Imaging: Guiding the Clinical Translation of Stem Cell Therapy

Stefanie Dimmeler, Douglas Losordo, Editors

**Abstract:** Stem cells have been touted as the holy grail of medical therapy, with promises to regenerate cardiac tissue, but it appears the jury is still out on this novel therapy. Using advanced imaging technology, scientists have discovered that these cells do not survive nor engraft long-term. In addition, only marginal benefit has been observed in large-animal studies and human trials. However, all is not lost. Further application of advanced imaging technology will help scientists unravel the mysteries of stem cell therapy and address the clinical hurdles facing its routine implementation. In this review, we will discuss how advanced imaging technology will help investigators better define the optimal delivery method, improve survival and engraftment, and evaluate efficacy and safety. Insights gained from this review may direct the development of future preclinical investigations and clinical trials. (Circ Res. 2011;109:962-979.)

**Key Words:** stem cell therapy ■ imaging ■ cardiovascular medicine

Heart failure remains the leading cause of death worldwide, accounting for 1 in 9 deaths in the United States in 2007.¹ Unfortunately, most current therapies do not address the issue of cardiomyocyte loss and serve only to delay progression or alleviate symptoms until potential orthotopic heart transplantation. Stem cell regenerative therapy has
emerged as a promising alternative to improve heart function and prevent the development of end-stage heart failure. New advances in stem cell technology have led to an explosion of preclinical and clinical studies using various delivery protocols and cell types, each touting their clinical advantages. Although results from early animal studies and pilot human trials were encouraging, findings from larger randomized studies have been underwhelming. Two meta-analyses of 18 and 10 trials showed only marginal benefit, and 2 large-scale randomized phase II trials using bone marrow–derived cells (BMCs) demonstrated conflicting results. The reasons for these puzzling findings remain unclear but may be further explored by applying imaging technology to guide stem cell therapy. In this review, we discuss how recent advances in imaging technology can help address the many challenges facing the clinical translation of stem cell therapy (Figure 1).

### Fundamentals of Imaging Stem Cell Therapy

Advanced imaging technology can provide anatomic and functional assessment (eg, conventional anatomic and functional imaging), as well as visualization of biological processes at the cellular and molecular level (eg, molecular imaging). Both disciplines use various imaging modalities such as radionuclide, optical, magnetic resonance, computed tomographic, and ultrasound technology. Selecting the most effective imaging strategy requires a determination of whether the imaging system can meet the necessary requirements for spatial and temporal resolution, sensitivity, and penetration depth for visualization of the imaging target.

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<td>• Tumorigenicity</td>
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<td>• Arrhythmogenicity</td>
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**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BLI</td>
<td>bioluminescence imaging</td>
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<tr>
<td>BMC</td>
<td>bone marrow–derived cell</td>
</tr>
<tr>
<td>ESC</td>
<td>embryonic stem cell</td>
</tr>
<tr>
<td>HSV-tk</td>
<td>herpes simplex virus thymidine kinase</td>
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<tr>
<td>iPSC</td>
<td>induced pluripotent stem cell</td>
</tr>
<tr>
<td>Luc</td>
<td>luciferase</td>
</tr>
<tr>
<td>MSC</td>
<td>mesenchymal stem cell</td>
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<tr>
<td>NIS</td>
<td>sodium-iodide symporter</td>
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*Figure 1. Application of advanced imaging to meet the challenges facing stem cell therapy for cardiac disease. Imaging can guide each step of stem cell therapy, from choosing the appropriate delivery method to evaluating the long-term safety of transplantation. CMs indicates cardiomyocytes; ECs, endothelial cells; VSMCs, vascular smooth muscle cells; EM, electromechanical; SSFP, steady state free precession; LVED, left ventricular end-diastolic; LVES, left ventricular end-systolic; and CE-MRI, contrast-enhanced MRI. aCourtesy of Rebecca Fahrig. bReprinted with permission from Tomkowiak et al. cvan den Borne et al. eGyöngyösi et al. and fCao et al.*
Table 1 details the advantages and limitations of different imaging modalities and can serve as a guide for selecting the most appropriate strategy for the chosen indication.

Although conventional anatomic and functional imaging can provide an overall road map for cell delivery, as well as an assessment of the effects of stem cell therapy, molecular imaging can be used to track stem cells in vivo and to study their potential mechanistic benefits. In molecular imaging, imaging probes are used to target the biological process of interest. These imaging probes consist of a carrier (ie, a cell, nanoparticle, and microbubble), which structurally binds a ligand designed to recognize the molecular target and a signal element to generate a detectable signal (Figure 2). The ideal imaging probe should have the following important properties: (1) high imaging specificity for tracking the desired biological process, (2) high imaging sensitivity for detection by available imaging modalities, (3) minimal cellular toxicity, and (4) minimal systemic toxicity.

In general, there are 2 main labeling approaches for cardiac stem cell imaging, each with its unique advantages and disadvantages: (1) direct labeling with radionuclides or iron nanoparticles and (2) reporter gene/probe labeling. Using the direct labeling approach, contrast agents (eg, signal elements) either bind to cell surface proteins or are transported into the cell by diffusion, endocytosis, or active transport (eg, radiolabeled indium oxine and superparamagnetic iron oxide particles; Figure 3A). In contrast, reporter gene/probe labeling requires cell transfection or transduction with a reporter gene that produces specific proteins (ie, membrane transport, surface receptor, and intracellular storage proteins, as well as intracellular enzymes) that can take up exogenously administered contrast agents. By far the most widely used reporter genes are firefly luciferase (Luc) and herpes simplex virus thymidine kinase (HSV-tk) and their mutants. After delivery of their respective substrates, these enzymes catalyze a chemical reaction that produces a detectable signal (Figure 3B).

