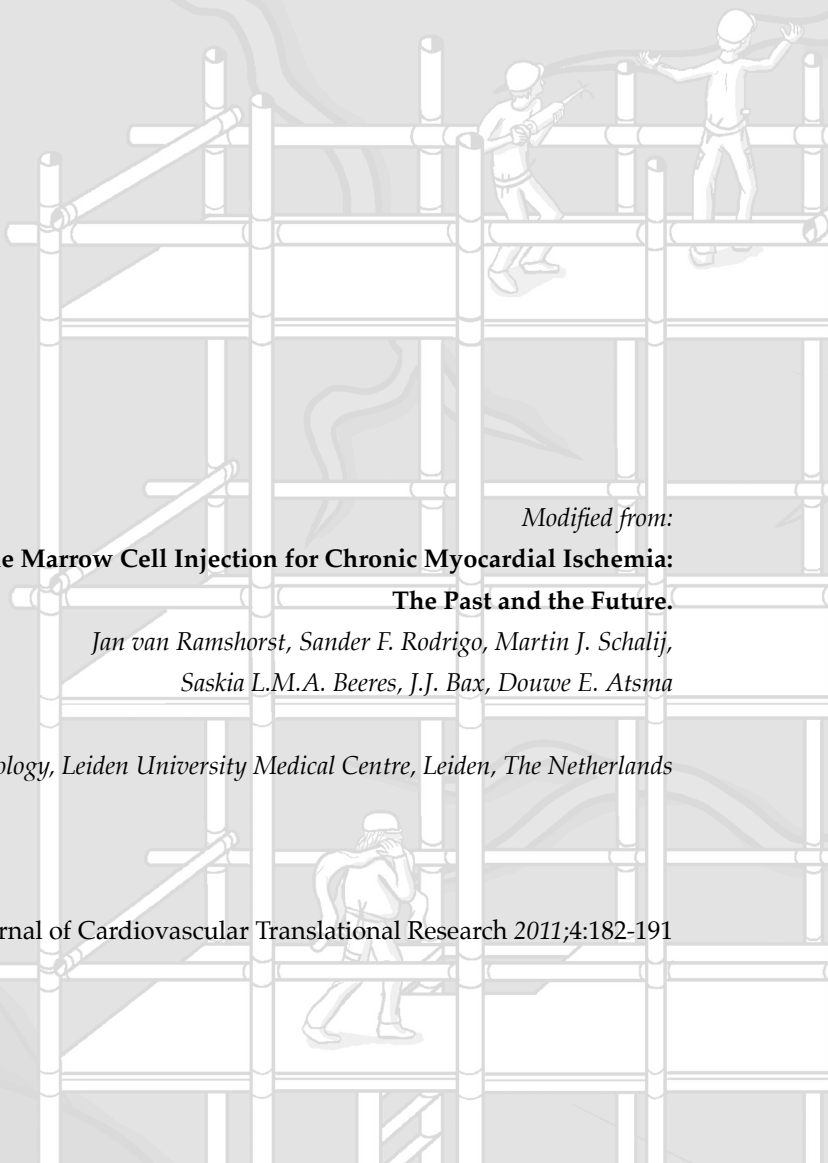
Chapter 1

General Introduction



Modified from:

Bone Marrow Cell Injection for Chronic Myocardial Ischemia:
The Past and the Future.

Jan van Ramshorst, Sander F. Rodrigo, Martin J. Schalij,
Saskia L.M.A. Beeres, J.J. Bax, Douwe E. Atsma

Department of Cardiology, Leiden University Medical Centre, Leiden, The Netherlands

Journal of Cardiovascular Translational Research *2011*;4:182-191

INTRODUCTION

Coronary artery disease is a major cause of mortality and morbidity in the western world. Despite major advances in surgical and percutaneous revascularization techniques, a large number of patients end up with end-stage coronary artery disease, not amenable for mechanical revascularization. These patients often have stress-inducible myocardial ischemia, resulting in disabling complaints of angina, refractory to medical treatment[1]. Furthermore, ischemic myocardial damage can result in chronic heart failure, due to reduced left ventricular function and subsequent remodeling of the left ventricle (LV).
Cell-based therapy is currently under investigation as a new therapeutic option to restore ischemically damaged myocardium and increase neovascularization. In numerous preclinical studies, it has been reported that various cell types have the capacity to promote cardiomyogenesis and new blood vessel formation through different mechanisms, resulting in improvements in cardiac function. On the basis of these encouraging findings, a large number of clinical studies have been performed in the last decade, generally demonstrating modest but significant clinical benefits. However, a large variability exists in the observed beneficial effects of cell therapy, which is likely to be related to differences in study design, patient population and cell characteristics. Therefore, many questions remain unanswered regarding the effect of cell-based therapy on damaged myocardium, including the exact mechanism of action, the optimal delivery method, and cell type and dose in various patient populations.
As an introduction to this thesis, an overview of the current status of cardiac cell therapy will be provided. First, cell types available for cardiac cell therapy will be described, along with the mechanisms through which these cells may improve myocardial perfusion and function. Furthermore, the different routes of cell delivery will be discussed and compared. Finally, the available experience from of experimental and clinical studies investigating cell therapy in patients with ischemic heart disease will be reviewed.

CELL TYPES FOR CARDIAC REPAIR

In vitro studies have been performed on various cell types to evaluate their potential to restore damaged myocardium. Below we discuss the origin of these cells, the proposed mechanism of action and potential safety concerns of each cell type. (Figure 1)

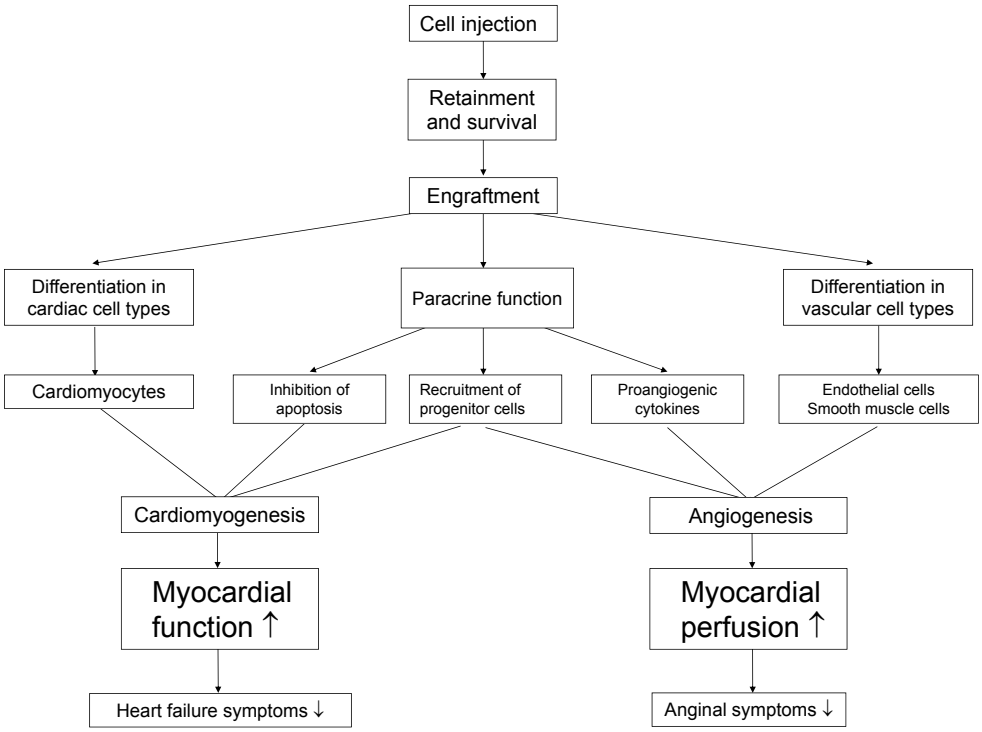


Figure 1: Mechanisms by which bone marrow cells may improve myocardial perfusion and contractile function.

HEMATOPOETIC STEM CELLS

Hematopoetic stem cells (HSCs) can be isolated from the bone marrow, and comprise 1 to 3% of the total mononuclear cell fraction. In peripheral blood, low numbers of circulating HSCs are detectable, although this may be augmented by granulocyte colony-stimulating factor (G-CSF) administration, resulting in mobilization of HSCs as well as other bone marrow stem and progenitor cells into the blood[2]. HSCs are commonly identified by the expression of CD34+ and CD133+ cell surface antigens and have the potential to differentiate into all types of blood cells.

In a landmark study by Orlic et al.[3], it was suggested that HSC were capable of replacing infarcted myocardium after differentiation into cardiomyocytes. However, since experimental studies yielded discordant results with regard to the presence of de novo myocardium formation[4-6], the ability of HSCs to differentiate into cardiac cells remains controversial. Nonetheless, in most randomized and non-randomized studies, transplantation of hematopoietic cells in ischemic myocardium resulted in beneficial

effects on cardiac function[4, 7-11]. Paracrine effects may account for these functional benefits, given that experimental studies demonstrated that CD34+ cells can secrete cytokines that may stimulate angiogenesis, inhibit apoptosis, recruit resident cardiac progenitor cells and change extracellular matrix composition[12, 13]. However, because the expression of CD34+ markers is overlapping with other hematopoietic cell types such as endothelial progenitor cells, the exact role of HSCs in myocardial improvement is still not fully determined.

ENDOTHELIAL PROGENITOR CELLS

Endothelial progenitor cells (EPCs) reside in the bone marrow, comprising 0.1 to 0.4% of mononuclear cells[14], and are detectable in peripheral blood in very low concentrations[15]. Classically, these cells are described as committed progenitor cells that can only give rise to endothelial cells and other vascular cell types (e.g. pericytes, smooth muscle cells, fibroblasts)[16]. In addition, EPCs possess the capacity to contribute to new vessel formation by secretion of pro-angiogenic cytokines[16]. Conditions such as cardiac ischemia or acute myocardial infarction (MI) may initiate mobilization of EPCs from the bone marrow into the blood where they can migrate to the site of injury[17].

EPCs were originally identified by their expression of the hematopoetic stem cell markers CD34, CD133 and VEGF receptor[18-20]. The cells are characterized by a high proliferative capacity, and have been demonstrated to incorporate into foci of neovascularization[21] and to differentiate into endothelial cells, and thus may be regarded as circulating angioblasts. However, the precise phenotype and nomenclature of EPC has been subject of debate[16] since other EPC populations have been identified in cultured EPCs such as 'early outgrowth EPCs', which express the monocyte marker CD14 in the absence of hematopoetic markers CD34 or CD45[22]. These cells possess a relatively low proliferative capacity and are thought to contribute to the process of angiogenesis predominantly by paracrine mechanisms.

Numerous observational clinical studies have documented that EPC numbers and function are related to the presence of cardiovascular disease. In patients with established cardiovascular disease, lower numbers and impaired function of circulating EPCs was documented[23]. Furthermore, the presence of cardiovascular risk factors was found to be related to circulating EPC numbers and endothelial function[24]. Moreover, reduced EPC levels were found to be an independent predictor of cardiovascular events in patients with coronary artery disease[15, 25], suggesting a relation between reduced levels of circulating

EPCs and atherosclerotic disease progression. Furthermore, the numbers and function of EPCs have shown improvement after life style alterations[26, 27] and pharmacological treatments such as statins[28-31]. Although the clinical usefulness of EPCs as therapeutic cell type may be influenced by their low numbers or decreased angiogenic potential in patients with cardiovascular disease, these cells are suggested to have potential for vascular regeneration.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) can be isolated from many tissues, including bone marrow, adipose tissue and umbilical cord blood. Although they are uncommon in bone marrow, making up only 0.001 to 0.01% of the total nucleated cells[32], MSCs can easily be expanded in vitro due to their extensive proliferative capacity. This cell type is characterized by expression of a specific set of membrane molecules (CD73, CD90, CD105), together with lack of expression of the hematopoietic markers CD14, CD34 and CD45 and human leucocyte antigen-DR. MSCs can differentiate into cells of the mesenchymal cell type, including osteoblasts, adipocytes and chondrocytes[33]. In addition, under specific in vitro conditions they can give rise to functional cardiomyocytes[34] and vascular cells[35]. Some experimental in vivo studies demonstrated differentiation of MSCs into cardiomyocyte-like phenotypes after intramyocardial injection[36, 37]. However, since other studies did not observe cardiomyogenic differentiation[35, 38, 39], the in vivo potential of MSCs to differentiate into cardiomyocytes remains unclear.

