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Current Cardiology Reviews, **2016,** *12,* **195-215**

REVIEW ARTICLE

Cellular Therapy for Heart Failure

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ARTICLE HISTORY

Received: November 30, 2015
Revised: December 18, 2015
Accepted: January 11, 2016

DOI: 10.2174/1573403X12666160606121858

Abstract: The pathogenesis of cardiomyopathy and heart failure (HF) is underpinned by complex changes at subcellular, cellular and extracellular levels in the ventricular myocardium. For all of the gains that conventional treatments for HF have brought to mortality and morbidity, they do not adequately address the loss of cardiomyocyte numbers in the remodeling ventricle. Originally conceived to address this problem, cellular transplantation for HF has already gone through several stages of evolution over the past two decades. Various cell types and delivery routes have been implemented to positive effect in preclinical models of ischemic and nonischemic cardiomyopathy, with pleiotropic benefits observed in terms of myocardial remodeling, systolic and diastolic performance, perfusion, fibrosis, inflammation, metabolism and electrophysiology. To a large extent, these salubrious effects are now attributed to the indirect, paracrine capacity of transplanted stem cells to facilitate endogenous cardiac repair processes. Promising results have also followed in early phase human studies, although these have been relatively modest and somewhat inconsistent. This review details the preclinical and clinical evidence currently available regarding the use of pluripotent stem cells and adult-derived progenitor cells for cardiomyopathy and HF. It outlines the important lessons that have been learned to this point in time, and balances the promise of this exciting field against the key challenges and questions that still need to be addressed at all levels of research, to ensure that cell therapy realizes its full potential by adding to the armamentarium of HF management.

Keywords: Angiogenesis, cardiac stem cells, heart failure, mesenchymal stem cells, pluripotent stem cells.

INTRODUCT1ION

Cardiomyopathy, resulting in the clinical syndrome of heart failure (HF), is a common and costly condition worldwide. In the United States alone, HF afflicts more than 5 million people, contributes to one in nine deaths and is responsible for an annual health care expenditure of over $30 billion [1]. The lifetime risk of developing HF is approximately one in five for men and women [2] and its prevalence will continue to increase with aging populations [3]. On an individual basis, HF impairs quality of life (QOL) more than other chronic diseases and carries poor prognosis, with approximately 50% of patients dying within five years of diagnosis [1]. This is despite numerous therapeutic advances spanning the key areas of home-care management, pharmacotherapy, device therapy, treatment and prevention of underlying diseases (e.g. coronary revascularization for myocardial infarction [MI] and ischemic heart disease [IHD]) and heart transplantation.

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Classifications of cardiomyopathy are commonly based on myocardial structural features (e.g. dilated, restrictive or hypertrophic) or underlying etiology (e.g. ischemic or nonischemic). Ischemic cardiomyopathy may be due to irreversibly damaged and scarred myocardium after MI, or due to dysfunctional but viable, hibernating myocardium when chronic coronary ischemia has caused prolonged and severe oxygen deprivation to cardiomyocytes. Numerous distinct etiologies may account for nonischemic cardiomyopathy, differing in their natural history of progression to HF, prognosis and responsiveness to contemporary treatments [4]. Despite being less prevalent, nonischemic cardiomyopathy has now superseded its ischemic counterpart as the more common cause of adult heart transplantation.

The development of clinical HF from ischemic and nonischemic cardiomyopathy arises from the progressive and complex process of myocardial remodeling. This begins with local compensatory changes at genomic, molecular, cellular and interstitial levels, that ultimately become maladaptive and lead to inexorable disturbances to cardiac metabolics, energomechanics and electromechanical coupling, with cardiac chamber dilatation and impairment in contractile function and relaxation. At the core of this, is the unreplaced loss of up to billions of cardiac cells, most critically cardiomyo-

1573-403X/16 $58.00+.00 © 2016 Bentham Science Publishers

cytes and vascular cells, with replacement by fibrotic tissue. Although it is now well established that the adult human heart is not post-mitotic, in that it contains a small content of actively cycling cells, along with its own stock of resident stem and progenitor cells [5], its intrinsic capacity for self-regeneration falls well short of compensating for the lost cell mass that underpins HF. Conventional therapies for end-stage HF are generally supportive in nature and do not overcome this primary problem, with the one exception of cardiac transplantation, which itself has numerous challenges, not least of which is a lack of donor organ availability. This unmet need of replacing cardiac cells has fueled great interest in regenerative cardiology over the past two decades, particularly the investigation of stem cell transfer for the prevention and cure of HF.

The field of cell-based therapy for cardiovascular diseases had its nascence in the late 1990s with early preclinical studies demonstrating the feasibility of transplanting fetal cardiomyocytes [6] and skeletal myoblasts (SkMs) [7] into damaged myocardium and fibrotic scars. Soon after, the inaugural reports of bone marrow (BM) cell transfer in murine models of MI were published [8, 9]. In contrast to the prolonged time-lines traditionally applied for clinical translation of novel pharmaceutical therapies, cell therapy was advanced to human studies with astonishing speed, with the first publications arriving in 2001 for SkM transplantation in patients with HF [10], and in 2002 for BM cell use in acute MI [11]. Since that time, many animal and human studies have been performed to evaluate various cell types for their capacity to mediate cardiac and vascular repair in the settings of MI, ischemic and nonischemic cardiomyopathy, cerebral and peripheral ischemia, valvular heart disease and cardiac conduction disorders.

While the greatest potential for *bona fide* tissue regeneration unequivocally rests with pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), the use of non-pluripotent adult cell preparations has predominated in human studies thus far. The most notable examples have been (1) autologous mononuclear cells (MNCs) and (2) proangiogenic (endothelial) progenitor cells from BM and peripheral blood, (3) autologous and allogeneic mesenchymal stromal/stem cells (MSCs) from BM, adipose and other tissue sources, (4) autologous SkMs, and (5) cardiac-derived cells, such as c-kit$^+$ cardiac stem cells (CSCs) and cardiosphere-derived stem cells (CDCs). Despite considerable biological differences between these distinct cell types, including markedly different levels of "stemness", proliferation capacity, differentiation repertoire and paracrine activity, each has been associated with positive data in preclinical models to indicate their cardiovascular therapeutic potential. However until now, this promise has not been fully realized in human studies and persistent questions remain unanswered as to how cell therapy might ultimately be best applied in the clinical setting. These especially relate to matching the optimal cell type to specific cardiovascular diseases, as well as using the optimal cell dose, mode, timing and frequency of administration to ensure durable treatment effect and minimize adverse outcomes.

This review will provide an update of the current status of cell-based interventions for cardiomyopathy and HF, by discussing the available experimental and clinical data in the field, and highlighting important controversies, challenges and future directions. Readers who are interested in other related topics, such as the mechanisms and scope for endogenous cardiac regeneration or the use of cell therapies for acute MI and chronic symptomatic angina, are referred elsewhere [5, 12].

SPECIFIC CELL TYPES EVALUATED FOR HF

Embryonic Stem Cells

As discussed below, many of the cell populations used in human studies of HF have fallen well short of meeting the primary objective of replacing scar tissue with new, functional cardiac cells, and therefore achieving actual myocardial regeneration. This is in large part due to the underwhelming retention and engraftment of cells in the recipient heart after their administration, combined with their limited ability to proliferate sufficiently *in vivo* and differentiate into mature cardiomyocytes and/or vascular cells. In contrast, ESCs and iPSCs have both enormous proliferative capacity and toti-differentiation potential. In theory, this makes them equipped to regenerate scarred and dysfunctional cardiac tissue with adequately sized, viable grafts that are well perfused and contractile.

ESCs are derived from the inner cell mass of the blastocyst (early-stage embryo) and can generate cells of all three of the germ cell layers (ectoderm, endoderm and mesoderm). Numerous articles have described the cardiopoietic potential of murine ESC lines and human ESCs, which were first successfully isolated from human blastocysts in 1998 [13]. Human ESC-derived cardiomyocytes (ESC-CMs) isolated from embryoid bodies behave structurally and functionally like cardiomyocytes, expressing characteristic morphology, cell marker and transcription factor expression, sarcomeric organization and electrophysiological properties, including spontaneous action potentials and beating activity [14]. Mouse and human cardiac-committed ESCs have been transplanted into small and large animal models of acute and old MI. Although these studies have demonstrated durable *in vivo* engraftment, proliferation and differentiation of ESC-CMs, as well as electromechanical integration with host cardiomyocytes [15-17], they have not universally shown improvement in myocardial remodeling and function [18, 19].

In addition there have also been reports of teratoma formation [20], most probably resulting from failure to exclude undifferentiated ESCs in the donor cell population. Despite ongoing refinements in ESC-CM preparation, the risk of tumorigenesis has remained a major hurdle to the clinical implementation of ESCs, as have concerns about immunorejection to allogeneic ESCs and ethical considerations, which can at least be obviated by the aternative use of autologous iPSCs, described below. Nevertheless, proponents of ESC transplantation continue to move forward with research efforts. Using an established monolayer technique for directed cardiomyocyte differentiation, Chong and co-workers were recently able to procure high numbers of human ESC-CMs (~$1x10^9$ cell doses) that were >70% positive for troponin-T and karyotypically normal [19]. These cells were surgically implanted by transepicardial injections into a small number

of immunosuppressed macaque monkeys, two weeks after myocardial ischemia-reperfusion injury. After 2-12 weeks, this resulted in donor cell grafts that were in the order of 1-5% of left ventricular (LV) size and 40% of infarct size, with perfusion from host vasculature and synchronized electromechanical connections with host myocardium, although cell treatment did not clearly improve LV ejection fraction (EF). Importantly, continuous ECG monitoring revealed episodes of non-fatal ventricular arrhythmia in all macaques that received ESC-CMs, the burden of which correlated with graft size. Despite the fact that these subsided by the first 4 weeks after cell delivery and did not cause animals obvious distress, it is nonetheless a cautionary finding.

The phase I ESCORT study (Transplantation of Human Embryonic Stem Cell-derived Progenitors in Severe Heart Failure; www.clinicaltrials.gov NCT02057900) has now been launched in Europe as the first human cardiovascular trial using ESC-based therapy in patients with severe HF [21]. In contrast to the approach undertaken by Chong *et al.* [19], a smaller number of ESC-derived $CD15^{+}Isl\text{-}1^{+}$ cardiac progenitor cells will be administered in a fibrin patch that will be secured to the epicardial surface of damaged myocardium by an autologous pericardial flap during open heart surgery. The intention is to provide a growth factor-rich, contractile scaffold to assist myocardial pumping, while also protecting donor cell viability, and preventing the electromechanical integration of donor cells with host tissue to mitigate risk of arrhythmia.

