37].

MSCs are readily expanded *in vitro* and have the capacity, as classically defined, to differentiate into osteoblasts, chondrocytes, and adipocytes [38, 39]. Studies also strongly support a role for MSCs in neovascularization, with the capacity for differentiation into both endothelial [40, 41] and vascular smooth muscle cells [40]. Finally, MSCs can differentiate into myocytes: skeletal myocyte differentiation is widely accepted, whereas there is ongoing controversy as to whether MSCs have a robust ability to form cardiomyocytes [40, 42–45].

No single cell surface marker specifically identifies MSCs. The International Society for Cellular Therapy has provided minimum criteria for defining multipotent human MSCs including (1) plastic-adherence under standard culture conditions; (2) expression of CD105, CD73, and CD90 and absence of hematopoietic cell surface markers, CD34, CD45, CD11a, CD19, and HLA-DR; and (3) *in vitro* differentiation into osteocytes, adipocytes, and chondrocytes under specific culture conditions [46]. However, MSCs can lose/acquire surface markers as they are isolated and expanded [47]. Furthermore, MSCs isolated from different tissues may exhibit a molecular fingerprint specific for their tissue of origin and thus vary in their differentiation capacity [48–50].

Bone marrow-derived MSC precursors (MPCs) have also been identified based upon specific cell surface marker expression, the most important being stromal precursor antigens (STRO-1, STRO-3) and CD271 [51–56]. *In vitro* studies suggest that the STRO-1 and STRO-3-enriched MPC populations have superior proliferative ability, multilineage regenerative capacity, and paracrine activity compared to MSCs [51, 54, 55], whereas $CD271^+$ selection significantly increases clonogenic outgrowth of MPCs [52]. Preclinical studies using large animals have shown the efficacy of MPCs in acute MI and chronic ischemic and non-ischemic models of cardiomyopathy. Intracoronary injection of allogeneic MPCs in sheep after acute MI produced a 40% decrease in scar size and a 50% increase in vascular density [57]. Similarly, using echocardiography to guide the catheter-based endomyocardial injection of allogeneic MPCs into sheep 4 weeks post-MI resulted in an increase in left ventricular ejection fraction (LVEF), wall thickness, and vascular density. In a model of non-ischemic cardiomyopathy, transendocardial administration of ovine allogeneic cells produced decreased left ventricular end-systolic volume, stabilization of LVEF, decreased myocardial fibrosis and increased myocardial regeneration [53].

2.1. Osteogenic, chondrogenic, and adipogenic differentiation

As mentioned above, MSCs can be readily expanded *in vitro* and can differentiate into osteoblasts, chondrocytes, and adipocytes [38, 39]. Various growth factors and molecules promote MSC differentiation. For instance, global gene expression profiling arrays were utilized to identify RNA transcripts, which led to the identification that TGF-β, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) signaling pathways regulate MSC differentiation into adipogenic, osteogenic, and chondrogenic lineages [58, 59]. Adipogenic and osteogenic differentiation of MSCs were enhanced *in vitro* upon inhibition of TGF-β signaling but prevented chondrogenic differentiation. In contrast, inhibition of PDGF signaling decreased osteogenic differentiation, whereas inhibition of FGF receptor signaling completely blocked osteogenic differentiation and reduced chondrogenic differentiation. Moreover, inhibition of any one of these pathways decreased MSC proliferation. Differentiation thus depends substantially on the microenvironment [60].

A key question regarding postnatal MSC function is the degree to which they participate in tissue homeostasis. For example, in the case of an osteogenic lineage, multiple investigators [61–63] have shown that exposure of MSCs to dexamethasone, β-glycerol phosphate, and ascorbic acid can lead to expression of alkaline phosphatase by the differentiated osteogenic cells with subsequent formation of a mineralized extracellular matrix [61]. Importantly, MSCs do retain the capacity for bone differentiation in vivo [38, 64]. For example, we have shown that subcutaneously implanting MSCs leads to osteoblast differentiation [38]. On the other hand, chondrogenic differentiation of MSCs can be achieved by treating MSCs with dexamethasone and TGF-β3 [58]. Similarly, dexamethasone together with insulin, indomethacin, and 1-methyl-3-isobutylxanthine can stimulate MSC differentiation into adipocytes, which express adipocyte-specific markers including peroxisome proliferator-activated receptor (PPAR)-γ [65].

Cao et al. [38] studied the regulation of MSC differentiation into adipocytes and osteoblasts with relation to PPAR-γ, an essential checkpoint regulator of the "adipogenesis-osteogenesis balance." The study showed that S-nitrosoglutathione reductase (GSNOR)-deficient mice have reduced adipogenesis and increased osteoblastogenesis compared to normal mice (**Figure 3**). Notably, GSNOR MSCs had improved differentiation capacity for bone and reduced propensity for adipocytes. This is due to higher levels of S-nitrosylated PPAR-γ protein with subsequent inhibition of its transcriptional activity, suggesting a negative feedback regulation by NO-mediated S-nitrosylation. In addition, S-nitrosylation of PPAR-y inhibits binding affinity to its downstream target fatty acid-binding protein 4 (FABP4) promoters (**Figure 4**). Importantly, the MSC differentiation affected the phenotype on the whole animal level. GSNOR deficient mice have lower body weight and fat mass, accompanied by elevated bone formation. In another study regarding osteogenic regulation, investigators found that modulation of specific microRNAs (-148b, -27a, and -489) plays a crucial role in MSC early osteogenic differentiation [66]. This has a tremendous corollary in bone diseases such as osteoporosis by providing both pathophysiological and therapeutic insights. Indeed, MSC differentiation into other cell lines of mesenchymal origin can offer further understanding into many other human disease processes, in support of future treatment strategies.

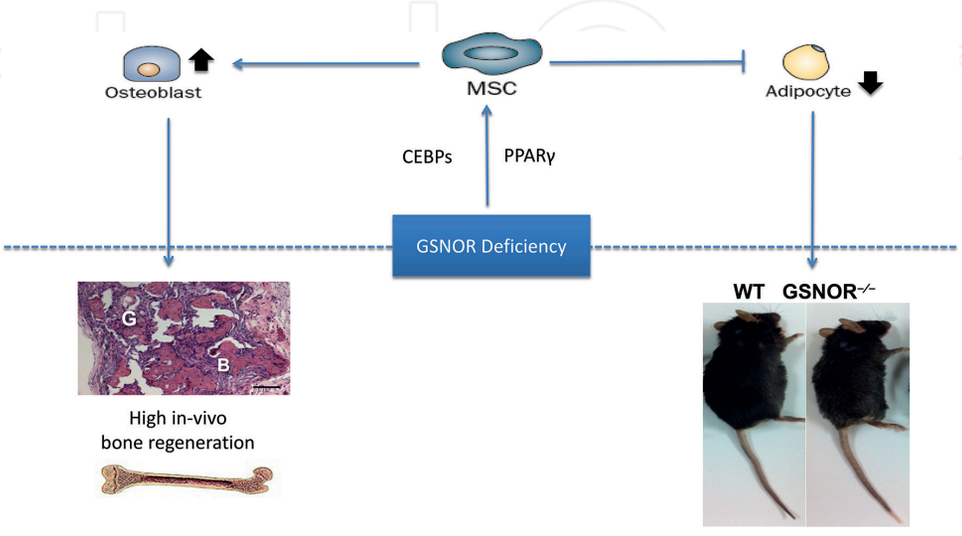

Figure 3. GSNOR deficient mice have reduced weight and body mass with increased bone formation.

