EXPERT REVIEWS

Stem cells for cardiac repair in acute myocardial infarction

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Despite recent advances in medical therapy, reperfusion strategies, implantable cardioverter-defibrillators and cardiac assist devices, ischemic heart disease is a frequent cause of morbidity and mortality worldwide. Cell therapy has been introduced as a new treatment modality to regenerate lost cardiomyocytes. At present, several cell types seem to improve left ventricular function in animal models as well as in humans, but evidence for true generation of new myocardium is confined to the experimental models. In the clinical perspective, myocardial regeneration has been replaced by myocardial repair, as other mechanisms seem to be involved. Clinical studies on adult stem cells suggest, at best, moderate beneficial effects on surrogate end points, but some applications may qualify for evaluation in larger trials. Complete regeneration of the myocardium by cell therapy after a large myocardial infarction is still visionary, but pluripotent stem cells and tissue engineering are important tools to solve the puzzle.

Keywords: bone marrow cells • cell therapy • MSCs • myocardial infarction • tissue engineering

In the last three decades, important advances such as acute thrombolytic and catheter-based reperfusion strategies, dual antiplatelet therapy, statins, β-blockers and ACE inhibitors have improved outcome for patients with acute myocardial infarction (AMI) [1]. With an increasing number of patients surviving acute coronary syndrome and an increase in life expectancy worldwide, the prevalence of heart failure is expected to increase by 25% from 2010 to 2030 in the USA, with a 300% increase in related healthcare expenses [2]. In severe heart failure, heart transplantation or mechanical assist devices can be lifesaving therapeutic options, but only for selected patients due to limited organ availability, high costs and risk of serious complications. Thus, mortality and morbidity in acute and chronic heart failure remain high and warrant continued scientific efforts to improve treatment and prognosis, first and foremost by improving cardiac function.

Most etiologies of severe heart failure – that is cardiomyopathy, myocarditis, myocardial infarction (MI) or cytotoxic drug therapy – share a common feature, namely the loss of functionally competent cardiomyocytes. Regeneration of heart tissue and restoration of cardiac function is a tremendous challenge, but encouraging results from preclinical studies have evoked significant interest in stem cell therapy as a potential therapeutic approach in patients with heart disease. Almost two decades have passed since the first preclinical studies on myocardial injections of skeletal myoblasts were published [3] and several clinical trials have been performed. However, due to a discrepancy in the results on the effect of treatment, cell therapy has still not been established in routine clinical cardiac care.

In this article, we will summarize the current evidence for cardiac cell therapy, discuss the results in clinical trials in the context of knowledge from preclinical studies and suggest future directions.

Myocardial self-renewal

For centuries, the heart was considered a terminally differentiated organ [4,5]. In the last decade, this paradigm has been challenged. A cardiac stem cell has been identified and proliferative activity has been documented. Bergmann *et al.* elegantly quantified ^{14}C incorporated at supranormal atmospheric levels during the nuclear bomb testing in the 1950s and 1960s and provided convincing data indicating that the heart is self-renewing, albeit at a slow rate [6]. Kajstura *et al.* suggest a more rapid turnover of cardiomyocytes based on histological staining for protein markers of cell division (Ki67 and phospho-H3) and apoptotic cell death ($p16^{INK4a}$) [7]. Differences in estimates of the rate of tissue turnover are probably explained by methodological differences, and both studies show that the

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heart is in a state of continuous self-renewal. However, clinical experience tells us that the intrinsic regenerative capacity of the heart is insufficient to compensate the substantial myocardial damage of a large MI, which may lead to the loss of more than a billion cardiomyocytes and cause inflammation, formation of a granulation tissue and then, finally, a fibrous scar. In addition, the change in myocardial wall stress and neuro-hormonal activation may induce complex maladaptive changes, also affecting the borderzone and remote myocardium, to alter global cardiac function and architecture in a process commonly known as remodeling. Nevertheless, the findings by Bergmann and Kajstura confirm the presence of proliferating cardiomyocytes in human adults and indirectly suggest that there is an operative infrastructure regulating cell death, cell removal and the formation, differentiation and integration of new cells within the myocardium. This infrastructure is not only a potential target for pharmacological intervention, but its presence may also be of utmost importance to facilitate engraftment of transplanted cells.

Myocardial repair

Several approaches have been applied experimentally to counteract the loss of cardiomyocytes:

- Pharmacological stimulation of circulating, migrating or cardiac resident progenitor cells;
- Cell therapy with pluripotent stem cells – that is, embryonic stem cells (ESCs) or induced pluripotent stem (iPS) cells;
- Tissue engineering – that is, transplantation of tissue constructs or patches including cardiomyocytes/myogenic cells and extracellular matrix cultured and assembled *ex vivo*;
- Cell therapy with adult stem cells.

In a clinical perspective, the current status of these approaches is as follows:

Statins and ACE inhibitors/ARBs are well-established drugs in postinfarction patient care, based on documented beneficial effects on cholesterol levels, vascular resistance, left ventricular (LV) afterload and neuro-hormonal status, with several randomized controlled trials confirming improved survival [8]. These drugs have also been shown to influence numbers and/or function of circulating stem cells and as such they may also influence myocardial repair [9–12]. However, the contribution of these regenerative 'side-effects' have not been addressed in prospective randomized trials.

Growth factor proteins such as EPO [13], FGF [14], G-CSF [15], HGF [16], IGF-1 [17], PTH [18], SDF-1 [19] and VEGF [20] have also been investigated in animal models, as recently reviewed by Segers and Lee [21]. They may act by stem cell mobilization or recruitment, angiogenesis, inhibition of apoptosis and/or stimulation of cell proliferation, and have all been shown to improve parameters of LV function in animal models. However, the human use of these polypeptides is hampered by technical challenges, such as the need for parenteral administration, short half-lives and the need for repeated dosing. These drugs stimulate complex signaling systems and clinically significant adverse effects are common when administered systemically. G-CSF is the only drug adequately clinically tested to address its potential for cardiac regeneration, as reviewed by Zohlnhofer *et al.* [22]. Although G-CSF provides up to a 25-fold increase in circulating $CD34^+$ cells, this meta-analysis did not support any significant beneficial effect of G-CSF on LV ejection fraction (LVEF) following AMI. G-CSF has been shown to not only mobilize stem cells, but also inhibit apoptosis. However, at present G-CSF has not been implemented in clinical cardiac care. VEGF has also been applied clinically, mainly to stimulate angiogenesis in patients with ischemic heart disease. A Phase II clinical trial on 178 patients with stable angina treated with intravascular infusions of VEGF, the Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis (VIVA) trial [23], failed to meet the primary end point – that is, an increase in treadmill exercise time after 60 days compared with placebo. Another trial with intramyocardial delivery of VEGF-expressing plasmids by a NOGA® guiding system and Myostar® catheter (Biosense Webster, Diamond Bar, CA, USA) documented a modest improvement in regional wall motion compared with placebo, but no difference between groups regarding myocardial perfusion or clinical symptoms [24]. Thus, further developments are warranted to improve the ratio between costs, risks and benefits in such interventions.

