Published in final edited form as:

J Mol Cell Cardiol. 2011 October ; 51(4): 619–625. doi:10.1016/j.yjmcc.2011.05.015.

Pharmacologic And Genetic Strategies To Enhance Cell Therapy For Cardiac Regeneration

Rosemeire M. Kanashiro-Takeuchi^a, Ivonne Hernandez Schulman^{a,b}, and Joshua M. Hare^a

^aInterdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL

^bNephrology-Hypertension Section, Miami Veterans Affairs Healthcare System, Miami, FL

Abstract

Cell-based therapy is emerging as an exciting potential therapeutic approach for cardiac regeneration following myocardial infarction (MI). As heart failure (HF) prevalence increases over time, development of new interventions designed to aid cardiac recovery from injury are crucial and should be considered more broadly. In this regard, substantial efforts to enhance the efficacy and safety of cell therapy are continuously growing along several fronts, including modifications to improve the reprogramming efficiency of inducible pluripotent stem cells (iPS), genetic engineering of adult stem cells, and administration of growth factors or small molecules to activate regenerative pathways in the injured heart. These interventions are emerging as potential therapeutic alternatives and/or adjuncts based on their potential to promote stem cell homing, proliferation, differentiation, and/or survival. Given the promise of therapeutic interventions to enhance the regenerative capacity of multipotent stem cells as well as specifically guide endogenous or exogenous stem cells into a cardiac lineage, their application in cardiac regenerative medicine should be the focus of future clinical research. This article is part of a Special Issue entitled 'Key Signaling Molecules Special Issue'.

Keywords

Chemokines; Growth Factors; Small molecules; Stem Cell; Cardiogenesis; Regeneration

1. Introduction

Despite substantial therapeutic advances over the past decade, heart failure (HF), due in large part to myocardial infarction (MI), affects approximately 5.8 million people in the United States and remains a leading cause of morbidity and mortality [1]. One of the most exciting new avenues for the 21st century treatment of chronic heart disease is regenerative medicine. Most focus on regenerative medicine is currently embodied by attempts to achieve cell-based tissue repair. Nevertheless, as this exciting field advances, it is worthwhile to

© 2011 Elsevier Ltd. All rights reserved.

Corresponding Author: Joshua M. Hare, M.D., Louis Lemberg Professor of Medicine, Professor of Biomedical Engineering and Cellular and Molecular, Pharmacology, Director, Interdisciplinary Stem Cell Institute, Biomedical Research Building, 1501 N.W. 10th Ave., Room 824 P.O Box 016960 (R125), Miami, FL 33101, Phone: 305-243-1999, Fax: 305-243-3906, jhare@med.miami.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures

All the authors declared no conflict of interest that could influence this work.

consider regenerative medicine more broadly, and in this context an examination of pharmacological approaches to augment tissue repair is important. This is crucial as emerging knowledge of the fundamental biology of survival and death as well as growth and regeneration of cardiomyocytes could yield novel therapeutic approaches [2, 3].

The field of cell-based therapy is advancing along several fronts [4]. First, the biology of pluripotent and multipotent stem cells is being rapidly unraveled. Inducible pluripotent stem (iPS) cells can be generated by the retrovirus-mediated transduction of four transcription factors (c-Myc, Oct3/4, SOX2, and Klf4) into mouse [5] or human [6, 7] fibroblasts and many efforts have been made to further improve the reprogramming efficiency to avoid tumorigenic risks [8] and enhance cardiogenic properties [9, 10]. Moreover, various growth factors and chemical compounds have also been found to improve the induction efficiency of iPS cells [11–13]. Second, insights into the biology of adult precursor cells are rapidly growing. In the case of the heart, compartments of precursor cells have been well characterized. The best-characterized adult cardiac stem cell is the c-kit⁺ cell [14–18]. Awareness of these cells raises the possibility that they could become a therapeutic target, and pharmacologic as well as genetic approaches that activate these cells or promote their differentiation are actively being sought [19, 20]. Here we review a number of emerging strategies to employ growth factors and small molecules to activate regenerative pathways in the injured heart.

2. Activation of stem cell homing

There is a growing body of evidence that growth factors and chemokines play an important role in stem cell signaling and homing to injured myocardium [21, 22]. In addition, there is a growing awareness that the heart has receptors for a wide range of growth factors and chemokines, activation of which can enhance myocyte survival and growth. Accordingly, administration of various stem cell homing factors has been increasingly explored by different groups (see Table 1). These different homing factors recruit different populations of stem cells. For example, stromal cell-derived factor-1 alpha (SDF-1 α) recruits chemokine (C-X-C motif) receptor 4 (CXCR4) expressing stem cells including hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), cardiac stem cells (CSCs), and CXCR4 expressing mesenchymal stem cells (MSCs) [23–27]. Monocyte chemoattractant protein-3 (MCP-3) homes MSCs [28], growth regulated oncogene-1 (GRO-1) homes EPCs [29], and hepatocyte growth factor (HGF) [30–32], fibroblast growth factor-2 (FGF-2) [33], and insulin growth factor-1 (IGF-1) [32, 34–36] activate CSCs.

Zhang et al [24] have shown that controlled release of SDF-1 α increases c-kit⁺ cell homing to the infarcted heart. Indeed, it has been recently shown that the regulation of bone marrow progenitor cell trafficking by SDF-1 α and CXCR4 is linked to Src-mediated c-kit phosphorylation [37]. The importance of the SDF-1 α /CXCR4 axis in stem cell recruitment to the heart following MI has been also demonstrated by Tang et al [38]. In addition, it has been recently demonstrated that vascular endothelial growth factor (VEGF) secreted by MSCs improves myocardial survival and the engraftment of implanted MSCs in infarcted hearts and promotes SDF-1 α /CXCR4-mediated recruitment of CSCs [39]. Chronic injections of human recombinant VEGF-A165 into the dystrophic hamstring muscle in a hereditary hamster model of muscular dystrophy and heart failure has been shown to stimulate skeletal muscle regeneration, production of growth factors, and mobilization of progenitor cells, resulting in attenuation of disease progression and substantial repair of the failing hamster heart [40].

