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## Randomized Clinical Trials in Stem Cell Therapy for the Heart - Old and New Types of Cells for Cardiovascular Repair

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### 1. Introduction

Recent advances in reperfusion strategies have dramatically reduced early mortality after acute myocardial infarction (AMI), but as a result there is a higher incidence of heart failure among survivors. Optimal medical therapy and device implantation can improve the prognosis and the quality of life of these patients. Nevertheless, mortality and rehospitalization rates are still high and entail an overwhelming cost.

The field of cardiac cell therapy has emerged as a new alternative in this situation, and has made rapid progress. Its final goal is to repair the damaged myocardium and to restore cardiac function. Nevertheless, this goal is hindered by the massive loss of cardiomyocytes after an AMI (in the order of 1 billion cells) and because ischemic myocardium loses cellular and extracellular signals which guide stem cells to the cardiac lineage or to the secretion of paracrine factors (Wollert & Drexler, 2010).

Studies evaluating this new approach during the last 15 years have overall succeeded to a greater or lesser extent, and evidence available so far is encouraging. Phase I and II randomized clinical trials (RCT) indicate that cell therapy is a safe treatment which can improve cardiac function after AMI and in the chronic phase of coronary artery disease (CAD). Trial results are not uniform, however, probably due 1) to a lack of standardization of cell isolation and delivery procedures, 2) to the absence of a universally accepted nomenclature, and 3) to the large number of stem cell types under investigation in different clinical settings. Nevertheless, these inconsistencies can be avoided or reduced if classical scientific methodology is followed. Although considered a relatively new field of research, stem cell experimentation must invariably walk on the path of the scientific method. Since Aristotle's time, scientific method has been used as a way to ask and answer scientific questions by making observations and doing experiments. It includes a series of steps, i.e. 1) asking a question, 2) doing background research, 3) constructing a hypothesis, 4) testing the hypothesis by doing an experiment, 5) analyzing the data and drawing a conclusion, and 6) communicating the results.

In the case of stem cell therapy, RCT started questioning if there was a possibility of repairing the heart after different types of tissular damage. Background evidence has already demonstrated that this possibility exists through stem cell administration in several preclinical models of cardiomyopathy. Thus, the key points in the design of present and future RCT in humans are 1) to formulate an adequate hypothesis, 2) to select the ideal population, cell type and delivery method and 3) to develop a correct and precise protocol. These decisions must be made in the light of previous evidence and with a translational mentality, in which experimental/preclinical data should help to design new RCT. Inversely, results of human studies should transfer new questions and hypothesis to the laboratory/bench side.

## **2. Types of stem cells in cardiovascular research. Preclinical background**

There are several types of stem cells which have been used with the aim of repairing damaged myocardium. Broadly speaking, stem cells can be subdivided into two large groups: embryonic and adult.

### **2.1 Embryonic stem cells**

Embryonic stem cells (ESC) are present in the earliest stage of embryonic development – the blastocyst (this is the stage before the embryo is implanted into the uterine wall, one week after fertilization). ESC can divide indefinitely in vitro and are pluripotent: they are capable of generating any terminally differentiated cell in the human body that is derived from any one of the three embryonic germ layers (Perin & Silva 2006).

Classic experimental studies have demonstrated cardiomyocyte obtention from ESC, with the same structural and functional properties as cardiomyocytes present in the cardiac muscle (Kehat et al., 2001), and showing even successful electromechanical coupling with host myocardium. After their transplantation into the infarcted myocardium, these cells can engraft and survive in the myocardial tissue network, providing an improvement in ventricular function in several small animal preclinical models (Klug et al., 1996; Min et al., 2002). On the other hand, in chronic heart failure models, ESC have been proved to differentiate into new cardiomyocytes and also into endothelial and smooth muscle cells forming new blood vessels (Yamashita et al., 2000).

Nevertheless, despite the enormous evidence available regarding ESC isolation, growth, differentiation and transplantation in animal models, there are several issues that have restricted their clinical applications in human RCT in most countries: ethical and legal considerations, limited numbers of cell lines, limited resources, the difficulty of obtaining autologous ESC, the need for immunosuppression in case of allogenic cells and the potential risk for tumorigenesis (Thomson et al., 1998).

### **2.2 Adult stem cells**

Adult stem cells are intrinsic to specific tissues of the postnatal organism into which they are committed to differentiate. Theoretically, adult stem cells are capable of self-renewal, yielding mature differentiated cells which are: 1) integrated into a particular tissue and 2) capable of performing the specialized function of that tissue. Adult-tissue specific stem cells are present in organs with self-renewal capacity, including the liver, pancreas, skeletal muscle, skin and bone marrow. These are the most investigated cells in regenerative medicine and include different cellular types:

### 2.2.1 Skeletal myoblasts

Skeletal myoblasts are satellite cells that remain in a quiescent stage between the basal lamina and the sarcolemma on the periphery of mature skeletal muscle fibers. They can be obtained from muscular biopsies and easily expanded in vitro (Murry et al., 1996; Taylor et al., 1998). This kind of stem cells has received considerable attention in the setting of chronic ischemic ventricular dysfunction, because these cells: 1) do not need a specific microenvironment to differentiate, 2) can be expanded without problems in undifferentiated stages, and 3) are highly resistant to ischemia and can multiply after injury. These cells are programmed to differentiate into myogenic lineages and, after their transplantation into scarred myocardium, they become myotubes and myocytes with the characteristics and function of skeletal muscle (Leobon et al., 2003; Pagani et al., 2003). In other words, transdifferentiation into cardiomyocytes, which was their first hoped mechanism of action, is an exceptional event rarely seen at the graft-host interface. Therefore, their beneficial effect is thought to be mediated through myocyte contraction, paracrine effects and by an increase in the infarcted wall resistance and stiffness, thus limiting ventricular dilatation and adverse remodeling. Safety concerns include basically the risk of ventricular arrhythmias observed in the first clinical trials, due to the absence of electromechanical coupling of the myoblasts with another cells or with host myocardium. As a consequence, in most of these protocols patients were treated at the same time with prophylactic implantable cardiac defibrillators and/or chronic amiodarone. Administration of skeletal myoblasts after certain culture conditions (with autologous human serum) could avoid this risk (Herrerros et al., 2003).

### 2.2.2 Bone marrow-derived cells

The bone marrow is one of the most investigated sources of adult stem cells. It is a complex organ with a specific geometric organization and an intricate system of cell-to-cell interaction and signaling, and it contains several cell subpopulations: 1) differentiated cells like stromal supporting mesenchymal cells, vascular cells, adipocytes, osteoblasts and osteocytes, and 2) progenitor cells. The most important subpopulation is the bone marrow mononuclear cell (BMMC) fraction, that includes mesenchymal stem cells (MSC), hematopoietic progenitor cells (HPC) and endothelial progenitor cells (EPC) (Shizuru et al., 2005; Suva et al., 2004). It also contains more committed cell lineages, such as natural killers, T and B lymphocytes. However, after the finding that MSC and EPC represent, respectively, only 0.01% and 1-2% of the total amount of cells included in BMMC fraction, culture techniques have been developed to select a specific type of cell and to expand it to obtain solutions with higher numbers of cells.

BMMC can be easily harvested, isolated, in vitro expanded and administered to the patient with several delivery devices. The plasticity of these cells has been demonstrated in classic preclinical studies, showing even transdifferentiation into mature cells from different germinal layers.

It has been observed that BMMC contribute to the formation of new cardiomyocytes and endothelial cells in ischemic areas when injected after an AMI in animal models. In treated animals, an improvement of hemodynamic parameters was demonstrated (Jackson et al., 2001; Orlic et al., 2001a).

Regarding HPC, no real specific surface antigen has been described to identify these cells. The antigens CD34, CD133 and CD117 and the surface markers c-kit, Sca-1 and Thy-1 have been used to sort this subtype of BMMC. C-kit<sup>+</sup> cells without hematopoietic markers (lin<sup>-</sup>) have been administered after AMI, with a noticeable increase in the number of

cardiomyocytes in the infarcted area, improvement of the ventricular function and survival benefits (Orlic et al., 2001b). However, other researchers have proved differentiation of these HPC into hematopoietic lineages and with the same results of reversing adverse remodeling and preventing post-infarction ventricular dysfunction (Balsam et al., 2004). Finally, improvements of myocardial perfusion, capillary density/collateral vessel formation and left ventricular ejection fraction have also been observed in other studies after HPC injection in AMI models, with a significant reduction of the infarct size (Kamihata et al., 2001).

EPC were first defined as cells positive for both hematopoietic stem cell markers such as CD34 and endothelial marker proteins such as vascular endothelial growth factor receptor 2 (VEGFR-2) or CD133. CD133 (also known as prominin or AC133) is expressed on HPC and on early stages of EPC, but is absent on mature EPC and monocyte cells. CD133+ cells promote angiogenesis in ischemic tissues, differentiating into adult endothelial cells and secreting angiogenic factors. The main drawback of CD133+ cells is their low number in the BMNC fraction (they represent less than 1% of the total population) and the impossibility of *in vitro* expansion. In fact, cytokine treatment with granulocyte colony-stimulating factor (G-CSF) is needed in order to mobilize a sufficient number of cells from the bone marrow (Virmani et al., 2006).

