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PRE-TRANSPLANTATION SPECIFICATION OF STEM CELLS TO CARDIAC LINEAGE FOR REGENERATION OF CARDIAC TISSUE

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Abstract

Myocardial infarction (MI) is a lead cause of mortality in the Western world. Treatment of acute MI is focused on restoration of antegrade flow which inhibits further tissue loss, but does not restore function to damaged tissue. Chronic therapy for injured myocardial tissue involves medical therapy that attempts to minimize pathologic remodeling of the heart. End stage therapy for chronic heart failure (CHF) involves inotropic therapy to increase surviving cardiac myocyte function or mechanical augmentation of cardiac performance. Not until the point of heart transplantation, a limited resource at best, does therapy focus on the fundamental problem of needing to replace injured tissue with new contractile tissue. In this setting, the potential for stem cell therapy has garnered significant interest for its potential to regenerate or create new contractile cardiac tissue. While to date adult stem cell therapy in clinical trials has suggested potential benefit, there is waning belief that the approaches used to date lead to regeneration of cardiac tissue. As the literature has better defined the pathways involved in cardiac differentiation, preclinical studies have suggested that stem cell pretreatment to direct stem cell differentiation prior to stem cell transplantation may be a more efficacious strategy for inducing cardiac regeneration. Here we review the available literature on pre-transplantation conditioning of stem cells in an attempt to better understand stem cell behavior and their readiness in cell-based therapy for myocardial regeneration.

Keywords

Stem cells; mesenchymal stem cells; in vitro lineage specification; myocardium infarct; cardiac differentiation; cardiac tissue recovery

The promise of stem cell-based therapy

The success in treatments for a variety of acute conditions has led to the development of a number of unmet clinical needs of patients with chronic end organ dysfunction. The ultimate goal of the treatment of end organ dysfunction is either to prevent the end organ dysfunction initially, or to regenerate the lost tissue to restore function. The increased interest in regeneration and stem cells (SC) during the last decades has suggested the feasibility of stem cell strategies to potentially regenerate lost tissue and repair end-organ function 14,15. It is

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necessary to optimize the generation of end-organ cells for the minimization of oncogenic potential 20 and therapeutic success 22, whether these approaches invoke the use of adult stem cells (ASC), embryonic stem cells (ESC), or induced pluripotent stem cells (iPS) strategies.

ESC are generated from the inner cell mass of the blastocyst, early after fertlization. ESC are totipotent and able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm 20. ESC can be maintained in their totipotent state when cultured under specific conditions such as the presence of a mouse embryonic feeder layer or culture in the presence of Leukemia Inhibitory Factor. However, when these support elements are removed, ESC spontaneously differentiate. This differentiation process has the potential to be manipulated by the addition of growth factors, morphogens, or gene transfection to direct the cells toward a certain lineage.

ASC are defined as undifferentiated progenitor cells from an individual after embryonic development 25. Multiple tissues have been shown to contain organ specific progenitor cells 26. In the heart, the cells have been shown to be Islet 1+ or c-kit+ and expressing cardiac myosin.27,28 Whether these progenitor cells are endogenous to the organ itself or ultimately derived from the bone marrow is still unclear 29,30. While these stem cells appear be mobilized to areas of tissue injury it is yet to be demonstrated whether they are capable of differentiating into sustained mature end-organ cells without some level of manipulation or intervention 29,31.

iPS, on the other hand, have recently arisen as promising alternatives to ESC without the ethical concerns. iPS are pluripotent stem cells artificially derived from adult somatic cells which have been induced to express a gene expression profile characteristic of embryonic stem cell in response to genetic engineering 32–35. iPS are thought to be potentially therapeutically equivalent to embryonic stem cells in many respects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability, but the full extent of their potential and possible toxicity are still being assessed 32,36.

Clinically the most studied form of stem cell therapy is the use of ASC. The most commonly implemented source of ASC to date is whole bone marrow transplantation for the treatment of blood borne malignancies 37. This treatment is widely accepted and results in the regeneration of the bone marrow. While successful, it is noteworthy for the purposes of this review that the stem cells used for this purpose do not require differentiation into other forms of tissue, as bone marrow transplantation uses donor adult hematopoietic stem cells to replace host hematopoietic stem cells. Thus, one can hypothesize that for other applications of stem cell therapy to be successful; we may need to effectively and efficiently differentiate stem cell populations into the end organ cells of interest.

Stem cell therapy for cardiac diseases

There are over 1 million patients with acute myocardial infarction (AMI) and over 400,000 new cases of chronic heart failure (CHF) annually in the United States. Our relative inability to significantly improve the outcomes in patients with CHF has led many to assess the ability of stem cell therapy to prevent and treat cardiac dysfunction 38,39. There is growing evidence that the infusion of ASC at the time of AMI and CHF is unlikely to lead to regeneration of myocardial tissue 40–43. Rather, the majority of data to date would suggest that the mechanism(s) of benefit seen are due to preservation of cardiac myocytes and revascularization of myocardial tissue. Ultimately to achieve true replacement of damaged myocardial tissue, we will need to achieve regeneration of endothelial cells, smooth muscle

cells and cardiac myocytes. While the regeneration of endothelial cells and smooth muscle cells, derived from either bone marrow derived progenitor cells or cardiac stem cells, seems well within our grasp, the ability to regenerate cardiac myocytes still remains to be achieved. Regeneration of cardiac myocytes requires the generation of cells with sarcomeric organization, the ability to contract and coordinate beating with the surrounding tissue. To date, these characteristics have been reached only by a small percentage of transplanted stem cells or cells derived from ESC. 3,4

Clinical Stem Cell Trials for the Prevention and Treatment of Cardiac Dysfunction

