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5.21 Stem Cell Therapy to Treat Heart Failure☆

Edit Gara* and Annamária Kosztin*, Heart and Vascular Center, Semmelweis University, Budapest, Hungary
Sian E Harding, National Heart and Lung Institute, ICTEM, Imperial College London, London, United Kingdom
Gábor Földes, National Heart and Lung Institute, ICTEM, Imperial College London, London, United Kingdom; and Heart and Vascular Center, Semmelweis University, Budapest, Hungary

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Glossary

Allogeneic stem cell transplantation A procedure in which a person receives stem cells from a genetically similar, but not identical donor.

Autologous therapy A tissue or single cells transplanted by autologous procedure is a situation in which the donor and recipient is the same person.

Cell proliferation The term is used in the contexts of cell development and cell division. It refers to growth of cell populations, where one cell grows and divides to produce two.

Crossover study It is a longitudinal study in which subjects receive a sequence of different treatments or exposures.

Double-blind, randomized study In a double-blind experiment, neither the individuals nor the researchers know who belongs to the control group and the experimental group. Random assignment of the subject to the experimental or control group is a critical part of double-blind research design. The key that identifies the subjects and which group they belonged to is kept by a third party until the study is over.

Patient and evaluator blinded study The study procedure requires unblinded study investigators. The patient and the investigators responsible for the follow up are blinded, similarly as in double-blind study. The blinded and unblinded study team is strictly separated.

Left ventricular ejection fraction In cardiovascular physiology, ejection fraction is the fraction of blood pumped out of a ventricle with each heartbeat. Calculation: EF = SV/EDV, SV = EDV − ESV SV: stroke volume, EDV: end-diastolic volume, ESV: end-systolic volume.

*These authors contributed equally.

☆*Change History:* January 2018. E. Gara (EG) and G Foldes (GF) have made minor changes in the synopsis. GF updated Acknowledgments, listing a new funding source. EG and GF have revised reference list and added 16 new references. There is a change in the order of authors of the revised version. Please see page 1. GF and EG revised "*Introduction. Cell-Based Therapy for Cardiac Disease*" section by adding new information on pluripotent stem cell sources as well as tissue engineering. EG updated Tables 1, 2, 3 and 4 with new information. EG added new sentences on pharmacological therapy in heart failure to "*Major Unmet and Compelling Clinical Need Drives Stem Cell Research and Trials in Heart Failure*" and updated information on HF statistics and etiology. GF revised the conclusions on section "*Mechanisms of Cardiac Regeneration*". EG added new sentence about mesenchymal stem cell trials to the "*1. Actions of Bone Marrow-Derived Haematopoietic Stem Cells. Paracrine Effects*" section. Also, paragraph "*Mesenchymal Stem Cells*" has also been rewritten. GF added some recently published information on endothelial progenitor cells. EG and GF has made major revision on "*Embryonic stem cells and derivatives*" part. Similarly, EG and GF made a major revision on cardiac progenitor cells, adding new details of the ongoing clinical trials. EG made minor changes in cell delivery and clinical trial sections.

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Myocardial infarction A severe, significant stenosis or total occlusion of arteries supplying the muscles of the heart, resulting in injury or necrosis of the heart muscle.

Paracrine Denoting a type of hormone function in which hormone synthesized in and released from endocrine cells binds to its receptor in nearby cells and affects their function.

Progenitor cell A parent cell that gives rise to a distinct cell lineage by a series of cell divisions. In contrast to stem cells, they are already far more specific and can only divide a limited number of times. Controversy about the exact definition remains and the concept is still evolving.

Reprogramming Methods for developing pluripotent stem cells from various type of adult somatic cells, i.e., fibroblasts. The reprogramming procedure delivers pluripotency genes into the genome of adult cells and forces pluripotent states.

Transdifferentiation It is a process when a non-stem cell transforms into a different type of cell, or when an already differentiated stem cell creates cells outside its already established differentiation path.

5.21.1 Introduction: Cell-Based Therapy for Cardiac Disease

Stem cell therapy has been available for several decades in medicine. It is routinely used in hemato-oncology patients and latest clinical trials also comprise ophthalmology, neuro- and muscle degenerative and diabetic care. With regard to coronary artery disease and heart failure, the widespread attention and substantial expectations about stem cell therapy are illustrated by over 1.5 million separate listings for "stem cell therapy in heart failure" on the Internet. Most of this therapy continues to be in the form of autologous bone marrow transplant strategies. Clinical allogeneic therapies currently utilize haematopoietic, mesenchymal, cartilage, adipose, myogenic, epithelial, and limbal stem cell transplants. Importantly, most of these cell sources lack the potency to differentiate into mature cardiovascular cells (i.e., cardiomyocytes and endothelial cells). The most promising alternative cell source to generate functional cardiac cells is embryonic and induced pluripotent stem cell derivatives. However, the application of these cells in cardiac tissue engineering is hampered due to the ethical problems and immune response. There are more steps that need to be optimized to realize successful clinical translation of than expected earlier. Furthermore, insufficient myocardial retention rate is a key challenge in optimizing these regenerative attempts.[1] In addition to exogenous cell therapy, recent basic and clinical studies have suggested that enhancing endogenous regeneration, e.g., by delaying remodeling and fibroblast proliferation might be another approach to improve or stabilize the function of the failing heart. Latest state-of-the-art preclinical studies use therefore 3D tissue engineering, immune-modulation, in vivo trans-differentiation and in vivo intramyocardial gene delivery to overcome these hurdles and repair failing hearts.

5.21.2 Major Unmet and Compelling Clinical Need Drives Stem Cell Research and Trials in Heart Failure

Heart failure is a leading cause of death in the developed world and remains one of the most expensive and disabling medical conditions. Patients with stage D heart failure suffer as much decrease in QOL as oncology patients do. It is estimated to affect over 11 million people worldwide.[2] In the United Kingdom alone, over 2 million patients live with coronary artery disease and over 500,000 patients suffer from heart failure, making this condition a major cause of hospitalisations, outpatient visits, chronic disability, decreased quality of life and mortality.[2a] The average prevalence of heart failure is around 2%–3%, the incidence of newly diagnosed patients is increasing.[3] Each year around 550,000 new cases are diagnosed in the UK only and some 300,000 patients die because of the progressive condition. Surveys show that heart failure is associated with a very poor prognosis: 5%–75% of patients die within 1 year after diagnosis[4] and 50% die within 5 years,[5] from worsening heart failure or from sudden cardiac death, mostly as a result of major ventricular arrhythmias.[5,6] Heart failure accounts for more consultations than angina, reflecting the severe symptoms and reduced well-being of heart failure patients.[7] Due to the evidence-based therapies the mortality of outpatients improved, recently new medication (neprilysin inhibitor sacubitril/valsartan) has been suggested by the revised European Guideline. This has proved to decrease number of hospitalization and mortality compared to ACEi in patients with HFrEF.[8] The number and survival of the inpatients however stayed unchanged.[9]

The majority of heart failure patients have underlying cardiovascular disorders that are often the precursors of their condition; left ventricular dysfunction is the most frequent etiology. In developed countries, it results mainly from coronary artery disease, hypertension, obesity and hyperlipidemia. Less frequent etiologies include degenerative valve diseases, idiopathic dilated cardiomyopathy, hereditary conditions, e.g., hypertrophic cardiomyopathy; toxic (alcohol-, drug-induced), viral and congenital cardiomyopathy.[10] Interestingly, immunization also influences development of the disease. Indeed, pneumococcal vaccination, which is highly recommended in heart failure patient population, was shown to decrease the risk of heart failure and cardiovascular mortality.[11] Acute ischemic injury and chronic cardiomyopathies both lead to metabolic impairment, cellular dysfunction and death of cardiomyocytes. Other causes of heart failure, including chronic high blood pressure, are also characterized by a gradual and permanent loss of cardiac tissue.[12] Heart failure results from the progressive deterioration of relaxation–contraction cycle, leading to inability of the heart to maintain sufficient cardiac output to match metabolic need of all tissues and organs. Cardiomyocyte relaxation (diastole) results in consumption of greater amount of intracellular energy than in cardiac contraction (systole), albeit the so called "heart failure with preserved ejection fractions" condition (diastolic heart failure) is difficult to recognize in clinical practice. In chronic heart failure the backward and forward failures of the ventricles cause widespread symptoms of these patients.

