Curr Heart Fail Rep (2013) 10:165–176
DOI 10.1007/s11897-013-0134-z

MANAGEMENT OF HEART FAILURE (TE MEYER, SECTION EDITOR)

Current Status of Cell-Based Therapy for Heart Failure

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Published online: 19 March 2013

Abstract In the last two decades, morbidity and mortality of patients with chronic heart failure could be further reduced by improved pharmacological and cardiac device therapies. However, despite these advances, there is a substantial unmet need for novel therapies, ideally specifically addressing repair and regeneration of the damaged or lost myocardium and its vasculature, given the limited endogenous potential for renewal of cardiomyocytes in adults. In this respect, cardiac cell-based therapies have gained substantial attention and have entered clinical feasibility and safety studies a decade ago. Different cell-types have been used, including bone marrow–derived mononuclear cells, bone marrow–derived mesenchymal stem cells, mobilized CD34+ cells, and more recently cardiac-derived c-kit+ stem cells and cardiosphere-derived cells. Some of these studies have suggested a potential of cell-based therapies to reduce cardiac scar size and to improve cardiac function in patients with ischemic cardiomyopathy. While first clinical trials examining the impact of cardiac cell–based therapy on clinical outcome have now been initiated, improved understanding of underlying mechanisms of action of cell-based therapies may lead to strategies for optimization of the cardiac repair potential of the applied cells. In experimental studies, direct in vivo reprogramming of cardiac fibroblasts towards cardiomyocytes, and microRNA-based promotion of cardiomyocyte proliferation and cardiac repair have recently been reported that may represent novel therapeutic approaches for cardiac regeneration that would not need cell-administration but rather directly stimulate endogenous cardiac regeneration. This review will focus mainly on recently completed clinical trials (within the last 2 years) investigating cardiac cell-based therapies and the current status of experimental studies for cardiac cell-based repair and regeneration with a potential for later translation into clinical studies in the future.

Keywords Cardiomyopathies · Myocardial infarction · Therapy · Regeneration · Bone marrow transplantation · Methods · Autologous transplantation · Stem cell transplantation · Stem cells · Heart failure · Left ventricular dysfunction · Remodeling

Introduction

Loss of myocardium rapidly after myocardial infarction and the ongoing death of cardiomyocytes thereafter frequently terminates in heart failure, as endogenous regeneration pathways cannot replace damaged myocardium and vasculature. Unlike in zebrafish [1, 2], division of differentiated cardiomyocytes (CM) is a rare event in humans [3].

In the last decade, numerous different human cell populations, including bone marrow-derived mononuclear cells and CD34+ cells, have been suggested to enhance cardiac function and repair in experimental animal models. Several clinical studies largely examining feasibility and safety have been performed and have yielded mixed results with respect to effects on cardiac function. Cell isolation procedures, cell types, number of transplanted cells, and the functional cardiac repair capacity of the transplanted cells are likely determinants of their effects on cardiac function [4–6]. Here, we describe recent experiences of cardiac cell-based therapies using different cell populations (Fig. 1, Table 1).