EPC have similar phenotypic and functional characteristics to those shown by fetal angioblasts. Indeed, they arise from a common hemangioblast precursor in the adult bone marrow (Virmani et al., 2006). Endothelial cell lineage markers used to sort these cells include CD34, Flk-1, VE-cadherin, platelet-endothelial cell adhesion molecule 1 (PECAM-1), von Willebrand factor (vWF) and E-selectin. Conversely to what happens with other stem cells, EPC have been proved to transdifferentiate into cardiomyocytes and smooth muscle cells *in vivo*. Besides, they increase capillary density after an AMI by means of angiogenesis and arteriogenesis mechanisms, reduce collagen deposits and cardiomyocyte apoptosis, and improve cardiac function (Kocher et al., 2001). Again, the ability to expand these cells is limited by their scarcity in peripheral blood. Furthermore, functional impairment of EPC has been observed in the elderly and in several pathologic conditions (i.e., diabetes mellitus). Recently, specific subpopulations of EPC have been described: CD14+/CD34- cells, which have shown very high plasticity (Yoon et al., 2005), and CD14+/CD34+ cells, which have been demonstrated to induce a paracrine response by releasing angiogenic growth factors.

MSC are one of the most promising types of stem cells for cardiac repair. They have been isolated from the bone marrow stroma (although they can also be found around blood vessels, in muscle, skin and adipose tissue) and exhibit unique characteristics, such as a multipotent differentiation potential capacity and the lack of surface markers. Indeed, they are CD34-, CD45- and CD133- cells that show an immunophenotype positive for adhesion proteins like CD29, CD44, CD71, CD90, CD105, CD106, CD117, CD120a, CD124, SH2, SH3 and SH4 (Pittenger & Martin, 2004). This is the reason why MSC can be isolated after density centrifugation by means of their ability to adhere to culture plates. Purified human MSC have been shown to migrate and differentiate to a cardiomyocyte phenotype and to endothelial cells in both healthy and infarcted myocardium (Toma et al., 2002). In the former MSC express cardiac surface markers, and in the latter they improve wall motion and prevent the adverse remodeling process. One advantage of MSC is that they are considered to be immuneprivileged. Thus, allogenic MSC have been successfully transplanted into murine hearts without the need for immunosuppression (Zimmet & Hare, 2005).

### 2.2.3 Adipose-derived stem cells

Recently, it has been shown that, besides committed adipogenic, endothelial and pluripotent vascular progenitor cells, the adipose tissue stroma contains multipotent adipose-derived stem cells (ADSC) (Sanz-Ruiz et al., 2009). These self-renewal cells possess high potentiality, and are capable of differentiating into myogenic, neural and cardiomyocytic lineages, in this last case showing even spontaneous beating. The phenotype of ADSC is similar to that of MSC (i.e., ADSC also express adhesion molecules in their surface) (Sanz-Ruiz et al., 2008). The similarities of both cell types in terms of potentiality and the fact that adipose tissue can be easily obtained in large amounts by means of a simple liposuction procedure, have pointed at the human adipose tissue as a novel promising alternative source of stem cells for cardiovascular repair that has shown encouraging results in the preclinical field (Fraser et al., 2004). In fact, in our center two first-in-man phase II randomized trials have been conducted with ADSC: with intracoronary administration after an AMI (APOLLO trial) and with transendocardial injection in “no-option” patients with advanced CAD (PRECISE trial).

### 2.2.4 Resident cardiac stem cells

A newly discovered population of resident cardiac stem cells (CSC) has been recently identified in the adult heart, which contributes to myocardial regeneration in animal models of AMI (Beltrami et al., 2003; Oh et al., 2003; Urbanek et al., 2003; Urbanek et al., 2005). These cells express surface markers such as c-kit, Sca-1 and Abcg2. However, the phenotypic characterization of this lineage is not definitely clear. Indeed, currently all CSC types are thought to be distinct from each other. In the adult human heart, these clusters of CSC have shown their capacity to differentiate into cardiomyocytes, to fuse with pre-existing cardiomyocytes and to differentiate also into smooth muscle and endothelial cells, providing both myocardial and vascular regeneration. A novel cardiac cell type has been identified using the transcription factor islet-1 (isl1). These isl1<sup>+</sup> cells have been proposed even for pacemaker cell and conduction system repair (Laugwitz et al., 2005). Finally, CSC have been isolated and expanded for the first time from human myocardial biopsies, offering another new source of stem cells in cardiovascular regenerative medicine (Messina et al., 2004).

### 2.2.5 Cord-blood derived stem cells

The umbilical cord blood contains a high proportion of hematopoietic and mesenchymal stem cells, and in higher numbers than in peripheral blood or in the bone marrow. Also known as “somatic non-restricted stem cells”, these cells are negative for c-kit, CD34 and CD45, and can differentiate into cardiomyocytes. They have been administered after an AMI in animal models, improving ventricular perfusion and contractility, and reducing the infarct size (Ma et al., 2005). Nevertheless, cord-blood stem cells have not been used in clinical trials so far.

## 2.3 Induced or “embryonic-like” stem cells

At the end of 2007, two groups reported for the first time the generation of pluripotent stem cell lines derived from adult human cells (Takahashi et al., 2007; Yu et al., 2007). Differentiated adult somatic human cells were successfully reprogrammed into a pluripotent state by transduction of four defined transcription factors. The resulting induced-pluripotent stem cells (iPS) were proved to have the same morphological

characteristics, surface markers, proliferative capacity and potentiality as those known in ESC. In the first study (Takahashi et al., 2007), the factors Oct3/4, Sox2, Klf4 and c-Myc were transduced in human dermal fibroblasts, and in the second one (Yu et al., 2007), Oct4, Sox2, NANOG and LIN28 were induced in the same type of adult cells. These iPS can differentiate into any kind of cell from any of the three embryonic germ layers, but two safety concerns have arisen: 1) the risk of mutations after viral transfection, and 2) the risk of teratoma formation. In other words, although these studies have meant a revolution in the field of regenerative medicine, we still have a long way to cover before their application in human pathology.

### **3. Host tissue and cell related issues. Understanding the ischemic myocardium**

The two main determiners of cardiovascular repair are stem cells and injured myocardial tissue in which these cells are delivered. Both play the central role that will establish the efficacy of the treatment, and knowledge of the molecular/cellular changes and interactions between them is crucial when designing new RCT.

After AMI, if blood flow is not restored quickly, cell death and myocardial necrosis are definitive. This activates a complement cascade with free radical and cytokine generation that recruit leukocytes and initiate the inflammatory response. Inflammation, while potentially detrimental to surviving cardiomyocytes, is necessary to clear away the debris (clearance of necrotic cells) and orchestrate downstream healing events. Chronic inflammatory cells such as macrophages and mast cells secrete cytokines and growth factors, which in turn activate fibroblasts to proliferate and synthesize collagen, a major component of the scar that replaces cardiomyocyte loss. Neovascularization is also stimulated by the release of growth factors from the inflammatory cells. Scar remodeling may continue for months to years, depending on the extent of the initial ischemic event (Lindsey et al., 2003).

Left ventricle (LV) remodeling, defined as post-AMI changes in wall structure, chamber geometry and pump function, is mainly caused by changes in extracellular matrix (ECM). Cardiac ECM not only supports and aligns cardiomyocytes, thereby preserving a fundamental mechanical relationship by which sarcomeric shortening is translated to muscle force contraction, but also has signaling functions. Indeed, ECM is a storage depot for growth factors, hormones and cytokines, and uses integrins to communicate with cells (Lindsey et al., 2003). All these functions are lost after myocardial ischemia due to the release from inflammatory and endogenous cells of matrix metalloproteinases (MMP) and cytokines. MMP degrade ECM, disengage integrins and stimulate reparative fibrosis. Cytokines like tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukins like IL-1 and IL-6 induce MMP synthesis and are related to the development of LV dysfunction, pulmonary edema, endothelial dysfunction and cardiomyocyte apoptosis (Dewald et al., 2004).

These cellular and signaling processes that constitute the proliferative phase of infarct healing in the myocardium influence and determine the fate of implanted stem cells. Ischemic myocardium constitutes an inflammatory hostile environment for stem cells, which is devoid of nutrients and oxygen and lacks survival signals from the ECM and cell-to-cell interactions. Indeed, only a small fraction of them survive in such adverse conditions. Nevertheless, some studies have shown that certain implanted stem cells may improve or counteract this situation. Intramyocardial transplantation of EPC after AMI induces

significant and sustained increase in angiogenic, antiapoptotic and chemoattractant factors, that are up-regulated in both transplanted and host cells (i.e., vascular endothelial growth factor-A [VEGF-A], fibroblast growth factor-2 [FGF-2], angiopoietin-1 [Ang-1], angiopoietin-2 [Ang-2], placenta growth factor [PIGF], hepatocyte growth factor [HGF], insulin-like growth factor-1 [IGF-1], platelet-derived growth factor-B [PDGF-B] and stromal cell-derived factor-1 [SDF-1]) (Cho et al., 2007). These humoral factors provide an additional favorable milieu for neovascularization and repair or regeneration of ischemic myocardium. Furthermore, there is a cross-talk between the heart and the bone marrow mediated by humoral effects that may improve this therapeutic effect: it has been proved that EPC transplantation further mobilizes endogenous BMMC into peripheral circulation, recruiting them into the ischemic myocardium (Cho et al., 2007).

Having these considerations in mind, new lines of research are being developed to improve cell survival rates in the ischemic myocardium, between them (Wollert & Drexler 2010):

1. Preconditioning of the myocardium to retain a higher number of cells: low-energy shock waves, ultrasound-mediated destruction of microbubbles in the coronary circulation and extracorporeal shock wave treatment have proved to increase retention of EPC, BMMC and MSC.
2. Activation or increase of chemotactic factors to attract cells to the damaged area: high mobility group box-1 (HMGB-1), SDF-1 or its receptor CXCR4,  $\beta_2$  integrin and endothelial nitric oxide synthase can be activated to increase the rate of homing of different types of stem cells (i.e., progenitor blood cells, EPC).

