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Cell-based cardiovascular repair and regeneration in acute myocardial infarction and chronic ischemic cardiomyopathy – current status and future developments

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ABSTRACT Ischemic heart disease is the main cause of death and morbidity in most industrialized countries. Stem- and progenitor cell-based treatment approaches for ischemic heart disease are therefore an important frontier in cardiovascular and regenerative medicine. Experimental studies have shown that bone-marrow-derived stem cells and endothelial progenitor cells can improve cardiac function after myocardial infarction, clinical phase I and II studies were rapidly initiated to translate this concept into the clinical setting. However, as of now the effects of stem/progenitor cell administration on cardiac function in the clinical setting have not met expectations. Thus, a better understanding of causes of the current limitations of cell-based therapies is urgently required. Importantly, the number and function of endothelial progenitor cells is reduced in patients with cardiovascular risk factors and/or coronary artery disease. These observations may provide opportunities for an optimization of cell-based treatment approaches. This review provides a summary of current evidence for the role and potential of stem and progenitor cells in the pathophysiology and treatment of ischemic heart disease, including the properties, and repair and regenerative capacities of various stem and progenitor cell populations. In addition, we describe modes of stem/progenitor cell delivery, modulation of their homing as well as potential approaches to "prime" stem/progenitor cells for cardiovascular cell-based therapies.

KEY WORDS: *stem and progenitor cell, myocardial regeneration, myocardial infarction*

Introduction

Coronary artery disease, i.e. acute myocardial infarction and ischemic cardiomyopathy, are the main causes of death in most of the developed countries and are a major socioeconomic healthcare problem (Landmesser *et al.* 2005). Despite improved pharmacological therapy and coronary revascularization procedures by either percutaneous coronary intervention PCI or coronary artery bypass surgery CABG there is still a major need for novel therapeutic approaches (Landmesser *et al.* 2005); (Ford *et al.* 2007). Whereas current treatment strategies aim largely to limit or delay progression of cardiac dysfunction (Landmesser *et al.* 2005; Landmesser *et al.* 2009; Segers *et al.* 2008) stimulation of vascular and cardiac repair mechanisms, such as those mediated by stem/progenitor cells, has become an important focus of cardiovascular research (Landmesser 2009). In fact, in patients with ischemic heart failure it is unlikely that the inhibition of novel neurohormonones other than catecholamines, angiotensin and aldosterone will further improve cardiovascular outcome, underlining the need for novel therapeutic concepts to promote cardiac repair (Landmesser *et al.* 2009).

Experimental and first small- to intermediate scale clinical studies have suggested the feasibility and safety of cell-based therapies in patients with ischemic cardiomyopathy (Landmesser 2009; Schachinger *et al.* 2006; Segers *et al.* 2008). Heterogeneous cell populations have been thoroughly investigated as potential sources of cardiac progenitors in cell based therapy for

Abbreviations used in this paper: CSC, cardiac stem cell; EPC, endothelial progenitor cell; ESC, embryonic stem cell; HSC, hematopoietic stem cell; iPS, induced pluripotent stem cell; MSC, mesenchymal stem cell; PCI, percutaneous coronary intervention.

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ischemic heart disease. To date, different autologous adult stem and progenitor cells, in particular several subtypes of bone marrow-derived cells, isolated adipose tissue-derived or cardiac-derived stem/progenitor cells are under preclinical and clinical evaluation. Additionally, embryonic stem cells and induced pluripotent stem cells provide regenerative capacity and improve cardiac function after ischemia in animal models (Nelson *et al.* 2009; van Laake *et al.* 2008). In an attempt to update the current field of cell-based therapy for ischemic heart disease, this review will give an overview on: (1) stem and progenitor cell populations in myocardial regenerative medicine, (2) routes of cell delivery, (3) current status of clinical trials, (4) mechanisms of adult stem and progenitor cell therapy, (5) limitations of current treatment strategies and (6) future developments of cell-based therapy.

Overview of cell types for cardiac repair

Over the past decade, early small and intermediate sized clinical trials have examined the effects of skeletal myoblasts (Menasche 2008; Menasche *et al.* 2003), circulating endothelial progenitor cells (Assmus *et al.* 2002; Hirsch *et al.* 2006), and bone marrow- derived mononuclear cell populations (Schachinger *et al.* 2006; Wollert *et al.* 2004) for treatment of ischemic heart disease. In addition, several progenitor and stem cell types have been studied in animal models to examine their potential use, including embryonic stem cells (ESCs) (Laflamme *et al.* 2007; van Laake *et al.* 2008), hematopoietic stem cells (HSCs) (Murry *et al.* 2004; Templin *et al.* 2008), mesenchymal stem cells (MSCs) (Mangi *et al.* 2003), endothelial progenitor cells (EPCs) (Aicher *et al.* 2003; Giannotti *et al.* 2010; Sorrentino *et al.* 2007), and resident cardiac stem cells (CSCs) (Beltrami *et al.* 2003; Laugwitz *et al.* 2005; Oh *et al.* 2003). Each cell types comprise unique profiles regarding isolation and culture, cell surface marker expression, transcription factors, expressed proteins, and ability to differentiate into different cell types:

Adult stem and progenitor cells

Bone-marrow-derived and circulating adult stem/progenitor cells: Stem/progenitor cells isolated from the bone marrow, peripheral blood, and other tissues have been used in cell-based treatments for ischemic heart disease. In contrast to pluripotent embryonic stem cells, adult stem cells present a limited and still controversial transdifferentiation capacity towards cardiomyocytes. The two major subsets of bone marrow-derived stem cells are HSCs and MSCs. The true bone-marrow stem cells comprise <0.01% of the total bone marrow cells (Abkowitz *et al.* 2002; Pittenger *et al.* 2004) and may be isolated by direct marrow aspiration or obtained from peripheral blood after cytokine mobilization. Compared with other stem cell types, these cells appear to be present in greater numbers *in vivo* and have been studied particularly well, at least in part due to the fact that they can be rather easily obtained. Other multipotent progenitor cells located in the bone marrow include side-population cells, which are characterised by their ability to efflux Hoechst dye (Challen *et al.* 2006).

Hematopoietic stem/progenitor cells express CD34 and CD133 cell surface antigens and have shown the ability to home to injured myocardium, but whether they differentiate into cardiomyocytes has been debated (Jackson *et al.* 2001; Murry *et al.* 2004).

Mesenchymal stem cells can be identified in adult tissues including the bone marrow and adipose tissue (Tomita *et al.* 1999). Criterions for their characterization have recently been summarized by a position statement of the international society for cellular therapy and include the expression of CD105, CD73 and CD90 and lack of expression of markers such as CD34, CD45, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules and their ability to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro* (Conget *et al.* 1999) (Dominici *et al.* 2006). MSCs can be isolated and expanded easily and have been suggested to improve left ventricular function after myocardial infarction (Makino *et al.* 1999; Schuleri *et al.* 2008; Toma *et al.* 2002) (Mangi *et al.* 2003). Furthermore, non-invasive multimodality imaging has suggested that therapy after myocardial infarction with allogeneic MSCs promotes active cardiac repair *in vivo* (Amado *et al.* 2006).

It has been proposed by experimental *in vitro* data that adipose tissue–derived MSCs may transdifferentiate into cardiomyocyte-like cells and endothelial cells (Planat-Benard *et al.* 2004; Planat-Benard *et al.* 2004). However, as discussed above, there is no definite proof as of today for a complete transdifferentation into cardiomyocytes. Adipose cells have been regarded as an attractive source because they are available in high quantities and easy to obtain.

Endothelial progenitor cells comprise a heterogenous circulating cell population likely derived largely from the bone marrow (Urbich *et al.* 2004). Different types of endothelial progenitor cells have been proposed, in particular "early" and "late" EPCs, based on their appearance in the culture of circulating mononuclear cells in endothelial medium (Hur *et al.* 2004). Early EPCs promote likely endothelial repair (Giannotti *et al.* 2010) and angiogenesis (Sieveking *et al.* 2008) largely by paracrine effects, whereas late EPCs, that are very low in number, may become endothelial cells. The differentiation potential of early EPCs into cardiomyocytes has been questioned (Gruh *et al.* 2006).

Endothelial progenitor cells isolated from patients with diabetes or hypertension display a reduced activity in promoting re-endothelialization of denuded arteries and blood flow recovery after ischemia when transplanted into nude mice (Giannotti *et al.* 2010; Landmesser *et al.* 2004; Sorrentino *et al.* 2007), pointing to an important limitation of current cell-based treatment approaches in these patients. The functional deficits that cause these reduced *in vivo* activities remain to be further characterized, but likely include reduced nitric oxide availability and an accelerated senescence (Giannotti *et al.* 2010; Sorrentino *et al.* 2007). Notably, assays of a reduced functionality of bone-marrow-derived mononuclear cells, such as impaired migration or diminished colony formation capacity *in vitro*, have been associated with a decreased functional benefit in cell therapy trials (Assmus *et al.* 2007).

Fetal and umbilical cord blood cells may possess greater plasticity than adult progenitor cells due to their prenatal origin. Umbilical cord blood contains a number of progenitor cell populations, including HSCs, MSCs, and unrestricted somatic stem cells, however, evidence of pluripotency after *in vitro* expansion is still lacking. Animal studies have shown an improvement in left ventricular function (Iwasaki *et al.* 2009; Kim *et al.* 2005).

Resident cardiac stem and progenitor cells are a relatively rare cell population in the heart, which have been classified according to surface marker or transcription factor expression (Beltrami *et*

al. 2003; Hierlihy *et al.* 2002; Oh *et al.* 2003). C-Kit$^+$ cells have the capacity for self-renewal, clonogenicity, and pluripotency through differentiation into myogenic, endothelial, and smooth muscle lineages *in vitro* and may contribute to repair of ischemic myocardium (Beltrami *et al.* 2003). A further population of cardiac stem cells that express stem cell antigen-1 (Sca-1) have been differentiated into cells expressing cardiac specific markers *in vitro* (Oh *et al.* 2003). Furthermore, it has been demonstrated that Isl1$^+$ cells can be differentiated into a cardiac phenotype with electrophysiological properties of mature cardiomyocytes (Laugwitz *et al.* 2005). Cardiospheres, which are spherical clusters of cells, are plated and grown in culture to yield cardiosphere-derived cells in addition to other populations of resident cardiac progenitors (Smith *et al.* 2008). Recently, Messina *et al.* demonstrated that cardiospheres could be isolated, and expanded to provide a potentially useful population of autologous cardiac stem cells (Messina *et al.* 2004). Several experimental studies using different preparations of cardiac-derived stem/progenitor cells have demonstrated positive effects on left ventricular function, remodeling, and infarct size; however, this has not been observed in all studies (Beltrami *et al.* 2003; Li *et al.* 2009; Oh *et al.* 2003). In particular, no long-term engraftment and benefit has been observed after transplantation of Sca-1-positive cardiac derived stem/progenitor cells.