5.21.3 Current Therapies in Heart Failure

Inhibitors of the renin–angiotensin–aldosterone pathway (i.e., angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers and neprilysin inhibitor), β receptor blockers and mineralocorticoid receptor antagonists are the most important disease-modifying therapies, improving symptoms, reducing hospital admissions and increasing survival. Treatment of underlying cardiovascular and related disorders that contribute to the development of heart failure, such as hypertension, myocardial ischaemia, atrial fibrillation, hyperlipidemia and diabetes, is also important.[13] In addition new device-based approaches have emerged for treating mild to severe heart failure such as biventricular pacemakers to resynchronize the ventricles with low ejection fraction[14] and implantable cardioverter-defibrillators to reduce risk of sudden cardiac death in patients who were receiving optimal medical treatment.[15] Ventricular assist devices are used as a bridge to transplantation and as destination therapies.[16] Development of artificial hearts and of novel ventricular splint and compressive devices continues. While these recent advances in therapy have been shown to slow heart failure progression and to improve clinical outcomes, none of them addresses the underlying cause of the disease, which is the damage and progressive loss of cardiomyocytes and/or the vasculature in the failing heart.[17] Orthotopic or heterotopic heart transplantation is considered as an only remaining option in selected patients. Heart transplant will however not fill the need given that the supply of donor hearts is limited and xenotransplantation remains experimental.[7] This continued health problem has prompted research into newer treatment modalities, including approaches to the protection, replacement, and regeneration of cardiac cells.

5.21.4 Mechanisms of Cardiac Regeneration

In mammalian hearts, cardiomyocytes around a myocardial infarction (MI) rarely divide, although transgenic overexpression of specific genes was shown to increase their cell division in mice. This is in contrast with other vertebrates; in the newt, after a substantial injury, remaining cardiomyocytes re-enter the cell cycle.[18] In zebrafish, mostly undifferentiated stem or progenitor cells from the epicardium may initiate heart regeneration.[19] Of interest, skeletal muscle in mammals can regenerate efficiently, even after extensive injury.[20,21] Recent elegant evidence from radiocarbon dating of postmortem cardiac tissue shows that a natural repair system of human myocyte also exists; however, it is limited as only less than 50% of cardiomyocytes are exchanged during a normal life span.[22] It has been recently shown that adult mammalian myocardium has a population of endogenous cardiac stem cells with the potential to give rise to multiple cell types and to reconstitute lost cardiomyocytes.[23–26] Resident stem cells may support basal turnover of mouse cardiomyocytes,[18] but this occurs at a very low rate in the absence of injury.[27] In addition, it has been showed that Y-chromosome-positive, male cardiomyocytes and endothelial cells can be detected in female donor human heart transplanted into male recipients, indicating cellular chimerism.[28,29] It is plausible, however, that under normal circumstances some of the male cells identified within a female heart may be of fetal origin from pregnancies with male offspring.[30] Similar chimerism, with a much lower frequency, was reported in cardiac tissue in patients receiving bone marrow transplants.[31] However, the spontaneous regenerative capacity of the human heart altogether appears to be insufficient to compensate for the loss of myocardium after MI or in chronic myocardial diseases. Recent studies have demonstrated the role of myocardial fibroblasts and macrophages after ischemic attack and showed that their activation was related to immune-modulatory and inflammatory mechanisms.[32] The initial concept that such patients could be treated by an exogenous supply of cells such as bone marrow-derived cells and other progenitor cells to improve cardiac function seemed to be a logical approach. However, position papers present a strong opinion that exogenous cells currently available for clinical use may not be sufficient alone to result in a significant cardiac improvement in patients. Paracrine effects may slow down myocardial remodeling, but novel immunomodulatory, small molecule-based or epigenetic-driven approaches are required for promoting cardiac regeneration.[1,33]

5.21.5 Which Stem Cells Type Can Be Suitable for Cardiac Cell Therapy?

Stem cells are defined by their ability to renew themselves and differentiate into a diverse range of committed cell types. Although stem cell therapy as a concept is attractive,[34–38] its use for heart failure is still in its early years. In preclinical and clinical studies, a wide variety of stem cells or their progeny have been considered as possible candidates for cell repair for the failing hearts, with mixed outcomes (**Table 1** and **Fig. 1**). Results from animal models indicate that transplantation of bone marrow-derived haematopoietic stem cells, mesenchymal stem cells, skeletal myoblasts, and embryonic stem cells have the potential to improve cardiac function after ischaemic injury. The latest cornerstone of regenerative medicine is denoted by Yamanaka and colleagues by developing human-induced pluripotent stem cells (hiPSC).[39] During the reprogramming method, pluripotency genes are integrated into the genome of adult somatic cells resulting in pluripotent fate. However, this new field of stem cell research is still under debate. The main advantages seem to be extraordinary attractive: autologous cell therapy supposedly without immunosuppression. Stem cells can exert beneficial effects on the failing heart by transdifferentiating into cardiac cell types or by providing a source of cardioprotective paracrine factors. Recent clinical trials using bone marrow-derived cells and skeletal myoblasts have also produced some encouraging results (**Table 1**). Cell transplantation is, however, hampered by suboptimal cell delivery, low survival of grafted cells, and their reduced proliferative and differentiation capacities. Ideal grafted cells should be easy to collect and expand; form stable grafts; be able to couple electromechanically with the host cardiomyocytes; and be devoid of arrhythmogenic or terato-oncogenic

Table 1 Advantages and disadvantages of various cell types isolated from different sources

Stem cells	*Cell source*	*Advantages*	*Disadvantages*	*Clinical trials*
Embryonic stem cells, induced pluripotent stem cells	Inner cell mass of the blastocyst, adult fibroblasts Allogeneic cell lines vs autologous somatic cells (iPSC)	Unlimited self-renewal capacity, pluripotency Ability to form functional cardiomyocytes	Ethical and legal considerations; potential teratoma formation from residual cells; immunological problems: graft versus host disease (not with iPSC)	One safety and feasibility transplantation study is recruiting with embryonic stem cell derivatives; none with iPSC
Bone marrow mononuclear cells, haemopoietic stem cells, circulating progenitor cells	Bone marrow, peripheral blood, umbilical cord, placenta	Easy to isolate, proven safety and feasibility to implant Potential for vasculogenesis	Lack of true cardiac differentiation Unknown underlying mechanisms	Medium-scale trials with modest or no benefits; significant reduction in subsequent cardiovascular events
Mesenchymal stem cells	Bone marrow (adherent cells), and other mesenchymal tissues such as adipose tissue	Easy to isolate and expand in culture, abundant supply, low immunogenicity, multipotency	Large heterogeneity; heterotopic differentiation (ventricular ossification) Unknown myocyte regenerative potential	Safety and feasibility studies and multi-centre, randomized clinical trials
Endothelial progenitor cells	Bone marrow, peripheral blood	Important in neovasculogenesis Mobilized from bone marrow or present in the peripheral blood	Heterogeneity; low circulating cell number; reduced cell number in patients with cardiovascular comorbidities	Safety and feasibility studies and multi-centre, randomized clinical trials, no significant benefit
Skeletal myoblasts	Mature skeletal muscle (between sarcolemma and basement membrane) Skeletal muscle biopsy specimen	Extensive scalability; resistance to ischemia; multipotent; no teratoma formation	Controversial data on arrhythmogenesis; lack cardiomyocyte differentiation (dyssynchronous beating)	Large scale clinical trials; no benefit observed
Cardiac resident stem cells and progenitors	Special niches within the myocardium	Resident cells; robust cardiovascular differentiation potential; reduced tumor formation; electrically integrated	Stem cell pool undergo senescence; unknown scalability	Safety/Efficacy studies in progress

effects. The ideal cell type that can fulfill these criteria has not yet emerged, and only few studies have compared stem cell types and lines directly.[40] Strict regulations and standardized operating cell handling protocols and standard clinical trial protocols are cornerstones to compare recent studies and to ensure reliable data in the future.[41] Here we list the potential cell types for cell therapy.