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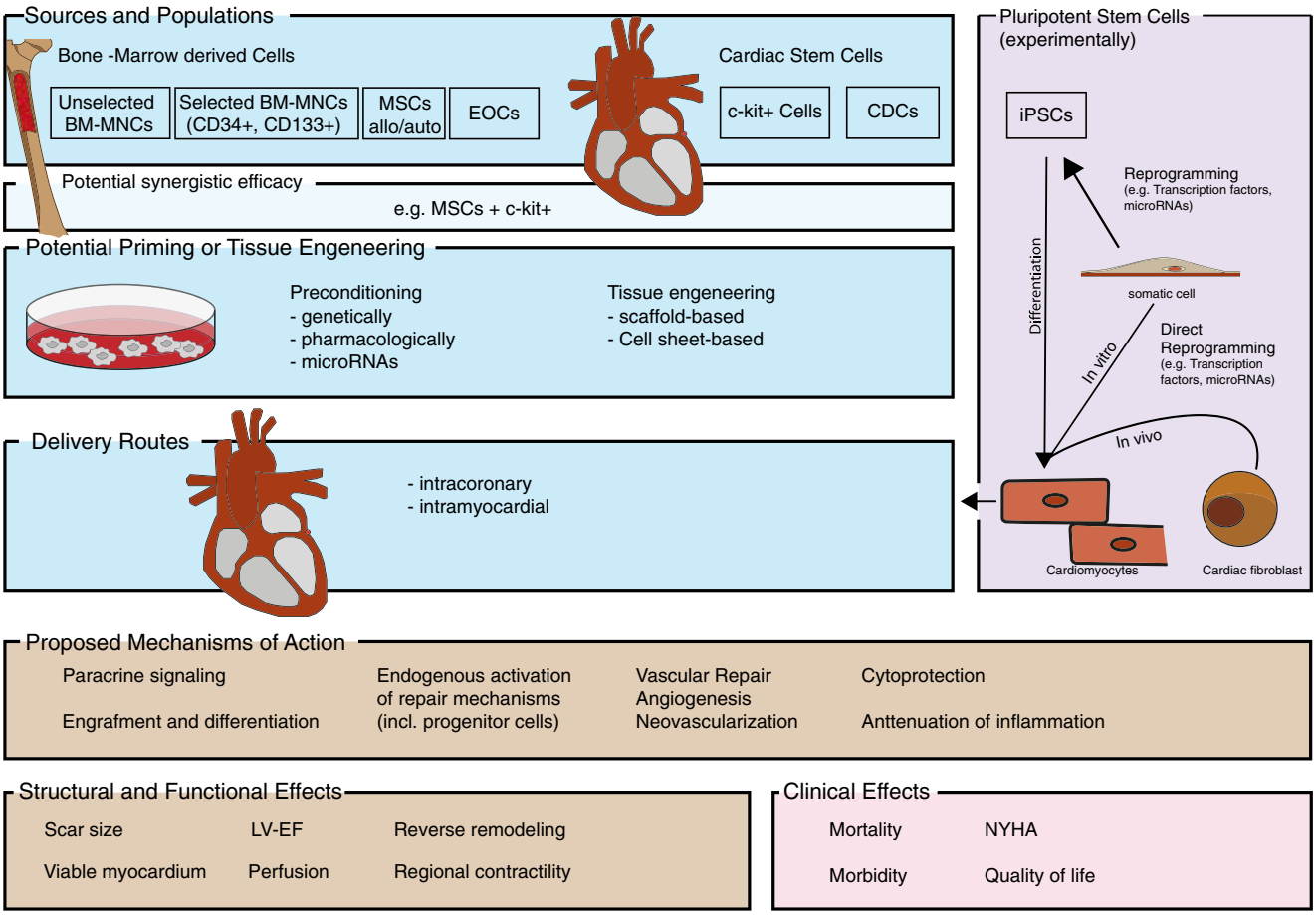

Fig. 1 Clinically and experimentally emerging cells for heart regeneration therapies. Allo allogeneic; Auto autologous; BM-MNC bone marrow mononuclear cells; CDCs cardiosphere-derived cells; EOCs early outgrowth cells (also known as circulating angiogenic cells (CACs) or early endothelial progenitor cells (EPCs); iPSC induced pluripotent stem cells; LV-EF left ventricular ejection fraction; MSCs mesenchymal stem cells; NYHA new york heart association

Embryonic Stem Cells and Induced Pluripotent Stem Cells

Embryonic stem cells (ESCs) share the ability to differentiate in all three germ layers (pluripotency) and can be infinitely expanded (clonogenicity and self-renewal) [7]. Therefore, ESCs can be considered as an infinite source to generate the desired tissue, particularly as numerous studies have shown differentiation into cardiomyocyte-like cells and endothelial cells, and improvement of cardiac function after transplantation in experimental cardiac injury models [8–10]. However, allogeneic transplantation is required, which may cause immunologic reactions after transplantation and, as they are obtained from blastocytes, i.e., an early embryonic stage, ethical concerns set an additional barrier for wider clinical applications. Moreover, because of their ability to expand clonogenically, there is a substantial risk of teratogenic potential, at least for undifferentiated ESCs. These aspects limit their current use for potential human heart regeneration therapies.

