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## **Clinical Applications of Stem Cells for the Heart**

Kai C. Wollert and Helmut Drexler

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This Review is part of a thematic series on **Stem Cells**, which includes the following articles:

Differentiation of Pluripotent Embryonic Stem Cells into Cardiomyocytes

Derivation and Potential Applications of Human Embryonic Stem Cells

Stem Cells for Myocardial Regeneration

Myocyte Death, Growth, and Regeneration in Cardiac Hypertrophy and Failure

Neural Stem Cells: An Overview

Mesenchymal Stem Cells and Their Potential as Cardiac Therapeutics

Clinical Applications of Stem Cells for the Heart

*Toren Finkel, Roberto Bolli, Editors*

## Clinical Applications of Stem Cells for the Heart

Kai C. Wollert, Helmut Drexler

**Abstract**—Repair of the heart is an old dream of physicians caring for patients with cardiac disease. Experimental studies suggest that cardiac transfer of stem and progenitor cells can have a favorable impact on tissue perfusion and contractile performance of the injured heart. Some researchers favor stable stem cell engraftment by fusion or transdifferentiation into cardiomyocyte or vascular cell lineages as likely explanations for these beneficial effects. Others have proposed that transient cell retention may be sufficient to promote functional effects, eg, by release of paracrine mediators. Although the mechanistic underpinnings of stem cell therapy are still intensely debated, the concept of cell therapy has already been introduced into the clinical setting, where a flurry of small, mostly uncontrolled trials indicate that stem cell therapy may be feasible in patients. The overall clinical experience also suggests that stem cell therapy can be safely performed, if the right cell type is used in the right clinical setting. Preliminary efficacy data indicate that stem cells have the potential to enhance myocardial perfusion and/or contractile performance in patients with acute myocardial infarction, advanced coronary artery disease, and chronic heart failure. The field now is rapidly moving toward intermediate-size, double-blinded trials to gather more safety and efficacy data. Ultimately, large outcome trials will have to be conducted. We need to proceed cautiously with carefully designed clinical trials and keep in mind that patient safety must remain the key concern. At the same time, continued basic research to elucidate the underlying mechanism of stem cell therapy is clearly needed. (*Circ Res.* 2005;96:151-163.)

**Key Words:** stem cells ■ myocardial infarction ■ myocardial ischemia ■ clinical trials

The dogma of the heart as an organ composed of terminally differentiated myocytes incapable of regeneration is being challenged. Evidence has been presented that a fraction of cardiomyocytes may be able to reenter the cell-cycle and that limited regeneration can occur through recruitment of resident and circulating stem cells.<sup>1</sup> Some clinicians may regard these new ideas as being mere curiosities, because of their everyday experience that endogenous repair mechanisms are overwhelmed in patients with acute

myocardial infarction (AMI), advanced coronary artery disease, and chronic heart failure. However, the existence of endogenous repair mechanisms suggests that cardiac repair may be achieved therapeutically in these clinical settings. Evidence to support this hypothesis will be reviewed in this article.

Another concept that has recently generated excitement by some, but disbelief by others, is the concept of adult stem cell plasticity.<sup>2,3</sup> Stem cells are capable of self-renewal, transfor-

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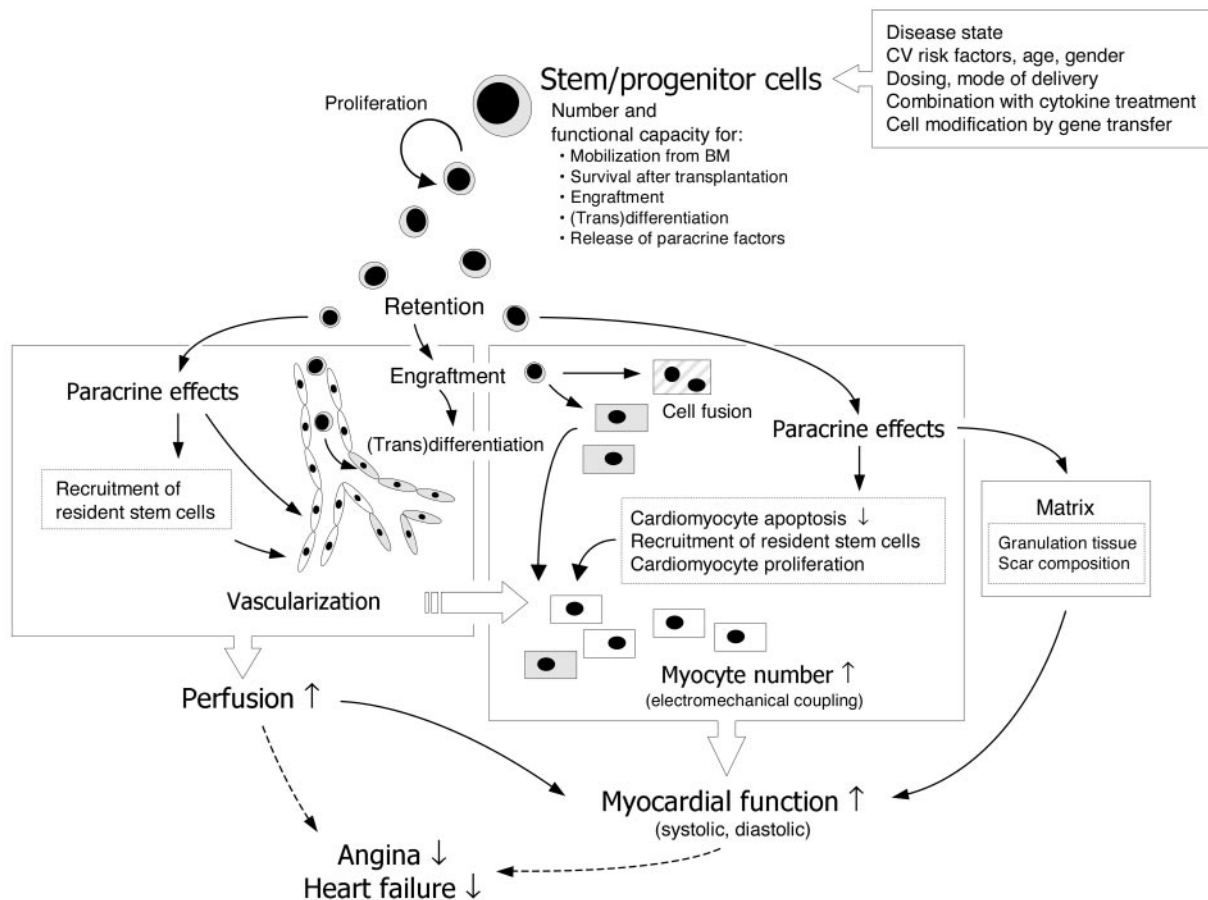
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Working hypothesis of therapeutic stem cell transplantation for myocardial regeneration. Stem and progenitor cell transplantation can have a favorable impact on tissue perfusion and contractile performance by promoting vascularization and myocyte formation. Improved vascularization may facilitate beneficial effects in the myocyte compartment. Depending on the stem cell type and local milieu, the relative contribution of cell incorporation (transdifferentiation and/or fusion) vs paracrine effects may vary. Stem and progenitor cell numbers and functional capacity are influenced by a patient's age, gender, cardiovascular risk factors, and underlying disease state (see text for details).

