# Comparison of Adult Versus Embryonic Stem Cell Therapy for Cardiovascular Disease: Insights from Molecular Imaging Studies

*Andrew S. Lee, BS, and Joseph C. Wu, MD, PhD*

**Corresponding author**
Joseph C. Wu, MD, PhD
Stanford University School of Medicine, Edwards Building R354, Stanford, CA 94305, USA.
E-mail: joewu@stanford.edu

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In the previous decade, cardiac cell replacement therapy has emphasized adult stem cells such as skeletal myoblasts, bone marrow mononuclear cells, and endothelial progenitor cells. Functional restoration of systolic function has been documented in most of these cases, but beneficial results have rarely persisted for significant lengths of time due to failure of cells to survive and the as yet controversial role of transdifferentiation into endogenous tissue. Future efforts at cell replacement therapy will likely focus upon cellular derivatives of embryonic stem (ES) cells, which can be induced to form any cell type of the body. Use of ES cells, however, presents several novel considerations such as teratoma formation and immune rejection. This review summarizes the current progress made in the field of cardiac cell replacement therapy and the role noninvasive imaging can play in realizing the therapeutic potential of stem cells.

## Introduction

Coronary artery disease is the leading cause of death in the Western world. Congestive heart failure (CHF) in particular has become an increasing cause for concern. Currently, 5.3 million Americans suffer from this condition, 20% of whom will die within 1 year of being diagnosed. The annual number of CHF hospital discharges in the United States has risen from 400,000 in 1979 to over 1 million in 2005 [1]. One major reason for the sharp increase in prevalence of CHF is improvements made in the treatment of acute coronary syndrome, which have raised patient survival rates post–myocardial infarction (MI). Patients who survive MI are at increased risk for developing CHF, as the heart has only limited capacity for intrinsic cellular regeneration. Ischemic insult to the myocardium causes cardiomyocyte death, leading to fibrous tissue deposition and ventricular remodeling to compensate for failure of the injured region of muscle to contract.

Stem cell therapy is a novel method of treating CHF through the replacement of injured myocardium with healthy cells capable of restoring contractility to the heart. Thus far, a number of cell candidates have been tested in both animal studies and human trials. The ideal cell type would not only result in repopulation of the infarct zone by cardiomyocytes, but should also stimulate revascularization of the injured region of heart by angiogenesis, and electrically reintegrate with the surrounding myocardium. As both preclinical and clinical trials have shown, however, not all of these conditions have to be met in order for restoration of function to occur. Conversely, many of these studies have produced conflicting results, prompting questions regarding the optimal cell type for therapy and the molecular mechanisms by which cardiac cell therapy can restore myocardial function [2•]. This review summarizes the progress that has been accomplished with regard to the different cell types used in cardiac cell replacement therapy and the role noninvasive imaging can play in evaluating each cell type.

## Adult Stem Cells

Clinically, adult stem cells carry the advantage of autologous harvest and can thereby bypass concerns of immune rejection. In addition, many adult stem cells can be transplanted into patients directly without in vitro expansion, thus avoiding culture conditions that could predispose cells to abnormal developments such as karyotype changes. Cells that require in vitro culture to attain high numbers (eg, skeletal myoblasts [SkMbs] and mesenchymal stem cells [MSCs]) can also be expanded rapidly without prolonged passage time.

## Skeletal Myoblasts

SkMbs were the first adult cell type used in cardiovascular transplantation studies. SkMbs are derived from muscle satellite cells, a self-renewing population of progenitor cells located within muscle that proliferate and give rise to myoblasts after muscular injury. The pioneering surgeries in which SkMbs were transplanted into injured hearts were conducted by Ray Chiu at McGill University in the early 1990s [3]. The first report demonstrating a restoration of myocardial function in postinfarction animal models was published in 1998 by Doris Taylor at Duke University [4]. Since then, several preclinical studies have shown the efficacy of SkMbs in aiding functional recovery after MI [5,6]. SkMbs engraft in the heart and form mature skeletal muscle [6]. The exact mechanisms by which SkMbs contribute toward the restoration of cardiac function are largely unknown, but they most likely result from either a direct physical scaffolding effect or the release of trophic factors such as hepatocyte growth factor, vascular endothelial growth factor (VEGF), or other paracrine agents. SkMbs carry the advantage of being ischemia resistant as compared to adult or fetal cardiomyocytes, and they largely differentiate into myogenic cells that can potentially supplement the injured region of myocardium. Because SkMbs form skeletal muscle, however, they are electrically isolated from surrounding myocardium and thus have been associated with arrhythmogenesis post-transplantation [5].