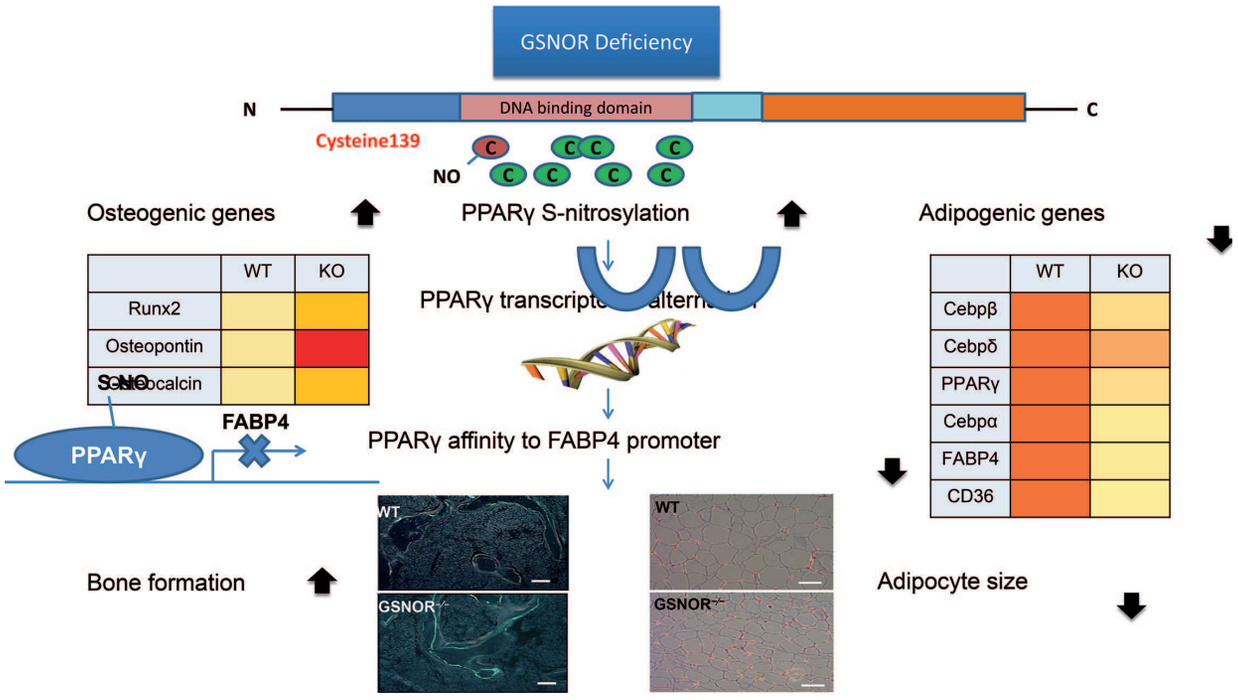

Figure 4. Regulation of adipogenesis-osteogenesis by MSCs. GSNOR deficiency with ensuing elevated levels of S-nitrosylated PPAR-γ leads to a decrease in PPAR- γ transcriptional activity and binding affinity to FABP4 promoter. This results in increased osteogenesis and decreased adipogenesis, which has strong implications in bone disease. *Reproduced from Cao Y et al., JCI, 2015.*

2.2. Cardiac differentiation

Cardiomyogenic differentiation of MSCs is of key interest for cardiac regenerative medicine, particularly ischemic and non-ischemic cardiomyopathy [40, 67, 68]. Treating MSCs with 5-azacytidine produces spontaneous, synchronous beating cells in culture with ventricular myocyte-like potentials, suggesting that MSCs are able to transdifferentiate into cardiomyocytes [43]. Alternative and potentially safer factors that induce differentiation into a cardiomyocyte phenotype include conditioned media containing bone morphogenetic protein-2 (BMP-2) and FGF-4 [69] as well as insulin, dexamethasone, and ascorbic acid [70]. The combination of these factors induces overexpression of cardiomyocyte-specific proteins, leading to cardiomyogenic differentiation for possible use in disease processes of injured myocardium [69–72]. Indeed, expression of myotubules, α-actinin, SERCA2 and other cardiac-related proteins in transdifferentiated cells may serve to attenuate cardiac infarct size and enhance perfusion, and regional function as suggested by early *in vivo* studies [73, 74]. Co-culture of mouse or rat MSCs with rat neonatal ventricular myocytes also stimulates MSC transdifferentiation into cardiomyocytes [75, 76]. The necessity of cell-to-cell contact [1, 75] versus secreted factors within the cardiac microenvironment [76] as a requirement for cardiomyogenic differentiation remains unclear.

MSC therapy promotes cardiomyogenesis not only by direct cardiomyocyte differentiation, but also by stimulating endogenous c-kit+ cardiac progenitors (CPCs) to proliferate, undergo lineage commitment, and form transient amplifying cells [1, 28, 29, 77–79]. We demonstrated

that transendocardial injections of allogeneic MSCs in swine following myocardial infarction (MI) results in cardiogenic differentiation of MSCs accompanied by increased proliferation and enhanced lineage commitment of endogenous CPCs, and reconstitution of niche-like structures [1]. This stimulation of endogenous CPCs by MSCs requires a complex molecular interaction and is a crucial component of the beneficial cell therapeutic effects [1, 28, 29, 77–79]. Histologic examination revealed chimeric clusters (niches) comprised of adult cardiomyocytes, transplanted MSCs and CPCs expressing connexin-43 gap junctions, and N-cadherin mechanical connections between cells. These findings support the notion that MSCs act both as progenitors for certain cell lineages and through their participation in niches, as supporting cells for other lineages [80].

Stimulation of endogenous precursors may be a general mechanism underlying MSC bioactivity. We recently showed that in humans with endothelial dysfunction MSCs can trigger endogenous EPC activation increasing their number and functional quality [81]. Thus MSCs can serve as a powerful therapeutic tool by reconstituting endogenous stem cell niches as well as enabling and augmenting the reparative abilities of endogenous stem cells.

2.3. Anti-fibrotic and proangiogenic effects

The hypothesis that exogenously delivered stem cells would promote organ regeneration through transdifferentiation into tissue-specific cells sparked interest in stem cell research and cell-based therapy and was originally supported by studies in the heart [82] where MSCs become cardiomyocyte-like cells and endothelial cells [40, 41, 43]. However, subsequent studies have revealed that the MSC-mediated regenerative process is more complex than was initially envisioned, and that several mechanisms underlie the ability of MSCs to reduce scar size and improve left ventricular structure and function after myocardial injury [33, 83, 84]. MSCs engraft and persist for several months in myocardium when delivered by transendocardial injection [1, 33, 40] and they reduce cardiac fibrosis and promote neovascularization and cardiomyogenesis [40, 77, 85, 86]. Importantly, cardiac magnetic resonance imaging (MRI) documented a reduction of infarct size, improvement in left ventricular shape (measured as sphericity index of the left ventricle), and improvement in tissue perfusion and regional contractility [87]. Together, these preclinical studies support the anti-fibrotic and proangiogenic role of MSCs in the repair of the injured myocardium.

2.4. Immunomodulatory, anti-inflammatory, and anti-microbial effects

Preclinical studies have demonstrated that MSCs can differentiate into cardiomyocytes and/or vascular structures in both allogeneic [1, 40, 87] and xenotransplantation [88] models, contributing to cardiac functional improvement and reduction of infarct size. Remarkably, there has been no evidence of rejection in animals subjected to allogeneic transplantation of MSCs [1, 29, 40, 87]. These studies reveal that allogeneic MSCs represent a unique cell population for cellular therapy due to their anti-proliferative, immunomodulatory, and anti-inflammatory effects [2, 33, 89]. The absence of major histocompatibility class (MHC) II antigens [90–92] and the secretion of T helper type 2 cytokines characterize MSCs as both immunoprivileged and immunosuppressive [2, 92–94]. MSCs fail to induce proliferation

of allogeneic lymphocytes *in vitro* [90, 92], and suppress proliferation of T cells activated by allogeneic cells or mitogens [91]. This immunomodulatory capacity supports the feasibility of using allogeneic MSCs for cardiovascular regeneration as well as other clinical applications [2, 95]. Furthermore, MSCs have been used to treat severe graft-vs-host disease (GVHD) [13, 96], decreasing the potential of graft rejection and/or GVHD, and supporting the concept that MSCs are a unique cell population for regenerative medicine with minimal immune reactivity. Allogeneic MSCs have proven both safe and effective [5, 7, 11, 29, 89], highlighting that MSCs engrafted in the cardiac tissue despite potential HLA mismatching. An advantage of allogeneic MSCs is their potential use as an "off-the-shelf" therapeutic agent, precluding the need to obtain and expand bone marrow or another tissue source from the patient, and providing consistency to the cell product [97]. In addition, autologous cells may have functional deficiencies due to the underlying diseases, co-morbidities, lifestyle, concomitant medications, or age [98–105]. Although allogeneic MSCs may be cleared more rapidly than autologous cells after differentiation [106], immunologic clearance might also offer the advantage of reducing any long-term risks of cell implantation [8, 94, 107].

