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Stem cells for cardiac repair: an introduction

Bastiaan C du Pré[1], Pieter A Doevendans[1,2], Linda W van Laake[1,3]

[1]Departments of Cardiology and Medical Physiology, Division of Heart and Lungs, University Medical Center Utrecht, P.O. box 85500, 3508 GA Utrecht, the Netherlands
[2]Interuniversity Cardiology Institute of the Netherlands, P.O. box 19258, 2501 DG Utrecht, the Netherlands
[3]Hubrecht Institute, P.O box 85164, 3508 AD Utrecht, the Netherlands

Abstract

Cardiovascular disease is a major cause of morbidity and mortality throughout the world. Most cardiovascular diseases, such as ischemic heart disease and cardiomyopathy, are associated with loss of functional cardiomyocytes. Unfortunately, the heart has a limited regenerative capacity and is not able to replace these cardiomyocytes once lost. In recent years, stem cells have been put forward as a potential source for cardiac regeneration. Pre-clinical studies that use stem cell-derived cardiac cells show promising results. The mechanisms, though, are not well understood, results have been variable, sometimes transient in the long term, and often without a mechanistic explanation. There are still several major hurdles to be taken. Stem cell-derived cardiac cells should resemble original cardiac cell types and be able to integrate in the damaged heart. Integration requires administration of stem cell-derived cardiac cells at the right time using the right mode of delivery. Once delivered, transplanted cells need vascularization, electrophysiological coupling with the injured heart, and prevention of immunological rejection. Finally, stem cell therapy needs to be safe, reproducible, and affordable. In this review, we will give an introduction to the principles of stem cell based cardiac repair.

1 Introduction

Repairing the injured body with its own tissue as a substrate has captured human fascination for a long time. In Greek mythology, the Lernaean Hydra was a serpent-like creature with multiple heads that regenerated each time they were cut off and Prometheus, a titan punished by Zeus for stealing fire, had a liver that was able to regenerate each night after it was eaten by an eagle. In 1740, Abraham Tembley discovered that microscopic, freshwater animals had the ability to regenerate their head after amputation, later followed by others who discovered that amphibians have the ability to regenerate their tails, limbs, jaws, and eyes.[1,2] It took scientists until 1933 before they discovered that some human organs, such as the liver, also have the ability to regenerate.[3]

Correspondence to: Linda W van Laake, MD, PhD, University Medical Center Utrecht, Department of Cardiology, Division Heart and Lungs, P.O. box 85500, 3508 GA Utrecht, the Netherlands.
E-mail: l.w.vanlaake@umcutrecht.nl
Telephone: +31-88-7557065   **Fax:** +31-30-2516396

Regenerative therapies are of major interest in cardiovascular medicine. Most cardiovascular diseases, including ischemic heart disease and cardiomyopathy, are associated with loss of functional cardiomyocytes and in other diseases, such as sick sinus syndrome, specific cardiac cell properties are missing. Unlike the Lernaean Hydra or the human liver, the heart does not have the ability to regenerate itself spontaneously once damaged. Cardiomyocytes are terminally differentiated and have a limited proliferative capacity. Lost cardiomyocytes are replaced by fibroblasts and connective tissue with the remaining cardiomyocytes becoming hypertrophic, which may eventually lead to heart failure. On the contrary, stem cells proliferate indefinitely and can be directed to differentiate into specialized cell types such as cardiomyocytes. The goal of stem cell-based regenerative medicine in cardiovascular disease, therefore, is to create healthy, functional cardiac cells that are able to integrate in the injured heart and restore its function.

In the past decades, several stem cell types have been discovered. These stem cells can be subdivided based on their differentiation capacity. Pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are able to differentiate into all three embry-

onic germ layers, whereas multipotent stem cells can differentiate into a number of closely related cell types of a single embryonic germ layer. Cardiomyocytes were derived from several stem cell sources (Figure 1). Other types of stem cells do not differentiate into cardiomyocytes themselves, but support cardiac repair by different mechanisms (Table 1). In this review, we will refer to all stem cell-derived cardiomyocytes and differentiated cell types enriched for cardiomyocytes as stem cell-derived cardiomyocytes (SCD-CMs), while we will refer to non-cardiomyocyte derivatives (such as vascular cells) as stem cell-derived cardiac support cells (SCD-CSCs).

In this review, we will give an introduction to the principles of stem cell-based cardiac repair. Our aim is to give a concise up-to date overview of the therapeutic possibilities of stem cells for cardiac injury. First, we describe general requirements for stem cell therapy. After that, we will discuss in more detail the different stem cell sources and their therapeutic effects, since these vary for each cell type.

2   Requirements for stem cell therapy

In order to be suitable for cardiac repair, stem cell-derived cardiac cells should resemble the original cardiac cell types and be able to integrate in the damaged heart. Integration requires administration of stem cell-derived cardiac
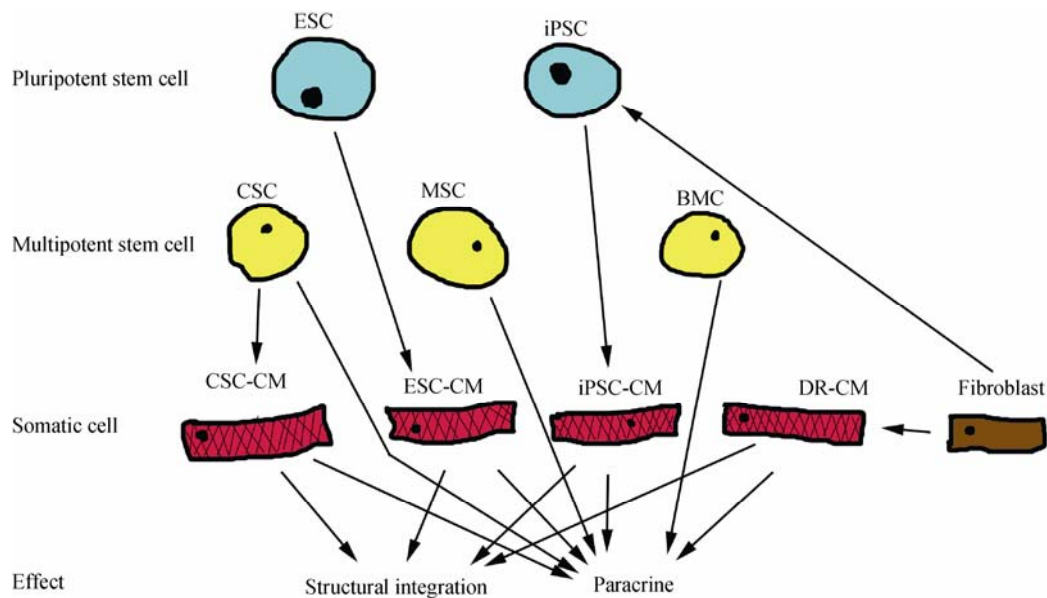