On the other hand, our group as well as others have shown that granulocyte-colony-stimulating-factor (G-CSF) and stem cell factor (SCF) ameliorate post-MI remodeling by

enhancing endogenous cardiac repair mechanisms, promoting stem cell mobilization, and decreasing cardiomyocyte apoptosis [41, 42]. However, these effects were significantly attenuated in aged animals [41], raising concerns regarding the efficacy of chemokine therapies in the elderly. More recently, we have shown that the growth hormone releasing hormone (GHRH) agonist, JI-38, exerts a cardioprotective role in vivo following MI [43]. Animals that received JI-38 manifested improved cardiac function and reduced infarct size and cardiac fibrosis, one of the major determinants of poor prognosis in HF. Moreover, we demonstrated that the expression of cardiac c-kit⁺ cells was highly upregulated, and the cardiac effects of this GHRH agonist were independent of the GH/IGF-1 axis, since it did not increase the circulating levels of these hormones. Nerve growth factor (NGF) has been shown to promote angiogenesis, increase endothelial cell and cardiomyocyte survival, and improve myocardial blood flow and cardiac function in a mouse model of MI [44]. Human NGF gene delivered to the murine peri-infarct myocardium also increased stem cell factor (SCF, the c-kit receptor ligand) expression, which resulted in higher myocardial abundance of c-kit⁺ cells. The therapeutic benefits of NGF were mediated by the prosurvival and proangiogenic Akt/Foxo pathway. Indeed, the best currently studied downstream signaling cascades activated during growth factor treatment that promote proliferation and stimulate anti-apoptotic mechanisms in stem cells include Akt and its downstream target Pim-1 [20, 45, 46]. The expression of PIM-1 has been reported in all cardiac cell types including cardiac progenitor cells [20, 46] and some studies suggest that PIM-1 may promote proliferation and survival of these cells [46] leading to enhanced cardiac repair following MI [47].

Estradiol has been shown to increase EPCs mobilization to the injured myocardium, indicating a potential mechanism for gender differences after MI [48]. HMG-CoA reductase inhibitors have also been shown to increase EPC mobilization, suggesting a potential pleiotropic mechanism for the benefits of statin therapy observed in patients with acute MI [49]. Both estradiol and HMG-CoA reductase inhibitors stimulate endothelial nitric oxide synthase (NOS3). NOS3 has been shown to be critical for the mobilization of stem cells after MI, at least in part via upregulation of matrix metalloproteinase (MMP)-9 [48, 50]. Homing of EPCs to the injured myocardium is also dependent on EPCs expressing CD18 and interacting with endothelial cell surface ICAM-1. Blockade of CD18–ICAM-1 binding through the administration of a CD18 neutralizing antibody has been shown to inhibit EPC engraftment after MI [51]. Erythropoietin (EPO) also mobilizes EPCs and enhances neovascularization, thereby potentially improving tissue ischemia [52, 53], whereas human chorionic-gonadotropin (hCG) stimulates cell proliferation. The hypothesis that the combination may have synergistic effects was tested in an animal model of MI. EPO, hCG, or their combination ameliorated cardiac remodeling post-MI. However, only hCG+EPO stimulated cardiac c-kit⁺ stem cell proliferation, suggesting that combining mobilization and proliferative agents may add to the durability and sustainability of chemokine-based therapies for remodeling post-MI [54].

In view of the multiple growth factors and chemokines that have been shown to promote endogenous stem cell mobilization, proliferation, and homing to the injured myocardium, the development of growth factor/chemokine “cocktails” seems an attractive therapeutic approach for enhancing cardiac regeneration.

3. Priming of stem cells

While results from an initial wave of studies employing bone marrow derived cells to treat ischemic cardiomyopathy revealed measurable improvement in cardiac performance after stem cell therapy [4, 55, 56], it remains clear that the current approach can be improved, generating interest in optimizing donor cell function. Whether additional therapeutic

interventions such as growth factor administration, gene therapy, or modulation of stem cells with small molecules would safely enhance cardiac regenerative capacity is the focus of intense investigation. In this regard, the process of modifying specific protein expression through ex-vivo modifications [57] or "priming" stem cells by pre-treating with growth factors [58–61] or by genetic modification has offered insight into the relevant receptors and molecules involved in stem cell paracrine signaling, homing, and survival.

3a. Priming of stem cells with growth factors

Strong evidence of a paracrine effect contributing to the beneficial results from stem cell therapy [21, 62] supports the hypothesis that the combination of chemokines with cell therapy may be synergistically favorable for cardiac regeneration post-MI. Several growth factors, including FGF-2, IGF-1, and bone morphogenetic protein-2 (BMP-2) have been implicated as promoting cardiomyocyte differentiation and cytoprotective potential in stem cells [58, 59, 61]. For example, in a canine model of chronic MI, autologous bone marrow derived MSCs that were driven into a cardiac lineage by pre-treating with FGF, IGF-1, and BMP-2 prior to injection resulted in a significantly larger increase in regional wall thickening of the infarcted territory and a greater myocardial functional recovery [61]. In further support of this concept, Behfar et al [60] using a murine chronic MI model demonstrated that a recombinant cocktail of cardiopoietic factors consisting of transforming growth factor-beta (TGF- β 1), BMP-4, activin A, retinoic acid, IGF-1, FGF-2, alpha-thrombin, and interleukin-6 (IL-6) enhanced the therapeutic benefits of bone marrow derived MSCs by promoting their differentiation into a cardiac progenitor. On the other hand, treatment of adult rodent bone marrow cells with platelet-derived growth factor (PDGF)-A and -B isoforms accelerated their differentiation into cardiac myocytes in vitro as well as in an in vivo model of MI [63]. However, the functional improvement of the hearts treated with the combination of cells and PDGF-AB was similar to those treated with cells or PDGF-AB alone. Of note, in the hearts injected with bone marrow cells and PDGF-AB there was evidence of disorganized bone marrow-derived cardiac myocyte bundles with decreased gap junction formation, whereas those that received bone marrow cells alone formed cardiac myocyte islands with gap junctions. Gap junctions are critical for intercellular communication and electromechanical coupling. These findings suggest that impaired formation of intercellular connections may limit the functional benefit from the combined injection of PDGF-AB and bone marrow cells.

3b. Priming of stem cells by genetic modification

Glycogen synthase kinase (GSK)-3 is a serine/threonine kinase that regulates various intracellular functions via phosphorylation of substrates such as β -catenin, glycogen synthase, GATA4, and myocardin [64] as well as key signaling proteins involved in cell growth and differentiation, including Wnt, Notch, and hedgehog [65–67]. Overexpression of GSK-3 β has been shown to induce expression of cardiomyocyte specific genes and proteins, in part through downregulation of β -catenin, whereas it prevents expression of non-cardiac markers in MSCs in vitro [65]. MSCs overexpressing GSK-3 β (GSK-3 β -MSCs) injected into murine hearts after MI resulted in improvement in mortality, MI size, and left ventricular remodeling and function compared with control MSCs and saline [68]. Moreover, GSK-3 β overexpression was shown to increase cardiomyocyte differentiation of MSCs, induce cell proliferation marker Ki67-positive myocytes and cardiac c-Kit⁺ cells, and increase capillary density via upregulation of VEGF. Similarly, Mangi et al has reported that transplantation of MSCs modified with the serine/threonine protein kinase Akt can prevent remodeling and restore cardiac function in a rodent model of MI [45].