Skeletal myoblasts transplantation into the heart as a cell-based strategy improved left ventricular function and reduced cardiac remodelling either to mechanical or scaffolding effects (Menasche 2008; Menasche *et al.* 2003). Unfortunatly, skeletal myoblasts do not transdifferentiate into cardiomyocytes (Menasche 2008). Remarkably, these cells lack electrical integrity and can therefore induce arrhythmias (Menasche *et al.* 2008). Moreover these cells fail to show long term beneficial effects on LV-function (Menasche *et al.* 2008). A small randomized controlled trial found that application through a 3-dimensional guided catheter system was favourable in terms of left ventricular function, quality of life and symptoms relief (Dib *et al.* 2009). However, the first randomized placebo-controlled study of myoblast transplantation (MAGIC trial) failed to improve cardiac function as assessed by echocardiography (Menasche *et al.* 2008).

Embryonic stem cells are undifferentiated, pluripotent cells obtained from the inner cell mass of blastocysts that have the most promising potential for organ regeneration (Segers *et al.* 2008; Smith 2001). Their unlimited capacity for differentiation has gained incremental interest for their use in regenerative cardiology. Previous studies of ischemia and reperfusion showed improved cardiac function, directly related to paracrine effects, after transplantation of undifferentiated murine ESCs (Crisostomo *et al.* 2008; Min *et al.* 2003). Of note, transplantation of undifferentiated murine ESCs can result in teratoma formation (Nussbaum *et al.* 2007). The risk can be reduced by transplanting pre-differentiated ESC-derived cardiomyocytes. In post-infarcted rat hearts, such cells ameliorate cardiac function and blunt left ventricular remodeling without teratoma formation (Caspi *et al.* 2007; Laflamme *et al.* 2007). Nevertheless, further investigations of tumor formation, immunologic responses and regenerative capacity are required to delineate the therapeutic potential of differentiated ESC. The isolation methods by which ESCs can be obtained have raised many ethical issues. This may form an obstacle for further discovery process both in preclinical and clinical setting (Murry *et al.* 2008; Passier *et al.* 2008).

Induced pluripotent stem cells (iPSCs) can be generated by retroviral transduction of so-called 'stemness' transcription factors (Geoghegan *et al.* 2008; Takahashi *et al.* 2007; Yu *et al.* 2007). Such cells can be maintained in culture for several months and induced to differentiate into lineages of all three germ layers, including cardiomyocytes, with electrophysiological properties and a gene expression profile that is similar to ESC-derived cardiomyocytes (Mauritz *et al.* 2008; Nelson *et al.* 2009). To reduce the risk of insertional mutagenesis following infection with retroviral vectors, the technique has recently been refined to incorporate virus-free approaches for gene delivery (Okita *et al.* 2008). Furthermore, a recent study was able to generate human-induced pluripotent stem cells by direct delivery of reprogramming proteins without DNA vectors (Kim *et al.* 2009). Also, the generation of functional cardiomyocytes from human induced pluripotent stem cells has been reported (Zhang *et al.* 2009). The strategy of reprogramming somatic cells could be also used to develop patient-specific stem cells, which could be a unique resource in studying genetic mechanisms of disease development, drug actions, and regenerative biology.

Routes of cell delivery

To date several routes of cell delivery are employed, including (1) intravenous (Barbash *et al.* 2003); (2) intracoronary (Strauer *et al.* 2002), (3) direct transepicardial (intramyocardial)"(Perin *et al.* 2006) or catheter-based transendocardial (intramyocardial) injection using electromechanical voltage mapping (Sherman *et al.* 2006), and (4) a recently established approach of transvenous injection into coronary veins (Thompson *et al.* 2003). Each delivery technique has its own risks and benefits, and their suitability may also depend on the cell type used.

(1) The least invasive technique is systemic intravenous infusion, which involves injecting progenitor cell suspensions into a vein followed by homing of the cells to the injured myocardium (Price *et al.* 2006). The primary disadvantage of this approach is that cells may be trapped in the pulmonary circulation before they reach the systemic circulation (Barbash *et al.* 2003; Templin *et al.* 2006).

