Challenges for heart disease stem cell therapy

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Abstract: Cardiovascular diseases (CVDs) are the leading cause of death worldwide. The use of stem cells to improve recovery of the injured heart after myocardial infarction (MI) is an important emerging therapeutic strategy. However, recent reviews of clinical trials of stem cell therapy for MI and ischemic heart disease recovery report that less than half of the trials found only small improvements in cardiac function. In clinical trials, bone marrow, peripheral blood, or umbilical cord blood cells were used as the source of stem cells delivered by intracoronary infusion. Some trials administered only a stem cell mobilizing agent that recruits endogenous sources of stem cells. Important challenges to improve the effectiveness of stem cell therapy for CVD include: (1) improved identification, recruitment, and expansion of autologous stem cells; (2) identification of mobilizing and homing agents that increase recruitment; and (3) development of strategies to improve stem cell survival and engraftment of both endogenous and exogenous sources of stem cells. This review is an overview of stem cell therapy for CVD and discusses the challenges these three areas present for maximum optimization of the efficacy of stem cell therapy for heart disease, and new strategies in progress.

Keywords: mobilization, expansion, homing, survival, engraftment

Introduction

The recovery of function after a myocardial infarction (MI) is dependent on increasing blood flow and regeneration of tissue. Stem cells (SCs) can provide cellular precursors for cardiomyocyte differentiation, endothelial and supporting cells, as well as signals for activation of cells and prevention of apoptosis. The results of clinical trials have been encouraging, however either no change or only small increments in recovery were found. Recent reviews of completed clinical trials (2002–2010) for SC therapy report improvements of 10% or less in about half of the studies.1–4 In the review by George,1 13 studies of SC therapy for acute MI were described. In the eight randomized controlled studies, bone-marrow (BM) cells were administered by intracoronary injection and left ventricular ejection fraction (LVEF) measured 3–6 months following the MI. In five of the randomized controlled trials, there was only an average increase of 6% (3%–12%) in cardiac function. Mozid et al2 reported two additional studies of BM SC therapy for acute MI,5,6 and only one study showed improvement (5%) of LVEF function. Mozid et al2 also described eight clinical trials of SC therapy for chronic ischemic heart failure. There was improvement in LVEF in three of the four studies in patients treated with BM SCs and improvement in two
of the four studies in patients transplanted with autologous skeletal myoblasts. Wen et al performed a meta-analysis of eight randomized controlled trials and concluded that BM cell therapy provided only moderate (6%–10%) but definite improvements in LVEF. SC therapy has the potential to provide gains not only for MI, but also for chronic ischemia and heart failure. Currently, there are 33 ongoing clinical trials described on the ClinicalTrials.gov Website (see Table 1). While autologous BM cells are still the major source of SCs in the ongoing studies, new SC sources are rigorously being investigated. SC therapy for cardiovascular disease (CVD) is an intensive area of research, and collective improvements in the source and number of SCs, and better mobilizing and homing agents, are needed to increase the effectiveness of this emerging therapy.

Challenges for SC therapy

Improved identification and expansion of autologous SCs and their role in cardiac recovery

In the 1960s, Till et al, while studying the components responsible for regenerating blood cells, defined two required properties of SCs: (1) self-renewal – the ability to go through numerous cycles of cell division while maintaining the undifferentiated state; and (2) potency – the capacity to differentiate into specialized cell types. SCs are identified by their capacity to form colonies in culture and by cell surface markers that are cell specific. The majority of clinical trials of SC therapy for heart disease have used BM cells, particularly the mononuclear cells (MNCs) (Figure 1). In the ongoing trials listed