5.21.5.1 Actions of Bone Marrow-Derived Haematopoietic Stem Cells: Paracrine Effects

Cardiac cell therapy using bone marrow-derived cells has already entered the clinical arena as part of ongoing early phase clinical trials. After being an area of considerable debate during the past years,[17,42,43] by now it seems that real cardiac muscle formation from bone marrow-derived haematopoietic stem cells is not likely. Subsequent studies have shown that what first was interpreted as trans-differentiation may have been the result of cell fusion of transplanted cells with host cardiomyocytes. Human bone marrow cells and recipient myocytes have been shown to fuse at a low frequency and express sets of cardiac and stem cell markers.[44–49] Yet, it was thought to be unlikely that new cardiomyocytes are being generated even by fusion events. Stem cells and progenitor cells, however, do enhance functional ventricular recovery after MI. A growing body of evidence suggests that the improvement in cardiac function is mostly independent of cardiac muscle regeneration. The predominant mechanism of action of transplanted cells is to secrete factors favourable for myocyte survival, cell cycle progression, decreased inflammation and fibrosis, calcium cycling, metabolism, and blood-vessel formation. The secretion of paracrine/autocrine factors can also stimulate resident cardiac stem cell differentiation, cell recruitment and immune system for a substantial preservation and regeneration of myocardium.[50–56] The agents used most frequently to elicit mobilization of stem cells and derivative progenitor cells are the myeloid cytokine granulocyte colony-stimulating factor (G-CSF) and chemokine receptor CXCR4 antagonists.[57]

Studies in rodent and large animal models showed induction of neovascularization and rescue of ischaemic myocardium even during the period of coronary occlusion and immediate reperfusion[52,58] and benefit without the formation of stable grafts of the transplanted cells.[59,60] After ischaemic injury, factors stimulate tissue recovery and reduce the infarct size.[54,61–63] Again, these beneficial effects have been attributed to specific factors released by the transplanted cells such as thymosin β4, which induces wound healing, or Wnt pathway inhibitor secreted frizzled-related protein-2, which shares an anti-apoptotic effect on hypoxic

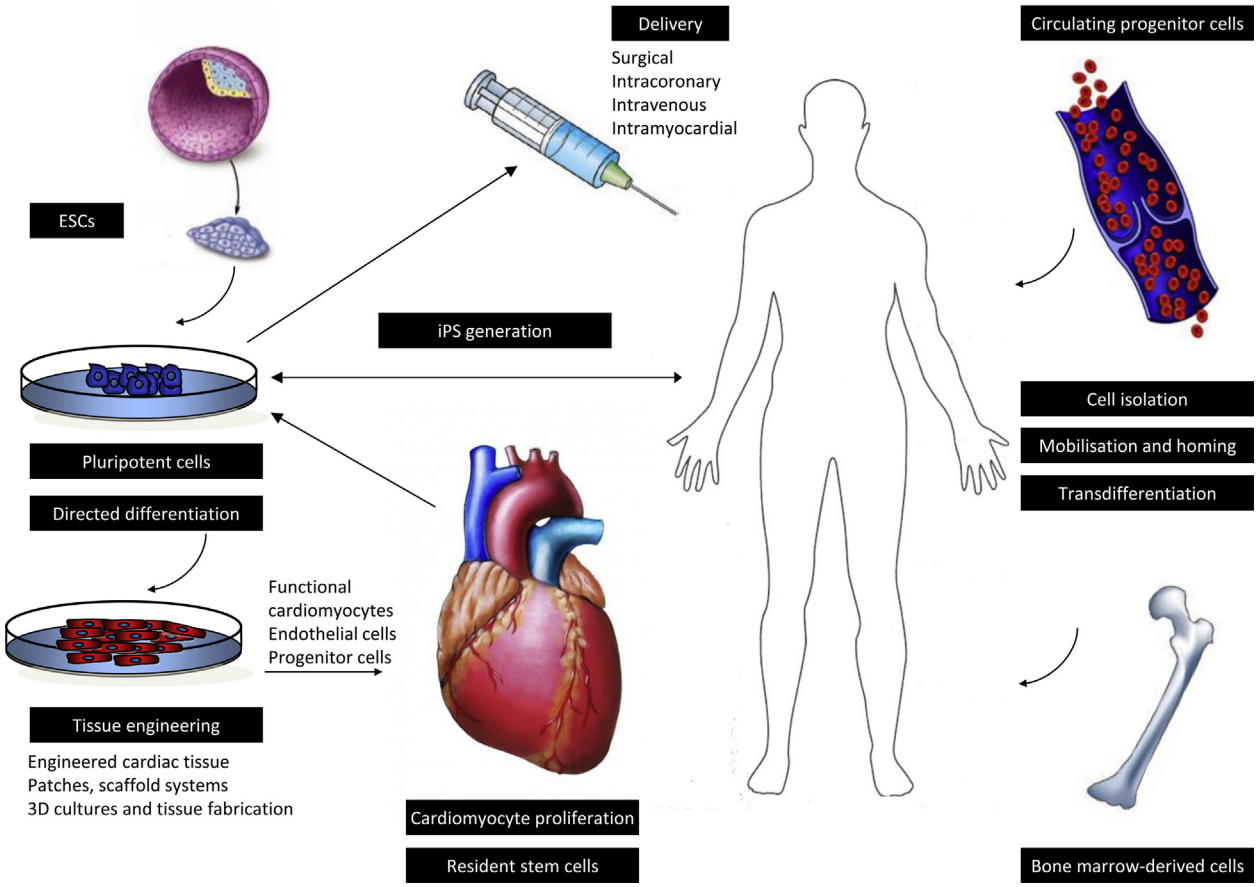

Figure 1 Experimental approaches to cell therapy in heart failure. Clinical trials have been performed with bone marrow-derived cells, circulating progenitor cells and skeletal myoblasts that have been delivered to the heart. Conceptually it would be also possible to achieve by purifying cells of the cardiac lineage from differentiating human embryonic stem cells or induced pluripotent stem cells to the relevant cell type, and thereafter transplanting such cells to the heart of the patient.

cardiomyocytes.[64–66] A large randomized phase III clinical trial is underway and has recruited 350 patients so far. The BAMI trial (NTC01569178) aims to evaluate effects of bone marrow-derived mononuclear cells on all-cause mortality in intracoronary infusion after myocardial ischemia.

Stem cell engraftment and survival are sensitive to the local hostile environment; its importance is underlined by the fact that clinically tested cell types are largely eliminated from the heart within 1 week after intracoronary infusion.[67] By using preconditioning and reprogramming of the target tissues and donor cells a better survival, retention, integration, and recruitment of transplanted cells can be reached. These can include the transduction of cells with prosurvival genes (e.g., protein kinase Akt, telomerase reverse transcriptase, vascular endothelial growth factor, or integrin-linked kinase) and the pretreatment of cells with statins, endothelial nitric oxide synthase, and small molecule inhibitors of p38-MAPK.[68,69] The injection of stem cells into the myocardium may also alter ventricular wall geometry and improve remodeling via a scaffolding effect.[70] This should be taken into account when injecting stem cells into the free wall. In addition, the newly formed muscle will provide passive mechanical support.