The major advantage of reporter gene/probe labeling, especially for in vivo cell tracking, is that cells must be viable and intact protein synthesis machinery in order to produce a detectable signal. In contrast, the signal produced by direct labeling with contrast agents can be diluted by cell division or dissipate after radioactive decay and/or may persist despite cell death because of the engulfment of dead cells by macrophages. Iron labeling by magnetic resonance imaging (MRI), for example, can remain in the injected site long after cell death, providing erroneous information on the long-term fate of cells despite its superiority in cellular localization.
Reporter gene/probe imaging is thus better suited for in vivo monitoring of cell viability. In one of the first clinical applications of reporter imaging, the positron emission tomography (PET) reporter probe HSV-tk was used to track and monitor “suicide gene” therapy for gliomas and hepatocellular carcinomas. More recently, Yaghoubi et al demonstrated that reporter gene imaging could track the fate of exogenously administered, genetically modified, and therapeutic cytolytic T cells in patients with glioblastoma. However, widespread application has been slowed by safety concerns, such as the potential risk of immunogenicity and tumorigenicity caused by random reporter gene integration, as well as limited sensitivity due to reporter gene/probe slicing.

Guiding Stem Cell Delivery

Both conventional and molecular imaging may help determine the best strategy for stem cell delivery. A lack of an optimized and standardized protocol for safe and effective stem cell delivery is a potential reason for the inconsistent results from previous trials. In preclinical and clinical studies, cells have been delivered via intravenous, intracoronary, or intramyocardial routes. Cells have also been administered as early as minutes and as late as a few months after infarction in acute and chronic ischemic models, respectively. Unfortunately, these significant protocol variations have impeded the accurate interpretation of preclinical and clinical trial results, because it is unclear whether the limitation lies with the regenerative capacity of stem cell therapy or with the techniques of delivery.

Determining the Most Optimal Delivery Method

Stem cells have been delivered via intravenous, intracoronary, and direct intramyocardial routes. Small-animal preclinical studies have been restricted to intravenous or intramyocardial delivery because of the small size of the murine and rat coronary arteries. Conversely, the intracoronary approach appears to be preferred in large-animal and human trials, despite the lack of evidence for its superiority. Only a few studies have directly compared the cellular retention and engraftment of various delivery routes using in vivo imaging. One such study compared the delivery efficiency of intracoronary versus peripheral intravenous injection of fluorescence and Tc-labeled autologous BMCs in a swine model of myocardial infarction. For intravenous injection, radioactivity was detected mainly in the lungs, with cardiac activity at only minimal levels 1 hour after injection. For intracoronary injection, 34.8 ± 9.9% of cells were detected in the heart 1 hour after injection, but the number of cells declined precipitously to 6.0 ± 1.7% at 24 hours. Cell detection, however, may be limited by the short half-life of the tracer.
Although intravenous or intracoronary injection is relatively easier and safer, direct intramyocardial injection bypasses the need for cell homing to the injured myocardium, which can be challenging. For example, in a previous clinical trial, only 1.3% to 2.6% of fluorine-18 fluorodeoxyglucose (18F-FDG)–labeled cells were detected in the infarcted myocardium 50 to 75 minutes after intracoronary injection.13 On the other hand, a higher number of cells have been found in the myocardium after direct myocardial injection, but the potential success of this technique may be less predictable.14 To address these limitations, a recent study has found that seeding cells within a fibrin porous biomatrix improved myocardial cell retention in an immunodeficient mouse model of left ventricular (LV) remodeling.15 Taken together, these findings suggest that intramyocardial injection with adjuvant agents to improve cell retention is the preferred delivery method. Greater enthusiasm for this approach may be generated with imaging guidance to accurately delineate the peri-infarct area and to safely deliver cells into the myocardium.

**Defining the Peri-Infarct Area During Direct Intramyocardial Injection**

One approach to ensure the safety and consistency of intramyocardial delivery is to use image-guided delivery. To derive the most benefit from direct intramyocardial injection, cells need to be physically placed near the target area of injury, and ideally in the border zone between viable and nonviable tissue, but not directly into the infarct. In this location, cells can provide the most benefit and have sufficient access to nutrients from the blood supply. Because the ischemic microenvironment may be less hostile in the peri-infarct area than in the infarct zone, cells may also have a relatively higher chance of survival and engraftment in the peri-infarct area.

Recent advances in imaging technology have enabled image-based navigation of stem cell injection. The best
established technique for 3-dimensional (3D) intracardiac navigation is intraventricular electromechanical mapping, which offers superior spatial orientation compared with x-ray fluoroscopy alone. Electromechanical mapping records point-by-point measures of electrophysiological and motion data, which enables the formation of an electroanatomic map to define the peri-infarct area.\textsuperscript{16} Although it has been applied successfully in a number of phase I clinical studies, including an ongoing multicenter trial of intramyocardial delivery of bone marrow mononuclear cells,\textsuperscript{17} the accuracy of electromagnetic mapping has been questioned because of overlap of electric and mechanical data between normal and abnormal myocardium, as well as positional artifacts such as heart motion, subject movement, and catheter-induced bundle-branch block.\textsuperscript{16} Other disadvantages include limited access to remote areas, radiation exposure, and lengthy procedure time that can take up to 40 minutes or longer for higher-resolution imaging because more points need to be mapped.\textsuperscript{16}

More recent advances in imaging technology have facilitated the development of 2 alternative techniques that may be used to define the peri-infarct area at the time of cell delivery. The first is the emergence of C-arm computed tomography (CT), which enables multiple serial ECG-triggered rotational acquisitions during slow intravenous contrast injection to achieve a 3D data cube with multiplanar reconstruction of the heart (Figure 1).\textsuperscript{18} Although this technique has been used to localize areas of injured myocardium induced by radiofrequency ablation,\textsuperscript{19} further study is needed to determine whether the C-arm CT can provide delayed-enhancement images of the infarcted myocardium similar to standard CT to enable imaging-based guidance of cell delivery.