Figure 1.   Summary of stem cells used for cardiac repair. BMC: bone marrow-derived cell; CSC: cardiac stem cell; CSC-CM: cardiac stem cell-derived cardiomyocyte; DR-CM: cardiomyocyte derived by direct reprogramming; ESC: embryonic stem cell; ESC-CM: embryonic stem cell-derived cardiomyocyte; iPSC: induced pluripotent stem cell; iPSC-CM: induced pluripotent stem cell-derived cardiomyocyte; MSC: mesenchymal stem cell.

Table 1.   Characteristics of stem cells studied for cardiac regeneration potential.

Stem cell	Origin	Stem cell type	Research stage	Primary intended effect	Immunological status cells	Remarks
Embryonic stem cell	Inner cell mass of blastocyst	Pluripotent	Pre-clinical	Structural integration	Allogenic/matched	Ethical and safety issues
Induced pluripotent stem cell	Somatic cell	Pluripotent	Pre-clinical	Structural integration	Autologous/matched	Safety issues
Cardiac stem cell	(Adult) Heart	Multipotent	Pre-clinical	Structural integration	Auto- and allogenic/matched	Limited availability
Mesenchymal stem cell	Bone marrow, fat, and cord blood	Multipotent	Clinical	Paracrine	Tolerated/autologous	No structural effects
Bone marrow cell	Bone marrow	Multipotent	Clinical	Paracrine	Tolerated/autologous	Heterogenous cell population, no structural effects
Directly reprogrammed cell	Somatic cell	No stem cell involved	Pre-clinical	Structural integration	Autologous	Safety issues, limited efficacy of differentiation

cells at the right time using the right mode of delivery. Once delivered, transplanted cells need vascularization, electrophysiological coupling with the injured heart, and prevention of immunological rejection. Ideally there would also be beneficial effects on the host myocardium, for example, by stimulating proliferation or differentiation of local progenitors, neovascularization or by inhibiting apoptosis. The minimum requirement for the donor cells is to have no adverse effects. Finally, stem cell therapy needs to be safe, reproducible, and affordable. Each of these requirements will be discussed separately. (Table 2)

Table 2. Requirements stem cell based cardiac regeneration.

Transplanted cells resemble original cardiac cell types
    Combination of different cardiac cells and extracellular matrix
    Mature electrophysiological phenotype
    Contractile function
Structural integration in damaged heart
    Delivery at right time
    Right mode of delivery
    Electrophysiological coupling recipient heart
    Vascularization
    Prevention of (immunological) rejection
Appropriate paracrine effect
    No adverse effects on host myocardium or even beneficial effects
Acceptable complications and ethical considerations
Reproducible methods on large scale
Affordable

2.1 Cell type

Appropriate cardiac function requires well-timed, successive contraction of different parts of the heart. These contractions are orchestrated by the cardiac electrical system, which consists of the sinoatrial (SA) node, the atria, the atrioventricular node, the His-Purkinje system, and the ventricles. Each part of the system has a different expression of ion channels, and thus a specific electrophysiological phenotype according to its function in the electrical system.[4] Regeneration therapy of the SA-node, therefore, requires SCD-CMs with a different cell mixture, contractile function, and ion channel profile compared to regeneration therapy of the ventricles.

Grafted cells should preferably possess characteristics similar to the original cells they replace. In literature, there is no consensus whether this is best achieved by transplantation of stem and progenitor cells, or of fully differentiated cardiac cells. Cardiac tissue is an organized and dynamic contractile tissue that consists of different kinds of myocytes, vascular smooth muscle cells, fibroblasts, and an extracellular matrix.[5] Undifferentiated stem and progenitor cells

have the advantage that they can migrate to injured areas and form all cardiac components; physiological signals in the heart, such as those created by the recently discovered telocytes, may regulate differentiation after transplantation.[6] On the other hand, since cardiac repair does not occur spontaneously in the damaged heart, cardiac signals might not be sufficient for regeneration therapy and an *in vitro* differentiated cell mixture might be preferable. *In vitro* differentiation also has the advantage that cells can be organized to optimize contractile function and that there is no risk of teratome formation associated with use of undifferentiated stem and progenitor cells.

Cardiac cells that were derived from stem cells *in vitro,* at present, consist of a heterogeneous population of cardiomyocytes, often accompanied by other SCD-CSCs like fibroblasts, vascular smooth muscle cells, or more problematic, undifferentiated cells.[7] Purification methods to remove unwanted cell types from the SCD-CMs population have been developed and the electrophysiological phenotype of SCD-CMs can be determined, although none of the clinically suitable methods to purify cells has been shown to be reproducible, efficient and safe.[8,9] In addition, there is no method to select SCD-CMs with a specific electrophysiological phenotype. Development of such methods will increase standardization of SCD-CMs and enable selection of SCD-CMs that have properties similar to original cardiac cells.