## Bone Marrow Mononuclear Cells

Bone marrow mononuclear (MN) cells are comprised of hematopoietic stem cells, MSCs, and endothelial progenitor cells (EPCs), all of which can contribute independently to restoration of cardiac function. After preliminary studies using MN cells for cardiac transplantation were conducted in 1999 [7], MN cells came to the forefront of cardiac cell replacement therapy in 2001, when two separate groups reported transdifferentiation of MN cells to cardiomyocytes upon engraftment in animal hearts [8,9]. Subsequent studies involving transplantation of MN cells into animal models of MI were not able to reproduce these results [10,11], but improved functional recovery was documented in animals that received these infusions, compared with animals that did not [10]. Benefits of MN cell transplantation have been attributed to the release of paracrine factors by these cells, such as VEGF, angiopoetin-1, and monocyte chemoattractant protein-1, which have been shown to increase collateral perfusion and restore cardiac function in animals [12]. MN cells are easily harvestable from the bone marrow of transplant recipients and do not need to be expanded in culture. Unlike SkMbs, MN cells do not form electrically insulated islands separate from the rest of the myocardium upon engraftment and thus do not contribute to arrhythmogenicity.

## Endothelial Progenitor Cells

EPCs are bone marrow–derived cells that enter the peripheral circulation after injury and contribute toward revascularization of the wounded area by angiogenesis [13]. EPCs can be mobilized into the peripheral circulation by administration of granulocyte colony-stimulating factor and collected directly from the blood for transplantation [14]. Alternatively, they may be harvested directly from bone marrow and expanded in culture. Following the isolation of EPCs in 1997 by Asahara et al. [15], these cells were shown to revascularize ischemic legs of rabbits and induce functional recovery [16]. The first studies using EPCs in the heart were published in 2001 and demonstrated that EPCs could contribute to functional regeneration of the myocardium via angiogenesis and neovascularization [17]. Although these cells are incapable of forming cells of myogenic lineages, they do restore function to the injured area of the heart by increasing access of injured regions of myocardium to oxygen and blood nutrients.

## Mesenchymal Stem Cells

Mesenchymal stem cells or marrow stromal cells (MSCs) are the most recent adult cell type to be tested in cardiac cell therapy. MSCs are found in numerous locations of the body, such as adipocytes, heart tissue, umbilical cord blood, and bone marrow, where they compose less than 0.01% of the total cell population [18]. MSCs can be isolated from the bone marrow and expanded in culture. They are capable of differentiating in vitro and in vivo into adipocytes, fat, bone, cartilage, skeletal muscle, and, more controversially, cardiomyocytes [19,20]. MSCs have several unique characteristics that make them an attractive candidate for adult stem cell therapy. Most importantly, MSCs are characterized by low expression of human leukocyte antigen class II proteins and a lack of B7 costimulatory molecules, making them potentially immunoprivileged and allowing for allogeneic transplantation [21]. Several groups have reported that MSCs can inhibit inflammatory responses, and research into the use of MSCs in ameliorating graft versus host disease and other inflammatory conditions is underway [22]. MSCs have also been shown to home to areas of injury and hence can be administered to recipients via intravenous infusion as opposed to via the coronary arteries [23]. Although several studies have shown MSCs are capable of differentiating into cardiomyocytes upon transplantation to the heart, this has only occurred in extremely low numbers and has not been documented within the region of infarct. One potential pitfall of MSCs is the large size of these cells. In particular, administration of MSCs into canine hearts has been shown to cause microinfarction [24].