The majority of clinical trials published to date have used whole bone marrow derived mononuclear cells (BMMNC). This population of cells includes endothelial progenitor cells (CD34+, CD133+) and mesenchymal stem cells. Clinical populations of interest have included patients with recent (within days) AMI or CHF. It is noteworthy that studies in patients with AMI and chronic angina, benefit has been shown from both BMMNC and more selective CD34+ cells; whereas, only BMMNC have demonstrated benefit in patients with CHF 44–54. A variation in homing ability may account for this difference as the paracrine factors necessary for the ability of different stem cell populations to migrate and engraft in injured verses remodeled tissue remains unclear.43,55 That said, it is clear that there are important differences between newly injured tissue and chronically remodeled tissue that will need to be addressed.56,57

While the presence of potential clinical benefit has been observed in some but not all of the clinical trials to date,51,58,59 the lack of myocardial regeneration from bone marrow derived mononuclear preparations has led others to study different populations of stem cells (for additional review of the findings of clinical cardiac stem cell trials see (60–63)). The multipotent adult progenitor cell is a bone marrow derived stem cell that has been shown to have the ability to differentiate into end organ cells of all three germ layers. While these cells may offer significant benefit and are under investigation in a Phase I clinical trial in patients with AMI, there is no compelling data that would suggest they can regenerate myocardium 64,65. Similarly, we and others have studied the potential use of umbilical cord blood stem cells as a non-controversial population of stem cell that could potentially regenerate myocardial tissue. While these cells too have shown benefit in preclinical models, there is no evidence that they can differentiate into cardiac myocytes 66.

Strategies of Generating Cardiac Myocytes

The inability to consistently derive cardiac myocytes form adult stem cells has led many to focus on the innate ability of embryonic stems cells (ESC), and more recently iPS cells, to differentiate into cardiac myocytes. Using the hanging drop method of ESC differentiation, a small mass of cells will spontaneously differentiate into cardiac myocytes and begin to beat. In an attempt to purify the cardiac myocytes generated from this strategy, ESC have been transfected with plasmid constructs encoding fluorescent markers or antibiotic resistance under control of the cardiac specific promoters 67–69. While these approaches have allowed for the efficient selection of newly generated cardiac myocytes, they do not increase the efficiency of cardiac myocyte generation, nor are they necessarily scalable to allow for use in clinical populations.

The cardiac differentiation potential of iPS is currently under intense investigation as a patient’s own somatic cells could be isolated and manipulated to possess pluripotency. Employing the same embryoid body formation method as is used with ESC, iPS can generate functional cardiac myocytes which contract, express cardiac lineage markers, and

display accurate electrophysiological properties 70. Selection for the mesodermal marker Flk-1 (vascular endothelial growth factor receptor-2) from differentiating iPS and then a further coculture with OP9 cells results in the induction of cardiac myocytes 71. These myocytes have also been demonstrated to be functionally and structurally similar to those derived from ESC but the yield is still low.

Pre-committing of stem cells for cardiac specification

A number of cells of different origins have been implemented for transplantation in infarcted cardiac tissue, including embryonic and neonatal cardiac myocytes, and cardiac myocyte cell lines as well as skeletal myoblasts, also from adult and perinatal sources, and established myoblast cell lines 72–74. Among these cell types, fetal and early postnatal cardiac myocytes have shown to effectively engraft, *in situ* coordinated beating and improvement in cardiac function 72,74–76. Studies comparing the arrhythmogenic effects of skeletal myoblasts and mesenchymal stem cells have demonstrated the arrhythmogenic potential of skeletal myoblasts and the importance of having connexin protein expression in vivo in order to minimize arrhythmogensis.31,56,77,78 The main caveat of the research performed with cardiac myocytes of embryonic origin is the graft rejection as the cells have to be isolated from allogeneic specimens. This barrier could be theoretically overcome by therapeutic cloning, ultimately though, the use of embryonic cardiac myocytes for human therapies is unlikely, at least in the near-term, due to the limiting sources and ethical implications of such a cell type.

Pre-transplantation differentiation of stem cells to cardiomyocytes

The lack of myocardial regeneration by ASC and the inefficient generation of cardiac myocytes from ESC has led to the concept that to achieve myocardial regeneration, manipulation of the cells prior to transplantation will be required 79,80. This could include: treating cells with small molecules or proteins to induce cardiac protein expression; cell based gene therapy 55,81,82 with transient or stable transfection of transplanted cells with siRNA or expression constructs; or co-transplantation of cells with cells engineered to express proteins capable of directing differentiation in vivo 3,12,83–85.

Precommitting Embryonic Stem Cells

Pre-transplantation conditioning/specification of cells to the cardiac phenotype has been widely explored in studies with ESC. The induction of spontaneous beating in vitro of ESC cultured as embryoid bodies with the addition of members of the transforming growth factor family proteins (TGFβ1, BMPs) appears to be a common approach. Coordinated beating areas in the cultures are then isolated, characterized for the expression of cardiac genes, and used for transplantation 67,86,87.

The in vitro generation of cardiogenic cells by this method was first detailed by Klug et al 67. In this early transplantation report, the authors generated an enriched culture of cardiac myocyte-like cells from mouse ESC by the utilizing the above mentioned methodology and then selecting the cells through antibiotic resistance driven by a cardiac promoter. These cardiac pre-conditioned ESC engrafted and integrated into the host heart effectively and were observed in the tissue up to 7 weeks after transplantation. Heart function was not assessed in this study. Importantly, while undifferentiated ESC form teratomas following transplantation into the heart, partially or fully differentiated ESC have not been shown to form teratomas following engraftment into the heart.88

Later, Kehat at al biochemically characterized the cardiac phenotype of ESC derived cells by the analysis of ultrastructural sarcomeric formation and electrophysiology in response to

calcium currents 86. The same methodology was then used in transplantation reports by Yang et al. 89. In this work, Yang and coworkers selected spontaneously beating ESC in vitro after several days in culture and, after transplantation, found improved cardiac function and cardiac tissue recovery in an experimental model of MI. Furthermore, overexpression of vascular endothelial growth factor in these cells increased cardiac function and capillary density demonstrating the potential improvements seen with cell based gene therapy.