Man-made dedifferentiated cells, which share similar properties with ESCs, are termed induced pluripotent stem cells (iPSCs). iPSCs have initially been reprogrammed from differentiated fibroblasts in 2006 [11, 12]. Since then, reprogramming protocols have been refined, thereby raising the efficiency and by transfection of recombinant proteins or RNA molecules, such as microRNAs, circumvented the initially required transfection procedures with stemness factors (i.e. transcription factors highly expressed in ESCs) via retroviruses [13–15]. As they can be directed to differentiate towards cardiomyocytes, iPSCs represent a potential resource of personalized heart tissue replacement and a valuable tool to further understand potential pathways towards cardiac regeneration. Using in vivo imaging, we have recently observed viability, tissue distribution and long-term engraftment of cellular iPSC-derived grafts in a large animal model of myocardial infarction [16].

However, iPSCs share the teratogenic potential with ESCs and recently the immuno-compatibility of undifferentiated autologous iPSCs has been questioned [17]. Moreover, as

Table 1 Selected published and ongoing cell-based clinical studies in patients with acute myocardial infarction and/or chronic heart failure

Study	Phase	*n* (treated/control)	Cell type	Condition	Donor type groups modification	Delivery	Placebo	Primary endpoints	Results	Clinical Trial Identifier	Citation
Selected published clinical trials in 2011/2012 using cell-based therapies for cardiac disease											
CPCs											
SCIPIO	I	16/7	c-kit+	post-CABG		IC	yes (standard care)	SAE	SAE -, LVEF↑, infarct size↓	NCT00474461	32
CADUCEUS	I	17/8	CDC	ICM		IC	yes (standard care)	SAE	SAE -, scar mass↓, LVEF -	NCT00893360	34
BM-MNCs											
FOCUS	II	61/31	BM-MNC	ICM		IM	yes (Placebo injection)	LVESV, MVO2, reversible defect	LVESV -, MVO2 -, reversible defect -	NCT00824005	57
TIME	II		BM-MNC	STEMI						NCT00684021	58
		41/22			3 days after PCI	IC	yes (Placebo injection)	LVEF, regional left ventricular function	LVEF -, regional left ventricular function -		
		34/15			7 days after PCI	IC	yes (Placebo injection)	LVEF, regional left ventricular function	LVEF -, regional left ventricular function -		
LateTIME	II	55/26	BM-MNC	AMI	2 to 3 weeks after PCI	IC	yes (Placebo injection)	LVEF, regional left ventricular function	LVEF -, regional left ventricular function -, LV volumes -, infarct size -	NCT00684060	60
SWISS-AMI	II	107/60	BM-MNC	STEMI			yes (standard care)			NCT00355186	61
					5-7 days after PCI	IC		LVEF	LVEF -, infarct size -,		
					3-4 weeks after PCI	IC		LVEF	LVEF -, infarct size -,		
ACT34-CMI	II	109/53	CD34	ICM (refractory angina)		IM	yes (Placebo injection)	angina pectoris frequency	angina frequency↓, execise tolerance↑	NCT00300053	70
MSCs											
POSEIDON	I/II	15/15 (allo/auto)	MSC	ICM	allogeneic and autologous	IM	no	SAEs	SAE -, auto/allo: infarct size↓, LVEF -; allogeneic: 6-min walk test↑, MLHFQ↑	NCT01087996	84
C-Cure	II/III	21/24	MSC	ICM	Pre-treatment with growth factors	IM	yes (standard care)	LVEF	LVEF↑	NCT00810238	96
Selected ongoing clinical trials using cell-based therapies for cardiac disease											
CPCs											
ALLSTAR	I/II		CDC	ICM	allogeneic	IC	yes (Placebo injection)	Infarct size, SAEs		NCT01458405	
ALCADIA	I		CDC	CABG	bFGF gelatin sheet	IM	no	SAEs		NCT00981006	
BM-MNCs											
REPEAT	II/III		BM-MNC	ICM	single/repeated delivery	IC	no	Mortality		NCT01693042	
BAMI	III		BM-MNC	STEMI		IC	yes (standard care)	All cause death		NCT01569178	
REGEN-AMI	II/III		BM-MNC	STEMI	Delivery within hours after PCI	IC	yes (Placebo injection)	LVEF		NCT00765453	
IMPACT-CABG	II		CD133+	CABG		IM	yes (Placebo injection)	SAEs, major arrhythmia		NCT01033617	

Table 1 (continued)

