Mending a broken heart: can gene modulation bolster therapeutic performance of adult stem cells?

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Myocardial infarction and the congestive heart failure (HF) that follows are poised to be the leading cause of death in the modern world. Although advances in technology and patient management have helped reduce the mortality rate, the ability to sustain long-term recovery of the heart post-infarction is nearly impossible. This is mainly due to the inability of the adult heart to repair the infarcted region as it has limited regenerative capacity. The implementation of adult stem cells (ASCs) for cardiac therapy has been associated with improvements in cardiac function in several preclinical HF models, however, their effectiveness in the clinical setting has been inconsistent with several large clinical trials reporting disappointing outcomes. To enhance the effectiveness of ASCs, the incorporation of transgenes that bolster their homing and engraftment, anti-apoptotic, anti-inflammation, and pro-angiogenic properties has been proposed and in support, recent findings show transgenic ASCs to be more effective than their naïve counterpart by attenuating disease progression and improving cardiac recovery. Moreover, in addition to improving the therapeutic performance of ASCs, gene modulation provides insight on the fundamental mechanisms underlying the effectiveness of the respective therapy. Here, we review the current status on ASC therapy for cardiac repair and discuss the implementation of transgenic ASCs for restoring the failing heart and improving clinical outcomes.

Keywords: Heart failure, Adult stem cells, Regeneration, Paracrine effects, Induced pluripotent stem cells (iPSCs), Bioengineering

Introduction

Congestive heart failure (HF) due to myocardial infarction (MI) is the leading cause of mortality and morbidity worldwide. Currently, an estimated 64.3 million people have been diagnosed with HF worldwide, and the incidence of HF ranges between 1 to 9 cases per 1000 person-years among Europe and the United States (Groenewegen et al., 2020). Coronary vessel occlusion (and MI) triggers a hypoxic environment that induces cardiomyocyte death via necrosis or apoptosis, resulting in irreversible damage to the myocardium. On the cellular level, ~26% of the 2-4 billion cardiomyocytes that are present in the human left ventricle (LV) could undergo apoptosis in just a few days following MI (Abbate et al., 2002; Ramachandra et al., 2020). The inflammation that ensues leads to the elimination of non-viable cardiomyocytes, which in turn causes fibrosis that replaces the damaged myocardium with a fibrous scar, comprising several extracellular matrix (ECM) components. This scarring process, though intended to support the weakened LV wall post-MI, renders the heart stiff, resulting in adverse LV remodeling with decreased contractility and increased workload on the surviving tissue, leading to HF.

The extremely limited proliferation ability of adult cardiomyocytes makes the heart one of the least regenerative organs in the human body. Though cardiac stem cells have been proposed to reside within the heart, their numbers are not sufficient to replace the damaged myocardium and restore normal heart function (Cruz-Samperio et al., 2021). A coronary artery bypass graft (CABG) could assist in restoring normal

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blood flow to the heart and prolong life-expectancy; however, it does not address the underlying condition, i.e., restoring the damaged myocardium (Konijnenberg et al., 2020). Heart transplantation is the most viable option for serious cases; however, lack of organ donors together with complications associated with immune suppression hinders this approach from being universally adopted. The lack of pharmacological and surgical intervention to restore the damaged myocardium has hence led researchers to find alternate ways to prevent HF and improve cardiac function.

Over the past few decades, adult stem cells (ASCs) have garnered much recognition as they have been implemented in several experimental and clinical studies for HF (Broughton and Sussman, 2018; Desgres and Menasche, 2019). The transplantation of skeletal myoblasts, bone marrow cells, mesenchymal stem cells, hematopoietic stem cells, and cardiac stem cells has shown much promise in several preclinical animal models of HF, where they improve cardiac function by enhancing hemodynamics and attenuate cardiac remodeling by either differentiation, neovascularization, cell fusion, or secretion of paracrine factors (Lancaster et al., 2018; Yang, 2018; Vazir et al., 2019). However, when it comes to myocardium repair and heart function restoration in humans (Banerjee et al., 2018; Ghiroldi et al., 2018; Sterner et al., 2018), ASC therapy has fallen short in providing substantial clinical benefits, despite being proven to be safe for transplantation. To overcome these disappointing findings, studies have focused on (i) the type and number of cells to be delivered, (ii) the timing of delivery, (iii) the route and frequency of administration, and (iv) their beneficial effects (Braunwald, 2018), and though there is a surplus of literature addressing these points, there is not much focus on the use of genetically modified ASCs, which are reported to be more effective than their naïve counterparts (Lionetti and Recchia, 2010). While the major concern over the use of transgenic ASCs is the incorporation of foreign genetic material, the implementation of *ex vivo* gene therapy strategies in clinical trials for other disorders (Evans et al., 2013; Evans and Huard, 2015; Holzinger et al., 2016), together with the disappointing outcomes from naïve ASCs, would suggest that it may be appropriate to consider transgenic ASCs for cardiac therapy. In this review, we highlight the current status of ASC-based cardiac therapy and discuss how transgenic ASCs have been instrumental in improving heart function in various models and why they could be considered for future clinical trials.

The current state of cardiac therapy

During first-generation stem cell clinical trials, skeletal myoblasts, mesenchymal stem cells, mesenchymal precursor cells, bone marrow cells, and hematopoietic stem cells were identified as potential candidates for improving clinical outcomes post-MI (Figure 1).

Skeletal myoblasts

Skeletal myoblasts (SMs) were the first ASC type considered for cardiac therapy as they could be easily obtained from patient muscle biopsies, be generated in large quantities *in vitro*, and demonstrated tolerance to hypoxic conditions. The success of SM therapy in experimental models led to their implementation in the first clinical trial in 2003, where intra-myocardial delivery of SMs into the scarred myocardium of patients with severe ischemic cardiomyopathy during CABG was reported to improve heart function (Menasche et al., 2003). Unexpectedly, SM transplantation was found to increase incidences of arrhythmia, which was attributed to the disruption of electrical coupling of engrafted SMs with resident cardiomyocytes (Fukushima et al., 2008). Spontaneous ventricular tachycardia was also observed in animal models due to the accumulation of SM clusters and immune cells

(Fukushima et al., 2008). Mechanistically, the global down-regulation of connexin 43 (Cx-43) post-transplantation has been proposed as the reason for arrhythmogenesis (Fukushima et al., 2008; Giraud et al., 2010), which has led to a clinical trial implementing SM overexpressing Cx-43 for treating advanced chronic HF (Gwizdala et al., 2017). These transgenic SMs were found to confer improvement in exercise capacity and myocardial viability of injected segments with no indication of significant ventricular arrhythmia, which could be attributed to better mechanical cooperation of injected cells and injured myocardium with subsequent improvements in electrical coupling. A limitation of this approach however, is that though a large number of cells were cultured from the harvested muscle biopsies, gene transfer of Cx-43 to myoblasts through electroporation led to a vast depletion of cells. To overcome such limitations, less strenuous gene delivery techniques need to be considered, if not, patients would need to undergo repetitive therapies with the provision of several biopsies. In summary, though this clinical trial did show the beneficial effects of transgenic SMs, it was underpowered (n = 13) and lacked a control group for comparison, necessitating the need for further studies to be conducted before the effectiveness of transgenic SM for cardiac therapy can be concluded (Gwizdala et al., 2017).