An important concern, and common exclusion criteria for participation in clinical trials is that the potential immunosuppressive effect of MSCs may lead to an increased risk of infection in patients who are already immunosuppressed due to medical therapy or concurrent chronic disease. In this regard, recent data has shown that MSCs exert significant anti-microbial effects through both direct and indirect mechanisms [108]. Indirect mechanisms include regulation of macrophages, neutrophils, phagocytes, and another pro- and anti-inflammatory cells of the immune system, whereas indirect mechanisms involve the secretion of anti-microbial peptides and proteins (AMPs) and the expression of indoleamine 2,3-dioxygenase, interleukin-17, and other molecules [94, 108]. Indeed, the anti-microbial effects of MSCs have been demonstrated in preclinical studies of sepsis, acute respiratory distress syndrome, and cystic fibrosis-related infections [108].

2.5. Enhancement of MSC therapy

Therapeutic interventions to optimize MSC function, such as growth factor administration [109–112], gene therapy [110], and modulation with small molecules or other pharmacologic approaches [110] are promising options under preclinical and clinical investigation to potentiate myocardial repair and regenerative capacity. For example, in the phase I cardiopoietic stem cell therapy in heart failure (C-CURE) trial and subsequent phase II/III congestive heart failure cardiopoietic regenerative therapy (CHART-1) study [72, 109, 113], autologous bone marrow-derived MSCs from patients with ischemic cardiomyopathy were treated ex-vivo with a cardiogenic cytokine cocktail to enhance their cardiac lineage commitment. In C-CURE, the authors reported significant improvement in cardiac function, physical performance, hospitalization, and event-free survival in the cell therapy group compared to controls [109]. However, the larger CHART-1 trial reported neutral results at 39 weeks of follow up with regards to composite and individual outcomes, including all-cause mortality, heart failure events, and surrogate cardiac structural and functional endpoints [113]. A sub-analysis of the CHART-1 study extended the follow-up period to 52 weeks at which point the anti-remodeling properties of the cardiopoietic MSCs became evident [72]. These findings are consistent with those of other clinical trials of MSC-based therapy for ischemic cardiomyopathy [7, 9, 114].

A potential approach to improve therapeutic potential is the combination of MSCs with c-kit+ CSCs [28, 29, 79]. Using a porcine model of chronic ischemic cardiomyopathy, the combination of autologous or allogeneic swine MSCs and c-kit+ CSCs provides greater reverse remodeling, scar size reduction, and functional improvements than MSCs alone [29, 79]. The demonstrated safety of cell-based therapy using MSCs [7, 9, 115, 116] and c-kit+ CSCs [117, 118] in patients with ischemic cardiomyopathy combined with these preclinical findings revealed important biological interactions between these two stem cell types that enhance therapeutic responses and led to the initiation of the Cardiovascular Cell Therapy Research Network (CCTRN)-sponsored, Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure (CONCERT-HF; NCT02501811) clinical trial.

2.6. MSC senescence and potential malignant transformation

There is evidence that senescence impairs the capacity of MSCs for multi-lineage differentiation, homing, immune modulation and wound healing [102, 103]. As stem cells age, they undergo a "quiescence-to-senescence switch" that impairs their function [102, 104, 119, 120] (**Figure 5**). The mechanisms underlying the age-related declines in stem cell function involve intrinsic aging as well as age-related changes in their tissue microenvironment, including extracellular matrix components and the stem cell niche [101, 104, 121], thereby adversely impacting self-renewal and therapeutic potential. This has implications when considering the age and comorbidities of patients and donors. For example, dysfunctional stem cell niches

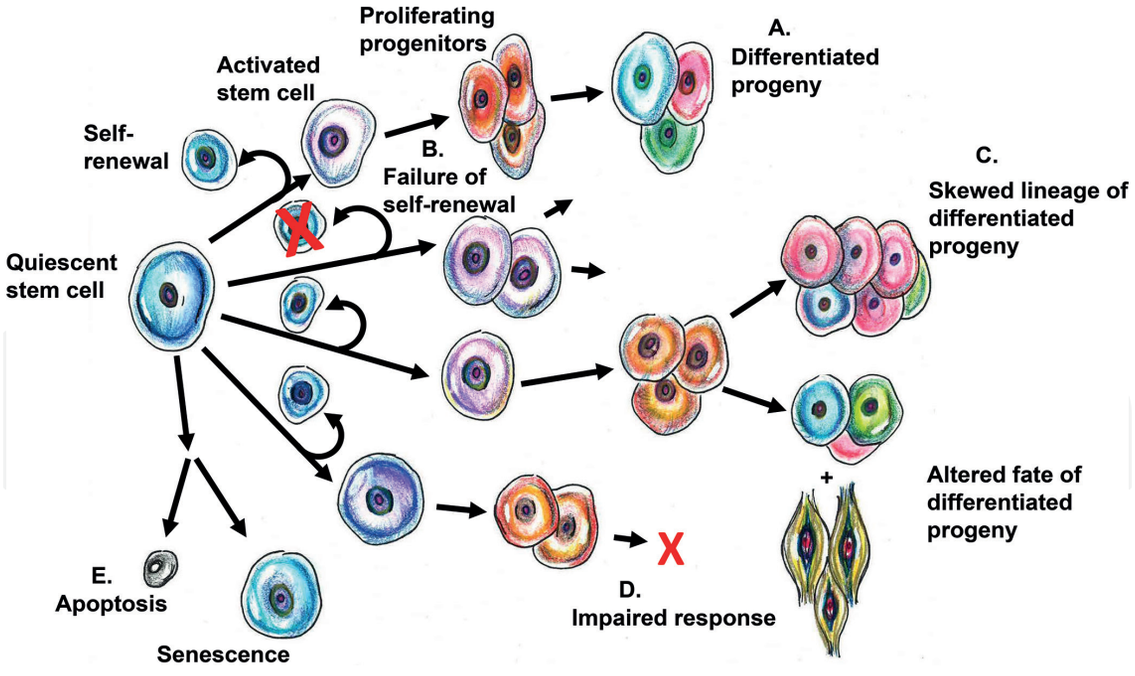

Figure 5. Proposed mechanisms of aging-induced stem cell dysfunction. (A). Normal stem cell function involves activation of a quiescent stem cell to divide asymmetrically giving rise to a new stem cell (self-renewal) and another daughter cell that undergoes proliferation and differentiation. (B). Failure of self-renewal involves differentiation of both daughter cells, leading to a gradual depletion of the stem cell pool. (C). Aberrant differentiation may result from the abnormal skewing of the distribution of progeny toward one fate instead of various potential fates. Another potential mechanism involves the daughter cells acquiring abnormal fates that are not part of the normal repertoire. (D). Impaired stem cell response may be due to a decline or impairment in extrinsic or intrinsic signals. (E). Senescence and apoptosis of the quiescent stem cell or among the progeny following activation has also been described in aging. Adapted from Jones DL et al., Nature Cell Biology, 2011.

have been implicated in the aging frailty syndrome, which is characterized by decreased strength, endurance, physiologic function, and reserve capacity in multiple organ systems [122, 123]. Moreover, aging, renal failure, C-reactive protein (CRP) levels, and other adverse health parameters have been shown to correlate significantly with poor angiogenic potency of bone marrow stem cells [105, 124]. These studies suggest that the therapeutic potential of autologous MSCs obtained from patients may be limited, whereas more robust repair and regeneration would occur by using allogeneic MSCs from young, healthy donors. Indeed, two clinical trials in patients with ischemic and dilated cardiomyopathy, respectively, compared autologous to allogeneic MSCs and found that although both provided benefits in cardiac structural endpoints, the allogeneic MSCs provided greater cardiovascular functional benefits [5, 7, 81]. On the other hand, a study on the impact of recipient age on the efficacy of MSC therapy found that older (>60 years of age) patients responded just as effectively as younger (<60 years of age) patients when administered either autologous or allogeneic MSC therapy for chronic ischemic cardiomyopathy [125]. This finding is highly significant since the majority of the population with cardiovascular disease requiring cell-based therapy is aged.