5.21.5.2 Mesenchymal Stem Cells

In addition to hematopoietic stem cells, other cells located in the bone marrow include mesenchymal stem cells (MSC), multipotent adult progenitor cells, and side-population cells. MSC can be separated from haematopoietic cells by an absence of hematopoietic stem cell markers and their ability to adhere to the culture dish and differentiation potency to chondrogenic, adipogenic and osteogenic lineage.[71] Bone marrow-derived MSC can be differentiated into cardiomyocyte precursors and other cell types,[72,73] resulting in an improved left ventricular function and remodeling.[74–76] MSCs are also present in adult tissues including adipose tissue stroma; mouse adipose tissue-derived MSCs are able to give rise to cardiomyocyte-like cells.[77] The magnitude of in vivo differentiation into cardiac cells, however, seems to be low,[72,73] but current evidence supports the engraftment and the differentiation of transplanted human MSC in sheep.[78] One of the advantages of MSCs is that they are less immunogenic than other lines[79]; this reduces the need for additional immunosuppression or autologous therapy. In addition, MSC can also modulate immune responses.[80] In mice after MI and in chronic ischemia, transplantation of MSC improved left ventricular function and reduced

infarct size.[76,79,81–83] To further increase the therapeutic potency of MSCs, they have been genetically modified to overexpress survival, angiogenic, growth, and stem-cell homing factors.[84,85] In a clinical study, autologous bone marrow MSC improved left ventricular function in 69 post-myocardial patients.[86] Results from a small phase II clinical trial Prochymal using intravenous allogeneic MSC hints at an improvement in ventricular function in treated patients at a 6-month midpoint.[87] Some of the concerns arise from the observations that implanted MSC can differentiate into bone-forming osteoblasts inside ventricular myocardium,[88,89] although this could have been calcification resulting from injection of foreign tissue. The phase III clinical trial C-CURE showed an improvement in clinical parameters such as longer distance in 6-min walk test and reduced left ventricular end-systolic volume in heart failure patients with ischemic history.[90,91] The CHART1 trial investigated the efficacy and safety of endomyocardial delivery of autologous bone marrow-derived cardiopoietic cells in patients suffering from severe ischaemic heart failure. The study proved safe and feasible implantation; however, it failed to meet primary endpoint at week 39 follow-ups (composite of cardiovascular mortality, 6-minute walk test, quality of life and hospitalizations). Publications outlined significant improvement in LVEDV and LVES at week 52 follow-up, furthermore advantageous (but not significant) trend of cell implantation in patients with LVEDV above 200 mLs. Efficacy were linked to number of cell injection, i.e., the less myocardial puncture the better results.[92–94]

5.21.5.3 Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are a controversial and heterogeneous cell type found to reside predominantly in the bone marrow.[95] Adult EPCs were first believed to be a rare population of CD34-expressing cells isolated from the blood of adult mice which could purportedly differentiate into endothelial cells in vitro.[96] EPCs were shown to express cell surface markers such as differentiation molecule 133 (CD133), vascular endothelial growth factor receptor 2 kinase (VEGFR-2 or KDR), CD34 and vascular endothelial cadherin. For identification, $CD34^+$ and $CD133^+$ cells are the most widely utilized, although these markers are also shared by haematopoietic stem cells.[96] Definitions of EPC, however, vary such that different studies use different cell types, making comparisons difficult. Some results in both mice[97] and humans[98] have led some scientists to question even the existence of EPC as no specific cell surface marker pattern or unique gene expression panel exists. Recent studies proved that EPCs are heterogeneous population and can be divided into early and late EPC population group. Mononuclear cells were isolated from human peripheral blood and the two populations of EPC gained different characteristics when cultured in vitro: early EPCs show spin like morphology, while late EPC take up cobblestone form. The two EPC populations differed in survival and proliferation rate and also in angiogenic properties. EPCs are mobilized from bone marrow in response to injuries, MI and cancer.[99] VEGF and granulocyte colony-stimulating factor (G-CSF) can also boost EPC mobilization.[100,101] It has clinical relevance that widely-used pleiotropic statins (3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors) can induce the mobilization of EPC.[102,103] They may promote neovascularization by secreting proangiogenic factors and by inducing re-endothelialisation[20,104]; some data further supports that in addition to vasculogenesis, $CD34^+$ cells may differentiate to cardiomyocytes as well.[105] It may be a clear therapeutic limitation that the stem cell pool for EPC is limited, and the scarcity needs to be overcome first by in vitro cell expansion.[106] The circulating number of EPC is lower in patients with cardiac comorbidities such as diabetes mellitus, hypertension and hypercholesterolemia.[107,108] Thus, EPC may act as a biomarker of these conditions. Additionally, a recent paper has outlined advantage of EPC as specific biomarker in the severity of hypertrophic cardiomyopathy.[109] After the assessment of clinical safety and feasibility,[110,111,2b] the results of several small trials have shown that injection of EPC into infarcted myocardium improves ventricular function and inhibits fibrosis, without adverse effects.[112–114] However, these effects were not sufficient for a significant functional improvement in clinical status. A correlation exists between the concentration of spontaneously mobilized $CD34^+$ cells in the circulation and subsequent improvements in left ventricular function and remodeling.[115] One of the related applications in interventional cardiology is bioengineered EPC-capture stents. These stents are coated with anti-CD34 antibodies, which can capture circulating EPC and thereby boost the endothelialization process to prevent in-stent restenosis.[116] The stents have already proven safe for implantation and current studies assess whether the restenosis rate could be reduced without the concern for in-stent thrombosis. The CATCH-AMI clinical trial (NCT01905475) and others are focused on activating the circulating endothelial progenitors and enhancing their homing into the temporary injured myocardial tissue. Despite the high expectations about the CATCH-AMI study, results have not yet been published.

5.21.5.4 Skeletal Myoblasts

Skeletal myoblasts (also known as satellite cells) can be located under the basal membrane of muscle tissue and are stimulated to proliferate by injury.[117] These cells can be easily harvested, expanded in culture, and then re-implanted autologous, thereby avoiding any problems from immunosuppression.[118] Skeletal myoblasts are resistant to ischaemia, one of the main obstacles to the proper function of stem cells in the infarcted myocardium.[119] They are multipotential and are capable of forming osteocytes, adipocytes and myocytes in vitro.[120] In animal models, their transplantation improved left ventricular function and decreased remodeling because the implanted cells form myotubules.[121,122] In rats, cells have also been shown to inhibit matrix breakdown in the peri-infarct and remote myocardium, which likely contributes to a reduced remodeling.[123] On the other hand, skeletal myoblasts transplanted into the myocardium cannot fully differentiate into cardiomyocytes[124] and the differentiated, contracting myotubules cannot operate in synchrony with the surrounding recipient cardiomyocytes.[125,126] This may be at least partially due to a lack of expression of connexin, a protein involved in the formation of gap junctions, and electrical integration with the neighboring myocardial cells.[127] Of note, the poor electrical coupling of skeletal myoblasts to resident cardiomyocytes can be improved with

skeletal cells overexpressing connexin-43.[128,129] Their survival is also poor: cellular death of the order of 90% within the first few days has been shown in mice[130]; similar cell death rates have been shown in humans.[119] In human, skeletal myoblasts were the first cells to be injected into the ischemic myocardium 8 years ago as part of a cell-based strategy. Initial clinical safety studies, however, proved the feasibility and safety of their implantation and showed that cells survive in the human heart. The safety results whether engraftment of unmodified skeletal myoblasts may generate arrhythmias in vivo have been conflicting.[131–133] While early studies reported rare cases in patients,[134,135] data from more recent large trials show that ventricular arrhythmic events did not differ after intramyocardial injection of skeletal myoblasts or placebo.[133,136] The most noteworthy large-scale randomized clinical trial to date was the MAGIC (Myoblast Autologous Grafting in Ischemic Cardiomyopathy) trial, which showed the lack of treatment efficacy. Other trials reported similar results.[119,135,137–139]

