Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/poamed

Review Article

Stem cells in clinical practice for cardiovascular diseases

Katarzyna Jezierska-Woźniak [a,c,*], *Joanna Wojtkiewicz* [b,c]

[a] *Department of Neurology and Neurosurgery, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Poland*
[b] *Department of Pathophysiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Poland*
[c] *Laboratory of Regenerative Medicine, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Poland*

ARTICLE INFO

Article history:
Received 17 February 2015
Received in revised form
26 May 2015
Accepted 21 September 2015
Available online 19 December 2015

Keywords:
Stem cells
Regenerative medicine
Cell therapy
Cardiovascular diseases

ABSTRACT

Introduction: According to a World Health Organization (WHO) report, 17.3 million people died from cardiovascular diseases (CVDs) in 2008, representing 30% of all global deaths, and almost 23.6 million people will die from CVDs by 2030. CVDs remain the predominant cause of mortality worldwide.
Aim: In this review, the authors discuss the current strategies and therapies targeting stem cells in CVDs.
Material and methods: In this paper we present an overview of stem cell therapy for CVD and discuss the challenges these three areas present for maximum optimization of the efficacy of stem cell therapy for heart disease, and new strategies in progress.
Discussion: Various kinds of therapeutic methods have been studied to improve prognosis in cardiovascular diseases. Stem cells comprise an enormous opportunity to rebuild damaged tissues. Most of the application and clinical trials involve the various types of stem cells derived mainly from bone marrow and others sources of mesenchymal stem cells. Early data from these trials have produced mixed results often showing minor or transitory improvements.
Conclusions: The divergences are attributed to differences in cell preparations, the large number of stem cell types under investigation in different clinical settings, timing, methods of cell administration and characteristics of patients.

1. Introduction

According to a World Health Organization (WHO) report, 17.3 million people died from cardiovascular diseases (CVDs) in 2008, representing 30% of all global deaths, and almost 23.6 million people will die from CVDs by 2030.[1] Although many drugs and medical devices have been developed, the incidence of CVDs remains high. The field of cardiac cell therapy has emerged as a new alternative in this situation, and has made

* *Correspondence to*: Warszawska 30, 10-082 Olsztyn, Poland. Tel.: +48 0895245338.
E-mail address: katarzyna.jezierska@uwm.edu.pl (K. Jezierska-Woźniak).

http://dx.doi.org/10.1016/j.poamed.2015.09.001

rapid progress. The use of stem cells to improve recovery of the injured heart is an important emerging therapeutic strategy.

Stem cells comprise an enormous opportunity to rebuild damaged tissues. To understand their functions, physiology and action, cells are tested not only *in vitro* but also *in vivo* in animal models. Stem cell therapy induces both therapeutic and side effects; therefore, extensive evaluation of the side effects is needed to decide if a treatment can be adopted in medical practice. Stem cell transplantation in human patients must ensure safety and therapeutic efficacy. Preclinical studies with animal models provide strong evidence for obtaining a relevant positive clinical outcome.

The main challenges of stem cell therapy for CVD are improved identification, recruitment, and expansion of autologous stem cells; the identification of mobilizing and homing agents that increase recruitment; and developmental strategies to improve stem cell survival during the engraftment of both endogenous and exogenous sources of stem cells.[2] During the past decade, multiple candidate cells have been proposed for cardiac regeneration. Moreover, the first clinical trials with cell-based therapies have been performed. At present, it is believed that stem cell therapy could lead to cardiac regeneration in various ways – differentiation of the administered cells into all of the cellular constituents of the heart, the release of paracrine factors, the stimulation of endogenous repair by injected cells, or a combination of these mechanisms.[3] The most likely route of action seems to be the paracrine influence, where the effectiveness of stem cells is related to the secretion of soluble factors that contribute to cardiac repair and regeneration. Moreover, cytokines and growth factors can induce cytoprotection and neovascularization. Additionally, as stem cells are released in a temporal and spatial manner, they exert various effects depending on the microenvironment after injury and may have an autocrine impact on the biology of stem cells themselves. Moreover, these stem cells may influence adjacent cells and exert their actions *via* several mechanisms.[3]

A myocardial infarction (MI) is the ischemic necrosis of the cardiac tissue and it is frequently triggered by severe coronary stenosis. The decrease in myocytes produces abnormal left ventricular (LV) remodeling, chamber dilatation and contractile dysfunction.[4] In patients after MI delivering naturally myogenic cells (i.e., skeletal myoblasts, cardiomyocytes, or any progenitor cell driven down a muscle lineage) seems to be a high priority. However, the formation of new myocardium has been established for embryonic stem cells (ESCs). In turn, bone marrow (BM) mononuclear cells (BMMCs) are an easily accessible source of adult stem cells. For patients with chronic ischemia, application cells with angiogenic potential, such as BMMCs, endothelial progenitor cells (EPCs), vascular progenitor cells or mesenchymal stem cells (MSCs), seem to have more therapeutic potential.

