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iGUIDE

RECOMMENDATIONS AND GUIDELINES

Cell Tracking and the Development of Cell-Based Therapies

A View From the Cardiovascular Cell Therapy Research Network

Martin Rodriguez-Porcel, MD,* Marvin W. Kronenberg, MD,† Timothy D. Henry, MD,‡ Jay H. Traverse, MD,‡ Carl J. Pepine, MD,§ Stephen G. Ellis, MD,‖ James T. Willerson, MD,¶ Lemuel A. Moyé, MD, PHD,# Robert D. Simari, MD*

Rochester and Minneapolis, Minnesota; Nashville, Tennessee; Gainesville, Florida; Cleveland, Ohio; and Houston, Texas

Cell-based therapies are being developed for myocardial infarction (MI) and its consequences (e.g., heart failure) as well as refractory angina and critical limb ischemia. The promising results obtained in preclinical studies led to the translation of this strategy to clinical studies. To date, the initial results have been mixed: some studies showed benefit, whereas in others, no benefit was observed. There is a growing consensus among the scientific community that a better understanding of the fate of transplanted cells (e.g., cell homing and viability over time) will be critical for the long-term success of these strategies and that future studies should include an assessment of cell homing, engraftment, and fate as an integral part of the trial design. In this review, different imaging methods and technologies are discussed within the framework of the physiological answers that the imaging strategies can provide, with a special focus on the inherent regulatory issues. (J Am Coll Cardiol Img 2012;5: 559–65) © 2012 by the American College of Cardiology Foundation

Cell-based therapies are being developed for cardiac dysfunction as well as refractory angina and critical limb ischemia. Promising results obtained in preclinical studies led to the translation of this strategy to clinical studies. To date, several clinical trials of cell therapy after myocardial infarction (MI) have been completed, providing initial evidence of the safety of stem cell delivery of many cell types including bone marrow cells (BMCs) (1) and mesenchymal stem cells (2). In terms of recovery of cardiac function, the initial results have been mixed: some studies have shown an improvement in cardiac function (3), whereas the results of others have been neutral (4) or associated with a transient improvement in the left ventricular (LV) ejection fraction (5). Meta-analysis of these trials (6,7) showed that cell therapy after MI has potential benefit, by increasing the LV ejection fraction, reducing LV end-systolic volume, infarct size, and a trend toward a decrease in major adverse cardiac events.

From the *Mayo Clinic, Rochester, Minnesota; †Vanderbilt University School of Medicine, Nashville, Tennessee; ‡Minneapolis Heart Institute Foundation at Abbott Northwestern Hospital, University of Minnesota School of Medicine, Minneapolis, Minnesota; §University of Florida College of Medicine, Gainesville, Florida; ‖The Cleveland Clinic Foundation, Cleveland, Ohio; ¶Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas; and the #University of Texas School of Public Health, Houston, Texas. All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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The Cardiovascular Cell Therapy Research Network (CCTRN) was established by the National Heart, Lung, and Blood Institute to develop, coordinate, and conduct multiple collaborative protocols testing the effects of stem cell therapy on cardiovascular disease. The initial step is to prove that these therapies are safe for use in patients and will not lead to adverse events, such as arrhythmias (as previously seen with skeletal myoblasts). The CCTRN builds on contemporary findings by the cell therapy basic science community, translating newly acquired information to the cardiac clinical setting in the phase I/II study paradigm (8).

The CCTRN is simultaneously conducting 2 trials in patients with acute MI, TIME (Transplantation in Myocardial Infarction Evaluation) (9) and LateTIME (Late Transplantation in Myocardial Infarction Evaluation) (10), and 1 trial in patients with chronic heart failure and ongoing ischemia, FOCUS (First Mononuclear Cells injected in the US) (11). In these initial studies, the CCTRN initial focus is on the clinical feasibility and safety of these strategies, together with measuring their effect on LV function. The variability in the response to cell transplantation underscores the importance of determining the fate of transplanted stem cells and whether it correlates with changes in cardiac function. There is a general consensus among the CCTRN and the scientific community that a better understanding of the fate of transplanted cells (e.g., cell homing and viability over time) (12,13) will be critical for the long-term success of these strategies and that future studies should include an assessment of cell homing, engraftment, and fate as an integral part of the trial design.