Some of the most relevant studies on ESC cardiac pre-conditioning before transplantation are summarized in Table 1. A noticeable work was performed by Kehat et al where the electrophysiology coupling of human ESC-derived cardiac myocytes (ESC-CM) is completely dissected with *in vitro* cocultures of these cells with rat postnatal cardiac myocytes and *in vivo* after transplantation in animal models of cardiac dysfunction 90.

LaFlamme et al (2007) took a two-tiered approach to ESC based therapy of acute MI by preconditioning the cells initially toward cardiac lineage and then treating the cells with a pro-survival cocktail 3. The directed differentiation of hESC by treatment with BMP4 and Activin increased the yield of myocytes to 30%. The prosurvival cocktail markedly increased cell engraftment and survival. This combination of treatments greatly augmented remuscularization and cardiac function.

It is also important to consider the xenogeneic transplantation studies of Menard at al. 7. Mouse ESC-CM were transplanted into infarcted sheep hearts with or without immunosuppression. ESC-CM were found engrafted in the host heart tissue and cardiac function was improved as determined by left ventricular ejection in both immunocompetent and immunosuppresed conditions. The implications of this work are of great interest for clinical approaches since the procedures were performed in a large clinically relevant preclinical model and provide evidences on the immunological privileges of ESC and their progeny. Whether the immune privilege of ESC extends to humans is unclear as recent studies have demonstrated that human ESC do not survive in a xenogenic model.91

While in vivo studies are of critical, significant advances regarding cardiac regeneration have been demonstrated using in vitro systems. The majority of studies assess the induction of cardiac lineage in ESC by antigenic detection regulated by miroRNAs; overepxression of the transcription factors MesP1, Mef2c and Nkx2.5, and GATA 4 or exposure to retinoic acid 84,92–95. It will be important to demonstrate in the future that these pretreatments not only increase the yield but also have the capacity of differentiated cells to function as end organ cardiomyocytes.

Pre-transplantation differentiation of Adult stem cells to cardiomyocytes

ASC pre-transplantation specification to the cardiac phenotype has received some attention, although not in the clinical realm. To date one could suggest that the ASC type showing the most potential for cardiomyogenic differentiation are stem cells derived from bone marrow – particularly mesenchymal stem cells. The multipotentiality of BMSC has been extensively studied and the potential for differentiation towards a cardiac phenotype has been suggested by some 96–98. Bone marrow derived stem cells include hematopoietic stem cells (HSC), which generate white and red blood cells and platelets, and mesenchymal stem cells (MSC), which generate a wide repertoire of cells of mesodermal origin. These include: Bone, cartilage, skeletal muscle, tendon, ligament, marrow stroma, fat and possibly cardiac myocytes 99. Importantly in the interest of cardiac transplantation, MSC have been shown to express basal levels of major cardiac proteins such as cardiac myosin, actinin and others 100. These cardiogenic features along with the ability to produce large amounts mesenchymal stem cells *in vitro* from bone marrow aspirates has fueled increased focus for their use in transplantation.

In general, injection of MSC in infarcted hearts results in MSC engraftment and survival after long periods of time 42,83,101–103. Cardiac function improvement has also been observed in key reports 21,42,83,101,102. MSC most likely have high levels of engraftment due to their capacity to readily occupy the infarcted tissue before cardiac fibroblast do, avoiding fibrotic scar formation and cardiac function degeneration over time 104. Engrafted MSC secrete survival factors that in turn maintain remaining cardiac myocytes viability and induce endogenous cardiac stem cells to differentiate and recover lost tissue/function 105. However, cardiac differentiation of MSC integrated into the infarcted cardiac tissue, in terms of sarcomeric organization and coordinated beating has not been reported to date. In general, as judged by MSC transplantation reports and early clinical trials, it is evident that the ability of these cells to induce therapeutic benefit may need to be enhanced and the key for this improvement may rely on pre-transplantation specification of MSC to the cardiac lineage.

Interestingly, there are a limited number of publications about pre-transplantation specification of MSC as a possible approach to enhance cardiac differentiation after engraftment. Early on, Tomita et al. investigated bone marrow derived cells with induced cardiogenic characteristics *in vitro* prior to transplantation 106,107. This particular study explored the in vitro differentiation of bone marrow stem cells to cardiogenic cells in response to growth factors (TGF-β1 or insulin) or the anti-cancer chemical 5-azacytidine before transplantation into cryoinjured-infarcted hearts. According to the authors, cardiac protein expression and myotubule formation, both characteristics of cardiac differentiation of bone marrow stem cells, was only observed in cells exposed to 5-azacytidine for 24 hrs and analyzed 7 days later. Furthermore, rats transplanted with 5-azacytidine-pre-treated cells showed cardiac function improvements but not the corresponding controls (untreated cells) or the MSC exposed to growth factors. From this first report on *in vitro* stem cell preconditioning and transplantation in infarcted hearts, it has become evident that limiting the stem cells (embryonic or adult) multipotentiality *in vitro* before transplantation by inducing transdifferentiation or specification to the cardiac myocyte lineage may enhance stem cell behavior towards effective integration in the heart tissue and subsequent heart function improvement.

5-azacytidine effects on stem cell cardiac differentiation have been reported in ESC as well as in ASC 108. The discovery of the cardiogenic induction capabilities of 5-azacytidine on MSC was first reported by Wakitani et al. who described myotubule formation and sudan-black nuclear staining in MSCs exposed to the drug, as indications of cardiac differentiation 109. Later studies by several groups confirmed the cardiogenic inducing potential of this drug and encouraged studies on the transplantation of preconditioned cells 19,110–112. While these biologically important studies have enhanced our understanding of stem cell differentiation and the potential for therapeutic benefit of stem cell preconditioning, unfortunately cells pre-exposed to such agents can not, for safety reasons, be transplanted to clinical populations 113,114.