2. Aim

In this paper we present an overview of stem cell therapy for CVD and discuss the challenges these three areas present for maximum optimization of the efficacy of stem cell therapy for heart disease, and new strategies in progress. We also discuss important questions that remain to be investigated to ascertain a successful translation of current experimental knowledge regarding cell therapy for myocardial repair/replacement.

3. Material and methods

3.1. Stem cells in clinical study

3.1.1. The bone marrow as a source of cardiogenic cells

The inflammatory process after myocardial ischemia stimulates the recruitment and homing to the cardiac the endogenous BM derived cells (BMDCs). It is connected with the mobilization a number of cytokines.[4,5] Preclinical studies of cell-based therapy with BMDCs showed impressive regeneration of lost myocarium, improvement of cardiac function and formation of new capillaries in both small and large animal models.[6] There are some early reports that BMDCs may transdifferentiate into skeletal muscle, hepatocytes or cardiomyocytes.[7,8] However, it is not completely known if improved cardiac function after therapy with those cells was caused by the paracrine theory of cardiac protection and regeneration. This paracrine action includes secretion of cardioprotective cytokines, angiogenic factors or factors which activate resident cardiac stem cells.[9]

After successful preclinical studies in animal models, the rapid transition to the clinical use of BMDCs took place. A significant contribution is the fact that BM can be easy accessed, is renewable, and contains a mixture of autologous cells with regenerative capacity. Much attention has been paid to the mononuclear cell fraction, mainly due to the full array of hematopoietic stem cells, MSCs, EPCs and side population cells. All of these cell types were shown to improve cardiac function if transplanted into infarcted myocardium in various animal studies.[6,10–13]

The first human clinical trial with stem cells in an acute myocardial infarction (AMI) patient was done using an intracoronary infusion of autologous BM unfractionated mononuclear cells. At 10 weeks after the stem cell transplantation, the infarct area had been reduced from 24.6% to 15.7% of LV circumference, while the ejection fraction, cardiac index and stroke volume had increased by 20%–30%.[14]

Several randomized trials showed measurable improvements that were comparable to established therapeutic regimes. Nonrandomized, smaller-scale trials also produced variable results, ranging from no significant changes in LV ejection fraction (LVEF) to a significant improvement. The meta-analysis of 18 randomized and non-randomized trials involving AMI and chronic ischemic cardiomyopathy patients found that transplantation of BMDSCs improved the LVEF by 5.40%, decreased infarct scar size by 5.49%, and lowered LV end-systolic volume by 4.80 mL.[15]

Application of BMMCs caused improved LV contractility in the infarct border zone and global LVEF by 6% in the BOOST trial,[16] 2.8% in the REPIR-AMI trial,[17] 5% in the FINCELL trial,[18] and 3% in the REGENT trial.[19] A high volume of this factor was obtained in a non-randomized trial undertaken by Srimahachota and co-workers[20] – 7%. By contrast, in the ASTAMI trial[21] no significant effects on LVEF, LV volumes, or infarct size were

observed after BMMCs transplantation. Similarly, in a trial undertaken by Janssens charge,[22] they observed only a reduction in the infarct volume and an improvement in regional contractility in the greatest transmural infarct. Intracoronary transfer of autologous BM cells within 24 h of optimum reperfusion therapy does not augment recovery of global LV function after MI, but could favorably affect infarct remodeling. In the HEBE trial, intracoronary infusion of mononuclear cells from BM and peripheral blood did not improve regional or global systolic myocardial function.[23]

Some trials have been completed in patients with advanced chronic myocardial ischemia, with no options for revascularization. BMMCs improved cardiac function in the PROTECT-CAD trial,[24] where improvements were observed of New York Heart Association (NYHA) functional class, exercise time, LVEF, wall thickening, and stress-induced perfusion defects. Similarly, in studies by van Ramshorst et al.,[25] improvement in LVEF, myocardial perfusion, angina functional class, exercise capacity and quality of life were observed. Losordo et al.[26] observed improved of angina frequency and exercise time, however no clear effects on myocardial perfusion were noted.

Penn et al.[27] used MultiStem in a patient after AMI. MultiStem is an allogeneic BM-derived adherent adult stem cell product that has shown efficacy in preclinical models of AMI. This trial showed MultiStem delivered *via* trans arterial adventitia using a microsyringe catheter was safe with improvement of cardiac function in a dose-dependent manner.