| Condition                          | Stem cells            | Phase | Acronym     | ClinicalTrials.gov NCTID |
|-----------------------------------|-----------------------|-------|-------------|--------------------------|
| Congestive heart failure          | Skeletal myoblasts    | II/III| MARVEL     | NCT00526253              |
| Old MI                            | Skeletal myoblasts    | II    | PERCUTANE   | NCT00908622              |
| Angina, coronary disease          | Bone marrow           | II    | REPAIR-ACS  | NCT00711542              |
| Ischemic heart disease            | Bone marrow           | II    | REGEN-AMI   | NCT00765453              |
| CAD, AMI                          | Bone marrow           | II    | REGEN-AMI   | NCT00747708              |
| MI, ischemia                      | Bone marrow           | II    | REGEN-AMI   | NCT01167751              |
| MI                                | Bone marrow/AC 133    | III   | POSEIDON-DCM| NCT01392625              |
| Chronic ischemic heart failure    | Bone marrow           | II    | POSEIDON-DCM| NCT01350310              |
| MI                                | Bone marrow           | II    | REGENERATE-DCM| NCT01302171            |
| Ischemic heart failure            | Bone marrow/PBC       | III   | ESCAPE      | NCT008419958             |
| Left ventricular dysfunction      | Bone marrow           | II    | TIME        | NCT00684021              |
| Left ventricular dysfunction      | MSC, bone marrow      | II    | TAC-HFT     | NCT00780666              |
| Ischemia, left ventricular dysfunction | Mesenchymal precursors | II    | MESAMI      | NCT01076920              |
| MI                                | Mesenchymal precursors | II    | ESTIMATION  | NCT00555828              |
| AMI, heart failure                | MSC                   | III   | ESTIMATION  | NCT01394432              |
| Chronic ischemic heart disease    | MSC                   | II    | My StromalCell| NCT01449032            |
| Congestive heart failure          | MSC                   | II    | My StromalCell| NCT00644410            |
| Dilated cardiomyopathy            | CD34+                 | II    | SELECT-AMI  | NCT00529932              |
| AMI                               | CD133+                | II    | PERFECT     | NCT00950274              |
| MI, CAD                           | CD133+                | II    | PERFECT     | NCT00950274              |
| CAD                               | CD133+                | II    | PERFECT     | NCT01049867              |
| MI, heart failure                 | CD133+                | II    | IMPACT-CABG | NCT01033817              |
| AMI                               | Adipose tissue-derived| II    | ADVANCE     | NCT01216995              |
| Heart failure                     | Cardiac progenitor     | I     | TICAP       | NCT01273857              |
| Congestive heart failure          | Cardiac               | I     | ALCADIA     | NCT00981006              |
| MI                                | Cardiospheres         | I     | CADUCEUS    | NCT00893360              |
| CAD, congestive heart failure     | Cardiac               | I     | SCIPIO      | NCT0047461               |

Source: ClinicalTrials.gov Website.7
Abbreviations: AMI, acute myocardial infarction; CAD, coronary artery disease; MI, myocardial infarction; MSC, mesenchymal stem cell; PBC, peripheral-blood cell.
in Table 1, other types of SCs are being tested, including specific BM, CD34+ or CD133+, and mesenchymal cells. One study tests adipose tissue-derived SCs, and three trials are testing cardiac progenitor/stem cells.

### Skeletal myoblasts

Skeletal myoblasts isolated from muscle biopsies were the first cells used for the SC therapy for cardiac recovery. In a comparison of rats with chronic MI, treated with human skeletal myoblasts or BM-derived CD133+ progenitors, improvements in cardiac function were similar with the two cell types. In trials of skeletal myoblast treatment in patients with chronic ischemic heart failure, there were improvements in LVEF in two of four studies (SEISMIC, TOPCARD-CHD). While the initial evaluation in cardiac studies of skeletal myoblast treatment showed there was improved function, the effect was not sustained, and the cells were not electrically integrated into the heart. Enthusiasm for this approach has waned. However, second-generation products are now being developed. Six trials of skeletal myoblast therapy have been discontinued, but currently there are two active trials with skeletal myoblasts (Table 1) for patients with an old MI (PERCUTANEO) or congestive heart failure (MARVEL).

