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Autologous Stem Cells for Cardiac Repair: New Insights on Clinical Trial Safety and Best Cell Source

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1. Introduction

Acute myocardial infarction (AMI) is a leading cause of death worldwide. (Wollert and Drexler, 2010) Despite improvements in survival rate after AMI with recent medical advances (Jackson, et al., 2001), the reduced heart function attributed to irreversible loss of viable cardiomyocytes remains a major clinical problem (Timmermans, et al., 2003). Typical MI results in large-scale loss of cardiac muscle, often a billion or more myocytes. (Laflamme and Murry, 2005) The loss of myocardial cell mass and the inability of remaining cells to adequately compensate through hypertrophic or hyperplastic responses result in the development of heart failure due to ventricular dysfunction (Jain, et al., 2005). Endogenous repair mechanisms of the adult human heart are not sufficient for meaningful tissue regeneration, so muscle lost is replaced by non-contractile fibrotic scar tissue, initiating progressive heart failure (Laflamme, et al., 2007). Heart failure is the number one cause of hospitalization in US citizens over 65 of age and has a significant economic impact (Laflamme and Murry, 2005). Despite recent advances in treatment options, mortality remains unacceptably high (Ertl, et al., 1993, Pfeffer and Braunwald, 1990, Towbin and Bowles, 2002) with >50% of all patients succumbing within 5 years of the initial diagnosis of heart failure (Jain, et al., 2005). Improved medical and surgical treatments for patients after AMI have led to the decrease in early mortality, but as a result there of a higher incidence of heart feailure. (Wollert and Drexler, 2010) In some patients with advanced disease, cardiac transplantation may be an option, but due to organ shortage, its practical use is limited to end-stage heart failure.

In the current clinical setting, early reperfusion by percutaneous coronary intervention (PCI) including intra-coronary stenting is the treatment of choice in the situation of acute MI (Topol, 2003). In patients with diffused coronary artery disease associated with severe ischemic cardiomyopathy, coronary artery bypass grafting (CABG) remains the optimal way of coronary revascularization, and is associated with improved survival and reduction of angina (Detre, et al., 1996, Pell, et al., 2004). Although surgical and catheter-based revascularization of ischemic myocardium can treat angina, reduce the risk of MI, and

improve cardiac function, the viability of severely ischemic myocardium, necrotic myocardium, or both cannot be restored. (Patel, et al., 2005) Current pharmacologic and interventional strategies are unsuccessful to regenerate dead myocardium and failed to address the clinical challenge caused by the early loss of cardiomyocytes (Charwat, et al., 2008). Moreover, none of our current therapies addresses the underlying cause of the remodeling process, namely the damage to the cardiomyocytes and the vasculature in the ischemic tissue (Wollert, 2008). Therefore, new cardiovascular therapies must be elaborated to promote myocardial repair and regeneration, this "Holy Grail" represents a major challenge in the treatment of ischemic cardiovascular diseases.

2. Stem cells for myocardial repair

One approach to counteract the effects of myocardial dysfunction could be replacement of damaged contractile cells by healthy myocytes or progenitor cells with the potential of becoming new functional cardiomyocytes (Evans, et al., 2007). There is growing evidence suggesting that heart muscle has the ability to regenerate through activation of cardiac resident stem cells (CSC), or through recruitment of progenitor stem cells population from other tissues, such as the bone marrow (BM) (Charwat, et al., 2008). Cellular transplantation is a potential approach to improve healing of the ischemic heart, to repopulate the injured myocardium (Dowell, et al., 2003), to treat heart failure (Dowell, et al., 2003, Raeburn, et al., 2002) and restore cardiac function (Hassink, et al., 2003, Orlic, et al., 2002). Experimental studies have shown that adult bone marrow stem cells (BMSC) are capable of differentiation (Blau, et al., 2001, Goodell, et al., 2001, Jackson, et al., 2002, Krause, et al., 2001), regeneration of infarcted myocardium, induction of myogenesis, as well as promotion of angiogenesis, ultimately leading to a better cardiac contractile performance (Dowell, et al., 2003, Kocher, et al., 2001, Orlic, et al., 2001, 2003, Orlic, et al., 2001, Orlic, et al., 2001, Shake, et al., 2002, Strauer, et al., 2002, Tomita, et al., 1999, Tomita, et al., 2002). A variety of embryonic and adult-derived cell types have been investigated for their capacity to mediate cardiac and vascular repair (Psaltis, et al., 2008). Nowadays, several cell candidates have been recently investigated for the treatment of ischemic cardiomyopathy such as fetal (Li, et al., 1997) and neonatal (Watanabe, et al., 1998) cardiomyocytes, embryonic stem cells (Min, et al., 2002, Min, et al., 2003), cardiac resident stem cells, skeletal myoblasts (Leor, et al., 1996, Menasche, et al., 2001, Menasche, et al., 2003, Murry, et al., 1996, Taylor, et al., 1998) and endothelial progenitor cells (Kawamoto and Losordo, 2008, Kawamoto, et al., 2003, Kocher, et al., 2001). The bone marrow is known to be an excellent reservoir for many adult stem cells and BMSC have been used to treat hematologic disorders for decades (Liao, et al., 2007). Bone marrow, which is easily accessible, is presently the most frequent source of cells used for clinical cardiac repair (Dimmeler, et al., 2005, Kocher, et al., 2007). Bone marrow-derived cells contain a complex assortment of progenitor cells, including hematopoietic stem cells (HSC), side population (SP) cells, mesenchymal or stromal, stem cells (MSC), and multipotent adult progenitor cells (MAPC), a subset of MSC (Barbash, et al., 2003, Jackson, et al., 2001, Perin, et al., 2003, Strauer, et al., 2002, Toma, et al., 2002). Other progenitor/stem cell populations investigated include (Kocher, et al., 2007): fat tissue-derived multipotent stem cells (Planat-Benard, et al., 2004), multipotential cells from bone marrow (Jiang, et al., 2002), skeletal muscle, somatic stem cells from placental cord blood (Kogler, et al., 2004), amniotic fluid-derived stem cells (De Coppi, et al., 2007), circulating endothelial progenitor cells (EPC), and cardiac resident progenitor cells (CPC) are already predisposed to adopt a cardiomyocyte

phenotype (Beltrami, et al., 2003, Oh, et al., 2003). Positive results from animal studies have prompted several clinical trials to ascertain the safety, feasibility and efficacy of cell therapy. Despite the fact that the exact mechanisms underpinning stem cell therapy have not been yet elucidated and are still intensely debated, cell therapy has already been introduced into the clinical setting (Dowell, et al., 2003, Weisel, et al., 2004, Wollert and Drexler, 2005).
Cell transplantation treatments are faced with many technical and practical issues, and are limited to autologous cells, which require bone marrow aspirations, muscle biopsies or blood sampling with cells sorting and/or culture. Moreover, they are unable to deliver large cell numbers that would survive the peri-transplantation period (Reinecke, et al., 1999, Shake, et al., 2002, Wollert and Drexler, 2005, Zhang, et al., 2001). Over the past several years, a surge of experimental data from pre-clinical and clinical studies has emerged providing both a proof of concept and therapeutic promises for post-MI cardiac repair and regeneration using these cell therapies (Jain, et al., 2005). Despite all the excitement in stem cell research resulting from initial experimental data and preliminary clinical trials, many crucial questions regarding stem cells therapy still remain to be answered. The most important ones being what are the exact mechanisms underlying their beneficial effects, and which cell type is the most appropriate for clinical application? A major obstacle to the identification of the optimal cell therapy is that the fate of the implanted cells and the nature of their beneficial effects are ill defined (Evans, et al., 2007). Moreover, a clear characterization of the cellular effects of stem cell transplantation is critical to avoid potentially adverse consequences and to improve the outcome (Evans, et al., 2007). A better understanding is fundamental for the development of new therapeutics, and to optimize stem cell applications in the treatment of ischemic cardiovascular diseases.

3. Various cell candidates for cardiac repair

A myriad of cell types have been tested experimentally, each of them being usually credited by its advocates of a high "regeneration" potential. This has led to a flurry of clinical trials entailing the use of skeletal myoblasts or bone marrow-derived cells either unfractionated or enriched in progenitor subpopulations. (Menasche, 2009) There is currently uncertainty as to which of the stem cell population is most potent in stimulating angiogenesis and cardiac repair. Theoretically, many stem and progenitor cell populations are potential candidates to be used for cardiac repair and to treat ischemic cardiomyopathy. Each cell type possesses its own profile of advantages, limitations and practicability issues (Wollert, 2008). It, appears that not "one cell fits all" but that the selection of the cell type should be tailored to the primary clinical indication. (Menasche, 2009) Studies comparing the regenerative capacity of distinct cell populations are scarce. (Wollert, 2008) As replacement therapy in humans, the estimates are that 10^8-10^9 cells might be needed to replace those lost after a moderate size AMI. Accordingly, an important issue in cell therapy is scalability and the ability to deliver a large amount of cells into the ischemic myocardium. It does not make sense to develop an "ideal" cell in a culture dish, if we remain unable to deliver it appropriately and to keep it alive, at least for a while, which requires to improve on the delivery techniques and to provide cells along with the vascular and extracellular matrix type of support necessary for their survival and patterning. (Menasche, 2009) Another issue which must be considered is autologous versus non-autologous source of donor cells. (Evans, et al., 2007) Autologous have the advantage of avoiding inflammatory response and immune rejection. However, to be useful for broad clinical application, cells must be readily available in sufficient number

or easily to harvest and isolate. Herein are some characteristics of the most commonly used adult stem cells for cardiac repair.

3.1 Primary remuscularization by myogenic cells

When contemplating how cell therapy might benefit the recently infarcted heart, perhaps the most obvious strategy is remuscularization, that is, the replacement of the necrotic myocardium with viable new muscle cells. (Laflamme, et al., 2007) Strong proof-of-principle data to support this approach were provided by studies in which committed cardiomyocytes from fetal (Li, et al., 1997) and neonatal (Watanabe, et al., 1998) sources were transplanted into murine or rat model of ischemic myocardium. The implanted myocytes formed stable intracardiac grafts and resulted in improvement in cardiac function. (Leor, et al., 1996, Li, et al., 1996) As expected for terminally differentiated cardiomyocytes, the implanted cells retained their contractile phenotype and expressed the necessary elements for intercellular electrical communication such as gap junction proteins. (Laflamme, et al., 2007) Another myogenic cell type, skeletal muscle satellite cells commonly referred to as skeletal myoblast has been extensively studied in animals and clinical trials. (Menasche, 2008, Menasche, et al., 2001, Menasche, et al., 2003, Murry, et al., 1996, Taylor, et al., 1998) Skeletal myoblasts have undergone extensive preclinical testing that has consistently demonstrated their ability to preserve postinfarct left ventricular function and to limit remodelling. (Menasche, 2008) Indeed, skeletal myoblasts, the progenitor cell that mediate normal regeneration of skeletal striated muscle, have a number of attractive properties for cardiac cell therapies, including relative resistance to ischemia, potentially autologous donor source from readily accessible muscle biopsies, paracrine effects by secretions of growth factors, and the capacity for tremendous in vitro expansion. (Dowell, et al., 2003, Laflamme, et al., 2007, Menasche, 2008, 2009) The primary disadvantage of skeletal myoblasts is simply that they differentiate into the wrong form of striated muscle, i.e. skeletal rather than cardiac, with the caveat of severe ventricular arrhythmias requiring the implantation of automatic implantable cardioverter-defibrillator (AICD) devices. (Laflamme, et al., 2007, Menasche, 2011, Menasche, et al., 2003)

3.2 Hematopoietic stem cells (HSC)

Commonly referred as bone marrow stem cells (BMSC), HSC constitute a small cell population of the bone marrow, perhaps as few as 1:10,000 bone marrow mononuclear cells. (Anversa, et al., 2004, Harrison, et al., 1988) HSC can be enriched using various technologies to high purity on the basis of surface markers (lineage$^-$, c-kit$^+$, Sca-1$^+$, CD38^{high}, CD34, or the more immature marker protein CD133) (Dimmeler and Zeiher, 2004, Orlic, et al., 2002). These cells represent the prototypic adult stem cell population. The ability of HSC to reconstitute the hematopoietic system of a myeloablated host led to the first clinical application of adult stem cells more than three decades ago. (Liao, et al., 2007) HSC can self-renew and were shown to trans-differentiate into multiple lineages, including endothelial cells (Nygren, et al., 2004, Orlic, et al., 2002, Urbich and Dimmeler, 2004), and can contribute to the regeneration of a variety of non-hematopoietic lineages in multiple organs, including myocardium (Jackson, et al., 2001, Orlic, 2003, 2004). The interest in stem cells for cardiac regeneration started in 2001 with the observation of Orlic et al who injected Lin$^-$ ckit$^+$ bone marrow-derived HSC that were able to repair acute MI in mice by transdifferentiating into

cardiomyocytes and vascular cells (Orlic, et al., 2001). To date, the HSC appear to be the most versatile stem cells across all lineages, since adult somatic HSC may share a similar developmental plasticity commonly seen in embryonic stem cells (Anversa, et al., 2004). The use of hematopoietic progenitors stem cells appears to uniformly induce neovascularization that seems to be a prerequisite of the successful functional repair. HSC can be used to repair infarcted hearts by regenerating new cardiomyocytes and vascular endothelium in response to ischemic injury (Jackson, et al., 2001, Kocher, et al., 2001, Nygren, et al., 2004, Orlic, et al., 2001).

3.3 Circulating stem cells

Endothelial progenitor cells (EPCs) are a heterogeneous group of endothelial cell (EC) precursors originating from the hematopoietic compartment of the bone marrow. (Asahara, et al., 1999) EPCs share many surface markers (hematopoietic markers CD133, CD34, vascular endothelial growth factor receptor-2 (Flk-1)) and biological properties with hematopoietic stem cell (HSC). (Asahara, et al., 1999, Gehling, et al., 2000, Shi, et al., 1998) EPCs are isolated from the bone marrow or from the peripheral circulation and expanded in vitro. (Liao, et al., 2007) After a few days in culture in endothelial cell medium supplemented with growth factors, EPC phenotype can be confirmed by direct fluorescent staining with 1,1′-dioctadecyl-3-3-3,3′-tetramethylindocarbocyanine (Dil)-labeled acetylated low-density lipoprotein (DiLDL) and fluorescein isocyanate (FITC)-labeled Ulex europaeus agglutinin-I (lectin). Dual stained cells positive for both DiLDL and lectin are considered EPC, see Fig. 1. EPCs are subdivided into “early”, and “late” also referred as outgrowth of EC (OECs). (Hur, et al., 2004, Smadja, et al., 2007, Yoon, et al., 2005) EPCs have the homing capacity to sites of active angiogenesis, where they participate in the repair of various tissues, including the heart. (Asahara, et al., 1999, Crosby, et al., 2000, Fujiyama, et al., 2003, Vasa, et al., 2001, Yoon, et al., 2005) Recent studies suggested that circulating EPC can home to site of tissue ischemia and injury, express endothelial antigens and play a significant role in new blood vessels formation and re-endothelialization (Asahara, et al., 1999, Crosby, et al., 2000, Fujiyama, et al., 2003, Vasa, et al., 2001). Normal adults have a small amount of circulating EPC in the peripheral blood (Choi, et al., 2004). In response to cytokine stimulation, ischemic insult, drugs, and under the influence of other pathological conditions, these cells are mobilized from the bone marrow (Asahara, et al., 1999, Choi, et al., 2004, Dimmeler, et al., 2001). For example, numbers and angiogenic functions of EPC have been reported to be reduced in patients with risk factors for coronary artery disease (Vasa, et al., 2001), [10], chronic renal failure (Choi, et al., 2004), older age (Scheubel, et al., 2003) and type II diabetics (Tepper, et al., 2002). In contrast, limb ischemia (Asahara, et al., 1999), acute myocardial infarction (Shintani, et al., 2001) and HMG-CoA reductase inhibitors (statins) (Dimmeler, et al., 2001) were associated with increased EPC in the circulation and better angiogenic potential. EPC accelerate re-endothelialization and improve vascular healing[14]. A reduction of their numbers and impaired function have been correlated with inflammatory states, adverse vascular events and death (George, et al., 2003, Lambiase, et al., 2004, Ruel, et al., 2005). The exact mechanism of EPCs-mediated neovascularization is unknown, and possibly the EPCs differentiate into EC, or by paracrine mechanisms via growth factors secretion. (Smadja, et al., 2007) There are limits of the EPCs clinical application. (Choi, et al., 2004, Kawamoto and Asahara, 2007) Amount of available EPCs is restricted, so large blood sample is required for cell preparation.