Embryonic stem cells and iPS cells are the only cell types that currently have the potential to generate *bona fide* cardiomyocytes on a scale that may potentially replace the cell numbers lost in a large MI [25]. ESCs in culture yield a mass of beating cardiac committed cells within a few days [26]. ESCs differentiated in a cardiomyogenic direction have been applied in animal models and they clearly engraft in the hearts, express markers of a cardiomyocyte phenotype and improve LV function [27,28]. However, several problems have to be addressed before the ESCs can make their way to human applications. First, the cells are pluripotent and may form teratomas [29,30]. Selection of more cardiac committed progeny and the use of recipients with a competent immune system seem to reduce tumor formation [31,32]. However, differentiated cells may have less potential for proliferation and integration. Thus, robust selection methods ensuring both the regenerative potential of the cells and control of their differentiation are mandatory to maintain the efficacy and safety of this type of treatment. Second, ESCs are allogenic, and immunosuppressive therapy will be required to reduce the risk of alloimmune rejection of transplanted cells. Third, the use of ESCs is ethically controversial. The recently discovered and rapidly evolving iPS (reprogramming) technique may provide cells harvested from adult patients themselves with an ESC phenotype, thus possibly circumventing alloimmune rejection and ethical controversies [33,34]. However, the risk of tumor formation may be even more pronounced [35], as pluripotency genes may also be oncogenes, such as *c-myc*. Reprogramming also introduces considerable changes in DNA methylation and the patterns of methylation seem to differ, not only between different iPS clones, but also between iPS cells and ESCs [36]. Both genetic and epigenetic heterogeneity have been documented [37]. Thus, although reprogramming without c-myc has been tested successfully [38], preclinical testing to ensure

homogenous cell products with genetic stability and the correct proteome is warranted [39]. There will be a tug of war between pluripotency and potential for proliferation versus lineage commitment and differentiation control, and thorough investigation in small and large animals must precede human Phase I trials with iPS cells to ensure an acceptable level of safety.

Tissue engineering is a complex approach, but it may turn out to be the only way large numbers of cells can be transplanted into a human heart with adequate engraftment and cell survival. 'Lost in translation' has been a frequently debated topic in cardiac stem cell therapy, as preclinical studies with a plethora of cell types generate encouraging effects in small animal models, while results in humans have been rather disappointing. Several factors are probably important to explain this phenomenon, but size is undoubtedly an issue. The human adult heart weight is approximately 300–350 g in comparison to the 0.5–1.0 g murine heart [40,41]. Accordingly, at least a 300-fold higher number of cells is needed to provide a comparable dose. In addition, the murine LV has a wall thickness of approximately 1 mm, in comparison to the human heart with an end-diastolic LV wall thickness normally within the range 7–11 mm. Thus, adequate layers of transplanted cells have to be at least tenfold thicker in humans than in rats, while the same maximum distance for passive diffusion of oxygen and nutrients to cells will apply in the two species. A cell tracking study in humans after intracoronary injection of autologous bone marrow cells (BMCs) indicate less than 7% initial cell retention and engraftment of only 2% of transplanted cells after 3–4 days [42]. Direct intramyocardial injection may increase the fraction of initially retained cells [43], but necessitates transendocardial injections by a guided catheter system or transepicardial injections during open heart surgery. Volumes up to 0.2 ml per injection are considered safe [44]. Cell concentrations up to 1×10^8 cells/ml have been injected in BMC studies [45], while only 4×10^6 cells/ml have been applied in the mesenchymal stem cell (MSC) studies [43,46] in order to retain high cell viability and avoid aggregation. Thus, feasibility may be challenged by the vast number of injections needed. Furthermore, although injected cells have been proliferating in culture, little evidence support further cell division after transplantation. In preclinical studies, the majority of transplanted cells engraft the infarct zone and the border zone, and the proportion of cells differentiating or transdifferentiating seems very modest [47–50]. The low engraftment in normal myocardium compared with the infarct zone is likely beneficial and several factors are probably involved. First, the infarct zone is characterized by ongoing inflammation, inducing endothelial activation and expression of leukocyte chemoattractants, cell adhesion molecules and increased vascular permeability to facilitate homing, migration and engraftment. Second, the impaired microcirculation in the infarct zone may reduce early washout of injected cells. Third, it is possible that neither the cells nor the extracellular matrix in intact myocardium provide sufficient ligands for retention and incorporation of injected cells from other tissues. On the other hand, the infarct zone will have a limited metabolic supply for the injected cells and the transplanted cells may well die for lack of oxygen or nutrients. These basic presumptions clearly suggest that successful transplantation of the ≥1 billion cells needed to replace lost cardiomyocytes in a medium-to-large sized MI [51] is more likely to succeed with the cotransplantation of extracellular matrix and functional microcirculation. Indeed, animal studies suggest higher engraftment rates when cells are transplanted on a collagen patch or with support from other cell types, such as a composite cell sheet with ESC-derived cells and adipose-tissue derived stem cells (ADSCs) [52–55]. Tissue engineering studies with contractile rings produced *ex vivo* from neonatal rat cardiomyocytes demonstrated the feasibility of transplantation of tissue constructs, including graft survival, electrical coupling and improved systolic and diastolic function [56]. However, again, the complexity increases with size and the tissue engineering approach has not yet been sufficiently validated in large animal models to see a progress towards human application.