(2) Percutaneous coronary cell delivery demonstrates the most frequent application route in the clinical setting. Under these conditions, cells are injected via an over-the wire balloon catheter into the vessel supplying the ischemic territory. The balloon is intermittently inflated to transiently stop coronary flow and allow cell distribution. Interestingly, a recent study in the porcine myocardial infarction model has suggested that prolonged balloon inflation is not necessary for the intracoronary approach using mononuclear bone-marrow-derived cells (Tossios *et al.* 2008). Notably, for intracoronary injection of mesenchymal stem cells in a dog model, however, induction of microinfarctions has been described (Vulliet *et al.* 2004). In some studies it was observed that the percutaneous intracoronary approach showed an increased engraftment of transplanted MSCs in pigs after myocardial infarction as compared to intramyocardial injection or intravenously transplanted cells (Freyman *et al.* 2006) (Moscoso *et al.* 2009). However, a recent study using bone marrow-derived mononuclear cells showed a 7-fold greater number of cells in the myocardium for the intramyocardial method and a 10-fold greater

number of cells in the lungs in the intracoronary group of pigs (Makela *et al.* 2009). The opposing results may be related to different cell populations. In any case, the intracoronary approach requires transmigration of the endothelial barrier, whereas after intramyocardial injection the cells are largely primarily in the interstial space.

(3) Direct intramyocardial injections can be applied through the epicardium into the underlying ischemic myocardium during cardiac surgery when the heart is fully exposed. An advantage of this approach is the ability to target specific areas of myocardium and scar under direct visualization. In contrast, the benefit of direct intramyocardial injection may be limited by poor cell diffusion (Melo *et al.* 2004) and applications for larger areas require multiple injections.

Percutaneous transendocardial delivery is performed through direct injection of cells into the myocardium using percutaneous catheters with small injection needles. Electromechanical mapping is an excellent technique supporting the percutaneous transendocardial approach to identify ischemic territories (Smits *et al.* 2003). Percutaneous coronary infusion and percutaneous transendocardial delivery are most likely more appropriate in patients without a planned surgical intervention. In an experimental study, intramyocardial but not intracoronary injection of bone-marrow cells after myocardial infarction was associated with an increased risk of ventricular tachycardias (Fukushima *et al.* 2007). The authors observed that the intramyocardial distribution of bone-marrow cells was more homogeneous after i.c. as compared to i.m. injection, and was associated with less inflammatory response (Fukushima *et al.* 2007). Therefore, the most appropriate route of cell application likely depends on the clinical setting (preferably i.c. in the acute myocardial infarction) and the cell type used.

Clinical trials

Therapeutic use of bone marrow derived cells (BMCs) in the setting of acute myocardial infarction has been studied in more than 1000 patients worldwide. These BMCs include hematopoietic and endothelial progenitor cells (approximately 2–4%), mesenchymal stem cells (MSC; ~ 0.1%) and a very small number of side population cells. To date four meta- analyses have been published (Abdel-Latif *et al.* 2007; Hristov *et al.* 2006; Lipinski *et al.* 2007; Martin-Rendon *et al.* 2008) suggesting the feasibility and safety of BMC application with a potential modest beneficial effects on left ventricular ejection fraction (LVEF) (an increase of approximately 3%). A reduction in ventricular volumes; a reduction in infarct or lesion size, ranging from 3.5% to 5.6%; and improved regional LV function (Lipinski *et al.* 2007). Although these effects on LV function are less than what was expected based on experimental studies in rodents, it should be noted that several of the established clinical therapies which do have an impact on prognosis in patients with ischemic cardiomyopathy, such as ACE inhibitor or beta-blocker therapy, are associated with a similarly small change in LV ejection fraction (Reffelmann *et al.* 2009). Furthermore, a patient with the greatest amount of myocardial damage displayed the greatest benefit (Janssens *et al.* 2006). In addition one study indicates that transplantation of bone marrow cells may have an impact on coronary flow reserve (Erbs *et al.* 2007). Finally, the number of injected cells may play a key role for the effects on LVEF (Martin-Rendon *et al.* 2008). Interestingly, the meta-analyses suggested a trend toward a reduction in recurrent MI (Martin-Rendon *et al.* 2008) and in the REPAIR-AMI (Intracoronary Progenitor Cells in Acute Myocardial Infarction) trial of 204 patients, even reported a significant reduction in mortality, rehospitalisation for heart failure, and repeated revascularization (Assmus *et al.* 2010; Schachinger *et al.* 2006). Of note, the overall benefit demonstrated in the meta-analyses with regard to left ventricular function needs to be tempered by the results of 3 other trials (Lunde *et al.* 2008; Meyer *et al.* 2006; Tendera *et al.* 2009), which showed either no benefit or an initial benefit that was not persistent beyond 6 months. In this regard, it has been suggested, that differences in cell isolation protocols may have an impact on the functional capacity of the cells in the REPAIR-AMI1 and ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction) trials and therefore may account for the conflicting results. Patients with reduced LV function may in fact have more benefit from BMC therapy as suggested by retrospective analyses of several of the aforementioned trials (Meyer *et al.* 2009) (Schachinger *et al.* 2006).

Additionally, there are also studies using enriched CD34+ or CD133+ hematopoietic and endothelial progenitor cells from bone marrow or after mobilization with the cytokine G-CSF (Losordo *et al.* 2007). Other studies used circulating blood-derived cells that have been isolated from mononuclear blood cells and selected *ex vivo* by culturing in endothelium-specific medium for 3 days. The two APOLLO trials aim to assess whether adipose tissue-derived cells enhances heart function in acute or chronic ischemia (APOLLO trials). Further clinical trials are underway investigating the use of c-kit+ cardiac stem cells in patients with chronic ischemic heart disease. Table 1 provides an overview of current ongoing cell therapy trials in patients with myocardial infarction / ischemic cardiomyopathy.