Still, improvements in cardiac function were observed in the majority of experimental studies[36, 38-40]. These functional improvements may be related to stimulation of angiogenesis, by differentiation of MSCs into endothelial cells and smooth muscle cells[35, 39] and secretion of pro-angiogenic cytokines[41]. Furthermore, MSCs may promote protection of ischemic tissue by production of a variety of growth factors and cytokines, which may beneficially affect post-infarct LV remodeling [41] In addition, MSCs are well amenable for enhancement of their therapeutic potential through pharmacological or genetic means. Overexpression of (prosurvival) genes such as Akt[42], GSK-3beta[43] and myocardin[44] augmented the ability of MSCs to restore cardiac function in acute MI models in an even higher degree as compared to non-transduced MSC treatment.

An unique characteristic of MSCs seems to be the interaction between these cells and the immune system. First, they are immunosuppressive to activated T-lymphocytes and can reduce inflammation by inhibiting T-cell proliferation without promoting

apoptosis[45, 46] In addition, allogeneic MSCs have been thought be capable of evading the host immune system since they express only very low levels of histocompatibility complex type II, making MSCs an attractive candidate for allogeneic cell use. However, induction of an immune response was described after administration of allogeneic MSCs in immunocompetent hosts[47, 48], although the clinical significance of this response for cardiac cell therapy remains unclear. Therefore, the clinical applicability of allogeneic MSC injection remains to be investigated.

Nonetheless, some safety issues have been raised with regard to MSC administration. Due to the heterogeneity among MSC and MSC-like populations and their broad differentiation capacity, administration of these cells carries the potential risk of unwanted differentiation of administrated cells. Although it is commonly assumed that the host tissue will direct the differentiation of transplanted cells, some studies observed osteogenic differentiation of implanted MSCs[49]. Of note, this observation was only made in 2 small studies comprising rodent animal models, and rodent-MSC. In both studies, extensive culturing had been performed (up to 11 passages). Although only scarce data are available, it is conceived that in human MSCs cultured for a normal duration (and even reaching 25 passages), the occurrence of transformation will be very unlikely [50]. Therefore, the risk of tumor formation after MSC transplantation in the clinical setting is considered to be very low [51].

Another safety concern arose from the study of Vulliet et al, which demonstrated the potential of MSCs to cause coronary obstruction and micro-infarction after intracoronary injection[52]. After injection of culture expanded canine MSCs in this canine MI model (about two-fold larger compared to MSCs used in human studies), microinfarcted regions containing high concentrations of injected MSC concentration were observed. In order to avoid this risk, clinical studies investigating MSC transplantation have focused on the intramyocardial transplantation method.

ADIPOSE TISSUE-DERIVED STEM CELLS

MSCs harvested from adipose tissue are referred to as adipose tissue-derived stem cells (ADSCs). They have shown to express surface markers similar to those observed on MSCs, though slight distinctions have been observed[53]. In contrast to bone marrow derived MSC, ADSCs can be harvested in large quantities from adipose tissue (on average 1 x 10^6 stem cells out of 100 ml) making extensive culturing unnecessary. ADSCs have the capacity of self-renewal and differentiation into various pluripotent endothelial

and vascular progenitor cells[54], comparable to MSCs. Although MSCs and ADSCs have a number of slight distinctions comprising surface phenotype and processes of cell homing, the consequences of these differences are not clear[53]. In vitro studies have shown that ADSCs have the capacity to develop into ventricle-like, atrial-like, and pacemaker-like cells displaying spontaneous action potentials, after 3 weeks of culturing[55]. Furthermore, ADSCs have been suggested to have the ability to engraft into injured myocardium and express specific cardiomyocyte markers[56]. In addition, ADSC transplantation in acute MI models resulted in significant improvements in left ventricular ejection fraction (LVEF), although no new cardiomyocyte formation was demonstrated [57, 58]. Since improved capillary density was observed, the beneficial effect was suggested to be attributable from vasculogenesis[57, 58]. Currently, the first-in-men study to explore safety and feasibility of ADSC transplantation in patients with AMI (APOLLO trial) is underway[53].

VERY SMALL EMBRYONIC-LIKE STEM CELLS

Recently, a novel population of rare multipotent cells (approximately 0.02% of the mononuclear cell population) has been described in adult bone marrow cells[59]. These cells, called very small embryonic-like stem cells (VSELs), are identified by Sca-1^{pos}/ Lin^{neg}/$CD45^{neg}$ and express the cardiac markers Nkx2.5/Csx, GATA-4, and MEF2C. In vitro, VSELs are able to differentiate into different cell types, including cell types of the cardiac and vascular lineages[59]. VSELs are mobilized into the peripheral blood after tissue ischemia[60] and home to the site of injury[61].

Intramyocardial injection of VSELs in mice models of MI resulted in improvement in cardiac function[62], which effect was further augmented by cardiogenic predifferentiation[63]. Isolated VSEL-derived cardiomyocytes and vascular cells were detected in the infarct region, although the number of VSEL-derived cells was too low to be responsible for the observed improvements. Therefore, it has been hypothesized that the secretion of paracrine factors by differentiating VSELs may plays an important role in improving myocardial perfusion and function, through comparable mechanisms as has been proposed for other cell types[64].

Because of their pluripotency, including the capacity to differentiate into cardiac myocytes, in combination with the suggestion of a substantial paracrine function, VSELs are attractive candidates for future therapeutic strategies. Nonetheless, although VSEL appear to be stable in vitro[65], limited in vivo data are available, requiring additional experimental studies to further explore the safety profile of this cell type.

(UNSELECTED) BONE MARROW-DERIVED MONONUCLEAR CELLS

Bone marrow mononuclear cells (BMNCs) represent a heterogeneous cell population containing hematopoietic and non-hematopoietic cells with diverse phenotypes. These cells include HSCs, EPCs, MSCs and various cell populations such as side population cells, multipotent adult progenitor cells, and VSELs[66]. Mononuclear cells can be isolated by direct marrow aspiration or can be obtained from the peripheral circulation. Because BMNCs are relatively easy to isolate in large numbers and do not require complex culture conditions, they have been used in the majority of clinical trials in cardiac patients. Apart from these practical considerations, BMNCs may have other advantages over selected cell types. Particularly, different cell populations may affect the function of each other, being more effective in combination as suggested by Suuronen et al[67].. In line with this suggestion, synergistic effects on neovascularisation were observed after transplanting different types of EPCs compared to administration of a single cell type[68]. Furthermore, after intramyocardial injection in a mouse MI model, BMNCs showed a more robust survival pattern as compared to MSC en skeletal myoblasts, resulting in reduced LV remodeling after MI[69]. Therefore, it has been suggested that the combination of mononuclear cells, as naturally present in the bone marrow, may be one of the most suitable and effective options for myocardial cell treatment. Combined with favorable safety profile, this cell population is suggested to be a qualified candidate for clinical application, although appropriate safety monitoring is still recommended and ongoing [70].

SKELETAL MYOBLAST CELLS

Skeletal myoblasts are tissue-committed progenitor cells which normally reside under the basal membrane of mature muscular fibers. These precursor cells are mobilized by injury, and have the capacity to regenerate muscle fibers by proliferation and fusion[71]. Skeletal myoblasts can be obtained by skeletal muscle biopsy and can be efficiently expanded in vitro, making them suitable for autologous application. Being committed precursor cells, skeletal myoblasts carry a low risk on ectopic differentiation. Another advantage of skeletal myoblasts is their resistance to ischemia and oxidative stress, facilitating survival in recently infarcted or poorly vascularized cardiac tissue[72].

Because of these appealing characteristics, skeletal myoblasts have been studied extensively as a potential source for cardiac cell therapy[73-76]. In animal models of MI, generation of functional multinucleated myotubes by the transplanted cells was

observed[77,78], often aligned parallel to the host cardiomyocytes. Moreover, administration of skeletal myoblasts into cryoinfarcted regions of rabbit hearts revealed formation of islands containing elongated striated cells that retained characteristics of both skeletal and cardiac cells[79]. However, the expression of cardiac markers is now recognized to be the result of fusion with host cardiomyocytes, and not from transdifferentiation[80]. Importantly, immunohistochemical results revealed a lack of connexin-43 expression, resulting in a lack of electrical coupling between engrafted myoblasts and the neighboring cardiomyocytes. This hampers synchronized mechanical activity, possibly attenuating the beneficial effect on myocardial function[81,82]. Moreover, this electric 'insulation' of transplanted cells is likely to create an arrhythmogenic substrate, increasing the risk of malignant arrhythmias[83]. Of note, the use of genetic modification to create myoblasts overexpressing connexin-43 may be a promising solution[84,85], although the precise effects of this approach on the differentiation and electrophysiological properties of the transplanted cells should be further explored[86].

In addition to their myogenic potential, skeletal myoblasts were also found to release paracrine factors that can stimulate angiogenesis[87], enhance cardiomyocyte survival[88] and decrease expression of matrix metalloproteinases[89], leading to reduced myocardial fibrosis. Therefore, skeletal myoblasts may contribute to a reduction in LV remodeling by paracrine mechanisms, which is in line with the results of the MAGIC trial that observed a decrease in LV end-diastolic and end-systolic volume which was not accompanied by an improvement in LV function[73].

The combination of myogenesis and significant paracrine function makes skeletal myoblasts interesting candidates for therapeutic purposes. However, further investigation is warranted to perform a comprehensive assessment of the safety profile and the potential benefits.

RESIDENT CARDIAC STEM CELLS

Traditionally, the heart was thought to be a postmitotic organ because mature cardiomyocytes withdraw from the cell cycle and cease to proliferate. Recent discovery of resident cardiac stem cells in the heart has demonstrated that, in contrast to long-standing belief, the heart has intrinsic regenerative potential. Cardiac stem cells consist of a heterogeneous cell population including several multipotent progenitor cells and adult cardiac stem cells, which can be differentiated by surface marker expression.

In 2003, Beltrami et al.[90] reported the identification of c-kit^{pos} cells negative for blood lineage markers (Lin^{neg}) in rat hearts, with the capacity to differentiate into cardiomyocytes, smooth muscle and endothelial cells. Transplantation of these cells in rodent and canine models of MI resulted in myocardial regeneration, with injected cells showing sarcomere formation and expression of N-cadherin and connexin-43. In addition, increased capillary density was observed[90, 91]. Recently, successful isolation and expansion of c-kit^{pos} cells from human myocardial biopsy specimens has been described, with comparable in vivo results after transplantation in a rat model. Despite these promising results, doubts have been raised with regard to the capacity of myocardial c-kit^{pos} cells from adult human hearts to undergo cardiomyogenic differentiation[92] and the availability of c-kit^{pos} cells in diseased human hearts[93].

Another type of cardiac stem cells has been identified based on the expression of stem cell antigen-1 (Sca-1)[94]. These cells were able to generate cardiomyocytes after treatment with oxybutine or 5-azacytidine[94, 95]. In experimental in vivo studies, these cells were retrieved in the infarct border zone and seemed to have differentiated into cardiomyocytes[96]. However, it has been suggested that these findings are mainly due to cell fusion with resident cardiac cells[94, 97].

An interesting population of multipotent progenitor cells which is present in the heart, but also in bone marrow, skin and muscle are side population cells. These cells are characterized by their cytoplasmatic exclusion of Hoechst dye[98] and have the capacity to generate functional cardiomyocytes in vitro[99]. Although residing in the heart in small amounts, side population cells can be mobilized from the bone marrow after acute MI. The therapeutic potential of side population cells remains to be determined, since no data are available on their clonogenic potential and capacity for self-renewal. Moreover, the ability of side population cells to improve myocardial perfusion and function in vivo is not yet clear.