In a recent publication pre-treatment of MSC with a combination of growth factors, basic fibroblast growth factor, insulin growth factor-1 and bone morphgenic protein-2 (FGF2/IGF-1/BMP2, respectively) induced cardiac protein expression and antiapoptotic signals in adjacent cardiac myocytes in vitro. Survival signals from MSC to cardiac myocytes appear to be mediated by cell-to-cell communication as determined by conexin-43 involvement in the process. These findings acquire clinical relevance when transplantation of growth factor pre-treated MSC the time of AMI induced by ligation of left ventricular artery, showed salvage of infarcted cardiac tissue and cardiac function improvement determined by shortening fraction 83. This study detailed the importance of gap junction proteins such as conexin-43, which has enhanced expression in hearts transplanted with growth factor pre-

treated MSC. The growth factors used in this particular study (FGF2/IGF-1/BMP2) have been implicated in several distinctly different aspects of cardiogenesis during the heart development. Interestingly, their combinatorial effect on MSC results in *in vitro* cardiac commitment of MSC. Since the studies performed in this report were carried out in relative short times after MI, it remains unknown whether the mechanisms of inducing survival of host cardiac myocytes in MSC transplanted hearts will have long-term benefits in clinical populations.

Another member of the transforming growth factor (TGFβ) family proteins closely linked to cardiac tissue formation is TGFβ1 115. This pleiotropic growth factor has also been used in cardiac differentiation studies in ESC and in MSC pre-conditioning 24,116,117. Enhanced expression of cardiac proteins such as Troponin-I and conexin-43 after 7 days of treatment with TGFβ1 was observed in immunogenic selected MSC expressing the oncogen c-Kit also known as CD117. Li et al., demonstrated that CD117+ cells transplanted into infarcted hearts improve cardiac function determined by cardiac wall thickness and shortening fraction.

A relevant aspect of this study is the inhibition of fibrosis in hearts receiving TGFβ1-pre-conditioned MSC as far as 90 days post-MI. Augmented wall thickness and decreased fibrosis suggest benefits following MSC engraftment and survival in the host tissue. It is of interest the fact that the authors selected CD117+ cells for this study. This marker defines the bone marrow cells as undifferentiated or lineage-negative cells showing highly proliferative capabilities 118. No experiments were performed with mixed bone marrow cell populations or only MSC cultures which could enhance the cardiogenic potential of the cells to be transplanted. Selection of cells from a variable population such as MSC could make the time of the procedure from bone marrow aspirates to transplantation inconveniently long, potentially decreasing the clinical value of the system particularly in patients with acute myocardial infarction prior to scarring.

Summary

It is clear that there is a significant unmet clinical need driving the development of cell based therapies for the prevention and treatment of cardiac dysfunction. With that goal in mind, a truly amazing level of knowledge has been amassed through initially preclinical and more recently clinical studies on the mechanisms and clinical potential of stem cell based myocardial repair. While there is evidence of some benefit to date 51, it is quite clear that we have not achieved our ultimate goal of replacing lost contractile tissue with functioning new cardiac myocytes. It would appear that simply placing stem cells that may have cardiac myocyte potential in the newly or remotely injured myocardial environment does not appear sufficient to direct cardiac myocyte differentiation. If so, and the preponderance of data to date would support that conclusion, then stem cells will need to be pre-conditioned to direct differentiation down a cardiac fate. This preconditioning will either induce cardiac differentiation as in ASC, or lead to more efficient cardiac myocyte development as in the case of ESC and iPS. Regardless, it is clear that unraveling the pathways responsible for stem cell transdifferentiation and cardiac differentiation is critically important at this juncture of the development of cell based therapies for the heart. The development of practical strategies either in the form of small molecules or gene therapy that allow for preconditioning and commitment of stem cells to a cardiac fate may be the next significant step forward in improving the outcomes of patients with heart disease.

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Literature Cited

1. Lai ZF, Chen YZ, Feng LP, et al. Overexpression of TNNI3K, a cardiac-specific MAP kinase, promotes P19CL6-derived cardiac myogenesis and prevents myocardial infarction-induced injury. Am J Physiol Heart Circ Physiol. 2008 August; 295(2):H708–H716. [PubMed: 18552163]
2. Rajasingh J, Bord E, Hamada H, et al. STAT3-dependent mouse embryonic stem cell differentiation into cardiomyocytes: analysis of molecular signaling and therapeutic efficacy of cardiomyocyte precommitted mES transplantation in a mouse model of myocardial infarction. Circ Res. 2007 October 26; 101(9):910–8. [PubMed: 17823373]
3. Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007 September; 25(9):1015–24. [PubMed: 17721512]
4. Tomescot A, Leschik J, Bellamy V, et al. Differentiation in vivo of cardiac committed human embryonic stem cells in postmyocardial infarcted rats. Stem Cells. 2007 September; 25(9):2200–5. [PubMed: 17540853]
5. Behfar A, Perez-Terzic C, Faustino RS, et al. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. J Exp Med. 2007 February 19; 204(2):405–20. [PubMed: 17283208]
6. Kofidis T, Lebl DR, Swijnenburg RJ, Greeve JM, Klima U, Robbins RC. Allopurinol/uricase and ibuprofen enhance engraftment of cardiomyocyte-enriched human embryonic stem cells and improve cardiac function following myocardial injury. Eur J Cardiothorac Surg. 2006 January; 29(1):50–5. [PubMed: 16337396]
7. Menard C, Hagege AA, Agbulut O, et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. Lancet. 2005 September 17; 366(9490):1005–12. [PubMed: 16168783]
8. Kofidis T, de Bruin JL, Yamane T, et al. Stimulation of paracrine pathways with growth factors enhances embryonic stem cell engraftment and host-specific differentiation in the heart after ischemic myocardial injury. Circulation. 2005 May 17; 111(19):2486–93. [PubMed: 15883216]
9. Kofidis T, de Bruin JL, Yamane T, et al. Insulin-like growth factor promotes engraftment, differentiation, and functional improvement after transfer of embryonic stem cells for myocardial restoration. Stem Cells. 2004; 22(7):1239–45. [PubMed: 15579642]
10. Mazo M, Planat-Benard V, Abizanda G, et al. Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. Eur J Heart Fail. 2008 May; 10(5):454–62. [PubMed: 18436478]
11. Hahn JY, Cho HJ, Kang HJ, et al. Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. J Am Coll Cardiol. 2008 March 4; 51(9):933–43. [PubMed: 18308163]
12. Grauss RW, van TJ, Steendijk P, et al. Forced myocardin expression enhances the therapeutic effect of human mesenchymal stem cells after transplantation in ischemic mouse hearts. Stem Cells. 2008 April; 26(4):1083–93. [PubMed: 18203678]
13. Plotnikov AN, Shlapakova I, Szabolcs MJ, et al. Xenografted adult human mesenchymal stem cells provide a platform for sustained biological pacemaker function in canine heart. Circulation. 2007 August 14; 116(7):706–13. [PubMed: 17646577]
14. Markel TA, Crisostomo PR, Manukyan MC, et al. Are Neonatal Stem Cells as Effective as Adult Stem Cells in Providing Ischemic Protection? J Surg Res. 2008 April 28.
15. Schierling W, Kunz-Schughart LA, Muders F, Riegger GA, Griese DP. Fates of genetically engineered haematopoietic and mesenchymal stem cell grafts in normal and injured rat hearts. J Tissue Eng Regen Med. 2008 August; 2(6):354–64. [PubMed: 18618869]

