[Frontiers in Bioscience, Elite, 5, 232-248, January 1, 2013]

Regenerative strategies for preserving and restoring cardiac function

Amanda Finan[1], Feng Dong[2], Marc S Penn[2,3]

[1]Department of Molecular Biology, Case Western Reserve University, Cleveland, OH, 44106, [2]Skirball Laboratory for Cardiovascular Cellular Therapeutics, Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, 44272, [3]Summa Cardiovascular Institute, Summa Health System, Akron, OH, 44304

TABLE OF CONTENTS

1. ABSTRACT

Over the past decade the cardiovascular regenerative medicine field has made significant advances in our understanding and treatment of injured myocardium. Prior to stem cell therapy, available treatments for cardiovascular disease were unable to repair or regenerate the damaged heart. Stem cell therapy is increasingly becoming a viable option to prevent and treat cardiac dysfunction. A number of exogenous stem cell populations have been examined for their ability to participate in cardiac repair. Their application in the clinical setting will be reviewed here. The molecular pathways that work in concert to orchestrate a systemic endogenous stem cell response to cardiac injury have also begun to be defined. A potential strategy for future therapeutics is the manipulation of these endogenous pathways via pharmacological or biopharmaceutical approaches. In this review we begin to formulate the discussion that the best future therapeutic option to regenerate end organ function will be a combination of programmed stem cells and biopharmaceuticals that modulate regenerative signaling to bolster the natural *in vivo* cellular and signaling mechanisms.

2. INTRODUCTION

Cardiovascular diseases (CVD) represent the most important cause of death in the United States and industrialized world (1). The most common cause of heart failure is acute myocardial infarction (AMI) (1). The chronic adverse remodeling following loss of cardiac myocytes at the time of AMI leads to heart failure. The success of present day therapies for acute myocardial infarction has increased the prevalence of chronic heart failure (2).

While there is an increasing prevalence of ischemic cardiomyopathy with the aging population, there is also a significant increase in the prevalence of aortic stenosis and dilated cardiomyopathies, both of which significantly contribute to the increased prevalence of chronic heart failure. These events result in pathological remodeling of the heart leading to increased ventricle size, altered ventricular shape, and decreased function. Current therapies for cardiac diseases include angiotensin-converting enzyme inhibitors, beta-blockers, angiotensin-receptor blockers, and diuretics (3). These treatments improve symptoms and prolong life, but none of these

Table 1. Summary of characteristics of different cell populations of interest for cardiac regenerative medicine.

Exogenous Cell Therapy	Pros	Cons
Skeletal Myoblasts	• Improve function	Do not become functional cardiac myocytes Potentially arrythmogenic
Fetal/Embryonic Myocytes	• Improve function • Stably engraft	Low engraftment Immature phenotype Ethical issues
Embryonic Stem Cells/Induced Pluripotent Stem Cells	• Improve function • Potential to become all cardiac cell types • Autologously derived (IPSC)	Cardiac cells have to be efficiently derived and purified Teratoma potential Ethical issues
Hematopoietic Stem Cells	• Improve function • Increase neoangiogenesis • Reduce apoptosis	Do not become functional cardiac myocytes
Mesenchymal Stem Cells	• Improve function • Low immunogenicity • Decrease scar size • Recruit endogenous stem cells • Increase neoangiogenesis	*In vivo* fate unclear Unlikely to replace lost cardiac myocytes
Multipotent Adult Progenitor Cells	• Improve function • Low immunogenicity • Participate directly in neoangiogeneis • Secrete cardioprotective signals	Do not become cardiac myocytes *in vivo*
Endothelial Progenitor Cells	• Improve Function • Participate directly in angiogenesis	Do not become cardiac myocytes
Cardiac Stem Cells	• Improve Function • Potential to become all cardiac cell types • Decrease Scar Size • Increase neoangiogenesis	No definitive marker Derivation for therapeutic application is inconvenient

therapies lead to improved tissue function or replacement of the lost cardiac myocytes (4).

As suggested above, a common theme in adverse ventricular remodeling and cardiac dysfunction is a significant loss of resident cardiac myocytes. As a result, considerable effort is being devoted to develop new cell and regenerative therapies. While available pharmacological treatments focus on alleviating symptoms or reducing cardiac workload, a regenerative approach provides the opportunity to repair or replace the injured tissue in order to re-establish cardiac function. Regenerative strategies have been both in the prevention of loss of cardiac myocytes in acute disease through blocking apoptosis and decreasing inflammation in an attempt to improve remodeling after AMI, or to improve cardiac remodeling in CHF through the induction of angiogenesis, activation of cardiac myocytes to enter cell cycle, or cell therapy to replace lost cells. Additional therapeutic targets are directed at improving stem and progenitor cell survival, restoring progenitor cell populations, and driving the cardiac fate of progenitor cells.

3. EXOGENOUS STEM AND PROGENITOR CELL THERAPY

The initial focus for regenerative therapy was directed at exploring exogenous stem cells to reconstruct the damaged myocardium. A number of sources have been used to isolate stem cells – embryonic, skeletal muscle, and bone marrow (Table 1). Each population has its benefits and downfalls. While the exact mechanisms associated with benefits associated with stem cell therapy remains to be elucidated, it would appear that a significant benefit of stem cell therapy to date is the induction of endogenous stem cell based repair. Importantly, several strategies involving exogenous cell transplantation appear to have therapeutic potential and are reviewed below.

3.1. Myogenic cell therapy

One of the earliest cells used for myocardial repair in ischemic cardiomyopathy was skeletal myoblasts. Myoblasts are easily isolated both from autologous skeletal muscle and have the ability to proliferate in culture to generate a number of cells appropriate for a therapeutic approach. Injection of skeletal myoblasts can improve cardiac function after AMI (5). There is no evidence, however, that the skeletal myoblasts have the ability to transdifferentiate into cardiac myocytes (6). Moreover, skeletal myoblasts were found to increase the arrythmogenic potential of myocardial scar (5, 7). This has been attributed to the inability of skeletal myoblasts to electrically couple with the myocardium due to a lack of *appropriate* connexin protein expression (5).

Another myogenic cell that has been implemented in pre-clinical studies is direct engraftment of cardiac myocytes. Fetal and neonatal cardiac myocytes when delivered directly into the myocardium after a cardiac injury can form stable grafts improving heart function (8, 9). However, they maintain an immature phenotype and have low engraftment levels contributing only a small percentage of viable cells to the heart (10).

3.2. Embryonic stem cells

Embryonic stem cells (ESC) have been proposed as an alternative source of cardiac myocytes. ESC are derived from the inner cell mass of the blastocyst. ESC can be cultured indefinitely in a stable undifferentiated state and have the ability to differentiate into cell types from the three primary germ layers (11, 12). This pluripotentiality constitutes a potential risk for the therapeutic use of these

cells. Injection of uninduced or unpurified ESC can generate teratomas (13). It is therefore necessary to isolate cardiac myocytes or their precursors from ESC cultures. Functional cardiac myocytes can be obtained from murine and human ESC. When ESC differentiation is induced using the standard embryoid body method, the cardiac myocyte yield is extremely low (human ESC <1% of total cells). Information learned through developmental biology has allowed researchers to attempt directed differentiation of ESC to a cardiac phenotype. Using human ESC, Laflamme *et al* demonstrated that serial administration of TGF- eta proteins, Activin A and BMP4, greatly increases the efficiency of cardiac myocyte differentiation (14). Activin A was also demonstrated to improve cardiac differentiation of ESC co-cultured with definitive endoderm, improving differentiation efficiency from 11 fold to 30 fold (15). It is important to note that individual ESC lines are sensitive to the levels of the Activin A and BMP4 and that specific protocols need to be optimized for each cell line (16). Injection of ESC into infarcted hearts, improves cardiac function by engraftment of the ESC-derived cardiac myocytes into functional myocardium (13-15). Interestingly, when ESC derived cardiac myocytes are used to treat chronic heart failure, although the cells engraft with the host myocardium, cardiac remodeling is not attenuated, suggesting cardiac myocytes cell therapy is more specific for an acute injury where a large number of cells are lost, that ESC derived cardiac myocytes need to integrated into contractile units through tissue engineering before being introduced to the injured myocardium, or that the effects of ESC engraftment are paracrine factor mediated and insufficient to induce endogenous repair in ischemic cardiomyopathy (17). Future studies will define which mechanism(s) are involved. That said cardiac myocytes derived from culturing of mesenchymal cells from the human amniotic membrane when transplanted into the heart following AMI significantly improve cardiac function through paracrine factor mechanisms (18, 19). These data strongly support induction of endogenous repair as potentially a primary mechanism of action even in the setting of exogenous cardiac myocyte engraftment.

As ESC have been at the center of ethical controversy, the field has recently focused on induced pluripotent stem cells (IPSC) as a source of cardiac myocytes. IPSC are somatic cells that have been reprogrammed by forcing the expression of four stem cell genes, acquiring an ESC-like phenotype (20-23). IPSC have an additional benefit in that they can be derived from autologous somatic cells, reducing the complication of immunogenicity, although recent data suggests this may not be the case. IPSC have the potential to become functional cardiac myocytes (24-26). The efficiency of differentiation can be improved as described for ESC (21, 27). Ieda, *et al*, began the reprogramming process with a more committed mesodermal tissue, forcing cardiac transcription factor expression to induce fibroblasts to take on a cardiac myocyte type phenotype *in vivo* (28). Injection of IPSC into an infarcted heart was shown to improve cardiac function, regenerating cardiac myocytes and smooth muscle cell (29). IPSC derived cardiac progenitor cells injected into an infarcted heart resulted in an overall functional benefit due to their *in vivo* differentiation to cardiac lineages (25). Therapeutic use of IPSC is not devoid of problems and a concern of their tumorogenic and immunogenic potential induced through the expression of non-self MHC proteins exists (30).

3.3. Bone marrow derived stem and progenitor cells

Bone marrow derived stem cells have also been actively investigated. This is due to their relative ease of isolation, lack of significant ethical concerns, and the ability for the cells to be autologous or allogeneic depending on the specific cell type. Bone marrow derived stem cells encompass a broad range of cells – Hematopoietic Stem Cells (HSC), Mesenchymal Stem Cells (MSC), Endothelial Progenitor Cells (EPC), Mononuclear Cells (MNC), and Multipotent Adult Progenitor Cells (MAPC).