The second alternative is the development of software that facilitates co-registration of CT or MRI delayed-enhancement images with x-ray fluoroscopy.\textsuperscript{20,21} The latter has the advantage of less radiation exposure, greater spatial resolution, and more validated protocols for viability imaging. Tomkowiak et al\textsuperscript{21} recently built customized software that has been shown to accurately register and overlay images acquired by MRI with those acquired from x-ray fluoroscopy by aligning discrete anatomic landmarks in a swine myocardial infarction model (Figure 1). Further study is required to demonstrate whether this technique can be applied to intramyocardial stem cell injections into the peri-infarct area.

In Vivo Monitoring of Stem Cell Fate

A potential reason for the marginal and inconsistent benefit in cardiac function noted in clinical trials is the variability in cell survival, engraftment, and differentiation. It is likely that functional improvement will require the presence of an adequate number of cells to differentiate into cardiac myocytes, recruit endogenous stem cells, and release paracrine factors to enhance the function of surviving cardiomyocytes. Molecular imaging has enabled in vivo monitoring of stem cell fate and may help investigators identify important strategies to improve cell survival, proliferation, engraftment, and differentiation.

Determining Survival, Proliferation, and Engraftment

Unlike MRI, which can provide only qualitative information on cell location and engraftment, reporter gene–based bioluminescence imaging (BLI) and nuclear imaging can quantify cell survival, proliferation, and engraftment. BLI, however, is limited to small animals because of poor optical transmission. An attractive alternative is PET imaging of $^{18}$F-FDG--labeled cells, which allows quantification of engraftment in vivo in small-animal and large-animal models, as well as humans.\textsuperscript{13,22} However, the half-life of $^{18}$F is only 110 minutes, and therefore, only acute evaluation of biodistribution immediately after transplantation is feasible. For serial monitoring, single-photon emission computed tomography (SPECT) imaging of $^{111}$In-labeled cells, which has a half-life of 2.8 days, can be used to monitor subjects 2 weeks after cell delivery.\textsuperscript{23} Perhaps the best long-term monitoring strategy for large animals and humans is PET reporter gene imaging with HSV-tk. Because the reporter gene is incorporated into the cell genome, which enables continuous transcription of the reporter enzyme if the cell remains viable, serial imaging can be performed after initial and repeated administrations of the radiolabeled substrate. An attractive alternative to the more commonly used HSV-tk is the sodium-iodide symporter (NIS), which promotes in vivo cellular uptake of $^{99m}$Tc or $^{124}$I for cell tracking by SPECT or PET, respectively. The advantage of NIS is that it is an endogenous mammalian gene that is potentially less immunogenic than HSV-tk. However, the feasibility of this technique has only been demonstrated in small animals.\textsuperscript{24}

Using these various in vivo imaging techniques, studies have revealed that only a limited number of cells engraft and most cells die shortly after transplantation. Specifically, cell-tracking studies have found that myocardial engraftment is less than 10\% within 48 hours irrespective of cell type, the number of cells implanted, and delivery route.\textsuperscript{25,26} The majority of cells are not found in the myocardium but are either trapped in the pulmonary vasculature or the microvasculature\textsuperscript{27,28} or localize to remote organs.\textsuperscript{14,29} Cell homing and retention in the infarcted myocardium, however, do occur. In a mouse model, Sheikh et al\textsuperscript{30} demonstrated that bone marrow mononuclear cells from a male donor injected intravenously preferentially home in on and are retained in the myocardium in female mice with ischemia-reperfusion injury compared with sham mice. The authors used in vivo cell tracking by BLI and ex vivo quantitative real-time polymerase chain reaction analysis for the male Sry gene 4 weeks after injection. Interestingly, in a dog model of myocardial infarction, Kraitchman et al\textsuperscript{31} showed that intravenous injection of mesenchymal stem cells (MSCs) resulted in redistribution of the MSCs from the lung to infarcted myocardium 1 day after injection, which further supports the idea that stem cells home to the injured tissue. Nevertheless, at present, the degree of cell homing and retention appears inadequate.

Even more disappointing is the fact that most cells die shortly after transplantation.\textsuperscript{6,32–36} After delivery of human embryonic stem cell (ESC)–derived cardiomyocytes in an immunodeficient mouse model of myocardial infarction, in vivo BLI demonstrated that 90\% of cells died within 3 weeks of delivery.\textsuperscript{34} Other investigators have also demonstrated failure of long-term survival (>8 weeks after delivery) for additional cell types, including MSCs,\textsuperscript{33,36} skeletal myo-
blasts,33 cardiac resident stem cells,35 bone marrow mononuclear cells,30 adipose stromal cells,36 and ESC-derived endothelial cells32 in small-animal models (Figure 4). Similarly, in a porcine model of myocardial infarction, Gyo¨ ngyo¨ s ie ta l could only detect faint focal activity in the myocardium 7 days after injection of porcine MSCs transfected with a PET reporter gene (Figure 5).37 Failure of cell detection beyond 7 days in large-animal models is likely due to limited resolution and sensitivity of PET for long-term monitoring. As the cell number declines over time, PET may be unable to detect the generated signal, especially given that only a fraction of cells are labeled by reporter genes because of variable viral transduction efficiency, as well as potential reporter gene silencing.10,26 Taken together, these findings emphasize the need to understand the reasons behind poor cell retention and survival to identify methods for their improvement.