Regarding stem cells administered to the myocardium, their functional activity is determined by age and cardiovascular risk factors. As a consequence, future phase II-III RCT will explore cell enhancement strategies intended to increase their therapeutic potential. Several strategies are currently under investigation (Wollert & Drexler 2010):

1. Pretreatment of the patients with drugs to stimulate cell potenciality: statins, rosiglitazone and nitric oxide synthase enhancer AVE9488 can improve the migratory, invasive and neovascularization capacity of EPC.
2. Strategies to prolong cell survival: between them, the use of a combination of growth factors to stimulate the expression of cardiomyocyte genes in MSC, the use of heat shock to increase the resistance of cells to external stressors and the pretreatment of ESC-derived cardiomyocytes with heat shock and a cocktail of survival factors, are being explored.
3. Genetic modification of the cells prior to administration: overexpression of antiapoptotic genes or genetic manipulation to maintain cell's functionality (i.e., capacity to secrete paracrine mediators, to connect with host myocardium or to differentiate into specialized cardiac cell types) can be achieved through genetic cell engineering.
4. Combined injection of cells and biomaterials: BMMC encapsulation within scaffolds (epicardial patches) or peptide nanofibers represents another strategy that needs further investigation.

#### 4. Patient selection and delivery methods

Patients with larger AMI or with severely depressed baseline left ventricular ejection fraction (LVEF) and stroke volumes, or those with transmural extent of the infarct seem to benefit the most after BMMC treatment (Wollert & Drexler 2010). Conversely, patients with

microvascular obstruction may not respond to intracoronary infusion of cells. Therefore, patient selection before conducting a RCT must take into account the pathophysiologic basis of the disease and baseline characteristics of the patients. For instance, it is well known that age, cardiovascular risk factors and previous heart failure have a negative impact on the potentiality and functional capacity of most types of stem cells in cardiovascular research. On the other hand, exploration of new delivery methods is mandatory, due to the low rate of cell retention, engraftment and survival in the myocardium with the present routes of administration. New devices include transcatheter arterial injection into the perivascular space, improvements in transendocardial injection needle design and the fusion of different imaging techniques for a more precise delivery (i.e., X-ray/MRI suites used in conjunction with electroanatomic maps of the LV).

5. Mechanisms of action

Nowadays, it is believed that stem cell therapy could lead to successful cardiac regeneration or repair by any or a combination of three main general mechanisms (figure 1): 1) differentiation of the administered cells into all of the cellular constituents of the heart (i.e., cardiomyogenesis and vasculogenesis processes), or, less probably, fusion of the administered cells with those, 2) release of factors capable of paracrine signaling from the administered cells and 3) stimulation of endogenous repair by injected cells, through stem cell cardiac niches activation (Mazhari & Hare, 2007).

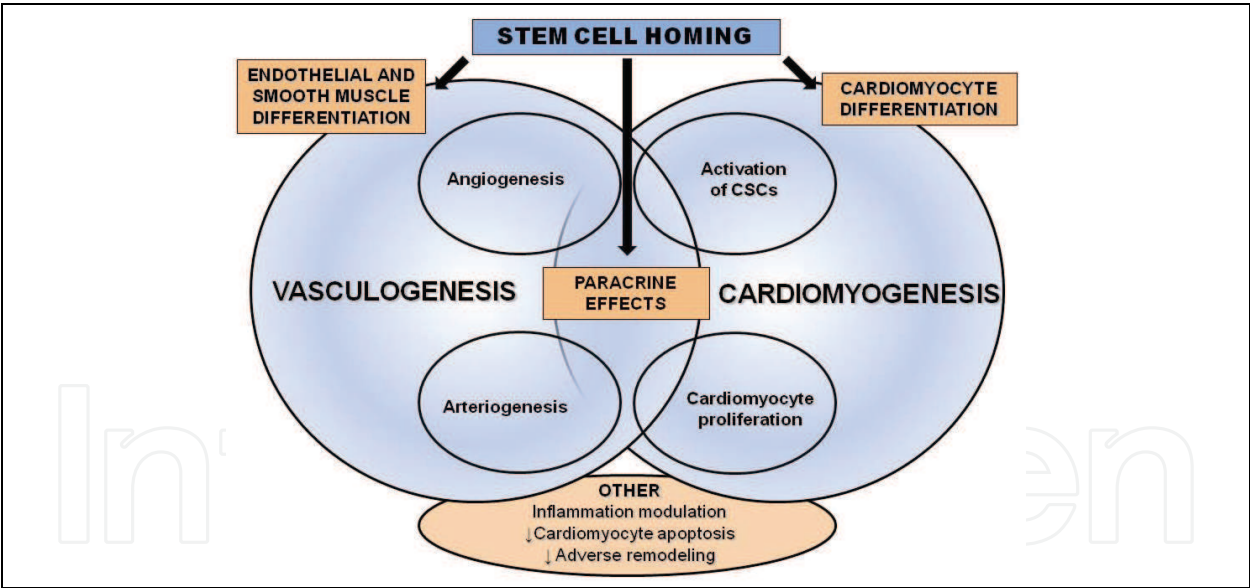


Fig. 1. Proposed mechanisms of stem cell function after homing into the damaged heart. Note that differentiation processes and paracrine effects activate a cascade of events that interact actively to create new blood vessels and cardiomyocytes, with the final objective of functional cardiac repair. CSCs: cardiac stem cells.

5.1 Cardiomyogenesis and vasculogenesis.

While in the classic studies of the beginning of the decade (trans)differentiation of BMMC into cardiomyocytes, smooth muscle cells and endothelial cells was postulated as the main mechanism that might explain the cardiac recovery resulting from stem cell therapy, this

phenomenon has been demonstrated in low proportions in more recent studies. Regarding cellular fusion of administered cells with host myocardial ones, to date there is little evidence to support this hypothesis.

## 5.2 Paracrine actions

Given that differentiation debate is still ongoing and that the number of newly generated cardiomyocytes and blood vessels is too low to explain significant functional improvements, the paracrine hypothesis is now considered the most plausible. According to this idea, the functional benefits of stem cells might be related to secretion of soluble factors that, acting in a paracrine fashion, protect the heart, attenuate pathological LV remodeling, induce neovascularization and promote regeneration (Gnecchi et al., 2008). BMMC and MSC have been extensively studied and proved to produce and secrete a broad variety of cytokines, chemokines and growth factors, between them VEGF, FGF, HGF, IGF, adrenomedullin, thymosin  $\beta$ 4 (TB4), SDF-1, PDGF and angiopoietin. These paracrine mediators are expressed/released in a temporal and spatial manner exerting different effects depending on the microenvironment after injury. In addition, these released factors may have autocrine actions on the biology of stem cells themselves (Gnecchi et al., 2008).

The paracrine factors may influence adjacent cells and exert their actions via several mechanisms, including:

1. Myocardial protection: MSC and BMMC in an ischemic environment release cytoprotective molecules that increase cardiomyocyte survival (VEGF, FGF, HGF, IGF-1, TB4, SDF-1, PDGF and IL-1).
2. Neovascularization: BMMC, MSC and EPC can give rise to vascular structures. The molecular processes leading to angiogenesis and arteriogenesis involve mediators such as nitric oxide, VEGF, SDF-1, FGF, HGF and angiopoietin.
3. Cardiac remodeling: paracrine factors released by transplanted stem cells may alter the ECM (i.e., inhibiting cardiac fibroblast proliferation and types I and III collagen synthesis), resulting in more favorable post-AMI remodeling and strengthening of the infarct scar. Stem cells (MSC) may also produce molecules that limit local inflammation, thus reducing the remodeling process.
4. Cardiac contractility and metabolism: it has been demonstrated that stem cell therapy limits infarct size and prevents LV dysfunction. On the other hand, MSC and BMMC secrete inotropic factors (i.e., IGF-1) that positively modulate cell contractility, and these cells attenuate the profound bioenergetic abnormalities found in the border zone of myocardial infarction.
5. Cardiac regeneration: differentiation and cell fusion with native cardiomyocytes occur in very low rates after stem cell administration. Therefore, it is now believed that exogenous stem cell transplantation may activate resident CSC and/or stimulate cardiomyocyte replication via paracrine action. Factors secreted by BMMC, MSC and EPC, including HGF, SDF-1, VEGF and IGF-1, enhance proliferation, mobilization, differentiation, survival and function of CSC or even restoration of cardiac stem cell niches.

## 5.3 Endogenous repair

As we have seen, clonogenic and self-replicating endogenous CSC have been isolated and cultured from human hearts. These CSC – located in cardiac stem cell niches – have the

capacity to differentiate into endothelial cells, smooth muscle myocytes and cardiomyocytes. Though insufficient for a complete repair of the myocardium after any kind of insult, these cells can be activated by extracardiac delivered cells. Thus, administered allogeneic MSC participate in maintaining stem cell niches, and through cell-to-cell interactions – apart from paracrine effects – may not only restore lost cellular constituents (differentiation) but also these niches with an ongoing and regulated self-replicating capacity (Mazhari & Hare, 2007).

## **6. Clinical scenarios in stem cell therapy. Evidence available**

Stem cell therapy has accumulated growing evidence in different pathophysiological conditions in small and large animal models, but human research has been almost limited to CAD. In this chapter we will focus on the largest and most relevant randomized placebo-controlled clinical trials in humans (tables 1 through 4).