Induced Pluripotent Stem Cells

The reprogramming of mouse and human somatic cells into highly proliferative iPSCs was first achieved by nuclear transfer of combinations of transcription factors, comprising Oct3/4, SOX2, c-Myc and Klf4 [22] or Oct4, SOX2, NANOG, and LIN28 [23] to dermal fibroblasts. iPSCs possess ESC characteristics, with similar cardiogenic potential, while circumventing the ethical objections against embryonic harvesting and bypassing the problem of immune rejection. Although their initial discovery arose from transduction with teratoma-forming oncogenes using genome-integrating retroviruses, advances in the field of iPSC engineering have quickly moved beyond the need for genomic integration or sustained exposure to reprogramming factors [24, 25] and various techniques have now been developed to generate iPSC-CMs [26].

While both undifferentiated iPSCs and iPSC-CMs have shown reparative benefits in animal studies of MI [27, 28], their therapeutic evaluation in HF models has not yet been described. In contrast, the utility of iPSCs has already been demonstrated as an experimental platform to model and gain new mechanistic insights into different subtypes of cardiomyopathy *in vitro* [29-31]. The development of clinical grade iPSC products for human HF treatment remains some distance away. Substantial efforts are needed to overcome important challenges, such as low efficiency of cellular reprogramming and cardiomyogenesis, variabilities between iPSC cell lines, and crucially the risk of teratoma formation. In recent times, the exciting prospect of direct cardiac reprogramming has also emerged. This "cell-free" approach involves the conversion of fibroblasts into mature, functional cardiomyocytes, by forced overexpression of combinations of reprogramming transcription factors (e.g. Gata4, Mef2c, Tbx5, Hand2) and/or exposure to cardiopoietic microRNAs (miR-1, miR-133, miR-208, miR-499) [32-34]. *In situ* reprogramming of cardiac fibroblasts has already been shown to reduce fibrosis, augment myocardial contractility and avert deleterious remodeling in murine models of acute and old MI [32, 35], sparking optimism for its therapeutic potential in established cardiomyopathy also.

Skeletal Myoblasts

SkMs are the progenitor cells located under the basal membrane of myofibers that normally proliferate and differentiate into myotubes to help generate new muscle fibers after muscle injury. Based on their ease of isolation from muscle biopsies and culture expansion, inherent contractile properties, and resistance to fatigue and ischemia, they were one of the first cell types to be investigated in preclinical and clinical studies of cardiomyopathy and HF [36]. Early data pointed to their ability to mitigate LV remodeling, reduce interstitial fibrosis and improve cardiac contractile and diastolic performance, in both ischemic and nonischemic cardiomyopathy, with evidence for sustained hemodynamic benefits [37-39]. However, it became clear that the mechanisms responsible for these benefits were not due to SkMs undergoing cardiomyocyte transformation, as these cells remained committed to the skeletal muscle phenotype [36, 40]. Despite fusion of donor SkMs with host cardiomyocytes, there was also failure of sufficient electromechanical coupling because of deficient expression of gap junctions and intercalated disc proteins (e.g. N-cadherin and connexin-43) by donor SkM grafts [41]. This has been thought to account for the concerning incidence of ventricular tachyarrhythmia that was observed after SkM transplantation in clinical studies [42]. In the absence of cardiac transdifferentiaton and electromechanical coupling, the reparative benefits of SkMs were largely ascribed to other mechanisms, ranging from their autonomous contraction to paracrine-mediated remodeling of extracellular matrix (ECM) and scar, and trophic stimulation of endogenous angiogenesis and cardiac repair pathways through the release of cytokines and growth factors (e.g. vascular endothelial growth factor, hepatocyte growth factor) [43-45].

Over the past decade, clinical studies of SkMs have generally targeted patients with chronic, scar-related cardiac dysfunction, delivering these cells to the damaged myocardium by surgical transepicardial [46] or percutaneous transendocardial [38, 47] injection. Early promise with regard to scar reduction and LV contractile improvement was tempered by safety concerns relating to arrhythmogenesis. Furthermore in early studies, the absence of a randomized, placebo control arm and the concurrent use of surgical bypass revascularization made it difficult to conclude that SkM transfer itself was actually responsible for these benefits [39, 48]. Subsequent results from well-conducted, randomized, controlled studies have been somewhat less encouraging. The phase II double-blind MAGIC trial randomized 97 patients with severe ischemic cardiomyopathy (LVEF $\leq$35%) to surgical injections of placebo or autologous SkMs at one of two cell doses ($4x10^8$ or $8x10^8$), administered transepicardially into and around LV scar tissue at the time of coronary

artery bypass grafting (CABG) [46]. All patients received an implantable cardioverter-defibrillator in the context of poor LV function and the existing concerns about SkM-induced ventricular arrhythmias. Six month data revealed no inter-group differences for global or regional cardiac function or major adverse cardiovascular events, although ventricular arrhythmias were more frequent in the cell-treated patients. High cell-dose treatment was however, associated with significant reduction of LV volumes relative to placebo.

Percutaneous intramyocardial delivery of SkMs was evaluated in the Phase IIa randomized, open-label SEISMIC trial. Despite improvement in 6-minute walking distance, SkM transfer was not associated with a significant benefit in global LV EF at six months [49]. The Phase I CAuSMIC study of 23 patients (12 SkM, 11 control) with previous MI and HF, reported favorable safety outcomes twelve months after SkM delivery by transendocardial injections, with benefits to New York Heart Association (NYHA) class, Quality of Life (QOL) scoring, LV end-diastolic and end-systolic dimensions and myocardial viability compared to control [47]. Unfortunately, two larger studies, MARVEL and CAuSMIC II, that were intended to more definitively investigate transendocardial SkM delivery were never completed [50]. In the wake of the negative MAGIC trial, and mixed results from other clinical studies, interest and momentum in the SkM field seems to have waned considerably, and it appears unlikely that these cells will ever assume a significant role in the treatment of HF.

Bone Marrow Mononuclear Cells

As the most extensively characterized adult stem cell niche, the BM contains hierarchies of stem and progenitor cell subpopulations for hematopoietic, mesenchymal and proangiogenic (endothelial) lineages. Due to accessibility, cost and ease of procurement, the unfractionated mononuclear cell (MNC) compartment of freshly isolated BM aspirates has been widely used for preclinical and clinical studies of MI and HF. BM MNCs can be readily separated from BM aspirates by manual or automated density gradient centrifugation, resulting in a heterogeneous preparation that contains a small minority of hematopoietic stem/progenitor cells (HSCs), $CD34^+$ and $CD133^+$ proangiogenic progenitor cells, and MSCs, along with more prevalent monocytes, lymphocytes, nucleated red cells, and immature B- and T-cell precursors.

Interest in the use of BM cells for cardiovascular repair was first sparked by a seminal study in mice that claimed that BM-derived HSCs could transdifferentiate into cardiomyocytes *in vivo* and thereby regenerate infarcted myocardium within a short time of cell transfer [8]. Although subsequent studies contended the validity of actual cardiomyocyte transdifferentiation [51], other data quickly emerged show-casing the capacity of BM-derived cells to support myocardial perfusion and stimulate endogenous myocardial repair processes [9, 52]. Freshly isolated BM MNCs were soon used in numerous human studies of cell therapy for acute MI, where the time required to culture autologous stem cell populations (e.g. MSCs) would preclude early cell delivery. These studies provided crucial reassurance of the safety of BM cell therapy, ruling out adverse outcomes such as arrhythmia, myocardial calcification, tumorigenesis, coronary restenosis and systemic inflammation. However, initial promise of the effectiveness of BM MNCs in the setting of acute MI [53] has not been upheld by recent randomized controlled or meta-analysis data [54, 55], and definitive results are now awaited from the large-scale, multicenter Phase III BAMI trial (NCT01569178) which is currently recruiting patients in Europe.

The ability of BM MNCs to facilitate new collateral blood vessel formation also has therapeutic appeal for HF. This most obviously applies to cardiomyopathy due to chronic myocardial ischemia and hibernation, but is also relevant to LV dysfunction from old infarct-related scar or nonischemic causes, where good vascular supply is essential for the recovery of injured cardiomyocytes and the viability of newly generated ones [56]. In different animal studies of post-MI cardiac dysfunction, direct injection of fresh autologous BM cells into scar tissue has had contrasting results of efficacy [57, 58], and this has also followed into the clinical setting. In the first non-randomized study of 21 patients with chronic ischemic cardiomyopathy (mean LV EF ~20%), Perin *et al.* used the NOGA® electromechanical navigation system to transendocardially deliver autologous BM MNCs into ischemic regions of myocardium which had preserved unipolar voltage amplitude and viability [59, 60]. After four months, cell treatment (n=14) was associated with improvement in reversible myocardial perfusion defects, regional and global LV systolic function, and clinical angina scores compared to the non-treatment control group (n=7) [59]. Although benefits to symptom burden and myocardial perfusion were sustained at one year, the improvement in LV EF was no longer significant [60]. In subsequent small, non-controlled studies, autologous BM MNC delivery appeared to have beneficial outcomes when cells were delivered into viable peri-scar myocardium [61], but not when injected directly into scar itself [62].

Intracoronary infusion was used as the delivery modality for autologous BM MNCs in the TOPCARE-CHD registry, involving patients with LV dysfunction due to an MI at least three months earlier [63]. In the first publication resulting from these data, a controlled-crossover design was used to compare treatment with no cells (n=23) to either administration of ~$2x10^8$ BM MNCs (n=28) or ~$2x10^7$ circulating progenitor cells (CPCs) (n=24). Three month analysis showed that BM MNCs led to an improvement in cardiac function in the order of 3 absolute LV EF units, which was not seen in the two other groups [63]. Contractile benefit from BM MNCs also involved enhanced regional wall function in the myocardial territory supplied by the infused coronary artery, and was also observed in patients who crossed over from the initial control or CPC arms. In a follow-up report of 121 consecutive patients enrolled into this registry, treatment benefit from BM MNCs was shown to also include reductions in serum levels for natriuretic peptides and all-cause mortality rate. The latter was particularly observed in those patients who had received a high percentage of BM progenitor cells with colony-forming capacity, a biological measure of "stemness" [64]. Other data from the TOPCARE-CHD cohort showed that BM MNC infusion also improved measures of cardiopulmonary exercise performance, particularly

in those patients who had the lowest baseline levels of exercise capacity prior to cell therapy [65].