The majority of clinical experience in cardiac cell therapy has been acquired from studies using autologous adult stem cells. Skeletal myoblasts (SMs), MSCs and BMCs were identified as potential agents for myocardial repair through experimental studies around the start of this millennium [49,57–59]. SM are robust and resistant to ischemia and readily engraft myocardium and scar tissue to form myotubes and myocytes after injection [60]. Unfortunately, it has appeared that the grafts do not couple electrically with adjacent cardiomyocytes [61]. Thus, excitation and contraction of the myoblasts depends on excitation of the cell membrane instead of the intercellular Ca^{2+} flux through gap junctions observed in normal myocardium. As expected, these islands of transplanted cells with different electrophysiological properties may serve as an arrhythmogenic substrate [62]. Indeed, in the largest clinical study to date, the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial [63], injection of myoblasts during surgical coronary artery bypass grafting (CABG) tended to increase the risk of ventricular arrhythmias and no significant beneficial effect on LVEF was observed. Given the modest beneficial effects and inherent risk profile of the myoblasts, further therapeutic use of these cells has not been proposed. The ultimate adult stem cell for cardiac regeneration could be the autologous cardiac stem cell (CSC). CSCs have been isolated from rodents [64] and cardiomyogenic cells have been isolated from human hearts via cardiospheres in culture [65]. CSCs isolated from biopsies in humans with cardiomyopathy have also been reported [66]. However, although a recent report indicates that newborn mammals have a substantial potential for cardiac regeneration, the capacity is lost by 7 days of age [67]. This study also indicated that the majority of regenerated myocardium was generated from preexisting cardiomyocytes. Thus, it remains controversial whether true CSCs can be isolated from biopsies from the diseased patient population in need of cell therapy and expanded to adequate numbers of cells feasible for human cell therapy within a reasonable period of time, and the role for CSCs in cell therapy is currently unresolved.

Mesenchymal stem cells

Mesenchymal stem cells were identified as plastic adherent, easily cultured cells with multilineage potential [58]. By definition,

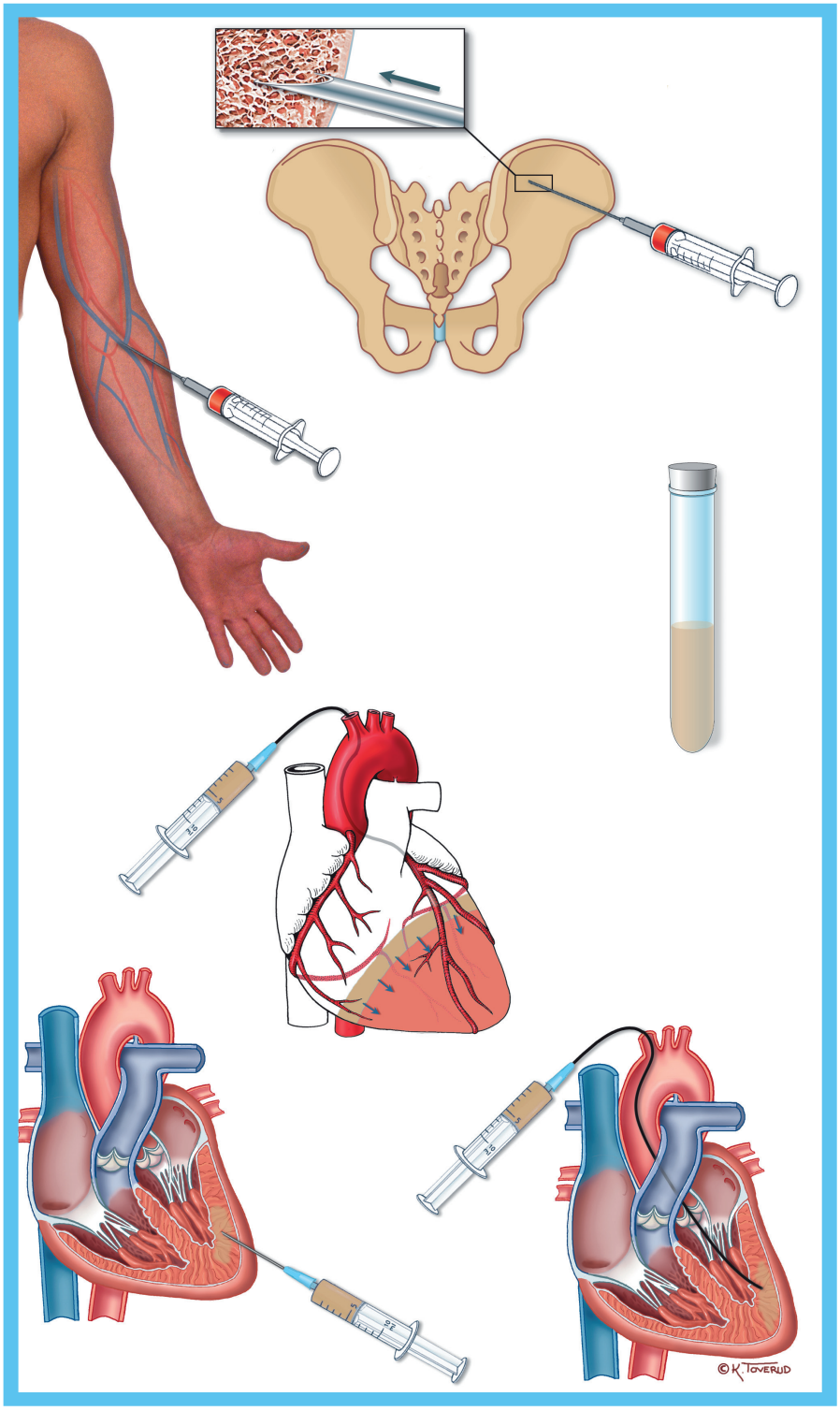

Figure 1. Stem cell harvesting and methods for cardiac administration.
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these cells form fat, cartilage and bone in conditioned growth media and have also been stimulated to express markers of endothelial, smooth muscle and even cardiomyocyte phenotypes in culture [49]. The first MSCs were isolated as a fraction of mononuclear BMCs (mBMCs), but cells with a MSC phenotype have now been isolated from other, more easily available, tissues like fat (ADSCs) and skeletal muscle [68]. Like the skeletal myoblasts, MSCs are relatively large and adhesive cells that may cause vascular obstruction, and they are therefore rarely infused in systemic arteries. Thus, these cells have been administered either by catheter-based transendocardial injections, by transepicardial injections during CABG surgery or as intravenous infusions [43,69]. Different routes for harvesting and injecting stem cells are illustrated in Figure 1. The majority of MSCs administered intravenous will be trapped in the pulmonary circulation [70]. Animal studies confirm negligible passage of cells to the systemic circulation, but the MSCs seem to stimulate macrophages in the lungs to secrete TSG-6, which has angiogenic and antiapoptotic effects in downstream injured organs such as the infarcted heart, thereby reducing infarct size and improving LV function [70]. These findings support the current opinion that MSCs act mainly through paracrine effects, as transdifferentiation to cardiomyocytes rarely occurs. Hare *et al.* have performed a clinical study with intravenous administration of allogenic MSCs in patients 1–10 days following reperfused AMI [69]. In this double-blind study of 53 patients randomized to receive high-dose MSCs, low-dose MSCs or placebo, the primary safety end point was met and provisional efficacy end points showed a lower occurence of ventricular arrhythmias and a trend for better LV function in the MSC groups. An off-the-shelf, easily administered application of cell therapy would be feasible for large numbers of patients and, assuming that paracrine effects are important for therapeutic efficacy, it seems logical that antiapoptotic, antifibrotic, proangiogenic and proregenerative effects have higher potential when activated early after an acute event. However, larger trials to confirm the efficacy and safety of this product should precede any clinical implementation.