MCP-3 is a ligand of C-C chemokine receptor type 1 (CCR1). Overexpression of CCR1 in MSCs has been shown to increase murine MSC migration and protect MSCs from apoptosis

in vitro [69]. Moreover, CCR1-expressing MSCs injected intramyocardially resulted in reduced infarct size, decreased cardiomyocyte apoptosis, and increased capillary density in the injured myocardium as well as prevented cardiac remodeling and restored cardiac function after MI. These results support the notion that overexpression of chemokine receptors enhances the migration, survival, and engraftment of MSCs, thus providing a potentially beneficial therapeutic strategy for the injured myocardium.

The neuregulin (NRG)-1/ErbB signaling pathway has been recently shown to regulate cardiac subtype differentiation of human embryonic stem (ES) cells into a working or nodal-like phenotype [70]. Thus, although the use of human ES cells for cell-based therapy is complicated by ethical, moral, and legal issues, the manipulation of this signaling pathway in adult stem cells has the potential to be a useful tool for the generation of working-type myocytes for infarct repair or nodal-like cells for use in a biological pacemaker. Genetic overexpression of pluripotency genes is also being studied as an approach to improve adult stem cell functions [71]. Isolated circulating mesoangioblasts (cMABs) from peripheral blood of children express the pluripotency markers Klf4, c-Myc, and Oct3/4, but not Sox2. Overexpression of Sox2 was shown to enhance pluripotency and facilitate differentiation of cMABs into cardiovascular lineages. Sox-MABs injected into nude mice after acute MI resulted in significantly improved cardiac function and increased number of differentiated cardiomyocytes, endothelial cells, and smooth muscle cells compared to mice treated with control cMABs [71]. In addition, Ieda et al [72] recently demonstrated that the combination of three developmental transcription factors (Gata4, Mef2c, and Tbx5) was sufficient to rapidly and efficiently reprogram mouse postnatal cardiac or dermal fibroblasts to generate spontaneously beating cardiomyocytes that expressed a cardiomyocyte-like gene profile. These promising findings suggest that it may be possible to directly reprogram endogenous cardiac fibroblasts into new functional cardiomyocytes to restore cardiac contractility after injury.

Together these findings indicate an opportunity to use individual chemokines, chemokine cocktails, or genetic approaches to optimize donor cell function with regards to improving cell engraftment, survival and differentiation and, consequently, enhancing the regenerative potential of the injured heart. It should be noted, however, that gene therapy [73, 74] has been criticized by a variety of shortcomings including poor transfection efficiencies, safety concerns due to the inability to target specific cells, and/or uncontrolled expression of the target gene/protein [75].

4. Novel small molecules and other pharmacologic approaches to promote cardiac regeneration

Recent studies have investigated whether modification of stem cells with small molecules and other pharmacological approaches can safely promote cardiac regenerative capacity. Manipulation of small molecules has been shown to promote ex-vivo cell proliferation, stability, mobilization, and thereby integration from tissue niches as well as improvement of the modulation of host responses to foreign cell grafts. Furthermore, multiple laboratories have reported that small molecules have the potential to modulate different cell signaling pathways involved in myocardial contractility, remodeling, survival, and repair (Table 2). By using a high-throughput screening system to identify bioactive small molecules that activate NKx2.5, an early cardiac lineage progenitor gene, Sadek et al [76] identified a family of sulfonyl-hydrazones (Shz) that not only potentially induce Nkx2.5 but also other cardiac markers, such as myocardin, troponin-I, and sarcomeric α -tropomyosin, in various embryonic and adult stem cells, including human mobilized peripheral blood mononuclear cells (PBMCs). Shz-pretreated human PBMCs injected in a rodent MI model enhanced engraftment of the cells and improved cardiac function. In a more recent study, treatment of

iPS cells with Shz enhanced cardiac marker expression and cardiomyocyte yield, thus providing a potentially feasible strategy to improve cardiac differentiation of iPS cells [77]. Using similar technology to identify small molecules that simulate the generation of cardiomyocytes, Takahashi et al [78] reported that ascorbic acid enhances the differentiation of ES cells into cardiomyocytes. Similarly, a recently discovered small molecule inhibitor of Wnt/ β -catenin signaling, XAV939, induces cardiomyogenesis in mouse ES cells [79]. The Wnt/ β -catenin signaling pathway is a major regulator of heart development as well as stem cell self-renewal. The study reports that administration of XAV939 immediately following the formation of mesoderm progenitor cells promotes cardiomyogenic development at the expense of other mesoderm-derived lineages, including endothelial, smooth muscle, and hematopoietic. On the other hand, pyrvinium, a drug that was originally identified as a Wnt inhibitor in a chemical screen for small molecules, has shown promise in reducing adverse cardiac remodeling and increasing cardiomyocyte proliferation after MI [80]. Pyrvinium inhibits Wnt signaling by activating casein kinase-1, which promotes degradation of β -catenin [80]. Regarding potential therapeutic targets in heart failure, small molecule inhibitors of $\beta\gamma$ -subunits of heterotrimeric G-proteins ($G\beta\gamma$) signaling have been identified that specifically bind to a $G\beta\gamma$ protein-protein interaction “hot spot” [81]. These inhibitors have been shown to normalize cardiac function and morphology as well as reduce interstitial cardiac fibrosis.

A mixed ester of hyaluronan with butyric and retinoic acid (HBR) has been recently developed that was shown to act as a cardiogenic and vasculogenic agent in human MSCs isolated from bone marrow, dental pulp, and fetal membranes of term placenta (FMhMSCs) [82]. HBR enhanced VEGF, KDR, and HGF gene expression, stimulated stem cell differentiation into endothelial cells, and also increased the transcription of the cardiac lineage-promoting genes GATA-4 and Nkx-2.5 and the yield of cardiac marker expressing cells. In a rodent and pig MI model, transplantation of HBR-preconditioned FMhM-SCs enhanced capillary density and decreased the infarct size compared to cells not HBR treated [82, 83].