More recently, the same investigators published the Phase I/II placebo-controlled CELLWAVE trial which studied the utility of shock wave pretreatment given 24h prior to intracoronary BM MNCs in 103 patients with chronic HF [66]. The purpose of shock wave therapy was to induce production of chemokines and homing signals in the treated myocardium to augment the retention of infused cells, which is otherwise consistently low. The combination of extracorporeal ultrasound shock wave treatment and BM MNCs resulted in a 3.2% absolute increase in LV EF after four months, which was significantly higher than the change in EF observed after shock wave and placebo infusion. In addition, the combination treatment group had significantly fewer major adverse cardiac events (hazard ratio 0.58) than did recipients of placebo shock wave and BM MNCs or active shock wave and placebo infusion.

As has been the case for acute MI, beneficial effects of BM MNCs have not been universally shown in other clinical studies of ischemic HF. In contrast to the German-based TOPCARE-CHD and CELLWAVE studies, the US Cardiovascular Cell Therapy Research Network (CCTRN) implemented the closed automated SEPAX system to procure density gradient separated BM MNCs for their multicenter cell therapy studies. This was done to standardize the cell preparation process across different study sites. The phase II FOCUS-CCTRN trial randomized 92 patients with LV EF ≤45%, a perfusion defect by single-photon emission computed tomography and coronary artery disease not amenable to revascularization, to transendocardial injection of 10^8 autologous BM MNCs or placebo [67]. Cell delivery resulted in no significant difference in the prespecified endpoints of LV end-systolic volume, maximal oxygen consumption, or myocardial reversible perfusion at six months, although exploratory analysis hinted at improvement in the non-prespecified outcomes of stroke volume and LV EF, especially in those who received a higher number of $CD34^+$ and $CD133^+$ progenitor cells.

Fewer clinical studies have specifically addressed the use of BM MNCs for non-ischemic HF. In the First-in-Man ABCD trial, 44 patients with severe dilated cardiomyopathy (EF≤35%) were randomized to non-placebo control or intracoronary delivery of BM MNCs [68]. The cell therapy group had an absolute increase in mean LV EF of 5.4% after six months, accompanied by an improvement in functional class of HF. Intracoronary infusion of BM MNCs was also associated with benefits in the TOPCARE-DCM pilot study of 33 patients, relating to regional wall contraction, microvascular function and serum levels of N-terminal pro-brain natriuretic peptide [69].

Overall, the use of BM MNCs in HF patients has met with inconsistent results. This firstly reflects the diversity in trial design and methodology between different studies (e.g. TOPCARE-CHD and FOCUS-CCTRN), encompassing differences in patient selection criteria and disease substrate, cell preparation, cell dose, delivery route, endpoints and their assessment. Secondly, there is the inherent heterogeneity of freshly isolated autologous BM MNCs. This has been highlighted by several publications, including a recent analysis of the CCTRN's biorepository made up of patient samples from its first series of MI (TIME, LateTIME) and HF (FOCUS-CCTRN) studies [70]. Improvement in LV EF was found to correlate positively with the $CD34^+$ progenitor cell content of BM, and negatively with the percentage of $CD11b^+$ monocytes and macrophages. These results speak to the importance of the quality of the BM cell product, and in particular its biologically active component of progenitor cells. This may be influenced by a variety of technical factors relating to BM aspiration, cell processing, density separation, red blood cell contamination and presence of anticoagulant (e,g. heparin), along with patient characteristics, such as age, comorbid status, risk factors, and medication usage [71-73].

The variability of BM MNCs can in part be overcome by using cultured BM cells, or alternatively more enriched progenitor/stem populations from BM or peripheral blood, such as proangiogenic progenitor cells or MSCs. Ixmyelocel-T is a culture-expanded, multicellular, autologous product from BM, that is derived using an automated, fully closed, cell processing system. The patented culture technique achieves depletion of lymphocytes and granulocytes from the starting MNC population, and amplification by several hundred fold for mesenchymal cells, monocytes and alternatively activated (M2) macrophages that are collectively purported to be helpful for tissue repair, angiogenesis and remodeling of fibrosis. This cell preparation has been used in the recently published IMPACT-DCM (NCT00765518) and ongoing (IDCM)-ixCell-DCM (NCT01670981) trials. IMPACT-DCM consisted of two randomized studies that administered Ixmyelocel-T cells intramyocardially by minithoracotomy or NOGA®-guided injection to patients with ischemic or nonischemic cardiomyopathy [74]. In total, 61 patients received cell treatment and 59 standard of care. Only the ischemic patients experienced a cell-related benefit in terms of fewer adverse cardiovascular events during follow-up, as well as improved NYHA class, 6-minute walk distance and Minnesota Living with Heart Failure Questionnaire scores.

Proangiogenic Progenitor Cells

Landmark descriptions of angiogenic endothelial progenitor cells (EPCs) isolated from peripheral blood and BM first appeared in the late 1990s [75]. Over the following two decades, a large body of research has been devoted to studying different subpopulations of these cells and their roles in physiological and pathological neovascularization, as well as their therapeutic potential for tissue repair. Various culture-based and immunoselection techniques have been used to isolate and characterize EPCs, including by detection of surface markers (e.g. CD34, CD133, VEGFR2) that are not definitive as they are shared between hematopoietic and endothelial cell lineages. Consequently, it has become apparent that many of the versions of EPCs that have been labeled as such, are in fact not *bona fide* progenitor cells for endothelial lineage, but rather hematopoietic subpopulations which possess angiogenic properties [76]. Nevertheless, progenitor cells expressing markers such as CD31, CD34 and CD133 have shown their ability to improve myocardial perfusion and function in preclinical models of ischemia and cardiac dysfunction, both by directly incorporating into newly developed blood vessels, and through the production and release of angiogenic cytokines [9, 77].

In the clinical setting, several studies have been published in the past two years pertaining to the use of autologous $CD133^+$ or $CD34^+$ progenitor cells in HF. In the double-blinded CARDIO133 trial, 60 patients with ischemic cardiomyopathy (LV EF <35%) were randomized to receive transepicardial injections of placebo or BM-derived $CD133^+$ cells into the hypokinetic infarct border zone, at the time of CABG [78]. Using cardiac magnetic resonance, no intergroup difference was observed in the primary endpoint of change in LV EF, nor in systolic or diastolic LV dimensions after six months. Similarly, cell therapy did not improve symptom class, walking distance or quality of life measures, although there was beneficial signal relative to the placebo group for scar mass, and segmental myocardial perfusion.

Recent studies by Vrtovec *et al.* have provided more encouraging results for the use of peripheral blood $CD34^+$ cells mobilized by granulocyte-colony stimulating factor and collected via apheresis. In a prospective crossover study of ischemic HF (n=33, NYHA III symptoms, LV EF <40%), patients were first stabilized on optimal pharmacotherapy for six months, before receiving an average of ~$9x10^7$ cells by NOGA®-guided catheter-based injections into segments of ischemic, hibernating myocardium [79]. Six months after cell transfer, substantial increments were seen in mean LVEF (+7.8%) and 6-minute walk distance (+85m), and these improvements were greatest in recipients of higher cell doses and more diffusely distributed cell injections.

Nonischemic cardiomyopathy is also associated with vascular flow abnormalities, and administration of $CD34^+$ cells has been accompanied by considerable benefits in this disease context too. In one series, cells delivered by the intracoronary route led to a 5% absolute improvement in LV EF and 126m increase in 6-minute walk distance, along with a significant reduction in 1-year mortality compared to the non-placebo control arm [80]. Follow-up data collected from 110 patients at 5 years, showed that these benefits were durable with rates of mortality (14% versus 35%) and pump failure (5% versus 18%) that were still much lower in cell recipients than controls [81]. In another study from the same group, transendocardial injection was shown to be more effective than intracoronary dosing, resulting in higher myocardial retention of $CD34^+$ cells (19.2% vs 4.4% at 18h), and greater improvements in LV function, N-terminal pro-brain natriuretic peptide and exercise capacity in patients with nonischemic HF [82].

Mesenchymal Stromal Cells

MSCs are a rare subset of non-hematopoietic progenitor cells that have a near ubiquitous tissue distribution, although they have been most widely studied in BM, where they contribute fundamentally to the hematopoietic stem cell niche [83]. They are usually isolated from the mononuclear fraction of their source tissue by their adherence to tissue culture plastic [84], or by antibody-based selection of their more primitive mesenchymal precursor cell (MPC) ancestors [85, 86]. During standard culture expansion they express a range of non-specific surface antigens (e.g. CD105, CD73, CD90) but are negative for HLA-DR, endothelial (CD31) and hematopoietic markers (e.g. CD45, CD34, CD14, CD11b). Although classically defined by their trilineage differentiation potential for bone, cartilage and fat, their multipotency also extends to other lineages and they can be stimulated *in vitro* to adopt properties of cardiomocytes, smooth muscle and endothelial cells. MSCs possess several other characteristics that make them attractive for use in tissue repair: (1) accessibility and ease of isolation from BM and adipose in particular; (2) proclivity for expansion in culture to high numbers; (3) immunoprivileged, immunosuppressive and anti-inflammatory disposition, allowing potential avoidance of host rejection after allogeneic transplantation; (4) extensive paracrine secretome and provision of support to other tissue-resident cells; and (5) propensity for stable manipulation *in vitro*, by cytokine/growth factor preconditioning or genetic engineering to augment reparative effectiveness [83, 87, 88].