HSC are multipotent cells with the ability to give rise to all blood cells. These cells are identified in mice as ckit+/sca1+/lin- cells and in humans as CD34+/lin- (31). HSC have been historically used in the treatment of hematological diseases. In 2001, Orlic, *et al*, directly injected HSC into infarcted myocardium and found a functional benefit, as well as the differentiation of the injected cells into de novo myocardium (32). The fate of HSC in the infracted heart is a matter of debate. Some groups concur with the original study; however, the majority of studies suggest they do not differentiate into cardiac myocytes, and other reports present data that HSC fuse with cardiac myocytes (33-36). Regardless of the differentiation capacity of HSC, they have repeatedly resulted in cardiac benefit that is now understood to be due to the release of paracrine factors. One paracrine factor expressed by HSC that can improve cardiac function is vascular endothelial growth factor (VEGF) (37, 38).

MSC are multipotent cells with the ability to differentiate into mesenchymal lineages, such as bone, cartilage, fat and can be induced to expression cardiac proteins but there exists little evidence that they can differentiate into functional cardiac myocytes (39, 40). MSC are identified as adherent cells lacking hematopoietic markers. Owing to low immunogenicity, MSC can be used in allogeneic therapy (41). MSC can migrate to endogenous signaling pathways (such as SDF-1/CXCR4) after cardiac injury and MCP-3 (42, 43). MSC improve cardiac function but do not participate directly in cardiac regeneration instead they preserve cardiac myocytes in the infarct zone, increase angiogenesis, increase endogenous stem cell numbers, and decrease scar size (42-44).

Multipotent adult progenitor cells (MAPC) are the only bone marrow derived progenitor cells that have the potential to generate all three germ layers (45). MAPC can be implemented in an autologous or allogeneic strategy since with either strategy they improve cardiac function after AMI equally (46). The benefits of MAPC are not due to cardiac regeneration but rather through liberation of paracrine factors that induce vascular growth, preserve cardiac myocytes and modulate inflammation (46-48).

Exogenous stem cell therapy overall results in an improvement in cardiac function through the modulation of endogenous stem cell based cardiac repair, regardless of whether the stem cells become functional cardiac myocytes or not. Generally, the number of cardiac myocytes generated from exogenous stem cells is too low to be associated with the observed benefit. Thus, it appears that the stem cells provide paracrine support through cytoprotection, neoangiogeneis, and activation of endogenous stem cells (49).

4. CLINICAL TRIALS WITH EXOGENOUS STEM CELLS

The results obtained using animal models triggered enthusiasm for a clinical application of stem cells even though the exact mechanisms of the benefit are still to be defined. The rapidity with which clinical trials have been executed highlights the true unmet clinical need that cardiac regenerative therapies are addressing and demonstrate the highly translatable nature of stem cell therapy. Most importantly these trials, with the exception of arrhythmogenic risk associated with skeletal myoblast engraftment, have demonstrated safety with cardiovascular cell therapy, and en mass have demonstrated some degree of efficacy.

The MAGIC trial, a skeletal myoblast transplantation study, described an increased number of post-operative arrythimias, while other trials did not report evidence of the myoblasts being arrhythmogenic (50-52). None of the trials showed any significant improvement in cardiac function after skeletal myoblast transplantation, considerably decreasing the initial enthusiasim for this cell type (50-52). Skeletal myoblasts do hold potential to be used concurrently with a gene therapy, although given the complexities of harvesting and expanding skeletal myoblasts and the fact that there are other, non-arrhythmogenic stem cell populations now under investigation, it seems the era of skeletal myoblast therapy may be coming to an end (5).

A large number of trials have tested the use of bone marrow derived cells in the treatment of acute MI. There is yet to be a common consensus in the definition of the cells that have been used (53). The majority of studies have tested autologous bone marrow derived stem cells injected intracoronary in patients following an acute MI. Meta analyses of the data to date suggest a benefit from bone marrow derived stem cell therapy, including improved cardiac function as seen by increased ejection fractions, decreases in infarct size, and enhancement in exercise capabilities of the patients (54-62). One analysis performed a systematic review of 9 clinical trials encompassing the treatment of 725 patients one year after therapy (63). They found that autologous bone marrow derived cell transplantation in patients with acute MI significantly improves ejection fraction and that the therapy works best in patients less than 55 years old and when cells are delivered 6-7 days post-MI. Trials that have investigated a relationship between the benefits of treatment and the initial extent of cardiac damage have consistently shown greater benefit in patients with more severe myonecrosis (55, 60, 61, 64).

Importantly, the efficacy of cell therapy in patients with chronic heart failure is somewhat heterogenous (65, 66). In the STAR trial, intracoronary bone marrow cells were used to treat chronic ischemic cardiomyopathy (67). The therapy led to improved exercise function and ejection fraction five years post treatment. In patients affected by non-ischemic dilated cardiac myopathy, bone marrow derived progenitor cells significantly increased ejection fraction early on and although elevated BNP levels were measured one year after, no indication was given if the benefit to cardiac function was long-term (68).

There are fewer trials testing specific populations of cells derived from the bone marrow. Autologous MSC injection after AMI or in ischemic cardiomyopathy was found to be safe and improved cardiac function (69, 70). Thus there is data that suggests that MSC can inhibit further adverse remodeling when delivered to patients with ischemic cardiomyopathy (70). Importantly in the setting of ischemic cardiomyopathy the delivery of CD34+ cells offered no significant benefit where as whole bone marrow did (71). One study has demonstrated the benefit of allogeneic adult human MSC in acute MI. The treatment was safe and tolerated by the patients and improved ejection fraction 12 months after treatment (69).

5. ENDOGENOUS STEM AND PROGENITOR CELLS

Several endogenous stem cell populations have been identified and their relevance to cardiac regeneration is being investigated and growing. We proposed over a decade ago that stem cell based repair of the myocardium after AMI was a natural process but clinically inefficient due to the lack of molecular signals that orchestrate the process. Consistent with this hypothesis there are data demonstrating that endogenous stem cells are inefficient in cardiac repair. It is as yet unknown if this is due to the down-regulation of critical molecular signals in the peri-infarct period and in ischemic cardiomyopathy, "dysfunction" of the endogenous stem cell population, or both. If we can fully identify the stem cell populations participating in the repair process and understand their role in CVD, these molecular signals and cell populations will be targets of future regenerative medicine strategies.

Stem and progenitor cell compartments have been identified in nearly all organs of the adult mammal including the bone marrow, blood, brain, skeletal muscle, intestines, liver, fat, dental pulp, and the heart. These stem cells can respond to pathological, stress conditions. Endogenous cardiac stem and progenitor cells (CSC, CPC) have received a significant amount of attention as have endogenous endothelial progenitor cell (EPC). Research is also demonstrating that there is a systemic response to injury not limited to the progenitor cells localized in the diseased organ (Figure 1). Identifying the molecular pathways that regulate this response and the progenitor

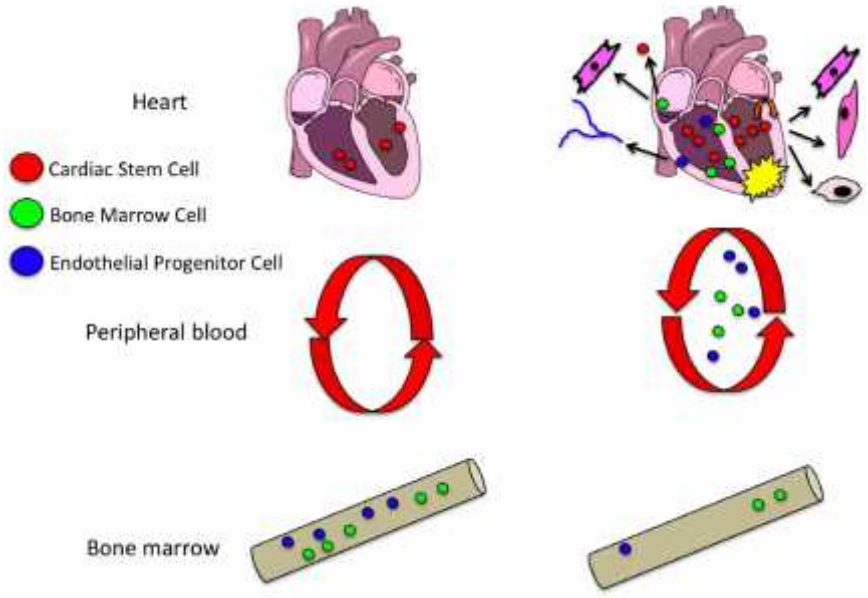

Figure 1. Systemic Endogenous Stem Cell Response to Cardiac Injury. Normally, cardiac stem cells (red), endothelial progenitor cells (blue), and bone marrow cells (green) are dormant. Signals released from a cardiac injury can induce a response from the endogenous stem cell populations throughout the body. The cardiac stem cells are activated to proliferate and likely differentiate into more committed cardiac cell fates. Endothelial progenitor cells increase their circulating numbers and participate in vasculogenesis. Bone marrow stem cells migrate to the site of injury and can take on a cardiac fate, either as a cardiac stem cell or a differentiated cardiac myocyte. All of the stem cell populations have paracrine effects to aid the myocardium in its recovery. Aging and disease can affect the ability of these cells to respond to the injury.

cells that generate the cell populations of interest are all critical goals of on-going research (72-74).

5.1. Cardiac stem and progenitor cells

Historically, the heart has been considered a post-mitotic organ, with no significant proliferation or regeneration. Primary tumors in the heart are very rare (.001-0.28% of all primary tumors), supporting the idea of limited cell cycling (75). However, this orthodoxy has recently been challenged and reports have suggested the heart to have regenerative capacity.

It has long been known that newts and zebrafish have robust cardiac regeneration. Cryoinjury or amputation of a zebrafish heart results in proliferation of cardiac myocytes, replacing the lost tissue (76-79). A similar phenomenon has been recently described in neonatal mice. Regeneration of partial surgical resection of one-day-old mice hearts mimics that seen in zebrafish (80). However, the regenerative capacity of the cardiac myocytes is lost by seven days after birth. It appears that there is a meager capacity in adults to regenerate myocardium by resident stem cells (81, 82). Finally, a very elegant study that carbon dated cardiac myocytes suggests that there is a 0.45-1% annual renewal of cells in the adult human heart throughout a normal life span (83).

C-kit, the tyrosine kinase receptor for stem cell factor, has been used to define one of the CSC populations and is probably the most studied (84). This population is more specifically defined as c-kit+/CD45-/lineage- (84). C-kit+ CSC have also been described to express the surface markers sca-1 and mdr1 (85, 86). Human c-kit+ CSC have been characterized to express endothelial lineage markers, suggesting their multipotentiality (87). When isolated, these cells are clonogenic and have the ability to differentiate into endothelial cells, smooth muscle cells, and cardiac myocytes both *in vitro* and *in vivo* (84, 88). C-kit+ CSC are located throughout the heart, including the atrium, ventricles, and in the epicardium (89-91).