The randomized clinical trial with 204 patients with AMI showed that intracoronary delivery of BM cells (at 3–7 days post-reperfusion therapy) decreased the incidence of MI and death, or revascularization, at a 12-month follow-up when compared with placebo.[17]

Now after more than 10 years of clinical experience, the use of cells from certain sources remains highly controversial. All that can be said is that the therapy is safe, but its clinical efficacy seems to be limited, as suggested by the latest meta-analyses. There is no meaningful reduction of cardiac injury, and, at best, there is a modest improvement of LVEF in the range of 3%.[28] New approaches to improve cell survival and preserve paracrine function are currently being explored.[29,30]

3.1.2. *Mesenchymal stem cells*

MSCs are adult stem cells traditionally found in the BM. They are multipotent stromal cells that can differentiate into a variety of cell types, including cardiomyocytes, if stimulated properly *in vitro*, and represent approximately 0.001%–0.010% of BM nucleated cells.[31,32] The data from experiments in animal models of acute and chronic ischemic cardiomyopathy indicate the cardiomyogenic and angiogenic differentiation capacity of MSCs.[33,34] The recovery of the heart is consistently reflected by improved LV remodeling, improved ejection fraction, and reduced scar size.[35] Similar to BMDSCs, paracrine secretome of MSCs is presumably more important for the observed beneficial effects on cardiac remodeling and function than transdifferentiation of MSCs into functional cardiac cells.[36]

Clinical phase I/II trials confirm the safety of MSC transplantation, both in AMI and ischemic cardiomyopathy.[37–39] In these trials reverse remodeling and improved regional contractility of the infarcted area were observed. In the multicenter, randomized cardiopoietic stem cell therapy in heart failure (or C-CURE) trial, MSCs after differentiation toward cardiomyocytes were used with the cardiogenic cocktail.[40] Derived cardiopoietic stem cells, were delivered by endomyocardial injections guided by LV electromechanical mapping. After 6 months, patients randomized to cell therapy exhibited significant improvement of cardiac function and reduction of adverse ventricular remodeling compared with patients receiving standard care. The C-CURE trial implements the paradigm of lineage guidance in cell therapy. Cardiopoietic stem cell therapy was found feasible and safe with signs of benefit in chronic heart failure, meriting definitive clinical evaluation.

Application of MSCs demonstrated an improvement in LVEF and perfusion with intracoronary infusion of these cells in a trial conducted by Chen.[37] Several imagining techniques demonstrated that MSC from BM significantly improved LV function. In another clinical trial[38] with intravenously administered allogeneic MSC, there was no higher rate of major adverse cardiac events and some benefits in terms of LVEF. This trial provides decisive safety and provisional efficacy data for allogeneic BMDSCs in post-infarction patients.

The safety of utilizing the allogeneic MSCs was confirmed in the POSEIDON trial.[41] In this early-stage study of patients with ICM, transendocardial injection of allogeneic and autologous MSCs without a placebo control were both associated with low rates of treatment-emergent serious adverse events, including immunologic reactions. In aggregate, MSC injection favorably affected patient functional capacity, quality of life, and ventricular remodeling. MSC injection affected patient functional capacity, quality of life, and ventricular remodeling favorably. The allogeneic MSC transplantation proved safe and was not associated with adverse immune response.

3.1.3. *Cardiac stem cells*

Another route of possible cardiac regeneration is stimulation of endogenous repair by injected cells, through stem cell cardiac niches activation.[42] Historically, the heart has been considered as a terminally differentiated organ without the capacity for self regeneration. Hierlihy et al.[43] described for the first time the presence of a stem cell-like population in the heart. In contrast to BM cells, cardiac stem cells (CSCs) isolated from the myocardium lack expression of hematopoietic markers, but express cardiac-specific transcription factors such as Nkx 2.5, GATA 4, and Mef2.[44] More recently, it has been reported that the adult heart contains cell populations with stem cell characteristics.[45–49] Hosoda et al.[50] indicate that the adult heart contains a pool of resident stem cells with a high rate of activity for differentiation to cardiomyocytes that can regulate cardiac homeostasis and repair. Other studies[51–53] suggest that cardiomyocytes have the ability to self-renew and new cardiomyocytes may be derived from the division of pre-existing cardiomyocytes. Senyo et al.[54] revealed that cell cycle activity during normal aging and after injury led to polyploidy and multinucleation, but also to new diploid, mononucleate cardiomyocytes. These data suggest that pre-existing cardiomyocytes are the dominant source of cardiomyocyte replacement in normal mammalian myocardial homeostasis as well as after myocardial injury. CSCs have the capacity to differentiate into endothelial cells, smooth muscle myocytes,