A heterogeneous population of progenitor cells can be derived from subcultures of postnatal human myocardial biopsy specimens, the so called cardiosphere-derived cells (CDCs). These cells form multicellular, self-adherent spherical clusters (cardiospheres) in culture[100] and are self-renewing and clonogenic. CDCs express antigenic characteristics of stem cells at each stage of processing (expressing KDR in human, flk-1 in mice, CD31, CD34, c-Kit, Sca-1), as well as proteins vital for cardiac contractile and electrical function[101]. These cells have the ability to undergo cardiac differentiation with spontaneous contractile activity, and can also give rise to endothelial and smooth muscle

cells[100]. Experimental studies have demonstrated beneficial effects of CDC injection in porcine and murine models of MI[101-103]. Since survival of injected cells was low, these improvements may partially be attributed to paracrine effects, such as recruitment of resident progenitor cells or inhibition of apoptosis[101, 102, 104].

EPICARDIUM-DERIVED CELLS

A subset of epicardial cells, referred to as epicardium-derived cells (EPDCs), undergo epithelial-to-mesenchymal transition (EMT) during cardiomorphogenesis and thereby acquire the ability to migrate into the subepicardial space and subsequently into the myocardium. Human EPDCs can be identified by receptor expression of CD44, CD90, CD105, HLA-ABC and CD46 at the plasma membrane, but are negative for CD34 and Sca-1[105]. Furthermore, human EPDCs express cardiac marker genes (cardiac troponin T, GATA4, dHand, Mef2C and connexin 43) and smooth muscle genes (ASMA, CNN1, SM22)[105]. These cells have the capacity to differentiate into multiple cell types, including coronary smooth muscle cells, subendocardial and atrioventricular cushion mesenchymal cells, adventitial fibroblasts and interstitial cardiac fibroblasts[106-112]. In addition, it is suggested that, under certain in vitro conditions, EPDCs can differentiate into cardiomyocytes[113], although their in vivo potential to do so is still subject of debate[114, 115]. Following epicardial biopsy, these cells can be cultured with specific factors, e.g. TGF-ß[116], myocardin[105] or Tß4[117] to promote epicardial EMT and subsequent differentiation. In vivo experiments have shown that intramyocardial injection of human EPDCs into ischemic myocardium of a MI mouse model preserved cardiac function and attenuated ventricular remodeling[118]. In addition, co-injection of EPDCs and CPCs synergistically improved cardiac function in MI mice[119]. Since no graft-derived cardiomyocytes were observed in this study, this improvement was suggested to result from complementary paracrine mechanisms. In line with these findings, EPDCs have shown to provide paracrine factors to enhance vascular recruitment[117, 118] and demonstrated to directly interact with cardiomyocytes, thereby inducing proliferation and correct mechanical and electrical coupling of cardiomyocytes[120]. Because of their regulating role in myocardial development, their ability to interact with surrounding myocardium and their potential to improve cardiac performance, this cell type might be a promising source for cell-based therapy.

Cardiac tissue-derived stem and progenitor cells show promising abilities in terms of proliferation and differentiation capacity. Due to their cardiac origin these cells may hold great potential as a source for myocardial regeneration. However, since only limited experimental data are available on most cell types, further pre-clinical investigations are necessary to assess the safety and possible therapeutic benefit of these cell types. Furthermore, cardiac stem cells for autologous purposes can only be obtained via endomyocardial biopsy or thoracic surgery (for example during coronary artery bypass grafting) which may be regarded as a disadvantage as compared to other adult stem cells.

EMBRYONIC STEM CELLS

Human embryonic stem cells (hESCs) are conventionally derived from pluripotent cells from the inner mass of a 5 day old human embryo at the blastocyst stage and have the potency of unlimited proliferation in vitro[121, 122]. hESCs can give rise to cells of all three primary germ layers and will spontaneously differentiate to form multicellular aggregates when maintained in an undifferentiated state[123]. By culturing in specific growth media, differentiation into specified cell types can be established. Studies have shown that hESCs can differentiate into cardiomyocytes possessing functional and electrophysiological characteristics similar to genuine cardiomyocytes. For example, immunohistological studies demonstrated that hESC-derived cardiomyocytes express early cardiac-specific transcription factors, sarcomeric proteins and gap junction proteins, whereas electrophysiological assessment revealed these cells to resemble human fetal ventricular myocytes that can propagate action potentials[124, 125]. Of note, recently, a method was demonstrated for obtaining >99% pure cardiomyocytes from hESCs[126]. In addition to cardiomyocyte differentiation, hESCs have the capacity to differentiate into endothelial and smooth muscle cells[127].

Because of their pluripotency, ESCs can form any cell type of the heart, providing an extensive regeneration potential. The ability to form cells from all three germ layers, however, is accompanied by the risk of ectopic differentiation at the implantation site, as observed after ESC transplantation in healthy animals and animal models of acute MI[128, 129]. Subsequently, increased interest has risen in methods to guide differentiation preceding implantation[130] to reduce the risk of ectopic differentiation. Several strategies have been used to guide differentiation of ESCs into cardiomyocytes, including co-culturing with endoderm-like cells or their conditioned medium, addition of growth

factors and hormones, and pre-treatment of cells with reagents aimed at blocking cell death[131]. To preclude unwanted differentiation of administrated ESCs, it is essential to produce homogenous populations of each of the cardiovascular cell types and exclude residual undifferentiated cells[122].

Besides the risk of teratoma formation, other disadvantages may hamper the use of ESCs for cardiac regeneration. Since ESCs have demonstated to express specific human leukocyte antigen (HLA) subclasses, the potential risk of graft rejection might necessitate immunosuppression. Because steroid use is known to be harmful to ischemic myocardium, research to diminish the immunogenicity of the cells for allogeneic transplantation is ongoing[132]. Finally, considerable ethical concerns surrounding ESC procurement from viable human embryos is an important limitation for the development of hESCs into a clinically applicable treatment for cardiac regeneration.

INDUCED PLURIPOTENT STEM CELLS

A promising method for obtaining multipotent stem cells is direct reprogramming of adult fibroblasts by overexpression of a limited set of defined transcription factors. By virus-mediated overexpression of these transcription factors, human somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells[133], which are practically similar to ESCs. During reprogramming, the expression of introduced exogenous transcription factors declines whereas the endogenous pluripotency network is upregulated, therefore it is proposed that the exogenous transcription factors only initiate the reprogramming[122]. In vitro, iPS cells have shown to differentiate into cell types of all three embryonic germinal layers exhibiting the morphology, proliferation, gene expression, epigenetic status and differentiation potential similar to ESCs[134, 135]. Functional cardiomyocytes with nodal-, atrial-, or ventricular-like electrophysiological phenotype have been derived from human iPS cells, using methods based on those effective for hESCs[136]. In addition, iPS cells have shown to be capable of differentiation into vascular progenitor cells and vascular cells[137]. Of note, the efficiency of cell reprogramming as well as the differentiation capacity of the cell is suggested to be influenced by the choice of cell origin used for reprogramming[122]. In addition to the strong differentiation potential of iPS cells, which is possibly approaching the capacity of ESC-derived cells, iPS cells are suitable candidates for autologous application[138]. Moreover, it has been proposed that iPS cells may be employed to generate unlimited numbers of identical, well-defined and genetically characterized, transplantable functional cells for therapeutic repair of cardiac tissue.

Few studies have investigated the effect of iPS transplantation into the heart. Nelson et al.[139] reported that the intramyocardial delivery of mouse iPS cells achieved regeneration of cardiac tissue and improvement of post-ischemic cardiac function. However, data concerning cell survival and engraftment rate are not yet available and these issues remain to be investigated.

Transplantation of iPS cells has several limitations in terms of their clinical applicability in cardiac regeneration. Genome-integrating viral vectors used for inducing iPS cells contain known oncogenes, thereby causing a risk for tumor formation. This problem can be overcome by reprogramming without viral integration using plasmids or direct reprogramming protein delivery[140-142]. Furthermore, human iPS cells are derived only at a low efficiency of about 0.01%, making the generation of a therapeutic amount of cells time-consuming. In addition, it is not clear whether human iPS cells have complete nuclear reprogramming and could therefore result in impaired differentiation of iPS cells into the required cell type[143], potentially contributing to reduced treatment efficacy.

In addition to cardiac repair, iPS cells can be used as models of cardiac disease, as these cells are more representative of disease phenotype compared to mouse models, and may theoretically even be used as a model representing a single patient. Thereby, iPS cells can be used to study mechanisms of disease and aid in the development of new therapeutic strategies[122].

CELL DELIVERY

In addition to finding the ideal cell type for cardiac repair, determining the optimal cell delivery method is crucial to maximize efficacy and minimize risks associated with cell treatment. Optimizing cell delivery methods requires targeted delivery of cells to minimize risk of extraneous diffusing and maximize survival and engraftment of transplanted cells. Also, the etiology of the cardiac disease influences the choice of delivery method. For example, chronic ischemic myocardial disease may be less attractive to approach via the intracoronary route because chronic occlusions or stenoses may hamper access to the target vessel, making the intramyocardial injection method a more feasible option. Currently available routes of administration include intramyocardial (epicardial or endocardial injection), intracoronary and intravenous infusion of cells (figure 2).

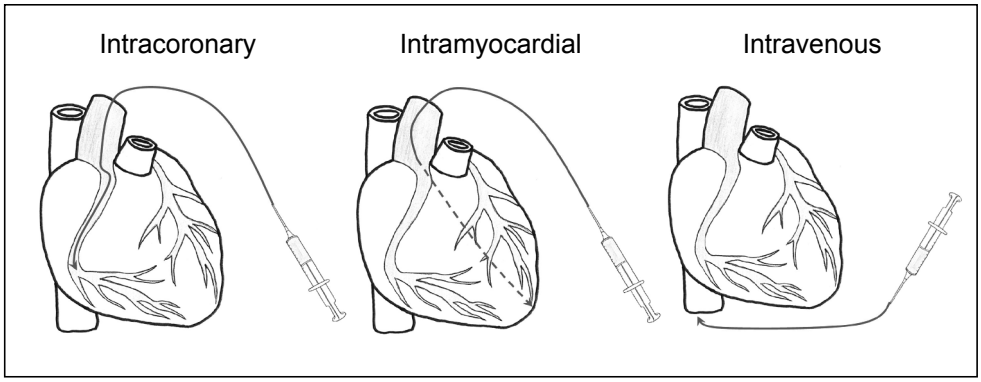


Figure 2: Possible routes of administration of therapeutic cells.

INTRAMYOCARDIAL INJECTION

Using the intramyocardial route, cells are directly injected into the myocardium. At present, direct intramyocardial injection has been described using a trans-endocardial, trans-epicardial and trans-venous approach.

Trans-endocardial delivery involves catheter-based targeted injection of cells into the myocardium guided by fluoroscopy or an electrophysiological cardiac mapping system. The most established 3D mapping system relies on electro-anatomical mapping to navigate the injection catheter inside the heart, accurately distinguishing viable, hibernating and infarcted myocardium[144]. The derived three-dimensional map is used to assess the viability of the myocardial target site followed by injection of the cells through a small extendable needle. This technique has the advantage of assessing viability of potential injection sites, allowing accurate targeting of cell injections into the infarct border zone[145] or ischemic myocardium[146-148]. Endocardial damage and ventricular perforation have been suggested as potential procedural risks. However, this method has successfully been used in several trials, with only 1 patient reported to have had pericardial effusion[146, 147, 149-151].

Trans-epicardial cell injection has been most commonly performed following sternotomy for concurrent coronary artery bypass grafting and has the advantage that it allows direct visualization of the myocardium. Due to the invasive nature of this approach, it's primarily used in pre-clinical trials. Since in patients trans-epicardial cell transplantation is conducted as an adjunct to coronary bypass surgery, the efficacy of cell injection by itself may be difficult to assess.

Trans-venous intramyocardial cell delivery is a catheter-based method which approaches the myocardium through the epicardial surface from the cardiac venous system. To achieve this, a catheter containing an intravascular ultrasound probe to localize the adjacent coronary artery and pericardium, is positioned in specific branches of the cardiac venous system. Using a small-caliber needle, the coronary vein is punctured and the needle is introduced into the ventricular wall followed by cell administration[152]. Procedural associated risks include damaging or perforating the venous wall facing the epicardium, resulting in pericardial hemorrhage. A disadvantage over the other intramyocardial routes is the inability to reach all myocardial territories due to the anatomy of coronary veins.