Fransioli, *et al* developed a c-kit-GFP transgenic mouse to monitor the ckit+ CSC population *in vivo* under normal and injury situations (92). The authors found that the number of c-kit+ cells in the heart is relatively high at birth compared to the adult heart and rapidly decreases within one to two weeks after birth. In the setting of AMI, c-kit-GFP+ CSC increase in number and as early as 1-2 weeks post-MI, localize in vessels and express cardiac transcription factors (92). In humans, AMI results in the elevation of c-kit+ CSC, with the majority located in the infarct border zone (86). Chronic pathologies of the heart result in up to a 14 fold increase in c-kit+ CSC compared to normal hearts but not to levels as seen in AMI (29 fold) (85, 86, 93). Females have more c-kit+ CSC than males, which may partially explain the female gender's advantage in CVD (91, 94). In aging adults, there is some inconsistency on the c-kit+ CSC population. Mishra, *et al* report that the number of c-kit+ CSC decreases with age whereas Kajstura, *et al* described an increase (94, 95). Both studies demonstrated an increase in cell senescence in the c-kit+ population (about 50%), suggesting there is a decrease in functionally competent CSC with age (94-96).

Stem cell antigen-1 (sca-1), a member of the Ly-6 family, has also been used as a marker for endogenous CPC. These cells have been described as either ckit+ or ckit- (97, 98). *In vitro*, sca-1 CPC express cardiac transcription factors but not structural genes and are clonogenic (97, 99). This population has the potential to differentiate into cardiac myocytes *in vitro* when treated with 5-azacytidine or oxytocin (97, 99). Either direct injection or intravenous infusion of sca-1 CPC after AMI results in incorporation of the cells into the myocardium and vessels with evidence of decreased scar and increased vasculogenesis (97, 99).

Islet -1 (Isl-1), a LIM-homeodomain transcription factor, has also been described to mark the resident CPC population. These cells are believed to be remnants of embryonic cardiac development (100). Isl-1+ CPC localize to the outflow tract, the atria, and the right ventricular regions of the heart corresponding to the secondary heart field (100, 101). Lineage tracing of Isl-1+ cells demonstrates that this population does contribute to the myocardium during normal development (102). Co-culture of Isl-1+ CPC with neonatal myocytes converts the stem cells to a mature cardiac phenotype (102). There is evidence of Isl-1 cells in humans as well (91, 103).

Side population cells are characterized by their ability to efflux Hoechst 33342 dye by the ATP-binding cassette transporter, ABCG2, and resident populations have been found in the heart and other organs (104, 105). Cardiac side population cells also generally express sca-1 but do not express CD45, CD44, CD34, or ckit, making them phenotypically different from bone marrow derived side population cells (106). Cardiac side population cells express cardiac transcription factors but not structural genes (106). Co-culture of cardiac side population cells with neonatal cardiac myocytes or treatment with oxytocin or trichostatin A, induces the progenitor cells to differentiate into beating cardiac myocytes (104, 106, 107). Cardiac side population cells can home to infarcted myocardium when injected intravenously and differentiate into cardiac myocytes, endothelial cells, and smooth muscle cells *in vivo* (107). A dynamic endogenous response to injury has been characterized in the setting of acute MI; the percentage of side population cells decreases one-day post MI and the population is fully reconstituted 7 days post-MI (108).

Cardiospheres are multipotent cardiac stem cells isolated from heart biopsies. Cardiospheres have been isolated from mice, rats, pigs, and humans (109, 110). Cardiospheres are a heterogenous population, expressing both ckit and sca-1 (110). In humans, the number of ckit+ cardiospheres decreases with age (95). Cardiospheres are clonogenic and have the ability to differentiate into cardiac myocytes, endothelial cells, and smooth muscle cells (109-111). Treatment of acute MI with cardiospheres results in a functional benefit, an increase in the amount of viable myocardium, increased vascular density, with the injected cells differentiating into cardiac myocytes and vasculature (110-112). Chimenti, *et al* suggested that 20-50% of the benefit from cardiosphere engraftment is from the generation of new cardiac myocytes with the majority of the effect being due to liberation of paracrine factors leading to decreased apoptosis, vasculogenesis and recruitment of endogenous stem cells (112). The potency of cardiosphere-derived cells has also been tested and found to result in a similar functional benefit as the cardiospheres themselves, however these cells are unable to attenuate adverse remodeling (113). Concerns exist about contamination of the cardiospheres accounting for the "differentiation" properties observed (114). Davis, *et al* responded to this concern, performing experiments using transgenic mice expressing GFP only in cardiac myocytes (109). The authors identified that 6% of the outgrowth of cardiospheres was ckit+/GFP- (stem cells not derived from a mature cardiac myocyte). However, they failed to demonstrate whether there were ckit+/GFP+ cells in the culture.

Cardiac stem cells defined as c-kit+ cells in the myocardium have been shown to induce myocardial repair when delivered in ischemic cardiomyopathy (115). Ultimately cardiac stem cells are bone marrow derived; however, it is likely they undergo some sort of maturation through their residence in the myocardial niches (116). We have previously demonstrated that cardiac stem cells are recruited to the infarct border zone in response to mesenchymal stem cell engraftment and that this effect can be enhanced by the local over expression of SDF-1 (117). Cardiac stem cells when engrafted in the infarct border zone do depolarize leading to propagation of the action potential into the infarct zone and may in part explain the anti-arrhythmic effect of mesenchymal stem cells (117, 118). Recently the potential of cardiac stem cells were demonstrated in the SCIPIO trial in which patients with ischemic cardiomyopathy with and without significant symptoms of chronic heart failure received autologous cardiac stem cells that were harvested and expanded 4 weeks earlier during coronary artery bypass surgery (119). These findings arguably serve as an important proof of concept study demonstrating the potential for myocardial repair through the induction of endogenous stem cell activation.

5.2. Endothelial progenitor cells

Endothelial progenitor cells (EPC) were first described in 1997 (120). Asahara, *et al* identified CD34+ cells in human blood capable of differentiating into endothelial cells. EPC originate from a primitive cell in the bone marrow, the hemangioblast (121-123). The latter are multipotent cells with the ability to generate hematopoietic and endothelial lineages. There is also evidence that EPC can be derived from monocytic origins as well (124).

Under normal conditions, the level of circulating EPC is very low (120). However in response to AMI, EPC are mobilized from the bone marrow into the blood within hours and their numbers remain elevated for at least a week; levels return to baseline two months post AMI (125, 126). EPC are reduced in heart failure (127). These differences suggest that there is a need to investigate the factors involved in the induction of EPC mobilization following AMI and the effects of co-morbidities on EPC

mobilization and function in CHF. EPC function decreases with age, though controversy exists on their baseline levels (128, 129). EPC from older patients proliferate less and have decreased migratory capacity and colony forming ability (128-130). Schuebel, *et al* measured lower EPC baseline in the elderly and scarce mobility to coronary artery bypass grafting, compared to younger patients (131). Thus, EPC offer a model system for evaluating the effects of aging and co-morbidities on endogenous stem cell based repair.

5.3. Bone marrow cells

Lately, the contribution of other systemic cells in the cardiac regeneration process has been evaluated. Recent studies have helped to define a role of bone marrow cells in response to injury. We recently demonstrated that heterochronic bone marrow transplants of young bone marrow (8 weeks) into old mice (>40 weeks) could attenuate the effects of trans-aortic constriction (132). The benefit of the young marrow was associated with decreased fibrosis and apoptosis, increased engraftment of bone marrow cells into the heart, and activation of resident cardiac progenitor cells. There were no changes in vascular density in this model although young marrow has been demonstrated to improve angiogenesis in old mice with cardiac allografts (133). Our analysis further showed that the number one predictor of myocyte hypertrophy to pressure overload was bone marrow age, not body age (132).

Similar benefit has been described in heterochronic parabiotic mice (134). When injury was induced in the skeletal muscle of old mice, only the mice parabiosed with young mice had restored activation of their satellite cells. Fazel, *et al*, demonstrated the importance of the bone marrow in a model of myocardial infarction (135). Mice carrying a mutation on the c-kit receptor had worse dysfunction after MI than their wild type counterparts. However, when normal bone marrow was transplanted into the mice carrying the c-kit mutation, the response was similar to that observed in wild-type mice.

There is evidence from clinical populations of bone marrow cells becoming CSC. The first reports were from gender-mismatched transplants. In males transplanted with female hearts, 7-10% of the cardiac myocytes, endothelial cells, and smooth muscle cells were found to be Y chromosome positive (90). Additionally, 12-16% of the CSC were Y+ chromosome. In females transplanted with male bone marrow, Y+ chromosome cells can be found as cardiac myocytes, and also skeletal muscle and hepatocytes (136). A third study examining both types of gender mismatched transplants confirmed the results by identifying 1% of cardiac myocytes as being bone marrow derived, regardless of the transplant type (137). In animal models of cardiac injury with labeled bone marrow cells (GFP, LacZ), a portion of the bone marrow cells can be identified as CSC, endothelial cells, or cardiac myocytes (108, 138-140). There is some debate on bone marrow cells taking on a cardiosphere phenotype (140, 141). These reports suggest that the bone marrow has some capacity to maintain and/or regenerate the CSC pool.

6. PATHWAYS OF STEM CELL BASED REPAIR

The benefit of exogenous stem cell transplantation has been described to be primarily paracrine factor mediated. Endogenous stem cells are activated and induced to respond to cardiac injury. The exact underlying mechanisms involved either in exogenous or endogenous stem cell based repair are unknown.

One of the first molecular signals that was identified and now carried through early clinical trials is stromal cell-derived factor (SDF)-1alpha (CXCL12). SDF-1 is a chemo-attractant for CXCR4 expressing stem cells. It has been demonstrated that cardiac SDF-1 and CXCR4 levels are elevated after AMI (42, 72, 142). SDF-1 levels are also increased in cardiac pressure overload (143). Increased SDF-1 expression in the heart recruits CXCR4+ stem and progenitor cells to the ischemic tissue (144). It has been reported that increased SDF-1 levels can enhance the homing of stem cells such as HSC, EPC**,** and ESC (145-147). The importance of stem cell homing for stem cell recruitment to myocardial tissue was highlighted by the recently published LATETIME trial which failed to demonstrate any benefit from bone marrow derived stem cells delivered via intracoronary infusion 2-3 weeks after acute myocardial infarction, a time point we previously demonstrated was devoid of SDF-1 expression (72, 148).