Natural history of CAD can be divided into acute (AMI) and chronic phases (chronic ischemic heart disease). In the latter stem cell therapy has been investigated in the subset of 1) ischemic heart failure (ventricular dysfunction) and 2) chronic myocardial ischemia (refractory angina).

In patients where restoration of contractile function is the clinical goal – such as those with end-stage ischemic heart failure or those early post-infarction – delivering cells with contractile potential may be of high priority. Under these conditions, naturally myogenic cells (i.e., skeletal myoblasts, cardiomyocytes or any progenitor cell driven down a muscle lineage) appear to be the first choice. However, on the one hand, formation of new myocardial mass has only been strictly established for ESC, and is a process that has been achieved in very few trials and in small percentages with adult stem cells. And on the other hand, most of the studies after AMI have used BMMC as an easily accessible source of adult stem and progenitor cells.

In conditions where chronic ischemia prevails, the angiogenic potential of the cells seems a more reasonable approach. In this case, BMMC, EPC, vascular progenitor cells or blood-derived multipotent adult progenitor cells and MSC may be better choices than myogenic precursors.

### **6.1 Stem cell therapy after acute myocardial infarction**

Several trials have evaluated stem cell therapy after AMI, some with positive results and some with neutral ones. All of them used the intracoronary route, once the patency of the infarct-related artery was restored, and most of them with the mononuclear fraction of the bone marrow (table 1). Four main RCT have been published with positive findings so far. In the BOOST trial (Wollert et al., 2004), BMMC were proved to improve left ventricular contractility in the infarct border zone and global LVEF by 6%. However, only patients with larger infarcts showed maintained benefits in terms of LVEF at long follow-up (18 months). In the REPAIR-AMI trial (Schachinger et al., 2006), infusion of BMMC promoted an increase in LVEF of 2.8% at 12 months. The FINCELL trial (Huikuri et al., 2008) reported an improvement of 5% in LVEF after BMMC delivery. Finally, in the REGENT trial (Tendera et al., 2009), patients treated with BMMC and with CXCR4<sup>+</sup>/CD34<sup>+</sup> BMMC showed an increase of 3% in LVEF which was not observed in the control group, but these differences were not significant between treated and control patients at 6-months follow-up. This trial was limited by imbalances in baseline LVEF and by incomplete follow-up.

Trial (year)	n	Cell type	Cell count	Days after AMI	Primary endpoint (follow-up)	Comments
Chen (2004)	69	MSC	9 x 10 <sup>9</sup>	18	Improved LVEF at 6m	LVEF by echocardiography
BOOST (2004)	60	BMMC	2 x 10 <sup>9</sup>	6±1	Improved LVEF at 6m	Effect diminished after 18/61m
REPAIR-AMI (2006)	187	BMMC	2 x 10 <sup>8</sup>	3-6	Improved LVEF at 4m	LVEF by ventriculography
Janssens (2006)	66	BMMC	2 x 10 <sup>8</sup>	1	No change LVEF at 4m	Improved regional contractility and reduction in infarct size
ASTAMI (2006)	97	BMMC	7 x 10 <sup>7</sup>	6±1	No change LVEF at 6m	LVEF ↑8% by SPECT, ↑1% by MRI
TCT-STAMI (2006)	20	BMMC	4 x 10 <sup>7</sup>	1	Improved LVEF at 6m	LVEF by echocardiography
FINCELL (2008)	77	BMMC	4 x 10 <sup>8</sup>	3	Improved LVEF at 6m	LVEF by ventriculography
Meluzin (2006)	66	BMMC	1 x 10 <sup>7</sup> (low d) 1 x 10 <sup>8</sup> (high d)	7	Improved LVEF at 3m in high dose group	LVEF by SPECT
Penicka (2007)	27	BMMC	3 x 10 <sup>9</sup>	9	No change LVEF at 4m	LVEF by echocardiography
HEBE (2008)	189	BMMC vs PBC	-	3-8	No changes in global or regional LV function	Final results pending
REGENT (2009)	117	BMMC (unselected, CD34 <sup>+</sup> /CXCR4 <sup>+</sup> )	2 x 10 <sup>8</sup> (unsel), 2 x 10 <sup>6</sup> (CD34 <sup>+</sup> )	3-12	Improved LVEF with both cell types	LVEF by MRI (in 117/200 patients)

MSC: mesenchymal stem cells (bone marrow origin); BMMC: bone marrow mononuclear cells; PBC: peripheral blood cells; LVEF: left ventricular ejection fraction; LV: left ventricle; SPECT: single-photon emission computed tomography; MRI: magnetic resonance imaging.

Table 1. Randomized clinical trials with stem cells in patients with acute myocardial infarction (intracoronary delivery).

On the other hand, three RCT resulted in neutral findings. Janssens et al. (Janssens et al., 2006) reported no changes in LVEF after BMMC infusion, but a reduction in the infarct volume and an improvement in regional contractility in the greatest transmural infarct cases were observed in treated patients. In the ASTAMI trial (Lunde et al., 2006) no significant

effects on LVEF, LV volumes, or infarct size were observed after BMMC administration. The smaller number of cells and differences in the cell isolation protocol were invoked to explain these findings. Finally, in the HEBE trial (van der Laan et al., 2008), presented at the AHA Scientific Sessions in 2008, no changes in global or regional LV systolic function were reported after BMMC and mononuclear cells isolated from peripheral blood injection.

So far, no safety concerns after BMMC intracoronary infusion have emerged. The risk of a higher rate of instant restenosis was not confirmed in the FINCELL trial (Huikuri et al., 2008) and in two recent meta-analyses (Lipinski et al., 2007; Martin-Redon et al., 2008). Moreover, none of the trials reported an increased incidence of malignant arrhythmias with BMMC (Wollert & Drexler, 2010).

Two trials have used MSC after AMI. The study by Chen et al. (Chen et al., 2004) demonstrated an improvement in LVEF and perfusion with intracoronary infusion of these cells, but these results have not been duplicated. Hare et al. (Hare et al., 2009) intravenously administered allogeneic MSC after an AMI with no higher rate of MACE and some benefits in terms of LVEF.

New types of cells are also being explored, like ADSC (figure 2). No evidence is available to date, but the first-in-man RCT with intracoronary administration of freshly isolated ADSC after AMI (the APOLLO trial) has been recently completed.

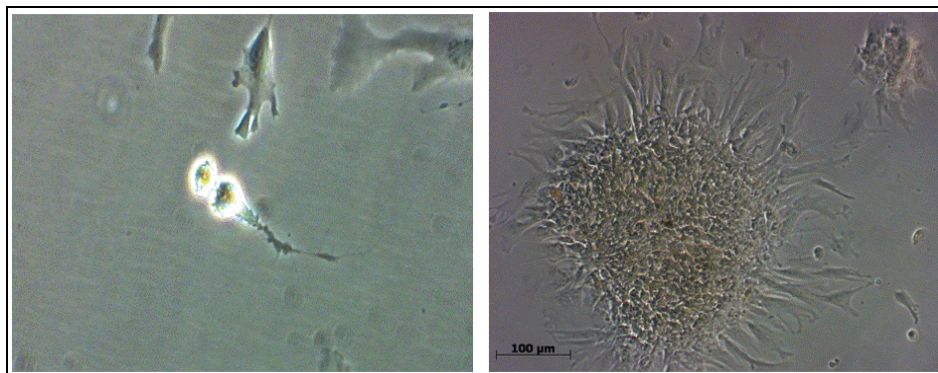


Fig. 2. Autologous adipose-derived mesenchymal stem cells during mitosis (left panel) and growing in colonies in the 6th day of culture (magnification  $\times 10$ , right panel). These cells were expanded from the adipose tissue stroma-vascular fraction under good manufacturing practice (GMP) conditions in our Cell Production Unit (Hospital Gregorio Marañón, Madrid).

Another approach for stem cell therapy after AMI is cell mobilization from the bone marrow with the administration of G-CSF. Several RCT have been published, but results have been somehow less encouraging (table 2). Only three trials have reported positive results. In the FIRSTLINE-AMI trial (Ince et al., 2005), the RIGENERA study (Leone et al., 2007) and in the study by Takano et al. (Takano et al., 2007), significant improvements in LVEF were observed. The rest of the trials showed negative findings.

Finally, the MAGIC trials used a combination of G-CSF and intracoronary injection of peripheral blood progenitor cells. In the first trial no differences in LVEF were noted, and an increase in instant restenosis rate was observed (G-CSF administration before bare-metal stent implantation) (Kang et al., 2004). Then the investigators changed the design and used drug-eluting stents. In the MAGIC 3-DES trial, positive results in terms of LVEF were found after mobilization and intracoronary injection of isolated cells (Kang et al., 2006).