INTRACORONARY INJECTION

Intracoronary infusion of cells is the most commonly used cell delivery method in clinical trials. Cells are infused through the central lumen of an over-the-wire balloon into the distal end of the infarct-related coronary artery. Transient low-pressure inflation of the balloon catheter prevents backflow of the cells, thereby enhancing myocardial delivery of cells. This technique is particularly suited for the infusion of cells into a specific coronary territory and is therefore mainly used in the setting of acute or chronic MI[8, 153-156]. Although isolated reports have observed potential risks of intracoronary cell infusion[157-159], meta-analyses have shown that this delivery technique is safe and is not associated with a higher incidence of overall clinical events[160, 161].

INTRAVENOUS INJECTION

Intravenous cell infusion is performed through a central or peripheral venous catheter and is technically the easiest method of cell delivery. Despite the advantage of the least invasive route and lowest risks, the effect of intravenous cell delivery heavily depends on stem cell homing signals and vascularisation at the target area. Animal studies indicated very low cell retention within the infarcted heart after intravenous infusion and indicated that most of the infused cells become trapped in the lungs, liver and spleen[162]. Furthermore, this technique may theoretically introduce clusters of larger cells (e.g. MSCs) in the circulation, causing microemboli in the vasculature of multiple organ systems.

COMPARING DELIVERY ROUTES

Amongst the various cell delivery techniques that have been tested in preclinical and clinical studies, intracoronary and intramyocardial catheter-based cell injection are considered most promising. However, until now, no delivery strategy has emerged as the most optimal cell delivery route for cell transplantation and variable retention rates are reported for all delivery routes[70].

At present, a comparison of different delivery routes has not been performed in humans. Using animal models, comparative studies have been performed to assess the engraftment of transplanted cells in the myocardium using different delivery routes. Freyman et al.[159] transplanted MSCs using a porcine infarction model and evaluated the engraftment rate after 14 days. The percentage of MSCs retained in the infarct zone was 6% after intracoronary infusion, 3% after intramyocardial injection and 0% after intravenous infusion. In contrast, Hou et al. [163] reported myocardial cell retention of 3% after intracoronary delivery, 11% after intramyocardial delivery and 3% after intravenous delivery in a swine model of MI, 6 days after BMNC transplantation. In a recent study, van der Spoel et al. found no difference between transendocardial and intracoronary injection in a pig model of ischemic cardiomyopathy, with both methods resulting in 11% cell retention[164].

Since cell engraftment and retention are also dependent of other variables, such as cell type and myocardial substrate, comparison of different studies is difficult. Therefore, more comparative studies will be necessary to determine the optimal delivery method for each situation. In deciding on which technique to use, factors such as cell type, presence of homing signals and anatomic location of target site could be taken into consideration.

EXPERIMENTAL STUDIES

ACUTE MYOCARDIAL INFARCTION

After the landmark study of Orlic et al in 2001, which demonstrated that locally delivered hematopoetic stem cells were capable of de novo myocardium formation after acute myocardial infarction[3], numerous experimental studies have been performed in animal models of myocardial infarction. Because of contradicting results of these studies[5, 165-167], the ability of bone marrow-derived cells to form de novo myocardium has been subject of discussion. However, in other experimental studies, other mechanisms were elucidated by which bone marrow cells may improve myocardial performance. Importantly,

stimulation of neoangiogenesis was observed by differentiation in vascular cells and excretion of pro-angiogenic substances[13, 35, 165, 168-170]. Thus, the subsequent improvement in myocardial perfusion may result in improved survival of host cardiomyocytes and attenuation of the post-infarction remodeling process. Furthermore, it has been suggested that bone marrow-derived cells such as HSC and MSC can secrete cytokines that may inhibit apoptosis, recruit resident progenitor cells and influence extracellular matrix composition[12, 13 41].

Skeletal myoblasts have been observed to form functional myotubes[77, 78], and islands of cardiomyocyte-like cells[79], but complete cardiomyogenic differentiation has not been observed. Of note, due to a lack of connexin-43 expression, electromechanical coupling between transplanted myoblasts and the host cardiomyocytes is absent. This may create an arrhythmogenic substrate[83] and may reduce the beneficial effect on myocardial function[81, 82].

Cardiomyogenic regeneration has been observed after injection of embryonic stem cells[171] and iPS[172]. Because of the risk of ectopic differentiation using these cells, transplantation of pre-differentiated, cardiac-committed embryonic stem cell- or iPS-cells has been investigated in a number of studies, resulting in cardiomyogenesis in vivo[173-175] and improvements in myocardial function[176].

Resident cardiac stem cells, especially the c-kit^{pos}Lin^{neg} cells and cardiosphere-derived cells, have also been demonstrated to be able to regenerate injured myocardium in vivo. For the other subtypes of resident cardiac stem cells, as well as mesenchymal stem cells and EPDC, the capacity for in vivo cardiomyogenesis remains subject of controversy. Nonetheless, transplantation of these cell types is associated with improvements in cardiac function, probably by other mechanisms, as described in the paragraphs about these cell types. Thus, for these cell types, it remains to be investigated to what extent cardiomyogenesis may contribute to clinically significant improvements of cardiac performance.

CHRONIC HEART FAILURE

For application of cell therapy in the clinical setting, assessment of the in vivo potential of stem and progenitor cells to restore ischemically damaged myocardium is of pivotal importance. Skeletal myoblasts were the first cells to be investigated in a rabbit model of MI, resulting in formation of elongated, striated cells with characteristics of both skeletal muscle and cardiac cells [79]. Numerous studies in small and large animal models

confirmed that myoblast injection was associated with improvements in myocardial function[177], but the exact mechanism of improvement remains unclear. Myotube formation by injected myoblasts was observed in several studies, however, since the number of surviving myocytes is relatively low[76, 78] and electrical integration is absent[81, 82], it has been questioned whether myogenesis in itself may account for the observed improvements. Accordingly, it has been hypothesized that the beneficial effects of myoblast injection are related to paracrine function of the injected cells[87-89] or a "packing effect" of engrafted cells, supporting the infarcted wall and increasing elasticity, thus attenuating remodeling[72].

Bone marrow-derived MSCs have mainly been investigated using models of acute MI. Most studies reported improvements of myocardial function after MSC transplantation, with discrepant results regarding cardiogenic differentiation[36, 38-40]. Of interest, in a porcine model of chronic MI, Tomita et al.[178] demonstrated that transplanted MSCs engrafted in infarcted myocardium, differentiating in cells expressing troponin I and containing organized sarcomeres and Z-bands. In addition, capillary density was increased at the injection sites. In this study, MSC transplantation resulted in enhanced left ventricular function and attenuation of left ventricular dilatation and pathologic thinning of the infarcted myocardium[178].

Bone marrow-derived mononuclear cells have extensively been investigated in models of acute MI, showing beneficial effects on myocardial perfusion and function, often in the absence of substantial cardiac regeneration[13, 165, 179]. Only a limited number of studies using BMNCs have been performed in models of chronic MI. In one of these studies, Mathieu et al.[180] injected BMNCs or MSCs into the infarct and border zone of a canine model of chronic MI to compare the efficacy of both cell types. They concluded that BMNC injection was superior to MSCs in improving cardiac function and reducing infarct size, and suggested that these improvements were mediated by a favorable angiogenic paracrine effect[180]. Similarly, after injecting chronic infarcted rat myocardium with BMNCs, Fukushima et al.[181] concluded that the small number of surviving donor-derived cells was unlikely to be responsible for the observed improvements in cardiac function, suggesting that the effect was mainly due to paracrine mechanisms. Overall, the majority of studies on BMC transplantation in chronic MI models demonstrated an improved myocardial function without the formation of new cardiomyocytes[180-183], suggesting that excretion of paracrine substances plays an important role in the mechanism by which bone marrow cells improve cardiac function.

Cardiac progenitor cells have shown promising results in models of acute MI, with transplanted cells differentiating toward cardiac lineages and enhancing cardiac performance after injection[90, 91, 184, 185]. In line with these findings, animal models of chronic MI demonstrated cardiac progenitor cells to differentiate into cells expressing markers specific for cardiac myocytes, endothelial cells, and vascular smooth muscle cells[186]. Furthermore, cell delivery was associated with beneficial effects on cardiac function, however, whether this was due to the effect of direct myocardial differentiation of the transplanted cells or caused by paracrine mechanisms is not clear[186]. Comparable results have been found after cardiosphere-derived cell administration into chronic MI models[13, 103, 165, 179, 187], although long-term engraftment was low, again suggesting a paracrine-mediated effect[102].

Theoretically, ESCs and iPCs are thought to posses great potential for cardiac repair. Delivery of hESCs in acute MI models has indeed shown to induce formation of new myocardium and improvement of myocardial function[128, 129, 188, 189]. In a study of Fernandes et al.[190], injection of these cells in chronic MI models also demonstrated formation of new cardiomyocytes, although no beneficial effects on cardiac function were observed. In this study, it was suggested that the effect of cell transplantation might be more pronounced in the acute of sub acute phase of MI[190]. Despite the beneficial effects following transplantation into animal myocardium, several studies also demonstrated the development of tumours[140, 191]. Currently, research is focusing on refining techniques for differentiation and purification to guide differentiation of these pluripotent cells into cardiomyocytes.

CHRONIC MYOCARDIAL ISCHEMIA

The majority of experimental studies investigating the effects of cell therapy have been performed in animal models of acute myocardial infarction. Only a minority of studies has been conducted using animal models of chronic myocardial ischemia, such as ameroid constrictor placement in the left anterior descending or circumflex coronary artery. Similar to studies in animal models of acute myocardial infarction models, comparison of different studies is difficult, since differences exist in cell isolation methods, cell dose, timing of delivery, and the characteristics of the animal model. Nonetheless, the results of these studies lead to the concept that bone marrow cell injection in ischemic myocardium may improve myocardial perfusion and function by stimulating angiogenesis, through differentiation into endothelial cells and smooth muscle cells, and secretion of pro-angiogenic cytokines (figure 1).

For example, the study of Kawamoto et al. reported macroscopic collateral formation and increased capillary density after intramyocardial cell injection in a swine model of myocardial ischemia[192]. In this study, adhesive CD 31+ cells isolated from peripheral blood were injected in ischemic myocardium using a 3D electromechanical mapping system, resulting in enhanced neovascularization which was accompanied by improvements in LV function. In line with these results, Silva et al. described increased capillary density and improved LV function after MSC injection using a canine ischemia model[35]. In addition, injected MSC were found to colocalize with endothelial cells and smooth muscle cells but not with cardiomyocytes, suggesting differentiation of MSC into these vascular cell types.

In contrast, Fuchs et al observed improved myocardial perfusion and enhanced contractility in the absence of microscopic or macroscopic collateral formation[193]. In this study, freshly aspirated, unselected bone marrow cells were injected into ischemic myocardium of pigs using a 3D electromechanical mapping system, Of note, this study demonstrated that the injected bone marrow cells secreted angiogenic factors which induced in vitro endothelial cell proliferation, suggesting that the observed improvements were mainly the result of paracrine function of the injected cells, possibly leading to changes in vascular diameters or decreased resistance to collateral flow. Importantly, since freshly aspirated, non-enriched bone marrow was injected in this study, the absence of collateral formation may be attributable to lower dose of progenitor cells as compared to studies using bone marrow mononuclear cells or enriched cell populations. Moreover, red blood cell contamination may have reduced progenitor cell function[194].

In a study focusing on the functional results of bone marrow cell injection, Schneider et al. compared intramyocardial injection of BMMC and MSC in a porcine model of chronic myocardial ischemia[195]. In all cell-treated animals, improved LV function, reduced fibrosis and increased vascular density were observed, with none of both cell types being superior. In addition, using strain rate imaging, a favorable effect on diastolic function was observed, as evidenced by improved parameters of filling pressure and myocardial relaxation.