Based on the above, autologous, allogeneic and xenogeneic MSCs and MPCs have been widely studied in different preclinical cardiovascular disease models, including chronic post-MI cardiac dysfunction [89], hibernating ischemic cardiomyopathy [90] and nonischemic cardiomyopathy [91]. These studies have corroborated the pleiotropic ability of MSCs to improve LV contractile and diastolic function, reverse or attenuate LV remodeling, reduce collagen deposition and myocardial fibrosis, and increase vascularity and perfusion. Although some groups have presented evidence that transplanted MSCs can do so by differentiating into cardiac-relevant cells *in vivo* [89, 90], others have emphasized the importance of their paracrine capacity to modulate myocardial inflammation, angiogenesis and extracellular matrix remodeling and facilitate endogenous cardiac repair by supporting mature cardiomyocytes and stimulating the proliferation and differentiation of CSCs [85, 91-93].

Hare *et al.* have been especially prominent in conducting preclinical and clinical studies of plastic adherence-isolated MSCs in HF. In the TAC-HFT study (Transendocardial Autologous Cells in Ischemic Heart Failure Trial) patients with a past history of MI and residual LV EF <50% were randomized to percutaneous transendocardial delivery of autologous BM MSCs (n=19), BM MNCs (n=19) or placebo, using ten myocardial injection sites [94]. Although cell transfer was not associated with significant benefits to LV volume or EF, both active treatment groups had better HF scores than their respective placebo arms after one year, and MSCs but not MNCs also significantly improved infarct size, regional myocardial function and 6-minute walk distance. These particular data therefore indicated the greater scope of benefit that may be achievable with culture-expanded MSCs compared to freshly isolated MNCs, whose limitations are described above.

The POSEIDON study (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis) compared between autologous and allogeneic BM MSCs administered in three different doses (20, 100 or $200x10^6$ cells) by catheter-based injection into infarct-border zones of patients with ischemic cardiomyopathy [95]. There were only five patients enrolled in each group, limiting the study's ability to detect significant differences. However, 1-year rates for serious adverse events, including arrhythmias, were numerically lower in recipients of allo-MSCs, and these patients also did not show evidence of an increased immune response, supporting the safety of allogeneic transplantation. Without available pla-

cebo comparisons, pooling of data from all MSC groups suggested that cell transfer did reduce LV volumes and scar size relative to baseline, as well as improve functional capacity and QOL scores, but not global LV EF. Subsequent imaging analysis highlighted that contractile benefit and scar size reduction were highest for segments where cells were actually injected, compared to non-injected sites [96].

Finally, the Phase I/II PROMETHEUS study set out to investigate transepicardial surgical injection of autologous MSCs into akinetic/hypokinetic myocardial segments that were not being revascularized at the time of CABG [97]. Although the study was prematurely suspended, published results from 6 patients suggested that LV EF improved by 10% after 18 months, with cardiac magnetic resonance showing that cell-treated segments had greater improvement in the end-points of scar size, perfusion and regional contraction. Taken together, these three small studies have each indicated benefits from intramyocardial BM MSC injection, primarily at sites of cell transfer.

Alternative mesenchymal cell preparations are also under clinical investigation for HF. The stromal vascular fraction of adipose tissue is much more enriched with MSCs than are BM MNCs, and can be easily isolated by liposuction. Transplantation of a monolayered sheet of adipose-derived MSCs reversed thinning of myocardial scar and improved contractile function in a rodent model of chronic MI [98]. Perin *et al.* administered transendocardial injections of autologous adipose-derived regenerative cells (ADRCs) to 21 no-option patients with ischemic cardiomyopathy in the PRECISE trial [99]. Compared to a small control group, ADRCs were well tolerated and led to significant improvements in maximal oxygen consumption, total LV mass and wall motion score index after 18 months.

An excellent example of translation from bench to bedside has been the discovery and refinement of cardiopoietic MSCs. Work from the Mayo Clinic, USA, first identified combinations of key signaling factors and mediators (e.g. transforming growth factor-β1, bone morphogenetic protein 4, activin A, retinoic acid, insulin-like growth factor-1, fibroblast growth factor-2, alpha-thrombin, interleukin-6) that can induce BM MSCs toward cardiac specification *in vitro* [100]. Durable effectiveness of human cardiopoietic MSCs was shown by Behfar *et al.* in a model of chronic MI in nude mice, in which these cells exhibited greater levels of *in vivo* engraftment, cardiomyocyte transformation and paracrine stimulation of endogenous c-kit$^+$ CSCs than did undifferentiated MSCs [101]. The long-term safety of these cells was then shown in the phase II C-CURE trial [102]. 21 patients with severe chronic ischemic cardiomyopathy were treated with NOGA®-guided injections of cardiac-specified autologous MSCs (0.6 to 1.2x10^9) and this led to marked improvements in LV EF (+7%), LV volumes and 6-minute walk distance, compared to no improvement in these parameters in patients who received standard medical care. Evaluation of allogeneic cardiopoietic MSCs, marketed as C3BS-CQR-1 (Cardio3 BioSciences), is now underway in the phase III CHART-1 study of 240 patients with advanced ischemic HF, using the novel C-Cath® injection catheter (www.clinicaltrials.gov NCT01768702). Various MSC preparations are being investigated in other ongoing trials of ischemic and nonischemic HF.

Cardiac-Derived Stem Cells

The discovery of resident stem cells in the heart has led to a second generation of studies involving their isolation, amplification and therapeutic administration back to the injured myocardium [103, 104]. Among the cardiac stem cell populations that have been identified in different mammalian species are: Islet-1$^+$ (Isl-1$^+$) [105], c-kit$^+$ [106, 107], Sca-1$^+$ [108] and Sca-1$^+$PDGFRα$^+$ CSCs [109]; side population (SP) CSCs that can efflux the dye, Hoechst 33342 [110]; epicardium-derived cells (EPDCs) [111]; and CDCs [112]. Each of these has displayed characteristics of self-renewal, clonogenicity and multilineage differentiation potential. While c-kit$^+$, Isl-1$^+$, Sca-1$^+$ and SP CSCs have shown plasticity for cardiomyocytes, endothelial cells, vascular smooth muscle cells and in some cases cardiac conduction tissue, the other subtypes (Sca-1$^+$PDGFRα$^+$, EPDCs and CDCs) appear to be analogous to cardiac-resident MSCs. Thus far, the greatest body of work has been done for c-kit$^+$ CSCs and CDCs which have both been progressed from preclinical models of MI and HF [107, 113] to be studied in human patients with ischemic HF [103, 104].

CSCs have been shown to have diminished functionality in patients with end-stage post-MI cardiomyopathy [114]. Their transplantation in small and large animal models of chronic MI has resulted in augmentation of myocardial function, mitigation of adverse remodeling and reduction of scar [115, 116]. However, some data have brought into question their actual capacity for cardiomyogenesis [117], and the extent to which they can contribute directly to cardiomyocyte replacement [118]. Therefore, like non-cardiac stem cells, at least some of their salutary effects for cardiovascular repair have been attributed to paracrine effects [115]. Based on the encouraging results shown in preclinical studies, autologous c-kit$^+$ CSCs were studied in the phase I SCIPIO trial (Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy) [103, 119]. Cells were procured and expanded from samples of right atrial appendage taken at the time of bypass surgery in patients with LV EF <40%. At an average of 4 months after CABG, up to one million CSCs were administered by the intracoronary route in 20 patients. Preliminary results showed the feasibility and safety of this strategy. Furthermore, unlike a non-placebo control group (n=13), recipients of CSCs showed substantial early improvement in global EF (+7%) and regional wall motion score for all myocardial segments, which were sustained in those patients followed out to 1 and 2 years. Additional benefits were shown for NYHA functional class, QOL scoring, scar size and viable myocardial mass [103, 119]. Positive preliminary data have also been presented for another Phase I study (ALCADIA, NCT00981006), in which autologous CSCs are being injected with adjuvant basic fibroblast growth factor. However, promise in the CSC field has been overshadowed in recent times by concerns as to the scientific integrity of some early preclinical data, along with criticisms relating to the study design and randomization of patients in SCIPIO [120].

Cardiospheres represent an undifferentiated population of self-adherent, heterogeneous cells obtained after enzymatic digestion and subculture of explanted cardiac tissue (e.g. from surgical or endomyocardial atrial or ventricular biopsies) [112]. Soon after their initial description, a plating method was developed to yield CDCs from human and porcine samples [121]. These cells showed *in vitro* plasticity to form electrically stable myocytes when co-cultured with rat ventricular cardiomyocytes, and when injected into a murine infarct model promoted cardiac regeneration and improved LV EF. Subsequently, confirmation of their safety and reparative potential was demonstrated after intracoronary infusion and intramyocardial injection in porcine studies of chronic MI [113, 122], and more recently in a transgenic mouse model of nonischemic cardiomyopathy [123]. The mechanisms of these benefits are again likely to be pleiotropic. Cardiospheres and CDCs actually contain a mixture of different cell types, comprising endothelial cells, a small percentage of stem cells that express c-kit, Sca-1 and CD34, and a large majority of mesenchymal ($CD90^+$, $CD105^+$) cells. Not surprisingly, their therapeutic potential is now predominantly attributed to their paracrine activity, which is mediated by production of soluble mediators and by cell-to-cell contact [124, 125]. In recent head-to-head comparison studies, the paracrine reparative properties of CDCs were found to be superior to those of BM MSCs, adipose MSCs and BM MNCs [126], and moreover augmented in CDCs obtained from patients with chronic ischemic HF, as compared to normal donors [127]. Furthermore, as an extension of their MSC-like properties, cardiospheres and CDCs also display immunotolerance and anti-inflammatory effects. This has enabled their allogeneic use in animal studies, without incurring significant rejection despite the absence of immunosuppressive treatment [128, 129].

Following comprehensive preclinical evaluation, the phase I CADUCEUS trial (CArdiosphere-Derived aUtologous Stem CElls to reverse ventricULar dySfunction) investigated intracoronary CDC delivery in a cohort of 17 patients with reduced LV EF up to 3 months after MI [104]. CDCs were obtained and prepared from endomyocardial biopsies, and expanded to allow comparisons of three doses (12.5, 17.3, 25 $x10^6$ cells). CDC infusion was well tolerated without significant adverse outcomes. Compared to standard of care (n=8), CDCs did not result in significantly better outcomes for LV EF, LV volumes, functional class or quality of life, but were associated with less scar burden, more viable LV mass and increased regional wall contraction after 6 and 12 months [130]. The double-blind, placebo-controlled, phase II ALLSTAR trial (ALLogeneic heart STem cells to Achieve myocardial Repair, n=274 patients, www.clinicaltrials.gov NCT01458405) and phase I DYNAMIC study (Dilated cardiomYopathy iNtervention with Allogeneic MyocardIally-regenerative Cells, NCT02293603) are now evaluating allogeneic CDCs, marketed as CAP-1002 (Capricor Therapeutics^{TM}) in patients with ischemic cardiac dysfunction and nonischemic HF, respectively.