Epigenetic modifications, such as DNA methylation and histone acetylation, are also being explored as potential approaches to promote stem cell differentiation and cardiac regeneration. Several studies have demonstrated an increase in cardiac protein expression as well as spontaneously beating cells after treatment of MSCs with 5-azacytidine, a DNA demethylation reagent [84, 85]. Studies have also demonstrated improvement in cardiac function after the transplantation of 5-azacytidine-treated MSCs compared to control MSCs [85–87]. Resveratrol (3,4',5-trihydroxy-trans-stilbene), a dietary polyphenol found in red wine and other sources, is a known activator of the NAD^+ -dependent histone deacetylase Sirtuin 1 that has been shown to modify the functions of EPCs, including attenuation of senescence and promotion of EPC adhesion, migration, and tube formation [88, 89]. The anti-senescence effect was accompanied by activation of telomerase through the Akt signaling pathway. On the other hand, the effect of resveratrol on EPC senescence was not abolished by inhibition of eNOS. More recently, the effect of resveratrol on survival and engraftment of implanted rat GFP-labeled CSCs was tested in a rodent model of MI [90]. Although there was improvement in cardiac function (left ventricular ejection fraction, fractional shortening, and cardiac output) in both the treated and control group after 7 days, only the resveratrol-modified stem cell group exhibited improvement in cardiac function at the end of one, two and four months time. The improvement of cardiac function was accompanied by enhanced stem cell survival and engraftment, as evidenced by the expression of the cell proliferation marker Ki67 and the expression of GFP up to four months after MI in the resveratrol treated stem cell group. Expression of SDF-1 and myosin suggested homing of stem cells in the infarcted myocardium, its regeneration presumably leading to improvement of cardiac function.

Accordingly, as with chemokines and growth factors, the development of small molecules and other pharmacologic agents, which potentially enhance stem cell differentiation and engraftment and promote myocardial repair by modulation of the host environment or regulation of genes and cell signaling pathways, points to an attractive new field of therapeutic targets to prevent or reverse left ventricular remodeling post-MI.

5. Clinical trials using cell-based and chemokine or gene therapy in cardiac repair

To date several strategies employing chemokines have been tested in early and late stage trials. The major clinical trials involving chemokines in the treatment of cardiovascular diseases are discussed below.

Granulocyte Colony-Stimulating Factor (G-CSF)

To date, various studies have investigated the administration of G-CSF alone or of G-CSF-mobilized cells into patients post-acute MI. One of the pioneering clinical studies using G-CSF was the open label Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion (MAGIC) trial [91]. In this study, parameters such as exercise capacity, myocardial perfusion, and systolic function were improved in the stem cell infusion group but there was a higher rate of in-stent restenosis in the group treated with G-CSF. In contrast, the randomized but not blinded Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) trial [92] reported that patients receiving G-CSF had increased mobilization of CD34+ cells and improved ejection fraction without enlargement of the left ventricular diastolic dimension. Notably, there was no evidence of restenosis or any other adverse effects in this group.

The encouraging results of the FIRSTLINE-AMI trial, however, were not validated in Stem Cell Mobilization in Acute Myocardial Infarction (STEM-AMI) [93, 94] and Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL-2) trials [95]. Although clinical trials have showed equivocal results and raised safety concerns, stem cell mobilization with G-CSF is still promising as a potential alternative therapy, mainly due to its feasibility and general applicability. Thus, as the underlying mechanisms are being elucidated, additional careful and well-designed clinical investigations are still necessary to examine whether or not this strategy is truly a potential approach for enhancing cardiac regeneration.

Erythropoietin (EPO)

A first pilot study conducted on EPO provided insights into its effects in humans [96], showing that EPO was safe and well tolerated [96]. Moreover, while the results of this study showed a substantial increase in EPCs (CD34+/CD45-) in the treated group compared with control, it did not show beneficial effects on cardiac function, suggesting that larger clinical trials are required to determine the efficacy of EPO in cardiac patients.

Subsequently, the HEBE-III study was initiated to assess the effects of EPO in a larger cohort (n= 529) of acute MI patients. The results from HEBE III study, conducted by Voors et al [97], showed that a single dose of EPO failed to improve left ventricular ejection fraction (LVEF), but the observed beneficial effects on the secondary endpoints suggested a substantial cardioprotective effect and a favorable clinical safety profile. Similarly, the Prospective, Randomized, Double-Blind, Placebo-controlled Trial of Erythropoietin in Patients With ST-Segment Elevation Myocardial Infarction Undergoing Percutaneous Coronary Intervention (REVIVAL-3) trial conducted by Ott I et al [98] reported no

improvement on cardiac function. Ongoing clinical trials, including the Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin after Large Myocardial Infarction (REVEAL) [99] trial and the Exogenous Erythropoietin in Acute Myocardial Infarction: New Outlook and Dose Association Study (EPAMINONDAS) [100], will provide an evaluation of the safety and efficacy of different doses of EPO following successful primary or rescue percutaneous coronary intervention in patients.

Despite the lack of beneficial effects of EPO on cardiac function in patients post-MI, evidence from in vivo and in vitro studies provide support for additional studies using EPO in combination with other chemokines [54] and/or stem cell therapy.

Adeno-associated viral vector based gene therapy

The first clinical test of a gene therapy for heart failure, The Calcium Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID), tested the administration of the sarcoplasmic reticulum Ca(2+) ATPase (SERCA2a) gene using an adeno-associated viral vector (AAV) in a single intracoronary dose. The gene therapy appeared to improve symptoms, functional status, and ventricular volumes in patients with severe HF [101]. Similarly, early results from a randomized, double-blinded, placebo-controlled, dose escalation trial of intracoronary administration of AAV1/SERCA2a (Phase I) in humans with advanced HF showed improvement of a number of HF parameters such as six-minute-walk distance, NYHA functional class, LVEF, left ventricular end-systolic volume, and natriuretic peptide levels. Moreover, Phase 2 of this trial which is placebo controlled, randomized, and blinded is currently under way [102].

Collectively, a number of clinical trials have already been committed to assess the safety and efficacy of several growth factors/chemokines and gene therapy. Although some of them seem promising and others have showed equivocal data, the importance of optimization of the study design and larger-scale studies for the evaluation of efficacy and safety of potential therapeutic strategies has been clearly demonstrated.

6. Conclusion

Cardiovascular medicine is poised to make a major leap forward with new strategies aimed at actual tissue regeneration. Novel discoveries in stem cell biology and the signaling pathways governing cellular survival, homing, and differentiation hold enormous promise to translate into innovative therapeutic approaches to repair the failing heart through the generation of new blood vessels and cardiac myocytes with appropriate ultrastructural organization. As reviewed here, the possibility to enhance cell-based therapy with pharmacologic and/or genetic means represents another new and transformative approach to achieving therapeutic tissue repair. As knowledge of the stem cell compartment and niche increase, so does the opportunity to manipulate these units of tissue repair using both chemokines and small molecules.