The use of allogeneic MSCs is facilitated by the fact that MSCs do not constitutively express MHC class II and are considered immune privileged. If MSCs are activated by IFN-γ, MHC class II expression will appear, but activated MSCs may also have immunosuppressive properties [71–74].

Table 1. Large mononuclear bone marrow cell trials and mononuclear bone marrow cell trials with long-term follow-up.

Study (year)	Design	Patients (n)	BM volume and dose (×10^6 cells)	Preparation	Timing (days after AMI)	Primary end point	Follow-up (mo)	Baseline EF	ΔEF (% points)	Δ LVEDV (ml)	Δ infarct size (MRI)	Other effects reported	Ref.
BOOST (2004)	Rnd, controlled	30 mBMC 30 control	50 ml 2460 ± 940	Succinate gel		LVEF by MRI	6	50.0 ± 10.0 51.3 ± 9.3	6.7 ± 6.5 0.7 ± 8.1 p = 0.003	7.6 ± 20.0 3.4 ± 11.1	-14.1 ± 13.0 ml -10.5 ± 10.6 ml	Improved diastolic function by echo in the mBMC group (not predefined end point)	[85]
BOOST (2005)						LVEF by MRI	18		5.9 ± 8.9 3.1 ± 9.6 p = 0.27	6.1 ± 20.3 3.6 ± 15.1	-12.8 ± 11.8 ml -10.1 ± 13.1 ml		[86]
BOOST (2008)						LVEF by MRI	60		-2.5 ± 11.9 -3.3 ± 9.5 p = 0.30	13.9 ± 25.4 7.5 ± 17.3	-15.0 ± 12.1 ml -14.6 ± 12.1 ml		[87]
ASTAMI (2006)	Rnd, controlled	50 mBMC 50 control	50 ml 68 (54–130)	Lymphoprep	6 (5–6)	LVEF by SPECT	6	41.3 ± 10.4 42.6 ± 11.7	8.1 ± 11.2 7.0 ± 9.6 p = 0.77	-11.2 ± 36.0 -1.8 ± 17.6	-2.4 ± 10.9 ml -5.8 ± 13.5 ml†	Improved exercise time in mBMC group after 6 months and 3 years (not predefined end point)	[93]
ASTAMI (2009)						LVEF by echo	36	45.7 ± 9,4 46.9 ± 9.6	2.2 ± 7.3 -0.4 ± 8.2 p = 0.28	2.1 ± 34.9 6.3 ± 35.8 p = 0.77	-11.4 ± 11.4 ml -13.8 ± 17.0 ml†		[94]
REPAIR-AMI (2006)	Rnd, controlled, double-blind	101 mBMC 103 placebo	50 ml 236 ± 174	Ficoll–Hypaque (Cambrex)	4.3 ± 1.3	LVEF by ventriculography	4	8.3 ± 9.2 46.9 ± 10.4	5.5 ± 7.3 3.0 ± 6.5 p = 0.01	12 ± 31 14 ± 33 p = 0.64	NA	MACE reduced in mBMC group (not predefined end point)	[88]
REGENT (2009)	Rnd, controlled	80 mBMC 80 SEL 40 control	mBMC: 50–70 ml 178 × 10^6 SEL: 100–120 ml 1.9 × 10^6	Ficoll–Hypaque (Cambrex)	7 (3–12)	LVEF by MRI	6	37 (19–44) 35 (12–45) 39 (23–44)	3% 3% 0% p = 0.19	10 13 -3‡ p = 0.33	NA		[89]

Numbers are mean ± SD or median (25th percentile – 75th percentile).
†Baseline MRI performed 2 weeks after inclusion/mononuclear bone marrow cell therapy.
‡Change in median, based on supplementary data.
AMI: Acute myocardial infarction; ASTAMI: Autologous Stem cell Transplantation in Acute Myocardial Infarction; BM: Bone marrow; BOOST: Bone marrow transfer to enhance ST-elevation infarct regeneration; EF: Ejection fraction; ic: Intracoronary; LV: Left ventricular; LVEDV: LV end-diastolic volume; LVEF: LV ejection fraction; MACE: Major adverse cardiac event; mBMC: Mononuclear bone marrow cell; mo: Months; mPBC: Mononuclear peripheral blood cell; NA: Not available; NS: Not significant; REGENT: Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction; REPAIR-AMI: Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction; Rnd: Randomized; SEL: $CD34^+CXCR4^+$ mBMCs; SPECT: Single-photon emission computed tomography.

Table 1. Large mononuclear bone marrow cell trials and mononuclear bone marrow cell trials with long term follow-up (cont.).

Study (year)	Design	Patients (n)	BM volume and dose ($\times 10^6$ cells)	Preparation	Timing (days after AMI)	Primary end point	Follow-up (mo)	Baseline EF	ΔEF (% points)	Δ LVEDV (ml)	Δ infarct size (MRI)	Other effects reported	Ref.
HEBE (2010)	Rnd, controlled	69 mBMC (ic) 66 mPBC (ic) 65 control	mBMC: 60 ml 296 ± 164 mPBC: 150–200 ml 287 ± 137	Lymphoprep	mBMC: 6 (4–7) mPBC: 5 (4–6)	Percentage of improved dysfunctional LV segments by MRI	4	43.7 ± 9.0 41.7 ± 9.1 42.4 ± 8.3	3.8 ± 7.4 4.2 ± 6.2 4.0 ± 5.8 p = 0.90	5.4 ± 13.4 5.3 ± 16.3 8.2 ± 13.5 p = 0.33	-7.7 ± 8.5 g -7.9 ± 6.5 g -9.4 ± 7.1 g	Trend for negative effect of cell therapy (p = 0.14) on primary end point	[90]

Numbers are mean ± SD or median (25th percentile – 75th percentile).
†Baseline MRI performed 2 weeks after inclusion/mononuclear bone marrow cell therapy.
‡Change in median, based on supplementary data.
AMI: Acute myocardial infarction; ASTAMI: Autologous Stem cell Transplantation in Acute Myocardial Infarction; BM: Bone marrow; BOOST: Bone marrow transfer to enhance ST-elevation infarct regeneration; EF: Ejection fraction; ic: Intracoronary; LV: Left ventricular; LVEDV: LV end-diastolic volume; LVEF: LV ejection fraction; MACE: Major adverse cardiac event; mBMC: Mononuclear bone marrow cell; mo: Months; mPBC: Mononuclear peripheral blood cell; NA: Not available; NS: Not significant; REGENT: Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction; REPAIR-AMI: Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction; Rnd: Randomized; SEL: $CD34^+CXCR4^+$ mBMCs; SPECT: Single-photon emission computed tomography.