Study	Phase	*n* (treated/control)	Cell type	Condition	Donor type groups modification	Delivery	Placebo	Primary endpoints	Results	Clinical Trial Identifier	Citation
PERFECT	III		CD133+	CABG		IM	yes (Placebo injection)	LVEF		NCT00950274	
PreSERVE-AMI	II		CD34+	STEMI		IC	yes (standard care)	SAE, myocardial perfusion		NCT01495364	
ENACT-AMI	II		EPC	STEMI	eNOS over-expression	IC	yes (Placebo injection)	LVEF		NCT00936819	
MSCs											
PROMETHEUS	I/II		MSC	post-CABG		IM	yes (Placebo injection)	SAEs		NCT00587990	
TAC-HFT	I/II		MSC or BMC	ICM		IM	yes (Placebo injection)	SAEs		NCT00768066	
POSEIDON DCM	I/II		MSC	DCM	allogeneic and autologous	IM	no	SAEs		NCT01392625	

AMI acute myocardial infarction, *bFGF* basic fibroblast growth factor, *BM-MNC* bone marrow mononuclear cells, *CABG* coronary artery bypass grafting, *CDCs* cardiosphere-derived cells, *DCM* dilated cadiomyopathy, *eNOS* endothelial nitric oxide synthase, *EPC* early endothelial progenitor cells, *IC* intracoronary delivery, *ICM* ischemic cardiomyopathy, *IM* intramyocardial, *LVEF* left ventricular ejection fraction, *LVESV* left ventricular end-systolic volume, *MLHFQ* minnesota living with heart failure questionnaire, *MSCs* mesenchymal stem cells, *MVO2* change in maximal oxygen consumption, *NYHA* new york heart association, *PCI* percutaneous coronary intervention, *SAE* serious adverse events, *STEMI* ST-elevation myocardial infarction

they are genetically modified and require prolonged cultivation times, iPSC-derived cells may have a risk for mutations. Hence, the use of iPSCs is challenging and not yet feasible for clinical applications. Direct reprogramming of cardiac fibroblasts into cardiomyocytes in vivo therefore represents a highly interesting perspective [18•, 19].

Cardiac-Derived Progenitor/Stem Cells

Amongst somatic progenitor cells, cardiac progenitor cells (CPCs) have been postulated to have the highest capacity to promote cardiac regeneration. The identification of c-kit+ cells [20] residing in stem cell niches [21] in the murine heart that can give rise to the main cellular components of the heart, namely cardiomyocytes, endothelial cells and smooth muscle cells have rendered the heart an organ with potential regenerative capacity. In experimental studies, transplantation of c-kit+ cells reconstituted the heart and improved cardiac function [20]. Next to c-kit+ cells, other populations as defined by surface markers or culture conditions have been suggested as an endogenous source of heart regeneration. Isl-1+ cells [22] derive from the second heart field, but can be rarely found in postnatal development stages (reviewed in [23]). Sca-1+ (stem cell antigen-1) cells [24] are restricted to murine hearts (no orthologue in human), but have also been suggested for heart regeneration [25]. Cardiosphere-derived cells (CDCs) are cultured from heart biopsies and are so named because of their ability to form spheroids in cell suspension [26, 27]. CDCs are multicellular clusters containing a mixed cell population, which comprise, next to cardiac progenitor cells with c-kit and CD105 surface marker expression, other cell types also, such as mesenchymal stem cells [26, 28]. Experimental data suggest that heart regeneration of CDCs depends on the release of paracrine factors, induction of endogenous regenerative capacity, and to a lesser extent on the differentiation into cardiomyocytes and endothelial cells in vivo (which has also been controversial) [26, 28–30].

All these adult cardiac-derived stem cells are suggested to have self-renewal capacities and the ability of multilineage differentiation. Importantly, adult cardiac stem cells have the potential to reconstitute damaged myocardium and improve cardiac function after heart injury [20, 25, 28, 29, 31]. Moreover, CPCs can be obtained by endomyocardial biopsies and sorted according to their surface markers and/or expanded in cell culture.

To date, two published clinical phase I trials have conducted transplantation of cardiac-derived cell products in patients with ischemic cardiomyopathy. In the randomized SCIPIO-trial, $1x10^6$ c-kit$^+$ cardiac stem cells were delivered intracoronary to patients with coronary artery bypass surgery (CABG) and left ventricular ejection fraction (LVEF) <40%. The control group received no cell therapy. After 4 months,

infarct size as assessed by cMRI decreased (with, however, a lack of a control group for comparison) and LVEF as assessed by echocardiography significantly increased in patients receiving cell therapy. In a subset of patients, cMRI and echocardiography measurements were performed after 12 months, which showed an even more-reduced scar size and improved LVEF. Moreover, although a low number of cells were injected, adverse events were comparable with standard care treated patients [32••, 33].