## Embryonic Stem Cell–Derived Cells

Recently, human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells have received considerable

attention as an alternate cell source for cardiac cell replacement therapy. Unlike adult stem cells, hES and iPS cells carry the potential for unlimited self-renewal and pluripotency. Although efforts to coax SkMbs and MN cells to transdifferentiate into cardiomyocytes upon cardiac transplantation have met with little success, hES cells can be differentiated into cardiomyocytes and administered directly. The primary pitfall of hES cell–derived therapy is the possible formation of teratomas in transplant recipients due to contamination by unpurified populations of undifferentiated hES cells. Immune rejection is a second concern in hES cell–based therapy, as it has been shown that undifferentiated hES cells can upregulate myosin heavy chain (MHC) class I molecules upon differentiation [25•]. In mice models, mouse ES (mES) cells that are differentiated into embryoid bodies (EBs) are rejected rapidly after transplantation [26]. Finally, expansion and differentiation of hES cells requires significant in vitro cell culture. This may predispose potential donor cells to acquire epigenetic changes, karyotype abnormalities, and mutations in both the nuclear and mitochondrial genomes [27].

**hES cell–derived cardiomyocytes**

hES cells form cell aggregates known as EBs when cultured without an inhibitory mouse embryonic feeder layer. Spontaneous differentiation of cardiomyocytes within the EB can occur by day 8, as evidenced by the presence of spontaneous beating cells. Cardiomyocytes can be isolated from the surrounding cells by Percoll density–based isolation, which has been shown to generate a 70% pure cardiac cell population for hES cells [28]. Genetic engineering can also be used to transfect hES cells with vectors carrying a cardiac-specific promoter such as the α-MHC, which drive expression of enhanced green fluorescent protein (EGFP) or antibiotics resistance gene. EGFP-positive or antibiotic-resistant cells can be separated from the background via fluorescence-activated cell sorting or antibiotic selection to produce a 99% pure cardiomyocyte population [29].

Recently, microarray data for cardiomyocytes responsible for EB beating have shown expression of cardiomyocyte genes at levels comparable to those of a 20-week-old fetal heart [30••]. Electrical activity in these cells is similar to both atrial and ventricular cardiomyocytes and is capable of restoring conductance in pig hearts characterized by atrioventricular blockage [31,32]. Thus far, only a few studies have used hES cell cardiomyocytes to treat animal models of MI. Although several of these studies have shown that hES cell cardiomyocytes align with host cardiac tissue upon transplantation and home in on areas of infarct [33], other reports indicate that hES cell cardiomyocytes integrate only minimally with surrounding tissue [30••,34•]. Functional restoration in myocardial contractility is still observed in these instances, however, and hES cell cardiomyocyte grafts persist longer than non-cardiomyocyte counterparts [29,34•].

**hES cell–derived endothelial cells**

hES cells can also be differentiated into grafts that promote restoration of cardiac function by forming blood vessels to supply oxygen and nutrients to injured myocardium. Although autologous EPCs can also be used for this purpose, heart failure patients receiving such grafts may suffer from comorbidities such as diabetes, hypertension, and hypercholesterolemia. In these patients, the regenerative capacity of EPCs may be compromised, and therefore hES cell–derived endothelial cells (ECs) may provide a better alternative source of vascular donor cells. Work in our group has shown that mES cell–derived ECs contribute toward functional recovery and neovascularization in the heart after MI [35•]. Recently, Wang et al. [36] reported that hES cell–derived ECs are capable of forming long-term grafts and blood-carrying microvessels in the heart that can last as long as 150 days.

## Clinical Trials

Early success in preclinical studies using adult stem cells led to a fast transition between bench and bedside for cardiac cell therapy, with a number of phase 1 clinical trials starting in the late 1990s and early 2000s. Randomized and controlled trials of MN, SkMb, and EPCs have shown mixed results, however (Table 1) [37–41]. The Bone Marrow Transfer to Enhance ST-Elevation Infarction Regeneration (BOOST) trial was the first randomized clinical trial using MN cells for cardiac replacement therapy [37]. Although significant benefits to left ventricular ejection fraction (LVEF) were reported 6 months after stem cell administration, differences between MN cell recipients and control patients were not significant by 18 months. The Autologous Stem Cell Transplantation in Acute MI (ASTAMI) trial was a second major randomized MN cell trial in which LVEF was monitored in cell and placebo recipients by single photon emission CT (SPECT), echocardiography, and MRI [39]. Unlike the BOOST trial, however, results from ASTAMI did not show any difference between patients who received cell infusions and those who did not. The Myoblast Autologus Graft in Ischemic Cardiomyopathy (MAGIC) trial was also recently completed and is the first double-blind, placebo-controlled stem cell trial using SkMb [41]. At 6 months, however, no functional differences were observed between control patients and recipients of cell infusions.