Importantly, none of the animal studies did pose any concerns with regard to the safety of intramyocardial bone marrow cell injection for chronic myocardial ischemia. Of note, no excessive necrosis was observed at the sites of intramyocardial injection[192, 193]. Furthermore, in a porcine model of chronic myocardial ischemia, Krause et al. demonstrated that 3D electromechanical mapping-guided injection of both bone marrow-derived mononuclear

cells and MSC into ischemic myocardium did not increase fragmentation and duration of endocardial electrograms[196]. These findings suggest that injection of either bone marrow cells or MSC is not likely to create a substrate for arrhythmias, and confirms observations from early clinical studies[197].

CLINICAL STUDIES

ACUTE MYOCARDIAL INFARCTION

The effect of intracoronary bone marrow cell transplantation in patients with acute myocardial infarction has been investigated in multiple clinical trials. Whereas the first landmark studies showed contradicting results[14, 198, 199], later studies have pointed out that bone marrow cell infusion is associated with moderate but significant effects on LV function[160, 161, 200]. Moreover, some studies presumed a beneficial effect on prognosis[201, 202], although a recent meta-analysis did not confirm this[203].

Of note, a large variability exists in the reported effects of bone marrow cell transplantation on myocardial function and infarct size. Several differences in the design of these studies may account for these variable findings. First, cell processing techniques have been suggested to account for the different results of these studies. Factors that have particularly been related to cell recovery and/or function are the separation protocol (lymphoprep versus ficoll separation), and the use of serum during incubation[204]. Furthermore, red blood cell[194] and platelet contamination[205] have been associated with impaired cell functionality after transplantation. Second, cell dose has been highly variable between studies, ranging from 12 million to over 240 million cells. Since a dose-response relationship has been suggested in a meta-analysis[200], this variability in cell dose may have contributed to the different outcomes. Third, several time intervals between myocardial infarction and cell transplantation have been studied. Of note, a recent meta-analysis suggests that the optimal time interval for bone marrow cell transplantation is within 7 days after acute myocardial infarction[200], whereas the REPAIR-AMI trial suggested that the beneficial effects of cell transplantation is limited to patients who receive bone marrow cells 4 or more days after acute myocardial infarction[14]. Thus, based on these findings, it may be supposed that 4-7 days after myocardial infarction is the optimal timing for bone marrow cell transplantation. Finally, different imaging techniques have been used for the evaluation of LV function and infarct size. Of note, LV ventriculography is often used, whereas only a part of the studies used magnetic resonance imaging or single-positron emission computed tomography.

Clinical studies using other cell types in patients with acute myocardial infarction are scarce. Recently, a phase I clinical trial demonstrated the safety of intracoronary infusion of cardiosphere-derived cells 2-4 weeks after acute myocardial infarction. In addition, a decrease in infarct size and improved regional systolic function were observed in CDC-treated patients.

In summary, the optimal conditions for bone marrow cell transplantation after acute myocardial infarction remain to be determined. Further studies are necessary to establish the optimal cell type, isolation protocol, cell dose and timing of cell transplantation in patients with acute myocardial infarction.

CHRONIC HEART FAILURE

Bone marrow cell therapy

Cell therapy for patients with ischemic heart failure has mainly been investigated using transcatheter intracoronary or intramyocardial injection of therapeutic cells, although some investigators used direct myocardial injection during surgical revascularization. A summary of currently available studies is provided in table1.

Early studies demonstrated that intracoronary administration of bone marrow-derived cells is safe and feasible in patients with ischemic heart failure[155, 206]. These initial studies reported no signs of cardiac or systemic inflammation, cardiac arrhythmias, or other short term complications after cell transplantation[155, 206]. In addition, preliminary efficacy analysis suggested that intracoronary cell infusion may improve myocardial function and perfusion, which was confirmed by the results of the TOPCARE-CHD trial[207]. In this trial, patients were randomly assigned to receive either BMCs, circulating progenitor cells or no cell infusion into the patent coronary artery supplying the most dyskinetic myocardial area. Transplantation of BMCs was associated with a 2.9% increase in LVEF as assessed by LV angiography, which was paralleled by an improvement in New York Heart Association (NYHA) class[207]. In line with these findings, Erbs et al.[208] documented that LVEF increased with 7.2% after intracoronary infusion of G-CSF mobilized cells as compared to placebo treatment. On the other hand, in a randomized controlled study by Yoa et al.[209], no improvements in LV systolic function were detected using magnetic resonance imaging. Nonetheless, echocardiographic tissue doppler analysis revealed modest improvements in diastolic function. In addition to these favorable effects on clinical and functional parameters, the STAR-heart study[210] reported an improvement in survival in patients receiving intracoronary BMC administration as compared to control

patients. However, this study comprised a non-randomized, open label study and the possible contribution of a placebo-effect could not be excluded.

Intramyocardial catheter-based injection of BMCs into ischemic myocardium[211] or the infarct border zone[145] with the use of a 3D-electromechanical mapping system is considered to be safe and feasible in patients with ischemic heart failure, although it must be noted that two studies reported one death in which an effect of the cell therapy could not be ruled out[145, 211]. In these non-randomized safety and feasibility studies, favorable effects on LVEF and perfusion were reported. A randomized trial conducted in 109 patients with ischemic heart failure confirmed these findings by demonstrating improvements in myocardial perfusion, LV function and anginal complaints after bone marrow cell injection into the infarct borderzone as compared to placebo-treatment. Moreover, bone marrow cell injection was associated with a significantly improved survival[212].

In contrast, a recently published multicenter trial investigating intramyocardial bone marrow cell injection in patients with ischemic heart failure did not detect improvements in their pre-specified endpoints end-systolic volume, maximal oxygen consumption, and reversibility on SPECT[213]. However, it must be noted that a significant improvement in LV ejection fraction was observed in the bone marrow cell group as compared to the placebo group, which was mainly driven by improvements in patients of 62 years old or younger. Additional subgroup analysis suggested that the amount of CD34 and CD 131 positive cells were associated with larger improvements in LV ejection fraction.

Clinical studies investigating surgical injection of bone marrow-derived cells in conjunction with bypass surgery have yielded mixed results. Initial safety and feasibility studies[214, 215] reported improvements in cardiac function after cell treatment, however interpretation of these results was hindered by the concomitant revascularization. Subsequently performed randomized controlled trials yielded discordant results: whereas CD34+ cell administration by Patel et al.[216] resulted in an improvement in LVEF of 8.9%, two other trials did not find[217;218] a beneficial effect on left ventricular function following BMC transplantation. A number of reasons may account for this disparity of which the biasing effect of bypass surgery may be the most abundant.

Table 1: Chronic heart failure

Reference	Number of patients	Delivery route	Cell type and number	Safety	LVEF	Perfusion	Clinical symptoms
TOPCARE-CHD	28 BMC vs 24 CPC vs 23 controls	Intracoronary	2 x 10^8 BMC 2 x 10^7 CPC	+	+	NA	↓NYHA
Erbs et al	13 vs 13 controls	Intracoronary	7 x 10^7 G-CSF mobilized cells	+	+	+	NA
Yao et al	24 vs 23 controls	Intracoronary	4 x 10^8 BMC	+	-	-	NA
Pokushalov et al	55 vs 54 controls	Intramyocardial	4 x 10^7 BMC	+	+	+	↓NYHA, ↓CCS
Patel et al	10 vs 10 controls	Intramyocardial during CABG	BMC (of which 22 x 10^6 $CD34^+$ cells)	+	+	NA	↓NYHA
Hendrikx et al	10 vs 10 controls	Intramyocardial during CABG	6 x 10^7 BMC	+	-	-	NA
Ang et al	21 IM vs 21 IC vs 20 controls	Intramyocardial or intracoronary during CABG	BMC (number NA)	+	-	NA	-
MAGIC phase II	30 HD vs 33 LD vs 34 controls	Intracoronary	8 x 10^8 or 4 x 10^8 skeletal myoblasts	+	-	NA	-
SEISMIC study	31 vs 16 controls	Intramyocardial	6 x 10^8 skeletal myoblasts	+	-	NA	-
CAuSMIC Study	12 vs 11 controls	Intramyocardial	Up to 600 $X10^6$ skeletal myoblasts	+	-	NA	↓NYHA, ↑QoL

LVEF: left ventricular ejection fraction; BMC: bone marrow cells; CPC: circulating progenitor cells; NA: not available; NYHA: New York Heart Association class; G-CSF: granulocyte-colony stimulating factor; CCS: Canadian Cardiovascular Society class; CABG: coronary artery bypass grafting; IM: intramyocardial; IC: intracoronary; HD: high dose; LD: low dose; QoL: quality of life score.

Skeletal Myoblast therapy

Since preclinical studies demonstrated the ability of skeletal myoblasts to generate functional myotubes in infarcted myocardium, clinical studies were initiated to estimate safety and efficacy of myoblast administration in patients with chronic heart failure. In most studies, skeletal myoblast transplantation has been carried out as an adjunct to routine surgical revascularization procedures, whereas a couple of studies performed direct catheter-based intramyocardial injection as a stand alone procedure.

Initial clinical studies evaluated safety and feasibility of intramyocardial skeletal myoblast transplantation in patients undergoing bypass surgery. Functional and clinical improvements were observed in these non-randomized studies[74, 219-222], however safety issues arose from these studies since ventricular arrhythmias including sustained monomorphic ventricular tachycardia and ventricular fibrillation were reported after cell transplantation[74, 219, 221, 222]. Although a direct relation between the observed arrhythmias and skeletal myoblasts transplantation is not proven, later studies performed concomitant internal cardioverter-defibrillator (ICD) implantation as a precaution.

Since the aforementioned studies lacked a control group, the attributed beneficial effect of cell transplantation over bypass surgery alone could not be determined. Therefore, the MAGIC clinical trial[223] was initiated to investigate efficacy of skeletal myoblast transplantation in heart failure patients undergoing bypass surgery. This multicenter, randomized, placebo-controlled trial administrated low dose (400 x 10^6), high dose (800 x 10^6), or placebo suspension in akinetic myocardium, accompanied by ICD implantation. Injection of high dose skeletal myoblasts resulted in reduced LV remodeling, as evidenced by decreased LV end-diastolic and -systolic volumes as assessed by echocardiography. However, no significant differences in regional or global LV function were observed[223].

Transplantation of skeletal myoblasts has also been performed using direct catheter-based delivery methods. In line with the findings of trans-epicardial myoblast administration, an increased occurrence of cardiac arrhythmias was observed following transendocardial delivery, emphasizing the need for prophylactic ICD implantation[74, 75, 221, 224-226]. Nonetheless, these small-sized clinical studies suggested that trans-coronary-venous[221] and transendocardial[74, 75, 224-227] cell delivery of skeletal myoblasts was associated with improvements in cardiac performance and clinical symptoms. The SEISMIC study[228] is a relatively small open-label randomized study in which patients were randomized to receive either skeletal myoblasts by trans-endocardial injection or optimal medical treatment. Cell therapy was associated with an increase in exercise

capacity, and an improvement in NYHA class. However, despite these beneficial effects, no significant improvement in global or regional LV function was detected in this study[228]. Unfortunately, the larger phase II/III randomized MARVEL trial was stopped because of financial reasons after inclusion of 23 patients[229]. In these patients, ventricular tachycardias were more frequent in cell-treated patients and no functional benefit could be demonstrated. Thus, larger randomized studies are necessary to assess the safety as well as the functional benefits of skeletal myoblast administration in patients with heart failure.

REFRACTORY ANGINA PECTORIS

Supported by encouraging preclinical data and an unmet clinical need, several clinical studies were initiated to investigate intramyocardial bone marrow cell injection as a novel therapeutic option for the treatment of chronic myocardial ischemia. In these studies, patients with refractory angina ineligible for conventional revascularization were treated with transendocardial bone marrow cell injection, performed during cardiac catheterization with the use of electromechanical mapping. In table 2, a summary of these studies is provided. Four studies included patients with angina [230-233], whereas one study included patients with heart failure[211]. The combined experience of these studies indicated that bone marrow cell injection is a safe and feasible treatment in patients with chronic myocardial ischemia. However, 1 patient in the study of Perin et al. died suddenly at 14 weeks follow-up. Although sudden cardiac death is a relatively common complication of ischemic heart failure, a cell-related cause of this event could not be ruled out. Of note, 2 studies demonstrated that intramyocardial bone marrow cell injection was not associated with progression of atherosclerosis[234] and did not alter the electrophysiological properties of the injected myocardium [197].