Acknowledgments

Dr. Hare is supported by NIH grants: RO1 HL094849, P20 HL101443, RO1 HL084275, RO1 HL107110, and U54 HL081028.

Reference List

1. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, de SG, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*. 2010; 121:e46–e215. [PubMed: 20019324]

2. Anversa P, Kajstura J, Leri A, Bolli R. Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation*. 2006; 113:1451–1463. [PubMed: 16549650]
3. Filipczyk AA, Passier R, Rochat A, Mummery CL. Regulation of cardiomyocyte differentiation of embryonic stem cells by extracellular signalling. *Cell Mol Life Sci*. 2007; 64:704–718. [PubMed: 17380311]
4. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. *Nature*. 2008; 451:937–942. [PubMed: 18288183]
5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
6. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
7. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
8. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*. 2008; 26:101–106. [PubMed: 18059259]
9. Martinez-Fernandez A, Nelson TJ, Yamada S, Reyes S, Alekseev AE, Perez-Terzic C, et al. iPS programmed without c-MYC yield proficient cardiogenesis for functional heart chimerism. *Circ Res*. 2009; 105:648–656. [PubMed: 19696409]
10. Martinez-Fernandez A, Nelson TJ, Ikeda Y, Terzic A. c-MYC independent nuclear reprogramming favors cardiogenic potential of induced pluripotent stem cells. *J Cardiovasc Transl Res*. 2010; 3:13–23. [PubMed: 20221419]
11. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol*. 2008; 26:795–797. [PubMed: 18568017]
12. Shi Y, Do JT, Despons C, Hahm HS, Scholer HR, Ding S. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell*. 2008; 2:525–528. [PubMed: 18522845]
13. Maherali N, Hochedlinger K. Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr Biol*. 2009; 19:1718–1723. [PubMed: 19765992]
14. Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De AA, et al. Human cardiac stem cells. *Proc Natl Acad Sci U S A*. 2007; 104:14068–14073. [PubMed: 17709737]
15. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003; 114:763–776. [PubMed: 14505575]
16. Zaruba MM, Soonpaa M, Reuter S, Field LJ. Cardiomyogenic potential of C-kit(+)-expressing cells derived from neonatal and adult mouse hearts. *Circulation*. 2010; 121:1992–2000. [PubMed: 20421520]
17. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003; 100:12313–12318. [PubMed: 14530411]
18. Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proc Natl Acad Sci U S A*. 2005; 102:8966–8971. [PubMed: 15951423]
19. Suzuki G, Iyer V, Cimato T, Canty JM Jr. Pravastatin improves function in hibernating myocardium by mobilizing CD133+ and cKit+ bone marrow progenitor cells and promoting myocytes to reenter the growth phase of the cardiac cell cycle. *Circ Res*. 2009; 104:255–264. 10p. [PubMed: 19096024]
20. Fischer KM, Cottage CT, Wu W, Din S, Gude NA, Avitabile D, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. *Circulation*. 2009; 120:2077–2087. [PubMed: 19901187]

21. Gneccchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res.* 2008; 103:1204–1219. [PubMed: 19028920]
22. Korf-Klingebiel M, Kempf T, Sauer T, Brinkmann E, Fischer P, Meyer GP, et al. Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction. *Eur Heart J.* 2008; 29:2851–2858. [PubMed: 18953051]
23. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet.* 2003; 362:697–703. [PubMed: 12957092]
24. Zhang G, Nakamura Y, Wang X, Hu Q, Suggs LJ, Zhang J. Controlled release of stromal cell-derived factor-1 alpha in situ increases c-kit⁺ cell homing to the infarcted heart. *Tissue Eng.* 2007; 13:2063–2071. [PubMed: 17518719]
25. Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation.* 2003; 107:1322–1328. [PubMed: 12628955]
26. Sasaki T, Fukazawa R, Ogawa S, Kanno S, Nitta T, Ochi M, et al. Stromal cell-derived factor-1alpha improves infarcted heart function through angiogenesis in mice. *Pediatr Int.* 2007; 49:966–971. [PubMed: 18045305]
27. Saxena A, Fish JE, White MD, Yu S, Smyth JW, Shaw RM, et al. Stromal cell-derived factor-1alpha is cardioprotective after myocardial infarction. *Circulation.* 2008; 117:2224–2231. [PubMed: 18427137]
28. Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, Popovic Z, et al. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. *Stem Cells.* 2007; 25:245–251. [PubMed: 17053210]
29. Kocher AA, Schuster MD, Bonaros N, Lietz K, Xiang G, Martens TP, et al. Myocardial homing and neovascularization by human bone marrow angioblasts is regulated by IL-8/Gro CXC chemokines. *J Mol Cell Cardiol.* 2006; 40:455–464. [PubMed: 16438981]
30. Nakamura T, Mizuno S, Matsumoto K, Sawa Y, Matsuda H, Nakamura T. Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF. *J Clin Invest.* 2000; 106:1511–1519. [PubMed: 11120758]
31. Wang Y, Ahmad N, Wani MA, Ashraf M. Hepatocyte growth factor prevents ventricular remodeling and dysfunction in mice via Akt pathway and angiogenesis. *J Mol Cell Cardiol.* 2004; 37:1041–1052. [PubMed: 15522281]
32. Rota M, Padin-Iruegas ME, Misao Y, De AA, Maestroni S, Ferreira-Martins J, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res.* 2008; 103:107–116. [PubMed: 18556576]
33. Rosenblatt-Velin N, Lepore MG, Cartoni C, Beermann F, Pedrazzini T. FGF-2 controls the differentiation of resident cardiac precursors into functional cardiomyocytes. *J Clin Invest.* 2005; 115:1724–1733. [PubMed: 15951838]
34. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res.* 2004; 94:514–524. [PubMed: 14726476]
35. Padin-Iruegas ME, Misao Y, Davis ME, Segers VF, Esposito G, Tokunou T, et al. Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. *Circulation.* 2009; 120:876–887. [PubMed: 19704095]
36. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De AA, et al. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res.* 2005; 97:663–673. [PubMed: 16141414]
37. Cheng M, Zhou J, Wu M, Boriboun C, Thorne T, Liu T, et al. CXCR4-mediated bone marrow progenitor cell maintenance and mobilization are modulated by c-kit activity. *Circ Res.* 2010; 107:1083–1093. [PubMed: 20847314]