and cardiomyocytes. CSCs are activated by paracrine signals that occur during ischemia.[55] Their amount is insufficient for a complete repair of the myocardium, but can be activated by extracardiac delivered cells. This way stem cell niches remain preserved, and through cell-to-cell interactions may restore lost cellular differentiation capacity.[42] Ma et al.[56] have used laser-patterned biochips to define cell-cell contact modes for systematic study of contact-mediated cellular interactions at the single-cell level. It allowed them to define the stem cell–cardiomyocyte contact mode formation. Those results can be used to determine specific cellular interactions, such as electrical coupling, mechanical coupling or mitochondria transfer.

In comparative studies of MSCs, cardiac (c-kit+) stem cells and cardiosphere derived cells,[57] the cardiomyogenic differentiation capacity was more effective with the cardiac derived cells than with MSCs. However, it is also necessary to determine the potential tropic influence of stem cell secretions or cytokines released at the site of injury and the degree of cardio-repair that may be clinically relevant.[58]

Innovative stem cell therapy has also found applications in the youngest patients. Simpson et al.[59] examined the regenerative capacity of CSCs in very young patients with nonischemic congenital heart defects. They showed that neonatal-derived CSCs demonstrated an increased number of cardiac progenitor cells expressing c-kit(+), flk-1, and Islet-1. Moreover, after transplantation into infarcted myocardium, neonatal-derived CSCs had a significantly higher ability to preserve myocardial function, prevent adverse remodeling, and enhance blood vessel preservation and/or formation when compared with adult-derived CSCs. Besides, neonatal-derived CDCs were more cardiomyogenic than adult-derived CSCs when cocultured with neonatal cardiomyocytes and displayed enhanced angiogenic function compared with adult-derived CSCs. This has important implications in the potential use of CSCs in future clinical trials.

3.1.4. *Induced pluripotent stem cells*

Because the regeneration and repair of the adult heart after MI are rather limited, attempts to force pluripotent stem cells (PSCs) into cardiomyogenic differentiation before intracardiac transplantation are currently being undertaken. Induced PSCs (iPSCs, also known as iPS cells) are a type of PSCs that can be generated directly from adult cells. The iPSC technology was pioneered by Shinya Yamanaka, who showed in 2006 that the introduction of four specific genes could convert adult cells to PSCs.[60] iPSCs can be generated by cell reprogramming technology from autologous fibroblasts and thus are not afflicted with ethical concerns.[61,62] Kawamura et al.[63] demonstrated therapeutic efficacy of human iPS-derived cardiomyocyte sheets for regenerative therapy in a porcine model of ischemic cardiomyopathy. In this study, cell transplantation improved cardiac performance significantly and attenuated LV remodeling. Moreover, cardiac fibroblasts can be converted directly into cardiac myocytes using advanced cell reprogramming technology.[64]

Induced PSCs also offer a unique tool for studying diseases and developing customized drug therapies *in vitro*. Understanding the basis for differential responses to drug therapies remains a challenge despite advances in genetics and genomics. Terrenoire et al.[65] used iPSCs differentiated into cardiomyocytes (iPSCs-CMs) to study the physiological basis for arrhythmias in a four-year-old child with long QT syndrome (LQTS). Using voltage clamp analyses of iPSCs-CMs derived from the affected child and his parents, the researchers determined that his arrhythmias were caused by the SCN5A mutation. Those results show promise for using *in vitro* iPSC techniques in the development of individualized drug therapies for patients with LQTS.

Recent evidence has shown that human iPSC-CMs offer a powerful tool to investigate disease mechanisms and to perform patient-specific drug screening. Lee et al.[66] designed a fluorescent imaging platform using LED illumination to measure the electrical activity of the cells. The new method can be readily applied for the study of arrhythmia mechanisms, testing new drug treatments and therapies to repair damaged heart muscle.

New reprogramming technologies open very promising avenues for cell-based therapies in cardiovascular medicine. However, further studies in large-animal models are needed, and important practical hurdles have to be overcome before translation of this approach into clinical practice.