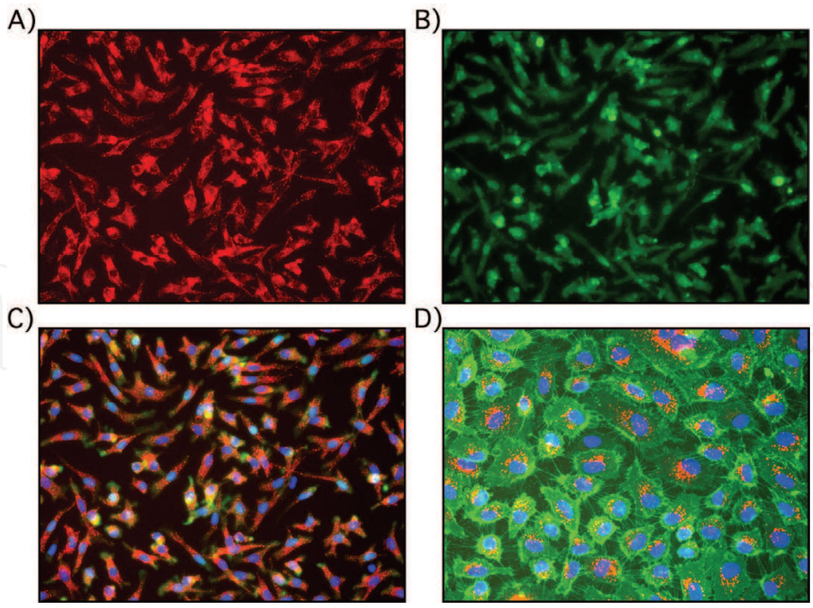


Fig. 1. Characterization of cultured EPC by direct fluorescent staining. A) Early EPC after a few days in culture, adherent cells are stained with (Dil)-labeled acetylated low-density lipoprotein (DiLDL, red) and B) Lectin staining is performed by incubation with fluorescein isocyanate (FITC, green)-labeled Ulex europaeus agglutinin-I. C) Nuclei are stained with Hoechst (blue) and typical merge image demonstrates dual stained cells positive for both DiLDL and lectin are considered EPC (Dimmeler, et al., 2001) (10X original magnification). After staining, samples are viewed with an inverted fluorescent microscope. D) After many days in culture some late EPC can adopt new phenotype from small spindle-like to large, round and flat cells characteristic of mature endothelial cells (10X original magnification). Dr Noiseux, unpublished data.

3.4 Mesenchymal Stem Cells (MSC)

Adult MSC are a population of stromal cells isolated from bone marrow-derived stem cells (BMSC) (Barbash, et al., 2003, Strauer, et al., 2002, Toma, et al., 2002). MSC have been isolated from many species including humans, and are present at a concentration several fold lower than their hematopoietic counterparts. MSC are present at a concentration several fold lower than their hematopoietic counterparts, representing approximatively 0.001-0.01% of the total nucleated marrow cell population. (Jain, et al., 2005, Meirelles Lda and Nardi, 2003) MSC are separated from other cells in culture by their preferential attachment to plastic surfaces (Colter, et al., 2000, Colter, et al., 2001, Meirelles Lda and Nardi, 2003). In their undifferentiated state, MSC do not express hematopoietic or endothelial cell surface markers (Annabi, et al., 2003, Colter, et al., 2000, Mangi, et al., 2003, Meirelles Lda and Nardi, 2003, Minguell, et al., 2001, Peister, et al., 2004) such as CD14, CD31, CD34, CD45, but may express CD-117 (c-kit) and the majority express CD29, CD44, CD90, and Sca-1 (Annabi, et al., 2003, Colter, et al., 2000, Mangi, et al., 2003, Meirelles Lda and Nardi, 2003, Minguell, et al., 2001, Peister, et al., 2004, Pittenger and Martin, 2004). MSC are identified by their specific antigens (SH2, SH3, SH4, STRO-1) and adhesion molecules (ALCAM, CD44). (Jain, et al., 2005) MSC are easily expandable in culture without losing their differentiation potential (Meirelles Lda and Nardi, 2003), and they constitute an

unlimited pool of transplantable cells, Fig. 2. MSC fulfilled the stem cells criteria including self-renewing cell division and potential for differentiation (Verfaillie, et al., 2002). MSC are multipotent (Grove, et al., 2004), and can differentiate into multiple lineages (Grove, et al., 2004, Mackay, et al., 1998, Meirelles Lda and Nardi, 2003, Pittenger, et al., 1999, Song and Tuan, 2004), including fibroblasts, osteoblasts (Liu, et al., 2001), chondroblasts and adipocytes. Differentiation of MSC to cardiomyocyte-like cells has been observed in vitro under specific conditions (Makino, et al., 1999, Rangappa, et al., 2003, Tomita, et al., 1999), and in vivo after injection into the myocardium (Dai, et al., 2005, Saito, et al., 2002, Shake, et al., 2002, Toma, et al., 2002, Tomita, et al., 1999, Wang, et al., 2000).

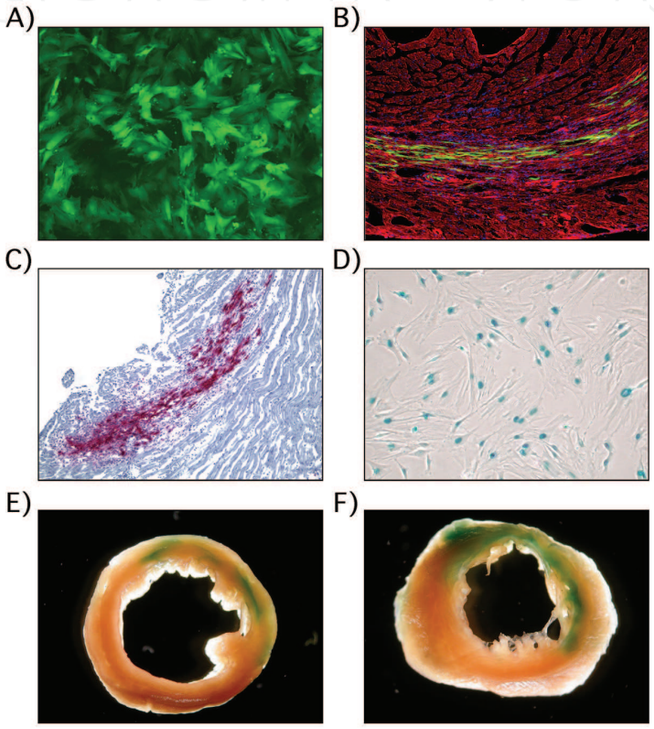


Fig. 2. A) MSC were retrovirally transduced to express GFP (Noiseux, et al., 2006), image under inverted fluorescent microscope. B) MSC were injected into ischemic heart in a mouse model of acute MI. GFP immunostaining (green) was used for tracking the implanted cells at 3 days post-MI, and cardiomyocytes were labeled in red by immunostaining for cardiac alpha-sarcomeric actin, and nuclei are identified by Hoechst staining (blue). C) MSC were stained in red by immonostaining for GFP 3 days after MI, demonstrating massive engraftment in the infarcted area. D) MSC were retrovirally transduced to express LacZ gene (nuclear localizating sequence), image under light microscope after X-gal staining demonstrating blue nuclei. E) Thick section of rat infarcted heart after X-gal staining demonstrating engraftment of MSC expressing LacZ (bleu) in the infarct border zone at the level of the papillary muscles. F) Same as in E, but in the apical segment of the left ventricle. 10X original magnification in A and B, 20X original magnification in B and C. Dr Noiseux, unpublished data.

MSC are known to secrete a wide spectrum of biologically active factors and angiogenic/arteriogenic cytokines (Heil, et al., 2004, Kamihata, et al., 2001, Kinnaird, et al.,

2004, Kinnaird, et al., 2004, Kinnaird, et al., 2004) that can be found in the culture-conditioned medium (MSC-CM) (Heil, et al., 2004, Kamihata, et al., 2001, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Kinnaird, et al., 2004), see Fig. 3. Under hypoxic culture conditions, the expression of several factors is significantly up-regulated (Gnecchi, et al., 2005, Gnecchi, et al., 2006, Kinnaird, et al., 2004, Noiseux N, 2004, Noiseux N, 2004). MSC implantation into the injured heart has been associated with improvement of cardiac performance and repair in animal studies and clinical trials. Because myocardial infarction leads to permanent loss of tissue with subsequent impaired function, the reports highlighting the capacity of MSC to differentiate into new cardiomyocytes have prompted new perspectives in the treatment of cardiovascular diseases using stem cell transplantation. Among adult stem cells, MSC possess unique properties that make them eligible for convenient and highly effective cell therapy (Jain, et al., 2005). MSC are particularly suitable for cellular therapy because of their multipotency, low immunogenicity, amenability to ex vivo expansion, and genetic modification. However, a recent study by Vulliet et al. raised concerns with the use of MSC, and their findings showed acute myocardial ischemia and sub-acute myocardial micro-infarction following intra-coronary injection of MSC into ischemic dog hearts due to the formation of small capillary emboli (Vulliet, et al., 2004).

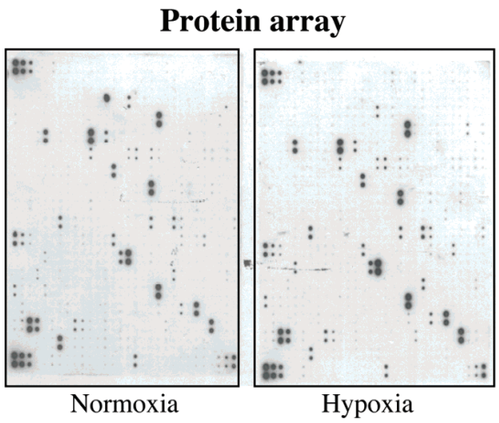


Fig. 3. Human MSC express genes encoding several cytokines and growth factors that are released in the MSC-conditioned medium. Hypoxic culture condition alters the production of several factors. Protein arrays from RayBiotech inc. allow the detection of several proteins simultaneously. Dr Noiseux, unpublished data.

3.5 Cardiac resident stem cells

Recently identified by Hierlihy and colleagues in the adult heart, these cardiac stem cell-like populations were identified based on their specific ability to efflux Hoechst dye (Hierlihy, et al., 2002). These progenitor cells, expressing c-kit, are capable of differentiation into cardiomyocytes and/or vascular tissue. They are self-renewing, clonogenic, and multipotent (Bearzi, et al., 2007). Recent studies have suggested that the heart has an inherent ability to replace its parenchymal cells continuously by these resident stem cells (Braun and Martire, 2007). These observations suggest the capacity of adult myocardium to maintain physiological homeostasis, at least partially, through resident cardiac stem cells (Liao, et al., 2007). The origin of these cardiac stem cells is unclear. These cells could either constitute the remaining endogeneous cardiac stem cells dormant in the myocardium or alternatively, cells

that have homed to the heart from another organ (maybe the bone marrow) or in response from injury (Lyngbaek, et al., 2007). These cells can be clonally expanded in vitro from an endomyocardial biopsy and be used for cardiac repair.

4. Insights on mechanisms of action and fate of the implanted cells

Over the last several years, there has been a surge of data detailing the use of cell-based therapies for post-MI treatment. Stem cells implantation has been shown to prevent deleterious remodeling and improve recovery of infarcted myocardium by cytoprotective effects, myogenesis and angiogenesis (Dai, et al., 2005, Davani, et al., 2003, Dowell, et al., 2003, Kocher, et al., 2001, Mangi, et al., 2003, Min, et al., 2002, Orlic, et al., 2002, Orlic, et al., 2001, 2003, Orlic, et al., 2001, Orlic, et al., 2001, Pittenger and Martin, 2004, Shake, et al., 2002, Strauer, et al., 2002, Tomita, et al., 1999, Tomita, et al., 2002, Wang, et al., 2001, Wollert and Drexler, 2005). The ultimate goal for cell therapy is the stem cell engraftment into the damaged heart and, ultimately, differentiation into new functional cardiomyocytes, vascular smooth muscle and endothelial cells. Although differentiation of stem cells into cardiomyocytes has been observed, there is much debate over the frequency of this phenomenon. Recent work has questioned the ability of stem cells to generate new cardiomyocytes. (Laflamme, et al., 2007, Murry, et al., 2004, Noiseux, et al., 2006) It remains unclear whether the beneficial effect results as a direct consequence of the transplanted cells participating and integrating in a functional syncytium with the host myocardium, or alternatively if the cells could benefit cardiac function without directly contributing to contraction (Dowell, et al., 2003). This indirect effect may be attributed to secretion of biologically active factors (Nguyen, et al., 2010) that could enhance the angiogenic process (Dowell, et al., 2003, Timmermans, et al., 2003), protect cardiomyocytes from apoptosis and induce proliferation, improve heart function with inotropic properties, or recruit resident cardiac stem cells (Wollert and Drexler, 2005). Implanted stem cells may also fuse with the native dysfunctional myocytes to augment function. (Noiseux, et al., 2006, Nygren, et al., 2004, Ying, et al., 2002) Indeed, mechanistic underpinnings of stem cell therapy appear to be far more complex that formerly anticipated, such indirect effects are referred to paracrine action, Fig. 4. Interestingly, while only few groups have observed differentiation of BMSC into cardiomyocytes, most groups have reported a beneficial effect on post-MI remodeling and cardiac functional recovery (Liao, et al., 2007). As such, these data are certainly encouraging, given the improvement in objective measures such as infarct size and contractility. However, they are also disappointing, since they fail to demonstrate cardiac differentiation.

The mechanisms by which stem cells can repair damaged myocardium or lead to improvement in cardiac function are still largely unknown. However, the foremost apparent and proposed pathways are: a) direct or indirect improvement in neovascularization, i.e., vasculogenesis, angiogenesis and arteriogenesis; and b) differentiation/fusion into cardiomyocytes and formation of regenerated myocardial tissue (Kocher, et al., 2007). To which extent these different mechanisms of action are involved may critically depend on the cell type and the clinical setting, such as acute versus chronic ischemic injury (Kocher, et al., 2007). For example, in patients presenting acute MI, stem cell transplantation is thought to significantly improve post-MI ventricular remodeling and function through enhanced neovascularization and reduced cardiomyocytes apoptosis, irrespective of long-term engraftment and trans-differentiation. On the other hand, these

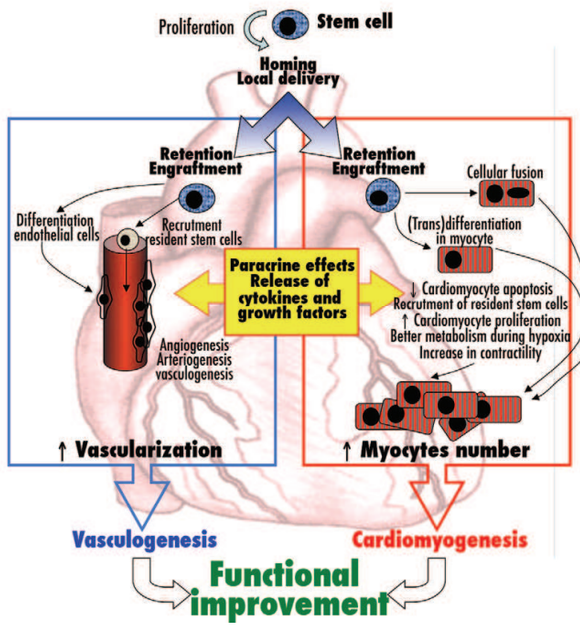


Fig. 4. Working hypothesis for mechanism of stem cell transplantation for the treatment of ischemic cardiomyopathy. Stem and progenitor cell transplantation can have a favorable impact on tissue perfusion and contractile performance by promoting vascularization and myocytes formation. Improved vascularization may facilitate beneficial effects in the myocyte compartment. Adapted from (Wollert and Drexler, 2005).

later mechanisms may have little or no benefit in patients with long established scars, apart from the functional rescue of hibernating cardiomyocytes. Virtually all currently available mechanistic insights have come from pre-clinical studies in animal models, whereas the human experience is rather limited (Laflamme, et al., 2007).

4.1 Stem cells (Trans)differentiation

The foremost purpose of cell-based therapies remains the regeneration of lost cardiac cells via stem cells (trans)differentiation into new contractile cardiomyocytes. (Liao, et al., 2007) Crucial proof-of-principle data in support of this contention were provided by early animal studies with transplantation of fetal and neonatal cardiomyocytes following acute MI (Laflamme, et al., 2007). Implanted myocytes formed stable intra-cardiac grafts and resulted in improved left ventricular function (Jia, et al., 1997, Li, et al., 1996, Li, et al., 1997, Sakai, et al., 1999, Scorsin, et al., 2000). Skeletal muscle satellite cells (referred commonly as myoblasts) have also been exhaustively examined as candidate cells for cardiac repair. Unlike native cardiomyocytes which form an electrical syncytium via gap junction coupling, implanted myoblasts are isolated and do not contract in synchrony with the host myocardium (Leobon, et al., 2003). Embryonic stem cells (ESC) are unambiguously cardiogenic cell type, capable of differentiating into cells with ventricular, atrial and pacemaker/conduction system phenotypes (He, et al., 2003, Kehat, et al., 2001). Cardiomyocytes derived from ESC are able to form stable intra-cardiac implant, and provide functional benefit in murine experimental infarct models (Hodgson, et al., 2004, Singla, et al., 2006).