mation into dedicated progenitor cells, and differentiation into specialized progeny. Traditionally, tissue-resident adult stem cells were believed to differentiate into progeny only within tissue lineage boundaries. Plasticity implies that stem cells can transdifferentiate into mature cell types outside their original lineage in response to microenvironmental cues. For example, hematopoietic stem cells (HSCs), when transplanted into the (murine) myocardium, may transdifferentiate into cardiomyocytes and blood vessels, thereby improving heart function and survival.<sup>4</sup>

Ironically, although cell therapy is already being introduced into the clinical setting, fusion of transplanted stem cells with resident cardiomyocytes has been offered as an alternative explanation for previous claims of transdifferentiation.<sup>5,6</sup> Moreover, the mechanistic underpinnings of stem cell therapy appear to be far more complex than previously anticipated. It has been proposed that stem cells release angiogenic ligands, protect cardiomyocytes from apoptotic cell death, induce proliferation of endogenous cardiomyocytes, and may recruit resident cardiac stem cells (Figure).<sup>7-11</sup> Regardless of the mechanisms, there appears to be general agreement that stem cell therapy has the potential to improve perfusion and contractile performance of the injured heart.<sup>4,7-9,11,12</sup>

### Potential Donor Cells

Conceptually, a variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings. Studies comparing the regenerative capacity of distinct cell populations are scarce. Many investigators have therefore chosen a pragmatic approach by using unfractionated bone marrow cells (BMCs),<sup>13-24</sup> which contain different stem and progenitor cell populations, including HSCs, endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs). Ease of harvest and lack of extensive requirement for ex vivo manipulation are additional advantages of using unselected BMCs.

### Endothelial Progenitor Cells

EPCs have originally been defined by their cell surface expression of the hematopoietic marker proteins CD133 and CD34 and the endothelial marker vascular endothelial growth factor receptor-2, and their capacity to incorporate into sites of neovascularization and to differentiate into endothelial cells in situ.<sup>25</sup> Increasing evidence suggests that culture-expanded EPCs also contain a CD14<sup>+</sup>/CD34<sup>-</sup> mononuclear cell population with "EPC capacity," which mediates its

angiogenic effects by releasing paracrine factors.<sup>26,27</sup> Notably, EPC numbers and their angiogenic capacity are impaired in patients with coronary artery disease, which may limit their therapeutic usefulness.<sup>28,29</sup>

### **CD133<sup>+</sup> Cells**

The cell surface antigen CD133 is expressed on early HSCs and EPCs, both of which collaborate to promote vascularization of ischemic tissues.<sup>30</sup> CD133<sup>+</sup> cells can integrate into sites of neovascularization and differentiate into mature endothelial cells. Because CD133 expression is lost on myelomonocytic cells, this marker provides an effective means to distinguish “true” CD133<sup>+</sup> EPCs from EPCs of myelomonocytic origin.<sup>26</sup> Less than 1% of nucleated BMCs are CD133<sup>+</sup>, and because these cells cannot be expanded *ex vivo*, only limited numbers of CD133<sup>+</sup> cells can be obtained for therapeutic purposes.

### **Mesenchymal Stem Cells**

MSCs represent a rare population of CD34<sup>-</sup> and CD133<sup>-</sup> cells present in bone marrow stroma (10-fold less abundant than HSCs) and other mesenchymal tissues.<sup>31</sup> MSCs can readily differentiate into osteocytes, chondrocytes, and adipocytes. Differentiation of MSCs to cardiomyocyte-like cells has been observed under specific culture conditions and after injection into healthy or infarcted myocardium in animals.<sup>32–34</sup> When injected into infarct tissue, MSCs may enhance regional wall motion and prevent remodeling of the remote, noninfarcted myocardium.<sup>34,35</sup> Little is known about the effects of MSCs on myocardial perfusion. It is interesting to note however, that cultured MSCs secrete angiogenic cytokines, which improve collateral blood flow recovery in a murine hind limb ischemia model.<sup>10</sup> Because MSC clones can be expanded *in vitro*, and reportedly have a low immunogenicity, they might be used in an allogeneic setting in the future.<sup>31</sup>

### **Skeletal Myoblasts**

Skeletal myoblasts, or satellite cells, are progenitor cells that normally lie in a quiescent state under the basal membrane of mature muscular fibers. Myoblasts can be isolated from skeletal muscle biopsies and expanded *in vitro*. Myoblasts differentiate into myotubes and retain skeletal muscle properties when transplanted into an infarct scar.<sup>36–39</sup> Although myotubes do not couple with resident cardiomyocytes electromechanically, myoblast transplantation has been shown to augment systolic and diastolic performance in animal models of myocardial infarction.<sup>40</sup>

### **Resident Cardiac Stem Cells**

The presence of resident cardiac stem cell (CSC) population(s) capable of differentiating into cardiomyocyte or vascular lineages suggests that these cells could be used for cardiac tissue repair.<sup>41–45</sup> Intriguingly, CSCs can be clonally expand from human myocardial biopsies.<sup>45</sup> It has been reported that intramyocardial injection of these cells after AMI in mice promotes cardiomyocyte and vascular cell formation and leads to an improvement in systolic function.<sup>45</sup> If these findings can be reproduced, CSCs hold great promise

for clinical applications, although it is conceivable that the bone marrow may contain a stem cell population with similar properties.<sup>46</sup>

### **Embryonic Stem Cells**

Embryonic stem (ES) cells are totipotent stem cells derived from the inner cell mass of blastocysts. Under specific culture conditions, ES cells differentiate into multicellular embryoid bodies containing differentiated cells from all three germ layers including cardiomyocytes. Human ES cell–derived cardiomyocytes display structural and functional properties of early-stage cardiomyocytes that couple electrically with host cardiomyocytes when transplanted into normal myocardium.<sup>47,48</sup> In theory, infinite numbers of cardiomyocytes could be obtained from human ES cell clones. However, unresolved ethical and legal issues, concerns about the tumorigenicity of the cells, and the need to use allogeneic cells for transplantation currently hamper their use in clinical studies. Eventually, nuclear transfer techniques may provide a means for generating an unlimited supply of histocompatible ES cells for the treatment of cardiac disease (therapeutic cloning).<sup>49</sup>

### **Modes of Cell Delivery**

The goal of any cell delivery strategy is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area. Retention may be defined as the fraction of transplanted cells retained in the myocardium for a short period of time (hours). The local milieu is an important determinant of cell retention, as it will influence short-term cell survival and, if a transvascular approach is used, cell adhesion, transmigration through the vascular wall, and tissue invasion.