38. Tang YL, Zhu W, Cheng M, Chen L, Zhang J, Sun T, et al. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. *Circ Res.* 2009; 104:1209–1216. [PubMed: 19407239]
39. Tang JM, Wang JN, Zhang L, Zheng F, Yang JY, Kong X, et al. VEGF/SDF-1 promotes cardiac stem cell mobilization and myocardial repair in the infarcted heart. *Cardiovasc Res.* 2011
40. Zisa D, Shabbir A, Mastri M, Suzuki G, Lee T. Intramuscular VEGF repairs the failing heart: role of host-derived growth factors and mobilization of progenitor cells. *Am J Physiol Regul Integr Comp Physiol.* 2009; 297:R1503–R1515. [PubMed: 19759338]
41. Lehrke S, Mazhari R, Durand DJ, Zheng M, Bedja D, Zimmet JM, et al. Aging impairs the beneficial effect of granulocyte colony-stimulating factor and stem cell factor on post-myocardial infarction remodeling. *Circ Res.* 2006; 99:553–560. [PubMed: 16873716]
42. Xiang FL, Lu X, Hammoud L, Zhu P, Chidiac P, Robbins J, et al. Cardiomyocyte-specific overexpression of human stem cell factor improves cardiac function and survival after myocardial infarction in mice. *Circulation.* 2009; 120:1065–1074. 9. [PubMed: 19738140]
43. Kanashiro-Takeuchi RM, Tziomalos K, Takeuchi LM, Treuer AV, Lamirault G, Dulce R, et al. Cardioprotective effects of growth hormone-releasing hormone agonist after myocardial infarction. *Proc Natl Acad Sci U S A.* 2010; 107:2604–2609. [PubMed: 20133784]
44. Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van LS, et al. Nerve growth factor promotes cardiac repair following myocardial infarction. *Circ Res.* 2010; 106:1275–1284. [PubMed: 20360245]
45. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med.* 2003; 9:1195–1201. [PubMed: 12910262]
46. Muraski JA, Rota M, Misao Y, Fransioli J, Cottage C, Gude N, et al. Pim-1 regulates cardiomyocyte survival downstream of Akt. *Nat Med.* 2007; 13:1467–1475. [PubMed: 18037896]
47. Cottage CT, Bailey B, Fischer KM, Avitable D, Collins B, Tuck S, et al. Cardiac progenitor cell cycling stimulated by pim-1 kinase. *Circ Res.* 2010; 106:891–901. [PubMed: 20075333]
48. Iwakura A, Shastry S, Luedemann C, Hamada H, Kawamoto A, Kishore R, et al. Estradiol enhances recovery after myocardial infarction by augmenting incorporation of bone marrow-derived endothelial progenitor cells into sites of ischemia-induced neovascularization via endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9. *Circulation.* 2006; 113:1605–1614. [PubMed: 16534014]
49. Walter DH, Zeiher AM, Dimmeler S. Effects of statins on endothelium and their contribution to neovascularization by mobilization of endothelial progenitor cells. *Coron Artery Dis.* 2004; 15:235–242. [PubMed: 15238818]
50. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med.* 2003; 9:1370–1376. [PubMed: 14556003]
51. Wu Y, Ip JE, Huang J, Zhang L, Matsushita K, Liew CC, et al. Essential role of ICAM-1/CD18 in mediating EPC recruitment, angiogenesis, and repair to the infarcted myocardium. *Circ Res.* 2006; 99:315–322. [PubMed: 16825578]
52. van der Meer P, Lipsic E, Henning RH, Boddeus K, van d V, Voors AA, et al. Erythropoietin induces neovascularization and improves cardiac function in rats with heart failure after myocardial infarction. *J Am Coll Cardiol.* 2005; 46:125–133. [PubMed: 15992646]
53. Prunier F, Pfister O, Hadri L, Liang L, del MF, Liao R, et al. Delayed erythropoietin therapy reduces post-MI cardiac remodeling only at a dose that mobilizes endothelial progenitor cells. *Am J Physiol Heart Circ Physiol.* 2007; 292:H522–H529. [PubMed: 16997893]
54. Kanashiro-Takeuchi RM, Takeuchi L, Hatzistergos C, Quevedo H, Selem S, Treuer AV, et al. Effects of Combination of Proliferative Agents and Erythropoietin on Left Ventricular Remodeling Post-Myocardial Infarction. *Clinical and Translational Science.* 2011 Ref Type: In Press.
55. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. 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.* 2009; 54:2277–2286. [PubMed: 19958962]
56. Williams AR, Trachtenberg B, Velazquez DL, McNiece I, Altman P, Rouy D, et al. Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling. *Circ Res.* 2011; 108:792–796. [PubMed: 21415390]
 57. Herrmann JL, Markel TA, Abarbanell AM, Weil BR, Wang M, Wang Y, et al. Proinflammatory stem cell signaling in cardiac ischemia. *Antioxid Redox Signal.* 2009; 11:1883–1896. [PubMed: 19187005]
 58. Hahn JY, Cho HJ, Kang HJ, Kim TS, Kim MH, Chung JH, 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; 51:933–943. [PubMed: 18308163]
 59. Hausenloy DJ, Yellon DM. Cardioprotective growth factors. *Cardiovasc Res.* 2009; 83:179–194. [PubMed: 19218286]
 60. Behfar A, Yamada S, Crespo-Diaz R, Nesbitt JJ, Rowe LA, Perez-Terzic C, et al. Guided cardiopoiesis enhances therapeutic benefit of bone marrow human mesenchymal stem cells in chronic myocardial infarction. *J Am Coll Cardiol.* 2010; 56:721–734. [PubMed: 20723802]
 61. Bartunek J, Croissant JD, Wijns W, Gofflot S, de LA, Vanderheyden M, 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; 292:H1095–H1104. [PubMed: 17056665]
 62. Behfar A, Zingman LV, Hodgson DM, Rauzier JM, Kane GC, Terzic A, et al. Stem cell differentiation requires a paracrine pathway in the heart. *FASEB J.* 2002; 16:1558–1566. [PubMed: 12374778]
 63. Xaymardan M, Tang L, Zagreda L, Pallante B, Zheng J, Chazen JL, et al. Platelet-derived growth factor-AB promotes the generation of adult bone marrow-derived cardiac myocytes. *Circ Res.* 2004; 94:E39–E45. [PubMed: 14963008]
 64. Hirotani S, Zhai P, Tomita H, Galeotti J, Marquez JP, Gao S, et al. Inhibition of glycogen synthase kinase 3beta during heart failure is protective. *Circ Res.* 2007; 101:1164–1174. [PubMed: 17901358]
 65. Cho J, Rameshwar P, Sadoshima J. Distinct roles of glycogen synthase kinase (GSK)-3alpha and GSK-3beta in mediating cardiomyocyte differentiation in murine bone marrow-derived mesenchymal stem cells. *J Biol Chem.* 2009; 284:36647–36658. [PubMed: 19858210]
 66. Jin YH, Kim H, Oh M, Ki H, Kim K. Regulation of Notch1/NICD and Hes1 expressions by GSK-3alpha/beta. *Mol Cells.* 2009; 27:15–19. [PubMed: 19214430]
 67. Riobo NA, Lu K, Ai X, Haines GM, Emerson CP Jr. Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. *Proc Natl Acad Sci U S A.* 2006; 103:4505–4510. [PubMed: 16537363]
 68. Cho J, Zhai P, Maejima Y, Sadoshima J. Myocardial injection with GSK-3beta-overexpressing bone marrow-derived mesenchymal stem cells attenuates cardiac dysfunction after myocardial infarction. *Circ Res.* 2011; 108:478–489. [PubMed: 21233455]
 69. Huang J, Zhang Z, Guo J, Ni A, Deb A, Zhang L, et al. Genetic modification of mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment, and capillary density in the injured myocardium. *Circ Res.* 2010; 106:1753–1762. [PubMed: 20378860]
 70. Zhu WZ, Xie Y, Moyes KW, Gold JD, Askari B, Laflamme MA. Neuregulin/ErbB signaling regulates cardiac subtype specification in differentiating human embryonic stem cells. *Circ Res.* 2010; 107:776–786. [PubMed: 20671236]
 71. Koyanagi M, Iwasaki M, Rupp S, Tedesco FS, Yoon CH, Boeckel JN, et al. Sox2 transduction enhances cardiovascular repair capacity of blood-derived mesoangioblasts. *Circ Res.* 2010; 106:1290–1302. [PubMed: 20185800]
 72. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell.* 2010; 142:375–386. [PubMed: 20691899]