Another, recently published trial, the Cardiosphere-Derived Autologous Stem Cells To Reverse Ventricular Dysfunction (CADUCEUS) trial [34••], used intracoronary infusion of cardiosphere-derived cells (CDCs) in patients with ventricular dysfunction 2–3 months after myocardial infarction.

The CADUCEUS trial [34••] suggested a reduction in scar mass and an enhanced viable heart mass at 6 and 12 months after transplantation. However, despite these beneficial effects, no significant change in LV-function could be observed.

The pilot data of these two clinical trials indicate that intracoronary delivery of heart-derived cell products is feasible and safe and may improve cardiac function in patients with ischemic cardiomyopathy. Although these trials, using either selected c-kit+ cells or CDCs (a mixed cell population), are not directly comparable because of different patient population and study designs, both suggest a reduction in scar size, which renders cardiac stem/progenitor cells to an interesting candidate for cell-based therapies. However as only a few patients were enrolled in the treatment arm (17 patients in the CADUCEUS trial and 16 patients in the SCIPIO trial), and control groups received only standard care, safety and efficacy has to be proven in a randomized-blinded, placebo-controlled study design.

Moreover, the finding that CDCs lack MHC II antigens, and therefore cause only a mild immune reaction after transplantation in the rat infarcted heart [35], initiated the ongoing randomized, double-blind, placebo-controlled ALLSTAR (NCT01458405) trial in patients with myocardial infarction and left ventricular dysfunction. With allogeneic cell transplantation, biopsies of patients would be needless; cells could be injected to a specific time point and circumvent the suggested impairment of adult progenitor cells [36, 37].

Bone-Marrow Derived Stem Cells

Mechanisms of Effects of Bone-Marrow Derived Cells on Cardiac Function Initially, bone-marrow mononuclear cell (BM-MNC)-transplantation was thought to yield its effects on cardiac function by transdifferentiation into cardiomyocytes and endothelial cells [38]. Later, this concept has been challenged [39, 40]. Experimental studies have indicated that direct transdifferentiation of BM-MNCs into cardiomyocytes or endothelial cells is (if it ever occurs) a very rare event [39, 40], and could not explain the observed effects on cardiac function. Early studies may therefore have observed cell fusions, rather than true transdifferentiation of BM-MNCs into cardiomyocytes [40]. It is more conceivable that BM-MNCs enhance cardiac repair by paracrine effects [41•, 42]. Release of growth factors from transplanted BM-MNCs are suggested to promote migration of endothelial cells and CPCs, and can exert cytoprotective effects on resident cardiomyocytes [43, 44]. Particularly, BM-MNCs support cardiac angiogenesis and neovascularization in the infarcted heart [45]. However, a recent study has also suggested that bone marrow–derived c-kit+ cells promote augmentation of cardiomyocyte progenitor activity, which may lead to cardiomyocyte formation [46].

Recently Published (Within Last 2 Years) Clinical Studies of Cardiac Cell-Based Therapies Using BM-MNCs in Patients with Myocardial Infarction and Ischemic Cardiomyopathy As BM-MNCs are an easily accessible cell source (via bone marrow aspiration), initial clinical studies have used transplantation of this heterogeneous cell population [47–50, 51••]. Clinical trials so far showed an excellent safety profile and feasibility. The effects observed in recent clinical studies on LV-function were more modest as expected [52–55], however, a meta-analysis of 1765 participants has suggested a significant improvement of LV-EF, both in short (3.26 %) and long term (3.91 %) follow-up [56].

In this regard, clinical trials were initiated by the Cardiovascular Cell Therapy Research Network (CCTRN) in patients with significant LV-dysfunction caused by ischemic cardiomyopathy and patients with ST-elevation myocardial infarction (STEMI) [57, 58•]. In the FOCUS-CCTRN trial, patients with ischemic cardiomyopathy were enrolled to receive BM-MNCs by transendocardial administration [57]. In this phase II randomized trial, at 6 months, LV end-systolic volume (as assessed by echocardiography) did not significantly differ between BM-MNCs administration and placebo group. However, exploratory analysis indicated a significant increase in LVEF (2.7 %) and stroke volume in the treatment group. Although this was the largest recent clinical trial conducted in patients with severe LV-dysfunction (LV-EF: 32.4 %) caused by ischemic cardiomyopathy, the sample size may have been too small.