As MSCs are readily available, easily cultured and show substantial plasticity, the possible cardiopoietic potential of these cells still deserves attention. Cardiomyogenic pretreatment of MSCs yielded promising results in an animal model [75] and the first results from clinical use (the C-CURE trial; n = 45) were recently presented by Bartunek *et al.* [76]. In patients with heart failure and New York Heart Association (NYHA) class II–III, catheter-based injections of autologous bone marrow-derived cardiopoietic MSCs provided significant improvement in LVEF (5.2 ± 0.6 vs 1.0 ± 0.7%; p < 0.01) and 6-min walking distance (+52 ± 19 m vs -21 ± 14 m; p < 0.01) compared with the control group. However, engraftment rate and formation of *de novo* myocardium can not be determined from this study. Catheter-based injections of MSC-derived cells have also been performed successfully in patients with coronary artery disease and refractory angina, relieving symptoms and improving LV function [46]. In general, these cells have been injected in viable ischemic or hypokinetic areas of the left ventricle, avoiding the LV apex and segments with wall thickness less than 5 mm to minimize the risk of perforation and tamponade.

Thus, current clinical evidence suggests that MSCs can improve symptoms, myocardial perfusion and LV function in patients with refractory angina or heart failure when administered transendocardially and, furthermore, improve LV function after AMI when administered intravenously. A larger clinical trial with intravenous administration of allogenic MSCs in AMI seems justified. The beneficial effects of MSCs administered by intracardiac injections seem modest compared with the risks and costs of the invasive procedure, but results from the Phase II of the C-CURE trial will provide important data to further evaluate this issue.

Mononuclear bone marrow cells

Bone marrow cells have been transplanted successfully to reconstitute the hematopoietic system in allogenic recipients after myeloablation in hematological diseases since the 1970s. Experimental data at the end of the 1990s suggested that stem cells in the mononuclear fraction of BMCs could differentiate to daughter cells in other lineages, such as liver cells [77], neurons [78] or even cardiomyocytes [79] – a phenomenon known as transdifferentiation. In 2001, Orlic *et al.* reported a high rate of engraftment and transdifferentiation leading to significant recovery of heart function when BMCs were injected into the hearts of mice with acute MI [57]. This landmark study evoked a great deal of enthusiasm, but also scientific controversy [80–82]. Nevertheless, the concept was rapidly translated to humans. The proof-of-principle cohort study by Strauer *et al.* was published in 2002 [83]. Ten patients with AMI had 40 ml of bone marrow harvested approximately 1 week post-MI. The mononuclear cell fraction was isolated by Ficoll gradient centrifugation and the cell product was infused in the infarct-related coronary artery through the central lumen of a percutaneous coronary intervention (PCI) catheter. The PCI balloon was inflated repeatedly three times to stop blood flow and allow more time for cells to adhere to the vessel wall and enter the infarcted tissue. Strauer's study supported that the method was feasible and safe, and a favorable decrease in perfusion defect,

infarct region and LV volumes was observed in the mBMC group compared with a control group consisting of AMI patients who refused to receive cell therapy. This study paved the way for several small-to-medium-sized clinical trials. These trials have been reviewed by Martin-Rendon *et al.* and a meta-analysis based on Cochrane methodology was published in 2008 [84]. The largest trials (>100 included patients) and trials with long-term follow-up are presented in Table 1.

As seen in Table 1, the Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration (BOOST) [85–87] and Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) studies [88] demonstrated a statistically significant improvement in LVEF 4–6 months after mBMC therapy. The other major trials did not provide positive results on the primary end point, but there is an overall trend suggesting larger improvement in LVEF in patients treated with mBMCs. This is also reflected in the meta-analysis by Martin-Rendon *et al.*, where an average treatment effect of 2.99% on LVEF was found after mBMC therapy. However, it should be noted that the large Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) [89] and HEBE [90] trials, both of which failed to prove significant benefit of mBMCs, were published after this meta-analysis and are therefore not part of the data basis. Furthermore, at least two other trials on mBMC treatment in AMI have been prematurely terminated based on negative preliminary results [91,92]. In the initially positive BOOST trial, no positive effect of mBMC therapy could be detected after 5 years [87]. The Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial was consistently neutral through 3 years of observation [93,94].

To summarize, small-to-medium-sized clinical trials suggest a treatment effect on LVEF within the range 0–3% when autologous mBMCs are harvested and injected approximately 1 week after AMI. The procedure is considered safe, based on summarized clinical end points in the meta-analysis by Martin-Rendon *et al.* Whether the modest effect on LVEF eventually translates into a clinically significant benefit is not clear. In the REPAIR-AMI study, a significant improvement in clinical outcome was observed after 1 year [95]. This was not the primary end point and the result was mainly caused by a high rate of coronary events in the placebo group, as discussed elsewhere [96]. A pan-European initiative to run a prospective randomized trial with 3000 patients, to obtain the statistical power to address effects on clinical end points, is in progress (The BAMI trial, Mathur A; Pers. comm.). However, several mechanistic and methodological issues in mBMC therapy are still unresolved. The currently used timing, dosing and modee of administration of mBMCs is not well founded on clinical evidence, but based mainly on theoretical assumptions, observations in small-animal models, *post hoc* analyses from small clinical trials and practical feasibility in the clinical setting. It is no longer assumed that mBMCs regenerate myocardium by transdifferentiation into cardiomyocytes and which cell type and what mediators are actually involved in the improvement of LVEF is still under investigation. Based on these considerations, a large clinical trial with mBMCs may seem premature. On the other hand, the alternative, 'optimal', cell for myocardial regeneration has not yet been identified and the complete optimization of such a cell for clinical application will take a long time. In perspective of the dismal prognosis for heart failure patients today, a larger clinical trial may be justified to clarify whether mBMC therapy can offer any effect on hard end points in selected high-risk patients following AMI.