Importantly, most of these initial clinical studies reported improvements in myocardial perfusion, LV function and anginal complaints after bone marrow cell injection. Since only preliminary conclusions could be drawn from these nonrandomized studies, several randomized trials were initiated to assess the efficacy of intramyocardial bone marrow cell injection.

In a small-sized randomized trial, Losordo et al. documented the feasibility and safety of intramyocardial injection of granulocyte colony-stimulating factor-mobilized (G-CSF) CD34+ stem cells[149]. No significant effect on angina frequency, exercise time, or Canadian Cardiovascular Society (CCS) score was observed, which may have been due to underpowering for these outcomes.

Table 2. Clinical studies of intramyocardial bone marrow cell injection for chronic myocardial ischaemia

	Study design	Number of patients	Patient population	Cell type	Cell dose	Follow up (months)	Safety	CCS/NYHA/QoL	Myocardial perfusion	LVEF
Perin et al, 2003	Observational, control +	14 vs. 7	Heart failure	BMC	3 x10^7	12	+	CCS -1.36, NYHA -1.07,	↓ Extent of ischemia = perfusion at rest	+ 9.0% at 3 months = at 12 months
Fuchs et al, 2006	Observational, control -	27	Angina pectoris	BMC	8 x10^7	12	+	CCS -1.0	↑ Stress perfusion in injected segments = stress perfusion in noninjected segments	=
Beeres et al, 2006	Observational, control -	25	Angina pectoris	BMC	8 x10^7	12	+	CCS -0.7 and QoL + 15% at 12 months	↓ Extent of ischemia	+ 6.0% at 3 months + 4.0% at 12 months
Briguori et al, 2006	Observational, control -	10	Angina pectoris	BMC	2 x10^8	12	+	CCS ↓ QoL ↑	↓ Extent of ischemia	=
Tse et al, 2006	Observational, control -	12	Angina pectoris	BMC	Up to 16 x10^7	44	+	NA	NA	=
Losordo et al, 2007	Randomised, sham +	18 vs. 6	Angina pectoris	CD34+ G-CSF mobilized cells/kg	Up to 5 x10^5 cells/kg	6	+	CCS -1.4	= Extent of ischemia	NA
PROTECT-CAD, 2007	Randomised, sham +	19 vs. 9	Angina pectoris	BMC	Up to 4.2 x10^7	6	+	CCS =, NYHA ↓	↓ Extent of ischemia in injected segments	+ 3.7%
Losordo et al, 2011	Randomised, control +	106 vs. 50	Angina pectoris	CD34+ G-CSF mobilized cells/kg	Up to 5 x10^5 cells/kg	12	+	Angina frequency↓ Exercise time =	Transient effect on stress myocardial perfusion	NA

BMC=bone marrow cells; G-CSF= granulocyte-colony stimulating factor; CCS= Canadian Cardiovascular Society angina class; NYHA= New York Heart Association functional class; QoL= quality of life score; NA= not available; LVEF= left ventricular ejection fraction.

This study was extended to a randomized, double-blind multicenter trial, which investigated the effect of intramyocardial injection of low dose ($1x10^5$ cells/kg) and high dose ($5x10^5$ cells/kg) G-CSF mobilized CD34+ cells in 167 patients with chronic myocardial ischemia[235]. In this study, intramyocardial CD34+ cell injection was associated with improvements in exercise tolerance and anginal symptoms, which were preserved after 12 months follow-up. However, SPECT imaging only demonstrated a transient increase in stress myocardial perfusion after 6 months which was not detectable at 12 months follow-up. Of note, the improvements were larger in the low dose group than in the high dose group, possibly suggesting a biphasic dose-response relationship, as suggested by the authors.

In the smaller PROTECT-CAD trial, Tse et al. evaluated the effect of intramyocardial bone marrow cell injection on myocardial perfusion, LV function and clinical parameters in 28 patients with chronic myocardial ischemia[150]. Patients were randomized in a 1:1:1 ratio to receive low dose bone marrow cells (n=9), high dose bone marrow cells (n=10), or placebo solution (n=9). Diabetes and previous percutaneous coronary intervention were more frequent in the placebo group than in the bone marrow cell groups, suggesting that baseline risks were not completely balanced between the groups. Although bone marrow cell injection was associated with a modest increase in exercise capacity and LV ejection fraction, no significant treatment effect on CCS class was observed, indicating no effect of bone marrow cell injection on anginal complaints. Moreover, the changes in myocardial perfusion did not differ significantly between the (pooled) cell group and the placebo group. Only when post hoc analysis was performed, a significant improvement in myocardial perfusion was observed in bone marrow cell-injected myocardial regions. Obviously, differences in cell type, dose and method of preparation may often account for variable treatment effects, but this would not explain the apparent discrepancy between clinical observations and SPECT parameters in the study of Losordo et al. Therefore, as suggested by the authors, it may be argued that the effect of CD34+ cell injection may be diffusely spread throughout the ischemic myocardium, resulting in subtle changes in myocardial blood flow which may not be detectable on SPECT imaging. It is theoretically conceivable that the improvement in myocardial perfusion after cell injection may tend to be more focal[236] or diffuse[35], depending on factors such as cell type, cell dose and injection technique. Possibly, new techniques such as positron-emission tomography imaging may provide more information about this mechanism[237].

Intracoronary infusion of BMC has been described in patient with chronic myocardial ischemia. However, adverse events possibly related to cell injection have been reported by Boyle et al.[238] in a small safety study. In contrast, a cohort consisting of 112 patients with severe coronary artery disease was described which underwent intracoronary cell transfusion without any complications[239]. Nevertheless, the safety of this administration route in patients with severe coronary artery disease remains to be further investigated.

AIM AND OUTLINE OF THE THESIS

The aim of this thesis was to investigate the effect of intramyocardial bone marrow cell injection in patients with chronic ischemic heart disease. The effect of bone marrow cell injection was assessed using conventional clinical measures such as CCS angina class, quality of life evaluation and exercise testing. Furthermore, various imaging techniques were used to evaluate the functional benefits of bone marrow cell injection in terms of myocardial perfusion, global and regional LV function, diastolic function, and cardiac sympathetic nerve function.

In **chapter 2**, the results are described of a randomized, placebo controlled, double-blinded trial investigating intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia. The results of the cross-over phase of this study are reported in **chapter 3.** In **chapter 4**, a substudy of this trial is presented in which the effect of bone marrow cell injection on diastolic function is evaluated. **Chapter 5** discusses the findings of myocardial innervation imaging using MIBG in patients which underwent bone marrow cell injection. In **chapter 6**, the results are described of a study which evaluated the effects of bone marrow cell injection in patients with ischemic heart failure. In particular, the effect on LV dyssynchrony was assessed. Finally, the long term results of bone marrow cell injection in patients with chronic myocardial ischemia are evaluated in **chapter 7**.

The last chapter concerns the summary and conclusions of the thesis.

REFERENCES

1. Yang EH, Barsness GW, Gersh BJ, Chandrasekaran K, Lerman A Current and future treatment strategies for refractory angina. *Mayo Clin Proc* 2004;79:1284-1292
2. Socinski MA, Cannistra SA, Elias A, et al. Granulocyte-macrophage colony stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. *Lancet* 1988;1:1194-1198
3. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701-705
4. Balsam LB, Wagers AJ, Christensen JL, et al. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428:668-673
5. 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
6. Nygren JM, Jovinge S, Breitbach M, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 2004;10:494-501
7. Kudo M, Wang Y, Wani MA, et al. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *J Mol Cell Cardiol* 2003;35:1113-1119
8. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106:1913-1918
9. 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
10. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation* 2003;108:2212-2218
11. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 2001;98:10344-10349
12. Yoshioka T, Ageyama N, Shibata H, et al. Repair of infarcted myocardium mediated by transplanted bone marrow-derived CD34+ stem cells in a nonhuman primate model. *Stem Cells* 2005;23:355-364
13. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7:430-436
14. Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;355:1210-1221
15. Schmidt-Lucke C, Rossig L, Fichtlscherer S, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 2005;111:2981-2987
16. Kovacic JC, Moore J, Herbert A, et al. Endothelial progenitor cells, angioblasts, and angiogenesis--old terms reconsidered from a current perspective. *Trends Cardiovasc Med* 2008;18:45-51
17. Takahashi T, Kalka C, Masuda H, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999;5:434-438
18. Gehling UM, Ergun S, Schumacher U, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000;95:3106-3112
19. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-967

20. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;95:952-958
21. Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001;103:634-637
22. Yoder MC, Mead LE, Prater D, et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;109:1801-1809
23. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89:E1-E7
24. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;348:593-600
25. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;353:999-1007
26. Muller-Ehmsen J, Braun D, Schneider T, et al. Decreased number of circulating progenitor cells in obesity: beneficial effects of weight reduction. *Eur Heart J* 2008;29:1560-1568
27. Croce G, Passacquale G, Necozione S, Ferri C, Desideri G Nonpharmacological treatment of hypercholesterolemia increases circulating endothelial progenitor cell population in adults. *Arterioscler Thromb Vasc Biol* 2006;26:e38-e39
28. Dimmeler S, Aicher A, Vasa M, et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001;108:391-397
29. Vasa M, Fichtlscherer S, Adler K, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 2001;103:2885-2890
30. Walter DH, Rittig K, Bahlmann FH, et al. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 2002;105:3017-3024
31. Westerweel PE, Visseren FL, Hajer GR, et al. Endothelial progenitor cell levels in obese men with the metabolic syndrome and the effect of simvastatin monotherapy vs. simvastatin/ezetimibe combination therapy. *Eur Heart J* 2008;29:2808-2817
32. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 1974;17:331-340
33. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-147
34. Makino S, Fukuda K, Miyoshi S, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999;103:697-705
35. Silva GV, Litovsky S, Assad JA, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation* 2005;111:150-156
36. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;105:93-98
37. Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100:II247-II256
38. Grauss RW, Winter EM, van TJ, et al. Mesenchymal stem cells from ischemic heart disease patients improve left ventricular function after acute myocardial infarction. *Am J Physiol Heart Circ Physiol* 2007;293:H2438-H2447
39. 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

40. 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
41. Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 2004;94:678-685
42. Mangi AA, Noiseux N, Kong D, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 2003;9:1195-1201
43. Cho J, Zhai P, Maejima Y, Sadoshima J Myocardial Injection With GSK-3{beta}-Overexpressing Bone Marrow-Derived Mesenchymal Stem Cells Attenuates Cardiac Dysfunction After Myocardial Infarction. *Circ Res* 2011;108:478-489
44. Grauss RW, van TJ, Steendijk P, et al. Forced myocardin expression enhances the therapeutic effect of human mesenchymal stem cells after transplantation in ischemic mouse hearts. *Stem Cells* 2008;26:1083-1093
45. Le BK, Ringden O Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med* 2007;262:509-525
46. Uccelli A, Moretta L, Pistoia V Immunoregulatory function of mesenchymal stem cells. *Eur J Immunol* 2006;36:2566-2573
47. Nauta AJ, Westerhuis G, Kruisselbrink AB, et al. Donor derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood* 2006;108:2114-2121
48. Eliopoulos N, Stagg J, Lejeune L, Pommey S, Galipeau J Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. *Blood* 2005;106:4057-4065
49. Breitbach M, Bostani T, Roell W, et al. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 2007;110:1362-1369
50. Bernardo ME, Zaffaroni N, Novara F, et al. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 2007;67:9142-9149
51. Sensebe L, Krampera M, Schrezenmeier H, Bourin P, Giordano R Mesenchymal stem cells for clinical application. *Vox Sang* 2010;98:93-107
52. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet* 2004;363:783-784
53. Meliga E, Strem BM, Duckers HJ, Serruys PW Adipose-derived cells. *Cell Transplant* 2007;16:963-970
54. Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation* 2004;109:656-663
55. Planat-Benard V, Menard C, Andre M, et al. Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. *Circ Res* 2004;94:223-229
56. Strem BM, Zhu M, Alfonso Z, et al. Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. *Cytotherapy* 2005;7:282-291
57. Valina C, Pinkernell K, Song YH, et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. *Eur Heart J* 2007;28:2667-2677
58. Schenke-Layland K, Strem BM, Jordan MC, et al. Adipose tissue-derived cells improve cardiac function following myocardial infarction. *J Surg Res* 2009;153:217-223
59. Kucia M, Reca R, Campbell FR, et al. A population of very small embryonic-like (VSEL) CXCR4(+) SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. *Leukemia* 2006;20:857-869