73. Penn MS, Mangi AA. Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circ Res.* 2008; 102:1471–1482. [PubMed: 18566313]
74. Gupta R, Tongers J, Losordo DW. Human studies of angiogenic gene therapy. *Circ Res.* 2009; 105:724–736. [PubMed: 19815827]
75. Mushtaq M, Oskoue BN, Hare JM. Cell therapy for heart disease: to genetically modify or not, that is the question. *Circ Res.* 2011; 108:398–401. [PubMed: 21335427]
76. Sadek H, Hannack B, Choe E, Wang J, Latif S, Garry MG, et al. Cardiogenic small molecules that enhance myocardial repair by stem cells. *Proc Natl Acad Sci U S A.* 2008; 105:6063–6068. [PubMed: 18420817]
77. Quattrocelli M, Palazzolo G, Agnolin I, Martino S, Bouche M, Anastasia L, et al. Synthetic sulfonyl-hydrazone-1 positively regulates cardiomyogenic microRNA expression and cardiomyocyte differentiation of induced pluripotent stem cells. *J Cell Biochem.* 2011
78. Takahashi T, Lord B, Schulze PC, Fryer RM, Sarang SS, Gullans SR, et al. Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. *Circulation.* 2003; 107:1912–1916. [PubMed: 12668514]
79. Wang H, Hao J, Hong CC. Cardiac Induction of Embryonic Stem Cells by a Small Molecule Inhibitor of Wnt/beta-Catenin Signaling. *ACS Chem Biol.* 2011; 6:192–197. [PubMed: 21077691]
80. Saraswati S, Alfaro MP, Thorne CA, Atkinson J, Lee E, Young PP. Pyrvinium, a potent small molecule Wnt inhibitor, promotes wound repair and post-MI cardiac remodeling. *PLoS One.* 2010; 5:e15521. [PubMed: 21170416]
81. Kamal FA, Smrcka AV, Blaxall BC. Taking the heart failure battle inside the cell: Small molecule targeting of Gbetagamma subunits. *J Mol Cell Cardiol.* 2011
82. Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I, 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; 282:14243–14252. [PubMed: 17363374]
83. Simioniuc A, Campan M, Lionetti V, Marinelli M, Aquaro GD, Cavallini C, et al. Placental stem cells pre-treated with a hyaluronan mixed ester of butyric and retinoic acid to cure infarcted pig hearts: a multimodal study. *Cardiovasc Res.* 2011
84. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest.* 1999; 103:697–705. [PubMed: 10074487]
85. Ye NS, Chen J, Luo GA, Zhang RL, Zhao YF, Wang YM. Proteomic profiling of rat bone marrow mesenchymal stem cells induced by 5-azacytidine. *Stem Cells Dev.* 2006; 15:665–676. [PubMed: 17105402]
86. Burlacu A, Rosca AM, Maniu H, Titorencu I, Dragan E, Jinga V, et al. Promoting effect of 5-azacytidine on the myogenic differentiation of bone marrow stromal cells. *Eur J Cell Biol.* 2008; 87:173–184. [PubMed: 18037191]
87. Yoon J, Min BG, Kim YH, Shim WJ, Ro YM, Lim DS. Differentiation, engraftment and functional effects of pre-treated mesenchymal stem cells in a rat myocardial infarct model. *Acta Cardiol.* 2005; 60:277–284. [PubMed: 15999467]
88. Huang PH, Chen YH, Tsai HY, Chen JS, Wu TC, Lin FY, et al. Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler Thromb Vasc Biol.* 2010; 30:869–877. [PubMed: 20093623]
89. Xia L, Wang XX, Hu XS, Guo XG, Shang YP, Chen HJ, et al. Resveratrol reduces endothelial progenitor cells senescence through augmentation of telomerase activity by Akt-dependent mechanisms. *Br J Pharmacol.* 2008; 155:387–394. [PubMed: 18587418]
90. Gorbunov N, Petrovski G, Gurusamy N, Ray D, Kim DH, Das DK. REGENERATION OF INFARCTED MYOCARDIUM WITH RESVERATROL-MODIFIED CARDIAC STEM CELLS. *J Cell Mol Med.* 2011
91. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet.* 2004; 363:751–756. [PubMed: 15016484]