Expert commentary & five-year view

Two decades of research have supported the biological potential of cell therapy in heart disease, but have also revealed the complexity in this field of medicine. Current evidence suggests that modest effects on symptoms and/or LV function can be achieved by the use of some applications of adult stem cells in patients with AMI, no-option angina and symptomatic ischemic heart failure. These cells probably provide their main effects through soluble 'paracrine' factors to stimulate angiogenesis, reduce apoptosis, inhibit fibrosis and activate/attract resident and/or circulating progenitor cells. Currently available cells and methods can be further optimized regarding dosing, timing and mode of administration. At present, the evidence does not justify implementation of cardiac cell therapy in routine clinical practice. True regeneration of cardiac tissue to restore cardiac systolic function will require further progress in research, probably on ESCs/iPS cells and tissue engineering, to ensure long-term engraftment of sufficient numbers of contractile cells. Over the next 5 years, we expect iPS technology to progress and replace ESCs as the source of pluripotent cells in human applications. Nongenomic reprogramming and further insight into the signaling pathways of differentiation will increase efficacy and safety. Engineered tissue patches with iPS-derived cardiomyocytes and vasculature embedded in a scaffold of biomaterials will be tested in small and larger animal heart failure models. However, in acute settings, iPS-based tissue engineering will be too time consuming and in no-option angina, angiogenesis to support already existing cardiomyocytes is preferable. In these applications, adult stem cells may still serve as the best available agent, but we believe that adult cell types with higher regenerative and/or angiogenic potential will replace the use of unfractioned mBMCs in these applications. The paracrine mechanisms will hopefully be defined and recombinant factors may replace adult stem cells in some applications. Clearly, there is a clinical demand for efficient cardiac regeneration and cell therapy has a great potential conceptually. However, convincing results from properly designed and conducted clinical trials will be pivotal for future clinical use.

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Key issues

- There is a great demand for therapeutic options to replace lost contractile myocardium in patents with heart failure. At present, cell therapy is a most promising approach.
- Myocardial regeneration in humans requires engraftment and long-term functional integration of large numbers of contractile cells.
- Injection of adult stem cells have not proved successful in generating sufficient numbers of *de novo* cardiomyocytes *in vivo*, but modest beneficial effects have been observed, probably related to paracrine factors with effects on angiogenesis, apoptosis, fibrosis and resident and/or circulating progenitor cells. Results from ongoing studies and/or further optimization of these applications may lead to clinical implementation.
- Pluripotent stem cells are the most reliable source for generation of new cardiomyocytes. Currently, tissue engineering with iPS-derived cells on a bioactive scaffold seems to be the most promising future application.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Lloyd-Jones D, Adams RJ, Brown TM *et al.* Heart disease and stroke statistics – 2010 update: a report from the American Heart Association. *Circulation* 121(7), e46–e215 (2010).

2 Heidenreich PA, Trogdon JG, Khavjou OA *et al.* Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 123(8), 933–944 (2011).

3 Marelli D, Desrosiers C, el-Alfy M, Kao RL, Chiu RC. Cell transplantation for myocardial repair: an experimental approach. *Cell Transplant.* 1(6), 383–390 (1992).

4 Linzbach AJ. Heart failure from the point of view of quantitative anatomy. *Am. J. Cardiol.* 5, 370–382 (1960).

5 Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ. Res.* 83(1), 15–26 (1998).

6 Bergmann O, Bhardwaj RD, Bernard S *et al.* Evidence for cardiomyocyte renewal in humans. *Science* 324(5923), 98–102 (2009).

• **Elegant study documenting cardiomyocyte renewal in humans.**

7 Kajstura J, Gurusamy N, Ogorek B *et al.* Myocyte turnover in the aging human heart. *Circ. Res.* 107(11), 1374–1386 (2010).

8 Van de Werf F, Bax J, Betriu A *et al.* Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur. Heart J.* 29(23), 2909–2945 (2008).

9 Dimmeler S, Aicher A, Vasa M *et al.* HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J. Clin. Invest.* 108(3), 391–397 (2001).

10 Landmesser U, Engberding N, Bahlmann FH *et al.* Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation* 110(14), 1933–1939 (2004).

11 Bahlmann FH, de Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* 45(4), 526–529 (2005).

12 Wang CH, Verma S, Hsieh IC *et al.* Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *J. Mol. Cell. Cardiol.* 41(1), 34–43 (2006).

13 Bahlmann FH, de Groot K, Spandau JM *et al.* Erythropoietin regulates endothelial progenitor cells. *Blood* 103(3), 921–926 (2004).

14 Yanagisawa-Miwa A, Uchida Y, Nakamura F *et al.* Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. *Science* 257(5075), 1401–1403 (1992).

15 Sugano Y, Anzai T, Yoshikawa T *et al.* Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction. *Cardiovasc. Res.* 65(2), 446–456 (2005).

16 Nakamura T, Mizuno S, Matsumoto K, Sawa Y, Matsuda H, Nakamura T. Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF. *J. Clin. Invest.* 106(12), 1511–1519 (2000).

17 Padin-Iruegas ME, Misao Y, Davis ME *et al.* Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. *Circulation* 120(10), 876–887 (2009).

18 Zaruba MM, Huber BC, Brunner S *et al.* Parathyroid hormone treatment after myocardial infarction promotes cardiac repair by enhanced neovascularization and cell survival. *Cardiovasc. Res.* 77(4), 722–731 (2008).

19 Saxena A, Fish JE, White MD *et al.* Stromal cell-derived factor-1α is cardioprotective after myocardial infarction. *Circulation* 117(17), 2224–2231 (2008).

20 Pearlman JD, Hibberd MG, Chuang ML *et al.* Magnetic resonance mapping demonstrates benefits of VEGF-induced myocardial angiogenesis. *Nat. Med.* 1(10), 1085–1089 (1995).

21 Segers VF, Lee RT. Protein therapeutics for cardiac regeneration after myocardial infarction. *J. Cardiovasc. Transl. Res.* 5(3), 469–477 (2010).

22 Zohlnhofer D, Dibra A, Koppara T *et al.* Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: a meta-analysis. *J. Am. Coll. Cardiol.* 51(15), 1429–1437 (2008).