Trial (year)	n	Dose	Timing after AMI	Follow-up	Comments
Valgimigli (2005)	20	5 µg/kg x 4 d	1 d	No change LVEF at 6m	LVEF by SPECT
FIRSTLINE -AMI (2005)	50	10 µg/kg x 6 d	90 min	Improved LVEF at 4m	LVEF by echocardiography
REVIVAL-2 (2006)	114	10 µg/kg x 5 d	5 d	No change LVEF at 5m	LVEF by MRI
STEMMI (2006)	78	10 µg/kg x 6 d	28 h	No change LVEF at 6m	LVEF by echocardiography and MRI
G-CSF- STEMI (2006)	44	10 µg/kg x 5 d	35 h	No change LVEF at 3m	LVEF by MRI
Ellis (2006)	18	5µg/kgx5d (low d), 10µg/kgx5d (high d)	< 30 h	Improved LVEF at 30d	LVEF by echocardiography
RIGENERA (2007)	41	10 µg/kg x 5 d	5 d	Improved LVEF at 6m	LVEF by echocardiography
Takano (2007)	40	2,5 µg/kg x 5 d	1 d	Improved LVEF at 6m	LVEF by SPECT
MAGIC (2004)*	27	10 µg/kg x 4 d; PBC: 1 x 10 <sup>9</sup>	1 d	No change LVEF at 6m	LVEF by SPECT
MAGIC 3- DES (2006)*	50	10 µg/kg x 3 d; PBC: 2 x 10 <sup>9</sup>	1 d	Improved LVEF at 6m	LVEF by MRI

Table 2. Randomized clinical trials with granulocyte colony-stimulating factor in patients with acute myocardial infarction (subcutaneous). \*: MAGIC trials used a combination of indirect mobilization (G-CSF) and direct intracoronary injection of peripheral blood cells (PBC); LVEF: left ventricular ejection fraction; SPECT: single-photon emission computed tomography; MRI: magnetic resonance imaging.

6.2 Stem cell therapy for chronic ischemic heart disease  
6.2.1 Ischemic heart failure

Skeletal myoblasts and BMSC have been used in heart failure patients (table 3). The MAGIC trial (Menasche et al., 2008), with transepical injection of skeletal myoblasts during coronary artery bypass graft surgery, reported no changes in global or regional contractility. However, a reduction in LV end-diastolic and end-systolic volumes was observed in the high-dose group. Moreover, a trend towards a higher incidence of ventricular arrhythmias was noted. Dib et al.(Dib et al., 2005) reported an improvement in LVEF and viability after myoblasts transendocardial injection, in contradiction with the SEISMIC trial (presented by Serruys at the 2008 ACC meeting) which showed no benefit of the same procedure at 6 months.

In the TOPCARE-CHD trial (Assmus et al., 2006), BMSC intracoronary delivery into the coronary artery supplying the most dyskinetic LV area showed an increase in LVEF of 2.9%,

whereas progenitor circulating cells infusion and controls did not show any positive change. No major adverse cardiac events (MACE) were reported in this trial. Another trial has been recently published with BMMC by the group of Strauer (the STAR-heart study) (Strauer et al., 2010). Although it lacks of a randomized design, this study represents the largest trial with BMMC in patients with severe LV dysfunction. Intracoronary transplantation improved haemodynamics at rest (including LVEF), exercise capacity, LV contractility and geometry (decrease in LV end-diastolic and end-systolic volumes, decrease in infarct size), and a remarkable reduction in mortality at 5-years follow-up was observed.

Trial	n	Cell type	Delivery	Timing	Primary endpoint	Comments
MAGIC	97	SM	epi	>4 weeks	No change LVEF	Reduction in LVEDV/LVESV
Dib	23	SM	endo	>10 years	Improved LVEF and viability	-
SEISMIC	47	SM	endo	chronic	No change LVEF	
TOPCARE-CHD	58	BMMC vs CPC	ic	81±72 months	Improved LVEF w/BMMC	-
STAR	391	BMMC vs controls	ic	8.5±3.2 years	Improved LVEF, LVEDV/LVESV , exercise capacity	No randomized design. Reduction of infarct size and mortality

Table 3. Randomized clinical trials in patients with chronic ischemic heart failure. SM: skeletal myoblasts; BMMC: bone marrow mononuclear cells; CPC: circulating progenitor cells; epi: transepical; endo: transendocardial; ic: intracoronary; LVEF: left ventricular ejection fraction; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume.

6.2.2 Chronic myocardial ischemia

Patients with advanced CAD and no further options of revascularization (“no-option” patients) have also been studied in stem cell therapy trials (table 4). Three RCT have been completed using the transendocardial route after electromechanical mapping of the LV, with BMMC or blood-derived progenitor cells. Losordo et al. (Losordo et al., 2007) studied peripheral CD34+ cells isolated after G-CSF injections. Angina frequency and exercise time were improved, but no clear effects on myocardial perfusion were observed. In the PROTECT-CAD trial (Tse et al., 2007), BMMC injections improved NYHA functional class, exercise time, LVEF, wall thickening and stress-induced perfusion defects. Finally, Van Ramshorst et al. (van Ramshorst et al., 2009) reported better LVEF, myocardial perfusion, angina functional class, exercise capacity and quality of life after BMMC administration. ADSC have also been studied in this type of patients. The PRECISE trial is a prospective, double blind, RCT that has randomised 27 patients with end-stage CAD not amenable for revascularization and with moderate-severe LV dysfunction to receive freshly isolated ADSC or placebo in a 3:1 ratio. The cells were delivered via transendocardial injections after

LV electromechanical mapping with the NOGA XP™ delivery system (BDS, Cordis Corporation, Johnson and Johnson) (figure 3), and results are still waiting for publication.

Trial	n	Cell type	Delivery	Timing	Primary endpoint	Comments
Losordo	24	CD34+	endo	chronic	Improved angina parameters	No clear perfusion benefit
PROTECT-CAD	28	BMMC	endo	chronic	Improved angina parameters	Improved LVEF and perfusion
Van Ramshorst	50	BMMC	endo	chronic	Improved angina parameters	Improved LVEF and perfusion

Table 4. Randomized clinical trials in patients with chronic myocardial ischemia. BMMC: bone marrow mononuclear cells; endo: transendocardial; LVEF: left ventricular ejection fraction.

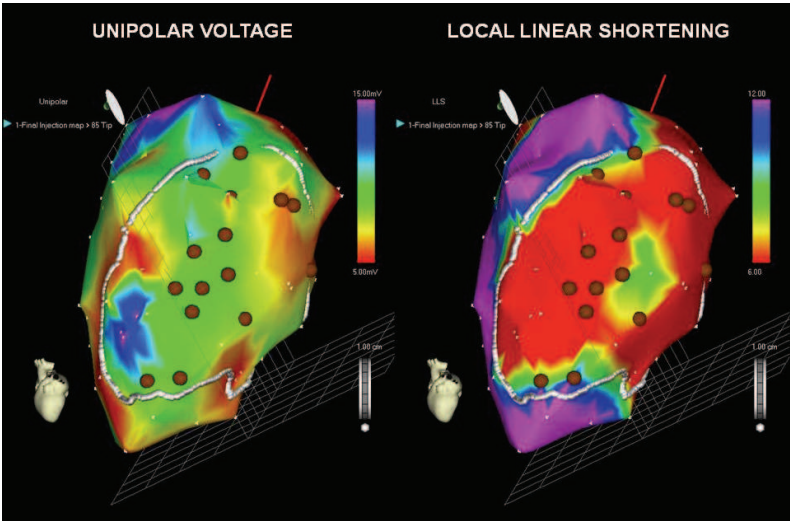


Fig. 3. Electromechanical mapping of the left ventricle with the NOGA XP™ System (BDS, Cordis Corporation, Johnson and Johnson) from a patient enrolled in the PRECISE trial in our centre. Myocardial areas with low contractility (red means most akinetic areas) and impaired endocardial voltage (red means necrotic myocardium, green hibernating tissue) are identified as viable and targeted for cell injection (brown dots).

7. Safety concerns of stem cell therapy

As a novel therapy, stem cell therapy has raised several safety concerns, which are listed below:

1. Arrhythmias: malignant arrhythmias can be induced 1) because transplanted cells do not engraft in a physiologic electromechanical way, forming functionally isolated clusters of cells, 2) because of intrinsic arrhythmogenicity of cells, 3) because reinnervation of the myocardium, 4) due to local damage or edema after cell injection or 5) because of the use of heterologous proteins in cell cultures. Ventricular arrhythmias

have been reported specifically with skeletal myoblasts (Menasche et al., 2003; Siminiak et al., 2004; Dib N 2005) and with CD133<sup>+</sup> cells (Bartunek et al., 2005), but without compromising the overall safety of this therapy.

2. Restenosis: the only study that reported a higher incidence of restenosis used G-CSF four days before stent implantation (Kang et al., 2004), which was considered to be a non-adequate methodology. This risk has not been observed in further trials.
3. Extracardiac engraftment: administered stem cells are located in the heart in low proportions, whereas kidneys, lungs and spleen receive the most of the cells. Local intramyocardial injections achieve higher retention rates in the heart. Nevertheless, clinical consequences of extracardiac engraftment are so far unknown or even beneficial.
4. Accelerated atherogenesis: some studies have shown a high proportion of de novo lesions in non-treated coronary arteries (Bartunek et al., 2005), but this issue needs further evaluation.
5. Microvascular obstruction: MSC have been proved to cause microinfarctions in canine models, but these findings have not been reproduced in human trials (Chen et al., 2004).
6. Oncogenicity: pluripotent and ESC have the risk of inducing cardiac tumors, but this risk is reduced if cells are in culture for no more than 6 or 8 weeks.

## 8. The consensus of the task force of the European society of cardiology for future trials

The Task Force of the European Society of Cardiology on stem cells and repair of the heart was created in 2006 to investigate and regulate the role of progenitor/stem cell therapy in the treatment of cardiovascular disease. It was almost four years ago that this group of experts and opinion leaders stated the type of studies needed (Bartunek et al., 2006):

1. Further large, double-blind, controlled RCT for the use of autologous BMMC in the treatment of AMI. The patient population should be all those presenting within 12 h of AMI and treated with immediate revascularization, be it primary angioplasty or fibrinolysis.
2. Double-blind, controlled RCT for the use of autologous BMMC in the treatment of AMI in those patients presenting late (>12 h) or who fail to respond to therapy (candidates for 'rescue' angioplasty). Although these groups may represent a small proportion of all patients with AMI, their prognosis remains poor.
3. Double-blind, controlled RCT for the use of autologous BMMC or skeletal myoblasts in the treatment of ischemic heart failure. At some stage, the role of autologous stem/progenitor cells in the treatment of cardiomyopathies (in particular, dilated cardiomyopathy) will need to be examined.
4. A series of well-designed small studies to address safety or mechanism to test specific hypotheses (i.e., studies with labelled cells or to investigate paracrine or autocrine mechanisms). Such hypotheses would have arisen from basic science experiments.
5. Studies to confirm the risk/benefit ratio of the use of cytokines alone (i.e., G-CSF) or in conjunction with stem/progenitor cell therapy.