**Imaging Cell Differentiation**

In addition to poor survival and engraftment, directed differentiation of pluripotent stem cells into functional cardiomyocytes remains challenging. Cardiac differentiation appears to be enhanced by prior in vitro induction, which was confirmed by a study using BLI and stem cells that expressed fluorescent and fLuc genes under control of the cardiac sodium-calcium exchanger-1 promoter.38 Cells that showed markedly enhanced fLuc expression on induction of differentiation in vitro continued to express fLuc for 2 to 4 weeks after transplantation in vivo. This finding has been supported by the emergence of several differentiation protocols for cardiomyocyte generation from pluripotent stem cells that use various growth factors, chemical stimuli, and physical stimuli.39 Despite these efforts, cardiomyocyte differentiation in vitro is largely uncontrolled and inefficient, with a success...
rate of <25% under most protocols,40 which highlights the need for better understanding of the mechanisms that regulate differentiation. Interestingly, one study has elucidated the role of STAT3 activity in ESC differentiation using BLI and a custom-designed STAT3 reporter construct driving a fluorescent protein and fLuc.41 Subject to further investigation, in vivo imaging of cardiac differentiation has the potential to provide valuable insight into the control of stem cell differentiation.

**Identifying Methods to Improve Cell Engraftment and Differentiation**

By providing greater understanding of the mechanism behind poor engraftment and inefficient differentiation, in vivo imaging can guide the development of techniques to address these limitations. In vivo imaging has evaluated the optimal timing for stem cell delivery (acute versus subacute infarction),42 determined the spatiotemporal kinetics of BMC homing,30 compared delivery of various stem cell types,33 and assessed the potential role of proangiogenic or prosurvival agents to meet these limitations.42,43,44 For example, Swijnenburg et al42 used in vivo cell tracking to determine whether timing of cell delivery would affect cell viability but found no significant difference in cell survival or cardiac function when cells were delivered immediately (acute) versus 7 days (subacute) after myocardial infarction (Figure 4C). In another study, acute donor cell death was seen in all cell types when the engraftment rates of bone marrow mononuclear cells, MSCs, skeletal myoblasts, and fibroblasts were compared (Figure 4B).33 More recently, Hu et al44 showed that the addition of a microRNA prosurvival cocktail (miR-21, miR-24, and miR-221) was associated with enhanced survival of cardiac progenitor cells by BLI and improved cardiac function by echocardiography.

Using BLI, investigators have also determined the kinetics of donor cell rejection across different immunologic barriers.45–47 Swijnenburg et al45 demonstrated that survival of transplanted human ESCs was significantly limited in immunocompetent as opposed to immunodeficient mice but could be mitigated by administration of immunosuppressive agents. Because these traditional regimens have shown only marginal improvement in survival, a recent study demonstrated that brief treatment with 3 costimulatory receptor-blocking agents (ie, cytotoxic T-lymphocyte–associated antigen 4 [CTLA4]-Ig, anti-CD40 ligand, and anti-lymphocyte function–associated antigen 1) induced long-term engraftment of mouse ESCs, human ESCs, mouse induced pluripotent stem cells (iPSCs), human iPSCs, and more differentiated ESC and iPSC deriv-
As an alternative to adjuvant immunosuppressive therapy, multiple investigators are exploring whether the administration of autologous iPSCs, which are reprogrammed from somatic cells, may be more immunoprivileged. The feasibility of transplantation of autologous iPSCs in large-animal models was reported recently using combined PET and MRI (Figure 6). Whether iPSCs are immune tolerated, however, has been challenged recently by a study that demonstrated that iPSCs could also induce a T-cell–dependent immune response in syngeneic recipients despite their autologous origins. Overall, these studies underscore the importance of in vivo imaging in developing strategies to enable the clinical translation of stem cell therapy.

Providing Mechanistic Insight Into the Benefits of Stem Cell Therapy

It was previously believed that the principal mechanism underlying the benefit of stem cell therapy was the regeneration of functional endothelial cells, vascular smooth muscle cells, and cardiomyocytes. Early studies in murine models of myocardial infarction have shown that cardiac stem cells appeared to integrate into the surrounding native myocardium and regenerate damaged tissue. With the advent of in vivo imaging, it has become evident that only a small fraction of injected cells survive, engraft, or proliferate beyond 8 weeks. Because the number of newly regenerated cells is too low to explain the significant functional improvement observed in most preclinical and clinical studies, it has been suggested that the functional benefit of therapy is related to secretion of soluble factors that protect the heart, induce neovascularization, and attenuate pathological remodeling (ie, paracrine effect). Although the exact mechanism associated with improvement in LV function remains unclear, molecular imaging may help elucidate some of these unknown factors.
Protecting the Myocardium
A potential application of in vivo imaging is to provide confirmation that stem cells release cytoprotective molecules that increase cardiomyocyte survival (ie, factors that reduce apoptosis and necrosis). Data in animal models suggest that even very low levels of apoptosis (23 myocytes per 10^5 cardiac nuclei) could result in progressive lethal dilated cardiomyopathy that may be prevented by the administration of stem cell therapy.50 The feasibility of in vivo imaging of cell death has been demonstrated with 99mTc-labeled annexin, albeit with difficulty differentiating necrosis from apoptosis.51 These limitations may be addressed with a combined annexin-labeled magnetofluorescent nanoparticle (AnxCLIO-cy5.5) with gadolinium-DTPA-NBD to detect apoptosis and necrosis, respectively.52 The incorporation of methods to image stem cell death may help develop future therapies to improve stem cell survival.

Inducing Neovascularization
Another important application of in vivo imaging is to verify that stem cells promote angiogenesis and arteriogenesis via release of paracrine factors, given that only a small number of vessels contain donor cells.49 In animal models of hindlimb ischemia and myocardial infarction, stem cell administration was associated with increased expression of proangiogenic factors that resulted in an increase in capillary density and collateral development, which can occur before any significant improvement in blood flow.53-55 In support of these findings, in vitro imaging using time-lapse microscopy has shown that co-culture of ESCs with endothelial cells results in endothelial cell migration, proliferation, lumen formation, and anastomosis to existing vasculature.56 The feasibility of in vivo imaging of angiogenesis in large animals and humans has been demonstrated by radionuclide probes targeting α5β3 integrin (ie, 18F-Galacto-RGD and 123I-Gluco-RGD),57,58 which are receptors that mediate endothelial cell migration, proliferation, and survival. Although it has yet to be applied directly to stem cell imaging, this technique has been used to demonstrate neovascularization after treatment with vascular endothelial growth factor in a porcine model of myocardial infarction, paving the way for its application to assess the benefits of stem cell therapy (Figure 7).59 It will be important to apply these imaging techniques in future studies to correlate the degree of angiogenesis with improvement in LV function.