### Hematopoietic progenitor/stem cell (HPSCs)

In clinical trials for MI or ischemic heart disease, BM, peripheral blood (PB), or umbilical cord blood (UCB) have been used as the source of SCs. Autologous BM and PB have an advantage over UCB cells since UCB cells may be at risk for immunological rejection. However, the UCB have a high proliferation potential. Autologous BM cells from aging individuals may have reduced transplant efficiency, and UCB cells would be advantageous. A limitation of the PB is the low yield of SCs. BM is the major source of SCs for reported and ongoing clinical trials. Currently, studies are underway that isolate subsets of the

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**Figure 1** Types of stem cells in use for heart disease therapy. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2011–2012. All Rights Reserved.
BM cells such as CD34+, and CD133+ for use in therapy. Whether these subsets of SCs will have an advantage in heart disease recovery remains to be seen.

Endothelial SCs

Stages of lineage development of endothelial SCs and their sites of origin are less well defined than those for the hematopoietic lineage.20 The endothelial progenitor cells (EPCs) found in the PB are thought to originate in the BM from a subset of SCs or from the myeloid precursors. There is considerable controversy with regard to the identification of the EPCs.21 Some investigators have identified the EPCs as CD34+ cells and/or CD133+ cells,22 while others view these cells as HPSCs.23,24 Recently,25,26 a consensus definition of EPC markers was suggested for cross-study comparisons and with the cell surface markers CD31+, CD34 bright, and CD45, AC133, CD14, CD14a, CD235a, Live/Dead Violet negative. Of importance for identification of the EPC is the ability to become endothelial cells (ECs) in culture. While CD34+ and/or CD133+ cells in culture may become ECs, the CD34+ and/or CD133+ cells could be a mixture of subpopulations. However, the cells identified as CD34+ and/or CD133+ may be more effective in providing paracrine factors and stimulating neovascularization than the commonly used BM MNCs. Tongers et al27 recently described the results of a clinical trial for patients with refractory angina treated with intramyocardial autologous CD34+ cells, finding significant improvements in angina frequency and exercise tolerance. There is one clinical trial currently underway for treatment with CD34+ in patients with dilated cardiomyopathy, and five clinical trials underway for the treatment of MI, CAD, and heart failure with CD133+ cells. One study, NCT01187654, will compare the treatment of CD133+ cells and BM MNC in MI patients. This comparison could be informative as to whether the CD133+ cells have an advantage over the more frequently used BM MNC. Bissels et al28 found that microRNAs were expressed differentially in CD133+, CD34+, and CD133- cells involved in differentiation, prevention of apoptosis, and cytoskeletal remodeling.

Mesenchymal SCs (MSCs)

The MSCs are found in the BM and other tissues. MSCs are positive for CD44, CD73, CD90 (Thy1), and CD105, and negative for the hematopoietic markers, CD45, lineage markers, EC (CD31), and macrophage (CD11b/MAC-1).29 The MSCs have advantages over HSCs.27,30 Compared with HSCs, MSCs are more abundant, readily proliferate in culture, and are easily differentiated into different cell types, such as adipocytes, fibroblasts, osteocytes, and myoblasts. Further, studies suggest that MSCs may be more potent for cardiac repair than HPSCs.31 Although the MSCs can be differentiated into cardiomyocytes, immortalization was important and could increase the potential of tumor formation.15 In addition to BM, adipose tissue can also be used as an abundant source of MSCs.32,33 The MSCs from UCB, adipose tissue, and BM expressed the same cell surface markers; however, there are some differences in the percentage of certain markers and colony heterogeneity. Gaebel et al34 compared treatment of MI in mice with MSCs from UCB, adipose tissue, and BM. Cells from BM, adipose tissue, and UCB CD105+ showed improvements in heart functions, decreased infarct size, and capillary density. UCB CD105 treated mice had reduced collagen deposition compared with BM and adipose tissue cells, and BM and UCB CD105 cells additionally had reduced apoptosis when compared with mice treated with adipose tissue cells. This study suggests that the function of the MSCs may be dependent on the source. Clinical trials with MSCs35–37 are promising, and currently there are 19 clinical trials underway.38 In a recent randomized, double blind, placebo-controlled study37 with MSC therapy after acute MI; there was improvement in the global assessment of cardiac function at 6 months in 45% of the patients.