This Task Force also underlined the necessity for studies with hard clinical endpoints, MACE, subjective benefit and economic gain (Bartunek et al., 2006). Another key point is standardization, both in outcome measures and in the processing of cells (better achieved in specialized centers following GMP routines), in order to derive meaningful comparisons. Since these trials will need to include thousands of patients, they should be multicentre and

ideally pan-European. On the other hand, the Task Force stated that small uncontrolled trials with BMMC should be avoided, as they are unlikely to add anything new to the field.

## 9. Next directions in stem cell therapy research.

Finally, next directions of cardiac cell therapy include:

1. The study of the array of bioactive molecules that are secreted by stem cells, which have been demonstrated to induce neovascularization, modulate inflammation, fibrogenesis, cardiac metabolism and contractility, increase cardiomyocyte proliferation and activate resident stem cells. The exhaustive analysis of this “secretomes” of BMMC, MSC or EPC would lead to a better understating of the mechanisms of action of the cells and to a hypothetical protein-based therapy (off-the-shelf, noninvasive, systemic and repetitive administration).
2. The use of different sources of pluripotent stem cells, like ESC, spermatogonial stem cells and oocytes. A new era has been initiated with the possibility of reprogramming adult cells (skin fibroblasts) to a pluripotent state by retroviral transduction (iPS) (Takahashi et al., 2007; Yu et al., 2007). New retroviral vectors and even nonviral vectors have been developed to reduce the risk of mutagenesis, and genetic modification of cells with suicide genes have been proposed to reduce the risk of tumor formation.
3. The creation of bioartificial hearts after a process of decellularization with detergents, obtention of the underlying extracellular matrix (cardiac architecture) and stem cell repopulation (Ott et al., 2008). The “acellular” heart can then be reseeded with cardiac stem cells or EPC, showing contractile activity in animal models. This new approach of tissue bioengineering has opened a fascinating era in cardiovascular medicine.

## 10. Conclusion

Although mixed results have emerged from the first stem cell therapy RCT in cardiovascular medicine, the overall data suggest that these procedures are feasible and safe in both acute and chronic scenarios of ischemic heart disease. After phase I-II RCT, it is clear that BMMC transfer after AMI has the potential to improve the recovery of LV systolic function beyond what can be achieved by current interventional and medical therapies. In chronic ischemic heart disease, skeletal myoblasts and BMMC have proved to improve myocardial perfusion and contractile performance.

New types of cells (including ADSC and iPS), improvements in delivery and imaging methods, strategies to enhance cell potentiality or to improve the myocardial pro-inflammatory microenvironment, and the creation of bioartificial hearts are the main new directions of research in the near future. Finally, large-scale, phase III, double-blind, controlled RCT performed under rigorous safety standards are being initiated to prove unequivocal clinical benefits, including improved survival. These trials will definitively establish the effectiveness of stem cell therapy in improving clinical outcomes, confirming the real potential of cardiac regenerative therapy.

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## 12. References

- Assmus, B., Honold, J., Schächinger, V., Britten, M.B., Fischer-Rasokat, U., Lehmann, R., Teupe, C., Pistorius, K., Martin, H., Abolmaali, N.D., Tonn, T., Dimmeler, S., & Zeiher, A.M. (2006). Transcoronary transplantation of progenitor cells after myocardial infarction. *The New England Journal of Medicine*, Vol.355, No.12, (September 2006), pp. 1222-1232, ISSN 1533-4406
- Balsam, L.B., Wagers, A.J., Christensen, J.L., Kofidis, T., Weissman, I.L., & Robbins, R.C. (2004). Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*, Vol.428, No.6983, (April 2004), pp. 668-673, ISSN 1476-4687
- Bartunek, J., Vanderheyden, M., Vandekerckhove, B., Mansour, S., De Bruyne, B., De Bondt, P., Van Haute, I., Lootens, N., Heyndrickx, G., & Wijns, W. (2005). Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. *Circulation*, Vol.112, No.9 suppl, (August 2005), pp. I178-183, ISSN 1524-4539
- Bartunek, J., Dimmeler, S., Drexler, H., Fernández-Avilés, F., Galinanes, M., Janssens, S., Martin, J., Mathur, A., Menasche, P., Priori, S., Strauer, B., Tendera, M., Wijns, W., & Zeiher, A.; task force of the European Society of Cardiology. (2006). The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. *European Heart Journal*, Vol.27, No.11, (June 2006), pp. 1338-1340, ISSN 0195-668X
- Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B., & Anversa, P. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, Vol.114, No.6, (September 2003), pp. 763-776, ISSN 0092-867
- Chen, S.L., Fang, W.W., Ye, F., Liu, Y.H., Qian, J., Shan, S.J., Zhang, J.J., Chunhua, R.Z., Liao, L.M., Lin, S., & Sun, J.P. (2004). Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *The American journal of cardiology*, Vol.94, No.1, (July 2004), pp. 92-95, ISSN 0002-9149
- Cho, H.J., Lee, N., Lee, J.Y., Choi, Y.J., Li, M., Wecker, A., Jeong, J.O., Curry, C., Qin, G., & Yoon, Y.S. (2007). Role of host tissues for sustained humoral effects after endothelial progenitor cell transplantation into the ischemic heart. *The Journal of Experimental Medicine*, Vol.204, No.13, (December 2007), pp. 3257-3269, ISSN 1540-9538
- Dewald, O., Ren, G., Duerr, G.D., Zoerlein, M., Klemm, C., Gersch, C., Tincey, S., Michael, L.H., Entman, M.L., & Frangogiannis, N.G. (2004). Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. *The American Journal of Pathology*, Vol.164, No.2, (February 2004), pp. 665-677, ISSN 0002-9440
- Dib, N., Michler, R.E., Pagani, F.D., Wright, S., Kereiakes, D.J., Lengerich, R., Binkley, P., Buchele, D., Anand, I., Swingen, C., Di Carli, M.F., Thomas, J.D., Jaber, W.A., Opie, S.R., Campbell, A., McCarthy, P., Yeager, M., Dilsizian, V., Griffith, B.P., Korn, R.,

- Kreuger, S.K., Ghazoul, M., MacLellan, W.R., Fonarow, G., Eisen, H.J., Dinsmore, J., Diethrich, E. (2005). Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. *Circulation*, Vol.112, No.12, (September 2005), pp. 1748-1755, ISSN 1524-4539
- Fraser, J.K., Schreiber, R.E., Zuk, P.A., & Hedrick, M.H. (2004) Adult stem cell therapy for the heart. *International journal of biochemistry & cell biology*, Vol.36, No.4, (April 2004), pp. 658-666, ISSN 1357-2725
- Gnecchi, M., Zhang, Z., Ni, A., & Dzau, V.J. (2008). Paracrine mechanisms in adult stem cell signaling and therapy. *Circulation research*, Vol.103, No.11, (November 2008), pp. 1204-1219, ISSN 1524-4571
- Hare, J.M., Traverse, J.H., Henry, T.D., Dib, N., Strumpf, R.K., Schulman, S.P., Gerstenblith, G., DeMaria, A.N., Denktas, A.E., Gammon, R.S., Hermiller, J.B. Jr., Reisman, M.A., Schaer, G.L., & Sherman, W. (2009). A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *Journal of the American College of Cardiology*, Vol.54, No.24, (December 2009), pp. 2277-2286, ISSN 1558-3597
- Herreros, J., Prosper, F., Perez, A., Gavira, J.J., Garcia-Velloso, M.J., Barba, J., Sanchez, P.L., Canizo, C., Rabago, G., Marti-Climent, J.M., Hernandez, M., Lopez-Holgado, N., Gonzalez-Santos, J.M., Martin-Luengo, C., & Alegria, E. (2003). Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. *European Heart Journal*, Vol.24, No.22, (November 2003), pp. 2012-2020, ISSN 0195-668X
- Huikuri, H.V., Kervinen, K., Niemelä, M., Ylitalo, K., Säily, M., Koistinen, P., Savolainen, E.R., Ukkonen, H., Pietilä, M., Airaksinen, J.K., Knuuti, J., & Mälikallio, T.H.; FINCELL Investigators. (2008). Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. *European Heart Journal*, Vol.29, No.22, (November 2008), pp. 2723-2732, ISSN 1522-9645
- Ince, H., Petzsch, M., Kleine, H.D., Schmidt, H., Rehders, T., Körber, T., Schümichen, C., Freund, M., & Nienaber, C.A. (2005). Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). *Circulation*, Vol.112, No.20, (November 2005), pp. 3097-3106, ISSN 1524-4539
- Jackson, K.A., Majka, S.M., Wang, H., Pocius, J., Hartley, C.J., Majesky, M.W., Entman, M.L., Michael, L.H., Hirschi, K.K., & Goodell, M.A. (2001). Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *Journal of Clinical Investigation*, Vol.107, No.11, (June 2001), pp. 1395-1402, ISSN 0021-9738
- Janssens, S., Dubois, C., Bogaert, J., Theunissen, K., Deroose, C., Desmet, W., Kalantzi, M., Herbots, L., Sinnaeve, P., Dens, J., Maertens, J., Rademakers, F., Dymarkowski, S., Gheysens, O., Van Cleemput, J., Bormans, G., Nuyts, J., Belmans, A., Mortelmans, L., Boogaerts, M., & Van de Werf, F. (2006). Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet*, Vol.367, No.9505, (January 2006), pp. 113-121, ISSN 1474-547X
- Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Tsutsumi, Y., Ozono, R., Masaki, H., Mori, Y., Iba, O., Tateishi, E., Kosaki, A., Shintani, S., Murohara, T., Imaizumi, T., & Iwasaka, T. (2001). Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of