60. Wojakowski W, Tendera M, Kucia M, et al. Mobilization of bone marrow-derived Oct-4+ SSEA-4+ very small embryonic-like stem cells in patients with acute myocardial infarction. *J Am Coll Cardiol* 2009;53:1-9
61. Wojakowski W, Kucia M, Liu R, et al. Circulating Very Small Embryonic-Like Stem Cells in Cardiovascular Disease. *J Cardiovasc Transl Res* 2010;
62. Dawn B, Tiwari S, Kucia MJ, et al. Transplantation of bone marrow-derived very small embryonic-like stem cells attenuates left ventricular dysfunction and remodeling after myocardial infarction. *Stem Cells* 2008;26:1646-1655
63. Zuba-Surma EK, Guo Y, Taher H, et al. Transplantation of expanded bone marrow-derived very small embryonic-like stem cells (VSEL-SCs) improves left ventricular function and remodeling after myocardial infarction. *J Cell Mol Med* 2010;
64. Zuba-Surma EK, Wojakowski W, Ratajczak MZ, Dawn B Very small embryonic-like stem cells: biology and therapeutic potential for heart repair. *Antioxid Redox Signal* 2011;
65. Shin DM, Zuba-Surma EK, Wu W, et al. Novel epigenetic mechanisms that control pluripotency and quiescence of adult bone marrow-derived Oct4(+) very small embryonic-like stem cells. *Leukemia* 2009;23:2042-2051
66. Dawn B, Abdel-Latif A, Sanganalmath SK, Flaherty MP, Zuba-Surma EK Cardiac repair with adult bone marrow-derived cells: the clinical evidence. *Antioxid Redox Signal* 2009;11:1865-1882
67. Suuronen EJ, Wong S, Kapila V, et al. Generation of CD133+ cells from CD133- peripheral blood mononuclear cells and their properties. *Cardiovasc Res* 2006;70:126-135
68. Yoon CH, Hur J, Park KW, et al. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation* 2005;112:1618-1627
69. van der Bogt KE, Sheikh AY, Schrepfer S, et al. Comparison of different adult stem cell types for treatment of myocardial ischemia. *Circulation* 2008;118:S121-S129
70. van Ramshorst J, Rodrigo SF, Schalij MJ, et al. Bone marrow cell injection for chronic myocardial ischemia: the past and the future. *J Cardiovasc Transl Res* 2011;4:182-191
71. Chou SM, Nonaka I Satellite cells and muscle regeneration in diseased human skeletal muscles. *J Neurol Sci* 1977;34:131-145
72. Menasche P Skeletal myoblasts and cardiac repair. *J Mol Cell Cardiol* 2008;45:545-553
73. Menasche P Skeletal myoblasts for cardiac repair: Act II? *J Am Coll Cardiol* 2008;52:1881-1883
74. Dib N, McCarthy P, Campbell A, et al. Feasibility and safety of autologous myoblast transplantation in patients with ischemic cardiomyopathy. *Cell Transplant* 2005;14:11-19
75. Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol* 2003;42:2063-2069
76. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *J Am Coll Cardiol* 2003;41:879-888
77. Ghostine S, Carrion C, Souza LC, et al. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. *Circulation* 2002;106:I131-I136
78. Maurel A, Azarnoush K, Sabbah L, et al. Can cold or heat shock improve skeletal myoblast engraftment in infarcted myocardium? *Transplantation* 2005;80:660-665
79. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998;4:929-933
80. Reinecke H, Minami E, Poppa V, Murry CE Evidence for fusion between cardiac and skeletal muscle cells. *Circ Res* 2004;94:e56-e60

81. 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
82. Rubart M, Soonpaa MH, Nakajima H, Field LJ Spontaneous and evoked intracellular calcium transients in donor-derived myocytes following intracardiac myoblast transplantation. *J Clin Invest* 2004;114:775-783
83. Hagege AA, Marolleau JP, Vilquin JT, et al. Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation* 2006;114:I108-I113
84. Abraham MR, Henrikson CA, Tung L, et al. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. *Circ Res* 2005;97:159-167
85. Roell W, Lewalter T, Sasse P, et al. Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. *Nature* 2007;450:819-824
86. Menasche P Stem cell therapy for heart failure: are arrhythmias a real safety concern? *Circulation* 2009;119:2735-2740
87. Payne TR, Oshima H, Okada M, et al. A relationship between vascular endothelial growth factor, angiogenesis, and cardiac repair after muscle stem cell transplantation into ischemic hearts. *J Am Coll Cardiol* 2007;50:1677-1684
88. Ebelt H, Jungblut M, Zhang Y, et al. Cellular cardiomyoplasty: improvement of left ventricular function correlates with the release of cardioactive cytokines. *Stem Cells* 2007;25:236-244
89. Murtuza B, Suzuki K, Bou-Gharios G, et al. Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. *Proc Natl Acad Sci U S A* 2004;101:4216-4221
90. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003;114:763-776
91. Dawn B, Stein AB, Urbanek K, et al. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci U S A* 2005;102:3766-3771
92. Zaruba MM, Soonpaa M, Reuter S, Field LJ Cardiomyogenic potential of C-kit(+)-expressing cells derived from neonatal and adult mouse hearts. *Circulation* 2010;121:1992-2000
93. Pouly J, Bruneval P, Mandet C, et al. Cardiac stem cells in the real world. *J Thorac Cardiovasc Surg* 2008;135:673-678
94. Oh H, Chi X, Bradfute SB, et al. Cardiac muscle plasticity in adult and embryo by heart-derived progenitor cells. *Ann N Y Acad Sci* 2004;1015:182-189
95. Matsuura K, Nagai T, Nishigaki N, et al. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem* 2004;279:11384-11391
96. Smits AM, van Laake LW, den OK, et al. Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. *Cardiovasc Res* 2009;83:527-535
97. Oh H, Bradfute SB, Gallardo TD, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A* 2003;100:12313-12318
98. Challen GA, Little MH A side order of stem cells: the SP phenotype. *Stem Cells* 2006;24:3-12
99. Pfister O, Oikonomopoulos A, Sereti KI, et al. Role of the ATP-binding cassette transporter Abcg2 in the phenotype and function of cardiac side population cells. *Circ Res* 2008;103:825-835
100. Messina E, De AL, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 2004;95:911-921
101. Smith RR, Barile L, Cho HC, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 2007;115:896-908
102. Chimenti I, Smith RR, Li TS, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. *Circ Res* 2010;106:971-980

103. Johnston PV, Sasano T, Mills K, et al. Engraftment, differentiation, and functional benefits of autologous cardiosphere-derived cells in porcine ischemic cardiomyopathy. *Circulation* 2009;120:1075-83, 7

104. Terrovitis J, Lautamaki R, Bonios M, et al. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. *J Am Coll Cardiol* 2009;54:1619-1626

105. van Tuyn J, Atsma DE, Winter EM, et al. Epicardial cells of human adults can undergo an epithelial-to-mesenchymal transition and obtain characteristics of smooth muscle cells in vitro. *Stem Cells* 2007;25:271-278

106. Mikawa T, Fischman DA Retroviral analysis of cardiac morphogenesis: discontinuous formation of coronary vessels. *Proc Natl Acad Sci U S A* 1992;89:9504-9508

107. Mikawa T, Gourdie RG Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. *Dev Biol* 1996;174:221-232

108. Gittenberger-de Groot AC, Vrancken Peeters MP, Mentink MM, Gourdie RG, Poelmann RE Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. *Circ Res* 1998;82:1043-1052

109. Vrancken Peeters MP, Gittenberger-de Groot AC, Mentink MM, Poelmann RE Smooth muscle cells and fibroblasts of the coronary arteries derive from epithelial-mesenchymal transformation of the epicardium. *Anat Embryol (Berl)* 1999;199:367-378

110. Perez-Pomares JM, Macias D, Garcia-Garrido L, Munoz-Chapuli R Contribution of the primitive epicardium to the subepicardial mesenchyme in hamster and chick embryos. *Dev Dyn* 1997;210:96-105

111. Perez-Pomares JM, Macias D, Garcia-Garrido L, Munoz-Chapuli R The origin of the subepicardial mesenchyme in the avian embryo: an immunohistochemical and quail-chick chimera study. *Dev Biol* 1998;200:57-68

112. Dettman RW, Denetclaw W, Jr., Ordahl CP, Bristow J Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intermyocardial fibroblasts in the avian heart. *Dev Biol* 1998;193:169-181

113. Kruithof BP, van WB, Somi S, et al. BMP and FGF regulate the differentiation of multipotential pericardial mesoderm into the myocardial or epicardial lineage. *Dev Biol* 2006;295:507-522

114. Christoffels VM, Grieskamp T, Norden J, et al. Tbx18 and the fate of epicardial progenitors. *Nature* 2009;458:E8-E9

115. Cai CL, Martin JC, Sun Y, et al. A myocardial lineage derives from Tbx18 epicardial cells. *Nature* 2008;454:104-108

116. Compton LA, Potash DA, Mundell NA, Barnett JV Transforming growth factor-beta induces loss of epithelial character and smooth muscle cell differentiation in epicardial cells. *Dev Dyn* 2006;235:82-93

117. Smart N, Risebro CA, Melville AA, et al. Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. *Nature* 2007;445:177-182

118. Winter EM, Grauss RW, Hogers B, et al. Preservation of left ventricular function and attenuation of remodeling after transplantation of human epicardium-derived cells into the infarcted mouse heart. *Circulation* 2007;116:917-927

119. Winter EM, van Oorschot AA, Hogers B, et al. A new direction for cardiac regeneration therapy: application of synergistically acting epicardium-derived cells and cardiomyocyte progenitor cells. *Circ Heart Fail* 2009;2:643-653

120. Weeke-Klimp A, Bax NA, Bellu AR, et al. Epicardium-derived cells enhance proliferation, cellular maturation and alignment of cardiomyocytes. *J Mol Cell Cardiol* 2010;49:606-616

121. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282:1145-1147
122. Freund C, Mummery CL Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models. *J Cell Biochem* 2009;107:592-599
123. Zhang F, Pasumarthi KB Embryonic stem cell transplantation: promise and progress in the treatment of heart disease. *BioDrugs* 2008;22:361-374
124. Xu C, Police S, Rao N, Carpenter MK Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. *Circ Res* 2002;91:501-508
125. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001;108:407-414
126. Hattori F, Chen H, Yamashita H, et al. Nongenetic method for purifying stem cell-derived cardiomyocytes. *Nat Methods* 2010;7:61-66
127. Odorico JS, Kaufman DS, Thomson JA Multilineage differentiation from human embryonic stem cell lines. *Stem Cells* 2001;19:193-204
128. 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
129. 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
130. Hao J, Daleo MA, Murphy CK, et al. Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells. *PLoS One* 2008;3:e2904
131. Dambrot C, Passier R, Atsma D, Mummery CL Cardiomyocyte differentiation of pluripotent stem cells and their use as cardiac disease models. *Biochem J* 2011;434:25-35
132. Joggerst SJ, Hatzopoulos AK Stem cell therapy for cardiac repair: benefits and barriers. *Expert Rev Mol Med* 2009;11:e20
133. Takahashi K, Yamanaka S Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-676
134. 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
135. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318:1917-1920
136. Zhang J, Wilson GF, Soerens AG, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009;104:e30-e41
137. Kane NM, Xiao Q, Baker AH, et al. Pluripotent stem cell differentiation into vascular cells: a novel technology with promises for vascular re(generation). *Pharmacol Ther* 2011;129:29-49
138. Choi KD, Yu J, Smuga-Otto K, et al. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells* 2009;27:559-567
139. 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
140. Okita K, Ichisaka T, Yamanaka S Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;448:313-317
141. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K Induced pluripotent stem cells generated without viral integration. *Science* 2008;322:945-949
142. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009;4:381-384