Overview of Clinical Data

Until recently the majority of cardiovascular cell therapy studies were performed in the setting of acute MI. Yet given its high mortality, morbidity and economic burden, it is arguably HF that carries the greater need for new therapeutic options, such as stem cell delivery. Preclinical evaluation of cell transplantation for chronic ischemic or nonischemic HF has been more challenging than for acute MI, as the relevant animal models are more time consuming and costly to establish, and less reproducible than those for acute coronary ligation or ischemia/reperfusion. Pleasingly over the past few years there has been something of a "catch-up" in the number of trials completed and registered for patients with established LV dysfunction and HF (Table **1**). After the initial emphasis on SkMs and BM MNCs, focus is shifting to the investigation of more specialized stem/progenitor cell populations, namely proangiogenic progenitor cells, MSCs and cardiac-derived stem cells (Table **2**). Given the typically small size of individual studies, not to mention their high degree of heterogeneity in terms of design, it has been difficult to draw general conclusions about the effectiveness of cell therapy in the HF setting. Furthermore, attention has been recently drawn to the high number of reporting discrepancies in cardiac cellular studies which may lead to confounding bias [120].

Among several meta-analyses that have evaluated cell therapies for HF, the most comprehensive and rigorous was recently published by Fischer *et al.*, who performed a systematic review of 31 randomized controlled trials (1521 participants: 882 cell-treated, 639 control) conducted in the presence of ischemic (28 trials) or nonischemic HF (3 trials) [131]. Trial sizes ranged from 14 to 120 randomized patients with duration of follow-up between 3 months and more than 5 years. 21 of the included trials administered BM MNCs, G-CSF mobilized MNCs or some subpopulation thereof (e.g. $CD34^+$, $CD133^+$, alcohol dehydrogenase-positive cells), 4 used BM MSCs, 4 SkMs, 1 CSCs and 1 ADRCs. Cell dose varied from $5x10^5$ for CSCs to as many as $8.4x10^8$ cells for BM MNCs and SkMs. Fifteen of the 31 trials had a placebo arm, 12 used the intracoronary route of cell delivery and the other 19 used intramyocardial injection. The primary composite endpoint of death and rehospitalization for HF was shown to be significantly improved in the cell therapy group, with a 52% mortality reduction at >12 month follow-up, and a 61% risk reduction for rehospitalization, compared to control [131]. The risk reduction for mortality did not differ between trials on the basis of route of stem cell administration or baseline cardiac function. Stem cell treatment was also associated with significant improvements for the secondary endpoints of LV EF, quality of life, exercise capacity and performance status, along with brain natriuretic peptide levels, without increasing the risk of arrhythmias. This meta-analysis was notable for performing a thorough evaluation of risk of performance, selection and reporting bias among the individual trials, which was found to be considerable. When only double blinded trials (n=5) with a low risk of performance bias were included, the beneficial effects of cell therapy on long-term mortality risk and short- and long-term NYHA class and LV EF were no longer statistically significant [131].

MECHANISMS OF CELL-MEDIATED REPAIR IN HF

The elucidation of mechanisms by which different cell types exert their actions in HF is a challenging task whose importance cannot been undersold, as it provides insights

Table 1. Selected randomized controlled clinical trials of cell therapy for cardiomyopathy and heart failure.

Study ID	Disease	Cell Type	Cell Dose	Delivery Route	Patient Number	Follow-up	Results
MAGIC [46]	ICM	SkM	LD $4x10^8$ HD $8x10^8$	TEp IM	Intended 120; Treated 97 LD 33, HD 34, Pl 30	6m	↔ EF, RWM ↓ ESV, EDV (HD) Arrhythmias
CAuSMIC [47]	ICM	SkM	0.3, 1, 3, $6x10^8$	TEn IM	Rx 12, Cont 11	12m	↑ QOL, viability ↓ NYHA, LV dimensions Arrhythmias
SEISMIC [49]	ICM	SkM	1.5-$8x10^8$	TEn IM	Rx 26, Pl 14	6m	↔ EF ↑ 6MWD Arrhythmias
MARVEL [50]	ICM	SkM	LD $4x10^8$ HD $8x10^8$	TEn IM	LD 7, HD 8, Pl 8	6m	↔ QOL ↑ 6MWD (trend)
TOPCARE-CHD [63]	ICM	BMMNC / CPC	BMMNC $2.05x10^8$ CPC $22x10^7$	IC	BMMNC 28, CPC 24, Cont 23	3m	↑ EF (BMMNC only) ↓ NYHA (BMMNC only)
First-in-Man ABCD [68]	NICM	BMMNC	$1.98x10^8$	IC	Rx 24, Cont 20	6m	↑ EF ↓ ESV, NYHA
Yao *et al*. [132]	ICM	BMMNC	$7.2x10^7$	IC	BMMNC 24, Cont 23	6m	↔ EF, EDV, ESV, perfusion, infarct size ↑ Diastolic parameters
Ang *et al*. [133]	ICM	BMMNC	IM $8.4x10^7$ IC $1.15x10^8$	TEp IM or IC	IM 21, IC 21, Cont 23	6m	↔ EF, EDV, ESV, RWM, infarct size
Zhao *et al*. [134]	ICM	BMMNC	$6.59x10^8$	TEp IM	Rx 18, Cont 18	6m	↑ EF, RWM, perfusion ↓ Angina, NYHA
Pokushalov *et al*. [135]	ICM	BMMNC	$4.1x10^7$	TEn IM	Rx 55, Cont 54	12m	↑ EF, QOL ↓ NYHA, mortality
FOCUS-HF [136]	ICM	BMMNC	$3x10^7$	TEn IM	Rx 20, Cont 10	6m	↔ EF ↑ QOL, perfusion ↓ Angina
Hu *et al*. [137]	ICM	BMMNC	$1.3x10^8$	IC (graft)	Rx 31, Cont 29	6m	↑ EF, RWM, 6MWD ↓ ESV index
Turan *et al*. [138]	ICM	BMMNC	$9.9x10^7$	IC	Rx 38, Cont 18	12m	↑ EF, RWM ↓ Infarct size
FOCUS-CCTRN [67]	ICM	BMMNC	$1x10^8$	TEn IM	Rx 61, Pl 31	6m	↔ ESV index, VO_2 max, perfusion, RWM, clinical status
CELLWAVE [66]	ICM	BMMNC	~$2x10^8$	IC	21 BMMNC 21 BMMNC + LDSW 21 BMMNC + HDSW 20 Pl + LDSW 19 Pl + LDSW	4m	↑ EF, RWM ↓ Infarct size, NYHA,

(Table 1) Contd….

Study ID	Disease	Cell Type	Cell Dose	Delivery Route	Patient Number	Follow-up	Results
Pätilä *et al*. [139]	ICM	BMMNC	$8.4x10^8$	TEp IM	Rx 20, Cont 19	12m	↔ EF, RWM, viability ↓ Infarct size
IMPACT-DCM & CATHETER-DCM [74]	ICM and NICM	Ixmyelocel-T	$0.35\text{-}2.95x10^8$	TEp IM TEn IM	TEp: Rx 25, Cont 14 TEn: Rx 15, Cont 7	12m	↔ EF, ESV ↑ NYHA, 6MWD, QOL (ICM only) ↑ MACE (ICM only)
Cardio133 [78]	ICM	$CD133^+$	$5.1x10^6$	TEp IM	Rx 30, Pl 30	6m	↔ EF, LV volumes, QOL, 6MWD ↑ Perfusion ↓ LV mass
Vrtovec *et al*. [81]	NICM	$CD34^+$	$1.13x10^8$	IC	Rx 55, Cont 55	5y	↑ EF, 6MWD ↓ NT-proBNP, mortality
Vrtovec *et al*. [82]	NICM	$CD34^+$	IM $1.05x10^8$ IC $1.03x10^8$	TEn IM, or IC	Rx 20, Cont 20	6m	TEn IM superior for cell retention, EF, 6MWD, NT-proBNP
Perin *et al*. [140]	ICM	$ALDH^+$	$2.37x10^6$	TEn IM	Rx 10, Cont 10	6m	↔ EF ↓ESV
TOPCARE-G-CSF [141]	ICM	G-CSF +/- CPC	$2.9x10^7$	IC	CPC 22, G-CSF only 10	5y	↔ EF, NYHA ↑ RWM
TAC-HFT [94]	ICM	BMMNC or MSC	~$2x10^8$	TEn IM	BMMNC 19, Pl 10 MSC 19, Pl 11	12m	↔ EF, LV volumes ↑ QOL ↑ 6MWD, RWM (MSC) ↓ Infarct size (MSC)
C-CURE [102]	ICM	BM MSC-Cardio	$7.33x10^8$	TEn IM	Rx 32, Cont 15	6m - 2y	↑ EF, 6MWD, composite clinical score (6m) ↓ ESV (6m)
PRECISE [99]	ICM	ADRC	$2.4x10^5$/kg	TEn IM	Rx 21, Pl 6	6m - 18m	↔ EF, LV volumes ↑ LV mass, RWM ↓ Inducible ischemia
CADUCEUS [104, 130]	ICM	CDC	$1.25\text{-}2.5x10^7$	IC	Rx 21, Pl 6	6m - 12m	↔ EF, LV volumes, QOL ↑ Viable mass ↓ Scar size
SCIPIO [103]	ICM	CSC	$0.5\text{-}1x10^6$	IC	Rx 20, Cont 13	4 - 24m	↑ EF, RWM, QOL, viable mass ↓ Scar size

Approximation of cell dose is shown based on the mean or median value reported by each study. **Abbreviations** ↔ no change; ↑: increased. ↓: decreased; 6MWD: 6-minute walk distance; ADRC: adipose-derived regenerative cells; ALDH: aldehyde dehydrogenase; BM: bone marrow; BMMNC: bone marrow mononuclear cells; CDC: cardiosphere-derived cells; Cont: control; CSC: $c\text{-}kit^+$ cardiac stem cells; CPC: circulating progenitor cells; EF: left ventricular ejection fraction; EDV: left ventricular end-diastolic volume; ESV: left ventricular end-systolic volume; G-CSF: granulocyte-colony stimulating factor; HD: high dose; HD(LD)SW: high dose (low dose) shock wave pre-treatment; IC: intracoronary; ICM: ischemic cardiomyopathy; IM: intramyocardial; LV: left ventricular; MACE: major adverse cardiovascular events; MI: myocardial infarction; MSCs: mesenchymal stem cells; m: months; MSC-cardio: cardiopoietic MSCs; NICM: nonischemic cardiomyopathy; NT-proBNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association; Pl: placebo; Rx: active cell treatment; RWM: regional wall motion; QOL: quality of life e.g. as measured by Minnesota Living with Heart Failure scale; SkM: skeletal myoblasts; TEn: transendocardial; TEp: transepicardial; y: years.