Despite these setbacks in randomized and controlled trials, most clinical trials have shown functional benefits at least in the short term after stem cell administration. Increases in LVEF for patients receiving cell infusion as compared to patients who do not receive infusion have yet to be reported at greater than 1 year post-therapy, which suggests benefits from cell replacement therapy may be limited to accelerated recovery. A phase 1 trial testing the use of allogeneic MSCs (Provacel; Osiris

**Table 1. Major randomized and controlled trials for cardiac cell replacement therapy**

| Study, year | Setting | Cell number and type | Study design | LVEF assessed by | Outcome |
|---|---|---|---|---|---|
| BOOST [37], 2004 | AMI | $2.5 \times 10^9$ MN cells | Randomized trial; 30 patients received MN cell infusion, 30 patients received no infusion | Cardiac MRI | 6.0% increase in LVEF for MN group over control group at 6 months; no difference in LVEF at 18 months |
| TOPCARE-CHD [38], 2004 | Chronic LV dysfunction | $2.5 \times 10^8$ MN cells or $2 \times 10^7$ EPCs | Randomized, crossover trial; 28 patients received EPC followed by MN cell infusion, 24 patients received MN cell infusion followed by EPC, 23 patients received no infusion | LV angiography, cardiac MRI | 4.0% increase in LVEF for patients receiving MN cells then EPCs over control group at 3 months; no statistical difference in LVEF was observed in patients who received EPC infusion followed by MN cell infusion |
| ASTAMI [39], 2006 | AMI | $7 \times 10^7$ MN cells | Randomized trial; 47 patients received MN cell infusion, 50 patients received no infusion | SPECT, echocardiography, cardiac MRI | No significant difference in LVEF between MN group and control group at 6 months |
| REPAIR-AMI [40], 2006 | AMI | $2.4 \times 10^8$ MN cells | Randomized, double-blind trial; 101 patients received MN cell infusion, 98 received placebo infusion | LV angiography | 2.5% increase in LVEF for MN group over control group at 4 months; reduction in adverse cardiovascular events for MN group as compared with control group at 1 year |
| MAGIC [41], 2008 | LV dysfunction, AMI, or indication for coronary surgery | $4 \times 10^8$ or $8 \times 10^8$ SkMb | Randomized trial; 33 patients received low dose of SkMb, 30 received high dose, 34 received no infusion | Echocardiography | No significant difference in LVEF between SkMb groups and control group at 6 months |
| Osiris Therapeutics Provacel Trial (phase 1 trial completed, publication forthcoming) | AMI | Dose-controlled MSC | Randomized, double-blind, 53-patient phase 1 trial | Cardiac MRI | 7.3% increase in LVEF for patients with "major anterior wall" MI over control patients with similar MI profiles at 6 months; 28% reduction in arrhythmic events for MSC group over control group at 6 months |

AMI—acute MI; ASTAMI—Autologous Stem Cell Transplantation in Acute MI Trial; BOOST—Bone Marrow Transfer to Enhance ST-Elevation Infarction Regeneration Trial; EPC—endothelial progenitor cell; LVEF—left ventricular ejection fraction; MAGIC—Myoblast Autologous Grafting in Ischemic Cardiomyopathy Trial; MI—myocardial infarction; MN—bone marrow mononuclear cell; MSC—mesenchymal stem cell; REPAIR-AMI—Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction; SkMb—skeletal myoblast; SPECT—single photon emission CT; TOPCARE-CHD—Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease.