23 Henry TD, Annex BH, McKendall GR *et al.* The VIVA trial: Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis. *Circulation* 107(10), 1359–1365 (2003).

24 Kastrup J, Jorgensen E, Ruck A *et al.* Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris. A randomized double-blind placebo-controlled study: the Euroinject One trial. *J. Am. Coll. Cardiol.* 45(7), 982–988 (2005).

25 Mummery CL, Davis RP, Krieger JE. Challenges in using stem cells for cardiac repair. *Sci. Transl. Med.* 2(27), 27ps17 (2010).

26 Vidarsson H, Hyllner J, Sartipy P. Differentiation of human embryonic stem cells to cardiomyocytes for *in vitro* and *in vivo* applications. *Stem Cell Rev.* 6, 108–120 (2010).

27 Menard C, Hagege AA, Agbulut O *et al.* Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. *Lancet* 366(9490), 1005–1012 (2005).

28 Laflamme MA, Gold J, Xu C *et al.* Formation of human myocardium in the rat heart from human embryonic stem cells. *Am. J. Pathol.* 167(3), 663–671 (2005).

29 Cao F, Lin S, Xie X *et al. In vivo* visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. *Circulation* 113(7), 1005–1014 (2006).

30 Cai J, Yi FF, Yang XC *et al.* Transplantation of embryonic stem cell-derived cardiomyocytes improves cardiac function in infarcted rat hearts. *Cytotherapy* 9(3), 283–291 (2007).

31 Qiao H, Zhang H, Yamanaka S *et al.* Long-term improvement in post-infarct left ventricular global and regional contractile function mediated by embryonic stem cell-derived cardiomyocytes. *Circ. Cardiovasc. Imaging* 4(1), 33–41 (2011).

32 Tomescot A, Leschik J, Bellamy V *et al.* Differentiation *in vivo* of cardiac committed human embryonic stem cells in post-myocardial infarcted rats. *Stem Cells* 9(25), 2200–2205 (2007).

33 Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature* 474(7350), 212–215 (2011).

34 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).

•• **First publication on induced pluripotent stem cells.**

35 Ahmed RP, Ashraf M, Buccini S, Shujia J, Haider HK. Cardiac tumorgenic potential of induced pluripotent stem cells in an immunocompetent host with myocardial infarction. *Regen. Med.* 6(2), 171–178 (2011).

36 Lister R, Pelizzola M, Kida YS *et al.* Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471(7336), 68–73 (2011).

37 Gore A, Li Z, Fung HL *et al.* Somatic coding mutations in human induced pluripotent stem cells. *Nature* 471(7336), 63–67 (2011).

38 Martinez-Fernandez A, Nelson TJ, Ikeda Y, Terzic A. c-MYC independent nuclear reprogramming favors cardiogenic potential of induced pluripotent stem cells. *J. Cardiovasc. Transl. Res.* 3(1), 13–23 (2010).

39 Pera MF. Stem cells: The dark side of induced pluripotency. *Nature* 471(7336), 46–47 (2011).

40 de la Grandmaison GL, Clairand I, Durigon M. Organ weight in 684 adult autopsies: new tables for a Caucasoid population. *Forensic Sci. Int.* 119(2), 149–154 (2001).

41 Sebkhi A, Zhao L, Lu L, Haley CS, Nunez DJ, Wilkins MR. Genetic determination of cardiac mass in normotensive rats: results from an *F344xWKY* cross. *Hypertension* 33(4), 949–953 (1999).

42 Schachinger V, Aicher A, Dobert N *et al.* Pilot trial on determinants of progenitor cell recruitment to the infarcted human myocardium. *Circulation* 118(14), 1425–1432 (2008).

• **Confirming the low rate of engraftment of mononuclear bone marrow cells (mBMCs) in human hearts.**

43 Perin EC, Silva GV, Assad JA *et al.* Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J. Mol. Cell Cardiol.* 44(3), 486–495 (2007).

44 Baldazzi F, Jorgensen E, Ripa RS, Kastrup J. Release of biomarkers of myocardial damage after direct intramyocardial injection of genes and stem cells via the percutaneous transluminal route. *Eur. Heart J.* 29(15), 1819–1826 (2008).

45 Krause K, Jaquet K, Schneider C *et al.* Percutaneous intramyocardial stem cell injection in patients with acute myocardial infarction. First-in-man study. *Heart* 95(14), 1145–1152 (2009).

46 Friis T, Haack-Sorensen M, Mathiasen AB *et al.* Mesenchymal stromal cell derived endothelial progenitor treatment in patients with refractory angina. *Scand. Cardiovasc. J.* 45(3), 161–168 (2011).

47 Mazo M, Planat-Benard V, Abizanda G *et al.* Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. *Eur. J. Heart Fail.* 10(5), 454–462 (2008).

48 Arminan A, Gandia C, Garcia-Verdugo JM *et al.* Mesenchymal stem cells provide better results than hematopoietic precursors for the treatment of myocardial infarction. *J. Am. Coll. Cardiol.* 55(20), 2244–2253 (2010).

49 Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105(1), 93–98 (2002).

50 Beitnes JO, Oie E, Shahdadfar A *et al.* Intramyocardial injections of human mesenchymal stem cells following acute myocardial infarction modulate scar formation and improve left ventricular function. *Cell Transplant.* (2011) (In Press).

51 Murry CE, Reinecke H, Pabon LM. Regeneration gaps: observations on stem cells and cardiac repair. *J. Am. Coll. Cardiol.* 47(9), 1777–1785 (2006).

52 Simpson D, Liu H, Fan TH, Nerem R, Dudley SC Jr. A tissue engineering approach to progenitor cell delivery results in significant cell engraftment and improved myocardial remodeling. *Stem Cells* 25(9), 2350–2357 (2007).

53 Sekiya N, Matsumiya G, Miyagawa S *et al.* Layered implantation of myoblast sheets attenuates adverse cardiac remodeling of the infarcted heart. *J. Thorac. Cardiovasc. Surg.* 138(4), 985–993 (2009).

54 Bel A, Planat-Bernard V, Saito A *et al.* Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells. *Circulation* 122(Suppl. 11), S118–S123 (2010).

55 Stevens KR, Pabon L, Muskheli V, Murry CE. Scaffold-free human cardiac tissue patch created from embryonic stem cells. *Tissue Eng. Part A* 15(6), 1211–1222 (2008).

56 Zimmermann WH, Melnychenko I, Wasmeier G *et al.* Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat. Med.* 12(4), 452–458 (2006).