3.1.5. *Cells from other sources*

Adipose derived stem cells (ADCs) have been used in the APOLLO trial in patients after AMI in the first human randomized clinical trial with intracoronary administration.[67] Within 36 h of the MI and no longer than 24 h after undergoing percutaneous coronary intervention, patients received an injection of either 20 million ADCs ($n = 10$) or a placebo ($n = 4$). The percentage of LV infarcted was reduced by 52% in the ADC-treated patients, as opposed to no change in the placebo-treated AMI patients. There was observed a significant improvement of the perfusion defect in ADC-treated at the 6-month follow-up as compared to a deterioration in the placebo group. LVEF improved by 4% as compared to a deterioration of 1.7% in the placebo group.[68]

A new approach in stem cell therapy is cell mobilization. Trials were conducted with the administration of granulocyte colony-stimulating factor (G-CSF), but results have been less encouraging. Only in the FIRSTLINE-AMI trial,[69] the RIGENERA study,[70] and in the study by Takano et al.,[71] significant improvements were observed, other trials showed negative findings. In some trials a combination of G-CSF mobilization and intracoronary injection of peripheral blood progenitor cells were used. In the MAGIC trials[72] no differences in LVEF were observed, merely an increase in the instant restenosis rate. However, after the design was changed and drug-eluting stents were used, the MAGIC 3-DES trial found positive results of LVEF.[73]

Although there is large variability of hemodynamic data after cell therapy, there is moderate improvement of cardiac performance by stem cell therapy that is more quantitatively effective than therapeutic interventions and pharmacotherapy.[17]

Skeletal myoblasts have been studied in ischemic heart diseases. Transepicardial injection of skeletal myoblast in MAGIC trial[74] reported no changes in global or regional contractility. Nonetheless, in the high-dose group reduction in LV end-diastolic and end-systolic volumes were observed.

Unfortunately, some incidences of ventricular arrhythmias were observed. Results obtained by Dib et al.[75] suggest that autologous myoblast transplantation offers a potential therapeutic treatment for end-stage heart disease.

The scientists from the Max Planck Institute for Heart and Lung Research in Bad Nauheim have identified a key molecule that plays a main role in regulating the function of stem cells in the heart. Using an animal model (the zebrafish), they investigated the effects of a defective switch on cardiac development. They have identified Ajuba, a transcription factor from the group of LIM proteins (named after their discovery in the proteins Lin11, Isl-1 and Mec-3[76]) as a crucial regulator of the second heart field progenitor cell specification and expansion. Understanding regulation of cardiac development, we will be able to consider therapeutic approaches – production of replacement cells from embryonic or stimulate stem cell activity by silencing Ajuba in the damaged heart and so cause the heart to regenerate itself.[77]

4. Discussion

The final goal of CSC therapy is the repair of the damaged myocardium and the restoration of cardiac function. Results of clinical trials have been reported as unusually promising, including cardiomyogenesis, neovascularization, and paracrine effect on injured myocardium. However, we have to remember that the loss of cardiomyocytes after an AMI is about 1 billion cells. Transplanted cells must be supplied together with cardiomyocytes, and the environmental signals which guide stem cells to the cardiac lineage or to the secretion of paracrine factors might be absent in damaged tissue.[58]

From a clinical point of view, an ideal cell source for cardiovascular regeneration should be easy to access, should have cardioprotective, cardiomyogenic, and angiogenic potential independent from patient age and cardiovascular risk factors, and should survive in harsh environments.

The results obtained during the last few years are not consistent for a few reasons. These well-designed randomized clinical trials generated very mixed results.[23,78–80] The divergences are attributed to differences in cell preparations, the large number of stem cell types under investigation in different clinical settings, timing, methods of cell administration and characteristics of patients.

Patient selection before conducting clinical trial must take into account the pathophysiologic basis of the disease and baseline characteristics. Each disease presents its own set of problems and complexities, and subsets of patients within diseases present differing challenges. Patients with larger AMI, much depressed baseline LVEF and stroke volumes seem to benefit the most after stem cell treatment, rather than patients with microvascular obstruction.[58]

Survival and engraftment of stem cells are the most important challenges for stem cells therapy, especially after a MI, where an enormous loss of cardiomyocytes is needed to be replaced. Using various *in vivo* imaging techniques revealed that only a limited number of cells engrafted and most cells died shortly after transplantation. It has been shown that more than 90% of injected cells during cell therapy die by apoptosis 24 h after transplantation.[81] Hence, when they are transplanted in an ischemia, hypoxia, and proapoptotic niche, most stem cells cannot survive.[82] Therefore, optimization of cell retention after injection and survival seem to be paramount to further define the optimal dose of cells to transplant. Currently to reduce this loss, large amount of cells must be injected. A dosage effect has largely been reported.[83] Moreover, many studies have focused on strategies to optimize migration of stem cells across injured myocardial tissue. Many agents, such as proteases, adhesion molecules, and integrin take part in regulating this migration and modulation of the connective tissue microenvironment to improve stem cells engraftment.[84–87] Specifically, cell-tracking studies have found that myocardial engraftment is less than 10% within 48 h irrespective of cell type, the number of cells implanted, and delivery route.[88] PET showed that only 1.3%–2.6% of the labeled stem cells migrated to the myocardium 2 h after injection, while the majority of the cells moved to the tissue outside of the heart muscle, including the liver, spleen, lung, bladder, and brain.[89] It seems that cells being cultured *in vitro* and administered in several doses at different times turned out to be indispensable.[90] Alternatively, combinations of two cell types with documented positive interactions might be a novel therapeutic strategy, which was proven in a porcine model of MI for the combination of human CSCs with human MSCs.[91] The combination of the two cell types turns out to be more effective at reducing infarct size and restoring cardiac function than either cell type alone. Other options may be transplantation of cell-free matrices being the supplier of cardiopoietic factors. Their controlled release may be deployed to enhance intracardiac paracrine effects and to activate the intrinsic regeneration capacity of the heart.