The effects of SDF-1 are not limited to stem cell homing. Our data suggest that SDF-1 can mediate cardiac tissue repair directly through the inhibition of cardiac myocytes apoptosis (42). Other groups have shown a similar cardioprotective effect of SDF-1 (149, 150). Another important mechanism is an SDF-1 dependent increase in the recruitment of endogenous cardiac stem cells (117, 151). Once the CXCR4 positive stem cells reach the area of injury, they inhibit cardiac myocyte apoptosis, increase vascular density and improve cardiac remodeling (72, 84, 117, 152). SDF-1 is down-regulated within days after AMI. Multiple studies have demonstrated that sustaining or re-establishing SDF-1 expression leads to improved cardiac function (7, 42, 72, 117). This has been investigated by transplantation of MSC (expressing SDF-1 naturally), transplantation of MSC engineered to overexpress SDF-1, or direct injection of SDF-1 DNA (7, 42, 72, 74, 151). In these studies in models of AMI and ischemic cardiomyopathy, CXCR4 positive cells, including CSC, were recruited to the site of SDF-1 expression, vessel density was increased, and cardiac function improved. The safety and utility of re-establishing SDF-1 expressing in patients with NYHA Class III heart failure has recently been completed. The biology of the SDF-1:CXCR4 axis is an example of the innovation and therapeutic potential that can be gained by defining the molecular mechanisms associated with endogenous stem cell repair of injured tissue (73, 153).

Granulocyte colony stimulating factor (G-CSF) is a hematopoietic cytokine. It acts by binding to the G-CSF receptors (GCSFR). GCSFR are found on cells of hematopoietic lineage, EPC, and cardiac myocytes (154,

155). G-CSF levels in the blood are elevated after AMI (156). Increased levels of G-CSF can mobilize bone marrow cells; therefore, G-CSF treatment has been studied as a therapeutic strategy to stimulate heart regeneration but largely has failed in clinical trials (154, 157, 158). AMD-3100 is a small molecule antagonist for the CXCR4 receptor that leads to stem cell mobilization (159). As G-CSF did, the administration of AMD-3100 has been shown to improve cardiac function in pre-clinical models (159). Whether it will have benefit in clinical trials remains to be determined.

A number of other proteins have been implicated in the recruitment of endogenous stem cells. High-mobility group protein B1 (HMGB1) is a cytokine released by necrotic cells (160). HMGB1 induces CSC migration and proliferation resulting in elevated levels of endogenous ckit+ CSC and newly formed cardiac myocytes (161-163). Monocyte chemotactic protein-3 (MCP3) is a chemokine that is elevated in the heart after an infarct or in cardiac pressure overload (43, 132). Overexpression of MCP-3 one-month post-MI, after the endogenous level has returned to baseline, efficiently recruits the migration of intravenously injected MSC (43). However, the role of MCP-3 as a homing factor for endogenous stem cells still remains to be determined. Thymosin beta 4 is an actin regulating peptide (164). Recently, it has been used in the treatment of MI and shows benefits in cardiac myocyte survival and function (165, 166). It has been proposed that thymosin beta 4 reminds the heart of its embryonic programming by stimulating the CSC and inducing cardiac migration (165, 167, 168).

Beyond factors that induce homing of endogenous stem cell populations to injured tissue, there is increasing evidence of molecular signals that regulate or inhibit endogenous stem cell differentiation. For example disabled-2 is a TGFbeta receptor adaptor protein that is rapidly up-regulated in cardiac myocytes following AMI (169). We have recently demonstrated that via miR-145 this up-regulation of disabled-2 results in down-regulation of cardiac programming in MSC (169). Whether inhibiting this naturally occurring signaling will result in improved stem cell based cardiac repair is the topic of on-going studies. If so, small molecule targeting of disabled-2 could become an intriguing pharmacological target for future therapeutics.

7. CONCLUSION

The advent of stem cell based tissue repair offers the potential for repairing and/or replacing lost end-organ function. The field has progressed rapidly with the translation of exogenous cell therapy for the prevention and treatment of cardiac dysfunction. As reviewed above and elsewhere multiple cell types and strategies have demonstrated safety and efficacy for cardiac regenerative medicine. Preclinical mechanism oriented studies from multiple groups on the effects of exogenous cell therapy have now clearly validated our early hypothesis that stem cell based tissue repair is a natural process. These data demonstrate an intricate, complicated and systemic stem cell based process that is activated in response to acute tissue injury, but is inefficient at preventing loss of end-organ function. Furthermore, the advancement of SDF-1 mediated gene transfer to clinical populations demonstrates that defining these pathways can lead to safe therapeutic strategies that leverage this endogenous repair process for therapeutic gain. Ultimately true tissue generation will likely require the combination of exogenous cell therapy from one or many of the populations reviewed above along with biologics that will manipulate the natural stem cell based repair process as well as induce specific states of differentiation or activation of the exogenous population itself.

8. REFERENCES

1. D. Lloyd-Jones, R. J. Adams, T. M. Brown, M. Carnethon, S. Dai, G. De Simone, T. B. Ferguson, E. Ford, K. Furie, C. Gillespie, A. Go, K. Greenlund, N. Haase, S. Hailpern, P. M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lackland, L. Lisabeth, A. Marelli, M. M. McDermott, J. Meigs, D. Mozaffarian, M. Mussolino, G. Nichol, V. L. Roger, W. Rosamond, R. Sacco, P. Sorlie, T. Thom, S. Wasserthiel-Smoller, N. D. Wong and J. Wylie-Rosett: Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*, 121(7), e46-e215 (2010)

2. Topol. E. and Penn MS: The challenge for stem cell therapy. In: *Stem Cells and Myocardial Regeneration*. Ed Penn. MS. Humana Press, New York (2007)

3. M. Jessup and S. Brozena: Heart failure. *N Engl J Med*, 348(20), 2007-18 (2003)

4. J. Tamargo, R. Caballero, R. Gomez, A. Barana, I. Amoros and E. Delpon: Investigational positive inotropic agents for acute heart failure. *Cardiovasc Hematol Disord Drug Targets*, 9(3), 193-205 (2009)

5. W. R. Mills, N. Mal, M. J. Kiedrowski, R. Unger, F. Forudi, Z. B. Popovic, M. S. Penn and K. R. Laurita: Stem cell therapy enhances electrical viability in myocardial infarction. *J Mol Cell Cardiol*, 42(2), 304-14 (2007)

6. H. Reinecke, V. Poppa and C. E. Murry: Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol*, 34(2), 241-9 (2002)

7. I. Deglurkar, N. Mal, W. R. Mills, Z. B. Popovic, P. McCarthy, E. H. Blackstone, K. R. Laurita and M. S. Penn: Mechanical and electrical effects of cell-based gene therapy for ischemic cardiomyopathy are independent. *Hum Gene Ther*, 17(11), 1144-51 (2006)

8. M. H. Soonpaa, G. Y. Koh, M. G. Klug and L. J. Field: Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science*, 264(5155), 98-101 (1994)

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

10. S. Etzion, A. Battler, I. M. Barbash, E. Cagnano, P. Zarin, Y. Granot, L. H. Kedes, R. A. Kloner and J. Leor: Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. *J Mol Cell Cardiol*, 33(7), 1321-30 (2001)

11. M. J. Evans and M. H. Kaufman: Establishment in culture of pluripotential cells from mouse embryos. *Nature*, 292(5819), 154-156 (1981)

12. G. R. Martin: Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A*, 78(12), 7634-8 (1981)

13. O. Caspi, I. Huber, I. Kehat, M. Habib, G. Arbel, A. Gepstein, L. Yankelson, D. Aronson, R. Beyar and L. Gepstein: Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol*, 50(19), 1884-93 (2007)

14. M. A. Laflamme, K. Y. Chen, A. V. Naumova, V. Muskheli, J. A. Fugate, S. K. Dupras, H. Reinecke, C. Xu, M. Hassanipour, S. Police, C. O'Sullivan, L. Collins, Y. Chen, E. Minami, E. A. Gill, S. Ueno, C. Yuan, J. Gold and C. E. Murry: Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*, 25(9), 1015-24 (2007)

15. J. R. Van Orman, K. Si-Tayeb, S. A. Duncan and J. Lough: Induction of Cardiomyogenesis in Human Embryonic Stem Cells (hESCs) by hESC-Derived Definitive Endoderm. *Stem Cells Dev* (2011)

16. S. J. Kattman, A. D. Witty, M. Gagliardi, N. C. Dubois, M. Niapour, A. Hotta, J. Ellis and G. Keller: Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell*, 8(2), 228-240 (2011)

17. S. Fernandes, A. V. Naumova, W. Z. Zhu, M. A. Laflamme, J. Gold and C. E. Murry: Human embryonic stem cell-derived cardiomyocytes engraft but do not alter cardiac remodeling after chronic infarction in rats. *J Mol Cell Cardiol*, 49(6), 941-9 (2010)

18. H. Tsuji, S. Miyoshi, Y. Ikegami, N. Hida, H. Asada, I. Togashi, J. Suzuki, M. Satake, H. Nakamizo, M. Tanaka, T. Mori, K. Segawa, N. Nishiyama, J. Inoue, H. Makino, K. Miyado, S. Ogawa, Y. Yoshimura and A. Umezawa: Xenografted human amniotic membrane-derived mesenchymal stem cells are immunologically tolerated and transdifferentiated into cardiomyocytes. *Circ Res*, 106(10), 1613-23 (2010)

19. M. S. Penn and M. E. Mayorga: Searching for understanding with the cellular lining of life. *Circ Res*, 106(10), 1554-6 (2010)

20. K. Takahashi and S. Yamanaka: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126(4), 663-676 (2006)

21. K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda and S. Yamanaka: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131(5), 861-72 (2007)

22. R. Blelloch, M. Venere, J. Yen and M. Ramalho-Santos: Generation of induced pluripotent stem cells in the absence of drug selection. *Cell Stem Cell*, 1(3), 245-247 (2007)

23. J. Yu, M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, Slukvin, II and J. A. Thomson: Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318(5858), 1917-20 (2007)