Table 2. Randomized clinical trials of cell therapy for cardiomyopathy and heart failure, registered with www.clinicaltrials.gov.

Study ID / Site	Disease	Cell Type	Design	Planned Recruitment Number	Delivery Route	Primary End-point	www.clinicaltrials.gov Identifier
REPEAT	ICM from old MI, EF ≤45%	BMMNC	Phase II/III ROL, Single vs Two doses 4m apart	676	IC	Mortality	NCT01693042
ASSURANCE	Cardiomyopathy with LVAD, EF <30%	BMMNC	Phase I/II RDBCT	24	TEp IM	Safety, Viability, Combined clinical event rate	NCT00869024
Spain	NICM, EF <50%	BMMNC	Phase II RDBCT	51	IC	EF	NCT02033278
REGEN-IHD *	ICM	G-CSF +/- BMMNC	Phase II	148	IC or TEn IM	Safety	NCT00747708
REVIVE-1, Harvest Technologies	EF ≤40%	BM Aspirate Concentrate	Phase I ROL	30	Cor Sinus	Safety	NCT01299324
ixCELL DCM, Aastrom Biosciences	ICM, EF ≤35%	IXMYELOCEL-T	Phase II RDBCT	108	TEn IM	Combined clinical event rate	NCT01670981
PERFECT	ICM, EF 25-50%	BM $CD133^+$	Phase III RDBCT	142	TEp IM	EF	NCT00950274
AlsterMACS, Asklepios proresearch	ICM, EF ≤45%	BM $CD133^+$	Phase I/II RSB, no control	64	TEn IM or IC	EF	NCT01337011
IMPACT-CABG	ICM from old MI, EF 25-45%	BM $CD133^+$	Phase II RDBCT	20	TEp IM	MACE, Safety/arrhythmias	NCT01033617
NOGA-DCM	NICM, EF <30%	Peripheral Blood $CD34^+$	Phase II RSBCT	90	TEn IM or IC	EF and dimensions	NCT01350310
REMEDIUM	NICM, EF 20-40%	Peripheral Blood $CD34^+$	Phase II/III RDBCT, Single v Two doses 6m apart	80	TEn IM	EF	NCT02248532
MSC-HF, Denmark	EF <45%	Auto-MSC	Phase I/II RDBCT	59	TEn IM	Safety, LV function	NCT00644410
POSEIDON-DCM	NICM, EF <40%	Allo- or Auto-MSC	Phase I/II, ROL	36	TEn IM	Safety, MACE	NCT01392625
TRIDENT	ICM, EF ≤50%	Allo-MSC	Phase II, RDBCT, Dose comparison	30	TEn IM	Safety	NCT02013674
Spain	NICM, EF 20-45%	Auto-MSC	Phase I/II RDBCT	70	TEn IM	Safety, MACE, NYHA class	NCT01957826
Teva Pharmaceutical Industries	ICM and NICM	Allo-MPC (CEP-41750)	Phase III RDBCT	1730	TEn IM	HF-MACE	NCT02032004
REVASCOR, Angioblast Systems	EF <40%	Allo-MPC	Phase II, RSBCT, Dose comparison	60	TEn IM	Safety, feasibility	NCT00721045 (Unknown status)

(Table 2) Contd….

Study ID / Site	Disease	Cell Type	Design	Planned Recruitment Number	Delivery Route	Primary End-point	www.clinicaltrials.gov Identifier
CHART-1, Cardio3 BioSciences	ICM, EF ≤35%	MSC-cardio (C3BS-CQR-1)	Phase III RDBCT	240	TEn IM	Combined clinical event rate	NCT01768702
ATHENA	ICM, EF ≤45%	ADRC	Phase II RDBCT	45	TEn IM	Safety, EF	NCT01556022
RIMECARD	ICM and NICM, EF ≤45%	Umbilical cord MSC	Phase I/II RDBCT	30	IV	Safety, EF	NCT01739777
DYNAMIC	ICM and NICM, EF ≤35%	Allo-CDC (CAP-1002)	Phase I RDBCT	42	IC	Safety	NCT02293603
ALLSTAR	ICM, EF ≤45%	Allo-CDC (CAP-1002)	Phase I/II RDBCT	274	IC	Safety, immune reaction, infarct size	NCT01458405

Non-randomized studies and those with 10 or fewer patients are not included. **Abbreviations** Allo: allogeneic; Auto: autologous; BM: bone marrow; BMMNC: bone marrow mononuclear cell; CDC: cardiosphere-derived cells; Cor sinus: retrograde coronary sinus infusion; EF: left ventricular ejection fraction; G-CSF: granulocyte-colony stimulating factor; HF-MACE: heart failure-related major adverse cardiovascular events; IC, intracoronary; ICM: ischemic cardiomyopathy; IM: intramyocardial; IV: intravenous; LVAD: left ventricular assist device; MACE, major adverse cardiovascular events; MI, myocardial infarction; MPC: mesenchymal precursor cells; MSC: mesenchymal stromal cells; MSC-cardio: cardiopoietic mesenchymal stromal cells; NICM: non-ischemic cardiomyopathy; NYHA: New York Heart Association; RD(S)CT, randomized double blinded (single blinded) controlled trial; ROL: randomized open label; TEn, transendocardial; TEp, transepicardial. * For REGEN-IHD trial, interim results for a pilot phase of 59 patients have been published showing no safety differences between three treatment arms [142].

into the potential and limitations of current cell therapy options, and ways in which they may be enhanced for optimal safety and benefit in the future. Broadly speaking, the potential mechanisms of action for any cell candidate can be classified as direct or indirect (Fig. **1**). The former relates to the holy grail objective that transplanted stem cells directly replace and repopulate damaged cells in the myocardium, by proliferating and directly transforming into new, functional cardiomyocytes or vascular cells. Although this may remain a realistic goal with pluripotent ESCs and iPSCs, it is no longer plausible to attribute the salubrious effects of the other cell types so far investigated to this ambitious mechanistic pathway. As described earlier, SkMs maintain their skeletal muscle commitment after *in vivo* transfer, without expressing cardiac-specific genes or proteins [40], while there have been conflicting data for the cardiac transdifferentiation of BM MNCs [8, 51] and proangiogenic $CD34^+$ progenitor cells [143, 144]. Even in the case of MSCs, CSCs and CDCs, which have more consistent evidence for adoption of cardiomyocyte properties *in vivo* [89, 121, 145], it is now generally conceded that this is a less important contributor to overall effect than these cells' paracrine actions [93, 116, 146]. This is firstly because the induced cardiac fate of these cells has typically been shown to be incomplete, resembling the size and phenotypic characteristics of fetal cardiomyocytes more so than adult cells. Secondly, on account of their early washout and subsequent attrition by ischemia, inflammatory clearance, oxidative damage and apoptosis, the retention and engraftment of transplanted cells in the host myocardium is notoriously poor. By numerical considerations alone, their contribution to new cardiac cell mass is therefore disproportionately small compared to the functional benefits that have been reported in many studies, and the enormous burden of lost cardiomyocytes that underpins cardiomyopathy.

The indirect effects of cells on the heart and its vasculature have been shown to be mediated by both cell-to-cell contact and the production and release of a broad range of paracrine signals, comprising microRNAs, transcription factors, cytokines, chemokines, growth factors and exosomes [92]. These may be influenced by cell-specific factors (e.g. cell type, tissue and donor source, culture status), as well as mechanical and biological factors in the recipient myocardial environment. Paracrine interactions have been shown to bring about myocardial restoration and repair through a diverse array of salutary processes that consist of: (1) activation of the proliferation and differentiation of endogenous cardiac progenitor cells [93, 101, 115]; (2) stimulation of neovascularization by the release of chemokines and proangiogenic factors to recruit and support endogenous endothelial cells and their progenitors [128, 147]; (3) inhibition of apoptosis [148] and cardiomyocyte hypertrophy [149]; (4) immune regulation and suppression of inflammation, such as through the polarization of macrophages to a reparative M2 phenotype [150]; (5) modulation of extracellular matrix remodeling, collagen deposition and fibrosis [149, 151]; and (6) positive effects on myocardial metabolism and electrophysiology [152, 153]. Notably many of these restorative effects have also been observed after administering conditioned media produced by cells in culture, or purified forms of their soluble products or exosomes [86, 93, 154, 155].

Key Considerations and Unanswered Questions

The effectiveness of cell therapy is intuitively dependent on the degree to which transplanted cells are retained and ultimately engraft in the target myocardium. This is influenced by the dose and biological properties of the cells, including their homing capacity, viability and durability to withstand ischemia, oxidative stress and inflammatory sig-

nals in the cardiac microenvironment, as the well as the method of cell delivery. Regrettably after 15 years of preclinical and clinical investigation, the questions of optimal cell type, dose and delivery route to treat different cardiovascular diseases remain essentially unanswered. Up until this point, relatively few groups have invested their energies to resolve these uncertainties by performing simple but carefully-designed comparison studies. Instead, there has been an ever growing wave of new publications reporting more and more modifications and refinements to the stem cell armamentarium. This has reflected admirable levels of scientific ingenuity, and has unquestionably led to important increments in knowledge in the cell therapy field. However, it has also arguably distracted researchers from addressing more fundamental and basic questions that are pivotal to advancing cell-based treatments to the clinical realm.