Potential mechanisms mediating effects of adult stem/progenitor cell-based therapy on cardiac function

There are many open questions at present with respect to the understanding of mechanisms of circulating or bone marrow-derived stem/progenitor cell-mediated cardiac repair (Burt *et al.* 2008; Landmesser 2009; Segers *et al.* 2008). Whereas initially, a rapid transdifferentiation of bone-marrow derived stem cells into cardiomyocytes was postulated to explain the effects on cardiac function (Orlic *et al.* 2001) several later studies have indicated that other mechanisms, in particular promotion of cardiac vascular growth, may mediate the observed beneficial effects on left ventricular (LV) function, likely at least in part due to paracrine effects of endothelial progenitor or bone-marrow-derived stem cells (Gnecchi *et al.* 2008; Murry *et al.* 2004). This concept was further supported by the observation that circulating endothelial progenitor cells and bone marrow-derived stem/progenitor cells may increase myocardial neovascularisation and perfusion in patients (Erbs *et al.* 2007). Furthermore, animal studies have suggested that bone marrow-derived progenitor cells (Templin *et al.* 2008; Templin *et al.* 2006) and human endothelial progenitor cells (Kocher *et al.* 2001) improve cardiac function in rodents with myocardial infarction by promotion of neovascularisation and prevention of apoptosis. It is well known from earlier studies, that myocardial capillary growth plays a critical role for maintenance of

TABLE 1

ONGOING CELL THERAPY TRIALS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION / ISCHEMIC CARDIOMYOPATHY

ClinicalTrials.gov Identifier	Trial name	Number of patients	Cell type	Primary end point	Route of cell delivery
			Acute coronary syndrome		
NCT00711542	REPAIR-ACS	100	Bone marrow-derived progenitor cells	Coronary flow reserve in the infarct vessel	Intracoronary
			Acute myocardial infarction		
NCT00355186	SWISS-AMI	150	Bone marrow mononuclear cells	LVEF	Intracoronary
NCT00984178	TECAM2	120	Bone marrow mononuclear cells	LVEF; Left ventricular end-systolic volume	Intracoronary
NCT00350766	EMRTCC	300	Bone marrow mononuclear cells	LVEF	Intracoronary
NCT00684021	The TIME Study	120	Bone marrow mononuclear cells	LVEF	Intracoronary
NCT00684060	The Late TIME Study	87	Bone marrow mononuclear cells	LVEF	Intracoronary
NCT00691834	ReNeW	50	Bone marrow mononuclear cells	LVEF; Occurence of arrhythmia, heart failure and death	Intracoronary
NCT00874354	REVI-TALIZE	30	Bone marrow mononuclear cells	Safety and feasibility; LVEF	Intracoronary
NCT00268307	-	60	Bone marrow mononuclear cells	Safety	Intracoronary
NCT00939042	-	40	Bone marrow mononuclear cells	LVEF	Intracoronary
NCT00765453	REGEN-AMI	102	Bone marrow-derived progenitor cells	LVEF	Intracoronary
NCT00437710	CARDIAC	50	Bone marrow-derived stem cells	Mortality; Mortality and Morbidity; Left ventricular function	Intracoronary
NCT00275977	-	10	Bone marrow-derived stem cells	LVEF	Intracoronary
NCT00529932	SELECT-AMI	60	CD133+ enriched bone marrow cells	Safety; Myocardial thickening in non-viable akinetic / hypokinetic LV wall segments	Intracoronary
NCT00725738	TRACIA STUDY	80	CD34+ cells	LVEF	Intracoronary
NCT00936819	ENACT-AMI	100	Early endothelial progenitor cells	LVEF	Intracoronary
NCT00501917	MAGIC Cell-5-Combicytokine Trial	116	Peripheral blood stem cells	LVEF	Intracoronary
NCT00555828	-	25	Mesenchymal precursor cells	Feasibility and safety	Transendocardial
NCT00877903 (Osiris Therapeutics)	-	220	Ex vivo cultured adult human mesenchymal stem cells	Left ventricular end systolic volume	Intravenous
NCT00893360	CADUCEUS	30	Cardiosphere-derived stem cells	Safety	Intracoronary
			Ischemic cardiomyopathy		
NCT00326989	Cellwave Study	100	Bone marrow progenitor cells	LVEF	Intracoronary
NCT00824005	FOCUS	87	Bone marrow mononuclear cells	maximal oxygen consumption, left ventricular end systolic volume, reversible defect size	Transendocardial
NCT00810238	C-Cure	240	Bone marrow-derived cardiopoietic cells	LVEF	Transendocardial
NCT00690209	-	30	Bone marrow-derived stem cells	Left ventricular volumes and contractility	Transepicardial during CABG
NCT00418418	-	60	Bone marrow-derived stem cells	LVEF	Transepicardial during CABG
NCT01049867	-	10	CD133+ Endothelial precursor cells	Regional and global myocardial contractility	Intracoronary
NCT01033617	IMPACT-CABG	20	CD133+ bone marrow stem cells	Freedom from major adverse cardiac event; Freedom from major arrhythmia	Transepicardial during CABG
NCT00950274	PERFECT	142	CD133+ bone marrow stem cells	LVEF	Transepicardial during CABG
NCT00462774	Cardio133	60	CD133+ marrow cells	LVEF	Transepicardial during CABG
NCT00346177	-	30	CD34+ cells	Safety, LVEF, heart failure symptoms	Transendocardial
NCT00620048	-	10	CD34+ cells	Safety, LVEF, heart failure symptoms	Transendocardial
NCT00221182	-	10	CD34+ cells	Myocardial perfusion abnormality/ Safety	
NCT00721045	-	60	Mesenchymal precursor cells	Safety and feasibility	Transendocardial
NCT01076920	MESAMI	10	Mesenchymal stem cells	Safety and feasibility	Transendocardial
NCT01087996	The POSEIDON-Pilot Study	30	Bone-marrow derived mesenchymal stem cells	TE-SAE	Transendocardial
NCT00768066	TAC-HFT	60	Mesenchymal cells / Bone marrow cells	TE-SAE	Transendocardial
NCT00587990	PROMETHEUS	45	Mesenchymal stem cells	Serious adverse events	Transepicardial during CABG
NCT00908622	PERCUTANEO	50	Skeletal myoblasts	LVEF; wall motion score index	Percutaneous Implantation
NCT00526253	MARVEL	390	Skeletal myoblasts	6-minute walk test; Quality of Life Questionnaire	Transendocardial
NCT00474461	SCIPIO	40	Cardiac stem cells	Safety/Efficacy Study	Intracoronary
NCT00981006	ALCADIA	6	Cardiac-derived stem cells	Safety	Transepicardial during CABG