Orlic et al were among the first to demonstrate in a mice model of AMI that bone marrow could provide an extra-cardiac source of progenitor cells with the ability to differentiate into cardiomyocytes and restore cardiac function (Orlic, et al., 2001, Orlic, et al., 2001, Orlic, et al., 2001). Studies in several species demonstrated that BMSC are stem cells found in various mesenchymal tissues, and precursors of peripheral tissue such as the heart muscle (Strauer and Kornowski, 2003). Cardiogenic potential has also been demonstrated for a surprisingly high number of adult progenitor cell types, including HSC, MSC, circulating EPC, resident cardiac progenitor cells, adipose-derived stem cells and others (Badorff, et al., 2003, Beltrami, et al., 2003, Laflamme, et al., 2007, Oh, et al., 2003, Planat-Benard, et al., 2004, Toma, et al., 2002). When BMSC are transplanted into an ischemic heart, they can express the cardiac specific markers troponin I and cardiac myosin, suggesting transformation into functional cardiomyocytes (Grigoropoulos and Mathur, 2006, Kajstura, et al., 2005, Yoon, et al., 2008).

Using genetic markers (e.g., Y chromosome) and/or labeled fluorescent dyes (e.g., Green fluorescent protein GFP), many groups reported the transdifferentiation of bone marrow-derived HSC into cardiomyocytes following implantation into ischemic heart (Jackson, et al., 2001, Liao, et al., 2007, Orlic, et al., 2001, Orlic, et al., 2001). However, these early observations have been called into question by others, who failed to identify similar cardiomyocytes derived from implanted stem cells (Balsam, et al., 2004, Murry, et al., 2004). Further experimental studies addressing the capacity of transplanted BMSC to differentiate into the cardiomyogenic lineage yielded conflicting results (Anversa, et al., 2007, Dimmeler, et al., 2008, Murry, et al., 2004).

4.2 Cellular fusion

Despite the initially observation that implanted HSC could generate new cardiomyocytes in the infarcted heart, several groups have subsequently revisited this hypothesis of stem cells differentiation (Laflamme and Murry, 2005, Laflamme, et al., 2007, Murry, et al., 2004, Noiseux, et al., 2006). Using state-of-the-art techniques and genetic markers, it has been suggested that rarely cardiomyocytes would display lineage markers of the transplanted stem cells, but that myocardial cells were derived from the fusion of implanted stem cells with damaged host cardiomyocytes (Alvarez-Dolado, et al., 2003, Laflamme, et al., 2007, Murry, et al., 2004, Nygren, et al., 2004). Fusion of transplanted stem cells with resident cardiomyocytes has been offered as an alternative for the previous claims of transdifferentiation (Alvarez-Dolado, et al., 2003, Balsam, et al., 2004, Murry, et al., 2004, Noiseux, et al., 2006, Nygren, et al., 2004, Wollert, 2008). Fused implanted cells may adopt phenotype of recipient cells, and without detailed genetic analysis, might be falsely interpreted as differentiation (Terada, et al., 2002). Fusion of BMSC with purkinje neurons, hepatocytes and cardiomyocytes has been reported by Alvarez-Dolado et al in 2003 (Alvarez-Dolado, et al., 2003). Using different genetic markers to track the fate of transplanted cells, fusion with recipient cardiomyocytes has been also reported for peripheral blood $CD34^+$ cells (Zhang, et al., 2004), hematopoietic stem cells (Murry, et al., 2004, Nygren, et al., 2004), cardiac progenitor cells (Oh, et al., 2003), and skeletal muscle cells (Reinecke, et al., 2004).

It is possible that many examples of putative stem cell plasticity reported in the literature are actually due to transplanted cells fusing with a different tissue type, followed by reprogramming of the donor and recipient cell genome (Vassilopoulos and Russell, 2003).

To address the issue of cellular fusion, it is imperative to use a method that allows the tracking of the implanted cells, but also a genetic marker that can be triggered exclusively by fusion event. A well-validated technique suitable for this purpose relies on the use of Cre/LoxP system. Using a model of AMI in transgenic mice, we previously demonstrated that transplanted MSC could fuse with recipient cardiomyocytes in the infarcted area (Noiseux, et al., 2006). MSC from wild type C57BL/6 mice were retro-virally transduced to express GFP as reporter gene and Cre recombinase. These MSC were transplanted into infarcted hearts of histocompatible R26R mice (similar C57BL/6 genetic background). In these transgenic mice, a loxP-flanked stop sequence is present 5′ of the LacZ expression cassette to prevent transcriptional read-through until selective excision by Cre mediated recombination from implanted MSC (Soriano, 1999). Thus, the LacZ gene is expressed exclusively after a donor MSC expressing Cre fuses with a recipient cell from the R26R mice. Consequently, X-gal staining is used to detect cellular fusion events (Alvarez-Dolado, et al., 2003, Nygren, et al., 2004) As early as 3 days following MSC injection into the ischemic heart, we observed cellular fusion with individual blue cells having typical cardiomyocyte morphology. Interestingly, the majority of the fusion events with $LacZ^+$ cells were detected within the infarct border zone, in areas with viable cardiomyocytes.

4.3 Indirect and paracrine effects

Despite the lack of significant myocardial regeneration by implanted stem cells through differentiation into new cardiomyocytes, numerous studies reported that cardiac function can be improved by cell therapy, suggesting further evidence for a non-myogenic pathway of cardiac repair (Laflamme, et al., 2007). Regardless of the mechanism responsible for the beneficial effects of stem cell therapy on post-MI, cardiac function improvement remains disproportionate to the degree of cardiomyogenic differentiation. (Liao, et al., 2007, Noiseux, et al., 2006) Indeed, the variable observations relating to cell transdifferentiation and fusion, as well as transient cell retention and survival in the ischemic heart (Noiseux, et al., 2006), have prompted a rethinking of the mechanisms that account for the functional benefits of cell therapy in cardiac repair (Psaltis, et al., 2008). It has been proposed that stem cell transplantation may improve myocardial function recovery predominantly by facilitating endogenous repair mechanisms, rather than through direct regeneration of lost cardiomyocytes and vascular cells. Thus, it is possible that implanted cells could secrete bioactive factors which may stimulate angiogenesis, suppress apoptosis of cardiomyocytes, increase efficiency of cardiomyocyte metabolism, improve inotropy of survival myocytes, modulate interstitial matrix composition and remodeling, and maybe even recruit cardiac resident stem cells (Gnecchi, et al., 2006, Kamihata, et al., 2001, Kinnaird, et al., 2004, Noiseux, et al., 2006, Wollert, 2008). The fact that it is possible to improve the heart's function without remuscularizing the infarct is already known from numerous pharmacological studies, e.g. with beta–adrenergic blocker or angiotensin-converting enzyme inhibitors and this has resulted in a search for other potential mechanisms of action (Laflamme, et al., 2007).

MSC are known to secrete a wide spectrum of biologically active factors, (Heil, et al., 2004, Kamihata, et al., 2001, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Kinnaird, et al., 2004) and under hypoxic culture condition and serum starvation, conditions that mimic myocardial infarct, the expression of several factors is significantly up-regulated (Gnecchi, et al., 2005, Gnecchi, et al., 2006, Kinnaird, et al., 2004, Noiseux N, 2004, Noiseux N, 2004). These growth

factors and cytokines are released in the culture medium (MSC-conditioned medium, MSC-CM), and can be concentrated and use therapeutically. We previously reported that transplantation of MSC into ischemic myocardium improved cardiac function recovery and repair as early as 72 hours, which is too early to be explained solely by myocardial regeneration from MSC (Gnecchi, et al., 2005, Gnecchi, et al., 2006, Noiseux, et al., 2006). Transplanted cells were found within the infarcted myocardium (detection of MSC by GFP immunoreactivity). Early massive engraftment was observed at 3 days, but the number of implanted MSC decreased significantly over time, and by 28 days post-MI very few cells remained. Since such an early recovery cannot be explained entirely by de novo myogenesis, we proposed that MSC could achieve cardioprotection by indirect effects, through paracrine mediators, rather than cardiac regeneration. Furthermore, to investigate how MSC could achieve protection of the ischemic myocardium by paracrine mediators and indirect effects, we demonstrated that MSC-CM reduced hypoxia-induced apoptosis, and also triggered vigorous spontaneous contraction of isolated hypoxic adult rat cardiomyocytes in vitro, suggesting the presence of anti-apoptotic and inotropic factors (Gnecchi, et al., 2006). Moreover, when injected directly into infarcted rat hearts, the MSC-CM limited infarct size as early as 72 hours and improved ventricular function (Gnecchi, et al., 2005, Gnecchi, et al., 2006) at levels comparable to those observed following MSC transplantation. These remarkable data strongly support the concept that the effects observed after intramyocardial injection of MSC into infarcted hearts are to a great extent attributable to paracrine protection and indirect effects on ischemic myocardium.

Recently, we investigated the effects of MSC-secreted growth factors on extent of early recovery from MI in a large animal pre-clinical model (Nguyen, et al., 2010). Swine subjected to acute MI by temporary balloon occlusion of the left anterior descending coronary artery using percutaneous techniques received intra-coronary injection of either concentrated MSC-derived growth factors or control medium. Treatment with MSC-derived factors significantly reduced cardiac tropinin-T elevation, and improved echocardiographic parameters, including fractional area shortening, stroke volume, and cardiac output. Quantitative evaluation of fibrosis by Verhoff staining revealed a reduction of the fibrotic area in the infracted zone at 7 days. Similarly, Masson's trichrome staining revealed reduced myocardial damage as demonstrated by areas of relatively preserved myocardium in the infracted area. TUNEL assay demonstrated less cardiomyocyte apoptosis. Protein array detected the presence of angiogenic (VEGF, endothelin, epiregulin), anti-apoptic (Galectin-3, Smad-5, sRFP-1), and anti-remodeling factors (TIMP). RT-PCR confirmed the expression of these factors. In summary, a single intra-coronary injection of concentrated biologically active factors secreted by MSC could achieve early protection of ischemic myocardium, improve cardiac repair, and partially preserve contractility. MSC-derived growth factors injection (rather than MSC themselves) should be evaluated as a novel therapy to treat ischemic heart disease, many practical and technical issues of cell therapy.

4.4 Healing response, inflammation and tissue perfusion

Following myocardial infarct, inflammation and cytokine production regulate myocytes survival/apoptosis, and trigger additional cellular responses, contributing to the healing and remodeling of the injured tissue, which may ultimately influence clinical outcome (Nian, et al., 2004). The immune system plays a crucial role during the acute phase of MI

and this constitutes a most appropriate timing where any immunomodulatory effects following stem cell implantation could exert their effects (Laflamme, et al., 2007). An adequate microenvironment supporting nutrient delivery and waste removal is necessary to sustain survival, growth and possibly differentiation of the transplanted cells (Timmermans, et al., 2003). Therefore, concurrent revascularization must keep pace with cell repopulation of the infarcted tissue (Luttun and Carmeliet, 2003). Transplanted cells may act in an indirect supportive role, optimizing the milieu for host vasculature and cells to respond to ischemia and healing. (Gnecchi, et al., 2005, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Noiseux N, 2004) Normal growth and ultimate stem cell fate depend on engraftment in an appropriate "niche". Thus, it appears that the fate of stem cells is determined by the environment in which they are engrafted rather than by an intrinsically programmed fate, but the mechanisms explaining how the local milieu influences stem cell differentiation are yet to be characterized (Strauer and Kornowski, 2003). Importantly, an angiogenic response has been observed in many studies following cell transplantation (Dowell, et al., 2003).

Much attention has been focused on the direct consequence of cell-based therapy by the renewal of lost parenchymal cells, but restoration of the extra-cellular matrix and vascular supply are also important issues since both components are essential for structural and functional support of the surviving cardiomyocytes (Timmermans, et al., 2003) in the ischemic myocardium. It is also important to consider other potential mechanisms by which transplanted cells could benefit cardiac function without directly contributing to systolic contraction. Blood vessel formation in the heart proceeds mainly through 2 mechanisms: angiogenesis and arteriogenesis. Arteriogenesis is by far the most efficient adaptative mechanism for survival of ischemic organs because of its ability to conduct relatively large blood flow (Buschmann and Schaper, 1999). Angiogenesis and arteriogenesis are complex processes sharing several common mechanisms of action, growth factors and cytokines dependency (Buschmann and Schaper, 1999). Many of these cytokines act not only in a co-ordinated time- and concentration-dependent manner, but one cytokine may potentiate (or inhibit) the effect of another (Kinnaird, et al., 2004), and the complexity of the process of collateral formation has led to speculate that multiple factor strategies could be used therapeutically to modulate vessel formation (Kinnaird, et al., 2003). The recruitment of monocytes that differentiate into macrophages and produce abundant angiogenic growth factors such as VEGF, NO, monocyte chemoatractant protein-1 (MCP-1) and other cytokines, is also essential and ultimately leads to endothelial and smooth muscle cell proliferation, migration, vessel remodeling and extra-cellular matrix synthesis.

The observation that BMSC contain a subpopulation of endothelial progenitor cells with the potential for angiogenesis suggests that these cells may contribute to cardiac repair by enhancing the local blood supply in ischemic myocardium (Grigoropoulos and Mathur, 2006). Some reports have suggested that MSC implantation into ischemic tissue promote collaterals development through paracrine mechanisms, but not through direct cell incorporation into growing vasculature (Dowell, et al., 2003, Fuchs, et al., 2001, Heil, et al., 2004, Hirata, et al., 2003, Kamihata, et al., 2001, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Kinnaird, et al., 2003, Kobayashi, et al., 2000, Li, et al., 2002, Mangi, et al., 2003, Strauer, et al., 2002, Ziegelhoeffer, et al., 2004). MSC express genes encoding a broad spectrum of cytokines with angiogenic properties including VEGF, HGF, FGF, MCP-1, PGF, IL-1 and IL-6, SDF-1, MMP-9 (Kinnaird, et al., 2004, Kinnaird, et al., 2004, Timmermans, et al., 2003). These cytokines can be found in the media of cultured cells, and have all individually been

shown to have positive effects on experimental blood flow recovery (Buschmann, et al., 2003, Heil, et al., 2004, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Kinnaird, et al., 2004, Timmermans, et al., 2003). Moreover, media collected from MSC cultures (MSC-derived conditioned medium, MSC-CM) promote in vitro proliferation and migration of endothelial cells (EC) and vascular smooth muscle cells (VSMC), and enhance in vivo collateral blood flow recovery when injected into ischemic hindlimb (Kinnaird, et al., 2004, Kinnaird, et al., 2004, Timmermans, et al., 2003). It is possible that neovascularization by cell therapy is leading to enhanced blood supply in the peri-infarct region and thereby promoting salvage of stunned, hibernating, or otherwise susceptible cardiomyocytes (Laflamme, et al., 2007). Controversy persists regarding the exact mechanisms through which cell transplantation may enhance repair and collateral development in ischemic tissue (Kinnaird, et al., 2004).

5. Lessons from early clinical trials: feasibility, efficacy and safety

Over the past few years, cell therapy has emerged as a potential new treatment of a variety of cardiac diseases, including AMI, refractory angina, and chronic heart failure. (Menasche, 2009) A variety of cell types have been tested experimentally, each of them being usually recognized by its beneficial "regeneration" potential. This has led to a flurry of clinical trials entailing the use of skeletal myoblasts or bone marrow-derived cells either unfractionated or enriched in progenitor subpopulations. Following acute MI, the observation that mobilization of BMSC occurs naturally to heal the myocardium led several groups to investigate their potential for cardiac repair (Shintani, et al., 2001). In patients with recent MI, Strauer et al demonstrated that IC administration of unselected BMSC was safe and associated with improved cardiac performance (Strauer, et al., 2002). In the same way, Zeiher et al reported that IC administration of BM progenitors after MI improved cardiac performance and enhanced the myocardial perfusion of the infarcted myocardium up to 1 year of follow-up (Assmus, et al., 2002, Britten, et al., 2003, Schachinger, et al., 2004). The landmark multi-center placebo-controlled REPAIR-AMI trial showed also a higher increase in the LVEF in the cell treated group compared to controls (Schachinger, et al., 2006), this effect was sustained up to 2 years and was associated with a significant reduction in the MACE rate (Assmus, et al., 2010). Recently, the FINCELL trial showed also that following acute MI, BMSC therapy significantly improved the LVEF recovery in treated patients as compared to controls (Huikuri, et al., 2008). Interestingly the randomized “BOOST” trial showed an improvement in the cardiac function following IC injection of autologous BMSC without adverse effect. Furthermore, this beneficial effect was sustained at 6 months (Wollert, et al., 2004) and 1 year of follow-up (Schaefer, et al., 2006), but not at 18 months15 raising questions about the potential transient effect of heterogeneous cells on cardiac repair (Meyer, et al., 2006).

Although many trials demonstrated a slight benefit on cardiac functional recovery, recent studies have yielded disparate results (Janssens, et al., 2006, Lunde, et al., 2006, Tendera, et al., 2009). In the randomized ASTAMI trial, the use of IC unselected BMSC following acute MI failed to demonstrate at 6 months any improvement in LVEF (Lunde, et al., 2006). Similarly, Janssens et al reported no significant difference in overall LVEF at 4 months, but showed a decreased infarct size and better regional function in cell-treated patients (Janssens, et al., 2006). Moreover, the REGENT acute MI trial failed to show any significant improvement in the LVEF, LVEDV and LVESV at 6 months in patients treated with

unselected mononuclear cells or selected CD34+ CXCR4+ cells as compared to controls. Finally, Lunde et al (Lunde, et al., 2008) failed to show any improvement in the LV function in patients with anterior MI treated with BMSC.