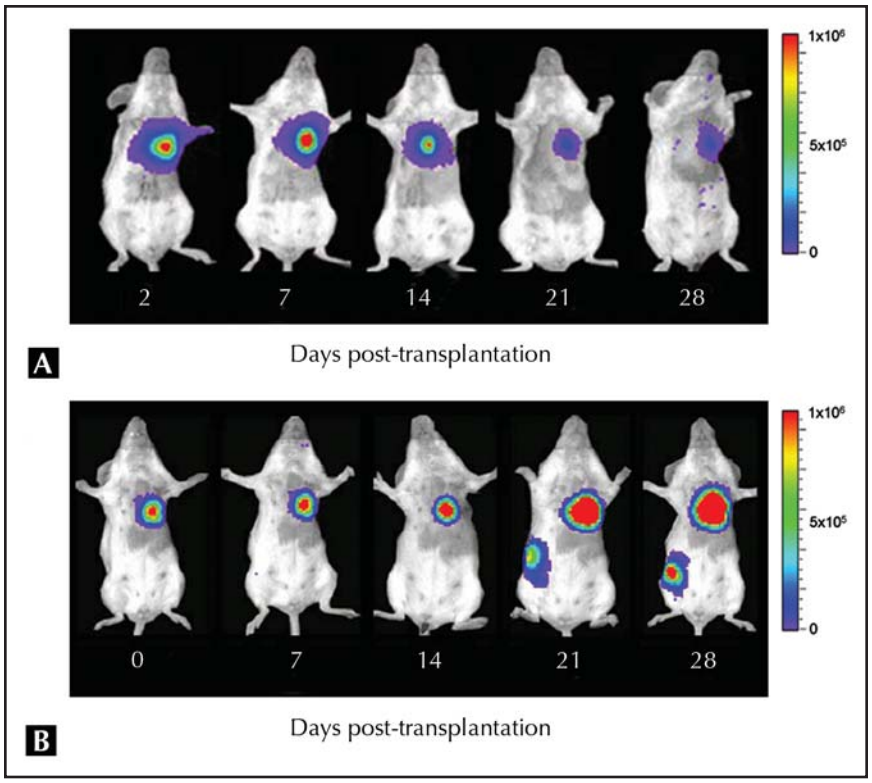


**Figure 1. A**, Reporter gene imaging for monitoring survival of $1 \times 10^6$ human embryonic stem (hES) cell–derived cardiomyocytes in murine model of myocardial infarction. Acute cell death occurs in first 3 weeks of transplantation followed by persistent level of signals indicating a stable graft. **B**, Imaging for $1 \times 10^6$ undifferentiated hES cells transplanted into a mouse heart. Intracardiac and extracardiac teratoma formation occurs by week 4. (*From* Cao et al. [30••]; with permission.)

Therapeutics, Columbia, MD) has also been completed and has shown promising results at the 6-month time point. It remains to be seen whether MSCs will be able to sustain restoration of function longer than other types of adult stem cells.

## In Vivo Tracking of Cardiac Stem Cells

The varied results in clinical trials have raised a number of questions regarding the molecular mechanisms by which different cell types contribute to the regeneration of function in injured myocardium. In that regard, noninvasive imaging provides a method by which cell survival, engraftment, proliferation, and migration within the body can be monitored. Currently, most cell therapy protocols rely on histological analysis of engrafted cells via reporter genes such as GFP and β-galactosidase (lacZ), which require animal sacrifice and preclude longitudinal monitoring. In human studies, changes in clinical measurements, such as LVEF, left ventricular end-systolic and diastolic volumes, and occurrence of cardiovascular events provide only indirect information about cell engraftment and function. To fully understand why stem cell therapy results in restoration of myocardial function, investigators must be able to monitor the biology and physiology of transplanted cells in living subjects over time. At present, the primary methods of monitoring cardiac cell transplantation can be divided into two modalities: direct labeling of stem cells with a physical compound (eg, radioactive tracers, iron particles, or quantum dots) and genetic labeling of stem cells with reporter genes.