Searching criteria's for http://www.clinicaltrials.gov (04/19/2010):- Cell therapy for myocardial infarction. - Cell therapy for ischemic cardiomyopathy

Abbrevations: CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; MACE: major adverse cardiac events; TE-SAE: define as composite of death, non-fatal MI, stroke, hospitalization for worsening heart failure, cardiac perforation, pericardial tamponade, ventricular arrhythmias >15 sec. or with hemodynamic compromise or atrial fibrillation

cardiac function (Giordano *et al.* 2001). In addition to enhanced neovascularization, paracrine factors released by the incorporated cells may beneficially influence cardiac repair by protecting cardiovascular cells from apoptotic stimuli or even by activating cardiac-resident stem cells to enhance the endogenous repair capacity (Uemura *et al.* 2006; Urbich *et al.* 2005). Paracrine mechanisms may additionally prevent inflammation, fibrosis and reactive hypertrophy (Burchfield *et al.* 2008). Moreover, the injection of conditioned medium from MSCs results in the improvements of left ventricular function and reduced apoptosis (Gnecchi *et al.* 2005). In a further article, SFRP2 (secreted frizzled-related protein II), which modulates the Wnt (winglesstype MMTV integration site family) pathway and the expression of antiapoptotic genes, was shown to be the main factor released by AKT-1(v-akt murine thymoma viral oncogene homolog 1)–enriched MSCs (Mirotsou *et al.* 2007). Recently, we analyzed the secreted proteome of a hematopoietic progenitor cell line which exert modulating effects on tissue repair and regeneration. In this study a subset of 95 different proteins were identified in a mass spectrometry based approach whereas the cytokines IL-6 and IL-13 and the chemokines MCP-1, MCP-3, MIP1-a, and MIP1-b were identified using an immunological approach (Luecke *et al.* 2010). Furthermore, experimental data have shown that interleukin 10 from transplanted bone marrow mononuclear cells may play a role in cardiac protection after MI (Burchfield *et al.* 2008). Additionally, other cytokines and growth factors from transplanted progenitor cells may exert important paracrine effects like vascular endothelial growth factor, stromal cell–derived factor, angiopoietin 1, hepatocyte growth factor, insulinlike growth factor 1, and periostin, among others (Kinnaird *et al.* 2004; Uemura *et al.* 2006; Urbich *et al.* 2005). Although the direct effects of cell therapy are not entirely understood, the majority of studies suggest that stem/progenitor cells may have a beneficial effect on cardiac function.

Limitations of current cell-based treatment approaches

There is currently a limited knowledge on the role of the required number and function of bone marrow cells needed for an optimal effect on cardiac repair. Low cell dosages might in fact limit the efficacy of bone marrow cell therapy. For example, in the ASTAMI trial, the median number of mononuclear cells injected was 68 x 10^6, and the median number of $CD34^+$ cells was 0.7 x 10^6. There were no significant differences between the BMC and control group in changes in LVEF, end-diastolic volume, or infarct size (Lunde *et al.* 2006). In the BOOST trial, the average number of mononuclear BM cells was 24.6 x 10^8, and the number of $CD34^+$ cells was 9.5 x 10^6. Six months after randomisation, global LVEF increased from 50.0% to 56.7% (P=0.0026), albeit this difference was not maintained at long-term follow-up (Wollert *et al.* 2004). In the TOPCARE-AMI trial, the average number of mononuclear BM cells was 24.5 x 10^7, and the number of $CD34^+$ cells was 7 x 10^6. In patients receiving progenitor cells, global LVEF increased from 51.6% to 60.1% (P=0.003) (Schachinger *et al.* 2006). To treat a patient of 80 kg with a high dose cell strategy as described in some animal studies (1 x 10^7 cells/25g) would require 32 x 10^9 $CD34^+$ cells (adjusted to body weight), which exceeds the number of HPCs used in clinical trials by a factor of ~3000 (Assmus *et al.* 2002). Therefore, strategies allowing rapid *ex vivo* progenitor cell expansion may improve cell-based clinical treatment regimens (Templin et al., 2008).