2.2 Mode of delivery

Although paracrine factor release from the transplanted SCD-CMs and SCD-CSCs is beneficial even if the donor cells do not survive on the long term, the ultimate success of regeneration by SCD-CMs transplantation depends on integration of the donor cells in the injured myocardium. Pre-clinical studies show that survival of transplanted SCD-CMs in the damaged myocardium is poor.[10] In order to solve this problem, several modes of transplantation have been developed.[11]

The first and most commonly used method is injection, which can be targeted at different locations. Injection in the center of the injured myocardium has the advantage that damage is most severe so the potential benefit of therapy is maximal. On the other hand, in the border zone of the injured heart, perfusion is still possible and signals of viable cardiac tissue in the proximity may be useful for survival, differentiation, and integration. This assumption is supported by the finding that after myocardial infarction (MI), a limited number of new myocytes is present in the border zone but not in the infarcted tissue itself.[12] Intramyocardial injection can be performed from the epicardial side of the

heart by surgical injection, or from the inside (transendocardially) with guidance using a NOGA system).[13] A third option is intravascular injection.[14–16] Intravenous injection is simple and has a low risk of mechanical damage by the injection, but requires adequate homing to the site of injury in the heart. Injection in one of the cardiac vessels (coronary arteries, veins or sinus) has the advantage that therapy can be combined with percutaneous coronary interventions (PCI), a therapy commonly used by intervention cardiologists during acute MI.

The application of biomaterials is a novel method to transplant SCD-CMs. Biomaterial application is based on the concept that in order to survive, transplanted cells require a biochemical and biophysical environment comparable to extracellular matrix in healthy myocardium. Extracellular matrix in cardiovascular disease plays a role in angiogenesis, differentiation and maturation of stem cells and mechanical and electrical engraftment of transplanted cells.[17] The goal of biomaterials, therefore, is to substitute healthy extracellular matrix to enable integration of SCD-CMs. In addition, biomaterials themselves also show beneficial effects, although mostly temporarily.[18–20] Several biomaterials have been developed for this aim, including alginate,[18,19] matrigel,[21] collagen,[22] fibrin,[23] and self-assembling peptides.[24]

In order to be used for regenerative therapy, biomaterials need to have mechanical properties like the cardiac extracellular matrix and be able to continuously and slowly send signals needed for integration, proliferation, and differentiation.[25] They need to be biodegradable in non-toxic metabolites after a period long enough to enable proper integration and the viscosity needs to be low enough to be transplanted and to permit migration, but high enough to prevent mechanical removal of the SCD-CMs. Especially in the area of signaling, significant progress has been made over the past few years. Biomaterials that slowly deliver proteins,[25] drugs,[26] plasmids,[27] viruses,[27] and microRNAs[28] to surrounding tissue are currently available.

2.3 Time of delivery

Regeneration therapy can be applied during several stages of cardiovascular disease. After MI, fast delivery of SCD-CMs has the advantage that remodeling, characterized by fibrosis and hypertrophy, has not yet developed and that regulatory mechanisms leading to these conditions can be altered. Anti-apoptotic, immune-modulatory, and pro-angiogenic effects of SCD-CMs can therefore be used immediately after MI.[29] On the contrary, ischemic and inflammatory conditions directly after MI do not favor cell survival, cell integration, and immunologic tolerance. For actual tis-

sue regeneration, strategies also including delivery at a later time-point might therefore achieve better results.

2.4 Vascularization

Oxygenation *via* vascularization is essential for the survival of cardiac tissues; both acute and chronic ischemia are associated with cardiovascular disease. After MI, ischemia induces pathophysiological mechanisms leading to cell death or hibernation and fibrosis, and termination of ischemia is believed to be the most important therapy after MI.[30]

In regenerative therapy, vascularization is both a prerequisite and a goal. Cardiomyocytes have a high oxygen demand and in order for SCD-CMs to survive and integrate sufficient oxygen and nutrient supply *via* blood vessels is required. Several strategies have been developed to promote blood vessel formation. This can be achieved by the formation of new blood vessels (vasculogenesis) and by extension of existing blood vessels (angiogenesis). Co-culture and co-transplantation of SCD-CMs with cardiac support cells, such as endothelial cells and fibroblasts, improves *in vivo* vascularization and cell survival.[31,32] In addition, paracrine factors secreted from the SCD-CMs themselves can enhance neovascularization.[17,33] Biomaterials designed with geometries that promote angiogenesis and vasculogenesis, or addition of angiogenic and vasculogenic growth factors and proteins to biomaterials were also developed to stimulate vascularization.[34–36]

2.5 Immunological reaction

Transplantation of genetically unrelated tissues usually results in foreign antigen recognition by T-lymphocytes, immune system activation and in most cases, graft rejection. Most *in vivo* experiments with SCD-CM use immunosuppressed animal models.[37] Before the use of SCD-CMs can become clinically applicable, however, the issue of graft rejection needs to be dealt with.

Generally, there are four strategies to prevent immune rejection. The graft and host can be genetically matched; the immune system can be adapted to tolerate the graft; the graft can be adapted to remain undetected by the immune system; or the immune system can be suppressed. SCD-CMs can be created using the host as a substrate. Adult stem cells and multipotent progenitor cells, but also induced pluripotent stem (iPS) cells, differentiated somatic cells that have been genetically reprogramed to resemble embryonic stem cells, can be derived from the host. From these stem cells, SCD-CMs can be developed that are genetically identical to the host. Alternatively, genetically matched stem cells from stem cell banks can be used.[38] Syngeneity of graft and host prevents detection and rejection of transplanted SCD-CMs

by the host's immune system, although it has been suggested that altering and reprogramming might enhance immunogenicity, even of autologous cells.[39] A second strategy to prevent immune rejection is tolerization. Immune tolerance is regulated by the acquired immune system, in which regulatory T cells play a central role. Some tissues that are not genetically identical to the host, e.g., an unborn child during pregnancy, are tolerated by the immune system of the host. Methods have been developed to induce donor-specific tolerance, for example, by preconditioning the host's immune system with tolerogenic immune cells, such as dendritic cells, before transplantation of SCD-CMs.[40] The third method to prevent immune rejection is to create SCD-CMs that remain undetected by the immune system.[41] Suppression of the immune system can be used in combination with all three previous strategies to further prevent immune rejection, but because of the side effects of immunosuppression, this strategy is unfavorable.