Although the evidence is conflicting [126–130], clinical trials of MSC therapy usually exclude patients with a history of cancer due to concerns regarding the MSCs' potential for carcinogenesis. It remains unclear whether MSCs have the potential to undergo spontaneous malignant transformation and/or whether they interact with surrounding tumor stromal elements [129–131]. Spontaneous malignant transformation of human bone marrow-derived MSCs has been shown in vitro during long-term cultures [127]. These MSCs underwent faster proliferation, failed to undergo complete differentiation, and exhibited altered morphology and phenotype. Moreover, when these altered MSCs were administered to immunodeficient mice rapid-growing tumors throughout the lung tissue were found. On the other hand, in a separate study [128], human bone marrow-derived MSCs were grown in culture and assessed at different time points for expression of various tumor-related proteins until they reached senescence or passage 25. A progressive decrease in proliferative capacity with shortened telomeres was observed in most cultured MSCs until they reached senescence. In addition, the MSCs did not express telomerase activity or telomerase reverse transcriptase transcripts, and no chromosomal abnormalities or alternative lengthening of telomeres were observed, supporting the safety of in vitro MSC expansion, and therapeutic use. Despite these encouraging findings, the functional, phenotypic, and genetic characterization of culture-expanded MSCs merits further careful study [129, 131, 132]. In addition, recent findings indicate that various direct (e.g., cell fusion) and indirect (e.g., exosome or vesicle-mediated) interactions between MSCs and cancer cells can produce functional interference and/or mutual acquisition of new cellular properties [130]. These functional and phenotypic cellular alterations can lead to changes in metastatic behavior and induce new cancer stem cell development. On the other hand, exosomes and vesicle-mediated mechanisms may be a promising therapeutic tool against cancer.

2.7. Sex differences in MSCs

Sex differences exist in many disease states as well as with respect to the role of MSCs in organ repair and regeneration after injury. There is evidence that female MSCs exhibit decreased apoptosis, interleukin-6, and tumor necrosis factor and increased endothelial growth factor and vascular endothelial growth factor expression compared to male donor MSCs [133].

Furthermore, in a mouse model of myocardial infarction, treatment with female MSCs produced greater improvement of cardiac functional endpoints than treatment with male MSCs [134]. Estradiol has been shown to contribute to these differences [135, 136]. A more complete understanding of how MSCs are influenced by donor sex and recipient hormonal environment is needed to address sex-related disparities in clinical outcomes as well as to optimize transplanted MSC function and survival.

3. MSCs as a regenerative therapeutic for cardiovascular diseases

The hypothesis that exogenously delivered stem cells would promote organ regeneration through transdifferentiation into tissue-specific cells sparked interest in stem cell research and cell-based therapy and was originally supported by studies in the heart [82] where MSCs become cardiomyocyte-like cells and endothelial cells [41, 43]. However, subsequent studies have revealed that the MSC-mediated cardiac regenerative process is more complex than was initially envisioned (**Figure 6**).

3.1. Clinical trials in cardiac disease

Multiple clinical trials suggest that MSCs can ameliorate left ventricular remodeling and improve cardiac function in patients with acute and chronic ischemic cardiomyopathy [7, 9, 11, 72, 84, 115, 116, 137–141]. The Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy (TAC-HFT) trial demonstrated reverse remodeling and improved regional contractility of the scar as well as improved functional capacity and quality of life over 1 year in patients with chronic ischemic cardiomyopathy treated with transendocardial injection of autologous bone marrow-derived MSCs versus bone marrow mononuclear cells or placebo [9, 142]. The mesenchymal stromal cells in chronic ischemic Heart Failure (MSC-HF) trial showed that intramyocardial injection of autologous bone marrow-derived MSCs in patients with severe ischemic cardiomyopathy improved ventricular function and myocardial mass [140]. The same group showed that intramyocardial delivery of autologous MSCs into patients with coronary heart disease and refractory angina provided a sustained effect (3-year follow-up) in improving exercise capacity and ventricular function, and reducing hospitalization rates and revascularizations [143]. As mentioned previously, the CHART-1 study also demonstrated the anti-remodeling properties of cardiopoietic MSCs at the 1-year follow-up [72]. Encouraging results from preclinical studies with combination therapy [28, 79] have led to the initiation of the CONCERT-HF (NCT02501811) trial by the Cardiovascular Cell Therapy Research Network (CCTRN) in an effort to examine the effects of the transendocardial delivery of a combination of autologous bone marrow-derived MSCs and cardiac progenitor cells into patients with ischemic cardiomyopathy.

Autologous adipose tissue-derived MSCs are also undergoing investigation in the cardiovascular field. The adipose-derived stromal cells for treatment of patients with chronic ischemic heart disease (MyStromalCell) trial was a phase II, first-in-man, single-center, double-blind, randomized, and placebo-controlled study of intramyocardial injections of autologous adipose-derived MSCs in patients with chronic ischemic heart disease and refractory angina but preserved ejection fraction [111, 112]. The MSCs were obtained from abdominal adipose tissue,

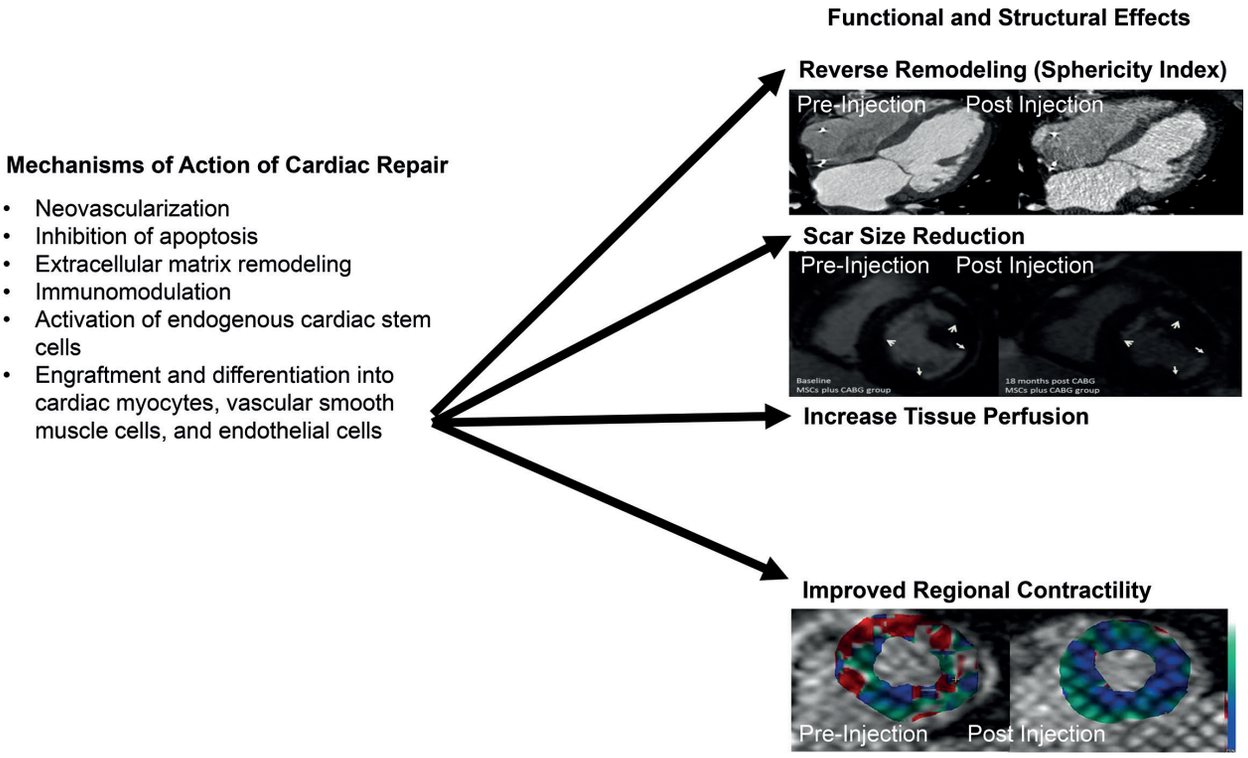

Figure 6. Effects Of mesenchymal stem cell therapy in heart disease.

culture-expanded in vitro and stimulated with vascular endothelial growth factor-A (VEGF-A) (165) the week before treatment. The six month follow-up results demonstrated safety, and although a significant increase in exercise capacity was observed in the patients treated with the MSCs but not with placebo, there was no statistically significant difference between the MSC and placebo treatment groups.