Cardiac progenitor cells (CPCs)

Although it had been believed for a long time that cardiac myocytes were terminally differentiated, dividing myocytes found in the heart implied that there are resident or noncardiac cardiomyocyte progenitor cells.39 There have been intensive efforts to identify the cardiomyocyte stem and progenitor cells in the last 10 years.39 Purified cardiomyocytes isolated from rodent hearts dedifferentiate and divide, expressing SC markers such as c-kit, Sca-1, Is1, and Abcg2.40–43 CPCs have been isolated from human myocardial biopsies.46,47 These same cells can organize into spheres and re-differentiate into myocytes and ECs.48 Yamada et al49,50 suggested that CD133+ cells from brown adipose tissue were highly effective in differentiation into cardiomyocytes compared with HPSCs, and that mouse BAT CD133+ cells efficiently induced BM SCs into cardiomyocytes (CD45- CD31- CD105+) differentiation. There are four ongoing clinical studies to test autologous CPCs (Table 1); one study (ALCADIA) will use cardiac-derived SCs to treat ischemic cardiomyopathy, and two studies will take advantage of the cardiosphere-derived stem/progenitor cells (derived from cell outgrowth of autologous cardiac biopsy) for patients with a recent MI (CADUCEUS) or heart failure (TICAP). In the SCIPIO trial, patients with
ischemic cardiomyopathy are treated with c-kit+lin− CPCs derived from the right atrial appendage, and initial results from 16 patients report that LVEF increased and infarct size decreased.51

Adipose tissue-derived SCs (ASCs)

Cells isolated from adipose tissue can be separated by centrifugation into adipocytes and stromal vascular cells. The stromal vascular fraction may contain preadipocytes, pericytes and EPCs, adult multipotent MSCs, circulating blood cells, fibroblasts, ECs, smooth-muscle cells, and immune cells. This stromal vascular fraction may differentiate into a number of cell lineages, including the adipocytes, cartilage, bone skeletal muscle, neuronal cells, ECs, cardiomyocytes, and smooth-muscle cells.52,53 ASCs are defined as CD44 and CD105 positive, and Cd11b, CD34, and CD45 negative cells. Although there is disagreement regarding the capacity of ASCs to differentiate into ECs, freshly isolated human ASCs also consist of EPCs (CD11b, CD34, and CD45 positive cells) and when cultured they have a cobblestone appearance and take up acetylated low-density lipoprotein. Bai et al54 found that human freshly isolated adipocytes or cultured adipose tissue-derived cells underwent cardiomyogenesis through a fusion-independent pathway. Takahashi et al55 reported that in rat femoral artery injury, ASCs did not differentiate into ECs, but were able to inhibit neointimal formation by the secretion of paracrine factors. There is one ongoing clinical trial (NCT01216995) testing adipose tissue-derived cells in patients after an acute MI.

Induced pluripotent stem (iPS) cells

Another potential source of SCs is iPS cells.56 This source relies on in vitro de-differentiation of adult cells to embryonic-like SCs and then reprogramming using specific culture conditions to induce cardiac lineage cells including cardiomyocytes, smooth-muscle cells, and ECs. Adult cells most commonly used for iPS cells are fibroblasts and may be derived from a variety of tissues such as dermal, liver, stomach, pancreas, and neural and hematopoietic cells. Endogenous non-BM SC and iPS cells have been characterized in animal models and some have been identified in adult humans. Defining these cells and their requirements for proliferation and mobilization will provide additional options for enhanced efficacy of SC therapy.

Embryonic SCs (ESCs)

The ESCs are the ideal SCs, due to the fact that cultures of embryonic cells when stimulated can develop into >200 adult cell types.58,57,59 Current efforts focus on establishing the conditions for directed differentiation of cells by altering the chemical composition of the culture medium, altering the culture surface, or inserting genes.59 A major challenge is the potential of uncontrolled differentiation when injected directly into an animal, and the potential for tumor formation. The promise of ESCs is to genetically modify lethal debilitating chronic disease. There are currently four clinical trials in progress of human ESCs for spinal cord injury and macular degeneration, but unfortunately none for cardiac disease.58