5. Conclusions

Over the last decade, cardiac cell therapy has been widely studied as a revolutionary approach to promote the non-pharmacological replacement of lost myocardium. Evidence obtained from human clinical trials in CVDs demonstrates the important role of stem cells. Despite diverse results, the data suggest that applied procedures are safe and feasible. Therefore, carefully designed clinical trials are needed to evaluate the potential of various SC sources to define the limitations in production, delivery, and clinical benefit.

Conflict of interest

None declared.

REFERENCES

1. WHO. *Cardiovascular Diseases*. 2015. http://www.who.int/cardiovascular_diseases/en Accessed 10.02.15.
2. Hoover-Plow J, Gong Y. Challenges for heart disease stem cell therapy. *Vasc Health Risk Manage*. 2012;8:99–113.
3. Sanz-Ruiz R, Gutiérrez Ibañes E, Arranz AV, Fernández Santos ME, Fernández PL, Fernández-Avilés F. Phases I–III

clinical trials using adult stem cells. *Stem Cells Int.* 2010;4:579142.
4. Mouquet F, Pfister O, Jain M, et al. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ Res.* 2005;97:1090–1092.
5. Fazel S, Cimini M, Chen L, et al. Cardioprotective c-kit1 cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest.* 2006;116: 1865–1877.
6. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001;410:701–705.
7. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. *Cell.* 2001;105:369–377.
8. 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.* 2000;290: 1779–1782.
9. Uemura R, Xu M, Ahmad N, Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ Res.* 2006;98:1414–1421.
10. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107:1395–1402.
11. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res.* 1999;85:221–228.
12. Dai W, Hale SL, Martin BJ, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation.* 2005;112:214–223.
13. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg.* 2002;73:1919–1925 [discussion 1926].
14. Strauer BE, Brehm M, Zeus T, et al. Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction. *Dtsch Med Wochenschr.* 2001;126:932–938.
15. Meyer GP, Wollert KC, Lotz J. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. *Eur Heart J.* 2009;30:2978–2984.
16. Abdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med.* 2007;167:989–997.
17. Schächinger V, Erbs S, Elsässer 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.* 2006;27:2775–2783.
18. Huikuri HV, Kervinen K, Niemelä M, et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. *Eur Heart J.* 2008;29:2723–2732.
19. Tendera M, Wojakowski W, Ruzyłło 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.* 2009;30:1313–1321.
20. Srimahachota S, Boonyaratavej S, Rerkpattanapipat P, et al. Feasibility and safety of intra-coronary bone marrow mononuclear cell transplantation in ST elevation myocardial infarction patients. *J Med Assoc Thai.* 2009 Dec;92:1591–1596.
21. Beitnes JO, Gjesdal O, Lunde K, et al. Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the ASTAMI randomised, controlled study. *Heart.* 2009;95:1983–1989.
22. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet.* 2006;367:113–121.
23. Hirsch A, Nijveldt R, van der Vleuten PA, 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.* 2011;32:1736–1747.
24. Tse HF, Thambar S, Kwong YL, et al. Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). *Eur Heart J.* 2007;28:2998–3005.
25. Van Ramshorst J, Bax JJ, Beeres SL, et al. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. *JAMA.* 2009;301:1997–2004.
26. Losordo DW, Schatz RA, White CJ, et al. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase 1/IIa double blind, randomized controlled trial. *Circulation.* 2007;115:3165–3172.
27. Penn MS, Ellis S, Gandhi S, et al. Adventitial delivery of an allogeneic bone marrow-derived adherent stem cell in acute myocardial infarction: phase I clinical study. *Circ Res.* 2012;110:304–311.
28. Zimmet H, Porapakkham P, Porapakkham P, et al. Short- and long-term outcomes of intracoronary and endogenously mobilized bone marrow stem cells in the treatment of ST-segment elevation myocardial infarction: a meta-analysis of randomized control trials. *Eur J Heart Fail.* 2012;14:91–105.
29. Mohsin S, Siddiqi S, Collins B, Sussman MA. Empowering adult stem cells for myocardial regeneration. *Circ Res.* 2011;109:1415–1428.
30. Jakob P, Landmesser U. Role of microRNAs in stem/progenitor cells and cardiovascular repair. *Cardiovasc Res.* 2012;93:614–622.
31. Xu M, Wani M, Dai YS, et al. Differentiation of bone marrow stromal cells into the cardiac phenotype requires intercellular communication with myocytes. *Circulation.* 2004;110:2658–2665.
32. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284:143–147.
33. Yang YJ, Qian HY, Huang J, et al. Combined therapy with simvastatin and bone marrow-derived mesenchymal stem cells increases benefits in infarcted swine hearts. *Arterioscler Thromb Vasc Biol.* 2009;29:2076–2082.
34. Dixon JA, Gorman RC, Stroud RE, et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. *Circulation.* 2009;120:S220–S229.
35. Halkos ME, Zhao ZQ, Kerendi F, et al. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. *Basic Res Cardiol.* 2008;103:525–536.
36. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res.* 2008;103:1204–1219.
37. Chen SL, Fang WW, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol.* 2004;94:92–95.