An important issue in this new field is whether MSCs can be used as an allograft [5, 7, 89], avoiding the need for bone marrow aspiration of patients and tissue culture delays prior to treatment. Furthermore, the function of autologous MSCs may be impaired in patients with comorbidities and/or advanced age [101–104]. A meta-analysis of 82 preclinical studies [144] demonstrated that allogeneic therapy is safe and at least as effective as autologous MSC therapy, suggesting that allogeneic MSCs are characteristically immunomodulatory, as discussed above.

The therapeutic benefit of allogeneic MSCs versus placebo delivered intravenously has been investigated in patients after acute MI [11, 145, 146]. Not only did these results show the safety of allogeneic MSC delivery to humans, but also moreover, echocardiography demonstrated a 6% increase in ejection fraction at 3 months for patients treated with MSCs. Moreover, the percutaneous stem cell injection delivery effects on neo-myogenesis (POSEIDON) trial compared allogeneic vs. autologous MSCs delivered by transendocardial stem cell injection in patients with chronic ischemic cardiomyopathy and showed that both MSC types are safe and clinically effective [7, 147]. Similarly, the percutaneous stem cell injection delivery effects on neo-myogenesis – dilated cardiomyopathy (POSEIDON-DCM) trial demonstrated safety and efficacy of transendocardial autologous vs. allogeneic MSC therapy in patients with non-ischemic, dilated cardiomyopathy, with a cardiac function efficacy preference toward allogeneic MSCs [5].

The transendocardial stem cell injection delivery effects on neomyogenesis study (TRIDENT) trial compared the safety and efficacy of two doses (20 million and 100 million) of allogeneic bone marrow-derived human MSCs delivered transendocardially in patients with ischemic cardiomyopathy [116]. Although both cell doses reduced scar size, only the 100 million doses increased LVEF, highlighting the crucial role of cell dose in the responses to cell therapy. In phase 2 dose-escalation study investigating immunoselected (Stro-1/Stro-3^+ enriched), allogeneic bone marrow-derived MPCs (25, 75, and 150 million cells) delivered transendocardially in patients with ischemic and non-ischemic heart failure, no differences were observed in LVEF at 12 months of follow-up, although the 150 million MPC group had a significant reduction in left ventricular end-systolic and end-diastolic volumes, a measure of reverse remodeling, at 6 months and a non-significant decrease of both ventricular volumes at 12 months [56]. These and other ongoing studies determining the optimal dose and delivery are essential to advance the field, decipher mechanism(s) of action, and enhance planning of pivotal Phase III trials [148–152].

A recent trial assessed the safety and preliminary efficacy of intravenously administered, allogeneic, ischemia-tolerant MSCs in patients with non-ischemic cardiomyopathy [153]. Ischemia-tolerant MSCs are grown under chronic hypoxic conditions and have been shown to better migrate toward wound healing-related cytokines and cytokines found in ischemic tissues and express higher levels of hypoxia-inducible factor-1 [154]. These studies suggested that ischemia-tolerant MSCs may be therapeutically more effective than MSCs grown under normoxic conditions. An increase in LVEF and reductions in end-systolic and end-diastolic volumes were observed at three months of follow up in the treated group but was not significantly different from the placebo group. Functional capacity and health status were significantly improved in the MSC treated group compared to placebo.

MSCs derived from umbilical cord (UC-MSCs) have also been tested in patients with heart failure. The randomized clinical trial of intravenous infusion umbilical cord mesenchymal stem cells on cardiopathy (RIMECARD) trial is a randomized, double-blind, placebo-controlled trial that evaluated the safety and efficacy of UC-MSCs administered intravenously in patients with heart failure of ischemic or non-ischemic origin [141]. Infusion of allogeneic UC-MSCs was safe, with no development of alloantigen directed antibodies post-infusion, and effective in improving LVEF, functional status, and quality of life. Intramyocardial delivery of UC-MSCs in patients with heart failure has also been shown to produce improvements in LVEF and end-systolic volume in patients with severe heart failure [155].

Ongoing clinical trials are assessing the safety and efficacy of allogeneic MSC therapy in patients with acute myocardial infarction, chronic ischemic and non-ischemic cardiomyopathy, and left ventricular assist devices. These studies will continue to pave the way for the development of allogeneic cell-based regenerative therapies for structural and functional disorders of the myocardium. The results from cardiovascular stem cell clinical trials are so far promising, with recent trials highlighting the vast therapeutic potential of allogeneic over autologous stem cells. However, many challenges remain, such as addressing long-term safety, serial stem cell injections, and optimal cell type, dose, and delivery route [148–152].

3.2. Vascular disease

Endothelial dysfunction is characterized by impaired endothelial vasodilation, a proinflammatory and prothrombotic state, and impaired bioactivity of EPCs and contributes to the

pathophysiology of most forms of cardiovascular disease, including hypertension, coronary artery disease, heart failure, peripheral vascular disease, kidney disease, diabetes mellitus, and metabolic syndrome [156, 157]. Endothelial function is implicated in heart failure [158] and we have studied the therapeutic potential of MSCs in restoring endothelial function in patients with ischemic and non-ischemic cardiomyopathy [81]. As mentioned above, individuals with heart failure received either autologous or allogeneic MSCs, and those in the allogeneic MSC group exhibit increased EPC colony formation and improved flow-mediated vasodilation (FMD), both of which strongly correlate with improved endothelial function [158, 159] (**Figure 7**). Moreover, patients who received allogeneic MSCs had reduced levels of VEGF. Elevated VEGF is associated with heart failure progression [160]. The concordant restitution of these parameters to near normal after allogeneic MSC therapy has significant clinical implications for the heart failure population and may play a critical role in the advancement of cardiovascular treatment modalities.