### **Transvascular Approaches**

Transvascular strategies are especially suited for the treatment of recently infarcted and reperfused myocardium when chemoattractants and cell adhesion molecules are highly expressed.<sup>50–52</sup>

### **Intracoronary Artery Infusion**

Selective intracoronary application delivers a maximum concentration of cells homogeneously to the site of injury during first passage. Unselected BMCs, circulating blood-derived progenitor cells, and MSCs have been delivered via the intracoronary route in patients with AMI and ischemic cardiomyopathy (Tables 1 and 3). In these studies, cells were delivered through the central lumen of an over-the-wire balloon catheter during transient balloon inflations to maximize the contact time of the cells with the microcirculation of the infarct-related artery. It is unknown whether this stop-flow technique is required to enhance cell retention within the infarcted area. In the hands of an experienced operator, intracoronary delivery is relatively easy to perform within less than an hour.

### **Intravenous Infusion**

In experimental models, intravenous delivery of EPCs or MSCs has been shown to improve cardiac function after AMI.<sup>7,31,53</sup> However, homing of cells to noncardiac organs

limits the clinical applicability of this approach.<sup>54,55</sup> Indeed, in a recent study in post-AMI patients, significant myocardial homing of unselected BMCs was observed only after intracoronary stop-flow delivery but not after intravenous application.<sup>56</sup>

#### **Mobilization of Stem and Progenitor Cells**

Considering that the acutely infarcted myocardium recruits circulating stem and progenitor cells to the site of injury,<sup>7,53,57,58</sup> stem and progenitor cell mobilization by cytokines may offer a noninvasive strategy for cardiac regeneration. This concept has been tested in animal models of AMI<sup>59–63</sup> and in pilot studies in patients with AMI and chronic myocardial ischemia.<sup>64,65</sup>

#### **Direct Injection in the Ventricular Wall**

Direct injection is the preferred route for cell delivery in patients presenting late in the disease process when an occluded coronary artery precludes transvascular cell delivery (patients with chronic myocardial ischemia) or when cell homing signals are expressed at low levels in the heart (scar tissue). However, direct injection of cells into ischemic or scarred myocardium creates islands of cells with limited blood supply and may lead to poor cell survival.<sup>66</sup> Direct injection techniques are especially suited for the application of large cells, such as MSCs or myoblasts, which may cause microembolization after intracoronary delivery. Direct injection techniques have been used in patients with advanced coronary artery disease (Table 2) and in patients with ischemic cardiomyopathy (Table 3). Cell delivery by direct injection may be technically challenging in patients with AMI, particularly if cells are to be injected into the border zone of the infarct. The safety of such an approach remains to be established because perforation of the friable necrotic tissue remains a matter of concern.

#### **Transendocardial Injection**

Using an injection needle catheter advanced across the aortic valve and positioned against the endocardial surface, cells can be directly injected into the left ventricular (LV) wall.<sup>21–24,67</sup> Electromechanical mapping of the endocardial surface can be used to delineate viable, ischemic, and scarred myocardium before cell injections. Average mapping and injection procedure times between 60 and 200 minutes have been reported.<sup>21–24</sup>

#### **Transepicardial Injection**

Transepicardial cell injection has been performed as an adjunct to coronary artery bypass grafting (CABG). Transepicardial cell injection during open heart surgery allows for a direct visualization of the myocardium and a targeted application of cells to scarred areas and/or the border zone of an infarct scar. The invasiveness of this approach hampers its use as a stand-alone therapy. Conversely, the efficiency of cell transplantation may be difficult to evaluate and ascertain if CABG is performed simultaneously.

#### **Transcoronary Vein Injection**

A catheter system incorporating an ultrasound tip for guidance and an extendable needle for myocardial access has been used to deliver BMCs through the coronary veins into normal

pig myocardium.<sup>68</sup> The same approach has been used in a pilot trial in patients with ischemic cardiomyopathy to deliver myoblasts to areas of nonviable myocardium.<sup>69</sup> In contrast to the transendocardial approach, where cells are injected perpendicular to the ventricular wall, the composite catheter system delivers cells parallel to the ventricular wall and deep into the injured myocardium. However, positioning of the injection catheter in a specific coronary vein is not trivial in all cases.<sup>69</sup>

## **Clinical Applications of Stem Cell Therapy**

### **Acute Myocardial Infarction**

Modern reperfusion strategies and advances in pharmacological management have resulted in an increasing proportion of AMI survivors at heightened risk of developing adverse LV remodeling and heart failure. None of our current therapies addresses the underlying cause of the remodeling process, ie, the damage of cardiomyocytes and the vasculature in the infarcted area.

### **Experimental Background**

In one of the earliest studies, HSCs were injected into the infarct border zone after coronary artery ligation in mice. Several days later, the infarcted area was replaced by newly formed myocardium with HSC-derived myocytes and vascular structures.<sup>4</sup> Transdifferentiation to cardiomyocytes and vascular structures has also been reported after transfer of CD34<sup>+</sup> cells into mice with AMI.<sup>58</sup> Recent studies questioning that HSCs can transdifferentiate to cardiomyocytes when transplanted into infarcted murine myocardium have ignited a heated debate.<sup>12,70,71</sup> Yet, although data have been presented to support and to refute this idea, both sides agree that HSC transplantation can improve cardiac function after AMI.<sup>4,12</sup> Improvement of cardiac function has also been observed after transplantation of unselected BMCs or EPCs. Although myocyte formation did not occur, cells were shown to secrete angiogenic ligands, to incorporate into foci of neovascularization, and to improve regional capillarization and blood flow.<sup>8,53,72</sup>

### **Clinical Trial Experience**

Inspired by the exciting experimental data, several trials were initiated to test whether cell therapy is safe and feasible in patients after AMI. Some have decried the clinical trials as being premature without a more complete understanding of the underlying mechanisms,<sup>71</sup> whereas others have pointed out that the clinical trials are justified by the potential benefits of cell therapy.<sup>73</sup> All clinical studies included patients with AMI who had undergone primary angioplasty and stent implantation to reopen the infarct-related artery, and cells were infused intracoronarily by using the stop-flow balloon-catheter approach. In this regard, the clinical studies differ significantly from the animal studies, where the infarct-related artery was not reperfused and cells were directly injected into the myocardium.<sup>4,8,12,53</sup> The clinical trials may be categorized into studies using unselected BMCs or selected cell populations (Table 1).