5.21.5.5 Embryonic Stem Cells and Derivatives

Human embryonic stem cells (ESCs), first derived from the inner mass of the embryo during the blastocyst stage,[140] are pluripotent cells capable of differentiating into any cell type present in the adult body, including cells of the heart. Human ESCs have emerged as one of the most promising sources of cardiac cells for transplantation purposes because of their capacity to efficiently undergo directed differentiation into genuine cardiomyocytes and supportive endothelial cells. A number of groups have successfully isolated cardiomyocytes or cardiac progenitor cells from differentiating ESC cultures grown either in 3D clusters termed embryoid bodies[141,142] or 2D cultures. Treatment with cytokines, such as Activin A and BMP4, recapitulate the natural embryonic milieu and increase the yield of differentiated cardiac cells.[143] These in vitro derived cardiomyocytes have been characterized extensively. Structural, electrophysiological, and contractility studies indicated that ESC-derived cardiomyocytes exhibit a phenotype reminiscent of fetal, rather than adult cardiomyocytes. In the animal transplantation models of cardiac disease (**Table 2**), use of ESC-derived cardiomyocytes has resulted in a significant improvement in ventricular function and structure. The cells appear to form gap junctions with host cardiac tissue; however, formation of protective fibrotic tissue around the grafts can prevent complete electrophysiological coupling.[144] Yet, transplantation also appears to normalize electrical conduction through the infarct zone, reducing susceptibility to arrhythmias.[145] The beneficial effects in MI have been reported 1 month after transplantation.[143] However, in a study with a longer follow up, no effect on cardiac function could be documented 3 months after transplantation of GFP-tagged cells into the hearts of immunodeficient mice.[146] Thus, post-transplantation expansion, maturation, survival, and long-term effects of grafted ESC-derived cardiomyocytes to injured myocardium need to be further evaluated. In a study by Murry and colleagues, myocardial infarction has been induced in non-human primates via LAD balloon-occlusion, followed by direct myocardial implantation of hESC-CM in open-chest surgery. The results proved promising electromechanical and histological coupling between the host and grafted human cells. At the same time, malignant ventricular arrhythmias (ventricular tachycardia and fibrillation) were registered which cast doubt on the success.[147] The same group made further direct comparison between hESC-CM, hESC-derived cardiovascular progenitor cells and bone marrow-derived mononuclear cells in a myocardial infarction model of nude rats. All the cell types were safe, but hESC derivatives were found to be superior to bone marrow derivatives as they improved left ventricular systolic function, while bone marrow derivatives only attenuated LV dilatation.[148] The latest preclinical data suggest that co-transplantation of hESC derived cardiovascular cells with mesenchymal stem cells may enhance the beneficial effects of hESC derivatives. MSC may support the engraftment and survival of implanted hESC derivatives via anti-inflammatory and immunosuppressive effects.[149] The first clinical trials have been initiated in purportedly immune privileged sites, the eye and spinal cord, although with covering immunosuppression in the latter. Retinal pigment epithelium cells recreated from human ESC[150] are being used to reverse vision loss after macular degeneration. The other phase I trial was designed to assess the safety and tolerability of human ESC-derived oligodendrocyte progenitor cells in patients with complete subacute thoracic spinal cord injuries.[151] The trial was discontinued due to financial reasons in 2011. The first clinical trials in cardiovascular disease are just started. Menasché and colleagues have differentiated a population of CD15- and Isl1-positive cardiac progenitors from human ESC. The ESCORT early trial

Table 2 Embryonic stem cell-derived cardiomyocyte transplantation studies in models of experimental myocardial infarction

	Number of cells injected	*Model*	*Injection time after infarction*	*Follow-up duration*	*LV function*	*Used control*	*References*
mESC (cardiac-committed)	5×10^7	Sheep	14 days	4 weeks	EF ↑	Medium	224
mESC-CM	$0.03–0.1 \times 10^6$	Mouse	0 days	3–4 weeks	EF ↑ EDV ↓	BMCs, fibroblasts and medium	225
hESC-CM	10^6	Mouse	0 days	12 weeks	EF ↑ (4 wks) ↔ (12 wks)	hESC-derived non-myocytes	146
hESC-CM	10^7	Rat	4 days	4 weeks	FS ↑ EDV ↓	hESC-derived non- myocytes and saline	143
hESC-CM	10^8	Guinea pig	10 days	4 weeks	FS ↑	Non-cardiac hESC derivatives	226
hESC-CM	10^9	Monkey	14 days	3 months	EF ↑ or ↔	Medium	147
hESC-CM	10×10^6	Rat	4 days	28 days	FS ↑ EDV ↓	hESC-CVP, BM- mononuclear cells	148

BMCs, bone marrow cells; EDV, end-diastolic volume; EF, ejection fraction; FS, fractional shortening; mESC-CM, mouse embryonic stem cell-derived cardiomyocytes; hESC-CM, human embryonic stem cell-derived cardiomyocytes; hESC-CVP, mesodermal cardiovascular progenitors.

(NCT02057900) recruits patients with ischaemic heart failure with indication for coronary-artery bypass graft or valvular surgery. During the operation, a fibrin-based cardiac patch, seeded with CD15 and Isl1 positive cardiopoietic cells, will be placed epicardially onto the infarcted area with a pericardial flap. The trial has enrolled six subjects so far, and estimated to run until late 2018.

5.21.5.6 Induced Pluripotent Stem Cells and Derivatives

Recent revolutionary experiments have shown that transient overexpression of a small number of transcription factors can reprogram differentiated cells into induced pluripotent stem cells (iPSC) that resemble hESC.[39,152–154] Similar to ESC, iPSC can be expanded over many passages in culture and give rise to all three germ layers, both under appropriate in vivo and in vitro differentiation conditions. The derivation protocol has been refined recently to reprogram without genetic selection,[155,156] or to incorporate virus-free approaches for gene delivery. Human iPSC derivation now can be achieved with transposon,[157,158] episomal,[159] and direct reprogramming protein delivery[160] systems. Human iPSC can differentiate into functional endothelial cells, smooth muscle cells and cardiomyocytes. Electrophysiology analyses indicate that iPSC have a capacity to differentiate into nodal-, atrial-, and ventricular-like cardiomyocyte phenotypes.[161] These hiPSC hold another great promise for cardiovascular medicine because they can generate patient-specific cell types for autologous cell replacement therapy and also produce in vitro models of disease, without the use of eggs or embryos. Indeed, numerous papers have reported the derivation and differentiation of a number of disease-specific human iPSC.[162] First cardiac studies described long QT syndromes or catecholaminergic VTs.[163–166] Inherited genetic disorders like arrhythmogenic right ventricular cardiomyopathy were also investigated by using hiPSCs.[167,168] In comparison with ESC, these iPSC can be more likely to produce teratomas if made with viral vectors, but newer methods may bypass this step. However their tumorigenic properties will be at least equal to ESC. Assays with hiPSCs have been improving. This can form a basis for surrogate endpoints in electrophysiological testing, as these cells share some similarities in structure and functions with mature cardiomyocytes. In vitro assays can be used in testing cardiotoxicity, contractile properties, drug-induced early afterdepolarization or QT prolongation individually. These advantages may translate into the safety, cost-effectiveness testing and terminate the ethical debates related to harvesting of stem cells. Transplantation immune-compatibility will be reduced with iPSC derivatives because the starting material such as skin fibroblasts can be obtained from the patient, but the logistics of using them therapeutically may not be as simple as hoped.[169] Large-animal models using monkeys, dogs and pigs demonstrated their feasibility and superiority to other ESC cells, partly due to lack of immunological issues with intramyocardial injection of autologous cells. These models investigated the changes in contractility after myocardial infarction (**Table 3**). Most recent preclinical studies aimed at developing tissue engineered grafts to repair failing heart. Engineered heart tissue (EHT) is fibrin-based contractile graft. Furthermore, complex tissue layers and cardiac patches are also being developed from hiPSC-derived cardiomyocytes, endothelial cells and smooth muscle cells.[170,234] Efforts are now focused on the development of well-sized hiPSC banks with a variety of diseases. Banking these cells similarly like the widespread umbilical cord blood banking may serve public health advantages in the future. Characterizing the human leukocyte antigen (HLA) pattern of the banked cells may further enhance their future clinical use.