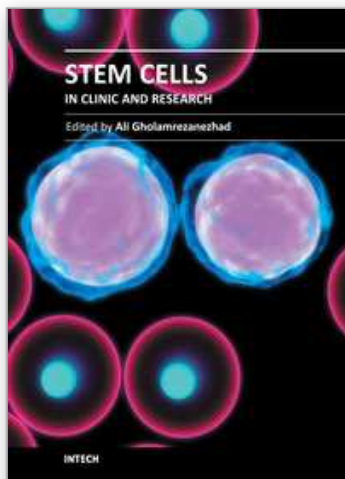
- angioblasts, angiogenic ligands, and cytokines. *Circulation*, Vol.104, No.9, (August 2001), pp. 1046-1052, ISSN 1524-4539
- Kang, H.J., Kim, H.S., Zhang, S.Y., Park, K.W., Cho, H.J., Koo, B.K., Kim, Y.J., Soo Lee, D., Sohn, D.W., Han, K.S., Oh, B.H., Lee, M.M., & Park, Y.B. (2004). Effects of intracoronary 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*, Vol.363, No.9411, (March 2004), pp. 751-756, ISSN 1474-547X
- Kang, H.J., Lee, H.Y., Na, S.H., Chang, S.A., Park, K.W., Kim, H.K., Kim, S.Y., Chang, H.J., Lee, W., Kang, W.J., Koo, B.K., Kim, Y.J., Lee, D.S., Sohn, D.W., Han, K.S., Oh, B.H., Park, Y.B., & Kim, H.S. (2006). Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. *Circulation*, Vol.114, No.1 Suppl, (July 2006), pp. I145-I151, ISSN 1524-4539
- Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J., & Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *Journal of Clinical Investigation*, Vol.108, No.3, (August 2001), pp. 407-414, ISSN 0021-9738
- Klug, M.G., Soonpaa, M.H., Koh, G.Y., & Field, L.J. (1996). Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *Journal of Clinical Investigation*, Vol.98, No.1, (July 1996), pp. 216-224, ISSN 0021-9738
- Kocher, A.A., Schuster, M.D., Szabolcs, M.J., Takuma, S., Burkhoff, D., Wang, J., Homma, S., Edwards, N.M., & Itescu, S. (2001). Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nature Medicine*, Vol.7, No.4, (April 2001), pp. 430-436, ISSN 1078-8956
- Laugwitz, K.L., Moretti, A., Lam, J., Gruber, P., Chen, Y., Woodard, S., Lin, L.Z., Cai, C.L., Lu, M.M., Reth, M., Platoshyn, O., Yuan, J.X., Evans, S., & Chien, K.R. (2005). Postnatal isl1<sup>+</sup> cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*, Vol.433, No.7026, (February 2005), pp. 647-653, ISSN 1476-4687
- Leobon, B., Garcin, I., Menasche, P., Vilquin, J.T., Audinat, E., & Charpak, S. (2003). Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proceedings of the National Academy of Sciences USA*, Vol.100, No.13, (June 2003), pp. 7808-7811, ISSN 0027-8424
- Leone, A.M., Galiuto, L., Garramone, B., Rutella, S., Giannico, M.B., Brugaletta, S., Perfetti, M., Liuzzo, G., Porto, I., Burzotta, F., Niccoli, G., Biasucci, L.M., Leone, G., Rebuzzi, A.G., & Crea, F. (2007). Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the rigenera study). *The American journal of cardiology*, Vol.100, No.3, (August 2007), pp. 397-403, ISSN 0002-9149
- Lindsey, M.L., Mann, D.L., Entman, M.L., & Spinale, F.G. (2003). Extracellular matrix remodeling following myocardial injury. *Annals of Medicine*, Vol.35, No.5, (2003), pp. 316-326, ISSN 0785-3890
- Lipinski, M.J., Biondi-Zoccai, G.G., Abbate, A., Khianey, R., Sheiban, I., Bartunek, J., Vanderheyden, M., Kim, H.S., Kang, H.J., Strauer, B.E., & Vetrovec, G.W. (2007).

- 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. *Journal of the American College of Cardiology*, Vol.50, No.18, (October 2007), pp.1761-1767, ISSN 1558-3597
- Losordo, D.W., Schatz, R.A., White, C.J., Udelson, J.E., Veereshwarayya, V., Durgin, M., Poh, K.K., Weinstein, R., Kearney, M., Chaudhry, M., Burg, A., Eaton, L., Heyd, L., Thorne, T., Shturman, L., Hoffmeister, P., Story, K., Zak, V., Dowling, D., Traverse, J.H., Olson, R.E., Flanagan, J., Sodano, D., Murayama, T., Kawamoto, A., Kusano, K.F., Wollins, J., Welt, F., Shah, P., Soukas, P., Asahara, T., & Henry, T.D. (2007). Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation*, Vol.115, No.25, (June 2007), pp. 3165-3172, ISSN 1524-4539
- Lunde, K., Solheim, S., Aakhus, S., Arnesen, H., Abdelnoor, M., Egeland, T., Endresen, K., Ilebakk, A., Mangschau, A., Fjeld, J.G., Smith, H.J., Taraldsrud, E., Grøgaard, H.K., Bjørnerheim, R., Brekke, M., Müller, C., Hopp, E., Ragnarsson, A., Brinchmann, J.E., & Forfang, K. (2006). Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *The New England journal of medicine*, Vol.355, No.12, (September 2006), pp. 1199-1209, ISSN 1533-4406
- Ma, N., Stamm, C., Kaminski, A., Li, W., Kleine, H.D., Muller-Hilke, B., Zhang, L., Ladilov, Y., Egger, D., & Steinhoff, G. (2005). Human cord blood cells induce angiogenesis following myocardial infarction in NOD/scid-mice. *Cardiovascular Research*, Vol.66, No.1, (April 2005), pp. 45-54, ISSN 0008-6363
- Martin-Rendon, E., Brunskill, S.J., Hyde, C.J., Stanworth, S.J., Mathur, A., & Watt, S.M. (2008). Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *European heart journal*, Vol.29, No.15, (August 2008), pp. 1807-1818, ISSN 1522-9645
- Mazhari, R., & Hare, J.M. (2007). Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. *Nature Clinical Practice Cardiovascular Medicine*, Vol.4, No.Suppl 1, (2007), pp. S21-26, ISSN 1743-4300
- Menasché, P., Hagège, A.A., Vilquin, J.T., Desnos, M., Abergel, E., Pouzet, B., Bel, A., Sarateanu, S., Scorsin, M., Schwartz, K., Bruneval, P., Benbunan, M., Marolleau, J.P., & Duboc, D. (2003). Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *Journal of the American College of Cardiology*, Vol.41, No.7, (April 2003), pp.1078 -1083, ISSN 0735-1097
- Menasché, P., Alfieri, O., Janssens, S., McKenna, W., Reichenspurner, H., Trinquart, L., Vilquin, J.T., Marolleau, J.P., Seymour, B., Larghero, J., Lake, S., Chatellier, G., Solomon, S., Desnos, M., & Hagège, A.A. (2008). The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation*, Vol.117, No.9, (March 2008), pp. 1189-1200, ISSN 1524-4539
- Messina, E., De Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., Salio, M., Battaglia, M., Latronico, M.V., Coletta, M., Vivarelli, E., Frati, L., Cossu, G., & Giacomello, A. (2004). Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circulation Research*, Vol.95, No.9, (October 2004), pp. 911-921, ISSN 1524-4571
- Min, J.Y., Yang, Y., Converso, K.L., Liu, L., Huang, Q., Morgan, J.P., & Xiao, Y.F. (2002). Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *Journal of Applied Physiology*, Vol.92, No.1, (January 2002), pp. 288-296, ISSN 8750-7587