16. Kruglyakov PV, Sokolova IB, Zin'kova NN, et al. In vitro and in vivo differentiation of mesenchymal stem cells in the cardiomyocyte direction. Bull Exp Biol Med. 2006 October; 142(4):503–6. [PubMed: 17415448]

17. Ventura C, Cantoni S, Bianchi F, et al. Hyaluronan mixed esters of butyric and retinoic Acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. J Biol Chem. 2007 May 11; 282(19):14243–52. [PubMed: 17363374]

18. Shiota M, Heike T, Haruyama M, et al. Isolation and characterization of bone marrow-derived mesenchymal progenitor cells with myogenic and neuronal properties. Exp Cell Res. 2007 March 10; 313(5):1008–23. [PubMed: 17289022]

19. Tomita Y, Makino S, Hakuno D, et al. Application of mesenchymal stem cell-derived cardiomyocytes as bio-pacemakers: current status and problems to be solved. Med Biol Eng Comput. 2007 February; 45(2):209–20. [PubMed: 17262204]

20. Martin GR. 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. 1981 December; 78(12): 7634–8. [PubMed: 6950406]

21. Bartunek J, Croissant JD, Wijns W, et al. Pretreatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. Am J Physiol Heart Circ Physiol. 2007 February; 292(2):H1095–H1104. [PubMed: 17056665]

22. Itskovitz-Eldor J, Schuldiner M, Karsenti D, et al. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med. 2000 February; 6(2):88–95. [PubMed: 10859025]

23. Yau TM, Li G, Zhang Y, Weisel RD, Mickle DA, Li RK. Vascular endothelial growth factor receptor upregulation in response to cell-based angiogenic gene therapy. Ann Thorac Surg. 2005 June; 79(6):2056–63. [PubMed: 15919309]

24. Li TS, Hayashi M, Ito H, et al. Regeneration of infarcted myocardium by intramyocardial implantation of ex vivo transforming growth factor-beta-preprogrammed bone marrow stem cells. Circulation. 2005 May 17; 111(19):2438–45. [PubMed: 15883211]

25. Hombach-Klonisch S, Panigrahi S, Rashedi I, et al. Adult stem cells and their trans-differentiation potential-perspectives and therapeutic applications. J Mol Med. 2008 July 16.

26. Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med. 2004 July; 8(3):301–16. [PubMed: 15491506]

27. Laugwitz KL, Moretti A, Lam J, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature. 2005 February 10; 433(7026):647–53. [PubMed: 15703750]

28. Oh H, Bradfute SB, Gallardo TD, et al. Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A. 2003 October 14; 100(21): 12313–8. [PubMed: 14530411]

29. Mouquet F, Pfister O, Jain M, et al. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. Circ Res. 2005 November 25; 97(11):1090–2. [PubMed: 16269652]

30. Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. J Clin Invest. 2003 July; 112(1):42–9. [PubMed: 12824456]

31. Unzek S, Zhang M, Mal N, Mills WR, laurita KR, Penn MS. SDF-1 recruits cardiac stem cell like cells that depolarize in vivo. Cell Transplant. 2007; 16:879–86. [PubMed: 18293886]

32. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 August 25; 126(4):663–76. [PubMed: 16904174]

33. Blelloch R, Venere M, Yen J, Ramalho-Santos M. Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell. 2007 September 13; 1(3):245–7. [PubMed: 18371358]

34. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007 July 19; 448(7151):313–7. [PubMed: 17554338]

35. Wernig M, Meissner A, Foreman R, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature. 2007 July 19; 448(7151):318–24. [PubMed: 17554336]