5.21.5.7 Cardiac Resident Stem Cells

Over the past years, several clusters of resident cardiac stem cells or progenitor cells have been identified in the adult hearts of humans and other mammalian species.[171] Cardiac stem cells appear to reside in specialized niches[172,173] and concentrated in

Table 3 Preclinical models with iPSC-derived cardiovascular cells

Source	*Cell type*	*Animal model*	*Delivery*	*Results*	*References*
Canine	Endothelial cells	SCID mice, post-MI	Intramyocardial	Improved contractility	227
Human	Cardiomyocytes Endothelial cells Smooth muscle cells	post-MI	Intramyocardial	Improved contractility	228
Porcine	Endothelial cells	SCID mice, post-MI	Intramyocardial	Improved contractility	229
Human	Endothelial cells	post-MI	Intramyocardial	Cells differentiated and detected up to 15 weeks	230
Human	Cardiomyocytes	Induced cardiomyopathy	Cell sheet	Improved contractility	231
Human	Cardiomyocytes	Porcine acute MI	Fibrin patch	Improved contractility, metabolism and reduced infarct size	232
Human	Cardiomyocytes	Post-MI	Cardiac patch	Reduced infarct size	233
Human	Cardiomyocytes	Guinea pigs post-MI	Engineered heart tissue strips	Improved left ventricular ejection fraction	234
Human	Cardiomyocytes + endothelial cells + smooth muscle cells	Swine post-MI	Engineered cardiac patches	Improved myocardial function, decreased scar and apoptosis	170

Post-MI, post myocardial infarction.

deep tissue at the atria and apex.[23] Different cardiac stem cell pools are small relative to the mature cardiomyocytes; they can still be the source of new cells in normal myocardium or after an ischaemic insult in mice.[26] Cardiac resident stem cells show a high proliferative and differentiation potential in vitro,[24–26] therefore expanding these autologous cells ex vivo, stimulating their regenerative capacity and mobilizing them in vivo all seem to be viable therapeutic options. Cell populations expressing stem cell marker proteins such as c-kit, stem-cell antigen 1 (SCA1) and multidrug resistance protein 1 (MDR1) have been identified in the human and mouse heart in minuscule quantities.

Side-population (SP) cells, identified by their ability to exclude Hoechst vital dye, were described in the bone marrow as being enriched in haematopoietic stem cells, but they are also present in murine skeletal muscle, adipose tissue[174] and heart.[25,175] Cardiac SP cells can differentiate to cardiomyocytes, suggesting that they represent cardiac progenitor cells; transplanted SP cells can form cardiomyocytes, endothelial cells and smooth muscle cells.[176,177] SP cells are mobilized after cardiac injury[178] but their regenerative potential is still unknown.

A second murine resident cardiac stem cell type expresses the stem cell antigen 1 (Sca-1$^+$).[26] Sca-1$^+$ cells home to infarcted myocardium and can give rise to novel cardiomyocytes around the injured area.[26] The Sca-1$^+$ cell subpopulation, which does not express CD31 antigen, differentiates into both cardiomyocytes and endothelial cells in culture.[179] Transplantation of Sca-1$^+$/CD31$^-$ cells in mice after MI improves ventricular function and promotes new blood vessel formation.[179]

Another putative resident progenitor cells express the stem cell factor receptor c-kit.[23] These c-kit$^+$ cells are thought to be originated from the bone marrow in minuscule quantities and form a part of a local innate immune surveillance system.[180] They are not cardiac progenitor cells but bone marrow-derived cells localized in small clusters in the heart and lack expression of transcription factors Nkx2.5 and islet-1, markers of the cardiac progenitor cells in the developing heart.[181,182] Yet, c-kit$^+$ cells were suggested to share regenerative potential after transplantation, giving rise to cardiomyocytes, endothelial cells and smooth muscle cells. c-kit$^+$ cell transplantation after ischaemic injury leads to an improvement in ventricular function, remodeling, and infarct size in animal models.[23,183] Their direct contribution in endogenous cardiac repair has yet to be unequivocally established.

As shown by lineage-tracing experiments, the LIM-homeodomain transcription factor islet (Isl)1-expressing cells can differentiate into endothelial, endocardial, smooth muscle, conduction system, right ventricular and atrial myogenic lineages during the development of the embryonic heart.[181] Isl1$^+$ cells are present in the adult mammalian heart, but they are limited to the right atrium, are found in much lower numbers than in embryonic hearts.[24]

Cardiac progenitor cells were isolated from mouse hearts by enzymatic digestion to obtain round shaped clusters of cells that form so-called cardiospheres in suspension.[184] Cardiosphere-derived cells can give rise to cardiomyocytes, endothelial cells and smooth muscle cells. Similar human cell population can be obtained with an endomyocardial biopsy. Human cardiospheres exhibit significant proliferation and differentiation capacity[184–186]; isolated cell populations can be differentiated into spontaneously beating aggregates of cardiomyocytes.[187] The injection of human cardiosphere-derived cells into injured myocardium showed some benefit in animal models mainly by improving left ventricular function.[185,187]

Finally, epicardium-derived progenitor cells have been recently described that show angiogenic potential.[188,189] Indeed, thymosin-β4 (an actin-binding protein that activates integrin-linked kinase and promotes cardiac cell migration and survival) was shown to modulate the migration of an endogenous epithelial progenitor cell population identified in human epicardium.[188] These epithelial cells can restore post-infarct function, by reducing dilatation of the heart chambers and increasing ejection fraction in immunodeficient mice upon transplantation.[189]

Cardiac resident stem cells can be obtained from myocardial biopsies and their potential regenerative capacity can be investigated by their myocardial re-implantation. Using this approach, the CADUCEUS phase II clinical trial published data on intracoronary re-infusion of cardiosphere-derived autologous stem cells in patients with ischemic heart failure. The trial concluded safe administration of the study product and beneficial clinical data (increased LV function and decreased scar) warranted phase III studies.[190]

A recently phase II clinical trial has investigated the effects of cardiac stem cell alone or in combination with MSC via endomyocardial implantation guided with NOGA catheter. This study, the CONSENT-HF (NCT02501811), recruits patients with ischemic HF and reduced ejection fraction. The study design comprises accurate implantation and follow-up strategy. Changes in LV function will be assessed with cMRI scans. Results are due in 2020.