Apart from its role in graft rejection, the immune system also gained attention in regeneration therapy as a potential therapeutic target. In cardiovascular disease, the immune system plays an important role. After MI, inflammation is accountable for a large part of cardiac damage.[42–44] Stem and progenitor cells have the ability to modulate immune responses and animal ischemia-reperfusion models suggest that these modulations have beneficial effects.[15,45]

2.6 Complications

The use of stem cells involves the risk of tumorigenesis. Stem cells have carcinogenic properties: they have the ability to self-renew, proliferate rapidly, lack contact inhibition, and have an extended life-time due to telomerase activity.[46] Studies show that several oncogenes that are highly expressed in teratomas are also found in ESCs.[47] The actual risk of tumorigenesis in stem cell therapy in humans was highlighted in 2009, when a child received fetal neural stem cells as a therapy for neurodegenerative disease, but developed multifocal glioneural tumors as a complication.[48] SCD-CMs are differentiated *in vitro* and therefore have a much smaller risk of tumorigenesis, but if not carefully selected, contaminating undifferentiated progenitor cells still form a risk. Appropriate cell selection is essential for primary prevention of tumorigenesis.

As secondary prevention, techniques have been developed to track and eliminate teratomas. A reporter can be added to SCD-CMs that allows tracking of transplanted SCD-CMs and selective targeting in case of teratoma formation.[49] Alternatively, molecular probes that attach to teratoma cell surface receptors were developed for *in vivo* tracking of teratoma formation.[50] Targeting of teratomas

using this technique is not yet possible, but would have the advantage that it circumvents genetic modification of SCD-CMs, which can induce tumor formation by itself.[46]

A second feared complication of cardiac regeneration therapy is the development of life-threatening arrhythmias. Transplanted SCD-CMs have a different action potential compared to host cardiomyocytes, are electrophysiologically poorly integrated in the host myocardium and in most cases display automaticity which leads to cardiac excitability and arrhythmias.[51–54] Experiments in rodent models show an increased risk of arrhythmias after SCD-CM transplantation and data from clinical trials reports ventricular arrhythmias as one of the main complications after myoblast transplantation.[55–57] On the contrary, some studies show that implantation of SCD-CMs prevents the occurrence of arrhythmias.[58] The risk of arrhythmias depends on the cell type used, the mode of delivery, and the host environment.[51] Improvement of electrophysiological integration, determination of the most suitable cell types, and improved modes of delivery are being developed in order to lower the risk of arrhythmia development.

3 Stem cell sources for cardiac regeneration

3.1 Pluripotent stem cells

3.1.1 Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells that are isolated from the inner cell mass of mammalian blastocysts. In 1981, murine embryonic stem cells were first isolated, followed by isolation of human ESCs in 1998.[59,60] Currently, most human ESC lines were derived from pre-implantation stage human blastocysts that were harvested for clinical use, but were no longer intended for that use and were donated after informed consent.[61] ESCs are pluripotent; they proliferate indefinitely, and can be differentiated into somatic cells of all three embryonic germ layers under specific culture conditions, including cardiovascular cell types such as fibroblasts, endothelial cells, smooth muscle cells, and cardiomyocytes.[62] Because of these properties, large quantities of cardiac cells which are necessary for regeneration can be created *in vitro*. Animal studies show that cardiomyocytes derived from embryonic stem cells (ESC-CMs) have the ability to integrate in the recipient heart.[33,63–67] A limited number of transplanted ESC-CMs survive, proliferate and mature *in vivo*. Several weeks after transplantation, ESC-CMs form desmosomes and gap-junctions and on the midterm cardiac function as measured by ejection fraction improves. These results are promising, but there are some setbacks. ESC-CMs have an im-

mature electrophysiological phenotype, there are still hurdles regarding cell integration and coupling, and functional cardiac improvement in the long term is not the result of cardiomyocytes specifically.[68] As described previously, the use of allogenous pluripotent stem cells also involves the risk of immunological rejection and teratoma formation. Finally, ESC use involves ethical and legal issues. So far, no clinical studies using ESC-CMs have been performed.

3.1.2 Induced pluripotent stem cells

As described above, pluripotent stem cells can differentiate into somatic cells. In 2006, Takahashi *et al.*[69] first showed that this process can be reversed *in vitro*. Fibroblasts were transduced with retroviral vectors (Oct 3/4, Sox2, Klf4, and c-Myc) which converted them to a cell type that is highly similar to the ESC, called induced pluripotent stem cell (iPSC).[69] Thus iPSCs have typical ESC markers, proliferate indefinitely, and are able to differentiate in somatic cells of all three embryonic cell lineages, including cardiomyocytes.[70,71] An advantage of iPSCs is that ethical issues of ES cells are irrelevant and that autologous cells can be created, preventing immunological rejection. A preclinical study performed in 2009 using iPS cell-derived cardiomyocytes reported promising beneficial effects on cardiac function.[72] However, there are issues that require further attention. The use of viral vectors introduces new potential side effects, as they can induce inflammation, cause cell rejection and, in rare cases, lead to a fatal systemic immune response.[73] Secondly, insertion of viral genomes at unwanted locations can disturb cellular function and cause oncogenic changes.[74] However, iPS generation methods are rapidly improving and recently it was shown that iPS cells can be created using non-integrating viruses and even without the use of viral vectors.[75,76] Finally, similar to ESC-CMs, there are still challenges regarding cell maturation, integration and coupling, functional cardiac improvement in the long term, and the risk of teratoma formation.

Recently, novel methods were developed to directly reprogram somatic cells into cardiomyocytes.[77–79] Using cardiac transcription factors (Gata4, Mef2c, Tbx5, with or without Hand2) fibroblasts were reprogrammed in cells that contracted spontaneously, had cardiac-specific markers and showed gene expression profiles comparable to adult cardiomyocytes. Using this technique cardiomyocytes can be created *in vitro,* but *in vivo* direct reprogramming of cardiac fibroblasts into CMs is also possible.[80] In direct reprogramming, the creation of pluripotent cells is bypassed, which reduces the risk of teratoma formation. Side effects related to viral vectors, however, are still possible with the currently available methods.