### Direct cell labeling

Direct cell labeling is the most straightforward approach to visualize transplanted cells in living subjects. Labeling is completed before cell administration and can be accomplished with superparamagnetic iron oxide (SPIO) particles for MRI, nanoparticle labeling for fluorescent imaging, or radionuclide labeling for SPECT or positron emission tomography (PET). Both MRI and radionuclide imaging have high spatial resolution (MRI >> PET ~ SPECT) and detection sensitivity (PET > SPECT >> MRI). These modalities of imaging can thus provide an accurate representation of where cells localize to within the body after administration. A number of studies have used both MRI [42,43] and radionuclide imaging [23,44] to visualize distribution of donor cells following transplantation. Direct labeling techniques share a common disadvantage, however, in that labels can continue to produce signal even after cell death. In addition, signal is diluted with cell division and hence cannot be used to monitor cell proliferation. SPIO agents suffer from the unique problem of being taken up by macrophages after donor cell death. A recent report by Li et al. [45] showed that MRI was unable to monitor cell proliferation and that SPIO agents remained in leg muscle long after labeled cells had died, giving a false signal.

Radionuclide labels are not as prone as SPIO agents to give off false signal due to their short half-life. Typical radionuclides such as Tc-99m, Cu-64 PTSM, or F-18 FDG are characterized by half-lives that are on the order of hours. Thus, the longest signal that can be detected using