Clinical data (and some later experimental observations) had suggested that timing of BM-MNC delivery after acute myocardial infarction may have an impact on its effects on cardiac function [51••, 59]. The TIME-trial [58•] focused on different time points of intracoronary BM-MNC delivery at day 3 and day 7 in patients with ST-elevation infarction treated with percutaneous primary intervention. However, no benefit on cMRI detected LV-performance could be observed 6 months after infusion of BM-MNCs in either group [58•].

Moreover, as assessed in the LateTIME [60•] and SWISS-AMI [61•] trial, BM-MNC administration 2 to 3 weeks after acute myocardial infarction did not significantly affect LV-function. However, although the SWISS-AMI trial was not optimized to evaluate this endpoint, subgroup analysis indicates a beneficial effect on LV-function after 4 months when revascularization therapy was performed less than 4.5 hours after symptom onset [61•]. In addition, these trials may not be geared to detect smaller changes in LV function.

Potential Impact of Cell Isolation Procedures and Impaired Functional Capacity of Adult Bone-Marrow Derived Cells Whereas all clinical trials have supported the safety of delivery of BM-MNCs, the lack of a significant beneficial effect after BM-MNC delivery on LV-function in some of these studies raises the question of whether this patient-derived cell population will be efficient enough for long-term improvement of cardiac function. Importantly, however, the mode of bone marrow–derived cell preparation may play a critical role that likely has been underestimated.

For example, certain agents, such as buffer and medium composition during cell isolation, have been shown to crucially alter cellular function. Heparin has been observed recently to exert detrimental effects on the functionality of BM-MNCs by interacting with the CXCR4/SDF-1 axis [62, 63]. The CCTRN–trials have been performed using an automated cell-sorting system for the isolation of BM-MNCs [64, 65]. However, whether these cells are efficient in an experimental myocardial infarction model in vivo has not been reported. Therefore, as isolation procedure steps may have a crucial influence on cell functionality, the functional properties of these cells after automatic separation may be a determinant for the in vivo effects.

In addition, our group could show that the cardiac repair capacity of angiogenic early outgrowth cells [EOCs, also known as circulating angiogenic cells (CACs)] is impaired in patients with chronic heart failure caused by ischemic cardiomyopathy as compared to healthy subjects in an experimental myocardial infarction model [36]. Together with other studies, which show an impairment of migration and angiogenic capacity of adult bone marrow–derived mononuclear cells [66, 67], this might contribute to the limited capacity of BM-MNCs in clinical trials to effectively impact on cardiac function. In this respect, a phase I trial was recently published using allogeneic bone marrow–derived cells [68] from healthy donors as an off-the-shelf product, which may circumvent impairment of autologous cell function in patients with cardiovascular disease.

Bone-Marrow-Derived and Mobilized CD34+ Cells for Cell-Based Cardiac Therapy Instead of unselected BM-MNCs, distinct cell populations with cardiac repair capacity can be isolated from the bone marrow. CD 34+ cells represent a rare subpopulation of BM-MNCs with an experimentally high potential of promoting angiogenesis and neovascularization in ischemic tissues [69]. In the ACT-34CMI trial, intramyocardial administration of low- or high-dose CD34+ cells was performed in patients with ischemic cardiomyopathy and refractory angina pectoris. Of interest, in the low-dose CD34+ cell group, a reduction in angina pectoris frequency and improvement in exercise tolerance was observed at 6 and 12 months after treatment [70•]. This study also delineates an example for trials, which not only takes functional endpoints into consideration, but focuses more on clinical endpoints in patients with chronic heart failure, and also questions whether higher numbers of intramyocardially applied cells are indeed more efficient.

In conclusion, it is noteworthy that no adverse events occurred in trials using BM-MNCs. In addition, a reduction of major adverse cardiovascular events was observed in the REPAIR-AMI trial, which maintained for 2 years after acute myocardial infarction [71]. This finding is underlined by a recently published meta-analysis [72•]. In this respect, large scaled phase 3 trials are on the way to identify the effects of BM-MNCs on clinical outcome and mortality in patients with acute myocardial infarction or ischemic cardiomyopathy (BAMI, NCT01569178; REPEAT, NCT01693042).