Attenuating Pathological Remodeling
In vivo imaging may also help us better understand how stem cell therapy attenuates pathological remodeling. Stem cell administration has been found to decrease fibrosis, resulting in improvement in LV dilatation and systolic and diastolic function.59 These positive effects on postinfarction remodeling may be mediated by the release of molecules that limit local inflammation (eg, monocyte chemoattractant protein) and factors that modulate proliferation of fibroblasts and synthesis of collagen and extracellular matrix (ie, metalloproteinases, tissue inhibitor of metalloproteinase, transforming growth factor, serine proteases, and serine protease inhibitors).60 Imaging of leukocyte trafficking, protease activation, collagen deposition, and myocardial fibrosis in animal models of myocardial infarction has already been achieved by using various imaging techniques, including fluorescence molecular tomography, MRI, and radionuclide imaging.60 Future studies evaluating these processes before and after stem cell therapy will improve our understanding of how stem cell therapy minimizes scar formation and prevents heart failure progression.

In addition to reducing scar formation, stem cell administration has been shown to reverse the abnormal myocardial energetics that have been associated with heart failure. Progressive LV dilatation results in increased LV wall stress, myocyte overstretching, and a shift toward expression of fetal contractile proteins, resulting in imbalance in ATP delivery and demand.61 Administration of BMCs has resulted in improvement of metabolism in both small- and large-animal models, as measured by nuclear magnetic resonance spectroscopy.62,63 The development of an ultrafast magnetization saturation transfer method may facilitate examination of ATP kinetics in humans in future clinical trials.64

Evaluating the Efficacy of Stem Cell Therapy
Over the last decade, significant resources have been invested in the development of stem cell therapy for cardiac regeneration, yet results from large-animal and human studies based on analysis of global left ventricular ejection fraction (LVEF) have been underwhelming. In a recent meta-analysis of 52 published large-animal preclinical studies (n=888 animals; median follow-up of 4 weeks, range 2–17 weeks) using various types of cardiac stem cells, treated animals showed improvement in LVEF by 7.5%, which resulted from a significant decrease in end-systolic volume.65 Important predictors of improvement in LVEF included the use ofMSCs, left anterior descending coronary artery infarction, chronic occlusion models, a higher number of cells (≥10^7), and cell injection at least 1 week after myocardial infarction. Animal type and route of delivery were not predictive. Similarly, 2 meta-analyses of clinical studies (10 studies with 698 patients and 18 studies with 999 patients) using BMCs showed an increase in LVEF of 3% to 4% and a reduction in end-systolic volume of 4 to 6 mL with median follow-up of 6 months (range 3–18 months), measured predominantly by echocardiography and MRI.2,3 As shown in Table 2, the primary end point of most clinical studies has been the evaluation of LV size and global LVEF. Perhaps the incorporation of additional functional and anatomic measures, such as regional systolic function, diastolic function, perfusion, infarct size, and viability, will further elucidate the benefits of stem cell therapy.

Cardiac Function
The majority of preclinical and clinical studies have measured changes in LV size and function before and after treatment to evaluate the effectiveness of cardiac regenerative therapy. The accurate and precise measurement of these parameters is critical given the relatively small changes observed after stem cell therapy. Unfortunately, M-mode (ie, fractional shortening) and 2-dimensional echocardiography (ie, Simpson’s rule) make geometric assumptions about the LV that can lead to inaccurate measurements, especially in the presence of regional wall motion abnormalities. Poor
Table 2. Effects of Cell Therapy on Ejection Fraction, Perfusion, Infarct Size, and Viability