OPTIMAL CELL TYPE

It is quite remarkable that so many cell types, with markedly distinct biological characteristics and functions, have been able to impart reparative effects in animal models of heart disease. Where direct comparisons have been performed, differences have certainly been observed between cell candidates in their homing ability, paracrine capacity and overall therapeutic effect. However, the main results from these studies have not been consistent with each other, and it remains impossible to draw definitive conclusions [58, 94, 126, 156-159]. This is particularly exemplified by the comparison of freshly isolated BM MNCs with culture-expanded MSCs, while there are further layers of complexity also introduced by considering that reparative function is influenced by the source of MSCs (e.g. BM versus adipose versus cardiac) [160] and their mode of isolation (plastic adherence versus immunoselection of MPCs) [85]. Moreover, for every cell population studied, there needs to be careful delineation of its dose-response relationship, and ideally this should be carried through to comparison studies between different cell types, so that they are actually being compared at equipotent doses.

Over time the limitations of SkMs, and to some degree BM MNCs, have declared themselves in clinical trials. More attention has shifted to fractionated progenitor cell populations from BM, blood and adipose, as well as cardiac-derived cells, while efforts have continued to intensify in the quest to make pluripotent stem cells clinically useable. Owing to their ability to evade or modulate the immune system, MSCs have earned their status as the prototype for allogeneic cell therapy [83]. A recent meta-analysis involving 82 preclinical studies for IHD showed similar improvement in mean LV EF for autologous (n=981 animals) and allogeneic (n=331) cell therapies, in the order of 7-8% [161]. There is now considerable scientific and commercial interest in the large-scale preparation of donor banks of allogeneic MSCs, MPCs and CDCs to provide "off-the-shelf" cell products with immediate availability, and this is reflected by a growing number of clinical trials that are investigating these cells in acute MI and HF.

Another conceptual strategy is to administer complementary cell types in combination to achieve an additive or synergistic increment in therapeutic effect compared to single-cell therapy. The best example of this has been the inclusion of progenitor cells with proangiogenic, trophic or immunomodulatory properties (e.g. MSCs, $CD34^+$ cells) to support the viability, engraftment and differentiation of cells that are more intrinsically myogenic (e.g. SkMs, CSCs) [162, 163]. This approach was used by Williams *et al.* who delivered human MSCs ($2x10^8$) and c-kit^+ CSCs ($1x10^6$) in combination to immunosuppressed swine 14 days after anterior MI [163]. By comparison to delivery of each cell type in isolation, combination therapy resulted in a seven-fold increase in donor cell engraftment, along with a marked reduction in infarct size and improvement in LV chamber compliance and contractility.

Optimal Cell Dose

A prerequisite of introducing any new therapy into clinical medicine is to rigorously assess and elucidate its dose effectiveness and safety profiles. The ability to do this definitively with biological therapies, such as stem cells, is challenged by their heterogeneity. This is most relevant for autologous cell therapy, where it is virtually impossible to standardize the quality of cell product and its content of functional progenitor cells. Robust and instructive data for dose comparisons are again in short supply. Among studies described in the sections above, the MAGIC [46], POSEIDON [95] and CADUCEUS [104] trials used a dose comparison design. Although it seems intuitive that higher doses of progenitor cells may result in more engraftment and by extension greater therapeutic benefit, this is by no means a given. Inverse relationships between cell dose and efficacy have been suggested by the results of the POSEIDON trial [95], and two ovine studies in which allogeneic STRO-3 selected MPCs were administered after induction of acute MI [151, 164]. One possible explanation for this, is that some cell types have a ceiling dose, beyond which they saturate and compromise the vascular and nutritional supply of the recipient myocardium. Results are now awaited from the ongoing REVASCOR trial (NCT00721045) which is assessing the transendocardial delivery of three doses (25, 75 and $150x10^6$) of allogeneic MPCs in patients with HF, and further dose comparison studies will hopefully follow.

An important upshot of the advent of allogeneic cell therapy is the opportunity it will provide for repeated cell dosing. Until now, preclinical and clinical studies of cardiovascular cell transfer have almost exclusively administered only a single cell dose, yet it seems unrealistic to expect that this would be sufficient to impart a significant, long-lasting effect on chronic disease conditions, like cardiomyopathy. This is especially given the suboptimal retention and engraftment of cells achievable with current delivery strategies, described below. The limited experience that is available for repeated cell delivery in preclinical models of MI has tended to indicate superior efficacy [165, 166], although a Danish study of 32 patients with ischemic HF failed to show benefit from intracoronary BM cell infusion given over two doses, four months apart [167]. Nevertheless the strategy of repeated cell dosing is worthy of future evaluation, and is being implemented in ongoing trials (Table **2**).

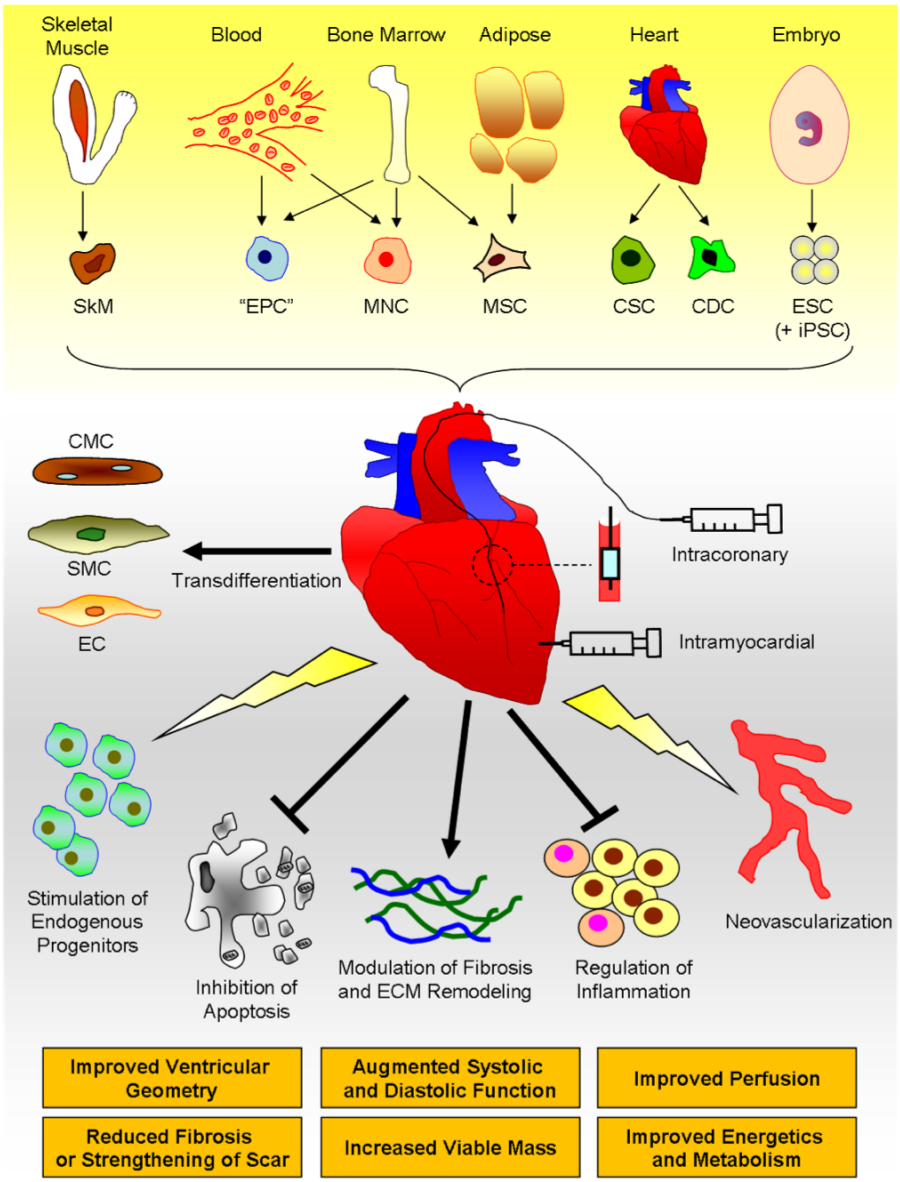

Fig. (1). Stem Cell Therapy for Heart Failure: cell types, delivery methods, mechanisms of action and beneficial effects.
Schematic depicting the main tissue sources and stem cell types that are under investigation for cardiomyopathy and heart failure. In this setting, cell delivery has been achieved predominantly by intracoronary infusion or direct intramyocardial injection, either transendocardially or transepicardially. The pleiotropic effects of cell transplantation are mediated by indirect and direct mechanisms of action, and provide benefit to myocardial structure, function, perfusion, fibrosis and metabolism. Abbreviations: CDC, cardiosphere-derived cells; CMC, cardiomyocytes; CSC, cardiac stem cells; EC, endothelial cells; ECM, extracellular matrix; EPC, endothelial progenitor cells, or more aptly proangiogenic progenitor cells; ESC, embryonic stem cells; iPSC, induced pluripotent stem cells; MNC, mononuclear cells; MSC, mesenchymal stromal cells; SkM, skeletal myoblasts; SMC, smooth muscle cells.

OPTIMAL DELIVERY ROUTE

The basic routes for cell administration to the heart can be summarized as: (1) systemic delivery by intravenous injection and/or growth factor mobilization, (2) regional infusion into one or more of the coronary arteries or veins, and (3) segmental injection directly into the myocardium either transendocardially or transepicardially. Although adopted occasionally after acute MI [168], the systemic route of delivery is not ideally suited to chronic HF, which lacks the upregulation of homing factors and chemoattractants required to recruit cells from the peripheral circulation into the myocardial territories at need.

Intracoronary injection provides higher first-pass delivery to the heart than systemic therapy. The usual strategy has been to infuse cells through the central lumen of an over-the-wire balloon catheter during brief balloon occlusions. These are designed to transiently stop coronary flow to limit antegrade washout of cells, and allow them enough time to extravasate through the microcirculation into the myocardial interstitium. By comparison to intramyocardial injection, the

coronary route has the advantages of lower cost and procedural time, less invasiveness and greater practicality and in turn accessibility for wider practice. While it theoretically distributes cells over a wider territory supplied by the treated coronary artery, they are still required to exit the circulation and mobilize toward homing factors expressed or released in the damaged myocardium. Cases of chronic total occlusion that have failed revascularization obviously preclude effective intracoronary delivery of cells to the downstream myocardium. Recently De Rosa *et al.* published an analysis of periprocedural complications and 30-day outcomes for 775 consecutive procedures in which the stop-flow technique of intracoronary administration was used for cell delivery. This included 562 patients with ischemic HF and 87 patients with nonischemic HF [169]. Overall safety results were acceptable with no procedural deaths. However, vascular injury requiring stenting occurred in 1.2% of cases, with 3.2% of HF procedures accompanied by a periprocedural rise in troponin T. Previously there has also been concern that intracoronary infusion of proangiogenic cells (e.g. from blood or BM) may accelerate atherosclerosis or in-stent restenosis [170], while large, adherent cell types (e.g. MSCs, CDCs, SkMs) may be prone to aggregating in the coronary microcirculation and accentuate ischemia [171, 172].