Bone marrow cells

Bone marrow cells (BMCs) include multiple stem cell populations, including mesenchymal stem cells, hematopoietic stem cells, and endothelial progenitor cells. BMCs can be easily obtained under GMP conditions by Ficoll density gradient centrifugation of bone marrow extracts. In animal models of HF, reports regarding the beneficial effects of BMCs are conflicting (Tomita et al., 1999; Perin et al., 2003) and while BMC transplantation has been shown to increase neovascularization, its effect on improving cardiac function remains controversial. In the first human trial, transendocardial BMC delivery seemed to improve ventricular function in patients with ischemic heart disease by improving blood circulation during a 2- and 4-month follow-up (Perin et al., 2003). However, a 6- and 12-month follow-up revealed no significant improvement in ventricular function, despite the patients exhibiting improvements in myocardial perfusion and exercise capacity (Perin et al., 2004). In other clinical studies, BMC transplantation was reported to significantly improve hemodynamics and ventricular function in patients with ischemic and non-ischemic HF (Hendrikx et al., 2006; Schachinger et al., 2006; Seth et al., 2006). Contrary to these findings, improvements in contractility and reduction in scar size were not observed in patients with chronic ischemic heart disease (Ang et al., 2008; Diederichsen et al., 2010; Perin et al., 2012). Similarly, the First Bone Marrow Mononuclear Cell United States Study in Heart Failure (FOCUS-HF) study (a randomized, double-blind study) reported that BMC transplantation had no significant effect on patients with ischemic cardiomyopathy (Perin et al., 2012). Though there are several advantages for using BMCs (e.g., relatively straight-forward isolation procedures, safe for delivery and negligible arrhythmia incidences), their implementation in cardiac therapy has been met with inconsistent and underwhelming results. To be reconsidered as a viable therapeutic option, improvements in BMC engraftment and differentiation potential are warranted.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells of non-hematopoietic origin, that can be isolated from diverse sources (e.g., bone marrow, adipose tissue, placenta, umbilical cord) (Bagno et al., 2018) and can be differentiated into various cell types, including adipocytes, chondrocytes, and osteocytes (Caplan and Dennis, 2006). In the infarcted myocardium they
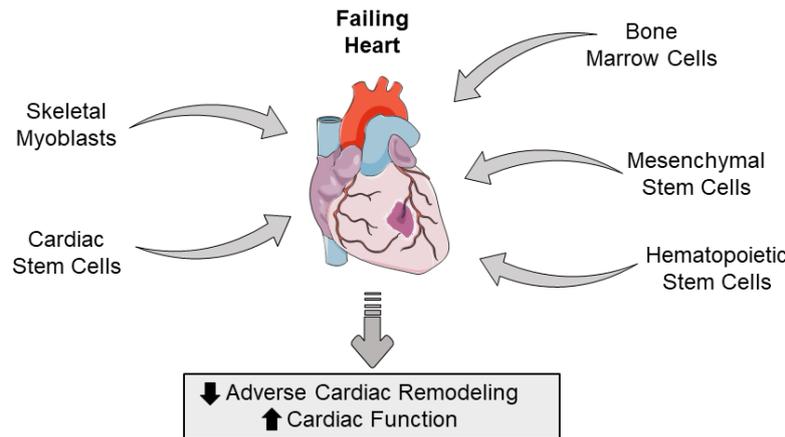
Figure 1. Schematic highlighting the types of adult stem cells that can be used to potentially restore the failing heart by attenuatiing adverse cardiac remodeling and improving cardiac function.

have been found to differentiate into smooth muscle cells and endothelial cells, though their cardiomyogenic potential remains debatable (Nagaya et al., 2005; Silva et al., 2005). The likely mode of action of MSCs in improving cardiac function has been attributed in large to paracrine effects as transplantation of these cells is reported to improve ventricular function via increased vascularization and reduced fibrosis with subsequent attenuation of cardiac remodeling (Mazo et al., 2008; Li et al., 2009; Schuleri et al., 2009; Mazo et al., 2010). Moreover, monolayered MSC sheets have been transplanted onto the scarred myocardium, but unlike fibrous cell sheets that weaken the ventricular wall, MSC sheets were found to improve hemodynamics and reduce mortality rates in rats (Miyahara et al., 2006). These encouraging results in animal models formed the basis for using bone marrow-derived MSCs in the POSEIDON clinical trial. In this study, though improvements in quality of life was observed, HF patients that received different doses of autologous or allogeneic MSCs failed to show drastic improvements in ventricular function, which was in stark contrast to what was previously observed in experimental models (Hare et al., 2012). Similarly, in the PRECISE trial that implemented adipose-derived MSCs, despite increases in patients' exercise capacity, an increase in ventricular function was not observed (Perin et al., 2014). It is important to note that MSCs derived from different sources are not identical and comprise distinct differentiating potential. Moreover, adipose tissue-derived MSCs (AT-MSCs) produce collagen (I, II, and III), whereas bone marrow-derived MSCs (BM-MSCs) possess greater immunosuppressive effects and exhibit heightened pro-angiogenic capacity (Bagno et al., 2018). Such discrepancies arising from the MSC source could be one of the reasons for the unexpected outcomes observed in the clinical trials.

Mesenchymal precursor cells (MPCs) are a restricted subset of MSCs, that when immunoselected for STRO-1 or STRO-3 demonstrate increased proliferative and developmental capabilities (Abdalmula et al., 2017). MPCs also have a unique multimodal mechanism of action that is proposed to result in the polarization of pro-inflammatory type 1 macrophages to an anti-inflammatory type 2 macrophage state in the heart, resulting in the reversal of cardiac and peripheral endothelial dysfunction, inhibition of maladaptive adverse ventricular remodeling, and recovery of deranged vasculature (Borow et al., 2019). In one study assessing the dose-response of allogeneic MPCs

in patients with ischemic or non-ischemic HF, a significant reduction of HF-related major adverse cardiac events, as well as improved remodeling was observed in the group that received 150 million MPCs (Perin et al., 2015). In another randomized Phase II clinical trial, intramyocardial injection of MPCs during left ventricular assist device (LVAD) implantation did not seem to improve successful temporary weaning from LVAD support at 6 months, but it did reduce the overall major bleeding rate (Yau et al., 2019). Lastly, in the highly awaited DREAM-HF study (a randomized Phase III study) that assessed the efficacy and safety of MPCs (Rexlemestrocel-L) in patients with advanced chronic HF, a 60% improvement in major adverse cardiac events such as stroke and cardiac arrest was reported regardless of patients' New York Heart Association (NYHA) class, albeit no impact on recurrent hospitalization (www.mesoblast.com). Mechanistically, MPCs are pericyte precursors, which produce high levels of angiopoietin-1, which in turn promotes vessel stability, and hence, these positive results could be attributed to anti-inflammatory effects and improved angiogenesis (Yau et al., 2019). Based on these findings, the implementation of MPCs for cardiac therapy does seem promising, however, their success relies heavily on invasive intra-myocardial delivery methods as intracoronary administration of MSCs/MPCs could lead to heightened risk of embolism with occlusion of coronary microvasculature (Freyman et al., 2006).