38. 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*. 2009;54:2277–2286.
39. Williams AR, Trachtenberg B, Velazquez DL, et al. Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling. *Circ Res*. 2011;108:792–796.
40. 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*. 2010;56:721–734.
41. Hare JM, Fishman JE, Gerstenblith G, et al. Comparison of allogeneic vs. autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA*. 2012;308:2369–2379.
42. Mazhari R, Hare JM. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. *Nat Clin Pract Cardiovasc Med*. 2007;4 (suppl 1):S21–S26.
43. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA. The post-natal heart contains a myocardial stem cell population. *FEBS Lett*. 2002;530:239–243.
44. Pfister O, Mouquet F, Jain M, et al. CD31 – but not CD311 cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res*. 2005;97:52–61.
45. Ellison GM, Torella D, Karakikes I, Nadal-Ginard B. Myocyte death and renewal: modern concepts of cardiac cellular homeostasis. *Nat Clin Pract Cardiovasc Med*. 2007;4:S52–S59.
46. Kajstura J, Urbanek K, Rota M, et al. Cardiac stem cells and myocardial disease. *J Mol Cell Cardiol*. 2008;45:505–513.
47. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003;114:763–776.
48. Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res*. 2004;95:911–921.
49. Barile L, Messina E, Giacomello A, Marbán E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis*. 2007;50:31–48.
50. Hosoda T, D'Amario D, Cabral-Da-Silva MC, et al. Clonality of mouse and human cardiomyogenesis in vivo. *Proc Natl Acad Sci U S A*. 2009;106:17169–17174.
51. Soonpaa MH, Field LJ. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. *Am J Physiol Heart Circ Physiol*. 1997;272:H220–H226.
52. Bergmann O, Bhardwaj RD, Bernard S. Evidence for cardiomyocyte renewal in humans. *Science*. 2009;324:98–102.
53. Walsh S, Pontén A, Fleischmann BK, Jovinge S. Cardiomyocyte cell cycle control and growth estimation in vivo—an analysis based on cardiomyocyte nuclei. *Cardiovasc Res*. 2010;86:365–373.
54. Senyo SE, Steinhauser ML, Pizzimenti CL, et al. Mammalian heart renewal by pre-existing cardiomyocytes. *Nature*. 2013;493:433–436.
55. Linke A, Müller P, Nurzynska D, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infracted myocardium, improving cardiac function. *Proc Natl Acad Sci U S A*. 2005;102:8966–8971.
56. Ma Z, Yang H, Liu H, et al. Mesenchymal stem cell–cardiomyocyte interactions under defined contact modes on laser-patterned biochips. *PLOS ONE*. 2013;8:56554.
57. Konincky R, Daniëls A, Windmolders S, et al. Mesenchymal stem cells or cardiac progenitors for cardiac repair? A comparative study. *Cell Mol Life Sci*. 2011;68:2141–2156.
58. Wollert KC, Drexler H. Cell therapy for the treatment of coronary heart disease: a critical appraisal. *Nat Rev Cardiol*. 2010;7:204–215.
59. Simpson DL, Mishra R, Sharma S, Goh SK, Deshmukh S, Kaushal S. A strong regenerative ability of cardiac stem cells derived from neonatal hearts. *Circulation*. 2012;126 (11 suppl 1):S46–S53.
60. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.
61. Okita K, Ichisaka T, Yamanaka S. Generation of germline competent induced pluripotent stem cells. *Nature*. 2007;448:313–317.
62. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
63. Kawamura M, Miyagawa S, Miki K, et al. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. *Circulation*. 2012;126:S29–S37.
64. Ieda M, Fu JD, Delgado-Olguin P, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010;142:375–386.
65. Terrenoire C, Wang K, Tung KW, et al. Induced pluripotent stem cells used to reveal drug actions in a long QT syndrome family with complex genetics. *J Gen Physiol*. 2013;141:61–72.
66. Lee P, Klos M, Bollensdorff C, et al. Simultaneous voltage and calcium mapping of genetically purified human induced pluripotent stem cell-derived cardiac myocyte monolayers. *Circ Res*. 2012;110:1556–1563.
67. *Randomized Clinical Trial of Adipose-Derived Stem Cells in the Treatment of Pts With ST-elevation Myocardial Infarction*. 2015. http://clinicaltrials.gov/show/NCT00442806 Accessed 15.02.15.
68. Houtgraaf JH, den Dekker WK, van Dalen BM, et al. First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2012;59:539–540.
69. Ince M, Petzsch M, Kleine HD, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). *Circulation*. 2005;112:3097–3106.
70. Leone AM, Galiuto L, Garramone B, et al. Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the Rigenera study). *Am J Cardiol*. 2007;100:397–403.
71. Takano H, Hasegawa H, Kuwabara Y, et al. Feasibility and safety of granulocyte colony-stimulating factor treatment in patients with acute myocardial infarction. *Int J Cardiol*. 2007;122:41–47.
72. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilized with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet*. 2004;363:751–756.
73. Kang HJ, Lee HY, Na SH, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC cell-3-DES randomized, controlled trial. *Circulation*. 2006;114:I145–I151.
74. Menasch'e 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*. 2008;117:1189–1200.