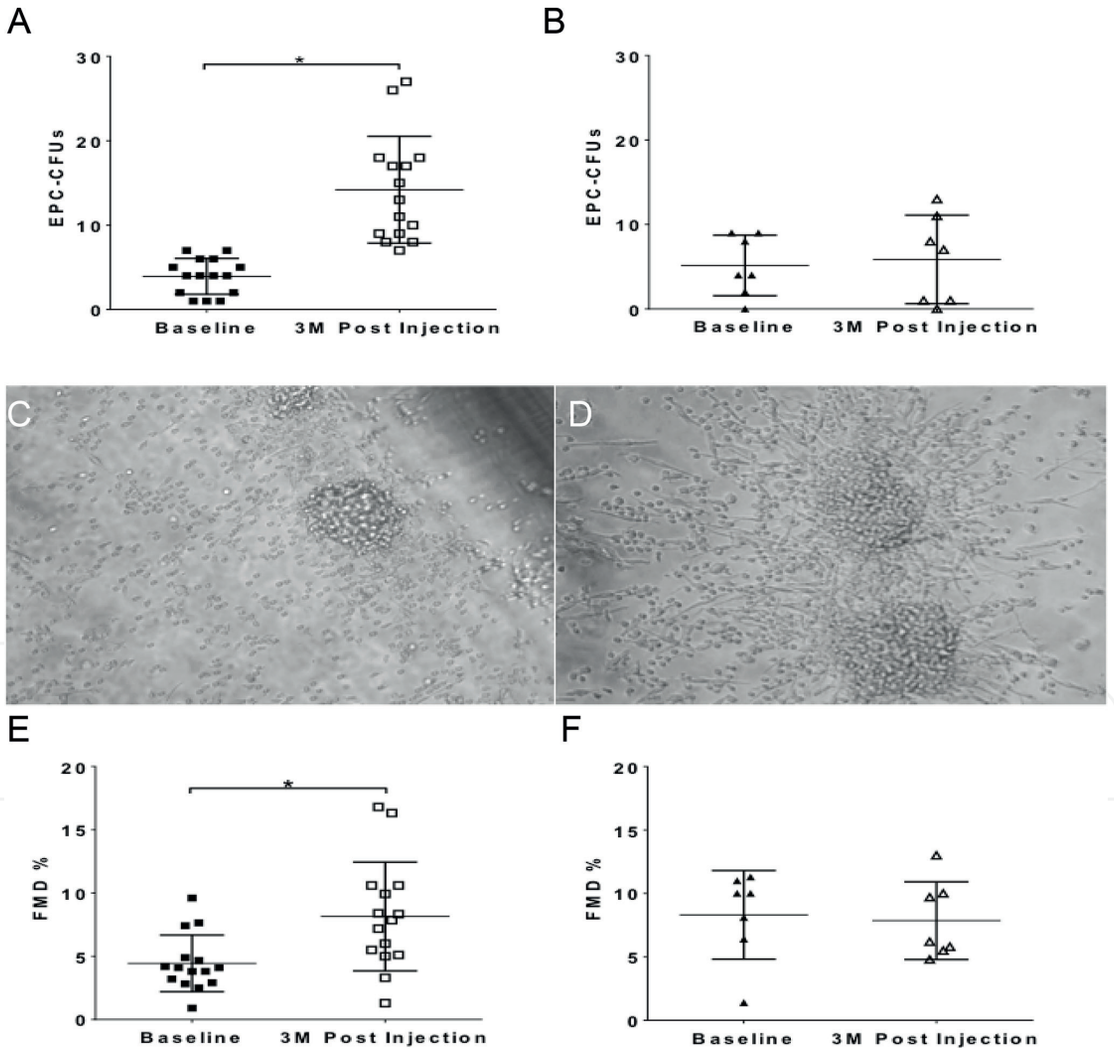

Figure 7. MSCs in vascular disease. Allogeneic mesenchymal stem cell therapy can help restore endothelial function in patients with cardiomyopathy by increasing EPC CFUs (A) and improving FMD (E) when compared to autologous therapy (B and F). Representative EPC-CFUs plated on fibronectin for 5 days before (C) and after (D) allogeneic MSC administration (magnification 20x). *Reproduced from Premer C et al., EBioMed, 2015.*

It is well established that cardiovascular disease is the leading cause of death and disability among people with type 2 diabetes mellitus [161] and has long been appreciated that endothelial dysfunction underlies the high rates of cardiovascular disease associated with long-term diabetes [162]. The persistent hyperglycemia and other metabolic abnormalities directly affect the endothelium, contributing to the pathophysiology of disease [163]. Based on our findings of improved endothelial function after allogeneic MSC treatment in patients with heart failure [81], we are conducting a clinical trial entitled, Allogeneic Mesenchymal Human Stem Cells Infusion Therapy for Endothelial Dysfunction in Diabetic Subjects (ACESO; NCT02886884) to investigate whether intravenously delivered MSCs restore endothelial function parameters, including FMD and EPC function, as well as decrease circulating inflammatory markers and improve clinical parameters of diabetes. Similarly, the Intravenous Infusion of Umbilical Cord Tissue (UC) Derived Mesenchymal Stem Cells (MSCs) Versus Bone Marrow (BM) Derived MSCs to Evaluate Cytokine Suppression in Patients With Chronic Inflammation Due to Metabolic Syndrome (CERES; NCT03059355) trial is testing MSC therapies to restore endothelial function.

Peripheral artery disease is generally caused by atherosclerosis in which cholesterol plaque builds up, ultimately weakening blood vessel walls and restricting blood flow, severely impairing endothelial function. The evaluation of cell therapy on exercise performance and limb perfusion in peripheral artery disease: The CCTRN patients with intermittent claudication injected with ALDH bright cells (PACE) Trial demonstrated safety but no improvement in peak walking time or capillary perfusion [164]. In patients with complete occlusion of femoral arteries, a post-hoc exploratory analysis suggested an improvement in the number of collateral arteries. Future clinical trials testing different cell types, doses, and administration routes are needed to optimize peripheral artery disease treatment.

4. MSCs as immunomodulatory, anti-Inflammatory, anti-fibrotic, and anti-rejection therapy

MSCs exhibit immune-privileged properties in vitro and in vivo [165] likely due to the absence of MHC II, B-7 costimulatory molecule, and CD40 ligand [90–92, 166] (**Figure 8**). The lack of costimulatory molecules prevents T-cell responses and also induces an immunosuppressive local microenvironment through the production of prostaglandins and other soluble mediators including nitric oxide, indoleamine 2,3-dioxygenase, and heme oxygenase-1 [92, 167–170]. MSCs reduce the respiratory burst that follows neutrophilic responses by releasing interleukin (IL)-6 [171]. They also inhibit the differentiation of immature monocytes into dendritic cells hence the antigen presentation to naïve T cells is greatly impaired [172]. In addition, MSCs release soluble factors, such as hepatocyte growth factor and transforming growth factor (TGF)-β1 [173], that suppress the proliferation of cytotoxic and helper T-(Th) cells. MSCs also stimulate Foxp3+ regulatory T cells with concurrent suppression of Th1, Th2, or Th17 responses [174]. These findings suggest that MSCs are an effective therapeutic strategy to induce tolerance in solid organ transplantation [175].

4.1. Transplantation

Le Blanc *et al.* first reported the clinical immunoregulatory response to MSCs in a case of severe, treatment-resistant grade IV acute graft-vs-host disease (GVHD) [13]. A multicenter phase 2 trials for steroid-resistant, severe acute GVHD confirmed this observation [12] and MSCs obtained from HLA-identical siblings, haploidentical third-party donors, or HLA-mismatched third-party donors were similarly effective. Recently, infusion of MSCs the day of hematopoietic cell transplantation (HCT) promotes engraftment and improves outcomes. A pilot study of allogeneic MSC infusion before nonmyeloablative HCT from HLA-mismatched donors showed sustained engraftment in 19 out of 20 patients, and the 1 year incidence of nonrelapse mortality, relapse, overall survival, progression-free survival, and death from GVHD was favorable compared to a historic control group [176]. In another pilot study evaluated the effect of infusion of MSCs at the time of dual transplant of cord blood and third-party donor mobilized hematopoietic stem cells regarding tolerance, cord blood engraftment, and effects on acute GVHD, both preventive and therapeutic [177]. MSC infusions were effective for treating severe acute GVHD, but no significant differences in cord blood engraftment and incidence of severe acute GVHD were observed. Although there is accumulating evidence of safety from these small pilot studies [96], randomized trials are necessary to establish efficacy.