Hematopoietic stem cells
Hematopoietic stem cells (HSCs) are rare multipotent, self-renewing cells capable of generating an entire hematopoietic system. HSCs can be isolated from peripheral blood, bone marrow, or cord blood by selecting for CD34^{+} and CD133^{+} cells (Ng and Alexander, 2017). In a randomized study, intramyocardial delivery of autologous CD34^{+} cells during CABG were associated with improved ventricular function in patients with congestive HF (Patel et al., 2005). Similarly, intracoronary infusion of CD133^{+} and CD133^{-}/CD34^{+} cells to an infarcted and non-viable region was found to improve ventricular function with favorable remodeling and increased myocardial perfusion in patients with chronic ischemic cardiomyopathy (Manginas et al., 2007). Bone marrow-derived progenitor cells have also been administered in patients with non-ischemic cardiomyopathy (Fischer-Rasokat et al., 2009; Vrtovec et al., 2011), which resulted in improved hemodynamics

and cardiac contractility. A similar, but larger study has also yielded promising results where patients with better myocardial homing of the CD34+ cells were reported to have a better response to the therapy as indicated by improvements in ventricular function (Vrtovec et al., 2013). In the PROGENITOR trial, delivery of CD133$^+$ cells in patients with refractory angina was reported to reduce the number of angina episodes per month (Jimenez-Quevedo et al., 2014), while in the ixCELL-DCM randomized phase II trial, transendocardial delivery of ixmyelocel-T in the setting of ischemic dilated cardiomyopathy was associated with significant reductions in adverse cardiac events (Patel et al., 2016). Contrasting findings were documented in other clinical trials that implement the same cells albeit, different delivery methods. For instance, intra-epicardial injection of CD133$^+$ was reported to have no effect on global LV function and clinical symptoms, although there was some improvement in scar size (Nasseri et al., 2014). Moreover, in the underpowered REGENT-VSEL study, transendocardial delivery of CD133$^+$ cells failed to improve myocardial perfusion, LV function, and angina symptoms in patients with refractory angina (Wojakowski et al., 2017), which was further confounded by the inability to recruit sufficient number of patients, thereby severely hampering the ability to conclude on the effectiveness of CD133$^+$ cell therapy. Larger controlled clinical trials are required to further investigate the effectiveness of HSC therapy. Considering that HSCs are not immune-privileged, the main bottleneck lies in finding ways to overcome low cell engraftment and immunological barriers, while minimizing the risk of graft-versus-host disease to yield improvements in cardiac function (Ng and Alexander, 2017).

In summary, due to several conflicting clinical trials, meta-analyses reviewing the effectiveness of first-generation ASCs have been unable to conclusively associate these cells with beneficial effects. Attention has therefore been given to second-generation ASCs, such as cardiac stem/progenitor cells where experimental studies have shown these cardiac-committed cells to possess heightened therapeutic effects as indicated by improvements in engraftment, cardiac function, angiogenesis, and scar size after delivery (Rossini et al., 2011; Li et al., 2012b; Oskouei et al., 2012; Zheng et al., 2013) (Table 1).

Cardiac stem cells

Cardiac stem cells (CSCs) are multipotent cells that express markers such as c-kit$^+$ (Beltrami et al., 2003; Messina et al., 2004) and Sca1$^+$ (Oh et al., 2003; Messina et al., 2004). When transplanted near the infarct zone, c-kit$^+$ CSCs were found to invade the scarred myocardium and differentiate into cardiomyocytes and coronary vessels, which in turn improved cardiac function (Rota et al., 2008). Alternatively, cardiosphere-derived cells (CDCs; a cardiac progenitor cell type) can be obtained from *ex vivo* heart biopsies and differentiate into 3 major cell types; cardiomyocytes, endothelial cells, and smooth muscle cells (Smith et al., 2007). The PERSESUS phase II clinical trial reported that intracoronary infusion of CDCs in patients with congenital HF, led to improvements in cardiac function and HF status. Moreover, these patients were found to exhibit reductions in cardiac fibrosis as a result of reverse remodeling. Despite these promising results, the PERSEUSUS study was an open-labeled study and the effectiveness of CDC therapy should be viewed with caution due to the small sample size (Ishigami et al., 2017). In the CADUCEUS study that assessed the intracoronary delivery of c-kit$^+$ CDCs in MI patients, improvements in heart function were not observed, though reduction in scarring and improvements in regional contractility was reported (Makkar et al., 2012). It can be speculated that the inability to generate target doses through biopsies and the delay in timing between CDC harvest and delivery are major limiting factors for this form of therapy.

Alternatively, consideration could be given for combination therapy as the synergistic action of CDCs and MSCs were found to improve cardiac function in a swine model of ischemia/reperfusion injury (Williams et al., 2013; Karantalis et al., 2015). Currently, the feasibility, safety, and efficacy of transendocardial delivery of MSCs and c-kit$^+$ CSCs is being assessed in the CONCERT-HF study, a 4-arm phase II, randomized and placebo-controlled clinical trial, which would allow for the direct comparison between MSCs and CSCs, as well as their combination in improving cardiac function in patients with chronic ischemic HF (Bolli et al., 2018). While we wait with anticipation for the outcome of this trial, it can be speculated that improvements in cardiac function (if any) may likely be attributed to the therapeutic properties of CSCs rather than MSCs, as the latter is associated with poor engraftment and reduced viability following administration (von Bahr et al., 2012; Wang et al., 2014).

In summary, evidence from experimental studies suggest that both first-generation and second-generation ASCs do have the potential for cardiac therapy, but are confounded by limitations reported in several clinical trials, which hampers their universal adoption as an intervention strategy for improving cardiac function and limiting adverse clinical outcomes in patients with HF. To overcome such obstacles, one strategy could be to bolster the properties of ASCs by introducing transgenes that render them more potent by enhancing their effectiveness during cardiac therapy (Figure 2).

Implementation of transgenic ASCs for cardiac therapy

Anti-apoptotic modifications

A major limitation in cardiac therapy is the poor survival and engraftment of transplanted cells and hence, attempts have been made to enhance such properties by preventing apoptosis mediated by oxidative stress and hypoxia. The enhanced benefits associated with the overexpression of pro-survival genes or through the modulation of microRNAs (miRNAs) that suppress apoptosis-related pathways are discussed below.

In a pioneering study, when MSCs overexpressing Akt1 (a pro-survival gene) were transplanted into the ischemic rat myocardium, profound effects on cardiac remodeling were noted in a dose-dependent manner together with restoration of myocardial volume by four-folds (Mangi et al., 2003). Similar observations were made in large animal models where Akt-overexpressing MSCs were found to be more resistant towards apoptosis and the resultant increase in viable cells post-administration was associated with enhanced repair of the injured myocardium and improved cardiac function (Lim et al., 2006; Yu et al., 2010). In other studies, preconditioning with heat shock proteins (Hsp) was shown to promote cell survival against oxidative stress. For instance, Hsp20 overexpressing rat MSCs were reported to withstand oxidative stress *in vitro* and exhibited a 2-fold increase in survival when transplanted into infarcted hearts by intracardial injection (Wang et al., 2009). Mechanistically, overexpression of Hsp20 was found to be associated with increased Akt activation and increased growth factor secretion (vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), insulin growth factor-1 (IGF-1)), which reduced fibrosis and enhanced neovascularization with subsequent improvements in cardiac function. Recently, aged MSCs that overexpress part of the shelterin complex, tripeptidyl peptidase 1 (TPP1) were reported to have heightened levels of Akt phosphorylation and meiotic recombination 11 (MRE11) (a double-strand break repair protein) that reduces oxidative stress-mediated apoptosis and improves DNA repair respectively (Yu et al., 2020). Moreover, transplantation of TPP1-MSCs led to augmented cell survival, improved cardiac function, and decreased fibrosis when transplanted into the peri-infarct area in mice.