In the most recently published SCAMI (Wohrle, et al., 2010) (Stem Cell therapy in patients with Acute Myocardial Infarction) study which used serial MRI for assessing results in 42 patients, of whom 29 were allocated to the treated arm), there was no either evidence for a positive effect of intracoronarily infused stem cells treatment versus placebo with regard to LVEF, volumes or infarct size. In this study, the centrifugation technique used for collecting mononuclear cells was similar to that used in the REPAIR-MI trial and a greater number of cells (381×10^6 versus 240×10^6 in REPAIR-MI) was injected at was has been identified as the optimal time point, i.e., at a median of 6.1 days after infarction, a noticeable difference between these two conflicting trials being the longer interval between symptom onset and revascularization in the SCAMI patients (14.3 hours versus 4.5 hours in REPAIRMI). However, a salient feature of the SCAMI protocol has been the rigorousness of the blinding since the control preparation consisted of autologous erythrocytes, which made the placebo syringes indistinguishable from those of the treated group), the design of the SCAMI trial strongly validates its conclusions.

Put altogether, these data clearly show that the potential benefit of bone marrow-derived stem cell therapy shortly after AMI still remains conflicting and the major lesson drawn from this first wave of clinical studies is therefore that there is a real need for a large, adequately powered trial incorporating some of the key findings of the previous studies regarding cell preparation, dosing and timing of delivery and focusing on clinically relevant "hard" end points such as mortality, re-infarction and heart failure. Four recent meta-analyses of BMC in the setting of acute MI incorporating 5, 10, 13, and 18 trials, helped to position results of individual trials into perspective (Abdel-Latif, et al., 2007, Hristov, et al., 2006, Lipinski, et al., 2007, Martin-Rendon, et al., 2008). Overall, results of these randomized placebo-controlled trials and cohort studies are promising in that they demonstrate feasibility, safety, and a slight but positive improved LVEF of 3.66%, a reduced infarct scar size of -5.49% and a reduced LVESV volume of -4.80 ml (Abdel-Latif, et al., 2007). Thus, available evidence suggests that BMSC transplantation is associated with modest improvement in physiologic and anatomic parameters in patients with both acute MI and chronic ischemic cardiomyopathy, but above and beyond conventional therapy (Abdel-Latif, et al., 2007) and could leads to significant and longstanding reduction in the mortality of patients after acute MI as demonstrated in the Balance study (Martin-Rendon, et al., 2008). Therefore, therapy with stem cells appears to be safe, but well-designed double-blinded and randomized studies are clearly needed to confirm promising findings from early studies.

To date, fewer randomized trials of IC trans-catheter transplantation of BMSC have been performed in the setting of chronic coronary artery disease and chronic heart failure (Assmus, et al., 2007, Erbs, et al., 2005, Tse, et al., 2007). Nonetheless, the results are similar to those in patients with acute MI, showing an improvement in LV function, perfusion, and relief of angina pectoris (Losordo, et al., 2007, van Ramshorst, et al., 2009). Assmus et al. reported a registry of 121 consecutive patients with chronic ischemic heart disease treated with intracoronary infusion of BMC (Assmus, et al., 2007). Importantly, they demonstrated that infusion of high number of colony forming cells is associated with a significantly lower mortality during further follow-up (Assmus, et al., 2007). Recently, Strauer et al. (Strauer, et al., 2010) reported in patients with chronic ischemic cardiomyopathy treated with

intracoronary BMSC therapy a significant improvement in the LVEF with a significant decrease in long-term mortality as compared to the control group over 3 months to 5 years of follow-up. These 2 studies were not blinded and randomized, and either lacked or only had a matched cases control groups.

5.1 What about safety?

As often in medicine, the hype generated by the early uncontrolled and small-sized clinical trials has been dampened by the marginally successful outcomes of the subsequent, more rigorously conducted randomized trials. (Menasche, 2009) Although they may have failed or succeed to achieve their primary functional end points, these trials have been positive in the sense that they have allowed to identify a major key issue regarding the safety and feasibility of this therapy. Cardiac cell therapy is overall safe in surgical studies where cells have been injected with hand-held syringes directly in the myocardium, no bleeding complications have been reported. Catheter-based endocardial injections have been equally safe without an increased risk of re-infarction, stent thrombosis or in-stent restenosis (Zhang, et al., 2009). Although the use of the intramyocardial injection of myoblasts was associated with the occurrence of sustained ventricular arrhythmias (Menasche, 2008), none of the trials testing the BMNC has reported an increased incidence of malignant arrhythmias (Menasche, 2011). Likewise, there has been no report of cell-derived tumor formation in the myocardium or elsewhere. This is clinically relevant as the longest follow-up periods now span almost 10 years (Menasche, 2009, Zhang, et al., 2009).

5.2 What about used endpoints in the previous trials?

So far, ejection fraction (EF) has usually been the gold standard for assessing outcomes in the first generation of clinical trials, regardless of the method on which its calculation was based (echocardiography, angiography, radionuclide imaging or magnetic resonance imaging which is likely the most reliable but may not be always possible because of a previously implanted ICD). There is mounting evidence that changes in EF may not be the most suited criteria for assessing the effects of cell therapy. Quantitative assessment of regional systolic function, such as echocardiographic strain rate, could be more sensitive than measuring global LVEF for the evaluation of cell therapy after AMI. (Wollert and Drexler, 2010) In trials exploring a new cell type in a limited number of patients, measurements in regional geometry and function with state of-the-art imaging modalities may be more useful than global assessments for establishing the proof of concept and providing mechanistic cues (Herbots, et al., 2009).

5.3 Selected vs. unselected cells for myocardial transfer?

There is currently uncertainty as to which of the stem cell population is most potent in stimulating angiogenesis and cardiac repair. Nevertheless, the use of hematopoietic progenitor stem cells appears to uniformly induce neovascularization that seems to be a prerequisite of the successful functional repair. The hematopoietic stem cells are characterized by the presence of the surface marker CD34. In addition, CD133 has been identified as a marker that is present on the stem cells that co-express not only CD34 but also other markers such as c-kit. It is hypothesized that use of $CD133^+$ cells may involve larger and more primitive group of stem cells than selection based only on the use of $CD34^+$ marker. The use of non-homogenous or un-selected stem cells may contribute to the

regeneration of necrotic myocardium and blood vessels, but does not allow the characterization of optimal cell type for cardiac repair. Moreover, different cell types may compete for the engraftment in the injured myocardium (Rosenzweig, 2006). The group of Drexler showed that selected BMSC displayed a 7-fold higher retention in the infarcted myocardium, when compared to unfractionated and unselected BMSC (Hofmann, et al., 2005). Furthermore, Stamm (Stamm, et al., 2003) used CD133+ cells for intramyocardial injections performed during an open-chest procedure, and observed an improvement in the tissue perfusion and LVEF during follow-up. Experimental studies demonstrated that selected, well-defined hematopoeitic stem cells contribute to cardiac repair of the acutely infarcted myocardium by inducing neovascularization, inhibition of apoptosis and cardiomyogenesis. Indeed, the hematopoietic CD133+ cells possess high engraftment, multipotent and angiogenic capacity, and appear to be valuable for cardiac repair in experimental myocardial infarction (Bhatia, 2001, Kuci, et al., 2003, Quirici, et al., 2001). Menasche showed in the situation of post-MI scars, transplantation of $CD133^{+}$-derived BMSC improves cardiac function, and benefit was similar to that afforded by myogenic cells. (Agbulut, et al., 2004)

Bartunek et al. tested in a phase I study the feasibility, safety and functional effects of intracoronary administration of selected $CD133^{+}$ BMSC in patients with recent MI. (Bartunek, et al., 2005) They noted a significant increase of 7% in the LVEF in the treated group compared to 3% in controls at 4 months of follow-up. Importantly, they showed in cell-treated patients that improvement of the LV function was paralleled with increased myocardial perfusion and viability (Bartunek, et al., 2005). However, they noted in the treated patients an increase of coronary events with greater in-stent proliferation and larger luminal loss in the non-stented segments of the infarct-related artery that resulted in a significant decrease in pressure-derived FFR (Fractional Flow Reserve) (Mansour, et al., 2006) mainly in early compared to late intracoronary CD133+ cells administration (Vanderheyden, et al., 2007). Yet, these data were obtained from retrospective analysis; they lack randomized controls and systematic use of intravascular ultrasound imaging to track changes in the vascular wall.

5.4 The COMPARE-AMI trial

Our group initiated the first Canadian clinical trial evaluating the intracoronary injection of autologous highly selected $CD133^{+}$ bone marrow-derived stem cells in patients presenting acute MI treated by percutaneous intervention and intra-coronary stent implantation: the COMPARE-AMI trial (Mansour, et al., 2010, Mansour, et al., 2009). This is a randomized, double blind, placebo controlled study. We are investigating the safety, feasibility and efficacy, and the change in the coronary atherosclerotic burden progression in the treated artery, in addition to the change in LVEF measured by MRI. For more details on the study, please visit http://www.anzctr.org.au, study#ACTRN12609001045202. We published a preliminary safety analysis on the first twenty patients that were successfully randomized and treated in the COMPAREAMI trial. The mean age was 52.2±8.9 years with a predominance of males (90%); culprit lesion was located on the left anterior descending artery in 90%, and peak troponin and CKMB were 10.5±8.3 Ug/L and 341±260 U/L, respectively suggesting large infarct. (Mansour, et al., 2011) To date, there is no protocol-related complication to report such as death, MI, stroke, or sustained ventricular arrhythmia. Re-PCI was necessary at 4 months of follow-up in three patients to treat bare-

metal stent restenosis. These patients were asymptomatic; however, silent ischemia was documented in the target territory. Baseline fractional flow reserve (FFR) was significantly lower in the stented culprit artery compared to the non-culprit artery at baseline: 0.88±0.05 vs 0.96±0.04, P<0.001. However, at 4 months of follow-up (n=20), no significant difference was found in the delta FFR compared to baseline in the culprit vs. non-culprit artery (−3.7%±5.4 vs −1.1%±4.6, respectively, P=0.148), suggesting no acceleration of the atherosclerosis by the treatment. Finally, at 4 months of follow-up, MRI assessment of the LVEF (n=18 patients) showed a significant improvement compared to baseline with LVEF 51.1%±2.5 vs 41.2%±1.1, respectively (P<0.001;). This improvement was sustained at 12-month followup (52.3% ± 2.0, P < .001 versus baseline, n = 13). Fig. 5. illustrates the procedure and typical results obtained in patients enrolled in our research protocol.

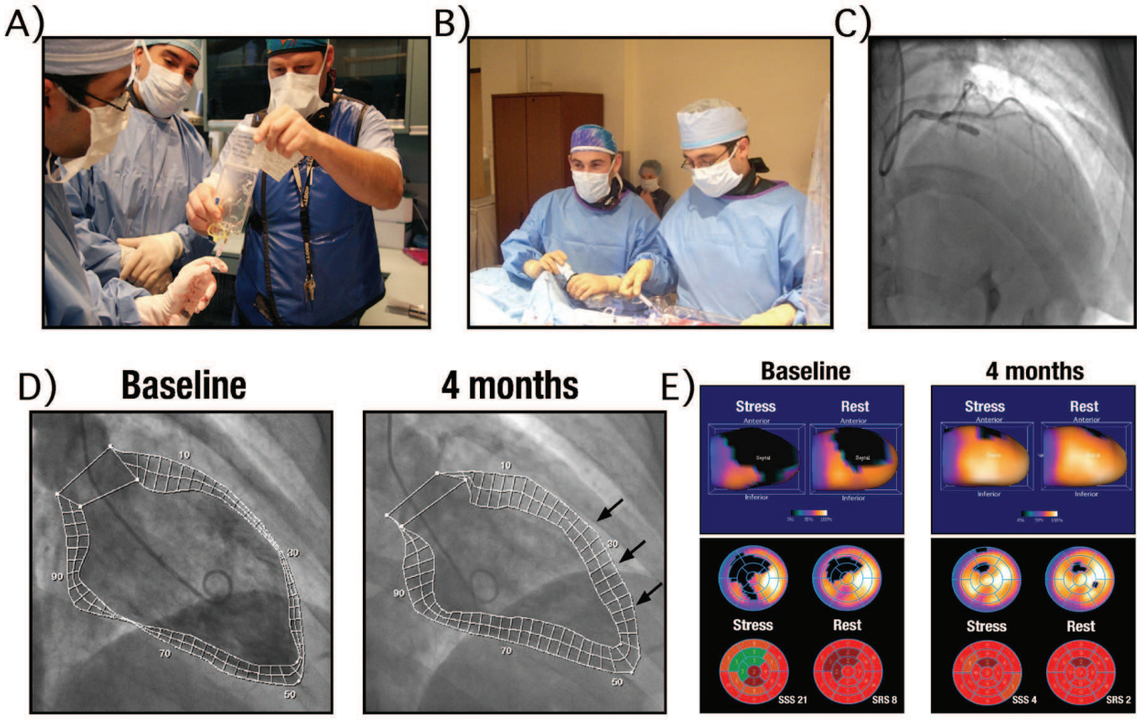


Fig. 5. A) Selected CD133+ bone marrow-derived cells or placebo is injected intracoronary in patients participating to the COMPARE-AMI trial. B) Using a small catheter under fluoroscopic guidance, the cells or placebo are injected in the stented coronary artery. C) During injection, a balloon is occluding the coronary artery proximally. D) A representative left heart ventriculography showing a near complete recovery of cardiac function in a 54-year-old man after his randomization in COMPARE-AMI trial. E) Technetium-99 m Sestamibi single-photon emission computed tomography (MIBI SPECT) scintigraphy looking at myocardial perfusion. The same patient as in D. A, B and C from Dr Noiseux and Dr Mansour, unpublished data. D and E, adapted from (Mansour, et al., 2010).

In conclusion, the findings of the present studies are as follows: i) BMC therapy after MI provide a faster improvement in systolic cardiac function, including LVEF, LVESV and LVEDV, compared with controls. These improvements were sustained after at least six months; ii) Statistically and clinically significant benefits were observed in the regional

cardiac anatomy, but these did not provide a physiological benefit; iii) In the baseline-impaired LVEF subgroup, LVEF improved after BMC therapy compared with the control treatment; iv) BMC therapy was safe, but a reduction in cardiovascular events was not observed; v) Subgroup analyses suggested that cell infusion after AMI had a positive effect on LVEF. Cell infusion within 4-15 days with a higher number of CD34+ cells may have a beneficial impact on LVEF; and vi) Selected BMC are safe and may have a beneficial effect on the healing of the infarcted myocardium.

Recently, we initiated a second study in our stem cell therapy program, looking at a different patient population presenting chronic ischemic cardiomyopathy associated with reduced LV function. IMPlantation of Autologous CD133+ sTem cells in patients undergoing coronary artery bypass grafting (CABG) surgery or IMPACT-CABG trial is a randomized, prospective, double blind, placebo-controlled phase II clinical trial designed a to assess the safety, feasibility and functional benefit of intramyocardial injection of autologous CD133+ BMSC as compared to placebo at the time of CABG surgery. The first 5 patients were treated in an open label fashion and received 10 millions autologous CD133+ cells with CABG. For more details, please visit http://clinicaltrials.gov, study #NCT01033617. To date, 7 patients were enrolled, and no protocol related complications were observed. Our preliminary work suggests the safety of CD133+ autologous cells for cardiac repair, and possibly beneficial effects on cardiac function are observed.