24. K. Schenke-Layland, K. E. Rhodes, E. Angelis, Y. Butylkova, S. Heydarkhan-Hagvall, C. Gekas, R. Zhang, J. I. Goldhaber, H. K. Mikkola, K. Plath and W. R. MacLellan: Reprogrammed mouse fibroblasts differentiate into cells of the cardiovascular and hematopoietic lineages. *Stem Cells*, 26(6), 1537-46 (2008)

25. C. Mauritz, A. Martens, S. V. Rojas, T. Schnick, C. Rathert, N. Schecker, S. Menke, S. Glage, R. Zweigerdt, A. Haverich, U. Martin and I. Kutschka: Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction. *Eur Heart J* (2011)

26. J. Zhang, G. F. Wilson, A. G. Soerens, C. H. Koonce, J. Yu, S. P. Palecek, J. A. Thomson and T. J. Kamp: Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res*, 104(4), e30-41 (2009)

27. G. Narazaki, H. Uosaki, M. Teranishi, K. Okita, B. Kim, S. Matsuoka, S. Yamanaka and J. K. Yamashita: Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation*, 118(5), 498-506 (2008)

28. M. Ieda, J. D. Fu, P. Delgado-Olguin, V. Vedantham, Y. Hayashi, B. G. Bruneau and D. Srivastava: Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*, 142(3), 375-386 (2010)

29. T. J. Nelson, A. Martinez-Fernandez, S. Yamada, C. Perez-Terzic, Y. Ikeda and A. Terzic: Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation*, 120(5), 408-416 (2009)

30. Y. Zhang, D. Wang, M. Chen, B. Yang, F. Zhang and K. Cao: Intramyocardial transplantation of undifferentiated

rat induced pluripotent stem cells causes tumorigenesis in the heart. *PLoS One*, 6(4), e19012 (2011)

31. I. L. Weissman and J. A. Shizuru: The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood*, 112(9), 3543-3553 (2008)

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

33. C. E. Murry, M. H. Soonpaa, H. Reinecke, H. Nakajima, H. O. Nakajima, M. Rubart, K. B. Pasumarthi, J. I. Virag, S. H. Bartelmez, V. Poppa, G. Bradford, J. D. Dowell, D. A. Williams and L. J. Field: Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*, 428(6983), 664-668 (2004)

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

35. E. T. Yeh, S. Zhang, H. D. Wu, M. Korbling, J. T. Willerson and Z. Estrov: Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells *in vivo*. *Circulation*, 108(17), 2070-2073 (2003)

36. S. Zhang, D. Wang, Z. Estrov, S. Raj, J. T. Willerson and E. T. Yeh: Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes *in vivo*. *Circulation*, 110(25), 3803-3807 (2004)

37. T. Yoshioka, N. Ageyama, H. Shibata, T. Yasu, Y. Misawa, K. Takeuchi, K. Matsui, K. Yamamoto, K. Terao, K. Shimada, U. Ikeda, K. Ozawa and Y. Hanazono: Repair of infarcted myocardium mediated by transplanted bone marrow-derived CD34+ stem cells in a nonhuman primate model. *Stem Cells*, 23(3), 355-364 (2005)

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

39. A. I. Caplan: Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol*, 213(2), 341-7 (2007)

40. M. E. Mayorga, F. Dong, S. Sundararaman, Y. Huang, Y. Jiang, P. H. Howe and M. S. Penn: Central role for disabled-2 in mesenchymal stem cardiac protein expression and functional consequences after engraftment in acute myocardial infarction. *Stem Cells Dev*, 20(4), 681-93 (2011)

41. F. P. Barry, J. M. Murphy, K. English and B. P. Mahon: Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft. *Stem Cells Dev*, 14(3), 252-65 (2005)

42. M. Zhang, N. Mal, M. Kiedrowski, M. Chacko, A. T. Askari, Z. B. Popovic, O. N. Koc and M. S. Penn: SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *FASEB J*, 21(12), 3197-207 (2007)

43. S. Schenk, N. Mal, A. Finan, M. Zhang, M. kiedrowski, Z. Popovic, P. M. McCarthy and M. S. Penn: Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. *Stem Cells*, 25(1), 245-251 (2007)

44. K. E. Hatzistergos, H. Quevedo, B. N. Oskouei, Q. Hu, G. S. Feigenbaum, I. S. Margitich, R. Mazhari, A. J. Boyle, J. P. Zambrano, J. E. Rodriguez, R. Dulce, P. M. Pattany, D. Valdes, C. Revilla, A. W. Heldman, I. McNiece and J. M. Hare: Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res*, 107(7), 913-22 (2010)

45. Y. Jiang, B. Vaessen, T. Lenvik, M. Blackstad, M. Reyes and C. M. Verfaillie: Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol*, 30(8), 896-904 (2002)

46. W. Van't Hof, N. Mal, Y. Huang, M. Zhang, Z. Popovic, F. Forudi, R. Deans and M. S. Penn: Direct delivery of syngeneic and allogeneic large-scale expanded multipotent adult progenitor cells improves cardiac function after myocardial infarct. *Cytotherapy*, 9(5), 477-87 (2007)

47. O. Agbulut, M. Mazo, C. Bressolle, M. Gutierrez, K. Azarnoush, L. Sabbah, N. Niederlander, G. Abizanda, E. J. Andreu, B. Pelacho, J. J. Gavira, M. Perez-Ilzarbe, S. Peyrard, P. Bruneval, J. L. Samuel, M. Soriano-Navarro, J. M. Garcia-Verdugo, A. A. Hagege, F. Prosper and P. Menasche: Can bone marrow-derived multipotent adult progenitor cells regenerate infarcted myocardium? *Cardiovasc Res*, 72(1), 175-83 (2006)

48. B. Pelacho, Y. Nakamura, J. Zhang, J. Ross, Y. Heremans, M. Nelson-Holte, B. Lemke, J. Hagenbrock, Y. Jiang, F. Prosper, A. Luttun and C. M. Verfaillie: Multipotent adult progenitor cell transplantation increases vascularity and improves left ventricular function after myocardial infarction. *J Tissue Eng Regen Med*, 1(1), 51-9 (2007)

49. M. Gnecchi, Z. Zhang, A. Ni and V. J. Dzau: Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*, 103(11), 1204-19 (2008)

50. N. Dib, J. Dinsmore, Z. Lababidi, B. White, S. Moravec, A. Campbell, A. Rosenbaum, K. Seyedmadani, W. A. Jaber, C. S. Rizenhour and E. Diethrich: One-year

follow-up of feasibility and safety of the first U.S., randomized, controlled study using 3-dimensional guided catheter-based delivery of autologous skeletal myoblasts for ischemic cardiomyopathy (CAuSMIC study). *JACC Cardiovasc Interv*, 2(1), 9-16 (2009)

51. H. J. Duckers, J. Houtgraaf, C. Hehrlein, J. Schofer, J. Waltenberger, A. Gershlick, J. Bartunek, C. Nienaber, C. Macaya, N. Peters, P. Smits, T. Siminiak, W. van Mieghem, V. Legrand and P. W. Serruys: Final results of a phase IIa, randomised, open-label trial to evaluate the percutaneous intramyocardial transplantation of autologous skeletal myoblasts in congestive heart failure patients: the SEISMIC trial. *EuroIntervention*, 6(7), 805-12 (2011)

52. P. Menasche, O. Alfieri, S. Janssens, W. McKenna, H. Reichenspurner, L. Trinquart, J. T. Vilquin, J. P. Marolleau, B. Seymour, J. Larghero, S. Lake, G. Chatellier, S. Solomon, M. Desnos and A. A. Hagege: The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation*, 117(9), 1189-1200 (2008)

53. M. S. Penn and A. A. Mangi: Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circ Res*, 102(12), 1471-82 (2008)

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

55. S. Janssens, C. Dubois, J. Bogaert, K. Theunissen, C. Deroose, W. Desmet, M. Kalantzi, L. Herbots, P. Sinnaeve, J. Dens, J. Maertens, F. Rademakers, S. Dymarkowski, O. Gheysens, J. Van Cleemput, G. Bormans, J. Nuyts, A. Belmans, L. Mortelmans, M. Boogaerts and F. Van de Werf: Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet*, 367(9505), 113-21 (2006)

56. J. H. Traverse, D. H. McKenna, K. Harvey, B. C. Jorgenso, R. E. Olson, N. Bostrom, D. Kadidlo, J. R. Lesser, V. Jagadeesan, R. Garberich and T. D. Henry: Results of a phase 1, randomized, double-blind, placebo-controlled trial of bone marrow mononuclear stem cell administration in patients following ST-elevation myocardial infarction. *Am Heart J*, 160(3), 428-34 (2010)

57. S. Charwat, I. Lang, M. Dettke, S. Graf, N. Nyolczas, R. Hemetsberger, S. Zamini, A. Khorsand, H. Sochor, G. Maurer, D. Glogar and M. Gyongyosi: Effect of intramyocardial delivery of autologous bone marrow mononuclear stem cells on the regional myocardial perfusion. NOGA-guided subanalysis of the MYSTAR prospective randomised study. *Thromb Haemost*, 103(3), 564-71 (2010)

58. M. F. Piepoli, D. Vallisa, M. Arbasi, L. Cavanna, L. Cerri, M. Mori, F. Passerini, L. Tommasi, A. Rossi and A. Capucci: Bone marrow cell transplantation improves cardiac, autonomic, and functional indexes in acute anterior myocardial infarction patients (Cardiac Study). *Eur J Heart Fail*, 12(2), 172-80 (2010)

59. J. O. Beitnes, E. Hopp, K. Lunde, S. Solheim, H. Arnesen, J. E. Brinchmann, K. Forfang and S. Aakhus: Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the ASTAMI randomised, controlled study. *Heart*, 95(24), 1983-1989 (2009)

60. T. Dill, V. Schachinger, A. Rolf, S. Mollmann, H. Thiele, H. Tillmanns, B. Assmus, S. Dimmeler, A. M. Zeiher and C. Hamm: Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. *Am Heart J*, 157(3), 541-7 (2009)

61. G. P. Meyer, K. C. Wollert, J. Lotz, J. Pirr, U. Rager, P. Lippolt, A. Hahn, S. Fichtner, A. Schaefer, L. Arseniev, A. Ganser and H. Drexler: Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. *Eur Heart J*, 30(24), 2978-84 (2009)

62. D. M. Leistner, U. Fischer-Rasokat, J. Honold, F. H. Seeger, V. Schachinger, R. Lehmann, H. Martin, I. Burck, C. Urbich, S. Dimmeler, A. M. Zeiher and B. Assmus: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. *Clin Res Cardiol* (2011)