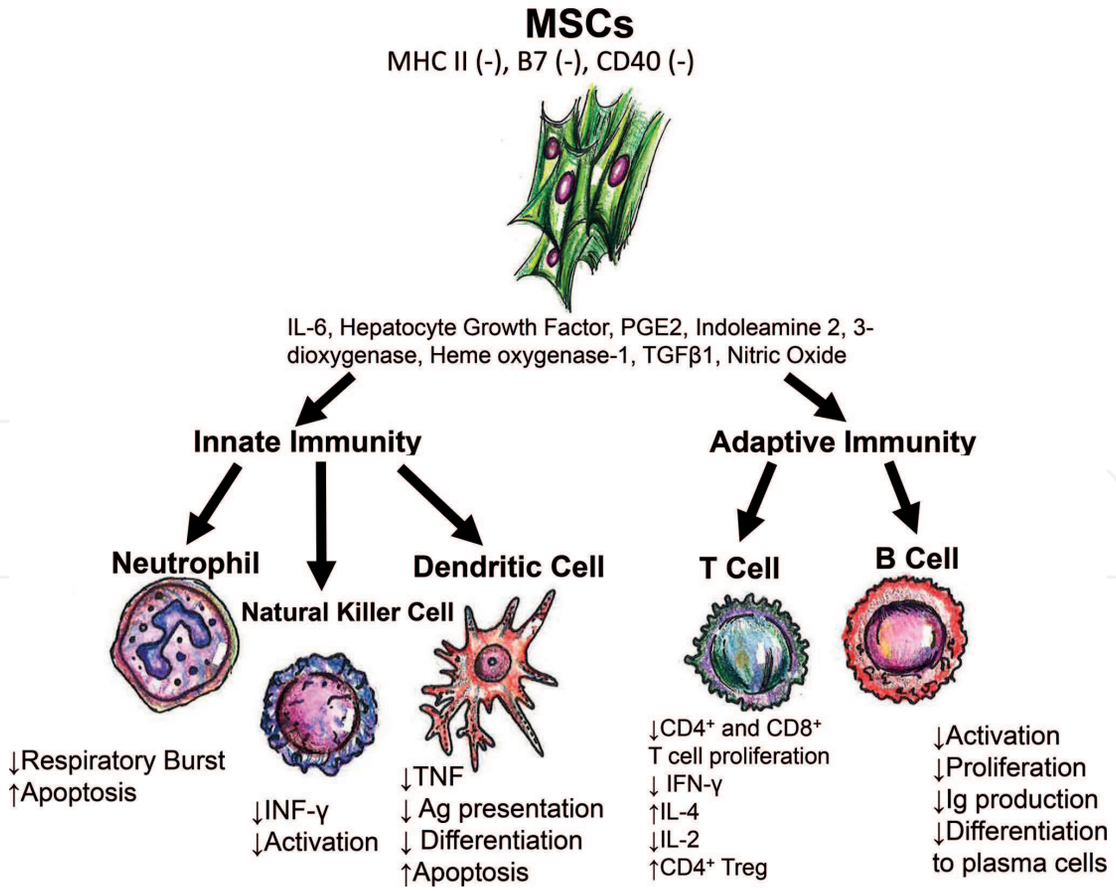

Figure 8. Immunomodulatory effects of mesenchymal stem cells. MSCs are immunoprivileged cells that inhibit both innate (neutrophils, dendritic cells, and natural killer cells) and adaptive (T cells and B cells) immune cells.

A single-site, open-label, randomized controlled clinical trial in 159 patients undergoing living-related donor kidney transplantation showed that induction therapy with autologous MSCs resulted in lower incidence of acute rejection, decreased the risk of opportunistic infection, and better estimated renal graft function at 6 months compared with anti-IL-2 receptor antibody induction therapy [16]. However, graft function and rejection rates were similar after 1 year [178]. Therefore, MSC therapy can safely replace induction immunotherapy, reducing opportunistic infections, without compromising graft function and survival [179].

Despite these encouraging results, the long-term safety of MSC transplants needs to be further investigated in chronically immunosuppressed patients that are at increased risk for opportunistic infections and tumors [132, 180]. In this regard, a clinical trial evaluated the safety and tolerability of third party MSC administration after liver transplantation. Patients enrolled in the experimental arm were infused with a single dose of 1.5 million MSCs/kg, 3(±2) days after the liver transplantation [181]. There was no impairment in liver transplant function and no increased rate of opportunistic infection or new cancer detected following MSC infusion. In addition, there was no difference in overall rates of rejection or graft survival. Weaning of immunosuppression in MSC recipients was not successful.

Issues needing further investigation include dose, timing and site of administration, interaction with immunosuppressive drugs, and whether MSCs are effective at preventing acute rejection and/or inducing tolerance. In a murine kidney transplant model, it was shown that MSC administration before (day -1) but not a few days after kidney transplantation avoided the acute deterioration of graft function while maintaining the immunomodulatory effect of MSCs [182]. Moreover, a clinical study found that autologous bone marrow-derived MSC infusion at day 7 post-kidney transplant induced acute kidney graft dysfunction, attributed to engraftment syndrome [183], although MSC infusion was associated with lower memory/effector CD8+ T cells, expansion of CD4+ regulatory T cells, and reduction of donor-specific CD8+ T-cell cytotoxicity compared with control kidney transplant recipients given the same induction therapy (basiliximab/low dose thymoglobulin) but not MSCs [184].

Islet cell transplantation combined with MSC therapy for type 1 diabetes in a cynomolgus monkey model provides clinical evidence for the anti-rejection effect of MSCs [185]. MSC treatment significantly enhanced islet engraftment and functions one month post-transplant, compared with animals receiving islets without MSCs. In addition, infusions of donor or third-party MSCs resulted in a reversal of rejection episodes and prolongation of islet function. Stable islet allograft function was associated with increased numbers of regulatory T cells in peripheral blood, suggesting that MSCs enhance islet engraftment, thereby decreasing the numbers of islets needed to achieve insulin independence.

4.2. Autoimmune diseases

Autologous MSC transplantation evaluated in clinical trials of amyotrophic lateral sclerosis [18] and multiple sclerosis [17, 186] is safe and associated with increased proportion of $CD4^+$ $CD25^+$ regulatory T cells, decreased proliferative responses of lymphocytes, and lower expression of co-stimulatory molecules ($CD40^+$, $CD83^+$, and $CD86^+$), and HLA-DR on myeloid dendritic cells within 24 hours of transplantation [17]. In a randomized, placebo-controlled, phase 2 trial of multiple

sclerosis, bone marrow-derived MSCs were also found to reduce inflammatory MRI parameters, supporting their anti-inflammatory and immunomodulatory properties [187]. Moreover, autologous and allogeneic MSC therapy showed evidence of benefit in other autoimmune disorders such as refractory Crohn's disease [188–191] and systemic lupus erythematosus [14, 192, 193], respectively. Although there are no clinical trial results in patients with rheumatoid arthritis (clinical trials are ongoing; NCT01851070), in vitro studies show that allogeneic MSCs or MSC-differentiated chondrocytes inhibit the proliferation and activation of collagen type II-stimulated T-cells and the secretion of proinflammatory cytokines, including IFN-gamma and TNF-alpha by CD4+ and CD8+ T cells, while increasing the secretion of IL-10 and restoring the secretion of IL-4 [194, 195]. These results suggest that the immunomodulatory and anti-inflammatory effects of MSCs offers an effective therapeutic modality for arthritic diseases [195], and several clinical trials are ongoing evaluating bone marrow, adipose, and UC-derived MSCs.

Transplanted MSCs exert a protective effect in type 1 diabetes mellitus [196]. MSCs localize to the pancreas after intravenous transplantation and lower blood sugar levels [197], similar to MSCs isolated from the Wharton's jelly of the umbilical cord, which differentiated into mature islet-like cell clusters and possessed insulin-producing ability in vitro and in vivo [198]. Transplanted MSCs lower blood sugar through secretion of trophic cytokines that promote endogenous pancreatic stem cells in the ductal epithelium to differentiate into new ß-cells and directly differentiate into functionally competent, new ß-cells [199]. Furthermore, MSCs produce a variety of cytokines and growth factors, which could promote survival of surrounding cells and improve the microenvironment of pancreas [200]. Based on these findings, clinical trials have been initiated to test safety and therapeutic efficacy. A pilot, randomized, controlled, and open-label trial investigated the potential benefits on metabolic control and safety of combined umbilical cord-derived MSCs and autologous bone marrow mononuclear cell transplantation without immunotherapy in patients with established type 1 diabetes [201]. The treatment was not only well tolerated, but at 1 year, metabolic measures, including hemoglobin A1C, fasting glycemia, and daily insulin requirements, improved in the treated patients, whereas it decreased in control subjects. In another clinical study, treatment with a single intravenous infusion of autologous MSCs was tested in new-onset type 1 diabetic patients and found to be safe and to show benefit in slowing disease progression and preserving β-cell function [202].