5.5 IMPACT-CABG trial: case report, presentation of the first treated patient

A 59 year-old male with angina at rest (CCS class 4) and congestive heart failure symptoms: dyspnea on slight exertion (NYHA functional class III) and edema of the lower extremities was referred for coronary artery bypass graft (CABG) surgery. He was an active smoker and known for hypertension, type II diabetes since 20 years treated with insulin and a slight chronic renal insufficiency (creatinine 134 umol/L, estimated glomerular filtration rate of 47 ml/min/1.73m^2). His coronary angiogram showed a left dominance system with 90% stenosis on the left anterior descending coronary artery (LAD), 80% on the first diagonal branch and 80% on the posterior descending artery (PDA). In addition, he was known to have a severe left ventricular (LV) dysfunction as assessed by left ventriculography (30%) and echocardiography (LVEF 35-40%). He consented to participate in the IMPACT-CABG study, a phase II clinical trial testing the safety and feasibility of selected CD133+ bone marrow stem cells. Pre-operative stress echocardiography and magnetic resonance imaging (MRI) depicted on the left ventricle necrosis of the apical segment with aneurysm, hypokinesia of mid and basal regions the antero-septal and antero-lateral segments, and akinesia of the infero-apical segments. On the morning of the surgery, the patient underwent bone marrow (BM) aspiration from the iliac crest under local anesthesia. Stem cells were prepared in the cell therapy laboratory and CD133+ cells were purified using the CliniMACS® CD133 Reagent System from Miltenyi Biotech Inc®. On the evening of the same day, the patient underwent CABG surgery and received the left internal thoracic artery on the LAD and a saphenous vein graft on the PDA. Immediately following distal anastomoses, autologous CD133+ cells (10 millions cells, 15 injection sites) were injected directly into the myocardium using a 26g needle in the anterior and lateral wall of the left ventricle (Figure 6). The aortic cross-clamp time and the total cardiopulmonary bypass (CPB) time were 29 minutes and 45 minutes respectively. The peri-operative course was uneventful without any in-hospital complication related to neither the research protocol nor the surgery. The patient

was discharged from the hospital after 7 days. At 6 months follow-up, the patient symptoms improved to NHYA class I and LVEF was increased to 60% assessed by echocardiography (Table 1). Regional motion also improved: contractility of the apical region enhanced significantly, and the left antero-septal segments were only slightly hypokinetic. The MRI study demonstrated a spectacular improvement of the perfusion in all territories with disappearance of the ischemia in the antero-apical and inferior territories and with mild

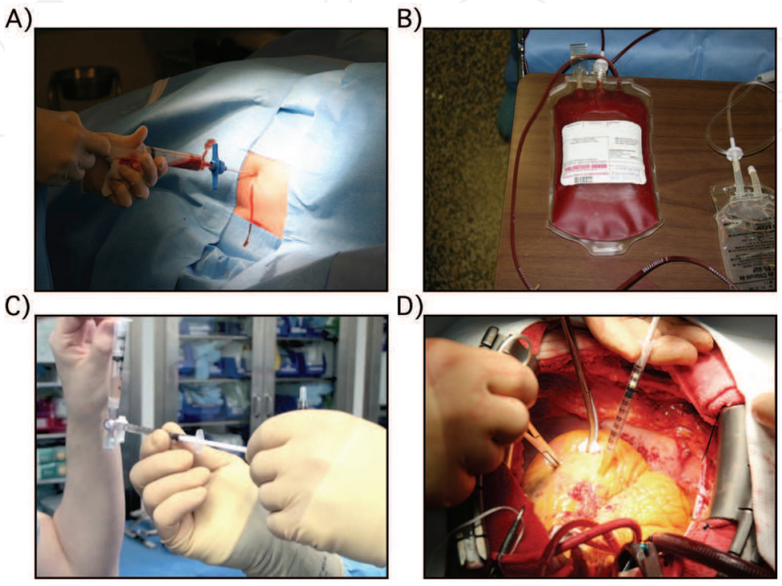


Fig. 6. A) Bone marrow aspiration in the iliac crest in the IMPACT-CABG trial protocol. B) Bone marrow is transferred into a blood collection bag with heparin. C) After CD133+ stem cells isolation and selection using the CliniMACS system from Miltenyi Biotech© cells are transferred into 1.0 ml syringe for injection. D) Intra-operative injection of the CD133+ stem cells into the infarcted area and infarct border zone. Dr Noiseux, unpublished data.

	Baseline	**6 months post-CD133+**
Echocardiography:		
LVEF bi plan %	41	60
WMS	37	22
WMSI	2.3	1.4
MRI:		
LVEDV ml (ml/m²)	179 (107)	178 (106)
LVESV ml (ml/m²)	111 (66)	94 (56)
LVEF %	38	48
LV mass gr (gr/m²)	118 (70)	140 (83)
Stroke volume ml	68	84

Table 1. Echocardiography and magnetic resonance imaging results at baseline and at 6 months post CD133+ injections. LVEF: left ventricular ejection fraction; WMS: wall motion score; WMSI: wall motion score index (normal=1); LVEDV: left ventricular end diastolic volume; LVESV: left ventricular end systolic volume; MRI: magnetic resonance imaging.

ischemia in the antero and inferoseptal basal segments. Moreover, the LV dilatation was reduced, with smaller volume and an increased myocardial mass. No arrhythmia was detected by 24 hrs Holter monitoring.

We believe $CD133^+$ stem cells to be amongst the most potent cells for myocardial repair. This work represents the first Canadian experience with $CD133^+$ stem cells for the treatment of chronic ischemic cardiomyopathy. The remarkable and encouraging results from the fist patient support the continuation of IMPACT-CABG trial, and by randomization between CABG combined to stem cell versus CABG alone, this trial will prove the safety of the procedure and possibly the beneficial effects of the cellular therapy. This novel therapy may become an important therapeutical adjunct to conventional treatment for coronary artery diseases.

6. Pitfalls and important issues on cell therapy

Although great enthusiasm was created by the possibility of reconstituting the damaged heart by cell therapy, the exact mechanism is still unclear, and it is possible that findings supporting myocardial regeneration by stem cells differentiation possibly result form technical artifacts. Controversy exists surrounding the ability of BMSC to undergo transdifferentiation, as some techniques that have been used to demonstrate this phenomenon have been questioned (Grigoropoulos and Mathur, 2006). Current failure to label the donor cells adequately and to follow them in vivo makes it very difficult to distinguish them from background tissue and could lead to misinterpretation (Hassink, et al., 2003). Indeed, the use of GFP reporter gene is attractive because it is compatible with a variety of imaging techniques, but dead and dying cardiomyocytes have an autofluorescent spectrum that partially overlaps with that of GFP. (Burdon, et al., 2011) After injury, autofluorescence increases due to accumulated lipofuscin, blood-derived pigments, and other intrinsic fluors such as flavins and reduced nicotinamide adenine dinucleotide (NADH) (Laflamme and Murry, 2005). Evidence for regeneration includes colocalization of GFP fluorescence from donor cells, with immunostaining for cardiomyocytes markers, including sarcomeric actin. Thus, it is possible to misidentify a GFP positive cardiomyocyte as the result from a donor GFP positive cell.

An important issue limiting cell therapy is the extensive cell death following transplantation into the ischemic heart. The survival of implanted cells is often limited in the infarcted tissue (Koc and Gerson, 2003, Zhang, et al., 2001) and cell death is worsened by the hostile environment caused by the reduced blood flow, hypoxemia, inflammation and scarring in the ischemic myocardium. Regardless of cell type, multiple studies suggest that more than 90% of cells successfully delivered to the heart will die within one week (Laflamme and Murry, 2005, Noiseux, et al., 2006, Zhang, et al., 2001). Most cells die within hours of transplantation because of interplay of ischemia, inflammation, and apoptosis. (Menasche, 2009, Rosenzweig, 2006) Recent clinical trials yielded inconsistent data in cardiac function reporting mixed results. (Janssens, et al., 2006, Lunde, et al., 2006, Schachinger, et al., 2006) These conflicting data have re-ignited interest in the unresolved questions regarding the biology of candidate cells, and how to improve these cells for clinical therapy. Our group investigated the over-expression of anti-apoptotic proteins such as Akt, to improve the cell survival, but also the reparative effects of MSC therapy in an animal model of acute MI (Gnecchi, et al., 2005, Gnecchi, et al., 2006, Mangi, et al., 2003, Noiseux, et al., 2006). We genetically engineered MSC to over-express the pro-survival gene Akt1 (Datta, et al., 1999,

Franke, et al., 2003, Franke, et al., 1997) (Akt-MSC). Akt over-expression resulted in better protective and anti-apoptotic effects against ischemia in vitro, but also in vivo as shown by the reduction of the proportion of apoptotic cells following implantation into rat infarcted myocardium and increased cell retention. Moreover, intra-cardiac injection of $5x10^6$ Akt-MSC inhibited the process of cardiac remodeling and resulted in normalization of cardiac function at 2 weeks, to a level indistinguishable from sham-operated animals (Mangi, et al., 2003). Although improvements in cardiac function were also observed in other studies, complete recovery of LV function occurred only following transplantation of MSC over-expressing Akt, and these results represent a new threshold in cardiac regeneration using cell therapy (Koc and Gerson, 2003). This cell-based therapy, combined with a gene-therapy approach, has the potential to address multiple issues in cell availability and scalability.

A clear understanding of events stimulated by and consequent of transplant of various cell types is critical to avoid potentially adverse consequences. (Evans, et al., 2007) In particular, appropriate electrical and mechanical coupling in the myocardium is essential for optimal cardiac function and avoiding lethal arrhythmias such as observed with skeletal myoblasts. An additional issue of possible concern may be tumorigenesis. (Evans, et al., 2007) Recent experimental study demonstrated that engraftment of undifferentiated embryonic stem cells into the myocardium could result in tumor formation. (Cai, et al., 2007)

The identification of the appropriate route for cell administration to the damaged heart is an essential prerequisite for successful tissue repair (Strauer and Kornowski, 2003). The goal of any cell delivery strategy is to transplant sufficient number of cells into the myocardial region of interest and to achieve maximum retention of cells within that area (Kocher, et al., 2007). Furthermore, the success of cell delivery is determined by the local milieu since it will influence short-term cell survival, cell properties in regard to cell adhesion, transmigration through the vascular wall, and tissue invasion. High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are required, therefore targeted and regional administration of cells are preferred (Strauer and Kornowski, 2003). Cell homing, adhesion, transmigration through the vascular wall, and tissue invasion involves many complex steps.

The most frequently used routes of cell delivery for ischemic cardiomyopathy are percutaneous intra-coronary injection, percutaneous endomyocardial using 3D-guiding systems, and direct intra-myocardial injection during open chest cardiac surgery procedure. Intra-coronary sinus or intravenous systemic deliveries have also been described, as well as stem cell mobilization following G-CSF treatment as described in the FIRSTLINE-AMI trial (Ince, et al., 2005)[5]. Intra-coronary infusion requires migration through the vessel wall into the ischemic heart tissue, which is helped by damaged and permeabilized endothelium as found after acute myocardial infarct. Cells like bone marrow-derived and blood-derived progenitor cells are known to extravasate and migrate efficiently into ischemic areas, whereas skeletal myoblasts do not (Kocher, et al., 2007). The intravenous route is the easiest, but the main disadvantage is that approximately only 3% of normal cardiac output will flow through the left ventricle via the coronary arteries, and is limited because of transpulmonary first-pass captation and sequestration (Strauer and Kornowski, 2003).

The intra-coronary route has been used safely and effectively to achieve selective administration and higher first-pass delivery to the heart than systemic therapy (Psaltis, et al., 2008). However, there have been reports of micro-circulation obstruction following intra-coronary infusion of satellite cells and MSC, resulting in embolic myocardial damage and sub-acute microinfarction (Vulliet, et al., 2004). Unlike intra-vascular infusion, direct intra-

myocardial injection targets specific regions of myocardium without relying on the up-regulation of inflammatory signals to assist transvascular cell migration and tissue invasion (Psaltis, et al., 2008). Pre-clinical and clinical results suggest that direct MSC injection may result in less non-cardiac cell entrapment than intra-coronary or systemic intravenous infusion, along with better retention culminating in greater benefit for cardiac function recovery (Freyman, et al., 2006, Heldman and Hare, 2008, Perin, et al., 2008). An intra-myocardial injection delivery approach appears specially well-suited for transplantation of larger and adherent cells (e.g. MSC) and particularly relevant to chronic myocardial diseases such as chronic ischemic cardiomyopathy or dilated failing heart (Psaltis, et al., 2008). After more than a decade since the beginning of cell therapy, no one has identified the technique for optimal administration of stem cell into the heart. As a result, more than 90% of the cells delivered to the heart through a needle are lost to the circulation or leak out of the injection site (Laflamme and Murry, 2005). Indeed, mechanical leakage and washout may account for a major proportion of cell loss after cell implantation (Kocher, et al., 2007). Experiments by Teng et al with microspheres revealed a retention rate of only 11% following direct intra-myocardial injection in the beating porcine heart versus 67% in the non-beating heart illustrate the complexity of the approach (Teng, et al., 2006).

7. Conclusion

The initial hypothesis underpinning cell therapy was that new muscle cells would be generated in the injured myocardium, and this would restore cardiac function through systolic force generation (Laflamme, et al., 2007). Although the prospect of cardiac tissue regeneration provided an initial stimulus for cell-based therapies, subsequent experimental work has questioned the ability of stem cells to effectively regenerate cardiomyocytes (Murry, et al., 2004, Noiseux, et al., 2006). Unfortunately, hardly any clinical studies demonstrated convincing evidence for electrical or mechanical activation of engrafted cells within the infarct. There is currently uncertainty as to the optimal stem cell population to use clinically for cardiac repair. It appears that not "one cell fits all" but that the selection of the cell type should be tailored to the primary clinical indication and expected outcome (Menasche, 2009). Despite recent significant progress, answer to basic questions such as the best cell type has not been addressed so far. Different cell type may compete for the engraftment in the injured myocardium. The fact that numerous cell types and preparations can enhance myocardial repair, when none of these cells can even generate new cardiomyocytes, suggests that multiple mechanisms may be implicated. Presently, the field of stem cell therapy in regenerative medicine has extremely limited insight about how any candidate cell or treatment may work (Laflamme, et al., 2007). Indeed, the mechanisms responsible for the improvement in cardiac function have not yet been elucidated, and clinical studies have suggested that only a small fraction of implanted cells survived in the heart (Rosenzweig, 2006). This lack of mechanistic understanding has not prevented rapid clinical translation of cellular therapy, and numerous trials have been recently initiated. Recent randomized studies of cell therapy represent a milestone in this rapidly developing field, while serving as a cogent reminder that many important clinical and fundamental questions have yet to be addressed in carefully designed human studies (Rosenzweig, 2006). While stem cell therapy trials in cardiovascular disease are promptly progressing from bench to bedside, more extensive and vigorous basic science and clinical research are clearly needed to thoroughly investigate the therapeutic merits and potential adverse effects of

stem cell transplantation (Jain, et al., 2005). Over the past few years, several promising results have been reported, but many hurdles remain before cell therapy can actually be commonly applied to treat patients with damaged hearts (Smits, et al., 2005). With a better understanding of adult stem cell biology and of the underpinning mechanisms involved in myocardial repair, we may eventually harness the therapeutic potential of these unique cells to better fulfill the beneficial promise of regenerative medicine in future years to come. Nevertheless, it appears that the exact mechanism underlying stem cell therapy is far more complex that previously anticipated, but so far cardiac cell therapy is feasible and overall safe in the clinical arena.

8. References

Abdel-Latif, A., Bolli, R., Tleyjeh, I. M., Montori, V. M., Perin, E. C., Hornung, C. A., Zuba-Surma, E. K., Al-Mallah, M. and Dawn, B. (2007). Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med*, 167. 10, pp.(989-97), 0003-9926 (Print).

Agbulut, O., Vandervelde, S., Al Attar, N., Larghero, J., Ghostine, S., Leobon, B., Robidel, E., Borsani, P., Le Lorc'h, M., Bissery, A., Chomienne, C., Bruneval, P., Marolleau, J. P., Vilquin, J. T., Hagege, A., Samuel, J. L. and Menasche, P. (2004). Comparison of human skeletal myoblasts and bone marrow-derived CD133+ progenitors for the repair of infarcted myocardium. *J Am Coll Cardiol*, 44. 2, pp.(458-63).

Alvarez-Dolado, M., Pardal, R., Garcia-Verdugo, J. M., Fike, J. R., Lee, H. O., Pfeffer, K., Lois, C., Morrison, S. J. and Alvarez-Buylla, A. (2003). Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature*, 425. 6961, pp.(968-73. Epub 2003 Oct 12.).

Annabi, B., Lee, Y. T., Turcotte, S., Naud, E., Desrosiers, R. R., Champagne, M., Eliopoulos, N., Galipeau, J. and Beliveau, R. (2003). Hypoxia promotes murine bone-marrow-derived stromal cell migration and tube formation. *Stem Cells*, 21. 3, pp.(337-47).

Anversa, P., Leri, A., Rota, M., Hosoda, T., Bearzi, C., Urbanek, K., Kajstura, J. and Bolli, R. (2007). Concise review: stem cells, myocardial regeneration, and methodological artifacts. *Stem Cells*, 25. 3, pp.(589-601), 1066-5099 (Print).

Anversa, P., Sussman, M. A. and Bolli, R. (2004). Molecular genetic advances in cardiovascular medicine: focus on the myocyte. *Circulation*, 109. 23, pp.(2832-8).

Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearne, M., Magner, M. and Isner, J. M. (1999). Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*, 85. 3, pp.(221-8).

Assmus, B., Fischer-Rasokat, U., Honold, J., Seeger, F. H., Fichtlscherer, S., Tonn, T., Seifried, E., Schachinger, V., Dimmeler, S. and Zeiher, A. M. (2007). 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*, 100. 8, pp.(1234-41), 1524-4571 (Electronic).

Assmus, B., Rolf, A., Erbs, S., Elsasser, A., Haberbosch, W., Hambrecht, R., Tillmanns, H., Yu, J., Corti, R., Mathey, D. G., Hamm, C. W., Suselbeck, T., Tonn, T., Dimmeler, S., Dill, T., Zeiher, A. M. and Schachinger, V. (2010). Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute

myocardial infarction. *Circ Heart Fail*, 3. 1, pp.(89-96), 1941-3297 (Electronic), 1941-3289 (Linking).

Assmus, B., Schachinger, V., Teupe, C., Britten, M., Lehmann, R., Dobert, N., Grunwald, F., Aicher, A., Urbich, C., Martin, H., Hoelzer, D., Dimmeler, S. and Zeiher, A. M. (2002). Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation*, 106. 24, pp.(3009-17).