75. Dib N, Michler RE, Pagani FD, et al. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. *Circulation*. 2005;112:1748–1755.
76. Bach I. The LIM domain: regulation by association. *Mech Dev*. 2000;91:5–17.
77. Witzel HR, Jungblut B, Choe CP, Crump JG, Braun T, Dobreva G. The LIM protein Ajuba restricts the second heart field progenitor pool by regulating Isl1 activity. *Dev Cell*. 2012;23:58–70.
78. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet*. 2006;367:113–121.
79. Roncalli J, Mouquet F, Piot C, et al. Intracoronary autologous mononucleated bone marrow cell infusion for acute myocardial infarction: results of the randomized multicenter BONAMI trial. *Eur Heart J*. 2011;32:1748–1757.
80. Perin EC, Willerson JT, Pepine CJ, et al. Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA*. 2012;307:1717–1726.
81. Hodgetts SI, Beilharz MW, Scalzo AA, Grounds MD. Why do cultured transplanted myoblasts die in vivo? DNA quantification shows enhanced survival of donor male myoblasts in host mice depleted of CD4+ and CD8+ cells or NKl, 1+ cells. *Cell Transplant*. 2000;9:489–502.
82. Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *J Am Coll Cardiol*. 2005;46:1339–1350.
83. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, et al. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. *Cardiovasc Res*. 2011;91:649–658.
84. Ip JE, Wu Y, Huang J, Zhang L, Pratt RE, Dzau VJ. Mesenchymal stem cells use integrin β1 not CXC chemokine receptor 4 for myocardial migration and engraftment. *Mol Biol Cell*. 2007;18:2873–2882.
85. Borg TK, Markwald R. Periostin: more than just an adhesion molecule. *Circ Res*. 2007;101:230–231.
86. Xiang G, Schuster MD, Seki T, Witkowski P, Eshghi S, Itescu S. Down regulated expression of plasminogen activator inhibitor-1 augments myocardial neovascularization and reduces cardiomyocyte apoptosis after acute myocardial infarction. *Am Coll Cardiol*. 2005;46:S336–S341.
87. Shimazaki M, Nakamura K, Kii I. Periostin is essential for cardiac healing after acute myocardial infarction. *J Exp Med*. 2008;205:295–303.
88. Wu JC, Abraham MR, Kraitchman DL. Current perspectives on imaging cardiac stem cell therapy. *J Nucl Med*. 2010;51 (suppl 1):128S–136S.
89. Hofmann M, Wollert KC, Meyer GP, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation*. 2005;111:2198–2202.
90. Lyon A, Harding S. The potential of cardiac stem cell therapy for heart failure. *Curr Opin Pharmacol*. 2007;164–170.
91. Williams AR, Hatzistergos KE, Addicott B, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. *Circulation*. 2013;127:213–223.