143. Yamanaka S A fresh look at iPS cells. *Cell* 2009;137:13-17

144. Kornowski R, Fuchs S, Tio FO, et al. Evaluation of the acute and chronic safety of the biosense injection catheter system in porcine hearts. *Catheter Cardiovasc Interv* 1999;48:447-453

145. Beeres SL, Bax JJ, Dibbets-Schneider P, et al. Intramyocardial injection of autologous bone marrow mononuclear cells in patients with chronic myocardial infarction and severe left ventricular dysfunction. *Am J Cardiol* 2007;100:1094-1098

146. Beeres SL, Bax JJ, Dibbets P, et al. Effect of intramyocardial injection of autologous bone marrow-derived mononuclear cells on perfusion, function, and viability in patients with drug-refractory chronic ischemia. *J Nucl Med* 2006;47:574-580

147. Fuchs S, Kornowski R, Weisz G, et al. Safety and feasibility of transendocardial autologous bone marrow cell transplantation in patients with advanced heart disease. *Am J Cardiol* 2006;97:823-829

148. Tse HF, Kwong YL, Chan JK, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;361:47-49

149. Losordo DW, Schatz RA, White CJ, et al. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation* 2007;115:3165-3172

150. 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

151. 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

152. Sherman W, Martens TP, Viles-Gonzalez JF, Siminiak T Catheter-based delivery of cells to the heart. *Nat Clin Pract Cardiovasc Med* 2006;3 Suppl 1:S57-S64

153. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002;106:3009-3017

154. Assmus B, Fischer-Rasokat U, Honold J, et al. Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. *Circ Res* 2007;100:1234-1241

155. Strauer BE, Brehm M, Zeus T, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. *J Am Coll Cardiol* 2005;46:1651-1658

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

157. Moelker AD, Baks T, Wever KM, et al. Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. *J Mol Cell Cardiol* 2007;42:735-745

158. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised 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

159. Freyman T, Polin G, Osman H, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J* 2006;27:1114-1122

160. 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

161. Lipinski MJ, Biondi-Zoccai GG, Abbate A, et al. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. *J Am Coll Cardiol* 2007;50:1761-1767

162. Barbash IM, Chouraqui P, Baron J, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* 2003;108:863-868

163. Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation* 2005;112:I150-I156

164. van der Spoel TI, Vrijsen KR, Koudstaal S, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischemic cardiomyopathy: A study on delivery efficiency. *J Cell Mol Med* 2012;

165. Kamihata H, Matsubara H, Nishiue T, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001;104:1046-1052

166. Balsam LB, Wagers AJ, Christensen JL, et al. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428:668-673

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

168. Shintani S, Murohara T, Ikeda H, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001;103:897-903

169. 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

170. Rehman J, Li J, Orschell CM, March KL Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003;107:1164-1169

171. 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

172. 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

173. 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

174. Yan P, Nagasawa A, Uosaki H, et al. Cyclosporin-A potently induces highly cardiogenic progenitors from embryonic stem cells. *Biochem Biophys Res Commun* 2009;379:115-120

175. Blin G, Nury D, Stefanovic S, et al. A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. *J Clin Invest* 2010;120:1125-1139

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

177. Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc Res* 2003;58:336-350

178. Tomita S, Mickle DA, Weisel RD, et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg* 2002;123:1132-1140

179. Shintani S, Murohara T, Ikeda H, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001;103:897-903

180. Mathieu M, Bartunek J, El OB, et al. Cell therapy with autologous bone marrow mononuclear stem cells is associated with superior cardiac recovery compared with use of nonmodified mesenchymal stem cells in a canine model of chronic myocardial infarction. *J Thorac Cardiovasc Surg* 2009;138:646-653

181. Fukushima S, Coppen SR, Lee J, et al. Choice of cell-delivery route for skeletal myoblast transplantation for treating post-infarction chronic heart failure in rat. *PLoS One* 2008;3:e3071

182. Muller-Ehmsen J, Krausgrill B, Burst V, et al. Effective engraftment but poor mid-term persistence of mononuclear and mesenchymal bone marrow cells in acute and chronic rat myocardial infarction. *J Mol Cell Cardiol* 2006;41:876-884

183. Waksman R, Fournadjiev J, Baffour R, et al. Transepicardial autologous bone marrow-derived mononuclear cell therapy in a porcine model of chronically infarcted myocardium. *Cardiovasc Radiat Med* 2004;5:125-131

184. Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. *Proc Natl Acad Sci U S A* 2007;104:14068-14073

185. Rota M, Padin-Iruegas ME, Misao Y, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res* 2008;103:107-116

186. Tang XL, Rokosh G, Sanganalmath SK, et al. Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. *Circulation* 2010;121:293-305

187. Lee ST, White AJ, Matsushita S, et al. Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. *J Am Coll Cardiol* 2011;57:455-465

188. Dai W, Field LJ, Rubart M, et al. Survival and maturation of human embryonic stem cell-derived cardiomyocytes in rat hearts. *J Mol Cell Cardiol* 2007;43:504-516

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

190. Fernandes S, Naumova AV, Zhu WZ, et al. Human embryonic stem cell-derived cardiomyocytes engraft but do not alter cardiac remodeling after chronic infarction in rats. *J Mol Cell Cardiol* 2010;49:941-949

191. Behfar A, Hodgson DM, Zingman LV, et al. Administration of allogenic stem cells dosed to secure cardiogenesis and sustained infarct repair. *Ann N Y Acad Sci* 2005;1049:189-198

192. Kawamoto A, Tkebuchava T, Yamaguchi J, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation* 2003;107:461-468

193. Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 2001;37:1726-1732

194. Assmus B, Tonn T, Seeger FH, et al. Red blood cell contamination of the final cell product impairs the efficacy of autologous bone marrow mononuclear cell therapy. *J Am Coll Cardiol* 2010;55:1385-1394

195. Schneider C, Jaquet K, Geidel S, et al. Transplantation of bone marrow-derived stem cells improves myocardial diastolic function: strain rate imaging in a model of hibernating myocardium. *J Am Soc Echocardiogr* 2009;22:1180-1189

196. Krause K, Schneider C, Lange C, et al. Endocardial electrogram analysis after intramyocardial injection of mesenchymal stem cells in the chronic ischemic myocardium. *Pacing Clin Electrophysiol* 2009;32:1319-1328

197. Beeres SL, Zeppenfeld K, Bax JJ, et al. Electrophysiological and arrhythmogenic effects of intramyocardial bone marrow cell injection in patients with chronic ischemic heart disease. *Heart Rhythm* 2007;4:257-265

198. Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006;355:1199-1209

199. 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

200. 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

201. 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

202. Assmus B, Rolf A, Erbs S, et al. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. *Circ Heart Fail* 2010;3:89-96

203. Clifford DM, Fisher SA, Brunskill SJ, et al. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev* 2012;2:CD006536

204. Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. *Eur Heart J* 2007;28:766-772

205. Prokopi M, Pula G, Mayr U, et al. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood* 2009;114:723-732

206. Katritsis DG, Sotiropoulou PA, Karvouni E, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv* 2005;65:321-329

207. Assmus B, Honold J, Schachinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006;355:1222-1232

208. Erbs S, Linke A, Adams V, et al. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. *Circ Res* 2005;97:756-762

209. Yao K, Huang R, Qian J, et al. Administration of intracoronary bone marrow mononuclear cells on chronic myocardial infarction improves diastolic function. *Heart* 2008;94:1147-1153

210. Strauer BE, Yousef M, Schannwell CM The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heARt failure: the STAR-heart study. *Eur J Heart Fail* 2010;12:721-729

211. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107:2294-2302

212. Pokushalov E, Romanov A, Chernyavsky A, et al. Efficiency of intramyocardial injections of autologous bone marrow mononuclear cells in patients with ischemic heart failure: a randomized study. *J Cardiovasc Transl Res* 2010;3:160-168

213. 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

214. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J* 2001;65:845-847

215. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;361:45-46

216. Patel AN, Geffner L, Vina RF, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J Thorac Cardiovasc Surg* 2005;130:1631-1638

217. Hendrikx M, Hensen K, Clijsters C, et al. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: results from a randomized controlled clinical trial. *Circulation* 2006;114:I101-I107

218. Ang KL, Chin D, Leyva F, et al. Randomized, controlled trial of intramuscular or intracoronary injection of autologous bone marrow cells into scarred myocardium during CABG versus CABG alone. *Nat Clin Pract Cardiovasc Med* 2008;5:663-670

219. Herreros J, Prosper F, Perez A, et al. Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. *Eur Heart J* 2003;24:2012-2020

220. Chachques JC, Herreros J, Trainini J, et al. Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. *Int J Cardiol* 2004;95 Suppl 1:S29-S33

221. Siminiak T, Kalawski R, Fiszer D, et al. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J* 2004;148:531-537

222. Menasche P, Hagege AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol* 2003;41:1078-1083

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

224. Ince H, Petzsch M, Rehders TC, et al. [Percutaneous transplantation of autologous myoblasts in ischemic cardiomyopathy]. *Herz* 2005;30:223-231

225. Sherman W Myocyte replacement therapy: skeletal myoblasts. *Cell Transplant* 2007;16:971-975

226. Biagini E, Valgimigli M, Smits PC, et al. Stress and tissue Doppler echocardiographic evidence of effectiveness of myoblast transplantation in patients with ischaemic heart failure. *Eur J Heart Fail* 2006;8:641-648

227. Dib N, Dinsmore J, Lababidi Z, et al. One-year follow-up of feasibility and safety of the first U.S., randomized, controlled study using 3-dimensional guided catheter-based delivery of autologous skeletal myoblasts for ischemic cardiomyopathy (CAuSMIC study). *JACC Cardiovasc Interv* 2009;2:9-16

228. Duckers HJ, Houtgraaf J, Hehrlein C, et al. Final results of a phase IIa, randomised, open-label trial to evaluate the percutaneous intramyocardial transplantation of autologous skeletal myoblasts in congestive heart failure patients: the SEISMIC trial. *EuroIntervention* 2011;6:805-812

229. Povsic TJ, O'Connor CM, Henry T, et al. A double-blind, randomized, controlled, multicenter study to assess the safety and cardiovascular effects of skeletal myoblast implantation by catheter delivery in patients with chronic heart failure after myocardial infarction. *Am Heart J* 2011;162:654-662

230. Fuchs S, Satler LF, Kornowski R, et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. *J Am Coll Cardiol* 2003;41:1721-1724

231. Tse HF, Kwong YL, Chan JK, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;361:47-49

232. Beeres SL, Bax JJ, Kaandorp TA, et al. Usefulness of intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with severe angina pectoris and stress-induced myocardial ischemia. *Am J Cardiol* 2006;97:1326-1331

233. Briguori C, Reimers B, Sarais C, et al. Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina. *Am Heart J* 2006;151:674-680

234. Beeres SL, Bax JJ, Roes SD, et al. Intramyocardial bone marrow cell transplantation and the progression of coronary atherosclerosis in patients with chronic myocardial ischemia. *Acute Card Care* 2007;9:243-251

235. Losordo DW, Henry TD, Davidson C, et al. Intramyocardial, Autologous CD34+ Cell Therapy for Refractory Angina. *Circ Res* 2011;

236. Thompson RB, Emani SM, Davis BH, et al. Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells. *Circulation* 2003;108 Suppl 1:II264-II271

237. Beeres SL, Bengel FM, Bartunek J, et al. Role of imaging in cardiac stem cell therapy. *J Am Coll Cardiol* 2007;49:1137-1148

238. Boyle AJ, Whitbourn R, Schlicht S, et al. Intra-coronary high-dose CD34+ stem cells in patients with chronic ischemic heart disease: a 12-month follow-up. *Int J Cardiol* 2006;109:21-27

239. Wang S, Cui J, Peng W, Lu M Intracoronary autologous CD34+ stem cell therapy for intractable angina. *Cardiology* 2010;117:140-147