Badorff, C., Brandes, R. P., Popp, R., Rupp, S., Urbich, C., Aicher, A., Fleming, I., Busse, R., Zeiher, A. M. and Dimmeler, S. (2003). Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation*, 107. 7, pp.(1024-32), 1524-4539 (Electronic).

Balsam, L. B., Wagers, A. J., Christensen, J. L., Kofidis, T., Weissman, I. L. and Robbins, R. C. (2004). Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*, 428. 6983, pp.(668-73).

Barbash, I. M., Chouraqui, P., Baron, J., Feinberg, M. S., Etzion, S., Tessone, A., Miller, L., Guetta, E., Zipori, D., Kedes, L. H., Kloner, R. A. and Leor, J. (2003). Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*, 108. 7, pp.(863-8).

Bartunek, J., Vanderheyden, M., Vandekerckhove, B., Mansour, S., De Bruyne, B., De Bondt, P., Van Haute, I., Lootens, N., Heyndrickx, G. and Wijns, W. (2005). Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. *Circulation*, 112. 9 Suppl, pp.(I178-83), 1524-4539 (Electronic).

Bearzi, C., Rota, M., Hosoda, T., Tillmanns, J., Nascimbene, A., De Angelis, A., Yasuzawa-Amano, S., Trofimova, I., Siggins, R. W., Lecapitaine, N., Cascapera, S., Beltrami, A. P., D'Alessandro, D. A., Zias, E., Quaini, F., Urbanek, K., Michler, R. E., Bolli, R., Kajstura, J., Leri, A. and Anversa, P. (2007). Human cardiac stem cells. *Proc Natl Acad Sci U S A*, 104. 35, pp.(14068-73), 0027-8424 (Print).

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. and Anversa, P. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, 114. 6, pp.(763-76).

Bhatia, M. (2001). AC133 expression in human stem cells. *Leukemia*, 15. 11, pp.(1685-8),

Blau, H. M., Brazelton, T. R. and Weimann, J. M. (2001). The evolving concept of a stem cell: entity or function? *Cell*, 105. 7, pp.(829-41).

Braun, T. and Martire, A. (2007). Cardiac stem cells: paradigm shift or broken promise? A view from developmental biology. *Trends Biotechnol*, 25. 10, pp.(441-7), 0167-7799 (Print).

Britten, M. B., Abolmaali, N. D., Assmus, B., Lehmann, R., Honold, J., Schmitt, J., Vogl, T. J., Martin, H., Schachinger, V., Dimmeler, S. and Zeiher, A. M. (2003). 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*, 108. 18, pp.(2212-8).

Burdon, T., Paul, A., Noiseux, N., Prakash, s. and Shum-Tim, D. (2011). Bone marrow stem cell derived paracrine factors for regenerative medicine: current perspectives and therapeutic potential. *Bone Marrow Research*, Vol 2011. Open acces.

Buschmann, I., Heil, M., Jost, M. and Schaper, W. (2003). Influence of inflammatory cytokines on arteriogenesis. *Microcirculation*, 10. 3-4, pp.(371-9).

Buschmann, I. and Schaper, W. (1999). Arteriogenesis Versus Angiogenesis: Two Mechanisms of Vessel Growth. *News Physiol Sci*, 14. pp.(121-125).

Cai, J., Yi, F. F., Yang, X. C., Lin, G. S., Jiang, H., Wang, T. and Xia, Z. (2007). Transplantation of embryonic stem cell-derived cardiomyocytes improves cardiac function in infarcted rat hearts. *Cytotherapy*, 9. 3, pp.(283-91), 1465-3249 (Print), 1465-3249 (Linking).

Charwat, S., Gyongyosi, M., Lang, I., Graf, S., Beran, G., Hemetsberger, R., Nyolczas, N., Sochor, H. and Glogar, D. (2008). Role of adult bone marrow stem cells in the repair of ischemic myocardium: current state of the art. *Exp Hematol*, 36. 6, pp.(672-80), 0301-472X (Print).

Choi, J. H., Hur, J., Yoon, C. H., Kim, J. H., Lee, C. S., Youn, S. W., Oh, I. Y., Skurk, C., Murohara, T., Park, Y. B., Walsh, K. and Kim, H. S. (2004). Augmentation of therapeutic angiogenesis using genetically modified human endothelial progenitor cells with altered glycogen synthase kinase-3beta activity. *J Biol Chem*, 279. 47, pp.(49430-8), 0021-9258 (Print), 0021-9258 (Linking).

Choi, J. H., Kim, K. L., Huh, W., Kim, B., Byun, J., Suh, W., Sung, J., Jeon, E. S., Oh, H. Y. and Kim, D. K. (2004). Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol*, 24. 7, pp.(1246-52).

Colter, D. C., Class, R., DiGirolamo, C. M. and Prockop, D. J. (2000). Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proc Natl Acad Sci U S A*, 97. 7, pp.(3213-8).

Colter, D. C., Sekiya, I. and Prockop, D. J. (2001). Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. *Proc Natl Acad Sci U S A*, 98. 14, pp.(7841-5).

Crosby, J. R., Kaminski, W. E., Schatteman, G., Martin, P. J., Raines, E. W., Seifert, R. A. and Bowen-Pope, D. F. (2000). Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. *Circ Res*, 87. 9, pp.(728-30).

Dai, W., Hale, S. L., Martin, B. J., Kuang, J. Q., Dow, J. S., Wold, L. E. and Kloner, R. A. (2005). Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation*, 112. 2, pp.(214-23).

Datta, S. R., Brunet, A. and Greenberg, M. E. (1999). Cellular survival: a play in three Akts. *Genes Dev*, 13. 22, pp.(2905-27).

Davani, S., Marandin, A., Mersin, N., Royer, B., Kantelip, B., Herve, P., Etievent, J. P. and Kantelip, J. P. (2003). Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation*, 108 Suppl 1. pp.(II253-8).

De Coppi, P., Bartsch, G., Jr., Siddiqui, M. M., Xu, T., Santos, C. C., Perin, L., Mostoslavsky, G., Serre, A. C., Snyder, E. Y., Yoo, J. J., Furth, M. E., Soker, S. and Atala, A. (2007). Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*, 25. 1, pp.(100-6), 1087-0156 (Print).

Detre, K. M., Rosen, A. D., Bost, J. E., Cooper, M. E., Sutton-Tyrrell, K., Holubkov, R., Shemin, R. J. and Frye, R. L. (1996). Contemporary practice of coronary revascularization in U.S. hospitals and hospitals participating in the bypass

angioplasty revascularization investigation (BARI). *J Am Coll Cardiol*, 28. 3, pp.(609-15), 0735-1097 (Print).

Dimmeler, S., Aicher, A., Vasa, M., Mildner-Rihm, C., Adler, K., Tiemann, M., Rutten, H., Fichtlscherer, S., Martin, H. and Zeiher, A. M. (2001). HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest*, 108. 3, pp.(391-7).

Dimmeler, S., Burchfield, J. and Zeiher, A. M. (2008). Cell-based therapy of myocardial infarction. *Arterioscler Thromb Vasc Biol*, 28. 2, pp.(208-16), 1524-4636 (Electronic).

Dimmeler, S. and Zeiher, A. M. (2004). Wanted! The best cell for cardiac regeneration. *J Am Coll Cardiol*, 44. 2, pp.(464-6).

Dimmeler, S., Zeiher, A. M. and Schneider, M. D. (2005). Unchain my heart: the scientific foundations of cardiac repair. *J Clin Invest*, 115. 3, pp.(572-83).

Dowell, J. D., Rubart, M., Pasumarthi, K. B., Soonpaa, M. H. and Field, L. J. (2003). Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc Res*, 58. 2, pp.(336-50).

Erbs, S., Linke, A., Adams, V., Lenk, K., Thiele, H., Diederich, K. W., Emmrich, F., Kluge, R., Kendziorra, K., Sabri, O., Schuler, G. and Hambrecht, R. (2005). Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. *Circ Res*, 97. 8, pp.(756-62), 1524-4571 (Electronic).

Ertl, G., Gaudron, P. and Hu, K. (1993). Ventricular remodeling after myocardial infarction. Experimental and clinical studies. *Basic Res Cardiol*, 88 Suppl 1. pp.(125-37).

Evans, S. M., Mummery, C. and Doevendans, P. A. (2007). Progenitor cells for cardiac repair. *Semin Cell Dev Biol*, 18. 1, pp.(153-60), 1084-9521 (Print).

Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A. and Sugimoto, C. (2003). PI3K/Akt and apoptosis: size matters. *Oncogene*, 22. 56, pp.(8983-98).

Franke, T. F., Kaplan, D. R. and Cantley, L. C. (1997). PI3K: downstream AKTion blocks apoptosis. *Cell*, 88. 4, pp.(435-7).

Freyman, T., Polin, G., Osman, H., Crary, J., Lu, M., Cheng, L., Palasis, M. and Wilensky, R. L. (2006). A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J*, 27. 9, pp.(1114-22), 0195-668X (Print).

Fuchs, S., Baffour, R., Zhou, Y. F., Shou, M., Pierre, A., Tio, F. O., Weissman, N. J., Leon, M. B., Epstein, S. E. and Kornowski, R. (2001). Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol*, 37. 6, pp.(1726-32).

Fujiyama, S., Amano, K., Uehira, K., Yoshida, M., Nishiwaki, Y., Nozawa, Y., Jin, D., Takai, S., Miyazaki, M., Egashira, K., Imada, T., Iwasaka, T. and Matsubara, H. (2003). Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. *Circ Res*, 93. 10, pp.(980-9).

Gehling, U. M., Ergun, S., Schumacher, U., Wagener, C., Pantel, K., Otte, M., Schuch, G., Schafhausen, P., Mende, T., Kilic, N., Kluge, K., Schafer, B., Hossfeld, D. K. and Fiedler, W. (2000). In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood*, 95. 10, pp.(3106-12).

George, J., Herz, I., Goldstein, E., Abashidze, S., Deutch, V., Finkelstein, A., Michowitz, Y., Miller, H. and Keren, G. (2003). Number and adhesive properties of circulating endothelial progenitor cells in patients with in-stent restenosis. *Arterioscler Thromb Vasc Biol*, 23. 12, pp.(e57-60).

Gnecchi, M., He, H., Liang, O. D., Melo, L. G., Morello, F., Mu, H., Noiseux, N., Zhang, L., Pratt, R. E., Ingwall, J. S. and Dzau, V. J. (2005). Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med*, 11. 4, pp.(367-8).

Gnecchi, M., He, H., Noiseux, N., Liang, O. D., Zhang, L., Morello, F., Mu, H., Melo, L. G., Pratt, R. E., Ingwall, J. S. and Dzau, V. J. (2006). Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *Faseb J*, 20. 6, pp.(661-9).

Goodell, M. A., Jackson, K. A., Majka, S. M., Mi, T., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., Entman, M. L., Michael, L. H. and Hirschi, K. K. (2001). Stem cell plasticity in muscle and bone marrow. *Ann N Y Acad Sci*, 938. pp.(208-18; discussion 218-20).

Grigoropoulos, N. F. and Mathur, A. (2006). Stem cells in cardiac repair. *Curr Opin Pharmacol*, 6. 2, pp.(169-75), 1471-4892 (Print).

Grove, J. E., Bruscia, E. and Krause, D. S. (2004). Plasticity of bone marrow-derived stem cells. *Stem Cells*, 22. 4, pp.(487-500).

Harrison, D. E., Astle, C. M. and Lerner, C. (1988). Number and continuous proliferative pattern of transplanted primitive immunohematopoietic stem cells. *Proc Natl Acad Sci U S A*, 85. 3, pp.(822-6).

Hassink, R. J., Brutel de la Riviere, A., Mummery, C. L. and Doevendans, P. A. (2003). Transplantation of cells for cardiac repair. *J Am Coll Cardiol*, 41. 5, pp.(711-7).

He, J. Q., Ma, Y., Lee, Y., Thomson, J. A. and Kamp, T. J. (2003). Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res*, 93. 1, pp.(32-9), 1524-4571 (Electronic).

Heil, M., Ziegelhoeffer, T., Mees, B. and Schaper, W. (2004). A different outlook on the role of bone marrow stem cells in vascular growth: bone marrow delivers software not hardware. *Circ Res*, 94. 5, pp.(573-4).

Heldman, A. W. and Hare, J. M. (2008). Cell therapy for myocardial infarction: Special delivery. *J Mol Cell Cardiol*, 44. 3, pp.(473-6), 1095-8584 (Electronic).

Herbots, L., D'Hooge, J., Eroglu, E., Thijs, D., Ganame, J., Claus, P., Dubois, C., Theunissen, K., Bogaert, J., Dens, J., Kalantzi, M., Dymarkowski, S., Bijnens, B., Belmans, A., Boogaerts, M., Sutherland, G., Van de Werf, F., Rademakers, F. and Janssens, S. (2009). Improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study. *Eur Heart J*, 30. 6, pp.(662-70), 1522-9645 (Electronic), 0195-668X (Linking).

Hierlihy, A. M., Seale, P., Lobe, C. G., Rudnicki, M. A. and Megeney, L. A. (2002). The post-natal heart contains a myocardial stem cell population. *FEBS Lett*, 530. 1-3, pp.(239-43), 0014-5793 (Print).

Hirata, K., Li, T. S., Nishida, M., Ito, H., Matsuzaki, M., Kasaoka, S. and Hamano, K. (2003). Autologous bone marrow cell implantation as therapeutic angiogenesis for

ischemic hindlimb in diabetic rat model. *Am J Physiol Heart Circ Physiol*, 284. 1, pp.(H66-70).

Hodgson, D. M., Behfar, A., Zingman, L. V., Kane, G. C., Perez-Terzic, C., Alekseev, A. E., Puceat, M. and Terzic, A. (2004). Stable benefit of embryonic stem cell therapy in myocardial infarction. *Am J Physiol Heart Circ Physiol*, 287. 2, pp.(H471-9), 0363-6135 (Print).

Hofmann, M., Wollert, K. C., Meyer, G. P., Menke, A., Arseniev, L., Hertenstein, B., Ganser, A., Knapp, W. H. and Drexler, H. (2005). Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation*, 111. 17, pp.(2198-202).

Hristov, M., Heussen, N., Schober, A. and Weber, C. (2006). Intracoronary infusion of autologous bone marrow cells and left ventricular function after acute myocardial infarction: a meta-analysis. *J Cell Mol Med*, 10. 3, pp.(727-33), 1582-1838 (Print).

Huikuri, H. V., Kervinen, K., Niemela, M., Ylitalo, K., Saily, M., Koistinen, P., Savolainen, E. R., Ukkonen, H., Pietila, M., Airaksinen, J. K., Knuuti, J. and Makikallio, T. H. (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. *Eur Heart J*, 29. 22, pp.(2723-32), 1522-9645 (Electronic) 0195-668X (Linking).

Hur, J., Yoon, C. H., Kim, H. S., Choi, J. H., Kang, H. J., Hwang, K. K., Oh, B. H., Lee, M. M. and Park, Y. B. (2004). Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol*, 24. 2, pp.(288-93), 1524-4636 (Electronic) 1079-5642 (Linking).

Ince, H., Petzsch, M., Kleine, H. D., Eckard, H., Rehders, T., Burska, D., Kische, S., Freund, M. and Nienaber, C. A. (2005). Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. *Circulation*, 112. 9 Suppl, pp.(I73-80), 1524-4539 (Electronic).

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. and Goodell, M. A. (2001). Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest*, 107. 11, pp.(1395-402).

Jackson, K. A., Majka, S. M., Wulf, G. G. and Goodell, M. A. (2002). Stem cells: a minireview. *J Cell Biochem Suppl*, 38. pp.(1-6).

Jain, M., Pfister, O., Hajjar, R. J. and Liao, R. (2005). Mesenchymal stem cells in the infarcted heart. *Coron Artery Dis*, 16. 2, pp.(93-7).

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. and 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*, 367. 9505, pp.(113-21), 1474-547X (Electronic).

Jia, Z. Q., Mickle, D. A., Weisel, R. D., Mohabeer, M. K., Merante, F., Rao, V., Li, G. and Li, R. K. (1997). Transplanted cardiomyocytes survive in scar tissue and improve heart function. *Transplant Proc*, 29. 4, pp.(2093-4).

Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W. C., Largaespada, D. A. and Verfaillie, C. M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, 418. 6893, pp.(41-9).

Kajstura, J., Rota, M., Whang, B., Cascapera, S., Hosoda, T., Bearzi, C., Nurzynska, D., Kasahara, H., Zias, E., Bonafe, M., Nadal-Ginard, B., Torella, D., Nascimbene, A., Quaini, F., Urbanek, K., Leri, A. and Anversa, P. (2005). Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ Res*, 96. 1, pp.(127-37. Epub 2004 Nov 29.).

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. and 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*, 104. 9, pp.(1046-52).

Kawamoto, A. and Asahara, T. (2007). Role of progenitor endothelial cells in cardiovascular disease and upcoming therapies. *Catheter Cardiovasc Interv*, 70. 4, pp.(477-84), 1522-1946 (Print).