Table 1: List of clinical trials that implemented adult stem cells for cardiac therapy

Study Type	Disease	Study Participants	Type of Therapy	Method of Cell Delivery	Follow-up	Outcomes	Reference
Prospective, non-randomised clinical trial (Phase I)	Ischemic Cardiomyopathy	10	Skeletal myoblasts	Intramyocardial implantation	10.9 months	Improved LVEF and NYHA functional class; 63% of cell implanted scars show systolic thickening; occurrence of ventricular arrhythmia in 4 patients	(Menasche et al., 2003)
Prospective, non-randomised clinical trial (Phase I)	Ischemic Cardiomyopathy (Heart Failure)	13	Skeletal myoblasts overexpressing connexin-43	Percutaneous Transcatheter	6 months	Improvement in NYHA class and exercise capacity	(Gwizdala et al., 2017)
Prospective, non-randomised open label study	Ischemic Cardiomyopathy	21 (14)	Autologous mononuclear bone marrow cells	Transendocardial	2 and 4 months	At 2 months, global LV function is improved and significant reduction in total reversible defect. At 4 months, there's improvement in EF and reduction in end-systolic volume	(Perin et al., 2003)
Prospective, non-randomised, open label study	Ischemic Cardiomyopathy	20 (11)	Autologous mononuclear bone marrow cells	Transendocardial	6 and 12 months	Total reversible defect was significantly reduced and at 12 months; exercise capacity was improved	(Perin et al., 2004).
Prospective, randomised study	Ischemic Cardiomyopathy	20 (10)	Bone marrow cells	Intramyocardial	4 months	Recovery of regional contractile function in previously non-viable scar; no significant difference in global LVEF between groups	(Hendrikx et al., 2006)
Prospective, randomised study	Dilated Cardiomyopathy	44 (24)	Bone marrow cells	Intracoronary	6 months	Slight improvement in EF and reduction in end-systolic volume	(Seth et al., 2006)
Prospective, randomised, double-blinded study	Ischemic Cardiomyopathy	204 (101)	Bone marrow cells	Intracoronary	12 months	Recurrence of MI and rehospitalisation for HF and death was reduced	(Schachinger et al., 2006)
Prospective, randomised, single-blinded study	Ischemic Cardiomyopathy	63 (42)	Bone marrow cells	Intramuscular/ Intracoronary	6 months	No improvement in contractility of nonviable scarred myocardium; no reduction in scar size and no improvement in LV function	(Ang et al., 2008)
Prospective, non-randomised, open label study	Chronic Ischemic Heart Failure	32	Bone marrow cells	Intracoronary	4, 8 and 12 months	Significant decrease in E/e' ratio and plasma NT-pro-BNP	(Diederichsen et al., 2010)
Prospective, randomised open-label study	Ischemic Cardiomyopathy	375 (185)	Bone marrow cells	Intracoronary	2 years	No difference in ability to reduce the time for all-cause mortality in patients with reduced LVEF	(Mathur et al., 2020)
Prospective, randomised, double-blinded, placebo-controlled study	Ischemic Cardiomyopathy	92 (61)	Bone marrow cells	Transendocardial	6 months	No improvement in LVESV, maximal oxygen consumption or reversibility on SPECT	(Perin et al., 2012)
Prospective, randomised clinical trial (Phase I/II)	Ischemic Cardiomyopathy	31	Mesenchymal stem cells	Transendocardial	13 months	Improvement in NYHA classs; lower MACE and hospitalisation rates	(Hare et al., 2012) POSEIDON
Prospective, randomised, double-blinded,	Ischemic Cardiomyopathy	27 (21)	Adipose-derived mesenchymal stem cells	Transendocardial	6, 12 and 18 months	Preservation of ventricular function, myocardial perfusion and improved exercise	(Perin et al., 2014) PRECISE

placebo-controlled study						capacity.	
Prospective, randomised, clinical trial (Phase II)	Ischemic/ Nonischemic Heart Failure	60 (45)	Mesenchymal progenitor cells	Transendocardial	3,6 and 12 months	Reduction in HF-related major adverse cardiac events and beneficial remodelling in 150 million MPC group	(Perin et al., 2015)
Prospective, randomised clinical trial (Phase II)	Advanced Heart Failure with LVAD support	159 (106)	Mesenchymal progenitor cells	Intramyocardial	6 months	Reduced overall major bleeding rate; did not improve successful temporary weaning from LVAD support	(Yau et al., 2019)
Prospective, randomised, sham-controlled clinical trial (Phase III)	Ischemic/ Nonischemic Heart Failure	565	Mesenchymal progenitor cells (Rexlemestrocel-L)	Transendocardial	5 years	60% improvement in major adverse cardiac events such as stroke and cardiac arrest; no impact on recurrent hospitalisations	www.mesoblast.com
Prospective, randomised study	Ischemic Cardiomyopathy	20 (10)	Autologous CD34+ hematopoietic stem cell	Intramyocardial	1,3 and 6 months	Improved ventricular function	(Patel et al., 2005)
Prospective (treatment)/ Retrospective (control), non-randomised study	Chronic Ischemic Cardiomyopathy	24 (12)	CD133+ and CD133-/ CD34+ hematopoietic stem cells	Intracoronary	28 months	Improve ventricular function with favourable remodelling; increased myocardial perfusion	(Manginas et al., 2007)
Prospective, non-randomised, open-labelled study	Nonischemic Dilated Cardiomyopathy	33	Bone-marrow derived progenitor cells	Intracoronary	12 months	Global LVEF improved and NT-proBNP serum levels decreased	(Fischer-Rasokat et al., 2009)
Prospective, randomised, open-labelled clinical trial (Phase I/II)	Dilated Cardiomyopathy	55 (28)	CD34+ hematopoietic stem cells	Intracoronary	12 months	Increase in LVEF, 6-minute walk distance, and a decrease in NT-proBNP	(Vrtovec et al., 2011)
Prospective, randomised, open-labelled clinical trial (Phase I/II)	Dilated Cardiomyopathy	110 (55)	CD34+ hematopoietic stem cells	Intracoronary	5 years	Improved ventricular function, exercise tolerance and long-term survival; homing is associated with better stem cell therapy response	(Vrtovec et al., 2013)
Prospective, randomised, double-blinded study	Refractory angina	28 (19)	CD133+ hematopoietic stem cells	Transendocardial	6 months	Reduced number of angina episodes per month, improvement in anginal function class	(Jimenez-Quevedo et al., 2014) PROGENITOR
Prospective, randomised, double-blinded, placebo-controlled clinical trial (Phase IIb)	Ischemic Dilated Cardiomyopathy	126 (66)	Autologous bone marrow stem cells (Ixmyelocel-T)	Transendocardial	12 months	Significant reduction in adverse cardiac event	(Patel et al., 2016) ixCELL-DCM
Prospective, randomised, double-blinded, placebo-controlled study	Ischemic Cardiomyopathy	60 (3)	CD133+ bone marrow cells	Intramyocardial	6 months	Slight improvement in scar size and regional perfusion, but no effect on global LV function and clinical symptoms.	(Nasseri et al., 2014)
Prospective, randomised, double-blinded, placebo-controlled study	Refractory Angina	31 (16)	CD133+ hematopoietic stem cells	Transendocardial	12 months	No improvement in myocardial perfusion, LV function and angina symptoms	(Wojakowski et al., 2017) REGENT-VSEL

| Prospective, randomised, open-label clinical trial (Phase II) | Hypoplastic Left Heart Syndrome | 41 (23) | Cardiosphere-derived cells | Intracoronary | 12 months | Reduction in cardiac fibrosis and associated with improved ventricular function | (Ishigami et al., 2017) PERSESUS |
| Prospective, randomised clinical trial (Phase I) | Acute Myocardial Infarction | 31 (23) | C-kit+ cardiosphere-derived cells | Intracoronary | 6 months | Reduction in scarring and improvements in regional contractility | (Makkar et al., 2012) CADUCEUS |