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>No. of Patients</th>
<th>Study Design</th>
<th>Number of Cells</th>
<th>F/U mo</th>
<th>EF</th>
<th>Perfusion</th>
<th>Infarct</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauer et al (2002)</td>
<td>10 BMSC vs 10 control</td>
<td>AMI/Obd</td>
<td>2.8 × 10^6</td>
<td>3</td>
<td>= (LVA, RNY)</td>
<td></td>
<td>(LVA, 201Tl SPECT; Rest)</td>
<td>= (99mTc SPECT: Stress-Rest)</td>
</tr>
<tr>
<td>TOPCARE-AMI (2004)</td>
<td>29 BMSC vs 30 CPC</td>
<td>AMI/Obd</td>
<td>2.1 × 10^6 BMSC, 1.6 × 10^6 CPC</td>
<td>12</td>
<td>≥ 9.3% (LVA, Cine MRI, 2D Echo)</td>
<td></td>
<td>(LVA, 201Tl SPECT; Rest)</td>
<td>= (99mTc SPECT: Stress-Rest; CE-MRI)</td>
</tr>
<tr>
<td>Fernandez-Aultes et al (2006)</td>
<td>20 BMSC vs 13 control</td>
<td>AMI/Obd</td>
<td>7.8 × 10^7</td>
<td>6</td>
<td>≥ 5.8% (Cine MRI)</td>
<td>N/A</td>
<td>(Cine MRI)</td>
<td>= Contractile reserve (O2 PET)</td>
</tr>
<tr>
<td>Chen et al (2004)</td>
<td>34 BMSC vs 35 control</td>
<td>AMI/Rnd/Obd</td>
<td>4.8–6.0 × 10^10</td>
<td>6</td>
<td>≥ 18% (LVA)</td>
<td>N/A</td>
<td>(LVA)</td>
<td>= (18F-FDG PET; EM linear local shortening and unipolar voltage)</td>
</tr>
<tr>
<td>MAGIC (2004)</td>
<td>14 BMSC vs 7 control</td>
<td>HF/Obd</td>
<td>3.0 × 10^7</td>
<td>12</td>
<td>= (LVA, 201Tl SPECT: Rest)</td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>= (99mTc SPECT: Rest)</td>
<td>N/A</td>
</tr>
<tr>
<td>Bartunek et al (2005)</td>
<td>19 BMSC vs 16 control</td>
<td>AMI/Obd</td>
<td>1.3 × 10^7</td>
<td>4</td>
<td>≥ 7% (LVA)</td>
<td></td>
<td>(LVA, 201Tl SPECT: Stress-Rest)</td>
<td>= (18F-FDG PET)</td>
</tr>
<tr>
<td>ICT (2005)</td>
<td>18 BMSC vs 18 control</td>
<td>CMI/Obd</td>
<td>9 × 10^7</td>
<td>3</td>
<td>≥ 8.0% (LVA)</td>
<td></td>
<td>(LVA, 201Tl SPECT: Stress-Rest)</td>
<td>= (18F-FDG PET)</td>
</tr>
<tr>
<td>Kvitris et al (2005)</td>
<td>11 BMSC, EPC vs 11 control</td>
<td>CMI/Obd</td>
<td>2–4 × 10^8</td>
<td>4</td>
<td>= (2D Echo, RNY)</td>
<td></td>
<td>(99mTc SPECT: Stress-Rest)</td>
<td>= (99mTc SPECT: Stress-Rest + (201Tl SPECT: Rest))</td>
</tr>
<tr>
<td>Erbs et al (2005)</td>
<td>13 CPC vs 13 control</td>
<td>CT/Obd</td>
<td>6.9 × 10^7</td>
<td>3</td>
<td>≥ 7.2% (Cine MRI)</td>
<td></td>
<td>CFR (intra coronary Doppler)</td>
<td>= (CE-MRI)</td>
</tr>
<tr>
<td>Janssens et al (2006)</td>
<td>33 BMSC vs 34 control</td>
<td>AMI/Rnd</td>
<td>4.8 × 10^8</td>
<td>4</td>
<td>= (Cine MRI)</td>
<td></td>
<td>(111C-acetate PET: Rest)</td>
<td>= (18F-FDG PET)</td>
</tr>
<tr>
<td>BOOST (2006)</td>
<td>30 BMSC vs 30 control</td>
<td>AMI/Rnd</td>
<td>2.4 × 10^9</td>
<td>18</td>
<td>= (Cine MRI)</td>
<td>N/A</td>
<td>= (CE-MRI)</td>
<td>= Oxidative metabolism (13C acetate PET)</td>
</tr>
<tr>
<td>REPAIR-AMI (2006)</td>
<td>101 BMSC vs 103 control</td>
<td>AMI/Rnd</td>
<td>2.4 × 10^9</td>
<td>4</td>
<td>≥ 5.5% (LVA)</td>
<td>N/A</td>
<td>= (CE-MRI)</td>
<td>N/A</td>
</tr>
<tr>
<td>ASTAMI (2006)</td>
<td>50 BMSC vs 50 control</td>
<td>AMI/Rnd</td>
<td>6.8 × 10^7</td>
<td>6</td>
<td>= (Gated SPECT, Cine MRI, Echo)</td>
<td></td>
<td>= (99mTc SPECT Rest)</td>
<td>= (99mTc SPECT Rest)</td>
</tr>
<tr>
<td>TOPCARE-CHD (2006)</td>
<td>28 BMSC vs 23 CPC vs 23 controls</td>
<td>CMI/Rnd</td>
<td>2.1 × 10^6 BMSC, 2.2 × 10^6 CPC</td>
<td>3</td>
<td>≥ 2.9% (LVA)</td>
<td>N/A</td>
<td>= (CE-MRI)</td>
<td>N/A</td>
</tr>
<tr>
<td>Fuchi et al (2006)</td>
<td>27 BMSC</td>
<td>CP/Obd</td>
<td>3.2 × 10^7, 2.8 × 10^7</td>
<td>3</td>
<td>= (2D Echo)</td>
<td></td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>N/A</td>
</tr>
<tr>
<td>Beeres et al (2006)</td>
<td>25 BMC</td>
<td>CP/Obd</td>
<td>8.4 × 10^7</td>
<td>12</td>
<td>≥ 4.0% (Gated SPECT)</td>
<td></td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>= (99mTc SPECT: Stress-Rest)</td>
</tr>
<tr>
<td>Li et al (2007)</td>
<td>35 PBSC</td>
<td>AMI/Obd</td>
<td>7.3 × 10^7</td>
<td>6</td>
<td>≥ 5.0% (2D Echo)</td>
<td>N/A</td>
<td>= (CE-MRI)</td>
<td>N/A</td>
</tr>
<tr>
<td>Chang et al (2008)</td>
<td>40 BMSC</td>
<td>AMI/Obd</td>
<td>7.0 × 10^8</td>
<td>6</td>
<td>≥ 7.0% (2D Echo, MRI)</td>
<td>N/A</td>
<td>= (CE-MRI)</td>
<td>N/A</td>
</tr>
<tr>
<td>MYSTAR (2009)</td>
<td>60 BMSC</td>
<td>AMI/Rnd</td>
<td>1.6 × 10^9</td>
<td>3–12</td>
<td>≥ 4.0% (Gated SPECT)</td>
<td></td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>= (99mTc SPECT: Stress-Rest, EM)</td>
</tr>
<tr>
<td>Hare et al (2009)</td>
<td>39 MSC vs 21 placebo</td>
<td>AMI/Rnd</td>
<td>0.5–5 × 10^8</td>
<td>6–12</td>
<td>= (2D Echo);</td>
<td></td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>&lt; (5.2% (MRI)</td>
</tr>
<tr>
<td>Plewka et al (2009)</td>
<td>40 BMSC vs 20 control</td>
<td>AMI/Rnd</td>
<td>1.4 × 10^9</td>
<td>6</td>
<td>≥ 5.0% (2D Echo)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>RESENT (2009)</td>
<td>160 BMSC vs 40 control</td>
<td>AMI/Rnd</td>
<td>1.8 × 10^9</td>
<td>6</td>
<td>≥ (MRI, LVA)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Grajek et al (2010)</td>
<td>31 BMSC vs 14 control</td>
<td>AMI/Rnd</td>
<td>1.2 × 10^9</td>
<td>12</td>
<td>= (2D Echo, RNY)</td>
<td></td>
<td>= (99mTc SPECT: Stress-Rest)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

(Continued)
interobserver and intraobserver variability also limit the use of these techniques for serial imaging. These limitations can be addressed by the use of 3D echocardiography and cardiac MRI, which provide improved accuracy and reproducibility.