Kawamoto, A. and Losordo, D. W. (2008). Endothelial progenitor cells for cardiovascular regeneration. *Trends Cardiovasc Med*, 18. 1, pp.(33-7), 1050-1738 (Print).

Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y. S., Milliken, C., Uchida, S., Masuo, O., Iwaguro, H., Ma, H., Hanley, A., Silver, M., Kearney, M., Losordo, D. W., Isner, J. M. and Asahara, T. (2003). Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*, 107. 3, pp.(461-8), 1524-4539 (Electronic).

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J. and Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest*, 108. 3, pp.(407-14), 0021-9738 (Print).

Kinnaird, T., Stabile, E., Burnett, M. S. and Epstein, S. E. (2004). Bone marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. *Circ Res*, 95. 4, pp.(354-63).

Kinnaird, T., Stabile, E., Burnett, M. S., Lee, C. W., Barr, S., Fuchs, S. and Epstein, S. E. (2004). 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*, 94. 5, pp.(678-85).

Kinnaird, T., Stabile, E., Burnett, M. S., Shou, M., Lee, C. W., Barr, S., Fuchs, S. and Epstein, S. E. (2004). Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation*, 109. 12, pp.(1543-9).

Kinnaird, T., Stabile, E., Epstein, S. E. and Fuchs, S. (2003). Current perspectives in therapeutic myocardial angiogenesis. *J Interv Cardiol*, 16. 4, pp.(289-97).

Kobayashi, T., Hamano, K., Li, T. S., Katoh, T., Kobayashi, S., Matsuzaki, M. and Esato, K. (2000). Enhancement of angiogenesis by the implantation of self bone marrow cells in a rat ischemic heart model. *J Surg Res*, 89. 2, pp.(189-95).

Koc, O. N. and Gerson, S. L. (2003). Akt helps stem cells heal the heart. *Nat Med*, 9. 9, pp.(1109-10),

Kocher, A. A., Schlechta, B., Gasparovicova, A., Wolner, E., Bonaros, N. and Laufer, G. (2007). Stem cells and cardiac regeneration. *Transpl Int*, 20. 9, pp.(731-46), 0934-0874 (Print).

Kocher, A. A., Schuster, M. D., Szabolcs, M. J., Takuma, S., Burkhoff, D., Wang, J., Homma, S., Edwards, N. M. and Itescu, S. (2001). Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med*, 7. 4, pp.(430-6).

Kogler, G., Sensken, S., Airey, J. A., Trapp, T., Muschen, M., Feldhahn, N., Liedtke, S., Sorg, R. V., Fischer, J., Rosenbaum, C., Greschat, S., Knipper, A., Bender, J., Degistirici, O., Gao, J., Caplan, A. I., Colletti, E. J., Almeida-Porada, G., Muller, H. W., Zanjani, E. and Wernet, P. (2004). A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med*, 200. 2, pp.(123-35), 0022-1007 (Print).

Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S. and Sharkis, S. J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, 105. 3, pp.(369-77).

Kuci, S., Wessels, J. T., Buhring, H. J., Schilbach, K., Schumm, M., Seitz, G., Loffler, J., Bader, P., Schlegel, P. G., Niethammer, D. and Handgretinger, R. (2003). Identification of a novel class of human adherent CD34- stem cells that give rise to SCID-repopulating cells. *Blood*, 101. 3, pp.(869-76).

Laflamme, M. A. and Murry, C. E. (2005). Regenerating the heart. *Nat Biotechnol*, 23. 7, pp.(845-56), 1087-0156 (Print) 1087-0156 (Linking).

Laflamme, M. A., Zbinden, S., Epstein, S. E. and Murry, C. E. (2007). Cell-based therapy for myocardial ischemia and infarction: pathophysiological mechanisms. *Annu Rev Pathol*, 2. pp.(307-39), 1553-4006 (Print).

Lambiase, P. D., Edwards, R. J., Anthopoulos, P., Rahman, S., Meng, Y. G., Bucknall, C. A., Redwood, S. R., Pearson, J. D. and Marber, M. S. (2004). Circulating humoral factors and endothelial progenitor cells in patients with differing coronary collateral support. *Circulation*, 109. 24, pp.(2986-92).

Leobon, B., Garcin, I., Menasche, P., Vilquin, J. T., Audinat, E. and Charpak, S. (2003). Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A*, 100. 13, pp.(7808-11), 0027-8424 (Print).

Leor, J., Patterson, M., Quinones, M. J., Kedes, L. H. and Kloner, R. A. (1996). Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation*, 94. 9 Suppl, pp.(II332-6).

Li, R. K., Jia, Z. Q., Weisel, R. D., Mickle, D. A., Zhang, J., Mohabeer, M. K., Rao, V. and Ivanov, J. (1996). Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg*, 62. 3, pp.(654-60; discussion 660-1), 0003-4975 (Print).

Li, R. K., Mickle, D. A., Weisel, R. D., Mohabeer, M. K., Zhang, J., Rao, V., Li, G., Merante, F. and Jia, Z. Q. (1997). Natural history of fetal rat cardiomyocytes transplanted into adult rat myocardial scar tissue. *Circulation*, 96. 9 Suppl, pp.(II-179-86; discussion 186-7).

Li, T. S., Hamano, K., Suzuki, K., Ito, H., Zempo, N. and Matsuzaki, M. (2002). Improved angiogenic potency by implantation of ex vivo hypoxia prestimulated bone marrow cells in rats. *Am J Physiol Heart Circ Physiol*, 283. 2, pp.(H468-73).

Liao, R., Pfister, O., Jain, M. and Mouquet, F. (2007). The bone marrow--cardiac axis of myocardial regeneration. *Prog Cardiovasc Dis*, 50. 1, pp.(18-30), 0033-0620 (Print).

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. and 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. *J Am Coll Cardiol*, 50. 18, pp.(1761-7), 1558-3597 (Electronic).

Liu, P., Kalajzic, I., Stover, M. L., Rowe, D. W. and Lichtler, A. C. (2001). Human bone marrow stromal cells are efficiently transduced by vesicular stomatitis virus-pseudotyped retrovectors without affecting subsequent osteoblastic differentiation. *Bone*, 29. 4, pp.(331-5).

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. and Henry, T. D. (2007). Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation*, 115. 25, pp.(3165-72), 1524-4539 (Electronic).

Lunde, K., Solheim, S., Aakhus, S., Arnesen, H., Abdelnoor, M., Egeland, T., Endresen, K., Ilebekk, A., Mangschau, A., Fjeld, J. G., Smith, H. J., Taraldsrud, E., Grogaard, H. K., Bjornerheim, R., Brekke, M., Muller, C., Hopp, E., Ragnarsson, A., Brinchmann, J. E. and Forfang, K. (2006). Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med*, 355. 12, pp.(1199-209), 1533-4406 (Electronic).

Lunde, K., Solheim, S., Forfang, K., Arnesen, H., Brinch, L., Bjornerheim, R., Ragnarsson, A., Egeland, T., Endresen, K., Ilebekk, A., Mangschau, A. and Aakhus, S. (2008). Anterior myocardial infarction with acute percutaneous coronary intervention and intracoronary injection of autologous mononuclear bone marrow cells: safety, clinical outcome, and serial changes in left ventricular function during 12-months' follow-up. *J Am Coll Cardiol*, 51. 6, pp.(674-6), 1558-3597 (Electronic).

Luttun, A. and Carmeliet, P. (2003). De novo vasculogenesis in the heart. *Cardiovasc Res*, 58. 2, pp.(378-89).

Lyngbaek, S., Schneider, M., Hansen, J. L. and Sheikh, S. P. (2007). Cardiac regeneration by resident stem and progenitor cells in the adult heart. *Basic Res Cardiol*, 102. 2, pp.(101-14), 0300-8428 (Print).

Mackay, A. M., Beck, S. C., Murphy, J. M., Barry, F. P., Chichester, C. O. and Pittenger, M. F. (1998). Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng*, 4. 4, pp.(415-28).

Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A. and Ogawa, S. (1999). Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest*, 103. 5, pp.(697-705).

Mangi, A. A., Noiseux, N., Kong, D., He, H., Rezvani, M., Ingwall, J. S. and Dzau, V. J. (2003). Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med*, 9. 9, pp.(1195-201. Epub 2003 Aug 10.).

Mansour, S., Roy, D. C., Bouchard, V., Nguyen, B. K., Stevens, L. M., Gobeil, F., Rivard, A., Leclerc, G., Reeves, F. and Noiseux, N. (2010). COMPARE-AMI trial: comparison of intracoronary injection of CD133+ bone marrow stem cells to placebo in patients after acute myocardial infarction and left ventricular dysfunction: study rationale and design. *J Cardiovasc Transl Res*, 3. 2, pp.(153-9), 1937-5395 (Electronic).

Mansour, S., Roy, D. C., Bouchard, V., Nguyen, B. K., Stevens, L. M., Gobeil, J. F., Rivard, A., Leclerc, G. and Noiseux, N. (2010). COMPARE-AMI Trial: Comparison of Intracoronary Injection of CD133+ Bone Marrow Stem Cells to Placebo in Patients After Acute Myocardial Infarction and Left Ventricular Dysfunction: Study Rationale and Design. *J. of Cardiovasc. Trans. Res.*, 3. 2, pp.(153-159).

Mansour, S., Roy, D. C., Bouchard, V., Stevens, L. M., Gobeil, J. F., Rivard, A., Leclerc, G. and Noiseux, N. (2011). One-Year Safety Analysis of the COMPARE-AMI Trial: Comparison of Intracoronary Injection of CD133+ Bone Marrow Stem Cells to Placebo in Patients after Acute Myocardial Infarction and Left Ventricular Dysfunction. *Bone Marrow Research*, 2011. pp.(1-6),

Mansour, S., Roy, D. C., Lemieux, B., Ouellet, C., Stevens, L. M. and Noiseux, N. (2009). Stem cell therapy for the broken heart: mini-organ transplantation. *Transplant Proc*, 41. 8, pp.(3353-7), 1873-2623 (Electronic).

Mansour, S., Vanderheyden, M., De Bruyne, B., Vandekerckhove, B., Delrue, L., Van Haute, I., Heyndrickx, G., Carlier, S., Rodriguez-Granillo, G., Wijns, W. and Bartunek, J. (2006). Intracoronary delivery of hematopoietic bone marrow stem cells and luminal loss of the infarct-related artery in patients with recent myocardial infarction. *J Am Coll Cardiol*, 47. 8, pp.(1727-30), 1558-3597 (Electronic).

Martin-Rendon, E., Brunskill, S. J., Hyde, C. J., Stanworth, S. J., Mathur, A. and Watt, S. M. (2008). Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *Eur Heart J*, 29. 15, pp.(1807-18), 1522-9645 (Electronic).

Meirelles Lda, S. and Nardi, N. B. (2003). Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. *Br J Haematol*, 123. 4, pp.(702-11),

Menasche, P. (2008). Skeletal myoblasts and cardiac repair. *J Mol Cell Cardiol*, 45. 4, pp.(545-53), 1095-8584 (Electronic).

Menasche, P. (2009). Cell-based therapy for heart disease: a clinically oriented perspective. *Mol Ther*, 17. 5, pp.(758-66), 1525-0024 (Electronic).

Menasche, P. (2011). Cardiac cell therapy: lessons from clinical trials. *J Mol Cell Cardiol*, 50. 2, pp.(258-65), 1095-8584 (Electronic) 0022-2828 (Linking).

Menasche, P., Hagege, A. A., Scorsin, M., Pouzet, B., Desnos, M., Duboc, D., Schwartz, K., Vilquin, J. T. and Marolleau, J. P. (2001). Myoblast transplantation for heart failure. *Lancet*, 357. 9252, pp.(279-80).

Menasche, P., Hagege, 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. and Duboc, D. (2003). Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol*, 41. 7, pp.(1078-83).

Meyer, G. P., Wollert, K. C., Lotz, J., Steffens, J., Lippolt, P., Fichtner, S., Hecker, H., Schaefer, A., Arseniev, L., Hertenstein, B., Ganser, A. and Drexler, H. (2006). Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. *Circulation*, 113. 10, pp.(1287-94), 1524-4539 (Electronic).

Min, J. Y., Sullivan, M. F., Yang, Y., Zhang, J. P., Converso, K. L., Morgan, J. P. and Xiao, Y. F. (2002). Significant improvement of heart function by cotransplantation of human mesenchymal stem cells and fetal cardiomyocytes in postinfarcted pigs. *Ann Thorac Surg*, 74. 5, pp.(1568-75).

Min, J. Y., Yang, Y., Converso, K. L., Liu, L., Huang, Q., Morgan, J. P. and Xiao, Y. F. (2002). Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol*, 92. 1, pp.(288-96).

Min, J. Y., Yang, Y., Sullivan, M. F., Ke, Q., Converso, K. L., Chen, Y., Morgan, J. P. and Xiao, Y. F. (2003). Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg*, 125. 2, pp.(361-9).

Minguell, J. J., Erices, A. and Conget, P. (2001). Mesenchymal stem cells. *Exp Biol Med (Maywood)*, 226. 6, pp.(507-20).

Murry, C. E., Soonpaa, M. H., Reinecke, H., Nakajima, H., Nakajima, H. O., Rubart, M., Pasumarthi, K. B., Virag, J. I., Bartelmez, S. H., Poppa, V., Bradford, G., Dowell, J. D., Williams, D. A. and Field, L. J. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*, 428. 6983, pp.(664-8).

Murry, C. E., Soonpaa, M. H., Reinecke, H., Nakajima, H., Nakajima, H. O., Rubart, M., Pasumarthi, K. B., Virag, J. I., Bartelmez, S. H., Poppa, V., Bradford, G., Dowell, J. D., Williams, D. A., Field, L. J., Shi, D., Torok-Storb, B. and Minami, E. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts Myogenic fusion of human bone marrow stromal cells, but not hematopoietic cells Evidence for fusion between cardiac and skeletal muscle cells. *Nature*, 428. 6983, pp.(664-8. Epub 2004 Mar 21.).

Murry, C. E., Wiseman, R. W., Schwartz, S. M. and Hauschka, S. D. (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest*, 98. 11, pp.(2512-23).

Nguyen, B. K., Maltais, S., Perrault, L. P., Tanguay, J. F., Tardif, J. C., Stevens, L. M., Borie, M., Harel, F., Mansour, S. and Noiseux, N. (2010). Improved function and myocardial repair of infarcted heart by intracoronary injection of mesenchymal stem cell-derived growth factors. *J Cardiovasc Transl Res*, 3. 5, pp.(547-58), 1937-5395 (Electronic).

Nian, M., Lee, P., Khaper, N. and Liu, P. (2004). Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res*, 94. 12, pp.(1543-53).

Noiseux N, G. M., Mirotsou M, Pratt R, Dzau VJ. (2004). Adult Mesenchymal Stem Cells Genetically Engineered to Overexpress Akt Exhibit Improved Resistance to

Apoptosis, Glucose Metabolism, and Angiogenic Potential. *Can J Cardiol*, 20. pp.((Suppl D):97D).

Noiseux, N., Gnecchi, M., Lopez-Ilasaca, M., Zhang, L., Solomon, S. D., Deb, A., Dzau, V. J. and Pratt, R. E. (2006). Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther*, 14. 6, pp.(840-50), 1525-0016 (Print).

Noiseux N, L.-I. M., Gnecchi M, Zang L, Solomon SD, Dzau VJ, Pratt RE. (2004). Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells Expressing Akt Into Infracted Murine Heart Produces Dramatic Improvement in Cardiac Function Despite Infrequent Cellular Fusion. *Circulation*, 110. (17), pp.((supplement III): III-68).

Nygren, J. M., Jovinge, S., Breitbach, M., Sawen, P., Roll, W., Hescheler, J., Taneera, J., Fleischmann, B. K. and Jacobsen, S. E. (2004). Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med*, 10. 5, pp.(494-501).

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. and Schneider, M. D. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*, 100. 21, pp.(12313-8).

Orlic, D. (2003). Adult bone marrow stem cells regenerate myocardium in ischemic heart disease. *Ann N Y Acad Sci*, 996. pp.(152-7).

Orlic, D. (2004). The strength of plasticity: stem cells for cardiac repair. *Int J Cardiol*, 95 Suppl 1. pp.(S16-9).

Orlic, D., Hill, J. M. and Arai, A. E. (2002). Stem cells for myocardial regeneration. *Circ Res*, 91. 12, pp.(1092-102).

Orlic, D., Kajstura, J., Chimenti, S., Bodine, D. M., Leri, A. and Anversa, P. (2001). Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci*, 938. pp.(221-9; discussion 229-30).

Orlic, D., Kajstura, J., Chimenti, S., Bodine, D. M., Leri, A. and Anversa, P. (2003). Bone marrow stem cells regenerate infarcted myocardium. *Pediatr Transplant*, 7 Suppl 3. pp.(86-8).

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. and Anversa, P. (2001). Bone marrow cells regenerate infarcted myocardium. *Nature*, 410. 6829, pp.(701-5).

Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D. M., Leri, A. and Anversa, P. (2001). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A*, 98. 18, pp.(10344-9).