Considering that oxidative stress is a critical mediator of apoptosis, enhancing antioxidant capacity could also be a viable option. In support, overexpression of sirtuin 3 (SIRT3) in aged MSCs led to improvements in antioxidant capacity attributed to up-regulation of Forkhead box O3 (FoxO3a), manganese superoxide dismutase (MnSOD), and catalase, which in turn promoted improvements in cardiac function and reduction in infarct size (Zhang et al., 2018).

H9C2 rat cardiomyoblasts that overexpress human B-cell lymphoma 2 (Bcl-2) were found to be associated with increased graft survival upon transplantation in MI rat models (Kutschka et al., 2006). Moreover, a study evaluating cell sheet technology reported that transgenic sheets comprising Bcl-2-expressing L6 rat skeletal myoblasts survived longer in the infarcted myocardium, increased production of pro-angiogenic factors, and improved cardiac function by reducing fibrosis and increasing angiogenesis in the infarct and border-zone regions in rats (Kitabayashi et al., 2010). In the same study, an increase in the number of proliferating c-kit+ cells were also observed. In the setting of hypoxia, MSCs overexpressing Bcl-2 were found to secrete 60% more VEGF *in vitro* and when injected into myocardium of MI rats, Bcl-2 expression conferred protection against apoptosis (Li et al., 2007). Interestingly, the capillary density in the infarct border zone was found to be 15% higher and the infarct size was 17% smaller in animals that received Bcl-2-MSC transplantation, thereby exhibiting remarkable functional recovery. In another study, overexpression of FGF-21 in bone marrow-derived MSCs was shown to effectively suppress caspase activation, which in turn protected MSCs from oxidative stress and inflammation mediated apoptosis (Linares et al., 2020), although it is yet to be determined if this approach can improve cardiac function *in vivo*.

Rat MSCs, which secrete human tissue kallikrein (TK), were reported to be more resistant to hypoxia-induced apoptosis as a result of reduced caspase-3, and transplantation of these cells into the myocardia of MI rats was associated with heightened heart function and improved neovascularization, together with a decrease in the accumulation of inflammatory cells and infarct size, as well as attenuated cardiac remodeling (Gao et al., 2013). In the same study, co-culture of cardiomyocytes with TK-MSC conditioned media was shown to suppress hypoxia-induced apoptosis and increased levels of phosphorylated Akt. The conditioned media was also found to contain elevated levels of VEGF that could stimulate the proliferation, migration, and tube formation of endothelial cells. Similarly, human endothelial progenitor cells (EPCs) that overexpress TK were shown to possess increased tolerance for oxidative stress that was attributed to reduced activation of caspase-3 and caspase-9 with subsequent activation of Akt (Yao et al., 2013). Moreover, as TK overexpression was found to stimulate secretion of VEGF, transplantation of TK-EPCs resulted in increased cardiac function, decreased cardiomyocyte apoptosis, and increased neovascularization and retention in the myocardium. Interestingly, transplanted TK-EPCs were also found to be incorporated into CD31$^+$ capillaries.

In the setting of hypoxia, MSCs that overexpress human heme oyxgenase 1 (HO-1) are reported to secrete several cytokines, including hepatocyte growth factor (HGF), basic FGF, transforming growth factor-beta (TGF-β), VEGF, and interleukin-1beta (IL-1β) (Zeng et al., 2008). Moreover, supernatant enriched with these cytokines was shown to reduce infarct size and cardiomyocyte apoptosis as well as increase angiogenesis in MI rats. Similar beneficial effects have been observed in larger animals, where transplantation of HO-1-overexpressing bone marrow stromal cells was found to improve cardiac function in a porcine ischemia/reperfusion injury model (Jiang et al., 2010). Mechanistically, HO-1-MSCs were found to modulate matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of matrix metalloproteinases (TIMP)) as an increase in TIMP-2 and TIMP-3 with corresponding decrease in MMP-2 and MMP-9 was observed post-transplantation, suggesting that these transgenic MSCs could reverse cardiac remodeling (Shu et al., 2010). Consistent with these findings, exosomes derived from TIMP2-modified human umbilical cord MSCs (huc-exoTIMP2) when injected at the peri-infarct zone of MI rats were shown to increase the expression of anti-apoptotic Bcl-2 and decrease expression of pro-apoptotic Bcl-2 associated X protein (Bax) and pro-caspase-9, which led to reduction of collagen deposition and improvements in cardiac function (Ni et al., 2019).

The overexpression of netrin-1 (a laminin-like protein) in bone marrow-derived Sca-1$^+$ cells has also been investigated. Rat cardiomyocytes and endothelial cells have been reported to express the netrin-1 receptor uncoordinated-5b and hence, when exposed to conditioned media from netrin-1 expressing Sca-1$^+$ cells, a decrease in oxidative stress-mediated apoptosis was observed (Durrani et al., 2012). In the same study, when transplanted to the center and border zone of the infarct, these transgenic cells were shown to reduce ischemia and preserve global heart function in MI rats that could be attributed to elevated activity of nitric oxide synthase (NOS) and increased angiogenesis. The role of apurinic/apyrimidinic endonuclease/redox factor (APE1), a multifunctional enzyme that can activate the DNA repair pathway and induce activation of redox-sensitive transcription factors has also been investigated in cardiac progenitor cells (CPCs) (Aonuma et al., 2016). In this study, APE1-CPCs exposed to H2O2 were found to inhibit reactive oxygen species production and suppress apoptosis via activation of transforming growth factor β-activated kinase 1 (TAK1) and nuclear factor kappa β (NF-κβ) pathway. Moreover, transplantation of APE1-CPCs into ischemic border zone of LV in MI mice was reported to improve LVEF by ~7% and decrease fibrosis by ~6%.