3.2 Multipotent stem cells

3.2.1 Bone marrow cells

Bone marrow-derived cells (BMCs) are stem cells that can be aspirated from the patient's bone marrow and consist of several stem cell types, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial stem/progenitor cells (EPCs).[81] They are different from previously discussed stem cell types, as they are transplanted directly without cardiomyocyte differentiation *in vitro* before transplantation. Some claim that BMCs have the ability to differentiate into non-hematopoietic cell types, such as the cardiac cells.[82,83] Others believe that stem cells found in adult organs, such as BMCs, are not truly pluripotent, but are restricted in their differentiative potential and that cardiomyocytes cannot originate from BMCs.[84–86] Therefore, BMCs are not considered candidates for "true" regeneration, but for the paracrine effects they might effectuate. At this moment, BMCs are the most widely used cell source for cardiac repair in clinical trials. Studies in animals and humans proved that transplantation is safe.[87–89] A recent meta-analysis concluded that transplantation of BMCs on average improves LV function, infarct size, and remodeling, and suggests effects on clinical end points, such as mortality and morbidity.[90] Nevertheless, most studies are small, have a relatively short follow-up period, show heterogeneous results, and some of the more recent conducted trials show no effects of BMCs.[91–93] Powered trials with long-term and patient centered outcomes are underway, but it also seems essential to clarify the mechanisms and use these to enhance the beneficial effects of BMC therapy.[94]

3.2.2 Mesenchymal stem cells (MSCs)

MSCs are multipotent stem cells that can be derived from several mammalian tissues, such as bone marrow, adipose tissue, and cord blood. In normal physiology, MSCs participate in organ homeostasis, wound healing, and successful aging.[95] They are easily isolated and cultured, do not have side-effects related to pluripotency and the use of viral vectors, are immunologically tolerated as allogeneic transplant, and have the potential to differentiate into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma.[96] Like BMCs, MSCs are generally not differentiated into cardiomyocytes before transplantation, although efforts have been undertaken to turn MSC into cardiac cells using a combination of growth factors.[97,98] Animal studies showed the ability of transplanted MSCs to engraft in the myocardium and to secrete paracrine factors.[99,100] Some even suggested that unmodified MSCs might differentiate into vascular cells and cardiomyocytes.[101–103] Generally, however, it is thought that the effects

of MSCs are not primarily based on trans-differentiation into cardiac cells, but on the secretion of paracrine factors by MSCs.[104] As such, true regeneration from unmodified MSC derived cardiomyocytes is not to be expected. Nevertheless, animal studies reported beneficial effects of MSC treatment on several outcome parameters, using multiple ischemia models, different kinds of MSC administration, directly and up to 4 weeks after cardiac injury.[104] Results of clinical trials using MSCs also show beneficial effects on cardiac function, although results are still preliminary and the question remains whether they will hold true for the long term.[29] Further clinical studies will determine whether the beneficial paracrine effects of MSCs found in animal models are reproducible in humans.

3.2.3 Cardiac stem cells (CSCs)

For a long time, it was believed that cardiac tissue is terminally differentiated. Recently, however, resident CSCs, progenitor cells in the cardiac lineage, were found in cardiac tissue. CSCs can be isolated from fetal and adult cardiac biopsies based on expression of stem cell marker proteins, such as lsl-1, c-kit, and Sca-1. They form cell lines that can be expanded in culture whilst keeping their progenitor state. Subsequently, they can differentiate in cardiovascular cell types, such as cardiomyocytes, endothelial cells, and smooth muscle cells.[105–107] After MI, cardiac stem cells and newly formed myocytes are found in the border area of the infarct, suggesting that the heart has some, although insufficient, regeneration capacity.[12] Transplantation of CSCs aims to use and enlarge the heart's own regeneration capacity. CSCs have the advantage that they can differentiate in cardiovascular cell types *in vivo* without the need of pre-implantation *in vitro* differentiation. Recently, results from the first phase 1 clinical trials using CSCs infusion were published.[108,109] Intracoronary autologous CSC injection appears to be safe and preliminary results showed an increase in viable myocardium, left ventricular ejection fraction, and other clinical parameters, although results differ between trials. Larger randomized, blinded trials with appropriate controls will have to be performed to fully analyze the clinical effect of CSC injection.

4   Clinical applicability

Results of pre-clinical studies using SCD-CMs for cardiac regeneration seem very promising. Animal studies show survival, maturation, integration, and sometimes functional coupling of SCD-CMs, and improvement of various cardiac functions, such as ejection fraction, after transplantation. However, there are reasons to be cautious.

Animal models used in preclinical studies do not always translate to human physiology. In a meta-analysis of 76 positive animal studies published in leading scientific journals, only 28 studies were replicated in humans. Of these 28 studies, only 8 therapies were subsequently approved for use in patients.[110]

In 2004, the NHLBI working group on the translation of therapies for protecting the heart from ischemia investigated the lack of translation of promising animal experiments into clinical practice. They concluded that numerous factors, including the use of imperfect animal models, the lack of reproducibility, standardized research protocols, randomized study design, and blinding of investigators resulted in unsuccessful translational research.[111]

In cardiac regeneration therapy specifically, ischemia in animal models is mostly achieved by coronary ligation in a healthy heart. On the contrary, cardiovascular diseases for which cardiac regeneration might be useful, such as heart failure and atherosclerosis that usually underlies a myocardial infarction, are chronic diseases in which the entire heart is affected. Non-ischemic cardiomyopathy represents yet another challenge for translational medicine as the disease seems to be based on entirely different, though often unknown, pathological mechanisms. Nevertheless, just as with ischemic cardiomyopathy, non-ischemic cardiomyopathy is characterized by a loss of functional cardiomyocytes and therefore, in theory amenable to stem cell-based repair. Pre-clinical studies using this model are scarce, although one clinical trial using BMCs has recently been reported.[112–114] Moreover, immunodeficient animal models allow transplanted SCD-CMs to survive, but skips the important step of immunotolerance which is necessary for translation to clinical applicability of allogeneic cells.

Most positive results are based on an increase in left ventricular ejection fraction, whereas results of cardiac regeneration therapy on patient-centred outcomes such as mortality and morbidity are absent. This requires longer follow-ups.

The follow-up period in pre-clinical regeneration studies is limited to a few months maximum and the few long-term studies that were published show less positive results.[115,116] This finding suggests that currently the results of cardiac regeneration are not predominantly attributable to structural integration of functional cardiomyocytes, but to temporary paracrine release of anti-apoptotic, immunemodulatory, and proangiogenic factors by SCD-CMs and SCD-CSCs. Although these and other paracrine effects of SCD-CMs are promising and clinical studies aiming to use these effects are currently being performed, the ultimate goal of regenerative medicine is to use stem cells to create healthy, func-

tional cardiac cells that are able to integrate in the injured heart and restore its function. Paracrine effects might reduce damage after cardiovascular disease such as MI, but are unlikely to repair the already damaged heart.