Expansion of SCs

A critical step for improved SC therapy is the expansion of accessible SCs (Figure 2). The homing of cells to injured tissues is very inefficient, and increasing the number of cells that are available for treatment would be beneficial. Autologous BM cells, adipose tissue, myocardial, and UCB are cultured ex vivo to increase the number of cells. Culturing the tissue also allows selection of specific cells. The ESCs and iPS cells require additional steps prior to expansion of a preparation. The iPS cells require de-differentiation as an initial step and then both iPS cells and ESCs are induced to differentiate prior to expansion. SCs in culture form colonies, and proliferation without differentiation requires a specific sequence and timing of the availability of growth factors and cytokines.59–66 In addition, these cells must maintain their pluripotency. Cells need to be free of feeder-cells, serum proteins, and microbial agents. Large-scale expansion with maintenance of pluripotency and transplant safety is required.58,67 Currently, effective cell culture proliferation is limited,61 and further studies are needed to understand the requirements for expansion. New approaches are being investigated including the use of nanofibers with growth factors, mesenchymal stromal cells in cultures of HSCs, and genetic manipulation of UCB HSC.68–72 To improve SC therapy, improved methods of SC ex vivo expansion are required.

Identify mobilizing agents with improved effectiveness SC niches

Intensive studies are underway to identify new sources of stem and progenitor cells for therapy. In addition to BM, SC niches have been identified (Figure 3) in heart. The SC niches are defined as a microenvironment with one or more SC that regulates self-renewal and progeny in vivo.73,74 Self-renewal occurs in all tissues and in addition to BM, niches of SCs have been identified in heart, arteries, veins, gonads, intestine, epidermal tissue, and neural tissue.73,75–77 The
non-BM SCs were initially defined by immunofluorescence in tissue, but given the number of markers needed, this became untenable, and isolation and identification of SCs by flow cytometry using multiple markers simultaneously has made it possible to isolate and investigate the function of these cells. Recently, lineage mapping has been utilized to locate niches in animal models by genetically labeling SC markers and identifying their location in adult tissue. An example of lineage mapping is the recent study of Tamura et al of neural crest-derived SCs found in the heart that migrate and differentiate into cardiomyocytes after MI. The lineage mapping has been utilized for locating SC niches in a variety of developing organisms. The number of quiescent SCs is small, and better detection methods are necessary. Further, identifying the regulation and recruitment of these endogenous SCs in adults is critical.

Mobilization of BM SCs
In the BM, SCs reside in an endosteal niche along with stromal cells, mesenchymal cells, and ECs. The SCs are retained in the BM with high concentrations of stromal-derived factor (SDF)-1, the major chemoattractant for SCs. The SDF-1 SC receptor, CXCR4, is found in low concentrations. Stimulation with cytokines or growth factors may interrupt ligand/receptor balance. With a decrease in SDF-1 and an increase in CXCR4 expression, a signaling gradient with the PB allows the egress of the SCs from the BM (Figure 4). Granulocyte colony-stimulating factor (G-CSF) is widely used clinically for SC mobilization and sometimes in conjunction with other factors including granulocyte-macrophage colony-stimulating factor, stem cell factor, fms-like tyrosine kinase (Flt)-3 ligand, and interleukin-1, -3, -6, -7, -8, -11, and -12 (Figure 3). AMD3100, an inhibitor that blocks SDF-1 binding to CXCR4; CTCE-0021, a CXCR4 agonist; recombinant human growth hormone, a pleiotrophic cytokine; parathyroid hormone; pegfilgrastim, pegylated G-CSF with a prolonged half-life, and thrombopoietin, a cytokine that regulates megakaryopoiesis, are also being investigated.