MicroRNAs, a type of non-coding RNA that play a key role in regulating gene expression by directly binding to the 3' untranslated region of their targeted mRNAs, have also been shown to modulate cardiac regeneration and repair (Tao et al., 2015), and much attention has been given to the link between miRNAs and apoptosis regulatory pathways as recently, pro- and anti-apoptotic miRNAs have progressively been identified (Sun et al., 2017). For instance, elevation of miR-133 is reported to be an effective strategy for treatment of MI by reducing hypoxia- and oxidative stress-mediated apoptosis *in vitro* (Zhang et al., 2012; Xu et al., 2014). Similarly, modified MSCs that overexpress miR-133 were found to drastically reduce their apoptosis when injected at the border zone of infarct in post-MI rats, with a subsequent increase in LVEF (~20%) (Chen et al., 2017). In other studies, MSCs that overexpress miR-25-3p,
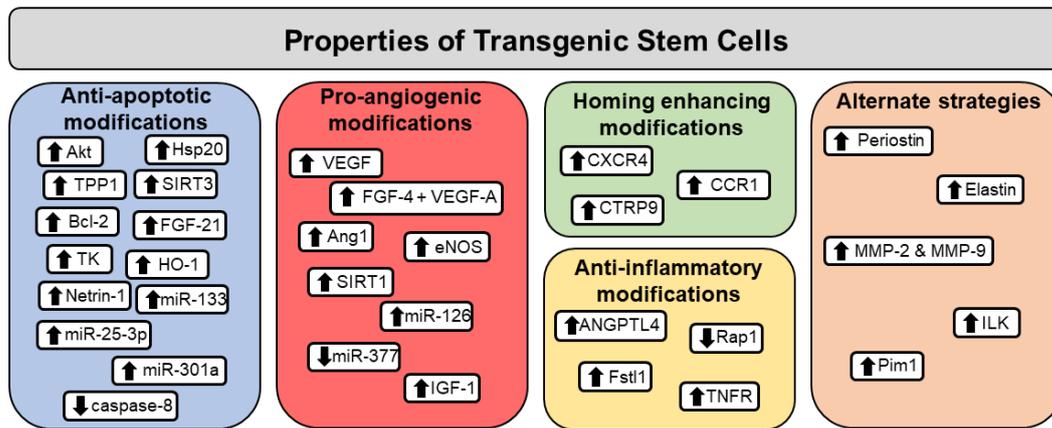
Figure 2. Schematic overview of transgenes that can bolster the therapeutic properties of adult stem cells (Up and down arrows indicate overexpression and suppression respectively).

were shown to alleviate MI by reducing the expression of pro-apoptotic genes, FAS ligand (FASL) and phosphatase and tensin homolog (PTEN) (Peng et al., 2020), while overexpression of miR-301a in human adipose-derived stem cells (hASCs) was shown to reduce apoptosis when transplanted into MI rats by down-regulating apoptosis signal-regulating kinase 1 (ASK1) (Lee et al., 2016). Mechanistically, miR-301a-hASCs were shown to inhibit ASK1 phosphorylation in the setting of hypoxia, which in turn suppressed the downstream p38/ c-Jun N-terminal kinase/NFκB axis.

Though most studies have focused on overexpressing transgenes, studies assessing the effect of gene silencing have also been conducted. For instance, caspase-8 shRNA-modified human MSCs were found to inhibit apoptosis and up-regulate HGF, IGF-1, and Bcl-2 *in vitro* (Liang et al., 2014). Moreover, when injected into the border zone of the infarcted region of the rat heart, these transgenic MSCs demonstrated enhanced survival and improved heart function with concomitant reduction in fibrosis and cardiomyocyte death.

In summary, these studies demonstrate that both overexpression and silencing of genes that regulate apoptosis could improve the survival and engraftment of transplanted ASCs, which could potentially lead to sustained improvements in cardiac function.

Pro-angiogenic modifications
Since most clinical studies have reported neovascularization to be the main contributor for restoring heart function, it seems appropriate to enhance these features in ASCs. In one study, when rat SMs overexpressing VEGF were seeded on scaffolds and implanted on rat myocardium, no functional recovery was observed, but an increase in vascularization was reported (von Wattenwyl et al., 2012). In a similar study, when MSCs overexpressing VEGF under hypoxic conditions were delivered into rat myocardium (Kim et al., 2011), an ischemia-responsive VEGF secretion was observed that led to increased neovascularization. In the same study, VEGF-MSCs were retained in greater numbers in the infarcted area and were associated with reduced cardiac remodeling. Simultaneous overexpression of FGF-4 and VEGF-A in human primary myoblasts was also shown to induce successful capillary formation, but more importantly did not alter their basic biological functions, including down-regulation of myogenic genes (Zimna et al., 2014).

It is important to note that when generating transgenic cell lines, the type of promoter determines the outcome as much as the transgene itself. For instance, the transplantation of bone marrow-derived EPCs overexpressing VEGF under the

control of a hypoxia promoter, was found to express more VEGF and induce more angiogenesis in rats as compared to cells overexpressing VEGF under the control of a constitute cytomegalovirus promoter (She et al., 2012). Moreover, careful consideration must be taken into account that regional higher levels of VEGF could potentially induce abnormal vessel growth, which may counteract its benefits. Therefore, efforts have been made to control VEGF secretion as evidenced by the generation of inducible VEGF-secreting human umbilical cord blood-derived MSCs (hUCB-MSCs) (Cho et al., 2017). These transgenic MSCs, when seeding onto a cardiac patch and grafted onto the injured epicardial anterolateral region in MI rats, were capable of secreting VEGF at physiological concentrations in a controlled setting and were associated with decreased infarct size as well as improved LVEF and fractional shortening.

Human MSCs have been modified to overexpress angiopoietin-1, which improved their survival in the infarcted rat myocardium, increased angiogenesis and arteriogenesis by 11-35%, reduced the infarcted area by 30%, and remarkably thickened the LV wall by 46% (Sun et al., 2007). Similarly, angiogenin modified MSCs were found to exhibit enhanced tolerance against hypoxia *in vitro* and improved viability in infarcted hearts, assisting in the preservation of contractile function and reducing cardiac remodeling through vasculogenesis as evidenced by the presence of factor VIII and α-smooth muscle actin (αSMA) (Liu et al., 2008). In another study, overexpression of SIRT1 was found to ameliorate the senescent phenotype of aged MSCs and improve their biological functions (Liu et al., 2014), likely mediated by increased expression of pro-angiogenic factors, including angiopoietin-1 and basic FGF.

The endothelial NOS (eNOS) signaling pathway is instrumental in regulating vasodilation and endothelial cell proliferation. In support, transplantation of eNOS overexpressing EPCs into the infarcted heart was shown to decrease tumor necrosis factor- alpha (TNF-α) and IL-1β that led to reductions in infarct size and increased angiogenesis in the peri-infarct region that culminated in improved cardiac function (Chen et al., 2013). Similarly, when human primary myoblasts and C2C12 mouse myoblasts were transfected with eNOS, both cell types were found to secrete large quantities of eNOS, which in turn induced capillary sprouting in human umbilical vein endothelial cells (HUVEC) cells (Janeczek et al., 2013). Unexpectedly, eNOS overexpression in human cells resulted in the down-regulation of myogenin, a transcriptional activator that is required for skeletal muscle development. Whether this down-regulation is deleterious remains to be

determined.

Recently, miRNAs have been found to be master regulators of angiogenesis (Ghodrat et al., 2021) and consistently, MSCs overexpressing miR-126 have been found to increase secretion of pro-angiogenic factors and also exhibit greater resistance to hypoxia *in vitro* (Huang et al., 2013). In the same study, miR-126-MSCs injected into the infarcted region demonstrated increased survival while promoting angiogenesis and improving heart function. Interestingly, these transgenic MSCs expressed elevated levels of delta-like-4 (Dll-4), which was shown to mediate tubulogenesis following transplantation. Importantly, the elevation of all miRNAs is not beneficial and this is shown from the observation that increased miR-377 in HF, is in fact detrimental to stem cell function. However, suppression of miR-377 in transplanted CD34+ cells was able to promote their angiogenic ability and reduce interstitial fibrosis, which resulted in improved LV function in a mouse model of ischemia/reperfusion injury (Joladarashi et al., 2015).