In order to clinically use stem cells for cardiac repair, more fundamental and translational research is necessary. Fundamental *in vitro* research, preferably using human cells (e.g., iPSC-CMs) will improve our knowledge of the mechanisms and requirements regarding differentiation of stem cells into functional cardiomyocytes, whereas translational research using appropriate animal models, long-term follow-up and relevant outcome measures, will hopefully result in clinically applicable cardiac repair.

5 Conclusion

Regenerative therapy using stem cells is a promising, relatively new modality for cardiac repair. Several stem cell types have been identified and investigated in pre-clinical and clinical research, generally with positive results. However, the degree of success has been variable and attributed to paracrine effects. Questions about optimal cell type, mode of delivery, time of delivery, integration in the heart, and safety have to be answered before true regeneration of cardiac tissue in patients will be possible. The field of regenerative medicine is rapidly developing and the first clinical studies are already being performed. In the meantime, many important fundamental questions are being addressed in pre-clinical research.

6 Acknowledgements

References

1   Lenhoff S, Lenhoff H. *Hydra and the birth of experimental biology, 1744: Abraham Trembley's Memoirs concerning the natural history of a type of freshwater polyp with arms shaped like horns*; Boxwood Press: Pacific Grove, California, USA, 1986.

2   Spallanzani L. *Prodromo di un opera da imprimersi sopra la riproduzioni animali (An essay on animal reproduction)*; T. Becket & de Hondt: London, UK, 1769.

3   Higgins G, Anderson R. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* 1933; 12: 186–202.

4   Schram G, Pourrier M, Melnyk P, *et al*. Differential distribution of cardiac ion channel expression as a basis for regional specialization in electrical function. *Circ Res* 2002; 90: 939–950.

5   Chien KR, Domian IJ, Parker KK. Cardiogenesis and the complex biology of regenerative cardiovascular medicine. *Science* 2008; 322: 1494–1497.

6   Gherghiceanu M, Popescu LM. Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. *J Cell Mol Med* 2010; 14: 871–877.

7   He JQ, Ma Y, Lee Y, *et al*. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res* 2003; 93: 32–39.

8   Wong SS, Bernstein HS. Cardiac regeneration using human embryonic stem cells: producing cells for future therapy. *Regen Med* 2010; 5: 763–775.

9   Dierickx P, Doevendans PA, Geijsen N, *et al*. Embryonic Template-Based Generation and Purification of Pluripotent Stem Cell-Derived Cardiomyocytes for Heart Repair. *J Cardiovasc Transl Res* 2012; 5: 566–580.

10   Segers VF, Lee RT. Stem-cell therapy for cardiac disease. *Nature* 2008; 451: 937–942.

11   Segers VF, Lee RT. Biomaterials to enhance stem cell function in the heart. *Circ Res* 2011; 109: 910–922.

12   Hsieh PC, Segers VF, Davis ME, *et al*. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med* 2007; 13: 970–974.

13   Rodrigo SF, van Ramshorst J, Beeres SL, *et al*. Intramyocardial injection of bone marrow mononuclear cells in chronic myocardial ischemia patients after previous placebo injection improves myocardial perfusion and anginal symptoms: an intra-patient comparison. *Am Heart J* 2012; 164: 771–778.

14   Madonna R, Geng YJ, De Caterina R. Adipose tissue-derived stem cells: characterization and potential for cardiovascular repair. *Arterioscler Thromb Vasc Biol* 2009; 29: 1723–1729.

15   Houtgraaf JH, Dejong R, Monkhorst K, *et al*. Feasibility of intracoronary GLP-1 eluting CellBead infusion in acute myocardial infarction. *Cell Transplant* 2012; 22: 535–543.

16   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.

17   van Laake LW, van Donselaar EG, Monshouwer-Kloots J, *et al*. Extracellular matrix formation after transplantation of human embryonic stem cell-derived cardiomyocytes. *Cell Mol Life Sci* 2010; 67: 277–290.

18   Landa N, Miller L, Feinberg MS, *et al*. Effect of injectable

alginate implant on cardiac remodeling and function after recent and old infarcts in rat. *Circulation* 2008; 117: 1388–1396.

19 Leor J, Tuvia S, Guetta V, *et al*. Intracoronary injection of in situ forming alginate hydrogel reverses left ventricular remodeling after myocardial infarction in Swine. *J Am Coll Cardiol* 2009; 54: 1014–1023.

20 Dobner S, Bezuidenhout D, Govender P, *et al*. A synthetic non-degradable polyethylene glycol hydrogel retards adverse post-infarct left ventricular remodeling. *J Card Fail* 2009; 15: 629–636.

21 Kofidis T, Lebl DR, Martinez EC, *et al*. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. *Circulation* 2005; 112: I173–I177.

22 Frederick JR, Fitzpatrick JR, 3rd, McCormick RC, *et al*. Stromal cell-derived factor-1alpha activation of tissue-engineered endothelial progenitor cell matrix enhances ventricular function after myocardial infarction by inducing neovasculogenesis. *Circulation* 2010; 122: S107–S117.

23 Danoviz ME, Nakamuta JS, Marques FL, *et al*. Rat adipose tissue-derived stem cells transplantation attenuates cardiac dysfunction post infarction and biopolymers enhance cell retention. *PloS One* 2010; 5: e12077.

24 Lin YD, Yeh ML, Yang YJ, *et al*. Intramyocardial peptide nanofiber injection improves postinfarction ventricular remodeling and efficacy of bone marrow cell therapy in pigs. *Circulation* 2010; 122: S132–S141.

25 Segers VF, Lee RT. Local delivery of proteins and the use of self-assembling peptides. *Drug Discov Today* 2007; 12: 561–568.

26 Stephan MT, Moon JJ, Um SH, *et al*. Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nat Med* 2010; 16: 1035–1041.

27 Penn MS, Mangi AA. Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circ Res* 2008; 102: 1471–1482.

28 Baker M. RNA interference: Homing in on delivery. *Nature* 2010; 464: 1225–1228.

29 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.