36. Nishikawa S, Goldstein RA, Nierras CR. The promise of human induced pluripotent stem cells for research and therapy. Nat Rev Mol Cell Biol. 2008 September; 9(9):725–9. [PubMed: 18698329]
37. Mathe G, Amiel JL, Schwarzenberg L, Cattan A, Schneider M. Haematopoietic chimera in man after allogeneic bone-marrow transplantation. Br Med J. 1963 December 28; 2(5373):1633–5. [PubMed: 14066188]
38. Tateishi K, Takehara N, Matsubara H, Oh H. Stemming heart failure with cardiac- or reprogrammed-stem cells. J Cell Mol Med. 2008 August 27.
39. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. Nature. 2008 February 21; 451(7181): 937–42. [PubMed: 18288183]
40. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature. 2004 April 8; 428(6983):664–8. [PubMed: 15034593]
41. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature. 2004 April 8; 428(6983):668–73. [PubMed: 15034594]
42. Zhang M, Mal N, kiedrowski M, et al. SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes following myocardial infarction. FASEB J. 2007; 21:3197–207. [PubMed: 17496162]
43. Charwat S, Gyongyosi M, Lang I, et al. Role of adult bone marrow stem cells in the repair of ischemic myocardium: current state of the art. Exp Hematol. 2008 June; 36(6):672–80. [PubMed: 18358589]
44. Losordo DW, Schatz RA, White CJ, et al. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. Circulation. 2007 June 26; 115(25):3165–72. [PubMed: 17562958]
45. Meluzin J, Janousek S, Mayer J, et al. Three-, 6-, and 12-month results of autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction. Int J Cardiol. 2008 August 18; 128(2):185–92. [PubMed: 17764767]
46. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation. 2002 December 10; 106(24):3009–17. [PubMed: 12473544]
47. bdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med. 2007 May 28; 167(10):989–97. [PubMed: 17533201]
48. Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet. 2004 July 10; 364(9429):141–8. [PubMed: 15246726]
49. Chen SL, Fang WW, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol. 2004 July 1; 94(1):92–5. [PubMed: 15219514]
50. Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med. 2006 September 21; 355(12):1199–209. [PubMed: 16990383]
51. Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 2006 September 21; 355(12):1210–21. [PubMed: 16990384]
52. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet. 2003 January 4; 361(9351):47–9. [PubMed: 12517468]
53. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure. Circulation. 2003 April 21.
54. Beeres SL, Bax JJ, bbets-Schneider P, et al. Sustained effect of autologous bone marrow mononuclear cell injection in patients with refractory angina pectoris and chronic myocardial ischemia: twelve-month follow-up results. Am Heart J. 2006 October; 152(4):684–6. [PubMed: 16996834]

55. Penn MS, Khalil MK. Exploitation of stem cell homing for gene delivery. Expert Opin Biol Ther. 2008 January; 8(1):17–30. [PubMed: 18081534]
56. Deglurkar I, Mal N, Mills WR, et al. Mechanical and electrical effects of cell-based gene therapy for ischemic cardiomyopathy are independent. Hum Gene Ther. 2006 November; 17(11):1144–51. [PubMed: 17069537]
57. Askari A, Unzek S, Popovic ZB, et al. Effect of stromal-cell-derived factor-1 on stem cell homing and tissue regeneration in ischemic cardiomyopathy. Lancet. 2003; 362:697–703. [PubMed: 12957092]
58. Assmus B, Honold J, Schachinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. N Engl J Med. 2006 September 21; 355(12):1222–32. [PubMed: 16990385]
59. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet. 2006 January 14; 367(9505):113–21. [PubMed: 16413875]
60. Penn MS. Stem-cell therapy after acute myocardial infarction: the focus should be on those at risk. Lancet. 2006 January 14; 367(9505):87–8. [PubMed: 16413856]
61. Yamahara K, Nagaya N. Stem cell implantation for myocardial disorders. Curr Drug Deliv. 2008 July; 5(3):224–9. [PubMed: 18673267]
62. Cogle CR, Madlambayan GJ, Hubsher G, et al. Marrow cell therapies for cardiovascular diseases. Exp Hematol. 2008 June; 36(6):687–94. [PubMed: 18375038]
63. Dimmeler S, Burchfield J, Zeiher AM. Cell-based therapy of myocardial infarction. Arterioscler Thromb Vasc Biol. 2008 February; 28(2):208–16. [PubMed: 17951319]
64. Pelacho B, Nakamura Y, Zhang J, et al. Multipotent adult progenitor cell transplantation increases vascularity and improves left ventricular function after myocardial infarction. J Tissue Eng Regen Med. 2007 January; 1(1):51–9. [PubMed: 18038392]
65. Van't HW, Mal N, Huang Y, et al. Direct delivery of syngeneic and allogeneic large-scale expanded multipotent adult progenitor cells improves cardiac function after myocardial infarct. Cytotherapy. 2007; 9(5):477–87. [PubMed: 17786609]
66. Leor J, Guetta E, Feinberg MS, et al. Human umbilical cord blood-derived CD133+ cells enhance function and repair of the infarcted myocardium. Stem Cells. 2006 March; 24(3):772–80. [PubMed: 16195418]
67. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embronic stem cells form stable intracardiac grafts. J Clin Invest. 1996 July 1; 98(1):216–24. [PubMed: 8690796]
68. Muller M, Fleischmann BK, Selbert S, et al. Selection of ventricular-like cardiomyocytes from ES cells in vitro. FASEB J. 2000 December; 14(15):2540–8. [PubMed: 11099473]
69. Huber I, Itzhaki I, Caspi O, et al. Identification and selection of cardiomyocytes during human embryonic stem cell differentiation. FASEB J. 2007 August; 21(10):2551–63. [PubMed: 17435178]
70. Mauritz C, Schwanke K, Reppel M, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. Circulation. 2008 July 29; 118(5):507–17. [PubMed: 18625890]
71. Narazaki G, Uosaki H, Teranishi M, et al. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. Circulation. 2008 July 29; 118(5):498–506. [PubMed: 18625891]
72. Koh GY, Soonpaa MH, Klug MG, et al. Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs. J Clin Invest. 1995 October; 96(4):2034–42. [PubMed: 7560097]
73. Koh GY, Soonpaa MH, Klug MG, Field LJ. Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. Am J Physiol. 1993 May; 264(5 Pt 2):H1727–H1733. [PubMed: 8498586]
74. Matsushita T, Oyamada M, Kurata H, et al. Formation of cell junctions between grafted and host cardiomyocytes at the border zone of rat myocardial infarction. Circulation. 1999 November 9; 100(19 Suppl):II262–II268. [PubMed: 10567314]
75. Li RK, Mickle DA, Weisel RD, et al. Natural history of fetal rat cardiomyocytes transplanted into adult rat myocardial scar tissue. Circulation. 1997 November 4.96(9 Suppl):II-86.