In addition to cytokines and growth factors, proteases such as neutrophil elastase, cathespin G, plasmin, and matrix metalloproteinase (MMP)-9 have been implicated in BM SC mobilization. After G-CSF treatment, these proteases increase in BM as well as in plasma; however, studies in mice deficient in neutrophil elastase or cathespin G suggest...
these two proteases are not required for HPSC mobilization. The results of studies in MMP-9 deficient mice are not consistent. While some studies report MMP-9 is not required, other studies suggest MMP-9 plays an important role. These differences may be due to the differences in genetic background of the mice and to differences in the dose of the mobilizing agent. In a recent study, the authors of this present paper report that plasmin/MMP-9 is a major proteolytic pathway required for SC mobilization from BM (Figure 5). Plasmin activation of MMP-9 regulates the SDF-1/CXCR4 signaling. In addition, plasmin also promotes direct degradation of the ECM during SC mobilization. G-CSF induced HSC MMP-9 degrades BM SDF-1. The increase in the number of SC mobilized with G-CSF treatment may not be sufficient for the cardiac remodeling after MI, and some patients are resistant to G-CSF. AMD3100, an inhibitor of CXCR4, is a promising HSC mobilizer under clinical investigation. Studies report mild and reversible side effects and that it works synergistically with G-CSF to increase CD34+ cells and total white blood count. However, Dai et al recently reported that chronic AMD100 exacerbates cardiac dysfunction after MI in mice.

Mobilization of CPCs

A number of cardiomyocyte progenitor pools have been identified that have common and unique markers, including: side population (SP) CPCs; c-kit+ CPCs; Sca-1+ CPCs; cardiospheres and cardiosphere-derived cells; stage specific-embryonic antigen-1+ (SSEA-1+) CPCs; LIM-homeodomain transcription factor+ (Islet-1+) CPCs; and epicardium-derived cells. The CPCs demonstrate greater proliferation potential in the infarct border compared with the necrotic core. These cells have the potential to differentiate into cardiomyocytes, smooth-muscle cells, and ECs, but the stimulatory factors for differentiation vary. The SP CPCs can be stimulated by SDF-1 and are both c-kit and Sca-1 positive, but are also positive for the ATP-binding cassette transporter (ABCG2). The cardiac SP cells are a mixture of subpopulations, and proof that these cells are SC is not definitive. The c-kit marker was used to identify and isolate HSCs, but their ability to differentiate into cardiomyocytes is controversial. Cells positive for c-kit isolated from human and rodent tissue express specific cardiac transcription factors, GATA4, GATA5, MEF2C, and KxX, and when cultured express mature cardiomyocyte markers, cardiac actinin, cardiac myosin, desmin, and connexin 43.

**Figure 3** Stem cell mobilization and homing. Growth factors and cytokines stimulate the mobilization of the stem cells from their niche to injured tissue. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2011–2012. All Rights Reserved. Notes: Flt-ligand is a growth factor; interleukins refer to interleukin-1, -3, -6, -7, -8, -11, and -12 cytokines; homing factors MCP-3, GRO-1, HGF, FGF-2, and IGF-1 are produced in the heart and promote endogenous and exogenous stem cells homing to the injured tissue; survival and implantation of stem cells in the tissue may result in differentiation, secretion of paracrine factors, and/or stimulation of angiogenesis to restore blood flow and remodel tissue. Abbreviations: G-CSF, granulocyte colony-stimulating factor; Gm-CSF, granulocyte-macrophage colony-stimulating factor; SCF, stem cell factor/c-kit ligand; SDF-1, stromal cell-derived factor 1; MCP-3, monocyte chemotactic protein-3; GRO-1, growth regulated oncogene 1; HGF, hepatic growth factor; FGF-2, fibroblast growth factor; IGF-1, insulin-like growth factor.
The CPCs may be stimulated by insulin-like growth factor-1 (IGF-1); hepatic growth factor (HGF); high-mobility group box protein-1 (HMGB1), a chromatin-binding protein secreted by necrotic cells, and SDF-1. The CPCs possess growth factor receptors and when activated increase proliferation, migration, and differentiation. Tamoxifen-treated double-transgenic mice expressed dedifferentiated cardiomyocytes that expressed CPC markers and ~2/3 expressed c-kit. Studies in zebrafish and mammalian development suggest the potential of the epicardium-derived cells, the epithelial cells in the outermost layer of the heart, to develop into cardiomyocytes in vivo. Smart et al reported that in mouse heart, thymosin β4 can release the quiescent EPDCs. Development of small molecules to release the cells is underway. Isl1+ CPCs are prominent during development, and in the postnatal rat, mouse, and human myocardium, Isl1+, c-kit+, Sca-1+, and CD31- cells have been defined as cardioblasts. Both iPSC and ESCs give rise to this lineage in vivo. The Isl1+ cells are rare in the myocardium and the possibility of endogenously recruiting or in vitro expansion appears to be limited. The SSEA-1+ CPCs give rise to myocardial and endocardial cells during development in the neonatal and adult rat heart, but can progress to more committed c-kit+, Sca-1, and abcg2+ cells. When transplanted into rat heart, improved regeneration of infarcted myocardium results. Sca-1+ CD31+ cells are found in the heart as small interstitial cells that lack the HSC lineage markers of c-kit, Flt-1, Flk-1, CD45, and CD34. Using transgenic mice, cardiac Sca-1+ cells were found to play a role in the regulation the signaling required for efficient myocardial regeneration. Studies with ESCs and their requirements for cardiomyocyte differentiation may shed light on the factors that induce differentiation and proliferation of the endogenous CPCs. A better understanding of SC mobilization from cardiogenic niches may lead to more effective agents for not only recruiting cells for ex vivo expansion, but to mobilize endogenous sources.