Another potential reason for discrepancies between experimental and clinical studies with respect to the impact of circulating or bone marrow-derived stem/progenitor cell therapy on cardiac function is related to the fact, that the effect of stem/progenitor cells obtained from young healthy rodents in experimental studies is compared with effects of stem/progenitor cells obtained from older patients with chronic coronary disease in clinical studies. In support of this concept, a substantially impaired *in vivo* vascular repair capacity of stem/progenitor cells derived from patients with cardiovascular risk factors as compared to healthy subjects has been observed (Giannotti *et al.* 2010; Sorrentino *et al.* 2007), and very recently a severely reduced vascular and cardiac repair capacity of stem/progenitor cells derived from patients with ischemic cardiomyopathy.

Additionally, cell therapy is currently limited by low rates of cell engraftment after intracoronary delivery and poor cell survival after intramyocardial injections (Hofmann *et al.* 2005; Menasche 2008; Schachinger *et al.* 2008). Furthermore, the amount of circulating progenitor cells in patients with cardiac ischemic disease comorbidities such as diabetes mellitus, hypertension and hypercholesterolemia is reduced (Imanishi *et al.* 2005; Vasa *et al.* 2001). This is problematic as this cohort is essentially the very one that would need to be treated with progenitor cells. These challenges require further research to enhance the therapeutic efficiency of stem and progenitor cells in the treatment of ischemic heart disease. This includes the use of more potent cells with a higher cardiac regeneration capacity (for instance induced pluripotent stem cells) and strategies for improving cell homing, survival,, engraftment and repair capacity, of transplanted cells.

Future directions of cell based-therapy for ischemic heart disease

To implement cell based-therapy for ischemic heart disease into clinical routine, many questions need to be addressed. Several studies have indicated a reduced cardiac and vascular repair capacity of patient-derived adult stem/progenitor cells as compared to cells obtained from healthy subjects (Giannotti *et al.* 2010; Sorrentino *et al.* 2007). Therefore, the mechanisms leading to stem/progenitor cell dysfunction need to be better characterized and will provide interesting novel approaches to optimize cell-based treatment approaches. Moreover, strategies to improve cardiac homing and engraftment of stem/progenitor cells may optimize the effect the results of this treatment approach (Pons *et al.* 2008; Pons *et al.* 2009; Tang *et al.* 2009; Zhao *et al.* 2009).

One interesting strategy for the improvement of current cell-based approaches may be the combination of cell- and gene therapy (Fig. 1). In this regard, we have demonstrated long-term self renewal and unlimited expansion of hematopoietic progenitor cells using human β-catenin gene transfer (Templin *et al.* 2008). Administration of defined β-catenin-HPCs after MI reduces infarct size and improves left ventricular function and dimensions in a threshold-dependent manner (Templin *et al.* 2008). This effect is associated with improved angiogenesis and reduced apoptosis in the infarct border zone. Furthermore, β-catenin-HPCs have greater therapeutic efficacy than control-transduced HPCs (GFP-HPCs), demonstrating a beneficial effect of β-catenin transduction on myocardial repair (Templin *et al.* 2008). Other recent studies have