TABLE 1. Cell Therapy Trials in Patients With Acute Myocardial Infarction

Study	[n]	Cell Type	Dose	Delivery	Time After AMI	Outcomes	
						Improved	No Change
Strauer et al <sup>13</sup>	10 treated, 10 controls*	MNC	2.8±2.2×10 <sup>7</sup>	IC	5–9 days	Regional wall motion†; Infarct size ↓; Perfusion†	Global LVEF; LVEDV†
TOPCARE-AMI <sup>14–16</sup>	29 MNC, 30 CPC, 11 controls*	MNC CPC	2.1±0.8×10 <sup>8</sup> 1.6±1.2×10 <sup>7</sup>	IC	5±2 days	Regional wall motion†; Global LVEF†; Infarct size ↓†; Coronary flow†	LVEDV†
Fernandez-Aviles et al <sup>17</sup>	20 treated, 13 controls*	MNC	7.8±4.1×10 <sup>7</sup>	IC	14±6 days	Regional wall motion†; Global LVEF†	LVEDV†
Kueth et al <sup>18</sup>	5 treated	MNC	3.9±2.3×10 <sup>7</sup>	IC	6 days		Regional wall motion†; Global LVEF†
BOOST <sup>19</sup>	30 treated, 30 controls	NC	2.5±0.9×10 <sup>9</sup>	IC	6±1 day	Regional wall motion; Global LVEF	LVEDV; Infarct size
Chen et al <sup>79</sup>	34 treated, 35 controls	MSC	4.8–6.0×10 <sup>10</sup>	IC	18 days	Regional wall motion; Global LVEF; Infarct size ↓; LVEDV ↓	
Vanderheyden et al <sup>81</sup>	12 treated, 10 controls*	CD133 <sup>+</sup>	6.6±1.4×10 <sup>6</sup>	IC	14±6 days	Regional wall motion†; Global LVEF†; Perfusion†	

MNC indicates bone marrow–derived mononuclear cells; CPC, circulating blood-derived progenitor cells; NC, bone marrow–derived nucleated cells; MSC, bone marrow–derived mesenchymal stem cells; CD133<sup>+</sup>, bone marrow–derived CD133<sup>+</sup> cells; IC, intracoronary; AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; \*Nonrandomized control groups; †Effects reported only within cell therapy groups. Study by Vanderheyden et al<sup>81</sup> has been presented in abstract form only. Values are means±SD.

### Unselected Bone Marrow Cells

The combined experience from more than 100 patients suggests that intracoronary delivery of unselected BMCs (all nucleated cells or mononuclear cell fraction only) is safe in the short- and mid-term (several months).<sup>13,14,16–19</sup> No bleeding complications were noted after bone marrow harvest. Intracoronary BMC infusions did not appear to inflict additional ischemic damage to the myocardium or to promote a systemic inflammatory reaction, because no further increases in serum troponin or CRP levels were observed. No increased rates of in-stent restenosis were observed after transfer of unselected BMCs.<sup>16,17,19</sup> It should be mentioned that one patient developed in-stent thrombosis of the target vessel three days after cell infusion. Two days later, this patient also developed in-stent thrombosis in an unrelated coronary artery and went into fatal cardiogenic shock. Although this patient may have had an intrinsic tendency to develop in-stent thrombosis, it cannot be excluded that this complication was somehow related to cell therapy.<sup>16</sup> Clinical surveillance, Holter monitoring, and data from an electrophysiological study indicate that intracoronary BMC transfer is not associated with an increased propensity to ventricular (or supraventricular) arrhythmias.<sup>13,14,16–19</sup> Direct injection of filtered nucleated BMCs into the acutely infarcted myocardium in rats has been found to induce intramyocardial calcifications.<sup>74</sup> No evidence for intramyocardial calcifications (or tumor formation) has been obtained in patients 12 to 18 months after intracoronary delivery of Ficoll or gelatin gradient-purified BMCs.<sup>16,75</sup>

Except for one study that included only five patients and no control group,<sup>18</sup> all trials indicate that intracoronary transfer of unselected BMCs enhances regional wall motion in the

infarcted area.<sup>13,14,17,19</sup> In the three largest studies, this was associated with an increase also in global LVEF.<sup>14,17,19</sup> In contrast to earlier trials that included nonrandomized control groups,<sup>13,14,17</sup> the Bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial included a randomized control group.<sup>19</sup> In the BOOST trial, BMC transfer resulted in an improvement of LVEF of six percentage points as compared with the control group after 6 months. For comparison, improvements of three to four percentage points are achieved by primary angioplasty and stent implantation in AMI and this results in better clinical outcomes as compared with thrombolytic strategies.<sup>76,77</sup> Improvement of LVEF was due mostly to improved regional wall motion in the infarct border zone.<sup>19</sup> Importantly, the effects of BMC transfer were observed on top of the benefits associated with established interventional and medical strategies to promote functional recovery after AMI.<sup>19</sup> In contrast to earlier nonrandomized studies,<sup>13,14</sup> a significant reduction of infarct size was not observed in the BOOST trial.<sup>19</sup> However, larger trials are required to further clarify the issue whether formation of new muscle tissue can be achieved by BMC transfer. So far, no trial has demonstrated a significant effect of BMC transfer on LV end-diastolic volumes, suggesting that unselected BMCs may have a limited impact on LV remodeling after AMI.<sup>13,14,17–19</sup> Again, larger studies are required to settle this issue. Follow-up data from the BOOST trial show that the improvement of LVEF is maintained after 18 months and indicate that BMC transfer prevents progression of diastolic dysfunction after AMI.<sup>75,78</sup>

### Selected Bone Marrow Cell Populations

The Transplantation Of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-

AMI) trial compared unselected mononuclear BMCs with circulating blood-derived progenitor cells (mostly EPCs). Both cell types appeared to have similar safety and efficacy profiles.<sup>14,16</sup>

The therapeutic effects of MSC transplantation after AMI have been investigated in one clinical trial.<sup>79</sup> No arrhythmias or other side effects were observed. Unfortunately, it was not reported whether intracoronary MSC delivery promoted ischemic damage to the myocardium,<sup>79</sup> a complication that has occurred after intracoronary MSC infusions in dogs.<sup>80</sup> Six months after MSC-transfer, regional wall motion and global LVEF were improved and LV end-diastolic volume was decreased compared with a randomized control group that had received an intracoronary infusion of saline.<sup>79</sup>

In another clinical trial using selected BMC populations, CD133<sup>+</sup> cells were infused into the infarct-related artery.<sup>81,82</sup> After 4 months, 6 of 14 patients had developed a significant in-stent restenosis or complete reocclusion, and two had developed a de novo lesion in the infarct-related artery.<sup>82</sup> These numbers are worrisome, but the study may be too small to establish that these side effects are causally related to CD133<sup>+</sup> cell transfer. Global LVEF, regional wall motion, and tissue perfusion increased in the cell transfer group but not in a cohort of matched control patients.<sup>81</sup> However, firm conclusions regarding efficacy cannot be derived from this small pilot trial.