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a subpopulation of bone-marrow mononuclear cells and can be cultured by repeated passaging on plastic surfaces [73]. Typically, MSCs are able to differentiate in cartilage, bone, or adipose tissue [73], but differentiation into cardiomyocyte-like cells has also been suggested [74], which has rendered them attractive for cardiac regeneration therapies. In addition, MSCs release growth factors, indicating a therapeutically important paracrine function and direct cell-cell interactions, which may additionally activate endogenous repair mechanisms [75–79]. Moreover, at least initially, MSCs prevent anti–donor T-cell responses and create an immunosuppressive milieu, thereby generating an immune-privileged state [80]. In this regard, experimental studies have demonstrated an improved LV-function after allogeneic transplantation of MSCs [81].

Both autologous and allogeneic MSC- administration was tested in clinical trials.

Chen et al [82]. recruited 69 patients after acute myocardial infarction (AMI) for a placebo-controlled trial using intracoronary delivery of autologous MSCs. Three months after administration, an improved LV-function and decreased left ventricular volumes were detected. In addition, a decrease in perfusion defect could be observed, indicating reverse remodeling and cardiac regeneration after autologous MSC administration. As an ‘off-the-shelf’ product, MSCs from

healthy volunteers were also transplanted allogeneically in patients with AMI [83]. Importantly, in this placebo-controlled trial using intravenous injection, safety outcomes did not differ in the treatment arm and furthermore, a decrease in ventricular arrhythmias was observed [83]. Notably, LVEF, as assessed with echocardiography, increased significantly in patients treated with allogeneic MSCs [83].

In the recently published POSEIDON trial [84••], based on pilot data [85], a head-to-head comparison between autologous and allogeneic transplantation of MSCs in a dose-escalating manner in patients with LV-dysfunction caused by ischemic cardiomyopathy was performed. Cell-based treatment associated adverse events were low, though a placebo-treated group was missing, and adverse events did not differ between autologous and allogeneic cell transplantation. Thirteen month after transplantation of MSCs, reverse remodeling (as assessed by LV sphericity index) could be observed, along with a reduction of myocardial infarction size. However, a significant change in LV-EF was not observed. Interestingly, it appeared that low doses of MSCs resulted in the greatest reductions in LV volumes.

New insights using an intramyocardial delivery approach in patients with ischemic cardiomyopathy are under way: TAC-HFT (NCT00768066), PROMETHEUS (NCT00587990), and pilot data from the TAC-HFT study (a placebo-controlled trial) suggest potential beneficial effects of MSCs in this patient population [85].

Priming/Preconditioning of Stem Cells

As it was reported that adult progenitor/stem cells are impaired in their functional cardiac repair capacity [36, 37, 66], next to advances in allogeneic cell transplantation described above, strategies to enhance functional capacity of autologous progenitor/stem cells emerge as promising applications. Preconditioning of progenitor cells by ischemic, pharmacological, or genetic manipulation to render them resistant to the hostile environment in ischemic tissues may enhance their functional properties that is currently intensely investigated [86, 87].

eNOS-Overexpression in EOCs Recruitment of angiogenic EOCs (also known as CACs) and dysfunction of endothelial cells is critically dependent on endothelial nitric oxide synthase (eNOS) [88–90]. In addition, eNOS-expression crucially alters cardiac repair capacity of bone marrow-derived progenitor cells in an experimental model of ischemic injury [91]. Based on these results, a randomized trial (ENACT-AMI (NTC00936819)) is currently under way to assess potential improvement after transplantation of EOCs transfected with human eNOS in patients with acute myocardial infarction [92].

Growth-Factor Treatment as a Strategy to Facilitate and Enhance Repair Capacity of Progenitor/Stem Cells Retention and engraftment of transplanted progenitor/stem cells is still an important issue, which is not resolved yet, as only few cells injected reside in the designated location [93]. Instead, they are flushed away or die because of a hostile milieu in the ischemic heart region. Therefore, in order to equip injected cells with a friendlier milieu, Takehara et al. [94] transplanted CDCs with a hydrogel controlling the release of bFGF (basic fibroblast growth factor), a compound that is known to facilitate differentiation, proliferation, and survival. CDCs injected with hydrogels releasing bFGF showed a superior engraftment and facilitate effects of CDCs in pigs with heart failure caused by myocardial infarction [94]. These results led to the initiation of the ongoing ALCADIA-trial (NCT00981006) in CABG-patients. Another strategy is to pretreat progenitor/stem cells to enhance their efficacy after transplantation. In this regard, Behfar et al. pretreated human MSCs with a growth-factor cocktail [95]. Thereby, differentiation of human MSCs towards a cardiopoietic lineage commitment has been achieved, leading to an improved cardiac function and structural benefits in infarcted murine hearts after cell transplantation [95]. These cardiopoietic MSCs were subsequently used in a clinical trial with patients with ischemic cardiomyopathy. Transplantation of cardiopoietic MSCs was safe and at 6 months, an increase of LVEF could be observed as compared to the control group with standard care [96].