**Figure 4** Bone marrow-derived stem cell mobilization. Bone marrow stem cells may be mobilized by reducing the ligand SDF-1 and increasing the stem cell receptor CXCR4 to create a chemotactic gradient with the peripheral blood. G-CSF treatment increases MMP-9 to regulate changes in SDF-1/CXCR4 pathway, which is dependent on plasmin activation of MMP-9.

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**Abbreviations:** CXCR4, C-X-C receptor 4; G-CSF, granulocyte colony-stimulating factor; MMP-9, matrix metalloproteinase-9; SDF-1, stromal-derived factor-1.

**Strategies for improving SC homing, survival, and engraftment in the injured heart**

**SC delivery**

Available routes of SC delivery include intravenous, intracoronary, epicardial, endocardial, and coronary sinus injection. While the intravenous injection of SCs is the least invasive
method of delivery, retention of cells in the lungs is problematic. After an MI, intracoronary injection through a catheter is the preferred method of delivery. The epicardial and transendocardial are more invasive, but the most reliable. The transendocardial administration uses a percutaneous catheter-based approach. The coronary sinus delivery provides access to the infarcted and ischemic tissue, but may not be available to all patients. In the clinical trials, SCs were delivered by either bolus or multiple intracoronary injections, but only a small percentage reached the heart. At least 90% of injected cells die by apoptosis. Alternative methods of delivery are being investigated, such as use of biodegradable scaffold-based engineered tissue. An advantage is the variable size, but problematic issues are thickness of the patch and toxicity of the degraded material. Only limited improvement in cardiac function has been noted. A recent study tested sheets of cardiomyocytes progenitor cell and reported an increase in cardiogenesis and improved function. The development of safe and more effective materials for use in SC delivery is necessary.

**Homing**

Homing is the migration of SCs from endogenous and exogenous sources through the blood or tissue to a destination where they

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**Figure 5** Plasminogen regulates CXCR4 after G-CSF stimulation. (A) CXCR4 immunostaining of bone marrow from Plg+/+ and Plg−/− mice treated with saline (control) or G-CSF. CXCR4 expressing cells (brown color) increased two fold after G-CSF treatment in Plg+/+ mice, but CXCR4 did not change in Plg−/− mice. (B) Lentivirus expression of act MMP-9 in Plg−/− restored CXCR4 expression. Plasminogen activation of MMP-9 is required for CXCR4 expression after G-CSF treatment.

**Note:** Reproduced with permission from Gong Y, Fan Y, Hoover-Plow J. Plasminogen regulates stromal cell-derived factor-1/CXCR4-mediated hematopoietic stem cell mobilization by activation of matrix metalloproteinase-9. Arterioscler Thromb Vasc Biol. 2011;31(9):2035–2043.