76. Hodgson DM, Behfar A, Zingman LV, et al. Stable benefit of embryonic stem cell therapy in myocardial infarction. Am J Physiol Heart Circ Physiol. 2004 August; 287(2):H471–H479. [PubMed: 15277190]
77. Mills WR, Mal N, Forudi F, Popovic ZB, Penn MS, laurita KR. Optical mapping of late myocardial infarction in rats. Am J Physiol Heart Circ Physiol. 2006 March; 290(3):H1298–H1306. [PubMed: 16214848]
78. Mills WR, Mal N, Kiedrowski MJ, et al. Stem cell therapy enhances electrical viability in myocardial infarction. J Mol Cell Cardiol. 2007 October 26.42(304):314.
79. Faustino RS, Behfar A, Perez-Terzic C, Terzic A. Genomic chart guiding embryonic stem cell cardiopoiesis. Genome Biol. 2008 January 9.9(1):R6. [PubMed: 18184438]
80. Arrell DK, Niederlander NJ, Faustino RS, Behfar A, Terzic A. Cardioinductive network guiding stem cell differentiation revealed by proteomic cartography of tumor necrosis factor alpha-primed endodermal secretome. Stem Cells. 2008 February; 26(2):387–400. [PubMed: 17991915]
81. Penn MS. Cell-based gene therapy for the prevention and treatment of cardiac dysfunction. Nat Clin Pract Cardiovasc Med. 2007 February.4 Suppl 1:S83–S88. [PubMed: 17230220]
82. Penn MS, Mangi AA. Genetic enhancement of stem cell engraftment, survival, and efficacy. Circ Res. 2008 June 20; 102(12):1471–82. [PubMed: 18566313]
83. Hahn JY, Cho HJ, Kang HJ, et al. Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. J Am Coll Cardiol. 2008 March 4; 51(9):933–43. [PubMed: 18308163]
84. Ivey KN, Muth A, Arnold J, et al. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. Cell Stem Cell. 2008 March 6; 2(3):219–29. [PubMed: 18371447]
85. Bian J, Popovic ZB, Benejam C, kiedrowski M, Rodriguez LL, Penn MS. Effect of cell-based intercellular delivery of transcription factor GATA4 on ischemic cardiomyopathy. Circ Res. 2007 June 8; 100(11):1626–33. [PubMed: 17495229]
86. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest. 2001 August 1; 108(3):407–14. [PubMed: 11489934]
87. Capi O, Gepstein L. Myocardial regeneration strategies using human embryonic stem cell-derived cardiomyocytes. J Control Release. 2006 November 28; 116(2):211–8. [PubMed: 17005287]
88. Laflamme MA, Gold J, Xu C, et al. Formation of human myocardium in the rat heart from human embryonic stem cells. Am J Pathol. 2005 September; 167(3):663–71. [PubMed: 16127147]
89. Yang Y, Min JY, Rana JS, et al. VEGF enhances functional improvement of postinfarcted hearts by transplantation of ESC-differentiated cells. J Appl Physiol. 2002 September; 93(3):1140–51. [PubMed: 12183512]
90. Kehat I, Khimovich L, Caspi O, et al. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. Nat Biotechnol. 2004 October; 22(10):1282–9. [PubMed: 15448703]
91. Swijnenburg RJ, Schrepfer S, Govaert JA, et al. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. Proc Natl Acad Sci U S A. 2008 September 2; 105(35):12991–6. [PubMed: 18728188]
92. Skerjanc IS, Petropoulos H, Ridgeway AG, Wilton S. Myocyte enhancer factor 2C and Nkx2-5 up-regulate each other's expression and initiate cardiomyogenesis in P19 cells. J Biol Chem. 1998 December 25; 273(52):34904–10. [PubMed: 9857019]
93. David R, Brenner C, Stieber J, et al. MesP1 drives vertebrate cardiovascular differentiation through Dkk-1-mediated blockade of Wnt-signalling. Nat Cell Biol. 2008 March; 10(3):338–45. [PubMed: 18297060]
94. Grepin C, Nemer G, Nemer M. Enhanced cardiogenesis in embryonic stem cells overexpressing the GATA-4 transcription factor. Development. 1997 June; 124(12):2387–95. [PubMed: 9199365]
95. Wobus AM, Kaomei G, Shan J, et al. Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes. J Mol Cell Cardiol. 1997 June; 29(6):1525–39. [PubMed: 9220339]

96. Ohnishi S, Ohgushi H, Kitamura S, Nagaya N. Mesenchymal stem cells for the treatment of heart failure. Int J Hematol. 2007 July; 86(1):17–21. [PubMed: 17675261]

97. Liao R, Pfister O, Jain M, Mouquet F. The bone marrow--cardiac axis of myocardial regeneration. Prog Cardiovasc Dis. 2007 July; 50(1):18–30. [PubMed: 17631435]

98. Burt RK, Loh Y, Pearce W, et al. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. JAMA. 2008 February 27; 299(8):925–36. [PubMed: 18314435]

99. Porada CD, Zanjani ED, meida-Porad G. Adult mesenchymal stem cells: a pluripotent population with multiple applications. Curr Stem Cell Res Ther. 2006 September; 1(3):365–9. [PubMed: 18220880]

100. Li X, Yu X, Lin Q, et al. Bone marrow mesenchymal stem cells differentiate into functional cardiac phenotypes by cardiac microenvironment. J Mol Cell Cardiol. 2007 February; 42(2):295–303. [PubMed: 16919679]

101. Schenk S, Mal N, Finan A, et al. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. Stem Cells. 2007 January; 25(1):245–51. [PubMed: 17053210]

102. Berry MF, Engler AJ, Woo YJ, et al. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. Am J Physiol Heart Circ Physiol. 2006 June; 290(6):H2196–H2203. [PubMed: 16473959]

103. Makkar RR, Price MJ, Lill M, et al. Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. J Cardiovasc Pharmacol Ther. 2005 December; 10(4):225–33. [PubMed: 16382259]

104. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg. 2002 June; 73(6):1919–25. [PubMed: 12078791]

105. Nagaya N, Kangawa K, Itoh T, et al. Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. Circulation. 2005 August 23; 112(8): 1128–35. [PubMed: 16103243]

106. Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. Cardiovasc Res. 2003 May 1; 58(2):336–50. [PubMed: 12757868]

107. Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation. 1999 November 9; 100(19 Suppl):II247–II256. [PubMed: 10567312]

108. Xu C, Police S, Rao N, Carpenter MK. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ Res. 2002 September 20; 91(6):501–8. [PubMed: 12242268]

109. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve. 1995 December; 18(12):1417–26. [PubMed: 7477065]

110. Fukuda K. Use of adult marrow mesenchymal stem cells for regeneration of cardiomyocytes. Bone Marrow Transplant. 2003 August.32 Suppl 1:S25–S27. [PubMed: 12931236]

111. Hakuno D, Fukuda K, Makino S, et al. Bone marrow-derived regenerated cardiomyocytes (CMG Cells) express functional adrenergic and muscarinic receptors. Circulation. 2002 January 22; 105(3):380–6. [PubMed: 11804996]

112. Rangappa S, Entwistle JW, Wechsler AS, Kresh JY. Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. J Thorac Cardiovasc Surg. 2003 July; 126(1):124–32. [PubMed: 12878947]

113. Cihak A. Effect of 5-azacytidine and its derivatives on gastric secretion. Pharmacol Ther. 1985; 28(1):103–6. [PubMed: 2414785]

114. Burlacu A. Can 5-azacytidine convert the adult stem cells into cardiomyocytes? A brief overview. Arch Physiol Biochem. 2006 October; 112(4–5):260–4. [PubMed: 17178600]

115. Xiao H, Zhang YY. Understanding the role of transforming growth factor-beta signalling in the heart: overview of studies using genetic mouse models. Clin Exp Pharmacol Physiol. 2008 March; 35(3):335–41. [PubMed: 18290874]

116. Lim JY, Kim WH, Kim J, Park SI. Involvement of TGF-beta1 signaling in cardiomyocyte differentiation from P19CL6 cells. Mol Cells. 2007 December 31; 24(3):431–6. [PubMed: 18182860]

117. Singla DK, Sun B. Transforming growth factor-beta2 enhances differentiation of cardiac myocytes from embryonic stem cells. Biochem Biophys Res Commun. 2005 June 24; 332(1): 135–41. [PubMed: 15896309]

118. Li TS, Hamano K, Nishida M, et al. CD117+ stem cells play a key role in therapeutic angiogenesis induced by bone marrow cell implantation. Am J Physiol Heart Circ Physiol. 2003 September; 285(3):H931–H937. [PubMed: 12915384]

Table 1

Stem Cell Type	Pretreatment	Species	Cell Status at Transplant	Delivery	Tracking	Heart Injury	Function Improvement	Cell Fate	Reference
P19CL6	Cardiac Troponin I- interacting kinase gene (TNNI3K) + DMSO	M/M	CM	CI	E	Occlusion	+	ND	1
ESC	LIF and BMP-2	M/M	CM	CI	V	Occlusion	+	CM, EC	2
ESC	Activin A and BMP-4	H/R	CM	CI	I	Occlusion	+	CM	3
ESC	BMP-2 and FGFR inhibitor	H/R	CM	CI	I	Occlusion	ND	CM	4
ESC	TGFB-1, BMP-2 and -4, activin-A, FGF-2 and -4, IGF-1 and -2, VEGF-A, EGF, TNFα	M/M	CM	CI	I	Normal	ND	CM	5
ESC	5-Azacytidine, allopurinol and ibuprofen orally	H/M	CM	CI	V	Occlusion	+	CM	6
ESC	LIF and BMP-2	M/S	CM	CI	E	Occlusion	+	CM	7
ESC	TGFbeta	M/M	SC	CI	E	Occlusion	+	CM	8
ESC	IGF-1	M/M	SC	CI	E	Occlusion	=	CM	9
ADSC	5-Azacytidine	M/R	CM	CI	T	Occlusion	E	UD	10
MSC	FGF-2, IGF-1, BMP-2	R/R	CM	CI	D	Occlusion	+	CM	11
MSC	Myocardin AdenoVirus	H/M	SC	CI	V	Occlusion	+	CM	12
MSC	HCN gene	H/D	SC	CI	E	Ablation	+	ND	13
MSC	5-Azacytidine	R/R	CM	IV	D	Occlusion	ND	CM	16
FMhMSCs	mixed ester of hyaluronan with butyric and retinoic acid	H/R	CM	CI	I	Occlusion	=	CM, EC	17
MPC	5-Azacytidine	M/M	CM	CI	V	Occlusion	+	CM	18
MSC	5-Azacytidine	M/M	CM	CI	Li, V	Normal	ND	CM	19
MSC	FGF-2, IGF-1, BMP-2	D/D	CM	CI	D	Occlusion	+	CM	21
BMC	VEGF, IGF-1 gene	R/R	CM	IV	CH	Occlusion	+	ND	23
BMC	TGF-beta	M/M	CM	CI	T	Occlusion	+	CM	24

Cell Type: ESC-embryonic stem cells; ADSC-adipose derived stem cells; MSC-mesenchymal stem cells; MPC-mesenchymal progenitor cells; FMhMSCs-fetal membranes of term placenta; BMC-bone marrow cells

Species: H-Human; M-Mouse; R-Rat; D-Dog

Cell status at transplant: CM-cardiomyocyte; SC-stem cell

Delivery: CI-cardiac injection; IV-intravenously

Tracking: E-electroporation; V-viral transfection; I-immunostain; T-transgenic; D-dye; Li-liposome gene delivery; CH-Y chromosome

Functional Improvement: +-improved; ND-not determined; =/-no improvement

Cell Fate: ND-not determined; CM-cardiomyocyte; EC-endothelial cell