63. S. Zhang, A. Sun, D. Xu, K. Yao, Z. Huang, H. Jin, K. Wang, Y. Zou and J. Ge: Impact of timing on efficacy and safetyof intracoronary autologous bone marrow stem cells transplantation in acute myocardial infarction: a pooled subgroup analysis of randomized controlled trials. *Clin Cardiol*, 32(8), 458-66 (2009)

64. M. S. Penn, S. Anwaruddin, R. Nair and S. Ellis: From mice to men. Commonalities in physiology for stem cell-based cardiac repair. *J Am Coll Cardiol*, 54(24), 2287-9 (2009)

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

66. E. C. Perin, H. F. Dohmann, R. Borojevic, S. A. Silva, A. L. Sousa, G. V. Silva, C. T. Mesquita, L. Belem, W. K. Vaughn, F. O. Rangel, J. A. Assad, A. C. Carvalho, R. V. Branco, M. I. Rossi, H. J. Dohmann and J. T. Willerson: Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation*, 110(11 Suppl 1), II213-8 (2004)

67. B. E. Strauer, M. Yousef and C. M. Schannwell: 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), 721-9 (2010)

68. U. Fischer-Rasokat, B. Assmus, F. H. Seeger, J. Honold, D. Leistner, S. Fichtlscherer, V. Schachinger, T. Tonn, H. Martin, S. Dimmeler and A. M. Zeiher: A pilot trial to assess potential effects of selective intracoronary bone marrow-derived progenitor cell infusion in patients with nonischemic dilated cardiomyopathy: final 1-year results of the transplantation of progenitor cells and functional regeneration enhancement pilot trial in patients with nonischemic dilated cardiomyopathy. *Circ Heart Fail*, 2(5), 417-23 (2009)

69. J. M. Hare, J. H. Traverse, T. D. Henry, N. Dib, R. K. Strumpf, S. P. Schulman, G. Gerstenblith, A. N. DeMaria, A. E. Denktas, R. S. Gammon, J. B. Hermiller, Jr., M. A. Reisman, G. L. Schaer and W. Sherman: A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*, 54(24), 2277-86 (2009)

70. A. R. Williams, B. Trachtenberg, D. L. Velazquez, I. McNiece, P. Altman, D. Rouy, A. M. Mendizabal, P. M. Pattany, G. A. Lopera, J. Fishman, J. P. Zambrano, A. W. Heldman and J. M. Hare: Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling. *Circ Res*, 108(7), 792-6 (2011)

71. B. Assmus, U. Fischer-Rasokat, J. Honold, F. H. Seeger, S. Fichtlscherer, T. Tonn, E. Seifried, V. Schachinger, S. Dimmeler and A. M. Zeiher: 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), 1234-41 (2007)

72. A. T. Askari, S. Unzek, Z. B. Popovic, C. K. Goldman, F. Forudi, M. Kiedrowski, A. Rovner, S. G. Ellis, J. D. Thomas, P. E. DiCorleto, E. J. Topol and M. S. Penn: Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*, 362(9385), 697-703 (2003)

73. M. S. Penn: Importance of the SDF-1:CXCR4 axis in myocardial repair. *Circ Res*, 104(10), 1133-5 (2009)

74. S. Sundararaman, T. J. Miller, J. M. Pastore, M. Kiedrowski, R. Aras and M. S. Penn: Plasmid-based transient human stromal cell-derived factor-1 gene transfer improves cardiac function in chronic heart failure. *Gene Therapy* (2011)

75. S. Neragi-Miandoab, J. Kim and G. J. Vlahakes: Malignant tumours of the heart: a review of tumour type, diagnosis and therapy. *Clin Oncol (R Coll Radiol)*, 19(10), 748-56 (2007)

76. J. M. Gonzalez-Rosa, V. Martin, M. Peralta, M. Torres and N. Mercader: Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development*, 138(9), 1663-1674 (2011)

77. K. Schnabel, C. C. Wu, T. Kurth and G. Weidinger: Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLoS One*, 6(4), e18503 (2011)

78. K. D. Poss, L. G. Wilson and M. T. Keating: Heart regeneration in zebrafish. *Science*, 298(5601), 2188-2190 (2002)

79. K. Kikuchi, J. E. Holdway, A. A. Werdich, R. M. Anderson, Y. Fang, G. F. Egnaczyk, T. Evans, C. A. Macrae, D. Y. Stainier and K. D. Poss: Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. *Nature*, 464(7288), 601-605 (2010)

80. E. R. Porrello, A. I. Mahmoud, E. Simpson, J. A. Hill, J. A. Richardson, E. N. Olson and H. A. Sadek: Transient regenerative potential of the neonatal mouse heart. *Science*, 331(6020), 1078-1080 (2011)

81. P. C. Hsieh, V. F. Segers, M. E. Davis, C. MacGillivray, J. Gannon, J. D. Molkentin, J. Robbins and R. T. Lee: Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med*, 13(8), 970-4 (2007)

82. M. S. Penn, M. E. Mayorga and F. Dong: Youth is wasted on the young. *Stem Cell Res Ther*, 2(3), 24 (2011)

83. O. Bergmann, R. D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabe-Heider, S. Walsh, J. Zupicich, K. Alkass, B. A. Buchholz, H. Druid, S. Jovinge and J. Frisen: Evidence for cardiomyocyte renewal in humans. *Science*, 324(5923), 98-102 (2009)

84. A. P. Beltrami, L. Barlucchi, D. Torella, M. Baker, F. Limana, S. Chimenti, H. Kasahara, M. Rota, E. Musso, K. Urbanek, A. Leri, J. Kajstura, B. Nadal-Ginard and P. Anversa: Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, 114(6), 763-776 (2003)

85. K. Urbanek, F. Quaini, G. Tasca, D. Torella, C. Castaldo, B. Nadal-Ginard, A. Leri, J. Kajstura, E. Quaini and P. Anversa: Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc Natl Acad Sci U S A*, 100(18), 10440-5 (2003)

86. K. Urbanek, D. Torella, F. Sheikh, A. De Angelis, D. Nurzynska, F. Silvestri, C. A. Beltrami, R. Bussani, A. P. Beltrami, F. Quaini, R. Bolli, A. Leri, J. Kajstura and P. Anversa: Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc Natl Acad Sci U S A*, 102(24), 8692-7 (2005)

87. J. Sandstedt, M. Jonsson, A. Lindahl, A. Jeppsson and J. Asp: C-kit+ CD45- cells found in the adult human heart represent a population of endothelial progenitor cells. *Basic Res Cardiol*, 105(4), 545-56 (2010)

88. S. Miyamoto, N. Kawaguchi, G. M. Ellison, R. Matsuoka, T. Shin'oka and H. Kurosawa: Characterization of long-term cultured c-kit+ cardiac stem cells derived from adult rat hearts. *Stem Cells Dev*, 19(1), 105-16 (2010)

89. F. Limana, A. Zacheo, D. Mocini, A. Mangoni, G. Borsellino, A. Diamantini, R. De Mori, L. Battistini, E. Vigna, M. Santini, V. Loiaconi, G. Pompilio, A. Germani and M. C. Capogrossi: Identification of myocardial and vascular precursor cells in human and mouse epicardium. *Circ Res*, 101(12), 1255-65 (2007)

90. F. Quaini, K. Urbanek, A. P. Beltrami, N. Finato, C. A. Beltrami, B. Nadal-Ginard, J. Kajstura, A. Leri and P. Anversa: Chimerism of the transplanted heart. *N Engl J Med*, 346(1), 5-15 (2002)

91. A. Itzhaki-Alfia, J. Leor, E. Raanani, L. Sternik, D. Spiegelstein, S. Netser, R. Holbova, M. Pevsner-Fischer, J. Lavee and I. M. Barbash: Patient characteristics and cell source determine the number of isolated human cardiac progenitor cells. *Circulation*, 120(25), 2559-2566 (2009)

92. J. Fransioli, B. Bailey, N. A. Gude, C. T. Cottage, J. A. Muraski, G. Emmanuel, W. Wu, R. Alvarez, M. Rubio, S. Ottolenghi, E. Schaefer and M. A. Sussman: Evolution of the c-kit-positive cell response to pathological challenge in the myocardium. *Stem Cells*, 26(5), 1315-1324 (2008)

93. H. Kubo, N. Jaleel, A. Kumarapeli, R. M. Berretta, G. Bratinov, X. Shan, H. Wang, S. R. Houser and K. B. Margulies: Increased cardiac myocyte progenitors in failing human hearts. *Circulation*, 118(6), 649-657 (2008)

94. J. Kajstura, N. Gurusamy, B. Ogorek, P. Goichberg, C. Clavo-Rondon, T. Hosoda, D. D'Amario, S. Bardelli, A. P. Beltrami, D. Cesselli, R. Bussani, F. del Monte, F. Quaini, M. Rota, C. A. Beltrami, B. A. Buchholz, A. Leri and P. Anversa: Myocyte turnover in the aging human heart. *Circ Res*, 107(11), 1374-86 (2010)

95. R. Mishra, K. Vijayan, E. J. Colletti, D. A. Harrington, T. S. Matthiesen, D. Simpson, S. K. Goh, B. L. Walker, G. Almeida-Porada, D. Wang, C. L. Backer, S. C. Dudley, Jr., L. E. Wold and S. Kaushal: Characterization and functionality of cardiac progenitor cells in congenital heart patients. *Circulation*, 123(4), 364-373 (2011)

96. C. Chimenti, J. Kajstura, D. Torella, K. Urbanek, H. Heleniak, C. Colussi, F. Di Meglio, B. Nadal-Ginard, A. Frustaci, A. Leri, A. Maseri and P. Anversa: Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res*, 93(7), 604-13 (2003)

97. H. Oh, S. B. Bradfute, T. D. Gallardo, T. Nakamura, V. Gaussin, Y. Mishina, J. Pocius, L. H. Michael, R. R. Behringer, D. J. Garry, M. L. Entman and M. D. Schneider: Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*, 100(21), 12313-8 (2003)

98. K. Matsuura, T. Nagai, N. Nishigaki, T. Oyama, J. Nishi, H. Wada, M. Sano, H. Toko, H. Akazawa, T. Sato, H. Nakaya, H. Kasanuki and I. Komuro: Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem*, 279(12), 11384-91 (2004)

99. K. Matsuura, A. Honda, T. Nagai, N. Fukushima, K. Iwanaga, M. Tokunaga, T. Shimizu, T. Okano, H. Kasanuki, N. Hagiwara and I. Komuro: Transplantation of cardiac progenitor cells ameliorates cardiac dysfunction after myocardial infarction in mice. *J Clin Invest*, 119(8), 2204-17 (2009)