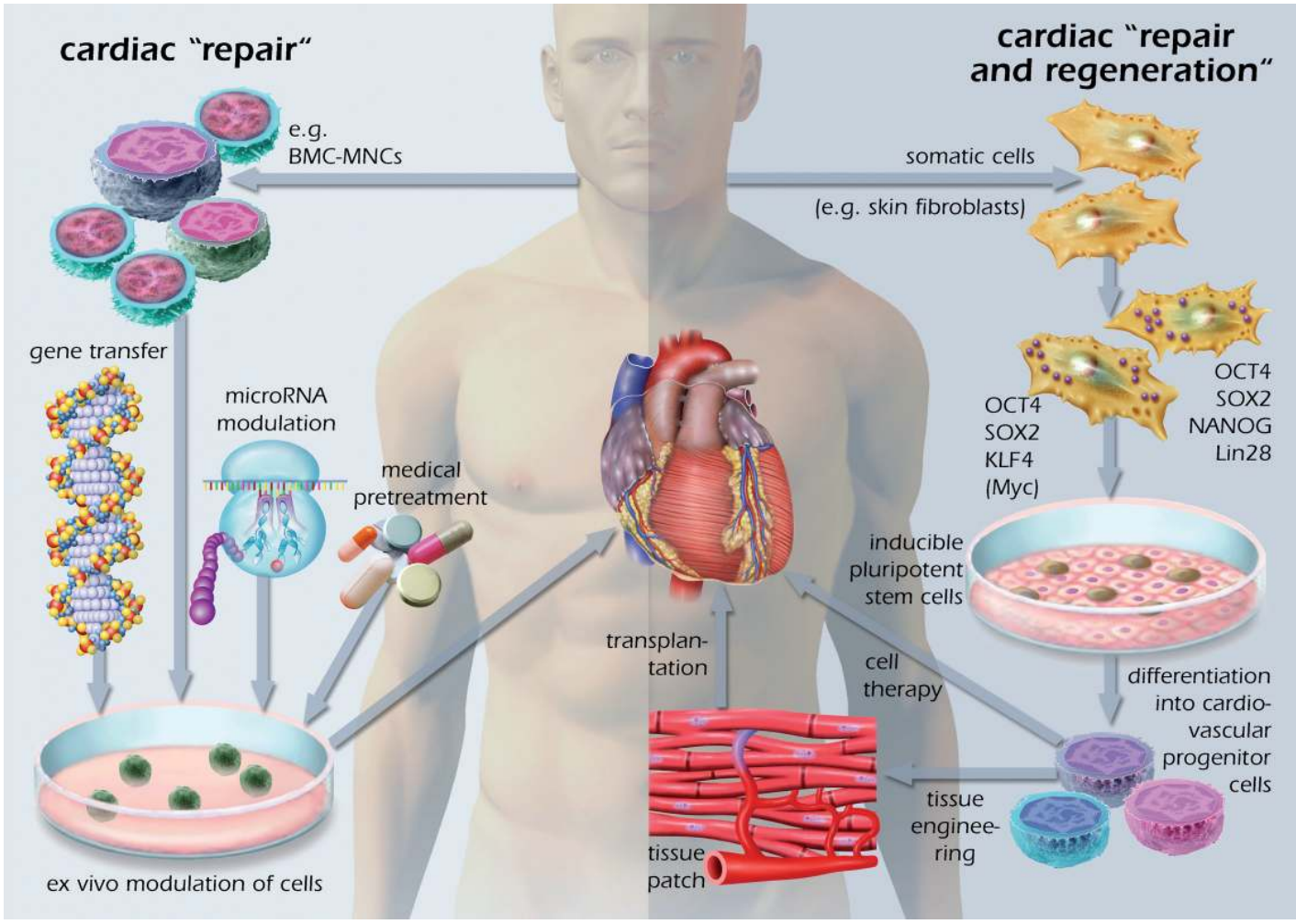

Fig.1. Future developments of cell-based therapy for ischemic cardiomyopathy (modified after Templin *et al.* 2010). *Ex vivo modulation (e.g. microRNA modulation or combined gene and cell-therapy) of bone marrow derived progenitor cells may be used to improve current adult cell treatment strategies. Inducible pluripotent stem cells have the ability to differentiate into cardiovasular cells and exhibits great potential for cardiac regeneration.*

investigated specific protein expression through *ex vivo* modifications of receptors and molecules involved in progenitor cell paracrine signaling, homing, and survival. In this regard, MSCs have been modified to overexpress growth factors (e.g. VEGF) (Yang *et al.* 2007) and ischemia protective proteins (e.g. heme oxygenase-1) (Tang *et al.* 2005), upregulate homing receptors (Cheng *et al.* 2008) or influence survival signaling pathways (e.g. Akt) (Mangi *et al.* 2003).

A recent interesting study combined a genetic and pharmacologic inhibition of dipeptidylpeptidase IV with G-CSF-mediated stem cell mobilization after myocardial infarction in mice. This approach leads to increased myocardial homing of circulating CXCR-4+ stem cells, and improved heart function and survival (Zaruba *et al.* 2009).

Pretreatment of endothelial progenitor cells with statins, eNOS-overexpression of PPAR-gamma agonists before transfer increases their migratory, invasive, and neovascularization capacity - effects that are mediated by activation of endothelial nitric oxide synthase (Shao *et al.* 2008; Spyridopoulos *et al.* 2004). Similary, own results showed that prestimulation of endothelial progenitor cells from diabetic individuals with the peroxisome proliferator activated receptor γ-agonist rosiglitazone, enhances nitric oxide availability and the *in vivo* endothelial repair capacity of these cells (Sorrentino *et al.* 2007). MicroRNAs have been identified as unexpectedly potent regulators in cardiovascular biology, controlling vascular growth, endothelial NO synthase and stem cell differentiation and may be interesting targets to optimize cell-based therapies (Suarez *et al.* 2007; Suarez *et al.* 2009; van Rooij *et al.* 2008).

Conclusion

Development of cell-based strategies for cardiac repair for the use in clinical routine is an exciting and challenging task. The concept of cardiac regeneration or rejuvenation and protection via paracrine mechanisms responses to reduce cardiomyocyte apoptosis and increase angiogenesis needs to be further developed and optimized, and represents one important line of current research. One such approach represents the combination of cell and gene therapy, by either transduction of genes into stem cells or modifying stem cells as vectors for drug delivery. Moreover, novel cell

types with a true cardiomyocyte transdifferentiation potential such as iPS provide another important line in the development of cell-based approaches for cardiac repair (Fig. 1). Upscaling and safety of such an approach represent important challenges to be solved.

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