Finally, bone marrow derived-EPCs overexpressing IGF-1 have been found to increase cardiomyocyte proliferation and increase vascularization in the peri-infarct region in MI rats (Sen et al., 2010). Moreover, human CD34$^+$ cells modified to enhance secretion of sonic hedgehog (Shh) were found to protect against ventricular dilation, reduce infarct size, and increase angiogenesis when transplanted into the border zone (Mackie et al., 2012). Interestingly, CD34$^+$ cells were found to store Shh in exosomes and upon secretion; these exosomes could transfer Shh to other cell types, eliciting the induction of canonical Shh signaling. In summary, transgenic modifications that conferred ASCs with more angiogenic ability reveal remarkable improvements to cardiac function and could potentially serve as a therapy for ischemic HF.

Homing enhancing modifications

While intramyocardial injections do guarantee delivery of ASCs to the heart, it is an invasive technique and hence, less-invasive approaches are sought (e.g., intravenous/intracoronary delivery). A major limitation of these approaches is that they are heavily dependent on ASCs to successfully home in on the damaged myocardial region. Hence, understanding the signaling cascades that regulate homing properties could help circumvent this issue. The stromal cell-derived factor 1/C-X-C- motif chemokine receptor 4 (SDF-1)/CXCR4) axis has been reported to be important for homing of progenitor cells to ischemic tissue (Cheng and Qin, 2012; Cheng et al., 2015), and in support, intravenous delivery of CXCR4 overexpressing MSCs were found to possess increased homing ability towards the infarct region and were able to reduce cardiac remodeling and improve ventricular function in rats (Cheng et al., 2008). These findings suggested that intravenous delivery of CXCR4-MSCs is a safe and non-invasive method for attainting functional recovery post-MI. On the contrary, intracoronary delivery of SDF-1α overexpressing EPCs have been found to only increase angiogenesis, unlike myocardial delivery, which resulted in reduced collagen content and improved cardiac function (Schuh et al., 2012). Moreover, although CXCR4 overexpressing human MSCs were capable of increasing cytosolic Ca2+ and activating certain mitogen-activated protein kinases in response to SDF-1α stimulation, they were unable to demonstrate improvements in cell migration *in vitro* (Wiehe et al., 2013), which could be attributed to the strong basal chemokinesis properties already present in naïve MSCs.

MSCs overexpressing C-C motif chemokine receptor 1 (CCR1) were found to possess augmented migration and anti-apoptotic properties that led to the reinstatement of cardiac function and prevented cardiac remodeling post-MI (Huang et al., 2010). While CCR1-MSCs were delivered via intramyocardial injection, it will be interesting to assess if similar functional improvements could be obtained through intravenous/intracoronary delivery. Moreover, migration, survival, and proliferation of adipose-derived mesenchymal stem cells (ADSCs) via an extracellular signal-regulated kinase 1/2-MMP-9 axis may also be enhanced by the cardiokine c1q/tumor necrosis factor-related protein-9 (CTRP9) to aid engraftment in addition to guarding against oxidative stress-mediated apoptosis by elevating SOD3 (Yan et al., 2017).

In summary, achieving successful homing and engraftment of ASCs through less-invasive approaches is still a considerable challenge and hence, further studies are needed to identify other mediators that could facilitate homing. The application of intravenous/intracoronary delivery of ASCs should also be viewed with caution as it could heighten the risk of embolism and disrupt microcirculation (Fiarresga et al., 2015). Alternatively, to circumvent the unsuccessful engraftment of cells, CD34-CD42b platelet-targeting bispecific antibodies (PT-BsAbs) have been developed to concurrently recognize both CD34$^+$ HSCs and CD42b$^+$ platelets so as to redirect cells from the lung to the damaged heart as an intervention strategy for MI (Liu et al., 2020a). Other novel strategies also include the usage of multilineage-differentiating stress enduring (Muse) cells that home in via the sphingosine-1-phosphate/ sphingosine-1-phosphate receptor 2 axis to the infarcted heart with corresponding decreases in infarct size and increased LVEF (Yamada et al., 2018).

Anti-inflammatory modifications

Apart from engraftment and survival, another challenge faced by transplanted ASCs is their clearance by the host immune system and hence, measures have been taken to circumvent such events. The highly expressed angiopoietin-like 4 (ANGPTL4) in MSCs is critical for suppressing the inflammatory responses and instigating cardiac repair as evidenced by increases in anti-inflammatory macrophages and decreases in pro-inflammatory macrophages in the setting of MI (Cho et al., 2019). Consistently, ANGPTL4-deficient MSCs were found to be unsuccessful in inhibiting the inflammatory response, while treatment with ANGPTL4 led to improvements in cardiac function (Cho et al., 2019). The therapeutic efficacy of MSCs could also be bolstered by inhibiting Ras-proximate-1 or Ras-related protein 1 (Rap1), as Rap1-KO was found to decrease production of pro-inflammatory cytokines and prevent apoptosis of cardiomyocytes *in vitro* (Zhang et al., 2015). In other studies, MSC survival and proliferation following transplantation has been enhanced by overexpression of follistatin like 1 (a cardiokine), which led to improvements in cardiac function post-MI in addition to restricting scar formation, diminishing inflammatory response and augmenting neovascularization (Shen et al., 2019). Finally, transplantation of MSCs overexpressing TNFR (an anti-inflammatory mediator) was found to reduce production of inflammatory cytokines (TNF-, IL-1β and IL-6) as well as inhibited cardiomyocyte apoptosis, which culminated in improved cardiac function (Bao et al., 2008; Bao et al., 2010). In summary, transgenic ASCs that display modulated inflammatory signaling cascades, either through suppression of pro-inflammatory responses or by enhancing the action of anti-inflammatory mediators could be considered for cardiac therapy.

Alternate strategies to bolster ASC properties

In addition to enhancing survival, engraftment, homing, angiogenic potential, and anti-inflammatory properties, other modifications could also be considered to facilitate the development of ASCs with augmented therapeutic properties. These strategies include modulation of genes that regulate cell adhesion and cell-matrix communications. For instance, transplantation of MSCs overexpressing periostin (a protein

assisting in cell adhesion) was found to enhance their survival in addition to preventing apoptosis of cardiomyocytes (Cho et al., 2012). Co-culture of cardiomyocyte with perisotin-MSCs or with their conditioned media led to better survival under hypoxic conditions where mechanistic studies attributed this to prevention of adhesion-related integrin reduction along with increases in PI3K and Akt phosphorylation. Since the loss of elastin is instrumental in cardiac remodeling, transplantation of elastin overexpressing bone marrow stromal cells into rats led to elastin deposits in the infarct, which in turn preserved myocardium structural integrity (Li et al., 2012a). In the same study, though reduction in infarct size was not observed, it was not found to expand either and more importantly, functional improvement was noted. Moreover, overexpression of fatty acid binding protein 3 (FABP3), a protein involved in early myocardial development and adult myocardial tissue repair has also been tested in human MSCs. However, despite improving MSC survival under hypoxic conditions, FABP3 expression unexpectedly had negative effects on cell growth and proliferation (Zhang et al., 2015), possibly by affecting the secretion of paracrine factors.