4.3. Pulmonary diseases

A recent randomized, double-blinded, placebo-controlled study demonstrated the safety of systemic administration of allogeneic MSCs in patients with moderate to severe chronic obstructive pulmonary disease (COPD) [15], however, there were no differences in the frequency of COPD exacerbations, pulmonary function tests, or quality of life after 2 years of follow up. A significant decrease in levels of circulating C-reactive protein (CRP) was observed in MSC-treated patients who had elevated CRP levels at study entry, suggesting a beneficial effect of MSC infusion on systemic inflammation [15].

Idiopathic Pulmonary Fibrosis (IPF) is a lung disease characterized by progressive interstitial fibrosis leading to hypoxemic respiratory failure for which no effective treatment exists [203]. Histologically, there is evidence of alveolar epithelial cell injury, interstitial inflammation,

fibroblast proliferation, and extracellular matrix collagen deposition. Because MSCs home to sites of injury, inhibit inflammation and contribute to epithelial tissue repair, they offer a potential therapy for IPF [203]. The phase 1 clinical trial entitled allogeneic human mesenchymal stem cells in patients with IPF via intravenous delivery (AETHER) demonstrated the safety of bone marrow-derived MSCs in nine patients with mild to moderate IPF [10]. A 3.0% mean decline in percent predicted forced vital capacity, and 5.4% mean decline in percent predicted diffusing capacity of the lungs for carbon monoxide was observed by 60 weeks post-MSC infusion, suggesting potential for efficacy.

Of note, a study has provided evidence of a resident c-kit+ multi-potent stem cell in the human lung [204]. These lung c-kit+ stem cells were shown to have the capacity to develop into bronchioles, alveoli, and pulmonary vessels, supporting the notion that they play an important role in lung homeostasis and tissue regeneration after injury. Although the therapeutic implications of these findings have not been investigated, we can infer from findings in ischemic heart disease models that there is the potential for MSCs to stimulate endogenous c-kit+ lung stem cell proliferation and differentiation, thereby facilitating lung tissue repair and regeneration.

4.4. Cutaneous wounds

Chronic, non-healing cutaneous wounds are a major cause of morbidity. The ability of MSCs to differentiate into various cell types and their capacity to secrete factors important in accelerating wound healing have made cell therapy a promising strategy for tissue repair and regeneration [24, 205]. Although both autologous and allogeneic MSCs appear to be well suited as wound healing therapies, allogeneic MSCs derived from young healthy donors may have an advantage over autologous sources where age and systemic comorbidities, such as diabetes, chronic renal failure, and arterial or venous insufficiency, are a contributing factor. The effects of aging and systemic illness on MSCs include impaired cell migration, reduced growth factor production, and poor tissue remodeling [24]. A study evaluated MSCs and fibroblasts derived from normal donors and chronic wound patients to characterize the induction of mobilization when these cells are mixed as well as examine the effect of soluble factors on fibroblast migration [206]. These studies showed that MSCs participate in skin wound closure by affecting dermal fibroblast migration in a dose-dependent manner, but impairments were noted in chronic wound patient fibroblasts and MSCs as compared with those derived from normal donors. These results support the notion that allogeneic MSCs from "healthy" donors provide greater efficacy for wound healing compared to autologous MSCs. Such promising findings have supported the use of MSCs in animal models of burn wound healing [207–209]. Consequently, a clinical trial entitled "Stem Cell Therapy to Improve Burn Wound Healing" (NCT02104713) is currently underway and is examining the efficacy of allogeneic MSCs in burn wound closure for patients with a 2nd degree burn wounds of less than 20% total body surface area.

4.5. Neurological diseases

MSCs are also considered a promising therapeutic strategy for acute injury and progressive degenerative diseases of the central nervous system [210], such as spinal cord injury [211, 212] ischemic stroke [21, 22, 213, 214] Parkinson's disease [215, 216] traumatic brain injury [217, 218] multiple sclerosis [17, 186, 219, 220] and multiple system atrophy [23]. Studies suggest that the

neuroprotective effect of MSCs is mediated by the production of various trophic factors, including brain-derived neurotrophic factors, nerve growth factor, and insulin-like growth factor-1, which contribute to recovering neurobehavioral function and stimulating endogenous regeneration [210, 212, 221]. In addition, MSCs home to injured brain tissues and exert immunoregulatory properties, reduce apoptosis, and improve neuronal cell survival [215, 217, 221]. However, it is unclear if MSCs differentiate into neural cells in vivo [210, 212].

4.6. Liver diseases

The anti-fibrotic properties of MSCs may exert therapeutic effects in liver regeneration and disease. MSCs inhibit activated fibrogenic cells such as hepatic stellate cells [222]. Numerous preclinical studies on bone marrow [223–225]. adipose tissue [226], and UC-derived [227] MSC treatment for improvement of liver fibrosis have been conducted and have reported reductions in liver fibrosis as well as improvements in hepatic function. Indeed, MSC based therapies for patients with end-stage liver disease, have shown promise in phaseIand II clinical trials [19, 20, 228]. MSC transplantation was safe and well-tolerated and hepatic function improved in patients with liver fibrosis [20]. Moreover, the biochemical hepatic index and model for end-stage liver disease (MELD) score were markedly improved from 2 to 3 weeks post transplantation [19]. However, the long-term hepatic function was not significantly enhanced in patients with liver failure caused by hepatitis B [19]. Notably, many of these clinical trials differ in MSC source, and liver pathology [229–232] and perhaps certain type of MSCs may serve as better therapeutic options for specific liver pathologies. These early stage studies and more recent clinical trials suggest that MSC transplantation is safe and may confer benefit to patients with liver cirrhosis and various kinds of liver diseases [233].

4.7. Aging frailty

Frailty is a medical syndrome that increases in prevalence with age and augments the risk for adverse health outcomes, including mortality, hospitalization, fall, and institutionalization. Markers of frailty include age-associated declines in lean body mass, strength, endurance, balance, walking performance, and activity; and are accompanied by declines in physiologic reserve in most organ systems. Together, these symptoms lead to the loss of homeostasis and the capability to withstand stressors and resulting vulnerabilities. Notably, there is a robust correlation between frailty and biomarkers of inflammation. There is also evidence that endogenous stem cell production decreases with age, likely contributing to reduce ability to regenerate and repair organs and tissues. Therefore, a regenerative treatment strategy could ameliorate signs and symptoms of aging frailty. Currently, there are no approved treatments for frail patients and therefore no established standard of care. There are specific features of the frailty syndrome that support the hypothesis that MSCs will also ameliorate or improve frailty. Indeed, in a pilot study and subsequently in a randomized, double-blind, dose-finding study, we demonstrated safety of intravenous infusion of allogeneic MSCs into elderly, frail individuals and found significant improvements in physical performance measures and inflammatory biomarkers [6, 234–235]. These findings suggest that frailty can ultimately be prevented or attenuated, and the link between frailty and inflammation offers a potential therapeutic target, addressable by cell therapy

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5. Conclusions

The promising cell-based therapy field has exploded in the past decade and currently, MSCs from various sources, mainly bone marrow and adipose-derived, are being evaluated in phase I and II trials for a myriad of chronic, disabling disorders with no currently effective therapies. Although preclinical studies provide mechanistic insights into therapeutic effects of MSCs and phase I/II studies provide evidence of safety in the short-term, questions regarding most effective dose, route of administration, interaction with other concurrent therapies, sustainability/durability of effect, and adverse effects, including opportunistic infections and tumor development or progression, remain to be resolved. Addressing these questions will require rigorously conducted, multicenter clinical trials with well-defined clinical outcomes, longer duration of follow up, and more patients [151, 236].

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Disclosures

JMH reported having a patent for cardiac cell-based therapy. He holds equity in Vestion Inc. and maintains a professional relationship with Vestion Inc. as a consultant and member of the Board of Directors and Scientific Advisory Board. JMH is the Chief Scientific Officer, a compensated consultant and advisory board member for Longeveron, and holds equity in Longeveron. JMH is also the co-inventor of intellectual property licensed to Longeveron. Longeveron LLC and Vestion Inc. did not participate in funding this work. The other author-reports no conflicts.

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