#### **Stem and Progenitor Cell Mobilization**

Stem cell mobilization with stem cell factor (SCF) and/or granulocyte colony-stimulating factor (G-CSF) has been proposed to stimulate myogenesis and angiogenesis in the infarcted area and to improve cardiac function after AMI in mice.<sup>59,60</sup> By contrast, treatment with SCF and G-CSF enhances vascularization of the infarcted area but does not improve cardiac function in baboons after AMI.<sup>63</sup> Perhaps, reperfusion of the infarct-related artery before cytokine therapy would have permitted better access of mobilized cells to the infarct center in this large animal model.<sup>63</sup> Of note, G-CSF may accelerate infarct healing by enhancing macrophage infiltration and matrix metalloproteinase activation<sup>62</sup> and suppress cardiomyocyte apoptosis by activating the cytoprotective STAT3 transcription factor,<sup>83</sup> suggesting that stem cell-independent mechanisms may contribute to the effects of G-CSF after AMI.

In a first clinical investigation, 10 patients presenting with myocardial infarction 2 to 270 days after symptom onset were treated with G-CSF at 10  $\mu\text{g}/\text{kg}$  body weight for 4 days. Patients then underwent angioplasty and stent implantation of the infarct-related artery. In seven of these patients, G-CSF mobilized peripheral blood-derived leukocytes were collected just before the intervention and infused into the infarct-related artery after stent placement. No deaths, substantial arrhythmias, aggravation of heart failure, or angina occurred during G-CSF administration and a 6-month follow-up period. However, cell infusions resulted in a 65% increase in serum creatine kinase-MB levels, indicative of mild myocardial damage. More seriously, 7 of the 10 patients developed in-stent restenosis at 6 months, which prompted a premature termination of the study.<sup>84</sup> It should be pointed out that

vascular injury by balloon angioplasty and stenting had been performed in this study while systemic leukocyte counts were greatly elevated. G-CSF has the potential to activate neutrophils, for example by stimulating adhesion to endothelial cells thereby influencing their recruitment at sites of inflammation and tissue injury.<sup>85</sup> These systemic effects of G-CSF may have contributed to excess neointima proliferation and restenosis. Although an improvement in LVEF was observed in patients receiving G-CSF and cell infusions,<sup>84</sup> interpretation of this finding is impossible without a control group.

In a more recent study, 15 patients with AMI were treated with G-CSF at 10  $\mu\text{g}/\text{kg}$  body weight for 6 days, starting 80 $\pm$ 30 minutes after primary angioplasty and stent implantation of the infarct-related artery.<sup>64,86</sup> G-CSF treatment after stent implantation was not associated with an enhanced rate of in-stent restenosis, or other serious adverse events.<sup>86</sup> Compared with a randomized control group, patients receiving G-CSF experienced a more pronounced recovery of global LVEF after 4 months. However, the beneficial effects of G-CSF were magnified by an unexpected decrease in LVEF in the control group.<sup>64</sup>

#### **Directions for Future Clinical Research**

Brief intermittent periods of ischemia applied at the onset of reperfusion reduce infarct sizes in animal models of myocardial ischemia and reperfusion (ischemic postconditioning).<sup>87</sup> In the cell therapy trials, stop-flow cell delivery was performed several days after reperfusion, making postconditioning an unlikely explanation for the effects of cell therapy. Nevertheless, future trials need to include control groups that undergo bone marrow harvest and an intracoronary sham infusion to unambiguously establish that cell transfer, and not bone marrow puncture or intracoronary manipulation, mediates the functional improvements.

Therapeutic effects of selected versus unselected BMC populations should be compared head-to-head. Assuming that unselected BMCs contain effective and ineffective cell populations, and that both may be recruited to the infarcted area by similar mechanisms,<sup>52</sup> purification and infusion of the effective cell population(s) only may allow more of the effective cells to transmigrate into the infarcted area.

Similar benefits have been reported after delivery of greatly variable numbers of mononuclear BMCs.<sup>13,14,17</sup> Along this line, the absolute numbers of transplanted nucleated cells, CD34<sup>+</sup> cells, and colony-forming stem cells did not correlate with subsequent improvements in LVEF in the TOPCARE-AMI and BOOST trials. This may be because the cell numbers infused were within a narrow range, or because differences in the functional capacity of the cells may override differences in cell numbers.<sup>15,19</sup> Intriguingly, labeling studies indicate that less than 3% of unselected BMCs are retained in the infarcted area after intracoronary delivery in patients.<sup>56</sup> Although this rate of retention was sufficient to improve LV systolic function in the BOOST trial,<sup>19</sup> dose-finding studies are required to define the optimum cell number.

Post hoc analyses of the BOOST trial database suggest that the effects of BMC transfer are consistent across several subgroups defined according to sex, age, infarct size and

territory, time from symptom onset to reperfusion, and baseline LVEF.<sup>19</sup> Specific subgroups need to be defined prospectively in future trials, eg, patients presenting late after symptom onset in whom little myocardial salvage can be expected from reperfusion therapy.<sup>88</sup> For safety concerns, patients with AMI and heart failure symptoms have been excluded from previous trials. Considering that these patients may benefit most from an improvement in LVEF, future trials should assess the effects of cell therapy in this patient subgroup.

G-CSF and other cytokines with stem-cell mobilizing and cardioprotective properties should be further evaluated as stand-alone therapy or in combination with cell transfer.<sup>89,90</sup> Depending on whether cytokine and stem cell therapy improve cardiac function via shared or distinct mechanisms, cytokines might evolve as a noninvasive alternative or as an adjunct to cell therapy.

Ultimately, a large outcome trial looking at a combined mortality and morbidity end point will have to be conducted. One way to limit the size of such a trial would be to include only patients with AMI complicated by severe LV systolic dysfunction or heart failure symptoms, ie, patients who carry a high risk of future adverse events. Still, thousands of patients will have to be randomized to demonstrate an added benefit of cell therapy on clinical outcomes on top of our current, interventional, and medical therapies.