Injection of conditioned medium from integrin-linked kinase ,(ILK) overexpressing MSCs into MI hearts has been found to inhibit cardiac fibroblast proliferation, reduce collagen type I and III gene expression as well as TIMP-1, TIMP-2, α-SMA, and connective tissue growth factor (Mao et al., 2013). In the same study, increased gene expression of MMP-2 and MMP-9 was also documented and accompanied by improvements in contractility, decreased infarct size, and fibrosis. Similar observations have been made in large animal studies, where ILK-MSCs demonstrated increased proliferation and suppressed apoptosis, which led to reductions in cardiac remodeling and restored heart function (Mao et al., 2014), likely mediated by increased neovascularization as well as cardiomyocyte proliferation. In other studies, mouse Sca-1+ cells overexpressing ILK have also shown increased viability, proliferation, and survival *in vitro* through activation of Akt and cyclin D (Ling et al., 2013). Interestingly, when injected into the myocardium, though long-term assessment of ILK-Sca-1+ cells did not seem to indicate better engraftment than controls, they did seem to induce reductions in infarct size and improved cardiac function.

When mouse Sca-1^{+} cells overexpressed a cocktail of growth factors including human IGF-1, VEGF, SDF-1α, and HGF, they were found to up-regulate multiple angiogenic and survival factors that enhanced their survival in lethal hypoxic conditions (Li et al., 2014). In the same study, transplantation of growth factor-Sca-1^{+} cells were found to exhibit better survival with heightened mobilization of c-Kit[+], multidrug resistance-1+, and CXCR4^{+} cells with histological analysis revealing extensive myofiber formation, increased gap junction proteins, and increased blood vessel density in both peri-infarct and infarct regions. Conversely, combined administration of MSCs overexpressing IGF-1 and HGF was unable to improve cardiac function in a porcine MI model (Kulandavelu et al., 2016). Although an increase in neovascularization was observed, this was also accompanied by an increase in fibrosis, suggesting that sustained exposure to growth factors could be beneficial as well as deleterious, while also highlighting the importance of validating initial findings in clinically relevant large animal models.

Future directions
The above studies support the use of transgenic ASCs for restoring cardiac function; however, careful consideration should be made when selecting a cell type for therapeutic intervention as patient age and cardiovascular risk factors are reported to weaken the effectiveness of ASCs (Zhao et al.,

2018). Alternatively, human induced pluripotent stem cells (hiPSCs) that are derived from patient somatic cells can be considered, as they are capable of differentiating into virtually every cell type. In support, hiPSCs overexpressing cyclin D2 when differentiated into cardiomyocytes were found to induce LV muscle regeneration and increased angiogenesis at the border zone in MI mice (Zhu et al., 2018). In another study, overexpression of endothelial cell-specific molecule 1 in hiPSC-derived endothelial cells was found to improve angiogenesis and neovascularization (Vila-Gonzalez et al., 2019). The temporary overexpression of Ang-1 in hiPSC-derived cardiomyocytes has also been associated with better engraftment and improved heart structure and function post-MI (Tao et al., 2020). Similarly, overexpression of cadherin 2 (N-cadherin) in hiPSC-derived cardiomyocytes was found to improve their survival, engraftment, and reparative potency with elevated LVEF and reduced infarct size in MI mice (Lou et al., 2020). Interestingly, extracellular vesicles released by hiPSC-derived cardiac progenitor cells have also been found to be cardioprotective in an acute infarction model that was attributed to the down-regulation of pro-inflammatory (cytokines IL-1α, IL-2, and IL-6, M1 macrophages, pro-inflammatory monocytes) and up-regulation of anti-inflammatory responses (cytokines IL-10, M2 macrophages) (Lima Correa et al., 2021). Based on these findings, not only are hiPSCs an unlimited source of therapeutic cells but also provides the flexibility for implementing various cell types for different therapeutic purposes, should the need arise.

Implementation of exosomes derived from MSCs are rapidly emerging as a cell-free alternative as concurrent delivery of both exosomes and stem cells have been found to improve cardiac function post-MI (Huang et al., 2019). Although the exact mechanism through which exosomes exert beneficial effects is unclear, it could be speculated that exosomes mainly function through modulation of miRNAs (Luther et al., 2018; Sun et al., 2020b). In support, treatment of aged MSCs with miR-136 (exosome cargo) significantly attenuated apoptosis and senescence of aged MSCs by down-regulating apoptotic protease activating factor-1 (Zhang et al., 2020). Other studies have also demonstrated the use of exosomes overexpressing macrophage migration inhibitory factor for improving cardiac function and reducing cardiomyocyte apoptosis, underscoring the crucial role exosomes could play in the treatment of MI (Liu et al. 2020b).

Consistent with the beneficial effects associated with gene modulation, overexpression of hypoxia-inducible factor 1-alpha in exosomes from MSCs was found to restore the impaired phenotype of HUVECs exposed to hypoxic conditions, in addition to preserving cardiac function by up-regulating pro-angiogenic factors and inducing neovascularization in MI rats (Sun et al., 2020a). Moreover, as inhibition of MMPs by TIMP-2 is a critical mediator of cardiac remodeling post-MI, exosomes obtained from TIMP2 overexpressing hUCB-MSCs were found to improve cardiac function by alleviating MI-induced oxidative stress and ECM remodeling (Ni et al., 2019). In other studies, exosomes secreted by SIRT1 overexpressing ADSCs (ADSCs-SIRT1-Exos) were found to improve cardiac function, reduce infarct size, attenuate LV remodeling and inflammation, as well as promote vasculogenesis, likely mediated through the recruitment of endothelial progenitor cells (Huang et al., 2020). Finally, exosomes derived from miR-146a-modified ADSCs and miR-126 overexpressing ADSCs have been found to be associated with beneficial effects post-MI (Luo et al., 2017; Pan et al., 2019).

In summary, though the use of both iPSC-derived progenies and exosomes could potentially serve as effective alternatives to ASCs, the requirement for large quantities of cells/exosome could be a hindrance towards their clinical adoption. While

these obstacles could be circumvented by devising efficient differentiation protocols (Mehta et al., 2014; Ramachandra et al., 2018) and by boosting exosome number through adiponectin treatment (Nakamura et al., 2020), further studies in large animal models are needed to draw conclusions on the therapeutic effectiveness of these approaches.

Conclusions

Several preclinical studies have reported the successful implementation of ASCs for restoring the failing heart. However, implementation of cardiac therapy in the clinical setting has been largely disappointing with only a few studies having been conducted with sufficient sample size and appropriate controls to demonstrate the effectiveness of ASCs for improving clinical outcomes. In reality, cardiac therapy faces several challenges; including (i) identifying the type/number of cell or combination of cells to be used, (ii) discerning the appropriate timing/frequency and route of cell delivery, (iii) the poor retention of injected cells or the low integration of cell sheets, and (iv) the limited effectiveness of the therapy. Recently, several experimental studies have revealed transgenic ASCs to be a viable alternative as it enables us to bolster key beneficial properties with the aim of augmenting their therapeutic potency for restoring the failing heart (Figure 2). These transgenic ASCs will probably face similar challenges as their naïve counterparts; however, their enhanced survival and engraftment, heightened angiogenic potential, increased homing, and reduced inflammatory responses are grounds for optimism. The increasing prevalence of HF and lack of restorative therapies does warrant an urgent need for novel intervention strategies and if transgenic ASCs are to be considered, they must be validated accordingly through standardized, large, multi-centered clinical trials.

Acknowledgements

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