ABBREVIATIONS AND ACRONYMS

BMC = bone marrow cell

CCTRN = Cardiovascular Cell Therapy Research Network

HSV1-tk = herpes simplex virus type 1 thymidine kinase

LV = left ventricular

MI = myocardial infarction

MRI = magnetic resonance imaging

PET = positron emission tomography

SPECT = single-photon emission computed tomography

SPIO = superparamagnetic iron oxide

In this review, the different imaging methods and technologies available are discussed within the framework of the physiological answers that they can provide. Furthermore, focus is placed on the advantages and disadvantages of each strategy and the inherent regulatory issues.

Unanswered Questions in Cell Therapy After MI

Currently, the evaluation of cell delivery for MI has been based on evaluating the recovery of cardiac function (14), as well as myocardial perfusion and ischemia (15). However, the efficacy of delivery, homing, and fate of these cells remains poorly understood. Hou et al. (16) delivered BMCs, labeled with indium 111 (^{111}In), to a swine model of myocardial ischemia and showed that cell retention varied with the delivery route with a high percentage of pulmonary cell trapping. Kraitchman et al. (17,18) confirmed these findings and showed that within days, cells ultimately homed in the myocardium and other organs. Furthermore, the effect of other factors, such as vascular leakage (19), extravasation, and lymphatic drainage can account for the variability observed in cell therapy studies.

The original premise was that BMC delivery after MI had a direct regenerative effect (20). More recently, it was postulated that the improvement can be achieved through a paracrine effect and by accelerating the healing process after MI (21). It is likely that the ratio of direct/paracrine beneficial effect depends, among other biological variables, on the cell type used and the conditions of the host tissue. Regardless of the mechanisms of the beneficial response, whether through a direct regenerative effect or a paracrine effect, the presence (even if brief) of transplanted cells in the damaged myocardium appears to be an important factor. Furthermore, numerous questions, such as the ideal timing, dose, and delivery route (e.g., intracoronary, intravenous, coronary sinus, intramyocardial) remain to be answered. To better understand these factors and to optimize the beneficial effect of these therapies, it is important to be able to monitor the presence of transplanted cells and the kinetics and biology of transplanted cells over time and to integrate this with the evaluation of LV structure and function.

Strategies to address these questions can be broadly divided into short- and long-term assessments of cell therapy. Short-term assessment can include the study of the retention and homing of transplanted cells. The long-term assessment includes the monitoring of the viability of transplanted cells over time as well as the post-engraftment biology of the transplanted cells. Understanding issues like the functionality of transplanted cells (e.g., differentiation, interaction of cells with the host tissue) will be of critical importance for the optimal translation of these approaches. However, short- and long-term assessment should not be considered separate concepts because they are closely connected. For example, the functionality of injected cells (long-term assessment) may not be relevant if those cells do not initially home and engraft (short-term assessment).

Short-Term Assessment of Transplanted Cells

To assess homing and engraftment, the most commonly used monitoring strategy is that of direct labeling (22,23), when different labeling agents are introduced into the cells exogenously (Fig. 1A) and cells are then transplanted and imaged in the living subject (Fig. 1). Imaging of the introduced molecules is performed, and the signal obtained is used as a surrogate for the number of stem cells. In direct-labeling strategies, signal originates from the labeling compounds and is independent of progenitor cell viability. Direct strategies have the advantage of the relative ease of labeling and that many probes are already used clinically (albeit for different purposes), facilitating their clinical translation. Notably, the signal from direct-labeling strategies may decrease over time due to cell division and "dilution," which will decrease the utility of the strategy for serial imaging. Imaging of direct labels may include magnetic resonance imaging (MRI) and nuclear techniques (single-photon emission computed tomography [SPECT] and positron emission tomography [PET]).

Monitoring of stem cells using MRI is based on the imaging of superparamagnetic iron oxide (SPIO) particles, which are highly magnetic particles that cause magnetic field perturbations that can be identified on T2*-weighted images (24,25) (Fig. 1B). The detected signal is used as a surrogate for the number of cells. However, SPIO particles may not stay inside the transplanted cells over time (26), but may be phagocytized by macrophages, resulting in an uncoupling between the MRI signal and the viability of stem cells (26,27). Furthermore, consideration should be given to the potential toxicity of ferromagnetic compounds and transfection agents (28,29) as well as the potential interaction between certain SPIO particles with metalloproteins (28). Because MRI has high spatial resolution, this strategy appears to be a good modality to define cardiac delivery and short-term (e.g., 1 to 2 days) homing of transplanted cells (Fig. 1B) (23,25). MRI labeling agents and/or the transfection agents used to introduce iron particles can affect cell viability of stem cells (27), whereas others have not (30), likely depending on the dose and cell type used. Although used in animal and small patient studies (29,31), direct-labeling MRI tracking has not yet been used in clinical studies.