5.21.6 Cell Delivery

Delivery of donor cells into the failing heart is feasible.[191–193] The goal of any of these delivery strategies is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area.[17] Upcoming trials should address further procedural issues such as the selection of optimal cell type, cell dosage, identification of the underlying disease substrate (ischemic vs non-ischemic heart failure), and timing of cell transfer.[17] With regards to procedures, pharmacokinetics and pharmacodynamics in cell therapy is a critical issue. Welt and Losordo have drawn attention to one of the problems of whether a dosage refers to the number of cells delivered, the number of cells retained, or the number of cells ultimately incorporated into the myocardium.[194] Experts have emphasized the importance of repeated cell delivery.[92] Potential delivery methods may include peripheral intravenous transfusion,[195] selective intracoronary transplantation,[196] transmyocardial by direct epicardial injection,[197] catheter-based transendocardial injections guided by advanced real-time imaging techniques such as MRI and electromechanical voltage mapping,[198–200] and a recently implemented approach of transvenous injection into coronary veins.

Percutaneous transluminal coronary catheters could be used for intracoronary administration of cells. Intracoronary administration into the infarct artery may allow the cells to incorporate into the areas bordering the infarct zone in a homogenous manner. Also, a catheter system incorporating an ultrasound tip for guidance and an extendable needle for myocardial access can be used to deliver cells through the coronary veins into the myocardium.[124] Systemic injection of the cells is not favourable despite of being a less invasive procedure. Unless cells are targeted directly to the injured myocardium, the retention rate remain very low and implanted cells escape to systemic circulation. Further imaging and guidance techniques include direct labeling with superparamagnetic agents, radioactive tracers (e.g., fludeoxyglucose F18 and indium-111-oxine), and molecular imaging using reporter-gene constructs via viral or non-viral vector to study of both engrafted cells and their progeny.[201–203]

5.21.7 Clinical Trials With Bone Marrow-Derived Stem Cells

Experimental studies have indicated that stem and progenitor cells enhance cardiac function especially in chronic heart failure or after myocardial infarction, which concept has been translated into clinical studies. There are four major subgroups of cells being studied in phase 1 and phase 2 clinical trials in patients with chronic HF: bone marrow-derived mononuclear cells, skeletal myoblasts, enriched subpopulations of bone marrow and resident cardiac stem cells. Evidence for a possible use of bone marrow-derived cells in the cardiovascular therapy first appeared in 2001.[43] In most of the studies intracoronary injection were performed, using various number of BMSC ($2–2500 \times 10^6$) cells after acute myocardial infarction, but early clinical trials can be described by their procedural heterogeneity, and a lack of standardization.[204] Intracoronary infusion of BMC was shown to improve left ventricular function in patients with acute MI in a number of larger controlled randomized trials evaluating the efficacy of BMC in MI patients with MI presenting mixed results, especially after longer follow-up times (**Table 4**). In a study by Lunde and colleagues, patients with acute ST-elevation MI of the anterior wall treated with percutaneous coronary intervention were randomized to the group that underwent intracoronary infusion of autologous mononuclear BMC or sham injections 3–5 days later.[205] After 6 months, echocardiography, ECG-gated SPECT, and MRI were used to assess left ventricular function, end-diastolic volume, and infarct size. With the largest volume, randomized REPAIR-AMI (Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction) trial included 204 patients with ST-elevation MI to an intracoronary infusion into the infarct-related artery with either heterogeneous population of haematopoietic, mesenchymal and mononuclear cells or placebo vehicle medium 3–7 days after reperfusion therapy.[206] At 4 month follow-up, patients treated with BMC had a significant improvement in LVEF compared with placebo (+5.5 vs +3.0%, absolute difference +2.5%). In further subgroup analyses, the improvement in LVEF was most prominent in patients with poorer baseline LVEF and in those treated ≥5 days after MI. The results also showed a reduction in the combined clinical endpoint of mortality, a trend toward a reduction in recurrent MI or repeated revascularisation.

Beneficial effects of BMC administration on cardiovascular outcome are preserved even after an observation period of 2 years after MI[207] and several trials showed further improvement in angina or left ventricular function in chronic ischaemic heart disease even with preserved or decreased ejection fraction. In a controlled crossover study patients with stable ischemic heart disease with at least 3 months after an MI were randomized to receive BMC, circulating progenitor blood cells, or placebo into the patent coronary artery.[208] This study suggested that intracoronary infusion of progenitor cells is safe in patients with previous MI and infusion of BMC is associated with moderate improvement in the LVEF. Patients receiving BMC had a 2 percentage point increase in LVEF, while the ones in placebo group had a 1% decrease in LVEF. Fewer randomized trials of transplants of blood- or bone marrow-derived stem cells have been performed in the setting of chronic coronary artery disease and chronic heart failure.[112,209,210] Some controversial results brought publication bias and clinical data from Strauer et al. were withdrawn due to publication fraud. Janssens and colleagues used infusion of autologous bone marrow mononuclear cells in patients with MI 24 h after successful percutaneous coronary intervention, showed no benefit in LVEF, but a significant reduction in infarct size and improved regional left ventricular function.[196] The BAMI study is due to end in 2018 and will clarify effects of these cells in intracoronary implantation after ischemic events.

Four recent meta-analyses[211–214] of these trials, incorporating 5, 10, 13, and 18 randomized and non-randomized trials, respectively, and involving altogether 999 patients with acute MI or chronic ischaemic cardiomyopathy help to place the results of individual trials into perspective. These meta-analyses showed that transplantation of BMC improved LVEF by 5.4%, decreased infarct scar size by 5.5% and lowered left ventricular end-systolic volume by 4.8 mL.[211] Other meta-analyses[215] indicated that BMC treatment is beneficial; however, the typical modest increase in ejection fraction is of uncertain clinical significance. The overall benefit on ventricular function demonstrated in the meta-analyses needs to be tempered by the ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction),[192] REGENT (Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction)[216] and BOOST (BOne marrOw transfer to enhance ST-elevation MI regeneration) trials showing either no benefit or a temporary improvement for less than 6 months. The post hoc analysis of data from the REPAIR-AMI and REGENT trials also revealed that BMC had substantial treatment effects only in areas with the greatest damage or extent of scarring.[196] Of note, LVEF may be an insensitive readout for assessing the long-term outcome of interventions and modest changes in LVEF may be translated into substantial improvements in clinical outcomes. Large clinical trials of primary coronary intervention or angiotensin-converting-enzyme inhibition are also associated with some 3%–4% improvement in LVEF 6–12 months after MI, but do show a significant effect on mortality and morbidity.[217,218] Other measurements such as brain natriuretic peptide levels, 6-minute walk test times, peak VO_2, infarct remodeling[219] or exercise capacity,[220] which may be more indicative of long-term outcome, were positively affected by BMC treatment, at least for the first 4–6 months, and could serve as more appropriate endpoints in cell therapy trials.