### Coronary Artery Disease With No Mechanical Revascularization Option

Despite significant advances in coronary revascularization techniques, some patients with coronary artery disease and myocardial ischemia have no revascularization option because of the diffuse nature of their disease. A number of these patients experience anginal symptoms despite maximal medical therapy. Chronic myocardial ischemia can be associated with a regional impairment of contractile function, which is partially reversible when tissue perfusion is restored (hibernating myocardium). Moreover, ischemia increases the risk of arrhythmias and sudden cardiac death. There is a clear need for new therapeutic strategies aimed at delivering oxygenated blood to the myocardium in these patients.

### Experimental Background

Transendocardial injection of unselected BMCs or EPCs enhances collateral flow, capillary density, and regional contractility in pigs with chronic myocardial ischemia.<sup>9,72</sup> The mechanisms how BMC injections enhance myocardial perfusion are unknown. Bone marrow–derived EPCs have been proposed to enhance tissue perfusion by differentiating into endothelial cells at sites of neovascularization.<sup>7,91,92</sup> Recent articles have highlighted the potential of BMCs to deliver a natural cocktail of angiogenic and arteriogenic cytokines to the myocardium.<sup>9,10,22,93</sup> In that regard, cell therapy may have advantages above previous single-cytokine gene therapy approaches to treat patients with chronic myocardial ischemia. It has also been reported that regional perfusion and contractile function of hibernating pig myocardium can be improved by G-CSF, suggesting that stem and (endothelial) progenitor cell mobilization may represent an alternative, less invasive therapeutic strategy.<sup>94</sup>

### Clinical Trial Experience

Unselected mononuclear BMCs have been used in several small studies in patients with coronary artery disease not amenable to conventional revascularization techniques (Table 2).<sup>20–24</sup> In a first study, five patients undergoing CABG received transepical BMC injections into an ischemic area with no graftable vessel. All patients had an uneventful postoperative course. No arrhythmias occurred, and no intramyocardial calcification or tumor formation was observed after 1 year, suggesting that the procedure may be safe.<sup>20</sup> Myocardial perfusion in the injected area improved in three of these patients.<sup>20</sup> In three additional studies, mononuclear BMCs were injected transendocardially into ischemic myocardium under electromechanical guidance.<sup>21–24</sup> No procedure-related complications were reported, and no sustained ventricular arrhythmias were observed up to 1 year after cell transfer.<sup>21–24</sup> One patient died suddenly 14 weeks after cell transfer.<sup>23</sup> Although sudden (cardiac) death is a typical complication in patients with severe ischemic heart disease, it cannot be ruled out that this death was related to cell injections. Improvements of anginal symptoms, exercise capacity, regional tissue perfusion, and LV systolic function

**TABLE 2. Cell Therapy Trials in Patients With Myocardial Ischemia and No Revascularization Option**

Study	[n]	LVEF	Cell Type	Dose	Delivery	Outcomes	
						Subjective	Objective
Hamano et al <sup>20</sup>	5 treated		MNC	0.3–2.2×10 <sup>9</sup>	Transepical (during CABG)		Perfusion ↑↑
Tse et al <sup>21</sup>	8 treated	58±11%	MNC	from 40 mL BM	Transendocardial (guided by EMM)	Angina ↓↑	Perfusion ↑↑; Regional wall motion ↑↑;
Fuchs et al <sup>22</sup>	10 treated	47±10%	NC	7.8±6.6×10 <sup>7</sup>	Transendocardial (guided by EMM)	Angina ↓↑	Perfusion ↑↑
Perin et al <sup>23,24</sup>	14 treated, 7 controls*	30±6%	MNC	3.0±0.4×10 <sup>7</sup>	Transendocardial (guided by EMM)	Angina ↓; NYHA class ↓	Perfusion ↑; Regional wall motion ↑↑; Global LVEF ↑

LVEF indicates left ventricular ejection fraction; MNC, bone marrow–derived mononuclear cells; NC, bone marrow–derived nucleated cells; BM, bone marrow; CABG, coronary artery bypass grafting; EMM, electromechanical mapping; NYHA, New York Heart Association; \*Nonrandomized control group; †Effects reported only within cell therapy groups. Values are means±SD.

have been reported after intramyocardial BMC injections (Table 2).

A recent study investigated the effects of G-CSF on symptoms and myocardial perfusion in 16 patients with intractable angina.<sup>65</sup> Treatment with G-CSF (10  $\mu\text{g}/\text{kg}$  body weight for 5 days) promoted a strong increase in circulating EPC numbers and an improvement in anginal symptoms. However, there was no objective evidence of enhanced myocardial perfusion or improved regional wall motion. Furthermore, two patients experienced myocardial infarctions, raising concerns about the safety of G-CSF in this patient population.<sup>65</sup>

### Directions for Future Clinical Research

Intramyocardial injection of unselected BMCs is feasible and appears to be safe in patients with chronic myocardial ischemia.<sup>21–24</sup> The efficacy of this approach is unknown because none of the previous trials included a randomized control group. Our experience with transmyocardial laser revascularization has highlighted the need for control groups undergoing sham-catheterization and intramyocardial sham-injections to control for the placebo effect typically observed in this patient population.<sup>95</sup> Still, the idea to improve myocardial perfusion by BMC injections is intriguing and should be tested prospectively in larger, randomized clinical trials. Because symptomatic improvement is the major goal of cell therapy in this patient population, it will be very important to establish the safety of procedure.

Although no adverse coronary events have been observed after short-term administration of G-CSF to normal volunteers,<sup>96</sup> G-CSF may not be safe in patients with advanced coronary artery disease.<sup>65</sup> Any future trial investigating the role of G-CSF in these patients should use a careful dose-escalating regime. Alternative strategies to promote EPC mobilization and, possibly, angiogenesis in ischemic myocar-

dium should be explored (eg, statins, cytokines, physical exercise). However, a limitation of any stem cell mobilizing strategy may be that circulating cells have insufficient access to severely ischemic myocardium.

## Ischemic Cardiomyopathy, Chronic Heart Failure

Chronic heart failure has emerged as a major worldwide epidemic. Recently, a fundamental shift in the underlying etiology of heart failure is becoming evident, in which the most common cause of heart failure is no longer hypertension or valvular disease, but rather long-term survival after AMI. Conceptually, replacement of akinetic scar tissue by viable myocardium should improve cardiac function and impede progressive LV remodeling.