57 Orlic D, Kajstura J, Chimenti S *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* 410(6829), 701–705 (2001).

•• **Pivotal animal study initiating the burst of interest in mBMC therapy.**

58 Pittenger MF, Mackay AM, Beck SC *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411), 143–147 (1999).

59 Taylor DA, Atkins BZ, Hungspreugs P *et al.* Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat. Med.* 4(8), 929–933 (1998).

60 Dowell JD, Rubart M, Pasumarthi KBS, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc. Res.* 58(2), 336–350 (2003).

61 Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc. Natl Acad. Sci. USA* 100(13), 7808–7811 (2003).

62 Fernandes S, Amirault JC, Lande G *et al.* Autologous myoblast transplantation after myocardial infarction increases the inducibility of ventricular arrhythmias. *Cardiovasc. Res.* 69(2), 348–358 (2006).

63 Menasche P, Alfieri O, Janssens S *et al.* The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 117(9), 1189–1200 (2008).

64 Beltrami AP, Barlucchi L, Torella D *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114(6), 763–776 (2003).

65 Smith RR, Barile L, Cho HC *et al.* Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 115(7), 896–908 (2007).

66 D'Amario D, Fiorini C, Campbell PM *et al.* Functionally competent cardiac stem cells can be isolated from endomyocardial biopsies of patients with advanced cardiomyopathies. *Circ. Res.* 108(7), 857–861 (2011).

67 Porrello ER, Mahmoud AI, Simpson E *et al.* Transient regenerative potential of the neonatal mouse heart. *Science* 331(6020), 1078–1080 (2011).

68 Crisan M, Chen CW, Corselli M, Andriolo G, Lazzari L, Peault B. Perivascular multipotent progenitor cells in human organs. *Ann. NY Acad. Sci.* 1176, 118–123 (2009).

69 Hare JM, Traverse JH, Henry TD *et al.* A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J. Am. Coll. Cardiol.* 54(24), 2277–2286 (2009).

• **Documentation of safety and feasibility of intravenous administration of allogeneic mesenchymal stem cells after acute myocardial infarction.**

70 Lee RH, Pulin AA, Seo MJ *et al.* Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 5(1), 54–63 (2009).

71 Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 103(12), 4619–4621 (2004).

72 Ren G, Zhang L, Zhao X *et al.* Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2(2), 141–150 (2008).

73 Le Blanc K, Frassoni F, Ball L *et al.* Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a Phase II study. *Lancet* 371(9624), 1579–1586 (2008).

74 Le Blanc K, Ringden O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 11(5), 321–334 (2005).

75 Behfar A, Yamada S, Crespo-Diaz R *et al.* Guided cardiopoiesis enhances therapeutic benefit of bone marrow human mesenchymal stem cells in chronic myocardial infarction. *J. Am. Coll. Cardiol.* 56(9), 721–734 (2010).

76 Bartunek J, Wijns W, Dolatabadi D *et al.* C-CURE multicenter trial: lineage specified bone marrow derived cardiopoietic mesenchymal stem cells for treatment of ischemic cardiomyopathy. *J. Am. Coll. Cardiol.* 57(Suppl. 14), E200 (2011).

77 Theise ND, Nimmakayalu M, Gardner R *et al.* Liver from bone marrow in humans. *Hepatology* 32(1), 11–16 (2000).

78 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290(5497), 1779–1782 (2000).

79 Tomita S, Li RK, Weisel RD *et al.* Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 100(19 Suppl.), II247–II256 (1999).

80 Balsam LB, Robbins RC. Haematopoietic stem cells and repair of the ischaemic heart. *Clin. Sci.* 109(6), 483–492 (2005).

81 Murry CE, Soonpaa MH, Reinecke H *et al.* Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 428(6983), 664–668 (2004).

• **Important methodological considerations regarding the fate of injected mBMCs.**

82 Nygren JM, Jovinge S, Breitbach M *et al.* Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat. Med.* 10(5), 494–501 (2004).

83 Strauer BE, Brehm M, Zeus T *et al.* Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106(15), 1913–1918 (2002).

•• **First human study on mBMC injections in acute myocardial infarction.**

84 Martin-Rendon E, Brunskill S, Doree C *et al.* Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst. Rev.* 4, CD006536 (2008).

85 Wollert KC, Meyer GP, Lotz J *et al.* Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 364(9429), 141–148 (2004).

86 Meyer GP, Wollert KC, Lotz J *et al.* Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration) trial. *Circulation* 113(10), 1287–1294 (2006).

87 Meyer GP, Wollert KC, Lotz J *et al.* Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. *Eur. Heart J.* 30(24), 2978–2984 (2009).

88 Schachinger V, Erbs S, Elsasser A *et al.* Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N. Engl. J. Med.* 355(12), 1210–1221 (2006).

• **The largest study to date on mBMC therapy in acute myocardial infarction.**

89 Tendera M, Wojakowski W, Ruzyllo W *et al.* Intracoronary infusion of bone marrow-derived selected $CD34^+CXCR4^+$ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. *Eur. Heart J.* 30(11), 1313–1321 (2009).

90 Hirsch A, Nijveldt R, van de V *et al.* Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary

intervention: results of the randomized controlled HEBE trial. *Eur. Heart J.* 32(14), 1736–1747 (2011).

91 Penicka M, Horak J, Kobylka P *et al.* Intracoronary injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction: a prematurely terminated randomized study. *J. Am. Coll. Cardiol.* 49(24), 2373–2374 (2007).

92 Wohrle J, Merkle N, Mailander V *et al.* Results of intracoronary stem cell therapy after acute myocardial infarction. *Am. J. Cardiol.* 105(6), 804–812 (2010).

93 Lunde K, Solheim S, Aakhus S *et al.* Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N. Engl. J. Med.* 355(12), 1199–1209 (2006).

• **One of the largest and most cited studies on mBMC therapy in AMI.**

94 Beitnes JO, Hopp E, Lunde K *et al.* Long term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction. The ASTAMI randomized, controlled study. *Heart* 95(24), 1983–1989 (2009).

95 Schachinger V, Erbs S, Elsasser A *et al.* Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. *Eur. Heart J.* 27(23), 2775–2783 (2006).

96 Arnesen H, Lunde K, Aakhus S, Forfang K. Cell therapy in myocardial infarction. *Lancet* 369(9580), 2142–2143 (2007).

97 Lunde K, Aakhus S. Bone marrow-derived stem cells for treatment of ischemic heart disease: background and experience from clinical trials. In: *Handbook of Cardiac Stem Cell Therapy.* Dimarakis I (Ed.). Imperial College Press, London, UK (2009).