- Murry, C.E., Wiseman, R.W., Schwartz, S.M., & Hauschka, S.D. (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. *Journal of Clinical Investigation*, Vol.98, No.11, (December 1996), pp. 2512-2523, ISSN 0021-9738
- Oh, H., Bradfute, S.B., Gallardo, T.D., Nakamura, T., Gaussin, V., Mishina, Y., Pocius, J., Michael, L.H., Behringer, R.R., Garry, D.J., Entman, M.L., & Schneider, M.D. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proceedings of the National Academy of Sciences USA*, Vol.100, No.21, (October 2003), pp. 12313-12318, ISSN 0027-842
- Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., Leri, A., & Anversa, P. (2001a). Bone marrow cells regenerate infarcted myocardium. *Nature*, Vol.410, No.6829, (April 2001), pp. 701-705, ISSN 0028-0836
- Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D.M., Leri, A., & Anversa, P. (2001b). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proceedings of the National Academy of Sciences USA*, Vol.98, No.18, (August 2001), pp. 10344-10349, ISSN 0027-8424
- Ott, H.C., Matthiesen, T.S., Goh, S.K., Black, L.D., Kren, S.M., Netoff, T.I., & Taylor, D.A. (2008). Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nature Medicine*, Vol.14, No.2, (February 2008), pp. 213-221, ISSN 1546-170X
- Pagani, F.D., DerSimonian, H., Zawadzka, A., Wetzel, K., Edge, A.S., Jacoby, D.B., Dinsmore, J.H., Wright, S., Aretz, T.H., Eisen, H.J., & Aaronson, K.D. (2003). Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *Journal of the American College of Cardiology*, Vol.41, No.5, (March 2003), pp. 879-888, ISSN 0735-1097
- Perin, E.C. & Silva, G.V. (2006). What are stem cells and what do they do?, In: *An essential guide to cardiac cell therapy*, E.C. Perin, G.V. Silva, J.T. Willerson (Ed), 3-12, Informa Healthcare, ISBN 978-1-84184-471-8, Houston, United States of America
- Pittenger, M.F., & Martin, B.J. (2004) Mesenchymal stem cells and their potential as cardiac therapeutics. *Circulation Research*, Vol.95, No.1, (July 2004), pp. 9-20, ISSN 1524-4571
- Sanz-Ruiz, R., Fernández-Santos, M.E., Domínguez-Muñoz, M., Ludwig Martín, I., Parma, R., Sánchez, P.L., & Fernández-Avilés, F. (2008). Adipose tissue-derived stem cells: the friendly side of a classic cardiovascular foe. *Journal of Cardiovascular Translational Research*, Vol.1, No.1, (March 2008), pp.55-63, ISSN 1937-5395
- Sanz-Ruiz, R., Fernández-Santos, M.E., Domínguez-Muñoz, M., Parma, R., Villa, A., Fernández, L., Sánchez, P.L., & Fernández-Avilés, F. (2009). Early translation of adipose derived cell therapy for cardiovascular disease. *Cell transplantation*, Vol.18, No.3, (March 2009), pp.245-254, ISSN 0963-6897
- Schächinger, V., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., Yu, J., Corti, R., Mathey, D.G., Hamm, C.W., Süselbeck, T., Assmus, B., Tonn, T., Dimmeler, S., & Zeiher, A.M.; REPAIR-AMI Investigators. (2006). Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *The New England Journal of Medicine*, Vol.355, No.12, (September 2006), pp. 1210-1221, ISSN 1533-4406
- Shizuru, J.A., Negrin, R.S., & Weissman, I.L. (2005). Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. *Annual review of medicine*, Vol.56, (February 2005), pp. 509-538, ISSN 0066-4219

- Siminiak, T., Kalawski, R., Fiszer, D., Jerzykowska, O., Rzeźniczak, J., Rozwadowska, N., & Kurpisz, M. (2004). Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. *American Heart Journal*, Vol.148, No.3, (September 2004), pp. 531-537, ISSN 1097-6744
- Strauer, B.E., Yousef, M., & Schannwell, C.M. (2010). The acute and long-term effects of intracoronary stem cell transplantation in 191 patients with chronic heart failure: the STAR-heart study. *European Journal of Heart Failure*, Vol.12, No.7, (July 2010), pp. 721-729, ISSN 1879-0844
- Suva, D., Garavaglia, G., Menetrey, J., Chapuis, B., Hoffmeyer, P., Bernheim, L., & Kindler, V. (2004). Non-hematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. *Journal of cellular physiology*, Vol.198, No.1, (January 2004), pp. 110-118, ISSN 0021-9541
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, Vol.131, No.5, (November 2007), pp. 861-872, ISSN 0092-8674
- Takano, H., Hasegawa, H., Kuwabara, Y., Nakayama, T., Matsuno, K., Miyazaki, Y., Yamamoto, M., Fujimoto, Y., Okada, H., Okubo, S., Fujita, M., Shindo, S., Kobayashi, Y., Komiyama, N., Takekoshi, N., Imai, K., Himi, T., Ishibashi, I., & Komuro, I. (2007). Feasibility and safety of granulocyte colony-stimulating factor treatment in patients with acute myocardial infarction. *International journal of cardiology*, Vol.122, No.1, (October 2007), pp. 41-47, ISSN 1874-1754
- Taylor, D.A., Atkins, B.Z., Hungspreugs, P., Jones, T.R., Reedy, M.C., Hutcheson, K.A., Glower, D.D., & Kraus, W.E. (1998). Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nature Medicine*, Vol.4, No.8, (August 1998), pp. 929-933, ISSN 1078-8956
- Tendera, M., Wojakowski, W., Rużyłło, W., Chojnowska, L., Kepka, C., Tracz, W., Musiałek, P., Piwowarska, W., Nessler, J., Buszman, P., Grajek, S., Breborowicz, P., Majka, M., Ratajczak, M.Z.; REGENT Investigators. (2009). Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. *European Heart Journal*, Vol.30, No.11, (June 2009), pp. 1313-1321, ISSN 1522-9645
- Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., & Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science*, Vol.282, No5391, (November 1998), pp. 1145-1147, ISSN 0036-8075
- Toma, C., Pittenger, M.F., Cahill, K.S., Byrne, B.J., & Kessler, P.D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*, Vol.105, No.1, (January 2002), pp. 933-938, ISSN 1524-4539
- Tse, H.F., Thambar, S., Kwong, Y.L., Rowlings, P., Bellamy, G., McCrohon, J., Thomas, P., Bastian, B., Chan, J.K., Lo, G., Ho, C.L., Chan, W.S., Kwong, R.Y., Parker, A., Hauser, T.H., Chan, J., Fong, D.Y., & Lau CP. (2007). Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). *European heart journal*, Vol.28, No.24, (December 2007), pp. 2998-3005, ISSN 0195-668X
- Urbanek, K., Quaini, F., Tasca, G., Torella, D., Castaldo, C., Nadal-Ginard, B., Leri, A., Kajstura, J., Quaini, E., & Anversa, P. (2003). Intense myocyte formation from

- cardiac stem cells in human cardiac hypertrophy. *Proceedings of the National Academy of Sciences USA*, Vol.100, No.18, (September 2003), pp. 10440-10445, ISSN 0027-8424
- Urbanek, K., Torella, D., Sheikh, F., De Angelis, A., Nurzynska, D., Silvestri, F., Beltrami, C.A., Bussani, R., Beltrami, A.P., Quaini, F., Bolli, R., Leri, A., Kajstura, J., & Anversa, P. (2005). Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proceedings of the National Academy of Sciences USA*, Vol.102, No.24, (June 2005), pp. 8692-8697, ISSN 0027-8424
- van der Laan, A., Hirsch, A., Nijveldt, R., van der Vleuten, P.A., van der Giessen, W.J., Doevendans, P.A., Waltenberger, J., Ten Berg, J.M., Aengevaeren, W.R., Zwaginga, J.J., Biemond, B.J., van Rossum, A.C., Tijssen, J.G., Zijlstra, F., & Piek, J.J. (2008). Bone marrow cell therapy after acute myocardial infarction: the HEBE trial in perspective, first results. *Netherlands Heart Journal*, Vol.16, No.12, (December 2008), pp. 436-439, ISSN 1568-5888
- van Ramshorst, J., Bax, J.J., Beeres, S.L., Dibbets-Schneider, P., Roes, S.D., Stokkel, M.P., de Roos, A., Fibbe, W.E., Zwaginga, J.J., Boersma, E., Schalij, M.J., & Atsma, D.E. (2009). Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. *JAMA*, Vol.301, No.19, (May 2009), pp. 1997-2004, ISSN 1538-3598
- Virmani, R., Kolodgie, F.D., & Ladich, E. (2006). Mechanistic insights into cardiac stem cell therapy from a histopathologic perspective, In: *An essential guide to cardiac cell therapy*, E.C. Perin, G.V. Silva, J.T. Willerson (Ed), 43-54, Informa Healthcare, ISBN 978-1-84184-471-8, Houston, United States of America
- Wollert, K.C., Meyer, G.P., Lotz, J., Ringes-Lichtenberg, S., Lippolt, P., Breidenbach, C., Fichtner, S., Korte, T., Hornig, B., Messinger, D., Arseniev, L., Hertenstein, B., Ganser, A., & Drexler, H. (2004). Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*, Vol.364, No.9429, (July 2004), pp.141-148, ISSN 1474-547X
- Wollert, K.C., & Drexler, H. (2010). Cell therapy for the treatment of coronary heart disease: a critical appraisal. *Nature Reviews Cardiology*, Vol.7, No.4, (April 2010), pp. 204-215, ISSN 1759-5010
- Yamashita, J., Itoh, H., Hirashima, M., Ogawa, M., Nishikawa, S., Yurugi, T., Naito, M., Nakao, K., & Nishikawa, S. (2000). Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature*, Vol.408, No.6808, (November 2000), pp. 92-96, ISSN 0028-0836
- Yoon, Y.S., Wecker, A., Heyd, L., Park, J.S., Tkebuchava, T., Kusano, K., Hanley, A., Scadova, H., Qin, G., Cha, D.H., Johnson, K.L., Aikawa, R., Asahara, T., & Losordo, D.W. (2005). Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *Journal of Clinical Investigation*, Vol.115, No.2, (February 2005), pp. 326-338, ISSN 0021-9738
- Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I., & Thomson, J.A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science*, Vol.318, No.5858, (December 2007), pp. 1917-1920, ISSN 1095-9203
- Zimmet, J.M., & Hare, J.M. (2005). Emerging role for bone marrow derived mesenchymal stem cells in myocardial regenerative therapy. *Basic Research in Cardiology*, Vol.100, No.6, (November 2005), pp. 471-481, ISSN 0300-8428



## **Stem Cells in Clinic and Research**

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigational more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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