An alternative approach, and perhaps a more sensitive marker, is to assess regional contractile function by measuring strain and strain rate by echocardiography or MRI. The strain and strain rate reflect the absolute deformation and the speed of deformation (shortening and thickening) of the myocardium from an applied force in a specific myocardial segment and layer (ie, endocardial and epicardial walls), respectively. This technique subtracts motion due to the effects of neighboring segments (eg, tethering) that can mask pathological deformation and impart abnormal motion to normal segments. Abnormal strain and strain rate imaging, for example, have been found in patients with diabetes mellitus, amyloidosis, and doxorubicin-induced cardiac injury who have normal LVEF.66,67 Similarly, a recent report in 67 patients with ST-elevation myocardial infarction who were randomized to intracoronary infusion with bone marrow progenitor cells or placebo showed significantly greater improvement in regional strain rate in the treatment group, with no significant differences in LVEF (Figure 8).67 Further application of this technique in preclinical and clinical studies may reveal a more impressive contribution of stem cell therapy for improving contractile function.

**Diastolic Function**

In addition to systolic dysfunction, an abnormal LV filling pattern is common in patients with ischemic heart disease and is a marker of poor prognosis. Moreover, patients who have improvement in LV filling after treatment have fewer symptoms and better survival.68 Despite this evidence, the evaluation of diastolic function has been performed in only a handful of clinical studies, unlike in animal studies, in which invasive pressure-volume loop measurements of LV relaxation are a mainstay. In an initial study, Schaefer et al69 reported improvement in mitral valve filling (E/A ratio) for up to 18 months in patients after acute myocardial infarction who were randomized to treatment with intracoronary infusion of bone marrow cell therapy, but the effect lasted less than 5 years. Other diastolic function parameters showed no difference. Diederichsen et al70 also showed improvement in diastolic function (ie, E/e’ ratio left atrial volume and plasma N-terminal pro-brain natriuretic peptide) in patients with chronic ischemic heart disease after repeat intracoronary administration of BMCs at 12-month follow-up. Although echocardiographic measures of diastolic function are noninvasive and therefore are preferred for serial evaluation in patients, they correlate only modestly with invasive pressure-volume loop analysis, the “gold standard” for evaluating LV relaxation. Perhaps further development and validation of strain imaging by echocardiography or MRI, which has the potential to be a more accurate and precise measure of LV relaxation properties, will generate greater interest in the incorporation of noninvasive diastolic function measures in clinical stem cell trials.71,72

**Myocardial Perfusion**

The potential for stem cell–induced vasculogenesis has prompted several studies to measure myocardial perfusion. Although the majority of studies appear to show improvement, more in-depth analysis reveals that the data are both limited and conflicting (Table 2). For example, only 6 studies evaluated both stress and rest phases,73–81 and only 2 studies incorporated PET data,82,83 which provide a more quantitative assessment of myocardial perfusion. Results have also been inconsistent. Although SPECT perfusion data at rest have shown a decrease in the number of segments with perfusion defects after treatment, studies incorporating both stress and rest phases only show a decrease in overall ischemia extent and not in the number of rest perfusion defects. Similarly, only 1 of the 2 studies incorporating PET perfusion showed improvement in overall myocardial blood flow in the treatment group compared with the control group. Interestingly, all studies that evaluated regional myocardial blood flow by intracoronary Doppler wire showed improvement in coronary flow reserve in the infarct-related vessel after treatment.84,85,86 The performance of serial evaluation with an intracoronary Doppler wire, however, is not without risk. Taken together, these findings suggest that further evaluation of regional and global myocardial perfusion reserve by PET should be performed to confirm that stem cell therapy contributes to detectable levels of vasculogenesis in the myocardium.
Infarct Size and Viability

Although a variety of methods can be used to assess the effects of stem cell therapy on infarct size and the amount of viable tissue, direct visualization by contrast-enhanced MRI, radionuclide imaging (ie, PET or SPECT), and electromechanical mapping are preferred over indirect approaches that evaluate the extent and severity of LV dysfunction, such as LV angiography, 2-dimensional echocardiography, or cine MRI. Because of its precise delineation of scar tissue and the ability to distinguish between subendocardial and transmural infarction, contrast-enhanced MRI is considered the most accurate for assessing infarct size. Interestingly, the majority of stem cell studies using contrast-enhanced MRI have failed to show a significant change in infarct size, whereas other techniques have demonstrated a significant decrease (Table 2).

The reasons for this discrepancy remain unclear because previous studies have provided only limited details on how contrast enhancement images were acquired and analyzed, 2 critical factors for determining changes in infarct size. Interestingly, the majority of stem cell studies using contrast-enhanced MRI have failed to show a significant change in infarct size, whereas other techniques have demonstrated a significant decrease (Table 2).