### Experimental Background

Among various indications for stem cell therapy that can be envisioned, repair of scar tissue is the most challenging. Transplanted cells will face limited blood supply and may not receive the environmental cues essential for transdifferentiation into vascular cells (or cardiomyocytes). Transplantation of myoblasts, which supposedly have a good tolerance to ischemia and are committed to differentiate along the myocyte lineage, may therefore be a valuable option in this setting. Indeed, injection of myoblasts into infarcted myocardium has been shown to improve LVEF and to ameliorate adverse LV remodeling in small and large animal species.<sup>36,97,98</sup> Although grafted myotubes may contract in response to electrical stimulation,<sup>37,38</sup> they do not express the intercalated disk proteins N-cadherin or connexin 43, indicating that they are not electromechanically coupled to their host cardiomyocytes.<sup>99</sup> Therefore, improvement of cardiac function observed in animal models after myoblast transplantation may not depend on synchronized contractile activity of the

**TABLE 3. Cell Therapy Trials in Patients With Ischemic Cardiomyopathy**

Study	[n]	LVEF	Cell Type	Dose	Time after MI	Delivery	Outcomes‡
Menasche et al <sup>104</sup>	10 treated	24±4%	Myoblasts	8.7±1.9×10 <sup>8</sup>	3–228 months	Transepical (during CABG)*	Regional wall motion ↑; Global LVEF ↑
Herreros et al <sup>105</sup>	11 treated	36±8%	Myoblasts	1.9±1.2×10 <sup>8</sup>	3–168 months	Transepical (during CABG)†	Regional wall motion ↑; Global LVEF ↑; Viability in infarct area ↑
Siminiak et al <sup>106</sup>	10 treated	25–40%	Myoblasts	0.04–5.0×10 <sup>7</sup>	4–108 months	Transepical (during CABG)†	Regional wall motion ↑; Global LVEF ↑
Chachques et al <sup>107</sup>	20 treated	28±3%	Myoblasts	3.0±0.2×10 <sup>8</sup>	not reported	Transepical (during CABG)*	Regional wall motion ↑; Global LVEF ↑; Viability in infarct area ↑
Smits et al <sup>67</sup>	5 treated	36±11%	Myoblasts	2.0±1.1×10 <sup>8</sup>	24–132 months	Transendocardial (guided by EMM)	Regional wall motion ↑; Global LVEF ↑
Stamm et al <sup>110,111</sup>	12 treated	36±11%	CD133 <sup>+</sup>	1.0–2.8×10 <sup>6</sup>	3–12 weeks	Transepical (during CABG)*	Global LVEF ↑; LVEDV ↓; Perfusion ↑
Assmus et al <sup>112</sup>	51 MNC, 35 CPC, 16 controls	40±11%	MNC CPC	1.7±0.8×10 <sup>8</sup> 2.3±1.2×10 <sup>7</sup>	3–144 months	IC	Global LVEF ↑; (only in MNC group)

LVEF indicates left ventricular ejection fraction; CD133, bone marrow–derived CD133<sup>+</sup> cells; MNC, bone marrow–derived mononuclear cells; CPC, circulating blood–derived progenitor cells; MI, myocardial infarction; CABG, coronary artery bypass grafting; EMM, electromechanical mapping; IC, intracoronary; LVEDV, left ventricular end-diastolic volume; \*CABG of noninjected territories only; †CABG of injected and noninjected territories; ‡Effects only within cell therapy groups. Study by Assmus et al<sup>112</sup> has been presented in abstract form only. Values are means±SD.

cells. In line with this hypothesis, it has been shown that the functional benefits of myoblast transplantation in a rat infarct model are sustained over time despite a progressive loss of engrafted cells.<sup>100</sup> Injected myoblasts do not appear to stimulate angiogenesis locally.<sup>101</sup> However, it has been hypothesized that myoblasts may release paracrine factors instructing neighboring cardiomyocytes to maintain their replicative potential or to favor differentiation of CSCs into cardiomyocytes.<sup>38</sup> Transplanted myoblasts can fuse with cardiomyocytes at the graft-host interface. Although fusion appears to be a rare event, the functional properties of these hybrid cells need to be further explored.<sup>102</sup>

Recent studies have investigated whether injection of BMCs can be used to regenerate recently infarcted myocardium. Injection of CD133<sup>+</sup> stem cells into the infarcted myocardium 10 days after coronary artery ligation in rats promoted an increase in LVEF.<sup>101</sup> By contrast, direct injection of unselected BMCs 3 weeks after coronary ligation in sheep did not enhance functional recovery,<sup>66</sup> emphasizing that the cell type and cell delivery method need to be carefully adapted to the underlying disease state.

## Clinical Trial Experience

### *Skeletal Myoblasts*

After an initial case report,<sup>103</sup> several small trials investigating the safety and feasibility of myoblast transplantation in patients with ischemic cardiomyopathy have been published (Table 3). These studies indicate that it is feasible to establish and expand myoblast cultures from skeletal muscle biopsies and to obtain target myoblast numbers within 2 to 3 weeks.<sup>39,67,104–107</sup> One major safety concern has arisen from these studies, ie, that myoblast grafts may represent an arrhythmogenic substrate.<sup>108</sup> In the first clinical trial,<sup>104</sup> 10 patients with severely reduced LVEF undergoing CABG received myoblast injections into scar tissue supplied by a totally occluded, nongraftable coronary artery. In four of these patients, sustained ventricular tachycardias occurred between 11 and 22 days after surgery. Two of these patients had additional episodes of ventricular tachycardias 5 and 9 months after the operation. All four patients were treated with an implantable cardioverter-defibrillator (ICD).<sup>104</sup> In two similar studies, 21 additional patients received myoblast injections. In these studies, surgical revascularization involved the noninjected and the injected areas.<sup>105,106</sup> Two patients developed ventricular tachycardias 1 day after surgery<sup>106</sup> and one patient on day 40.<sup>105</sup> In another report, myoblasts were injected into scarred myocardium in five patients with end-stage ischemic heart failure undergoing LV assist device implantation as a bridge to heart transplantation. Two of these patients had ventricular tachycardias in the immediate postoperative period, one of whom already had a history of ventricular arrhythmias.<sup>39</sup> Finally, myoblasts have been injected transendocardially as a stand-alone procedure in five patients with heart failure after an anterior wall AMI.<sup>67</sup> In one patient, a minor elevation of serum troponin levels was noted after the procedure. More seriously, another patient had ventricular tachycardias 6 weeks after myoblast injections and underwent ICD implantation.<sup>67</sup> Two sudden deaths and three ventricular arrhythmias have occurred in eight addi-

tional patients treated by transendocardial myoblast injections.<sup>67</sup> Episodes of ventricular tachycardias were also observed in one of nine patients with postinfarction heart failure receiving transcatheter vein injections of myoblasts.<sup>69</sup> It is likely, therefore, that myoblast injections increase the risk of ventricular arrhythmias in this patient population.<sup>109</sup> In the absence of electromechanical coupling, the underlying mechanisms remain uncertain. It has been proposed that the ability of myoblasts to fire bursts of action potentials may induce deleterious extrasystoles, even in the absence of electromechanical coupling, through electrotonic interaction.<sup>38,108</sup> Moreover, local tissue injury could be responsible for arrhythmogenesis.<sup>104,108</sup> In a recent study, no serious arrhythmias were observed in 20 patients during a mean followup of 14 months after injecting myoblasts that had been expanded in autologous rather than fetal bovine serum,<sup>107</sup> leading to the hypothesis that trace contamination with xenogenic proteins may provoke an arrhythmogenic immune reaction at the injection site.<sup>107</sup>