Intramyocardial injection by transepicardial or transendocardial means, can be used to direct cell therapy to specific regions of need, even if the supplying coronary artery is completely occluded. As described earlier, studies have generally suggested that most of the benefit from intramyocardial cell injection occurs at the actual sites of cell delivery [96]. The implication is that this strategy is most effective when targeting discrete regions of myocardium, such as in, or around chronic scar from previous MI, or in areas of hibernating myocardium. Although often assumed to be a global process, the pathology of nonischemic cardiomyopathy can in fact be segmental and patchy [173], and this has lent itself to transendocardial delivery of cells in preclinical [91] and clinical studies [82]. Just as the effectiveness of infusing cells into the coronary vasculature is challenged by problems of cell washout, direct intramyocardial injection is also hampered by early injectate loss [174]. In spite of this, preclinical studies have shown that cell retention in the myocardium is up to several-fold higher after intramyocardial injection compared to intracoronary infusion, with similar levels achieved between the transepicardial and transendocardial routes [175]. This in turn is associated with less entrapment of cells in other organs (e.g. lungs, liver) and possibly greater therapeutic effect [171, 172, 176]. A theoretical downside of direct injection is that it tends to deposit cells as clumps, which are less uniformly distributed than after coronary arterial delivery, and which may cause small disturbances to myocardial architecture or create niduses for arrhythmia.

Transepicardial injection is usually performed under direct visualization at the time of open chest surgery, video-assisted thoracoscopic surgery or pericardioscopy. Therefore its invasiveness generally limits its application to patients who have an indication for another procedure in addition to stem cell transfer (e.g. CABG, valve surgery, ventricular assist device implantation). Percutaneous trans-coronary-venous injection is another method of transepicardial delivery that requires accessing the coronary sinus from peripheral venous entry [177]. Although not featured in many studies, it may be a suitable option for patients with advanced HF whose myocardium is very thinned, and for safely repeating cell injections. Retrograde coronary sinus infusion is a different strategy again, and may be better tolerated than coronary arterial delivery in patients with severe LV dysfunction, as the use of transient occlusions in the coronary venous system does not cause as much temporary ischemia. It also distributes cells more evenly and without the clustering associated with intramyocardial injections, and allows access to ischemic territories that are not accessible from chronically occluded coronary arteries [178].

Most catheter-based cell delivery for HF has been via the transendocardial approach. This typically entails femoral artery access, with retrograde passage of the delivery catheter across the aortic valve into the LV cavity. There are various commercially available injection catheters, reviewed previously [179]. Catheter manipulation and selection of injection sites are most commonly navigated by fluoroscopic guidance or electromechanical mapping. The MyoStar™ injection catheter is designed for use with the NOGA® XP electromechanical mapping system (Biologics Delivery Systems, Irwindale, CA, USA) [180]. This technology allows accurate and reproducible tracking of the customized catheter inside the LV as it contacts different points on the endocardial surface, acquiring spatial, electrophysiological and mechanical data in real-time. The resulting three-dimensional color-coded reconstruction of the endoventricular surface provides a visual representation of segmental electrical viability and mechanical contractility, that in turn can be used to identify areas of scar and fibrosis, reduced perfusion and hibernation, and contractile dysfunction. Endoventricular electromechanical mapping has been validated against the gold standard of histology and against other myocardial imaging modalities in both ischemic and nonischemic cardiomyopathy [173, 181], and has been used widely in preclinical and clinical studies of cell therapy. Although the MyoStar™/NOGA® delivery system has an acceptable safety profile, its main limitations are its high cost, time consuming nature and considerable demand on operator expertise, training and accreditation. Alternative catheter systems, such as the BioCardia Helical Infusion Catheter (HIC-BioCardia Inc. San Carlos, CA, USA) are used in combination with fluoroscopy. While this is sufficient to guide catheter placement and cell injection in cases of peri-infarct or scar treatment, it lacks the subtlety and precision to navigate cell delivery to regions of hibernation or patchy fibrosis. Injection catheters have also been coupled with real-time MR fluoroscopy [182] and high-resolution three-dimensional echocardiography [183], although unlike NOGA® XP and fluoroscopic guidance these have been used in very few studies.

Despite the importance of other factors, such as disease substrate and cell type, much of the choice between different delivery modalities has come down to cost, availability, practicality, operator expertise and preference. Although not definitive, data from comparison studies have indicated more favorable cell retention after direct intramyocardial injection. However, across the board all current delivery techniques fall well short of achieving adequate levels of cell retention and engraftment to enable maximal therapeutic effect. Re-

ports consistently show that for different cell types and delivery routes, less than 10% of cells are retained in the first 24 hours after delivery, and less than 2% after a few weeks [176, 184, 185]. Recent innovations have focused on the adjuvant use of natural or synthetic biomaterials to promote cell retention, engraftment and biological function, such as by embedding cells in an epicardial patch or scaffold, that may itself provide additional mechanical, structural and paracrine support to underlying scar tissue [21, 186]. There have also been improvements in injection catheter design, epitomized by the recent development of the C-Cath® transendocardial catheter which has a nitinol curved needle that can reportedly achieve almost 40% cell retention in the setting of chronic infarction, compared to approximately 10% with the traditional straight needle [187]. Further progress in the cell therapy field hinges as crucially on optimizing the mechanical process of cell delivery, as it does on enhancing the biological properties of the transplanted cells [87].

Perspective and Future Directions

As outlined in the sections above, there remain significant questions to address and challenges to meet before the promise of stem cell transplantation is converted into clinical reality as part of the armamentarium of therapeutic approaches to treat HF. Despite divergent results, clinical studies to date have provided reassuring safety outcomes for a variety of different adult-derived cell preparations. There is continued progress at the scientific bench to counter the critical problem of inadequate cell retention and engraftment, and optimize both cell biology and delivery for enhanced therapeutic efficacy [87]. Among the plethora of strategies being undertaken, are: (1) the preconditioning and modification of cells with small molecules, growth factors, cytokines, genes and pharmacological agents to augment their viability, differentiation capacity and paracrine repertoire; (2) the targeting of myocardial substrate with techniques such as ultrasound shock wave therapy; and (3) the integration of stem cell delivery with tissue engineering and biomaterials.

The rational design and competent execution of clinical trials are just as crucial in moving the field forward as are basic scientific discoveries and advances. Mistakes of past studies must be heeded to avoid ongoing confusion relating to the key issues that are barricading further progress. This means that clinical studies must be collaborative and held to fundamental standards to minimize the different sources of bias that compromise data interpretation. Appropriate selection of patient groups that have the greatest need and scope for benefit from cell therapy is of utmost importance, as is the thoughtful matching of cell type, dose and delivery route to the specific disease context. Up until this point, most attention has been understandably directed to the application of cell therapy in IHD, where the proangiogenic properties of BM and blood-derived progenitor cells especially, have obvious appeal. In recent years there has been more acknowledgement of the potential for cell transfer to also restore myocardial performance in nonischemic cardiomyopathy, and in time stem cell therapy will also be investigated for diastolic HF and cardiomyopathy secondary to other causes (e.g. valvular heart disease).

Going forward, clinical trials must be capable of objective and standardized acquisition of meaningful endpoints that are biologically instructive and clinically relevant. Owing to their small size, most previous studies have focused on surrogate endpoints, such as global EF, regional wall motion, LV volumes, exercise capacity, natriuretic peptide levels and myocardial perfusion. Future trials should be adequately powered to measure hard endpoints, such as cardiac and all-cause mortality, repeat hospitalizations and other adverse events, as well as to assess the cost-effectiveness of cell therapies in the presence of conventional HF management. In the case of measuring safety outcomes and surrogate parameters for myocardial function and remodeling, there is tremendous opportunity to implement state-of-the-art imaging techniques that can provide novel insights relating to cell distribution and retention, long-term fate and mechanism of action [188]. Another topic of interest that has not yet been properly explored, is the potential for injected cells to have off-target effects, positive or negative, on other organs (e.g. kidneys, lungs, skeletal muscle) to which they distribute, and which are often compromised in patients with advanced HF. Particularly high standards of methodological rigor will also be required if, or when, pluripotent cells and their derivatives are studied in human patients. This should initially proceed in highly selected, end-stage patients with otherwise limited long-term outlook, in whom cell fate can be scrutinized very accurately, including by histology. One such context that has previously been studied with SkMs and MPCs, is that of severe cardiomyopathy requiring LV assist device (LVAD) implantation and/or listing for cardiac transplantation [48, 189].

CONCLUSIONS

A considerable body of experimental data has accumulated relatively quickly to show promising results from cell-based intervention in cardiomyopathy and HF, mediated by a broad range of pleiotropic actions. Positive effects have been observed for a variety of distinct cell types and delivery methods, and investigators have been spoilt for choice in these areas. As studies have progressed from the preclinical setting to human patients, the incremental gains that cell therapy may provide over current standard of care, have been less consistent and more modest. Now more than ever, the research community must apply common sense, patience and utmost scientific rigor to ensure that the field does not lose precious momentum, but advances steadily toward realizing its potential of bringing much-needed benefit to the growing number of patients burdened by HF. As remarkable discoveries and developments continue at the bench, others will need to prioritize the fundamental questions of optimal cell type, dosing regime and delivery method, while clinical trialists are encouraged to pool their energies and resources together to execute large-scale, definitive studies. Although some way from prime time, the use of stem cells in the wider clinical practice of HF management remains a realistic goal for the future.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

ACKNOWLEDGEMENTS

Dr Psaltis receives funding from the National Health and Medical Research Council (PG1086796) and Heart Foundation (FLF100412) of Australia. Professor Nicholls has received funding support from Novartis for heart failure-based research.

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