The reasons for this discrepancy remain unclear because previous studies have provided only limited details on how contrast enhancement images were acquired and analyzed, 2 critical factors for determining changes in infarct size. One reason may be that prior studies failed to control for the time interval between contrast injection and image acquisition, which is known to affect infarct size, especially in acute settings in which gadolinium can enhance the area at risk and the infarct core. Additionally, previous studies have used traditional methods of image analysis, which have poor reproducibility. Finally, these studies did not evaluate changes specific to the peri-infarct area, which has been shown to have independent prognostic value beyond that of LV infarct size, volume, or ejection fraction. Perhaps a more standardized and comprehensive approach to image acquisition and analysis will provide clearer evidence that stem cell therapy reduces the size of both the peri-infarct and infarct zones.

To determine viability, 18F-FDG PET, which measures glucose metabolism, is considered the "gold standard" for its superior sensitivity and specificity compared with other functional measures of viability, including stress echocardiography and SPECT. All clinical studies using 18F-FDG PET have consistently shown improvement in myocardial viability (Table 2). In contrast to glucose metabolism, improvement in contractile reserve measured by dobutamine echocardiography has not been shown in all studies. This is not surprising, because myocardium that is severely dysfunctional because of extensive cellular damage usually does not have intact contractile reserve but may have preserved glucose metabolism. Additional studies incorporating both these measures will be important to further delineate the benefits of stem cell therapy in improving myocardial viability.

Evaluating the Long-Term Safety of Stem Cell Therapy

In addition to establishing the efficacy of stem cell therapies, routine clinical implementation of novel cell-based treatment will depend on the successful resolution of important safety concerns, such as cell migration, tumorigenicity, and arrhythmogenicity. Particularly challenging is the monitoring of cell biodistribution, because administered cells may be essentially indistinguishable from host cells. As discussed previously, short-term in vivo cell monitoring has already been achieved in clinical trials using radionuclide-based imaging techniques.
Safety concerns, however, continue to impede regulatory approval of iron oxide– and reporter gene–labeling techniques for longer-term monitoring. Also problematic is the detection of the low number of cells that may migrate to nontarget organs. This need potentially may be met by a recently approved class of fluorocarbon-based reagents designed to safely and efficiently label cells ex vivo without the use of transfection agents.  

The ability to track stem cells in vivo is also vital for detecting the formation of inappropriate ectopic tissues and for guiding their elimination. This is especially critical for human ESCs and iPSCs, which have the potential to form teratomas (tumors composed of a haphazard array of somatic cell types) and teratocarcinomas (a malignant tumor composed of a teratoma mixed with embryonal carcinoma and/or choriocarcinoma).  

Although multimodality approaches have been applied to image teratoma formation in vivo with MRI and reporter gene–based optical imaging, the former is limited by sensitivity and the latter by poor tissue penetration. An alternative approach is to use the PET reporter probe $^{64}$Cu-radiolabeled cyclic RGD peptides to image αvβ3 integrin expression, the upregulation of which is known to play a key role in angiogenesis and metastasis (Figure 1).  

Cao et al recently demonstrated the superiority of this technique for monitoring tumorigenicity after ESC transplantation compared with more commonly used PET tracers ($^{18}$F-FDG and $^{[18]F}$-FLT), which are less sensitive in detecting teratoma formation. Finally, molecular imaging can also monitor suicide gene ablation of teratomas with HSV-tk used as both a PET reporter gene and a suicide gene, respectively.  

Quantification and localization of cell engraftment will be important to limit arrhythmogenic risk. Although higher levels of cell engraftment may be associated with improved efficacy, focal grafts can create inhomogeneities within the myocardium that predispose the heart to reentrant arrhythmias. In support of this theory, in vitro studies using skeletal myoblasts, neonatal cardiomyocytes, and MSCs have found that the risk of reentrant arrhythmias appears to be dose dependent. Results from subsequent in vivo phase I clinical trials, however, have been conflicting, with an increase in arrhythmogenic risk associated with skeletal myoblasts, whereas minimal risk has been found with transplantation of MSCs and BMCs. However, with so few cells surviving long-term in vivo, it would be premature to conclude that the arrhythmogenic risk of stem cells other than skeletal myoblasts is low. Overall, these studies in cell migration, tumorigenicity, and arrhythmogenicity demonstrate that in vivo imaging can help investigators monitor recipients after transplantation.

**Future Directions**

With parallel developments in conventional and molecular imaging technology, investigators are well positioned to apply and extend current capabilities to facilitate the clinical translation of stem cell therapy. These invaluable tools have already helped researchers study issues related to poor engraftment, identify possible mechanisms of benefit, and characterize potential safety concerns. With this knowledge in hand, investigators can now focus on finding solutions to the challenges facing the clinical implementation of regenerative therapy.

In the next several years, we anticipate that investigators will gravitate toward cell delivery under direct visual guidance using advanced imaging modalities, including MRI and CT. In vivo tracking of cells during and shortly after delivery will enable identification of techniques to improve initial engraftment, such as administration of agents that optimize cell adhesion, control hemodynamics, and prevent migration to nontarget areas. Longer-term tracking of cells remains a challenge, especially in humans, given the limitations of direct cell labeling and the risks of insertional mutagenesis or immunogenicity associated with reporter gene labeling. Greater focus on the development of a safe, robust technique to label and follow cell viability, differentiation, and migration is needed before routine implementation. In the meantime, currently available techniques will be used to identify the optimal cell type, timing of delivery, and immunosuppressive regimens in animal models to improve cell engraftment and survival. Future studies will also likely apply advanced imaging technology to provide mechanistic insight to determine whether regulation of these processes may augment the benefits of stem cell therapy. Finally, we encourage investigators to provide a more comprehensive evaluation of the benefits of stem cell therapy beyond an evaluation of global systolic function. We firmly believe that application of the advanced imaging techniques reviewed here will be instrumental to the successful clinical implementation of this novel therapy.

**Acknowledgments**

We thank Amy Morris for preparing the illustrations. Because of space limitations, we were unable to include all of the important papers relevant to stem cell imaging; we apologize to those investigators whom we omitted here.

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None.

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