**Abbreviations:** CXCR4, C-X-C receptor 4; G-CSF, granulocyte colony-stimulating factor; MFI, mean fluorescence intensity; MMP-9, matrix metalloproteinase-9; Plg, plasminogen.
Survival/engraftment
Survival and engraftment of SCs is perhaps the most important challenge for SC therapy, and the factors necessary for effective survival and engraftment are not necessarily the same as those required for homing. After an MI, there is an enormous loss of cardiomyocytes and supporting cells that need to be replaced. The environmental signals that may guide SCs to the cardiomyocyte lineage or to the secretion of paracrine factors may be absent in the infarcted tissue, and SCs may provide these signals. Many studies have focused on strategies to optimize SC migration through injured myocardial tissue. Proteases, adhesion molecules, and integrins are important in regulating SC migration through injured myocardial tissue and modulation of the connective tissue microenvironment to improve SC engraftment.133–136

Several proteases have been identified to have significant effects on SC mobilization or SC migration and engraftment in cardiac tissue. SDF-1 and other factors induce the secretion of matrix metalloproteinase MMP-2 and MMP-9.137–139 Of significant interest, proteolytic enzymes, including neutrophil elastase, cathepsin G, and MMP-2/9, also negatively regulate cell migration by cleaving the N-terminal region of SDF-1 or cleaving CXCR4.90,139–142 Those proteolytic enzymes are involved in spatial temporal changes in the locomotion machinery of SCs, thus mediating SC recruitment and engraftment.

Integrins are also key factors for adhesion, rolling and transmigration of SCs across the endothelium. The HSCs express several adhesion molecules including multiple integrins. In particular, a dominant role for the α4β1 integrin very-late antigen [VLA]-4 interaction with vascular cell adhesion molecule (VCAM)-1 has been suggested by studies in which exposure to blocking antibodies to VLA-4 or VCAM-1 significantly reduced the engraftment of transplanted HSCs.143–145 CD18 expression by the EPCs is necessary for its interaction with EC surface ICAM-1, and a CD18 neutralizing antibody significantly inhibits SC engraftment after acute MI.146 These studies suggest the potential targets for the genetic enhancement of SC recruitment and engraftment.

Several other strategies have been proposed: identifying natural mediators; pre-translational directed differentiation of SCs to cardiomyocytes; activation of growth factors (FGF-2, IGF-1a)132 and antiapoptotic factors (p-Akt, SDF-1, BCl-1, and PDGF); and genetically engineered SCs.125,132 The challenge to improve survival in SC therapy is to identify effective ways to increase the number of cells that reach and survive in the injured heart area.

Assessment of SC therapy
The goals of SC therapy are to: replace lost cardiomyocytes; increase ECs to improve blood flow; provide paracrine cytokines and growth factors; and improve measurable cardiac function, including an increase in LVEF; decrease left ventricular end-diastolic diameter; increase myocardial perfusion; and importantly increase exercise capacity. In clinical trials, methods to measure cardiac function include echocardiography, single photon emission computed tomography, and magnetic resonance imaging (MRI).1,3,7,147–149 These methods are well established, but more sensitive methods are necessary to evaluate SC homing and engraftment. Techniques to evaluate the timing and specific role of narrow populations of cells, such as MR150–152 and SC labeling with genetic153,154 and immunofluorescence detectable tags155 are being investigated in animal models. The lineage/fate mapping156–158 has proved to be an informative tool, and further studies in animal models and ex vivo SC labeling of cells for therapy will continue to be valuable.

Conclusion
SC therapy is an exciting and dynamic area of research with the potential to improve recovery of CVD, the leading cause of
death. While animal models clearly show benefits of SC therapy to improve cardiac function after MI and ischemic heart failure, clinical trials have been disappointing. However, the results of clinical trials are promising. Better methods are needed to improve the isolation and identification of SCs, increase ex vivo expansion of SCs, and increase delivery effectiveness. A clearer understanding of mobilization and homing of SCs is needed to identify new and more effective agents. Delineating the function of specific SCs in remodeling injured tissue and how resident cardiac SCs may be enhanced is needed to improve SC engraftment and survival.

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Disclosure

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