100. C. L. Cai, X. Liang, Y. Shi, P. H. Chu, S. L. Pfaff, J. Chen and S. Evans: Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell*, 5(6), 877-89 (2003)

101. R. Genead, C. Danielsson, A. B. Andersson, M. Corbascio, A. Franco-Cereceda, C. Sylven and K. H. Grinnemo: Islet-1 cells are cardiac progenitors present during the entire lifespan: from the embryonic stage to adulthood. *Stem Cells Dev*, 19(10), 1601-15 (2010)

102. K. L. Laugwitz, A. Moretti, J. Lam, P. Gruber, Y. Chen, S. Woodard, L. Z. Lin, C. L. Cai, M. M. Lu, M. Reth, O. Platoshyn, J. X. Yuan, S. Evans and K. R. Chien: Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*, 433(7026), 647-653 (2005)

103. L. Bu, X. Jiang, S. Martin-Puig, L. Caron, S. Zhu, Y. Shao, D. J. Roberts, P. L. Huang, I. J. Domian and K. R. Chien: Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature*, 460(7251), 113-117 (2009)

104. C. M. Martin, A. P. Meeson, S. M. Robertson, T. J. Hawke, J. A. Richardson, S. Bates, S. C. Goetsch, T. D. Gallardo and D. J. Garry: Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev Biol*, 265(1), 262-75 (2004)

105. M. Huls, F. G. Russel and R. Masereeuw: The role of ATP binding cassette transporters in tissue defense and organ regeneration. *J Pharmacol Exp Ther*, 328(1), 3-9 (2009)

106. O. Pfister, F. Mouquet, M. Jain, R. Summer, M. Helmes, A. Fine, W. S. Colucci and R. Liao: CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res*, 97(1), 52-61 (2005)

107. T. Oyama, T. Nagai, H. Wada, A. T. Naito, K. Matsuura, K. Iwanaga, T. Takahashi, M. Goto, Y. Mikami, N. Yasuda, H. Akazawa, A. Uezumi, S. Takeda and I. Komuro: Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes *in vitro* and *in vivo*. *J Cell* Biol, 176(3), 329-41 (2007)

108. F. Mouquet, O. Pfister, M. Jain, A. Oikonomopoulos, S. Ngoy, R. Summer, A. Fine and R. Liao: Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. Circ Res, 97(11), 1090-2 (2005)

109. D. R. Davis, Y. Zhang, R. R. Smith, K. Cheng, J. Terrovitis, K. Malliaras, T. S. Li, A. White, R. Makkar and E. Marban: Validation of the cardiosphere method to culture cardiac progenitor cells from myocardial tissue. PLoS One, 4(9), e7195 (2009)

110. E. Messina, L. De Angelis, G. Frati, S. Morrone, S. Chimenti, F. Fiordaliso, M. Salio, M. Battaglia, M. V. Latronico, M. Coletta, E. Vivarelli, L. Frati, G. Cossu and A. Giacomello: Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res, 95(9), 911-21 (2004)

111. R. R. Smith, L. Barile, H. C. Cho, M. K. Leppo, J. M. Hare, E. Messina, A. Giacomello, M. R. Abraham and E. Marban: Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation, 115(7), 896-908 (2007)

112. I. Chimenti, R. R. Smith, T. S. Li, G. Gerstenblith, E. Messina, A. Giacomello and E. Marban: Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res, 106(5), 971-80 (2010)

113. S. T. Lee, A. J. White, S. Matsushita, K. Malliaras, C. Steenbergen, Y. Zhang, T. S. Li, J. Terrovitis, K. Yee, S. Simsir, R. Makkar and E. Marban: Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. J Am Coll Cardiol, 57(4), 455-65 (2011)

114. D. C. Andersen, P. Andersen, M. Schneider, H. B. Jensen and S. P. Sheikh: Murine "cardiospheres" are not a source of stem cells with cardiomyogenic potential. Stem Cells, 27(7), 1571-1581 (2009)

115. Q. Li, Y. Guo, Q. Ou, N. Chen, W. J. Wu, F. Yuan, E. O'Brien, T. Wang, L. Luo, G. N. Hunt, X. Zhu and R. Bolli: Intracoronary administration of cardiac stem cells in mice: a new, improved technique for cell therapy in murine models. Basic Res Cardiol, 106(5), 849-64 (2011)

116. F. Mouquet, O. Pfister, M. Jain, A. Oikonomopoulos, S. Ngoy, R. Summer, A. Fine and R. Liao: Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ.Res.*, 97(11), 1090-1092 (2005)

117. S. Unzek, M. Zhang, N. Mal, W. R. Mills, K. R. Laurita and M. S. Penn: SDF-1 recruits cardiac stem cell-like cells that depolarize *in vivo*. *Cell Transplant*, 16(9), 879-86 (2007)

118. W. R. Mills, N. Mal, M. J. Kiedrowski, R. Unger, F. Forudi, Z. B. Popovic, M. S. Penn and K. R. laurita: Stem cell therapy enhances electrical viability in myocardial infarction. *J Mol.Cell Cardiol.*, 42(304), 314 (2007)

119. R. Bolli, A. R. Chugh, D. D'Amario, J. H. Loughran, M. F. Stoddard, S. Ikram, G. M. Beache, S. G. Wagner, A. Leri, T. Hosoda, F. Sanada, J. B. Elmore, P. Goichberg, D. Cappetta, N. K. Solankhi, I. Fahsah, D. G. Rokosh, M. S. Slaughter, J. Kajstura and P. Anversa: Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*, 378(9806), 1847-57 (2011)

120. T. Asahara, T. Murohara, A. Sullivan, M. Silver, R. van der Zee, T. Li, B. Witzenbichler, G. Schatteman and J. M. Isner: Isolation of putative progenitor endothelial cells for angiogenesis. *Science*, 275(5302), 964-7 (1997)

121. E. Ciraci, S. Della Bella, O. Salvucci, C. Rofani, M. Segarra, C. Bason, A. Molinari, D. Maric, G. Tosato and A. C. Berardi: Adult human circulating CD34-Lin-CD45-CD133- cells can differentiate into hematopoietic and endothelial cells. *Blood* (2011)

122. A. Caprioli, T. Jaffredo, R. Gautier, C. Dubourg and F. Dieterlen-Lievre: Blood-borne seeding by hematopoietic and endothelial precursors from the allantois. *Proc Natl Acad Sci U S A*, 95(4), 1641-6 (1998)

123. E. Pelosi, M. Valtieri, S. Coppola, R. Botta, M. Gabbianelli, V. Lulli, G. Marziali, B. Masella, R. Muller, C. Sgadari, U. Testa, G. Bonanno and C. Peschle: Identification of the hemangioblast in postnatal life. *Blood*, 100(9), 3203-3208 (2002)

124. C. Urbich, C. Heeschen, A. Aicher, E. Dernbach, A. M. Zeiher and S. Dimmeler: Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation*, 108(20), 2511-2516 (2003)

125. M. Massa, V. Rosti, M. Ferrario, R. Campanelli, I. Ramajoli, R. Rosso, G. M. De Ferrari, M. Ferlini, L. Goffredo, A. Bertoletti, C. Klersy, A. Pecci, R. Moratti and L. Tavazzi: Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood*, 105(1), 199-206 (2005)

126. S. Shintani, T. Murohara, H. Ikeda, T. Ueno, T. Honma, A. Katoh, K. Sasaki, T. Shimada, Y. Oike and T. Imaizumi: Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*, 103(23), 2776-2779 (2001)

127. C. K. Kissel, R. Lehmann, B. Assmus, A. Aicher, J. Honold, U. Fischer-Rasokat, C. Heeschen, I. Spyridopoulos, S. Dimmeler and A. M. Zeiher: Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. *J Am Coll Cardiol*, 49(24), 2341-9 (2007)

128. G. L. Hoetzer, G. P. Van Guilder, H. M. Irmiger, R. S. Keith, B. L. Stauffer and C. A. DeSouza: Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *J Appl Physiol*, 102(3), 847-52 (2007)

129. C. Heiss, S. Keymel, U. Niesler, J. Ziemann, M. Kelm and C. Kalka: Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol*, 45(9), 1441-8 (2005)

130. E. I. Chang, S. A. Loh, D. J. Ceradini, S. E. Lin, N. Bastidas, S. Aarabi, D. A. Chan, M. L. Freedman, A. J. Giaccia and G. C. Gurtner: Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation*, 116(24), 2818-29 (2007)

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

132. N. A. Sopko, B. A. Turturice, M. E. Becker, C. R. Brown, F. Dong, Z. B. Popovic and M. S. Penn: Bone marrow support of the heart in pressure overload is lost with aging. *PLoS One*, 5(12), e15187 (2010)

133. J. M. Edelberg, L. Tang, K. Hattori, D. Lyden and S. Rafii: Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. *Circ Res*, 90(10), E89-93 (2002)

134. I. M. Conboy, M. J. Conboy, A. J. Wagers, E. R. Girma, I. L. Weissman and T. A. Rando: Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*, 433(7027), 760-764 (2005)

135. S. Fazel, M. Cimini, L. Chen, S. Li, D. Angoulvant, P. Fedak, S. Verma, R. D. Weisel, A. Keating and R. K. Li: Cardioprotective c-kit+ cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest*, 116(7), 1865-77 (2006)

136. A. Deb, S. Wang, K. A. Skelding, D. Miller, D. Simper and N. M. Caplice: Bone marrow-derived cardiomyocytes are present in adult human heart: A study of gender-mismatched bone marrow transplantation patients. *Circulation*, 107(9), 1247-1249 (2003)

137. R. A. de Weger, I. Verbrugge, A. H. Bruggink, M. M. van Oosterhout, Y. de Souza, D. F. van Wichen, F. H. Gmelig-Meyling, N. de Jonge and L. F. Verdonck: Stem cell-derived cardiomyocytes after bone marrow and heart transplantation. *Bone Marrow Transplant*, 41(6), 563-9 (2008)

138. P. Muller, A. Kazakov, A. Semenov, M. Bohm and U. Laufs: Pressure-induced cardiac overload induces upregulation of endothelial and myocardial progenitor cells. *Cardiovasc Res*, 77(1), 151-9 (2008)

139. K. A. Jackson, S. M. Majka, H. Wang, J. Pocius, C. J. Hartley, M. W. Majesky, M. L. Entman, L. H. Michael, K. K. Hirschi and M. A. Goodell: Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest*, 107(11), 1395-402 (2001)