30 Iyer RK, Chiu LL, Reis LA, *et al*. Engineered cardiac tissues. *Curr Opin Biotechnol* 2011; 22: 706–714.

31 Radisic M, Park H, Martens TP, *et al*. Pre-treatment of synthetic elastomeric scaffolds by cardiac fibroblasts improves engineered heart tissue. *J Biomed Mater Res A* 2008; 86: 713–724.

32 Sekine H, Shimizu T, Hobo K, *et al*. Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. *Circulation* 2008; 118: S145- S152.

33 van Laake LW, Passier R, den Ouden K, *et al*. Improvement of mouse cardiac function by hESC-derived cardiomyocytes correlates with vascularity but not graft size. *Stem Cell Res* 2009; 3: 106–112.

34 Saif J, Schwarz TM, Chau DY, *et al*. Combination of injectable multiple growth factor-releasing scaffolds and cell therapy as an advanced modality to enhance tissue neovascularization. *Arterioscler Thromb Vasc Biol* 2010; 30: 1897–1904.

35 Jay SM, Shepherd BR, Andrejecsk JW, *et al*. Dual delivery of VEGF and MCP-1 to support endothelial cell transplantation for therapeutic vascularization. *Biomaterials* 2010; 31: 3054–3062.

36 Madden LR, Mortisen DJ, Sussman EM, *et al*. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. *Proc Natl Acad Sci USA* 2010; 107: 15211–15216.

37 Bradley JA, Bolton EM, Pedersen RA. Stem cell medicine encounters the immune system. *Nat Rev Immunol* 2002; 2: 859–871.

38 Zimmermann A, Preynat-Seauve O, Tiercy JM, *et al*. Haplotype-based banking of human pluripotent stem cells for transplantation: potential and limitations. *Stem Cells Dev* 2012; 21: 2364–2373.

39 Zhao T, Zhang ZN, Rong Z, *et al*. Immunogenicity of induced pluripotent stem cells. *Nature* 2011; 474: 212–215.

40 Lui KO, Waldmann H, Fairchild PJ. Embryonic stem cells: overcoming the immunological barriers to cell replacement therapy. *Curr Stem Cell Res Ther* 2009; 4: 70–80.

41 Zijlstra M, Bix M, Simister NE, *et al*. Beta 2-microglobulin deficient mice lack CD4-8+ cytolytic T cells. *Nature* 1990; 344: 742–746.

42 Zuidema MY, Zhang C. Ischemia/reperfusion injury: The role of immune cells. *World J Cardiol* 2010; 2: 325–332.

43 Vilahur G, Juan-Babot O, Pena E, *et al*. Molecular and cellular mechanisms involved in cardiac remodeling after acute myocardial infarction. *J Mol Cell Cardiol* 2011; 50: 522–533.

44 Timmers L, Pasterkamp G, de Hoog VC, *et al*. The innate immune response in reperfused myocardium. *Cardiovasc Res* 2012; 94: 276–283.

45 Di Nicola M, Carlo-Stella C, Magni M, *et al*. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; 99: 3838–3843.

46 Kooreman NG, Wu JC. Tumorigenicity of pluripotent stem cells: biological insights from molecular imaging. *J R Soc Interface* 2010; 7 (Suppl 6): S753-S763.

47 Blum B, Bar-Nur O, Golan-Lev T, *et al*. The anti-apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. *Nat Biotechnol* 2009; 27: 281–287.

48   Amariglio N, Hirshberg A, Scheithauer BW, *et al*. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med* 2009; 6: e1000029.

49   Cao F, Drukker M, Lin S, *et al*. Molecular imaging of embryonic stem cell misbehavior and suicide gene ablation. *Cloning Stem Cells* 2007; 9: 107–117.

50   Cao F, Li Z, Lee A, *et al*. Noninvasive de novo imaging of human embryonic stem cell-derived teratoma formation. *Cancer Res* 2009; 69: 2709–2713.

51   Menasche P. Stem cell therapy for heart failure: are arrhythmias a real safety concern? *Circulation* 2009; 119: 2735–2740.

52   Leobon B, Garcin I, Menasche P, *et al*. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A* 2003; 100: 7808–7811.

53   Makkar RR, Lill M, Chen PS. Stem cell therapy for myocardial repair: is it arrhythmogenic? *J Am Coll Cardiol* 2003; 42: 2070–2072.

54   Rubart M, Soonpaa MH, Nakajima H, *et al*. Spontaneous and evoked intracellular calcium transients in donor-derived myocytes following intracardiac myoblast transplantation. *J Clin Invest* 2004; 114: 775–783.

55   Mills WR, Mal N, Kiedrowski MJ, *et al*. Stem cell therapy enhances electrical viability in myocardial infarction. *J Mol Cell Cardiol* 2007; 42: 304–314.

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

57   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.

58   Shiba Y, Fernandes S, Zhu WZ, *et al*. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* 2012; 489: 322–325.

59   Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; 292: 154–156.

60   Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al*. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282: 1145–1147.

61   Shiba Y, Hauch KD, Laflamme MA. Cardiac applications for human pluripotent stem cells. *Curr Pharm Des* 2009; 15: 2791–2806.

62   Mummery C, Ward-van Oostwaard D, Doevendans P, *et al*. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* 2003; 107: 2733–2740.

63   Caspi O, Huber I, Kehat I, *et al*. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* 2007; 50: 1884–1893.

64   Xue T, Cho HC, Akar FG, *et al*. Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers. *Circulation* 2005; 111: 11–20.

65   van Laake LW, Passier R, Doevendans PA, *et al*. Human embryonic stem cell-derived cardiomyocytes and cardiac repair in rodents. *Circ Res* 2008; 102: 1008–1010.

66   van Laake LW, Passier R, Monshouwer-Kloots J, *et al*. Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. *Stem Cell Res* 2007; 1: 9–24.

67   Laflamme MA, Chen KY, Naumova AV, *et al*. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007; 25: 1015–1024.

68   Jonsson MK, Vos MA, Mirams GR, *et al*. Application of human stem cell-derived cardiomyocytes in safety pharmacology requires caution beyond hERG. *J Mol Cell Cardiol* 2012; 52: 998–1008.