Patel, A. N., Geffner, L., Vina, R. F., Saslavsky, J., Urschel, H. C., Jr., Kormos, R. and Benetti, F. (2005). Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J Thorac Cardiovasc Surg*, 130. 6, pp.(1631-8), 1097-685X (Electronic).

Peister, A., Mellad, J. A., Larson, B. L., Hall, B. M., Gibson, L. F. and Prockop, D. J. (2004). Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood*, 103. 5, pp.(1662-8).

Pell, J. P., Pell, A. C., Jeffrey, R. R., Jennings, K., Oldroyd, K., Eteiba, H., Hogg, K. J., Murday, A., Faichney, A., Colquhoun, I., Berg, G., Starkey, I. R., Flapan, A. and Mankad, P. (2004). Comparison of survival following coronary artery bypass grafting vs. percutaneous coronary intervention in diabetic and non-diabetic patients: retrospective cohort study of 6320 procedures. *Diabet Med*, 21. 7, pp.(790-2), 0742-3071 (Print).

Perin, E. C., Geng, Y. J. and Willerson, J. T. (2003). Adult stem cell therapy in perspective. *Circulation*, 107. 7, pp.(935-8).

Perin, E. C., Silva, G. V., Assad, J. A., Vela, D., Buja, L. M., Sousa, A. L., Litovsky, S., Lin, J., Vaughn, W. K., Coulter, S., Fernandes, M. R. and Willerson, J. T. (2008). Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J Mol Cell Cardiol*, 44. 3, pp.(486-95), 1095-8584 (Electronic).

Pfeffer, M. A. and Braunwald, E. (1990). Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation*, 81. 4, pp.(1161-72).

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S. and Marshak, D. R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, 284. 5411, pp.(143-7).

Pittenger, M. F. and Martin, B. J. (2004). Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res*, 95. 1, pp.(9-20).

Planat-Benard, V., Menard, C., Andre, M., Puceat, M., Perez, A., Garcia-Verdugo, J. M., Penicaud, L. and Casteilla, L. (2004). Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. *Circ Res*, 94. 2, pp.(223-9), 1524-4571 (Electronic).

Psaltis, P. J., Zannettino, A. C., Worthley, S. G. and Gronthos, S. (2008). Concise review: mesenchymal stromal cells: potential for cardiovascular repair. *Stem Cells*, 26. 9, pp.(2201-10), 1549-4918 (Electronic).

Quirici, N., Soligo, D., Caneva, L., Servida, F., Bossolasco, P. and Deliliers, G. L. (2001). Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol*, 115. 1, pp.(186-94).

Raeburn, C. D., Zimmerman, M. A., Arya, J., Banerjee, A. and Harken, A. H. (2002). Stem cells and myocardial repair. *J Am Coll Surg*, 195. 5, pp.(686-93).

Rangappa, S., Fen, C., Lee, E. H., Bongso, A. and Wei, E. K. (2003). Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg*, 75. 3, pp.(775-9).

Reinecke, H., Minami, E., Poppa, V. and Murry, C. E. (2004). Evidence for fusion between cardiac and skeletal muscle cells. *Circ Res*, 94. 6, pp.(e56-60. Epub 2004 Mar 4.).

Reinecke, H., Zhang, M., Bartosek, T. and Murry, C. E. (1999). Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation*, 100. 2, pp.(193-202).

Rosenzweig, A. (2006). Cardiac cell therapy--mixed results from mixed cells. *N Engl J Med*, 355. 12, pp.(1274-7), 1533-4406 (Electronic).

Ruel, M., Suuronen, E. J., Song, J., Kapila, V., Gunning, D., Waghray, G., Rubens, F. D. and Mesana, T. G. (2005). Effects of off-pump versus on-pump coronary artery bypass grafting on function and viability of circulating endothelial progenitor cells. *J Thorac Cardiovasc Surg*, 130. 3, pp.(633-9).

Saito, T., Kuang, J. Q., Bittira, B., Al-Khaldi, A. and Chiu, R. C. (2002). Xenotransplant cardiac chimera: immune tolerance of adult stem cells. *Ann Thorac Surg*, 74. 1, pp.(19-24; discussion 24).

Sakai, T., Li, R. K., Weisel, R. D., Mickle, D. A., Jia, Z. Q., Tomita, S., Kim, E. J. and Yau, T. M. (1999). Fetal cell transplantation: a comparison of three cell types. *J Thorac Cardiovasc Surg*, 118. 4, pp.(715-24), 0022-5223 (Print).

Schachinger, V., Assmus, B., Britten, M. B., Honold, J., Lehmann, R., Teupe, C., Abolmaali, N. D., Vogl, T. J., Hofmann, W. K., Martin, H., Dimmeler, S. and Zeiher, A. M. (2004). Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol*, 44. 8, pp.(1690-9).

Schachinger, V., Erbs, S., Elsasser, A., Haberbosch, W., Hambrecht, R., Holschermann, H., Yu, J., Corti, R., Mathey, D. G., Hamm, C. W., Suselbeck, T., Assmus, B., Tonn, T., Dimmeler, S. and Zeiher, A. M. (2006). Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*, 355. 12, pp.(1210-21), 1533-4406 (Electronic), 0028-4793 (Linking).

Schaefer, A., Meyer, G. P., Fuchs, M., Klein, G., Kaplan, M., Wollert, K. C. and Drexler, H. (2006). Impact of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: results from the BOOST trial. *Eur Heart J*, 27. 8, pp.(929-35), 0195-668X (Print), 0195-668X (Linking).

Scheubel, R. J., Zorn, H., Silber, R. E., Kuss, O., Morawietz, H., Holtz, J. and Simm, A. (2003). Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting. *J Am Coll Cardiol*, 42. 12, pp.(2073-80).

Scorsin, M., Hagege, A., Vilquin, J. T., Fiszman, M., Marotte, F., Samuel, J. L., Rappaport, L., Schwartz, K. and Menasche, P. (2000). Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function. *J Thorac Cardiovasc Surg*, 119. 6, pp.(1169-75), 0022-5223 (Print).

Shake, J. G., Gruber, P. J., Baumgartner, W. A., Senechal, G., Meyers, J., Redmond, J. M., Pittenger, M. F. and Martin, B. J. (2002). Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg*, 73. 6, pp.(1919-25; discussion 1926).

Shi, Q., Rafii, S., Wu, M. H., Wijelath, E. S., Yu, C., Ishida, A., Fujita, Y., Kothari, S., Mohle, R., Sauvage, L. R., Moore, M. A., Storb, R. F. and Hammond, W. P. (1998). Evidence for circulating bone marrow-derived endothelial cells. *Blood*, 92. 2, pp.(362-7).

Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Honma, T., Katoh, A., Sasaki, K., Shimada, T., Oike, Y. and Imaizumi, T. (2001). Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*, 103. 23, pp.(2776-9).

Singla, D. K., Hacker, T. A., Ma, L., Douglas, P. S., Sullivan, R., Lyons, G. E. and Kamp, T. J. (2006). Transplantation of embryonic stem cells into the infarcted mouse heart: formation of multiple cell types. *J Mol Cell Cardiol*, 40. 1, pp.(195-200), 0022-2828 (Print).

Smadja, D. M., Cornet, A., Emmerich, J., Aiach, M. and Gaussem, P. (2007). Endothelial progenitor cells: characterization, in vitro expansion, and prospects for autologous cell therapy. *Cell Biol Toxicol*, 23. 4, pp.(223-39), 0742-2091 (Print)0742-2091 (Linking).

Smits, A. M., van Vliet, P., Hassink, R. J., Goumans, M. J. and Doevendans, P. A. (2005). The role of stem cells in cardiac regeneration. *J Cell Mol Med*, 9. 1, pp.(25-36), 1582-1838 (Print).

Song, L. and Tuan, R. S. (2004). Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. *Faseb J*, 18. 9, pp.(980-2).

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet*, 21. 1, pp.(70-1).

Stamm, C., Westphal, B., Kleine, H. D., Petzsch, M., Kittner, C., Klinge, H., Schumichen, C., Nienaber, C. A., Freund, M. and Steinhoff, G. (2003). Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet*, 361. 9351, pp.(45-6).

Strauer, B. E., Brehm, M., Zeus, T., Kostering, M., Hernandez, A., Sorg, R. V., Kogler, G. and Wernet, P. (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation*, 106. 15, pp.(1913-8).

Strauer, B. E. and Kornowski, R. (2003). Stem cell therapy in perspective. *Circulation*, 107. 7, pp.(929-34).

Strauer, B. E., Yousef, M. and 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. *Eur J Heart Fail*, 12. 7, pp.(721-9), 1879-0844 (Electronic)1388-9842 (Linking).

Taylor, D. A., Atkins, B. Z., Hungspreugs, P., Jones, T. R., Reedy, M. C., Hutcheson, K. A., Glower, D. D. and Kraus, W. E. (1998). Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med*, 4. 8, pp.(929-33).

Tendera, M., Wojakowski, W., Ruzyllo, W., Chojnowska, L., Kepka, C., Tracz, W., Musialek, P., Piwowarska, W., Nessler, J., Buszman, P., Grajek, S., Breborowicz, P., Majka, M. and Ratajczak, M. Z. (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. *Eur Heart J*, 30. 11, pp.(1313-21), 1522-9645 (Electronic).

Teng, C. J., Luo, J., Chiu, R. C. and Shum-Tim, D. (2006). Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: implications for cellular cardiomyoplasty. *J Thorac Cardiovasc Surg*, 132. 3, pp.(628-32), 1097-685X (Electronic).

Tepper, O. M., Galiano, R. D., Capla, J. M., Kalka, C., Gagne, P. J., Jacobowitz, G. R., Levine, J. P. and Gurtner, G. C. (2002). Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation*, 106. 22, pp.(2781-6).

Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D. M., Nakano, Y., Meyer, E. M., Morel, L., Petersen, B. E. and Scott, E. W. (2002). Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature*, 416. 6880, pp.(542-5).

Timmermans, F., De Sutter, J. and Gillebert, T. C. (2003). Stem cells for the heart, are we there yet? *Cardiology*, 100. 4, pp.(176-85).

Toma, C., Pittenger, M. F., Cahill, K. S., Byrne, B. J. and Kessler, P. D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*, 105. 1, pp.(93-8).

Tomita, S., Li, R. K., Weisel, R. D., Mickle, D. A., Kim, E. J., Sakai, T. and Jia, Z. Q. (1999). Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation*, 100. 19 Suppl, pp.(II247-56).

Tomita, S., Mickle, D. A., Weisel, R. D., Jia, Z. Q., Tumiati, L. C., Allidina, Y., Liu, P. and Li, R. K. (2002). Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg*, 123. 6, pp.(1132-40),

Topol, E. J. (2003). Current status and future prospects for acute myocardial infarction therapy. *Circulation*, 108. 16 Suppl 1, pp.(III6-13), 1524-4539 (Electronic).

Towbin, J. A. and Bowles, N. E. (2002). The failing heart. *Nature*, 415. 6868, pp.(227-33),

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. and Lau, C. P. (2007). Prospective randomized trial of direct endomyocardial implantation of bone marrow cells for treatment of severe coronary artery diseases (PROTECT-CAD trial). *Eur Heart J*, 28. 24, pp.(2998-3005), 0195-668X (Print).

Urbich, C. and Dimmeler, S. (2004). Endothelial progenitor cells functional characterization. *Trends Cardiovasc Med*, 14. 8, pp.(318-22).

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. and Atsma, D. E. (2009). Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. *JAMA*, 301. 19, pp.(1997-2004), 1538-3598 (Electronic).

Vanderheyden, M., Vercauteren, S., Mansour, S., Delrue, L., Vandekerckhove, B., Heyndrickx, G. R., Van Haute, I., De Bruyne, B., Timmermans, F., Wijns, W. and Bartunek, J. (2007). Time-dependent effects on coronary remodeling and epicardial conductance after intracoronary injection of enriched hematopoietic bone marrow stem cells in patients with previous myocardial infarction. *Cell Transplant*, 16. 9, pp.(919-25), 0963-6897 (Print).

Vasa, M., Fichtlscherer, S., Aicher, A., Adler, K., Urbich, C., Martin, H., Zeiher, A. M. and Dimmeler, S. (2001). Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res*, 89. 1, pp.(E1-7).

Vassilopoulos, G. and Russell, D. W. (2003). Cell fusion: an alternative to stem cell plasticity and its therapeutic implications. *Curr Opin Genet Dev*, 13. 5, pp.(480-5), 0959-437X (Print).

Verfaillie, C. M., Pera, M. F. and Lansdorp, P. M. (2002). Stem cells: hype and reality. *Hematology (Am Soc Hematol Educ Program)*, pp.(369-91).

Vulliet, P. R., Greeley, M., Halloran, S. M., MacDonald, K. A. and Kittleson, M. D. (2004). Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*, 363. 9411, pp.(783-4).

Wang, J. S., Shum-Tim, D., Chedrawy, E. and Chiu, R. C. (2001). The coronary delivery of marrow stromal cells for myocardial regeneration: pathophysiologic and therapeutic implications. *J Thorac Cardiovasc Surg*, 122. 4, pp.(699-705).

Wang, J. S., Shum-Tim, D., Galipeau, J., Chedrawy, E., Eliopoulos, N. and Chiu, R. C. (2000). Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. *J Thorac Cardiovasc Surg*, 120. 5, pp.(999-1005).

Watanabe, E., Smith, D. M., Jr., Delcarpio, J. B., Sun, J., Smart, F. W., Van Meter, C. H., Jr. and Claycomb, W. C. (1998). Cardiomyocyte transplantation in a porcine myocardial infarction model. *Cell Transplant*, 7. 3, pp.(239-46).

Weisel, R. D., Fazel, S., Fedak, P. W. and Li, R. K. (2004). Cardiac restoration by cell transplantation. *Int J Cardiol*, 95 Suppl 1. pp.(S5-7).

Wohrle, J., Merkle, N., Mailander, V., Nusser, T., Schauwecker, P., von Scheidt, F., Schwarz, K., Bommer, M., Wiesneth, M., Schrezenmeier, H. and Hombach, V. (2010). Results of intracoronary stem cell therapy after acute myocardial infarction. *Am J Cardiol*, 105. 6, pp.(804-12), 1879-1913 (Electronic)0002-9149 (Linking).

Wollert, K. C. (2008). Cell therapy for acute myocardial infarction. *Curr Opin Pharmacol*, 8. 2, pp.(202-10), 1471-4892 (Print).

Wollert, K. C. and Drexler, H. (2005). Clinical applications of stem cells for the heart. *Circ Res*, 96. 2, pp.(151-63).

Wollert, K. C. and Drexler, H. (2010). Cell therapy for the treatment of coronary heart disease: a critical appraisal. *Nat Rev Cardiol*, 7. 4, pp.(204-15), 1759-5010 (Electronic)
1759-5002 (Linking).

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. and Drexler, H. (2004). Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*, 364. 9429, pp.(141-8).

Ying, Q. L., Nichols, J., Evans, E. P. and Smith, A. G. (2002). Changing potency by spontaneous fusion. *Nature*, 416. 6880, pp.(545-8).

Yoon, C. H., Hur, J., Park, K. W., Kim, J. H., Lee, C. S., Oh, I. Y., Kim, T. Y., Cho, H. J., Kang, H. J., Chae, I. H., Yang, H. K., Oh, B. H., Park, Y. B. and Kim, H. S. (2005). Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation*, 112. 11, pp.(1618-27), 1524-4539 (Electronic)0009-7322 (Linking).

Yoon, J., Choi, S. C., Park, C. Y., Choi, J. H., Kim, Y. I., Shim, W. J. and Lim, D. S. (2008). Bone marrow-derived side population cells are capable of functional cardiomyogenic differentiation. *Mol Cells*, 25. 2, pp.(216-23), 1016-8478 (Print).

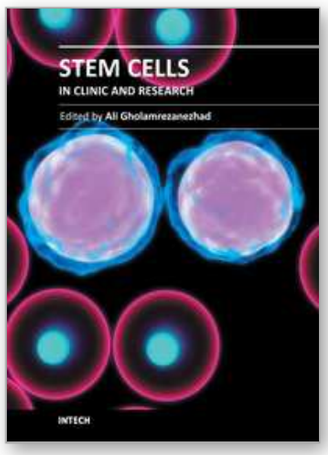
Zhang, M., Methot, D., Poppa, V., Fujio, Y., Walsh, K. and Murry, C. E. (2001). Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol*, 33. 5, pp.(907-21).

Zhang, S., Wang, D., Estrov, Z., Raj, S., Willerson, J. T. and Yeh, E. T. (2004). Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo. *Circulation*, 110. 25, pp.(3803-7).

Zhang, S. N., Sun, A. J., Ge, J. B., Yao, K., Huang, Z. Y., Wang, K. Q. and Zou, Y. Z. (2009). Intracoronary autologous bone marrow stem cells transfer for patients with acute

myocardial infarction: a meta-analysis of randomised controlled trials. *Int J Cardiol*, 136. 2, pp.(178-85), 1874-1754 (Electronic) 0167-5273 (Linking).

Ziegelhoeffer, T., Fernandez, B., Kostin, S., Heil, M., Voswinckel, R., Helisch, A. and Schaper, W. (2004). Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res*, 94. 2, pp.(230-8).



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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 investigationally 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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