140. L. Barile, F. Cerisoli, G. Frati, R. Gaetani, I. Chimenti, E. Forte, L. Cassinelli, L. Spinardi, C. Altomare, E. Kizana, A. Giacomello, E. Messina, S. Ottolenghi and M. C. Magli: Bone marrow-derived cells can acquire cardiac stem cells properties in damaged heart. *J Cell Mol Med*, 15(1), 63-71 (2011)

141. A. J. White, R. R. Smith, S. Matsushita, T. Chakravarty, L. S. Czer, K. Burton, E. R. Schwarz, D. R. Davis, Q. Wang, N. L. Reinsmoen, J. S. Forrester, E. Marban and R. Makkar: Intrinsic cardiac origin of human cardiosphere-derived cells. *Eur Heart J* (2011)

142. U. Agarwal, W. Ghalayini, F. Dong, K. Weber, Y. R. Zou, S. Y. Rabbany, S. Rafii and M. S. Penn: Role of cardiac myocyte CXCR4 expression in development and left ventricular remodeling after acute myocardial infarction. *Circ Res*, 107(5), 667-76 (2010)

143. Y. L. Tang, W. Zhu, M. Cheng, L. Chen, J. Zhang, T. Sun, R. Kishore, M. I. Phillips, D. W. Losordo and G. Qin: Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. *Circ Res*, 104(10), 1209-16 (2009)

144. M. M. Zaruba, H. D. Theiss, M. Vallaster, U. Mehl, S. Brunner, R. David, R. Fischer, L. Krieg, E. Hirsch, B. Huber, P. Nathan, L. Israel, A. Imhof, N. Herbach, G. Assmann, R. Wanke, J. Mueller-Hoecker, G. Steinbeck and W. M. Franz: Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction. *Cell Stem Cell*, 4(4), 313-323 (2009)

145. K. W. Christopherson, 2nd, G. Hangoc and H. E. Broxmeyer: Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. *J Immunol*, 169(12), 7000-8 (2002)

146. M. Dabusti, F. Lanza, D. Campioni, B. Castagnari, A. Tieghi, S. Moretti, M. Punturieri, C. De Angeli, R. Spanedda, E. Ferrazzi and G. Castoldi: CXCR-4 expression on bone marrow CD34+ cells prior to mobilization can predict mobilization adequacy in patients with hematologic malignancies. *J Hematother Stem Cell Res*, 12(4), 425-34 (2003)

147. Y. Guo, G. Hangoc, H. Bian, L. M. Pelus and H. E. Broxmeyer: SDF-1/CXCL12 enhances survival and chemotaxis of murine embryonic stem cells and production of primitive and definitive hematopoietic progenitor cells. *Stem Cells*, 23(9), 1324-1332 (2005)

148. J. H. Traverse, T. D. Henry, S. G. Ellis, C. J. Pepine, J. T. Willerson, D. X. Zhao, J. R. Forder, B. J. Byrne, A. K. Hatzopoulos, M. S. Penn, E. C. Perin, K. W. Baran, J. Chambers, C. Lambert, G. Raveendran, D. I. Simon, D. E. Vaughan, L. M. Simpson, A. P. Gee, D. A. Taylor, C. R. Cogle, J. D. Thomas, G. V. Silva, B. C. Jorgenson, R. E. Olson, S. Bowman, J. Francescon, C. Geither, E. Handberg, D. X. Smith, S. Baraniuk, L. B. Piller, C. Loghin, D. Aguilar, S. Richman, C. Zierold, J. Bettencourt, S. L. Sayre, R. W. Vojvodic, S. I. Skarlatos, D. J. Gordon, R. F. Ebert, M. Kwak, L. A. Moye, R. D. Simari and R. Cardiovascular Cell Therapy: Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. *JAMA*, 306(19), 2110-9 (2011)

149. X. Hu, S. Dai, W. J. Wu, W. Tan, X. Zhu, J. Mu, Y. Guo, R. Bolli and G. Rokosh: Stromal cell derived factor-1 alpha confers protection against myocardial ischemia/reperfusion injury: role of the cardiac stromal cell derived factor-1 alpha CXCR4 axis. *Circulation*, 116(6), 654-63 (2007)

150. A. Saxena, J. E. Fish, M. D. White, S. Yu, J. W. Smyth, R. M. Shaw, J. M. DiMaio and D. Srivastava: Stromal cell-derived factor-1alpha is cardioprotective after myocardial infarction. *Circulation*, 117(17), 2224-31 (2008)

151. J. M. Tang, J. N. Wang, L. Zhang, F. Zheng, J. Y. Yang, X. Kong, L. Y. Guo, L. Chen, Y. Z. Huang, Y. Wan and S. Y. Chen: VEGF/SDF-1 promotes cardiac stem cell mobilization and myocardial repair in the infarcted heart. *Cardiovasc Res*, 91(3), 402-11 (2011)

152. M. Majka, A. Janowska-Wieczorek, J. Ratajczak, M. A. Kowalska, G. Vilaire, Z. K. Pan, M. Honczarenko, L. A. Marquez, M. Poncz and M. Z. Ratajczak: Stromal-derived factor 1 and thrombopoietin regulate distinct aspects of human megakaryopoiesis. *Blood*, 96(13), 4142-51 (2000)

153. M. S. Penn: SDF-1:CXCR4 axis is fundamental for tissue preservation and repair. *Am J Pathol*, 177(5), 2166-8 (2010)

154. B. R. Avalos: The granulocyte colony-stimulating factor receptor and its role in disorders of granulopoiesis. *Leuk Lymphoma*, 28(3-4), 265-73 (1998)

155. G. Srinivas, P. Anversa and W. H. Frishman: Cytokines and myocardial regeneration: a novel treatment option for acute myocardial infarction. *Cardiol Rev*, 17(1), 1-9 (2009)

156. A. M. Leone, L. Galiuto, S. Rutella, M. B. Giannico, S. Brugaletta, B. Garramone, V. De Stefano, G. Liuzzo, M. L. Calcagni, F. Cirillo, A. Giordano, G. Niccoli, L. M. Biasucci, A. G. Rebuzzi, G. Leone and F. Crea: Safety of granulocyte-colony-stimulating factor in acute myocardial infarction (the Rigenera study). *Heart*, 92(12), 1850-1 (2006)

157. B. R. Avalos: Molecular analysis of the granulocyte colony-stimulating factor receptor. *Blood*, 88(3), 761-777 (1996)

158. S. G. Ellis, M. S. Penn, B. Bolwell, M. Garcia, M. Chacko, T. Wang, K. J. Brezina, G. McConnell and E. J. Topol: Granulocyte colony stimulating factor in patients with large acute myocardial infarction: results of a pilot dose-escalation randomized trial. *Am Heart J*, 152(6), 1051 e9-14 (2006)

159. K. Jujo, H. Hamada, A. Iwakura, T. Thorne, H. Sekiguchi, T. Clarke, A. Ito, S. Misener, T. Tanaka, E. Klyachko, K. Kobayashi, J. Tongers, J. Roncalli, Y. Tsurumi, N. Hagiwara and D. W. Losordo: CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. *Proc Natl Acad Sci U S A*, 107(24), 11008-13 (2010)

160. P. Rovere-Querini, A. Capobianco, P. Scaffidi, B. Valentinis, F. Catalanotti, M. Giazzon, I. E. Dumitriu, S. Muller, M. Iannacone, C. Traversari, M. E. Bianchi and A. A. Manfredi: HMGB1 is an endogenous immune adjuvant released by necrotic cells. *EMBO Rep*, 5(8), 825-30 (2004)

161. F. Limana, A. Germani, A. Zacheo, J. Kajstura, A. Di Carlo, G. Borsellino, O. Leoni, R. Palumbo, L. Battistini, R. Rastaldo, S. Muller, G. Pompilio, P. Anversa, M. E. Bianchi and M. C. Capogrossi: Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. *Circ Res*, 97(8), e73-83 (2005)

162. F. Limana, G. Esposito, D. D'Arcangelo, A. Di Carlo, S. Romani, G. Melillo, A. Mangoni, C. Bertolami, G. Pompilio, A. Germani and M. C. Capogrossi: HMGB1 Attenuates Cardiac Remodelling in the Failing Heart via Enhanced Cardiac Regeneration and miR-206-Mediated Inhibition of TIMP-3. *PLoS One*, 6(6), e19845 (2011)

163. A. Rossini, A. Zacheo, D. Mocini, P. Totta, A. Facchiano, R. Castoldi, P. Sordini, G. Pompilio, D. Abeni, M. C. Capogrossi and A. Germani: HMGB1-stimulated human primary cardiac fibroblasts exert a paracrine action on human and murine cardiac stem cells. *J Mol Cell Cardiol*, 44(4), 683-93 (2008)

164. D. Safer, M. Elzinga and V. T. Nachmias: Thymosin beta 4 and Fx, an actin-sequestering peptide, are indistinguishable. *J Biol Chem*, 266(7), 4029-32 (1991)

165. I. Bock-Marquette, A. Saxena, M. D. White, J. M. Dimaio and D. Srivastava: Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature*, 432(7016), 466-472 (2004)

166. N. Sopko, Y. Qin, A. Finan, A. Dadabayev, S. Chigurupati, J. Qin, M. S. Penn and S. Gupta: Significance of thymosin beta4 and implication of PINCH-1-ILK-alpha-

parvin (PIP) complex in human dilated cardiomyopathy. *PLoS One*, 6(5), e20184 (2011)

167. D. Srivastava, A. Saxena, J. Michael Dimaio and I. Bock-Marquette: Thymosin beta4 is cardioprotective after myocardial infarction. *Ann N Y Acad Sci*, 1112, 161-70 (2007)

168. S. Shrivastava, D. Srivastava, E. N. Olson, J. M. DiMaio and I. Bock-Marquette: Thymosin beta4 and cardiac repair. *Ann N Y Acad Sci*, 1194, 87-96 (2010)

169. M. E. Mayorga and M. S. Penn: miR-145 is differentially regulated by TGFbeta1 and ischemia and targets disabled-2 expression and wnt/beta-catenin activity. *J Cell Mol Med* (2011)

Key Words: Cardiovascular Disease, regenerative Medicine, Endogenous Stem Cells, Exogenous Stem Cells, Cell Therapy, Review

Send correspondence to: Marc Penn, Director of Research, Summa Cardiovascular Institute, Summa Health System, Professor of Medicine and Integrative Medical Sciences, Northeast Ohio Medical University, Tel: 216-835-8503, Fax: 866-299-7071, E-mail: mpenn2@neomed.edu