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

70   Zhang J, Wilson GF, Soerens AG, *et al*. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104: e30-e41.

71   van Laake LW, Qian L, Cheng P, *et al*. Reporter-based isolation of induced pluripotent stem cell and embryonic stem cell-derived cardiac progenitors reveals limited gene expression variance. *Circ Res* 2010; 107: 340–347.

72   Nelson TJ, Martinez-Fernandez A, Yamada S, *et al*. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation* 2009; 120: 408–416.

73   Raper SE, Chirmule N, Lee FS, *et al*. Fatal systemic inflammatory response syndrome in an ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 2003; 80: 148–158.

74   Thrasher AJ, Gaspar HB, Baum C, *et al*. Gene therapy: X-SCID transgene leukaemogenicity. *Nature* 2006; 443: E5-E6; discussion E-7.

75   Stadtfeld M, Nagaya M, Utikal J, *et al*. Induced pluripotent stem cells generated without viral integration. *Science* 2008; 322: 945–949.

76   Zhou H, Wu S, Joo JY, *et al*. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009; 4: 381–384.

77 Ieda M, Fu JD, Delgado-Olguin P, *et al*. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010; 142: 375–386.

78 Efe JA, Hilcove S, Kim J, *et al*. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. *Nat Cell Biol* 2011; 13: 215–222.

79 Song K, Nam YJ, Luo X, *et al*. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* 2012; 485: 599–604.

80 Qian L, Huang Y, Spencer CI, *et al*. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 2012; 485: 593–598.

81 Dawn B, Bolli R. Adult bone marrow-derived cells: regenerative potential, plasticity, and tissue commitment. *Basic Res Cardiol* 2005; 100: 494–503.

82 Orlic D, Kajstura J, Chimenti S, *et al*. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; 410: 701–705.

83 Kucia M, Dawn B, Hunt G, *et al*. Cells expressing early cardiac markers reside in the bone marrow and are mobilized into the peripheral blood after myocardial infarction. *Circ Res* 2004; 95: 1191–1199.

84 Wagers AJ, Sherwood RI, Christensen JL, *et al*. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 2002; 297: 2256–2259.

85 Chien KR. Stem cells: lost in translation. *Nature* 2004; 428: 607–608.

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

87 Yousef M, Schannwell CM, Kostering M, *et al*. The BALANCE Study: clinical benefit and long-term outcome after intracoronary autologous bone marrow cell transplantation in patients with acute myocardial infarction. *J Am Coll Cardiol* 2009; 53: 2262–2269.

88 Martin-Rendon E, Brunskill SJ, Hyde CJ, *et al*. Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *Eur Heart J* 2008; 29: 1807–1818.

89 Price MJ, Chou CC, Frantzen M, *et al*. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. *Int J Cardiol* 2006; 111: 231–239.

90 Jeevanantham V, Butler M, Saad A, *et al*. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. *Circulation* 2012; 126: 551–568.

91 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 randomised, controlled study. *Heart* 2009; 95: 1983–1989.

92 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* 2009; 30: 2978–2984.

93 Traverse JH, Henry TD, Pepine CJ, *et al*. Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. *JAMA* 2012; 308: 2380–2389.

94 BAMI trial. http://clinicaltrials.gov/show/NCT01569178 (accessed Jan 22, 2013).

95 Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. *Circ Res* 2011; 109: 923–940.

96 Pittenger MF, Mackay AM, Beck SC, *et al*. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143–147.

97 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.

98 C-Cure Trial. http://clinicaltrials.gov/ct2/show/NCT00810238 (accessed Jan 22, 2013).

99 Quevedo HC, Hatzistergos KE, Oskouei BN, *et al*. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci USA* 2009; 106: 14022–14027.

100 Gnecchi M, Zhang Z, Ni A, *et al*. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res* 2008; 103: 1204–1219.

101 Toma C, Pittenger MF, Cahill KS, *et al*. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002; 105: 93–98.

102 Wang T, Xu Z, Jiang W, *et al*. Cell-to-cell contact induces mesenchymal stem cell to differentiate into cardiomyocyte and smooth muscle cell. *Int J Cardiol* 2006; 109: 74–81.

103 Xu W, Zhang X, Qian H, *et al*. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Exp Biol Med* (*Maywood*) 2004; 229: 623–631.

104 Gnecchi M, Danieli P, Cervio E. Mesenchymal stem cell therapy for heart disease. *Vascul Pharmacol* 2012; 57: 48–55.

105 Goumans MJ, de Boer TP, Smits AM, *et al*. TGF-beta1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes in vitro. *Stem Cell Res* 2007; 1: 138–149.

106 Smits AM, van Laake LW, den Ouden K, *et al*. Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. *Cardiovasc Res* 2009; 83: 527–535.

107 Noort WA, Sluijter JP, Goumans MJ, *et al*. Stem cells from in- or outside of the heart: isolation, characterization, and

potential for myocardial tissue regeneration. *Pediatr Cardiol* 2009; 30: 699–709.

108 Bolli R, Chugh AR, D'Amario D, *et al*. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet* 2011; 378: 1847–1857.

109 Makkar RR, Smith RR, Cheng K, *et al*. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet* 2012; 379: 895–904.

110 Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. *JAMA* 2006; 296: 1731–1732.

111 Bolli R, Becker L, Gross G, *et al*. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res* 2004; 95: 125–134.

112 Dhein S, Garbade J, Rouabah D, *et al*. Effects of autologous bone marrow stem cell transplantation on beta-adrenoceptor density and electrical activation pattern in a rabbit model of non-ischemic heart failure. *J Cardiothorac Surg* 2006; 1: 17.

113 Nair M, Mukherjee S, Ahluwalia NS, *et al*. Feasibility and safety of autologous intracoronary stem cell transplantation in patients of non ischemic dilated cardiomyopathy. *Indian Heart J* 2007; 59: 511–514.

114 Vrtovec B, Poglajen G, Lezaic L, *et al*. Effects of Intracoronary CD34+ stem cell transplantation in nonischemic dilated cardiomyopathy patients: 5-year follow-up. *Circ Res* 2013; 112: 165–173.

115 Passier R, van Laake LW, Mummery CL. Stem-cell-based therapy and lessons from the heart. *Nature* 2008; 453: 322–329.

116 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.