microRNA-Based Pre-treatment to Optimize Cell-Based Cardiovascular Repair Capacity Key regulators, which are already therapeutically used in patients with hepatitis C in a clinical trial (NCT01200420), but have not been translated yet in clinical applications for cell-based cardiac therapies, are microRNAs. These small RNAs [97], which regulate gene expression at the post-transcriptional level mostly by degradation of mRNAs, have a highly attractive potential to regenerate damaged myocardium in experimental studies after viral delivery [98]. Interestingly, dysregulation of microRNAs has been observed in bone marrow-derived cells from patients with cardiovascular diseases [36, 66, 99]. Overexpression of the proangiogenic microRNA-126 [36] or blocking of microRNA-21 or microRNA-34a [66, 100] may enhance functional capacity of impaired adult circulating or bone-marrow derived mononuclear cells. Moreover, several microRNAs have been transfected into progenitor cells and improved their biological functions [101].

These applications may potentiate and/or restore the functional capacities of applied progenitor/stem cells. Thus, preconditioning and priming of cells used for cell-based therapies may have not only an important impact on their

own functional ability to improve cardiac function, but also to enhance the activation of endogenous repair mechanisms by paracrine signaling.

Conclusions and Future Directions

A decade ago, the first in-man administration of BM-MNCs was performed in a patient with myocardial infarction [93]. Since then, thousands of patients have been enrolled in clinical trials examining cardiac cell-based therapies. Safety and feasibility of bone marrow–derived cells have so far been excellent, and beneficial effects on cardiac function, reverse remodeling, and scar size have been observed in some studies. The main focus is still to unravel the ideal approach to regenerate the heart in different cardiovascular disease conditions.

However, reconstitution of the myocardium and sufficient neovascularization after cardiac injury may require more than a single injection and/or a combination of progenitor/stem cells. In this regard, recently, synergistic effects of simultaneously injected MSCs and c-kit+ cells on cardiac function have been observed after myocardial infarction in a swine model [102] and a clinical trial with repeated injections of BM-MNCs is planned (REPEAT (NCT01693042)).

iPSCs have a clear potential for cardiac regeneration, but substantial safety and practical hurdles are an important limitation. Direct reprogramming of cardiac fibroblasts into cardiomyocytes, thereby skipping the induction of pluripotent stem cells with the associated risks, represents a highly interesting direction of research [18•, 19]. Recently, systemic application of a microRNA-cocktail [19] or 3 cardiac transcription factors (Gata4, Mef2c and Tbx5 (GMT)) [18•] in a murine model of myocardial infarction has been reported to directly reprogram cardiac fibroblasts into cardiomyocyte-like cells in vivo, leading to an improved cardiac function [18•].

Recently published clinical trials with cardiac-derived stem cells and the non-inferiority of allogeneic versus autologous MSCs-transplantation represent interesting avenues worth to pursue in the future. Furthermore, phase III clinical trials are under way to examine the effects of BM-MNCs administration on all-cause mortality in patients with ischemic LV-dysfunction (BAMI, REPEAT). In addition, ex vivo preconditioning to enhance the cardiac repair potential of autologous cells for cardiac cell-based therapies may improve their efficacy, in particular in heart failure patients.

Acknowledgments This work was supported by Swiss National Research Foundation grants (310030–122339, 33CM30-124112/1), the German Research Foundation (DFG-LA-1432/3-1), the Swiss Heart Foundation, Uniscientia Foundation, the Zurich Center for Integrative Human Physiology and the Clinical Research Focus Program of the University of Zurich.

Conflict of Interest Philipp Jakob declares he has no conflict of interest.

Ulf Landmesser declares he has no conflict of interest.

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- • Of importance
- •• Of major importance

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