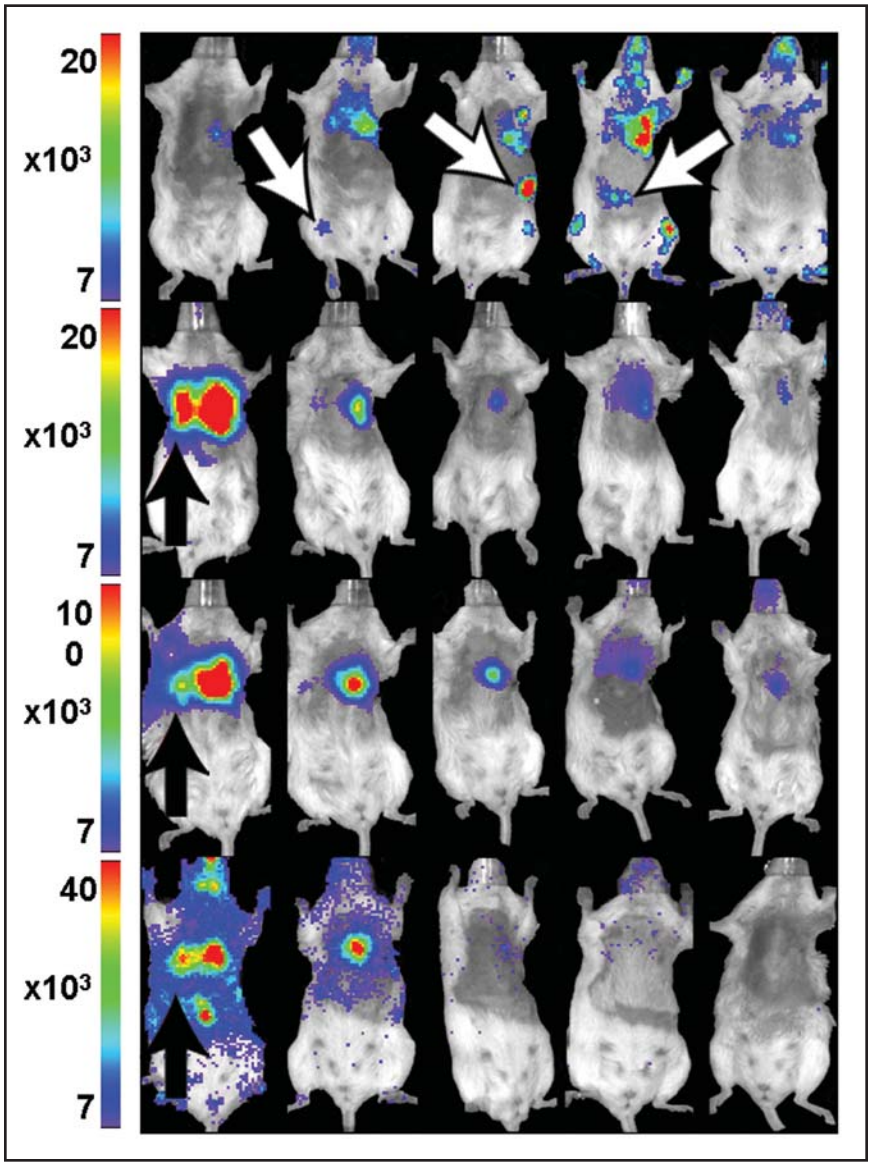


**Figure 2.** Head-to-head comparison of cell survival, engraftment, and migration for bone marrow mononuclear (MN) cells, skeletal myoblasts (SkMbs), mesenchymal stem cells (MSCs), and fibroblasts. *White arrows* reveal extracardiac migration of MN cells to the femur, spleen, and liver. *Black arrows* show early localization of SkMbs, MSCs, and fibroblasts to the lung. MN cell grafts persisted at higher signal intensity than SkMbs, MSCs, or fibroblasts. (*From* van der Bogt et al. [47•]; with permission.)

radionuclide-labeled cells is usually limited to under a week. Additionally, radionuclide tracers have a high nonspecific uptake by liver and kidney cells, which results in high background signals for these organs. High background signals are problematic because they prevent accurate tracking of extracardiac cellular migration, which is important in safety monitoring of off-target sites.

### Reporter gene imaging

Reporter gene imaging is an alternative approach to monitoring cells in vivo. Although reporter genes can be transfected or transduced into cells for visualization by MRI and SPECT, at present the two most commonly used modalities are bioluminescence imaging (BLI) and PET. In BLI, the firefly luciferase (Fluc) gene is delivered into donor cells via a viral or nonviral vector. The reporter gene can integrate into the host cell's genome, where it undergoes transcription into mRNA and translation into the reporter protein. Interaction of the Fluc reporter protein with its substrate D-luciferin produces light that is detectable by an ultrasensitive cooled charge-couple device camera. PET imaging uses a herpes simplex virus thymidine kinase (HSV-tk) gene or its variants, such as mutant thymidine kinase (HSV-sr39tk), truncated thymidine kinase (HSV-ttk), or human mitochondrial thymidine kinase 2 (hTK2). Donor cells are transfected or transduced with the reporter gene. After cell transplantation, the different types of PET reporter probe (eg, 9-(4-[18F]fluoro-3-(hydroxymethylbutyl)guanine; [18F]-FHBG) can be administered intravenously. The HSV-tk (or variant) can phosphorylate [18F]-FHBG, which emits high-energy photons (511 keV) that are detected by the PET camera.

In contrast with direct labeling, cells that produce signals in reporter gene imaging must be alive, as transcription is a prerequisite for creation of the reporter protein. In addition, because the reporter gene integrates into the host cell's