Table 4 Clinical trials with stem cell and progenitor cells

Cell type	*Clinical setting*	*Study design*	*n*	*Method of cell transplantation*	*Cell number transplanted*	*Mean follow-up duration (months)*	*% Change in LVEF versus control*	*References*
BMMNC	AMI	R-SB	60	Intracoronary	1 × 108	12	+7.0%; *p*=.03	235
BMMNC	AMI	R-SB	66	Intracoronary	1 × 108	3	+3%; *p*=.04	236
BMMNC	AMI	R-SB	60	Intracoronary	2.5 × 109	18	+2.8%; NS	237
BMMNC	ICMP	R-SB	51	Intracoronary	2 × 108	3	+4.1%; *p*<.001	238
BMMNC	AMI	R-SB	204	Intracoronary	2.4 × 108	12	Lower mortality	239
BMMNC	AMI	R-SB	204	Intracoronary	2.4 × 108	4	+2.5%; *p*=.01	206
BMMNC	AMI	R-SB	20	Intracoronary	4 × 107	6	+6.7%; NS	240
BMMNC	ICMP	R-SB	20	Intramyocardial	6 × 107	4	+2.5%; NS	241
BMMNC	AMI	R-DB	67	Intracoronary	1.7 × 108	4	+1.2%; NS	196
BMMNC	AMI	R-SB	100	Intracoronary	8.7 × 107	6	−3.0%; *p*=.05	205
BMMNC	AMI	Cohort	36	Intramyocardial	3 × 108	3	+4%	242
BMMNC	AMI	Cohort	20	Intramyocardial	2.6 × 107	12	+8.1; NS	243
CPC	AMI	Cohort	54	Intracoronary	5 × 109	6	+6%; *p*=.04	244
CPC	AMI	Cohort	73	Intracoronary	2 × 109	6	+2.8; NS	245
CPC	ICMP	R-SB	47	Intracoronary	2.2 × 107	3	+0.8%; NS	238
CPC	AMI/ICMP	R	82	Intracoronary	1.4 × 109	6	−0.2%; NS	246
CPC	AMI	Cohort	70	Intracoronary	7.3 × 107	6	+5.5%; *p*=.04	247
CPC	ICMP	SB	26	Intracoronary	7 × 107	3	+7.2%; NS	112
CD133	ICMP	Cohort	27	Intramyocardial	Not available	6	Not available	248
CD133	ICMP	Cohort	55	Intramyocardial	6 × 106	6	+6.3%; *p*=.02	110
CD133	ICMP	Cohort	35	Intracoronary	1.3×107	4	+2.8%; NS	111
CD34	ICMP	R-DB	24	Intramyocardial	5 × 104,105,5 × 105	6	Not available	2b
SMB	ICMP	R-DB	97	Intramyocardial	4 × 108 or 8 × 108	6	−1.0%/+0.8%; NS	137
SMB	ICMP	Cohort	26	Intramyocardial	2.5 × 108	12	+14.5%; *p*<.01	249
SMB	ICMP	Cohort	12	Intramyocardial	2.1 × 108	12	+11.6%; *p*<.05	250
MSC	ICMP	R	45	Intracoronary	5 × 106	12	−3.0%; NS	251
MSC	AMI	R-SB	69	Intracoronary	6 × 1010	6	+12.0%; *p*=.01	86
MSC+EPC	ICMP	Cohort	22	Intracoronary	3 × 106	4	+0.3%; NS	252
BMSC	AMI	R-SB	20	Intracoronary	Not available	6	+9.2%; *p*<.05	253
BMSC	ICMP	R-DB	92	Intramyocardial	100 × 106	24	+2.7% *p*=.03	254
BMSC	DCM	R-SB	30	Intramyocardial	100 × 106	6	+3% NS	243
BMSC	CAD	R-SB	100	Intracoronary	not available	6	+4.1 NS	192
BMSC	CAD	R-SB	200	Intracoronary	178 × 106	6	+3% *p*=.01	216
MSC	ICMP	R-DB	55	Intramyocardial	Not available	6	6.2% *p*=.0001	1a
MSC	ICMP	R-DB	240	Intramyocardial	600 × 10 (6)	13	+4% NS	92

BMSC, bone marrow-derived stem cells; Cohort, non-randomised/blinded study; CPC, circulating progenitor cells; DB, double-blinded; BMSC, bone marrow-derived stem cells; Cohort LVEF, left ventricular ejection fraction; R, randomized; SMB, skeletal myoblast.

5.21.8 Conclusions and Future Challenges

Stem cell therapy holds the promise of replacing damaged myocardium in patients with heart failure. The clinical experience from the approximately 1000 patients who have already received stem cell therapy indicates a favourable safety profile and a significant capability for cardiac repair, improvement in cardiac function and structural remodeling in the setting of MI and chronic heart failure. Clinical data to date suggest, however, that these are still early times for cell-based therapy for heart failure, with a number of negative, minor, and transient effects recorded in larger randomized double-blind trials. There is a strong argument for the continuation of clinical trials though[221–223]; more than 100 new clinical trials were listed in the US and Europe at January 2018 (http://clinicaltrials.gov/ct2/results?term=stem+cell+heart+failure). Most of these trials focus on MSC, BM-MNC or their combination. New approaches include implantation of HUVECs and hiPSC-derivatives. Tailored patient selection and early identification of those who are responder to stem cell therapy will be a cornerstone of clinical translation. Clearly, stem cells are very complex therapeutic products which significantly differ from drug-based clinical medicine. Introducing stem cells into the clinical routine practice through cell-based therapy protocols requires better understanding of their interesting basic biological processes. Intense research in this area is expected to resolve current biological and technical problems and satisfy all criteria for a future use of stem cells for heart failure therapy (**Table 5**). Among others, improvements in scalable manufacturing processes, selection of the most favourable cell type(s), optimizing in vivo delivery and engraftment systems, and finding new ways to promote the stem cell differentiation to functional myocytes and integrated vascularization would allow researchers to overcome barriers that stem cell therapy faces.

Table 5 Challenges and requirements for a clinical application of stem cells and derivatives

Proliferative and production issues	Appropriate candidate stem or progenitor cell types and cell sources
	Control of cell and tissue growth, different cardiac subtypes. Role of proliferative enhancers. Need for standard operating procedures
	Need for effective differentiation of the culture and purification from contaminating cells that yield purified cell populations for transplantation[255]
	Approaches to drive cardiomyocytes toward electrophysiological maturation[256,257]
	Issues on scalability and need for large scale ESC cultures for sheer number of cells that needs to be replaced (up to 10^9 myocytes are lost in MI),[258] need for HLA classified hiPSC banks
	Ethical and safety concerns of genetically modified regenerative products
Xenogeneic laboratory conditions	Xeno-, DMSO- and serum-free conditions for maintenance
	Characterization of defined matrix components
	Importance of recombinant enzymes for passaging
Preclinical studies. Model systems	Use of suitable, clinically relevant large animal models
	Control of graft size in infarction models. Cell survival in grafts and cell integration into the host tissue
	Inducible but no spontaneous cellular contractility, stable resting cell membrane potential
Administration. Standardization of methods	Timing
	Standardized cell harvest, scalable isolation, preparation and storage procedures
	Number and type of cells transplanted
	Methods and optimal sites and route of delivery and tracking of implanted cells
	Augmentation of cell homing, retention, integration, alignment/spatial distribution and survival of transplanted cells in recipient heart
Immunogenicity	Allogeneic nature of the cells. Immune rejection and suppression
	Cell banks of donors with different HLA types[259]
Safety concerns, side effects and biodistribution issues	Aberrant cell differentiation for mesenchymal stem cells[260]
	Arrhythmogenicity.[261–263] Effects of cell delivery routes on arrhythmia[263] and the presence of underlying ischemic substrate[127]
	Tumor formation with ESC/iPSC-derived populations. Lowering of tumorigenic risk by guided lineage specification or selection of early progenitors
	Multiorgan seeding in the spleen, lungs, and liver[264,265]
	iPS reprogramming procedure. Use of integrating viral vectors.
	Increased restenosis in MI patients after stenting and mobilization bone marrow cells by granulocyte colony-stimulating factor (G-CSF).[266]
	Possibility of accelerated atherosclerosis in patients undergoing percutaneous coronary intervention in acute MI.[111]
	No serious adverse effects on restenosis or other adverse outcomes.[267,268]
	Prevention of the development of chromosomal abnormalities
Other	Ethical and legal issues. Regulatory issues in the European Union surrounding new cardiovascular therapies, and procedures in which cells are manipulated/engineered.[269]
	Patient selection criteria. Can etiology of heart failure define the cell type used? Impact of co-morbid conditions such as age, diabetes, smoking, and hypertension. Repeated implantation.

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