In most trials, improvements of regional wall motion and global LVEF have been noted after myoblast injections.<sup>67,104–107</sup> Moreover, evidence for enhanced viability in the injected myocardial areas has been obtained in some of these reports.<sup>105,107</sup> However, because of the small number of patients, lack of control groups, and the confounding effect of concomitant revascularization, no firm conclusions regarding efficacy can be drawn at this time.

### *Bone Marrow Cells*

In one trial, CD133<sup>+</sup> BMCs were injected transeptically into the infarct border zone in 12 patients undergoing CABG of noninjected myocardial areas.<sup>110,111</sup> In contrast to the myoblast trials, infarcts had occurred fairly recently in the patients in this study (Table 3). No procedure-related complications were reported, and no serious ventricular arrhythmias were observed up to 14 months.<sup>111</sup> After 6 to 8 months, perfusion of the cell-injected area and LVEF were improved. However, because there was no control group, the efficacy of this approach remains uncertain.

In a recent trial, 86 patients with ischemic cardiomyopathy received intracoronary infusions of unselected mononuclear BMCs or of circulating blood-derived progenitor cells by the stop-flow balloon catheter technique. The procedure was safe.<sup>112</sup> After 3 months, LVEF in the BMC group was improved by three percentage points, but did not change significantly in control patients and in the progenitor cell group.<sup>112</sup>

## Directions for Future Clinical Research

Randomized, double-blind trials are needed to rigorously evaluate the safety and efficacy of cell therapy in patients with ischemic heart failure. It may be advisable to restrict the use of myoblast transplantation to patients with an ICD. The monitoring function of the ICD will provide critical information on the natural course of myoblast-induced arrhythmias.<sup>108</sup> If the implantation of an ICD will be routinely required in patients receiving myoblast injections, the procedure might not be cost-effective.

Postmortem studies indicate that only a small fraction of injected myoblasts survive in scarred human myocardium.<sup>39,113</sup> Accordingly, preimplantation antiapoptotic treatments or coinjection of angiogenic growth factors may enhance myoblast survival after transplantation.<sup>109,114</sup> Another future strategy may involve the ectopic expression of connexin 43 in myoblasts, which may permit electrical coupling with resident cardiomyocytes.<sup>115</sup>

It is interesting to note that intracoronary infusions of mononuclear BMCs or blood-derived progenitor cells promoted greater improvements of LVEF in patients with AMI as compared with patients with ischemic cardiomyopathy.<sup>14,112</sup> Because cell retention may be limited after intracoronary delivery into chronically infarcted myocardium, pharmacological or genetic approaches to enhance cell retention and engraftment should be explored.

Considering that functional benefits of cell transplantation have also been observed in animals with dilated cardiomyopathy,<sup>116</sup> future trials may want to explore the role of cell therapy in patients with nonischemic heart failure. In this regard, a pilot study suggests that intracoronary BMC-transfer may be safe and potentially effective in patients with Chagas cardiomyopathy.<sup>117</sup>

### Issues to Be Addressed in Future Studies

So far, a flurry of small, mostly uncontrolled clinical studies exploring the safety and feasibility of stem cell therapy have been conducted. These studies have used a myriad of different cell types and preparations, each in a small number of patients with different disease states. In the aggregate, this preliminary clinical evidence suggests that stem cell therapy might work. Although these initial clinical studies have generated a great deal of hope, we should take into account the lessons learned from the translation of therapeutic angiogenesis into clinical studies, where great expectations raised by open studies have not been confirmed by subsequent randomized trials. We advocate to no longer perform studies involving small numbers of patients, but rather to conduct intermediate-size, double-blind, randomized-controlled clinical trials to establish the effects of stem cell therapy on surrogate markers, like LVEF, myocardial perfusion, or exercise capacity. Upcoming trials should also address procedural issues such as the optimal cell type, cell dosage, and timing of cell transfer. These trials may also look at combined morbidity and mortality end points, although they may be too small to be conclusive in this regard. Safety remains the key concern as we proceed.

Although these studies are underway, fundamental questions need to be addressed experimentally. What is the fate of the injected cells after transplantation? How long do they survive? Do the cells incorporate, or is transient retention sufficient to promote functional effects? Genetic and transgenic markers should be used to determine the lineage commitment of engrafted cells. We would encourage laboratories that have arrived at discrepant conclusions regarding cell fate and lineage commitment to share their experimental protocols. Cell labeling and imaging techniques need to be developed to track stem cell fate in patients and correlate cell retention and engraftment with functional outcomes. Emerg-

ing evidence suggests that transplanted stem cells may interact with resident CSCs to enhance their regenerative potential. What is the nature and functional relevance of this interaction? Can CSCs be used for cardiac repair, or is their potential similar to cells obtained from bone marrow? Can the regenerative capacity of transplanted stem cells be enhanced by drugs, cytokines, or gene therapy approaches? Pharmacological and genetic strategies may help to enhance stem cell retention, engraftment, differentiation, and paracrine capability.<sup>34,118–120</sup>

In the era of evidenced-based medicine, effects on surrogate markers will not suffice to establish stem cell therapy as a valid therapeutic option for patients with cardiovascular disease. Outcome trials will have to be conducted. Funding is an issue, unless intellectual property is involved or public programs are being developed. Support from governmental organizations or charities will be required to ensure that cell therapies, which may be efficacious but commercially less attractive (eg, unselected BMCs), will undergo much-needed further clinical testing. Support may also come from device manufacturers, if a specific cell therapy approach relies on an optimized cell delivery system, or if a device is developed that allows cardiologists to obtain cell preparations “at the bedside” without the requirement for a local GMP facility. It can be anticipated that cell preparation and delivery devices will undergo considerable development once the clinical benefit of cell therapy is clearly established. Some companies have already started to develop and market cell therapy products, for example, culture-expanded autologous skeletal myoblasts for use in patients with ischemic cardiomyopathy, or cryopreserved allogeneic MSCs as an “off-the-shelf” therapeutic for patients with AMI.

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