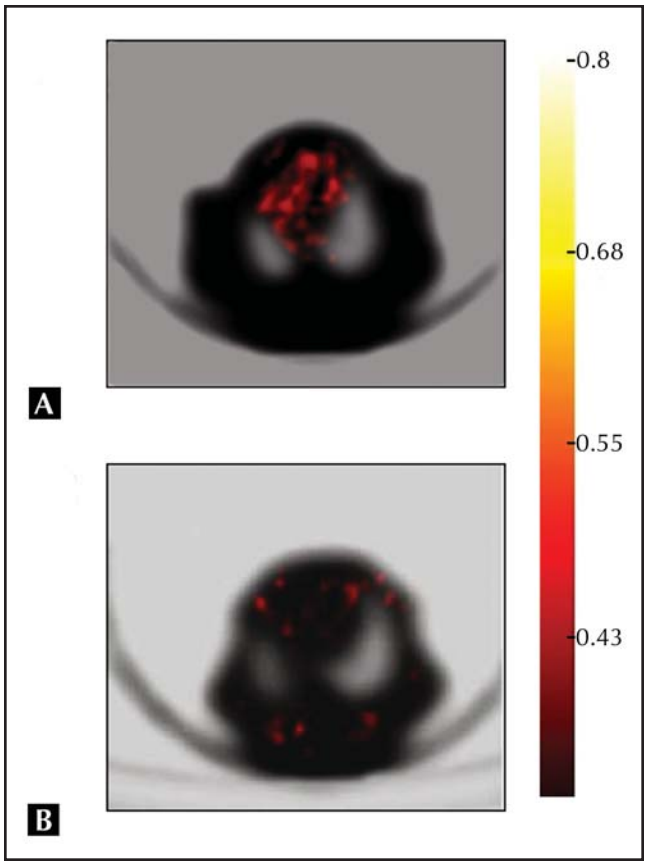


**Figure 3. A**, Reporter gene imaging of mesenchymal stem cells (MSCs) transduced with an HSV-ttk reporter gene 30 hours after cell infusion into a porcine heart. The substrate of the ttk reporter protein is [18F]-FHBG. **B**, Reporter gene imaging of same MSCs 7 days after infusion. (*From* Gyöngyösi et al. [48•]; with permission.)

chromosome, the reporter gene is passed on from the mother cell to daughter cell. Genetic inheritance of the reporter gene allows for monitoring of donor cell proliferation and, in cases of ES cells, detection of teratoma formation [46••].

Recently, Cao et al. [30••] used BLI to track hES cell–derived cardiomyocyte engraftment in the post-MI heart for 8 weeks. Results showed that administration of $1 \times 10^6$ hES cell–derived cardiomyocytes resulted in the loss of over 90% of the donor population within the first 3 weeks of delivery, after which stable grafts were formed for more than 6 months. In contrast, transplantation of $1 \times 10^6$ undifferentiated hES cells resulted in teratoma formation with cellular migration to extracardiac locations (Fig. 1). The acute donor cell death that occurred in the initial weeks after transplantation suggests that hES cell–derived cardiomyocytes may contribute only temporarily to improved systolic function in cell recipients. In a separate study by Li et al. [35•], the formation of mES cell–derived EC grafts was monitored in murine hearts that had undergone coronary artery occlusion. Although improved function was noted in animals that received mES cell–derived EC infusion, BLI imaging revealed that less than 2% of the transplanted cells remained in the myocardium by 8 weeks.

In another study, head-to-head comparison of cardiac engraftment of SkMb, MN, and MSCs was performed in a murine model of MI [47•]. Imaging by BLI showed significant donor cell death by week 3 in both the SkMb and MSC groups. MN cells, on the other hand, engrafted in significant numbers until week 6. BLI imaging was able to visualize extracardiac migration of MN cells to the femur, liver, and spleen (Fig. 2). These results suggest MN cells engraft better than SkMbs and MSCs. However, BLI signals confirmed by TaqMan PCR at week 6 showed less than 1% of the initially injected cells were still alive. One major drawback of BLI is that deep tissue attenuates the signals created by the interaction of Fluc and D-luciferin, thereby limiting this technology to small animals. PET imaging, on the other hand, has recently been shown to be applicable to large animals due to the higher energy photons that are emitted from the phosphorylation of [18F]-FHBG (Fig. 3) [48•]. Successful application of these technologies to human trials should elucidate reasons why certain cell regimens result in functional benefit and others do not.

## Conclusions

The efficacy of adult stem cells in restoring cardiac function post-MI has been demonstrated in a number of preclinical and clinical studies. However, the mechanisms by which cell transplantation confers these benefits to cell recipients are not clear. Furthermore, cardiac cell therapy has not been shown to affect measures of systolic function more than 1 year after transplantation in human trials. Preclinical studies suggest that this is probably due to the inability of grafted adult stem cells to integrate with the host myocardium effectively and to survive for significant periods of time. Despite the capacity of hES cells for pluripotency and self-renewal, clinical trials involving hES cell–based therapies are still years away. As with trials involving adult stem cells, longitudinal noninvasive imaging will play an increasingly important role in determining the efficacy of hES cell–derived cardiomyocytes or hES cell–derived ECs to engraft, survive, and proliferate in the heart. Imaging modalities should also be able to monitor cellular misbehavior (eg, teratoma formation) that can arise from transplantation of these cells. Currently, no single imaging modality has the capability to monitor all of these biological issues in living subjects. Thus, the ongoing development of sensitive, noninvasive, and multimodality imaging technologies will be vital in establishing a timely transition from bench to bedside in this field.

## Disclosures

No potential conflicts of interest relevant to this article were reported.

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