92. Ince H, Petzsch M, Kleine HD, Schmidt H, Rehders T, Korber T, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). *Circulation*. 2005; 112:3097–3106. [PubMed: 16275869]
93. Ripa RS, Jorgensen E, Wang Y, Thune JJ, Nilsson JC, Sondergaard L, et al. Stem cell mobilization induced by subcutaneous granulocyte-colony stimulating factor to improve cardiac regeneration after acute ST-elevation myocardial infarction: result of the double-blind, randomized, placebo-controlled stem cells in myocardial infarction (STEMMI) trial. *Circulation*. 2006; 113:1983–1992. [PubMed: 16531621]
94. Ripa RS, Haack-Sorensen M, Wang Y, Jorgensen E, Mortensen S, Bindslev L, et al. Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: results from the Stem Cells in Myocardial Infarction (STEMMI) trial. *Circulation*. 2007; 116:I24–I30. [PubMed: 17846310]
95. Zohlnhofer D, Ott I, Mehilli J, Schomig K, Michalk F, Ibrahim T, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. *JAMA*. 2006; 295:1003–1010. [PubMed: 16507801]
96. Lipsic E, van der Meer P, Voors AA, Westenbrink BD, van den Heuvel AF, de Boer HC, et al. A single bolus of a long-acting erythropoietin analogue darbepoetin alfa in patients with acute myocardial infarction: a randomized feasibility and safety study. *Cardiovasc Drugs Ther*. 2006; 20:135–141. [PubMed: 16761193]
97. Voors AA, Belonje AM, Zijlstra F, Hillege HL, Anker SD, Slart RH, et al. A single dose of erythropoietin in ST-elevation myocardial infarction. *Eur Heart J*. 2010; 31:2593–2600. [PubMed: 20802250]
98. Ott I, Schulz S, Mehilli J, Fichtner S, Hadamitzky M, Hoppe K, et al. Erythropoietin in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomized, double-blind trial. *Circ Cardiovasc Interv*. 2010; 3:408–413. [PubMed: 20736448]
99. Melloni C, Rao SV, Povsic TJ, Melton L, Kim RJ, Kilaru R, et al. Design and rationale of the Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin after Large Myocardial Infarction (REVEAL) trial. *Am Heart J*. 2010; 160:795–803. [PubMed: 21095264]
100. Andreotti F, Agati L, Conti E, Santucci E, Rio T, Tarantino F, et al. Update on phase II studies of erythropoietin in acute myocardial infarction. Rationale and design of Exogenous erythroPoietin in Acute Myocardial Infarction: New Outlook aNd Dose Association Study (EPAMINONDAS). *J Thromb Thrombolysis*. 2009; 28:489–495. [PubMed: 19533304]
101. Jaski BE, Jessup ML, Mancini DM, Cappola TP, Pauly DF, Greenberg B, et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail*. 2009; 15:171–181. [PubMed: 19327618]
102. Lipskaia L, Chemaly ER, Hadri L, Lompre AM, Hajjar RJ. Sarcoplasmic reticulum Ca(2+) ATPase as a therapeutic target for heart failure. *Expert Opin Biol Ther*. 2010; 10:29–41. [PubMed: 20078230]

Table 1

Potential ligands and receptors used as therapeutic approaches for cardiac repair following myocardial infarction.

Ligand/Receptor	Action	Model	Effect	Study
Growth Hormone Releasing Hormone (GHRH)	Stem cell mobilization	Rat	Improves cardiac function, reduces infarct size	[43]
Insulin Growth Factor-1 (IGF-1)	CSC proliferation and survival	Mouse, Rat	Improves cardiac function	[32, 34–36]
Stromal Cell-Derived Factor-1 α (SDF-1 α)	Stem cell recruitment Angiogenic, anti-apoptotic	Mouse, Rat	Improves cardiac function	[23–27, 38]
Nerve Growth Factor (NGF)	CSC proliferation Angiogenic, anti-apoptotic	Mouse	Improves cardiac function and neovascularization	[44]
Stem Cell Factor (SCF)	C-kit ⁺ stem cell proliferation, mobilization, and survival	Mouse, Rat	Improves cardiac function and reduces mortality	[41, 42]
Granulocyte-Colony Stimulating Factor (G-CSF)	Stem cell mobilization Angiogenic, anti-apoptotic	Mouse, Rat, Human	Improves cardiac function and reduces cardiomyocyte apoptosis	[41, 42] [91–95]
Fibroblast Growth Factor-2 (FGF-2)	Stem cell mobilization Angiogenic	Mouse, Canine	Improves cardiac function	[33, 60–61]
Platelet-Derived Growth Factor-AB (PDGF-AB)	Cardiomyogenic differentiation	Rat	Improves cardiac function	[63]
Erythropoietin (EPO)	Stem cell mobilization Angiogenic, anti-apoptotic	Rat, Human	Improves contractility (Rat)	[52–54] [96–100]
Vascular Endothelial Growth Factor (VEGF)	Stem cell mobilization Angiogenic	Rat, Hamster	Improves cardiac function and neovascularization	[39–40]
Hepatocyte Growth Factor (HGF)	Chemotactic on CSCs Angiogenic, anti-apoptotic	Mouse, Rat	Reduces infarct size and mortality and improves ejection fraction	[30–32]
Neuregulin	Mitogenic	Mouse	Improves ejection fraction, reduces infarct size	[70]
C-C chemokine receptor type 1 (CCR1)	Stem cell mobilization Angiogenic, anti-apoptotic	Mouse	Reduces apoptosis, improves cardiac function, reduces infarct size	[69]
GSK-3 β	Cardiomyogenic differentiation CSC proliferation Angiogenic	Mouse	Improves cardiac function, reduces infarct size and mortality	[64–66, 68]

Table 2

Overview of small molecule approaches for myocardial regeneration.

Intervention	Action	Study
Sulfonyl-Hydrazones (Shz)	Promote myocardial repair/regeneration by activating cardiac differentiation in mobilized peripheral blood mononuclear cells and improve cardiac differentiation of inducible pluripotent stem cells.	[76, 77]
Ascorbic acid	Enhances cardiomyogenesis in embryonic stem cells.	[78]
Wnt / β -Catenin Signaling Inhibitor (XAV939)	Induces cardiomyogenesis in mouse embryonic stem cells.	[79]
Wnt Inhibitor (Pyrvinium)	Promotes wound repair and prevents cardiac remodeling. Increases cardiomyocyte proliferation post-myocardial infarction.	[80]
G $\beta\gamma$ Inhibitors	Normalize cardiac function, reduce interstitial cardiac fibrosis, and enhance cardiac contractility. Reduce GRK2 expression in heart failure.	[81]
5-azacytidine	Increases MSC cardiac protein expression and improves cardiac function.	[84–87]