Radionuclide labeling of cells has also been used for direct cell labeling and imaging (Figs. 1C and 1D) (22,32). The half-life of the radionuclides used (e.g., 6 h for technetium-99m [^{99}Tc], 109 min for fluorine 18 [^{18}F]) determines the duration of time that cells can be monitored after labeling.

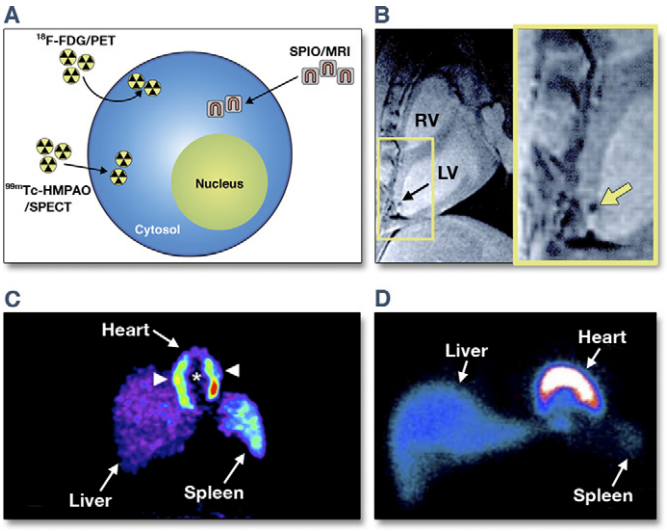

Figure 1. Direct Cell Labeling Strategies

(A) Labeling agents (for either magnetic resonance or radionuclide imaging) are first introduced into the stem cells exogenously and are then transplanted to the tissue and/or organ of interest. Noninvasive imaging is subsequently performed. **(B)** Mesenchymal stem cells (2.8×10^7), labeled with superparamagnetic particles (Feridex [ferumoxides], 25 μg Fe/ml), were imaged after direct transmyocardial delivery using a 1.5-T MRI unit. The black signal **(yellow arrow)** represents the superparamagnetic signal, which has been used to monitor the delivery of stem cells. **(C)** Bone marrow cells (1.25×10^8) (BMCs), labeled with ^{18}F-FDG (100 MBq), were delivered to the myocardium via intracoronary injection, and then imaged using PET. The **white arrowheads** point to the transplanted cells in the heart. There is also liver and spleen uptake (route of tracer elimination). **(D)** BMCs (8×10^8) were labeled with ^{99}Tc-HMPAO (100 MBq/1×10^8 cells) and infused via intracoronary injection in patients with chronic ischemic cardiomyopathy and imaged with SPECT at different times after delivery (shown is a representative image obtained 1 h after cell delivery). ^{18}F-FDG = fluorine 18–labeled fluorodeoxyglucose; LV = left ventricle; MRI = magnetic resonance imaging; PET = positron emission tomography; RV = right ventricle; SPECT = single-photon emission computed tomography; SPIO = superparamagnetic iron oxide particles; ^{99m}Tc-HMPAO = technetium 99m–labeled hexamethylpropylenamineoxime. Adapted, with permission, from Kraitchman et al. (23), Gousettis et al. (32), and Hofmann et al. (22).

SPECT and PET are more sensitive (nano- and femto-molar detection, respectively) compared with SPIO MRI (micromolar) (12,13,33). However, the cellular detection sensitivity should be considered together with the spatial resolution (MRI > SPECT or PET). The recent development of integrated PET–computed tomography and SPECT–computed tomography provides a better anatomic guide for the location of the PET or SPECT signal.

Hofmann et al. (22), using ^{18}F-fluorodeoxyglucose as the label and PET as the imaging modality, monitored cells after intravenous or intracoronary delivery of unselected BMCs or CD34-enriched cells (Fig. 1C), demonstrating that intracoronary delivery, especially of CD34-enriched populations, enhanced

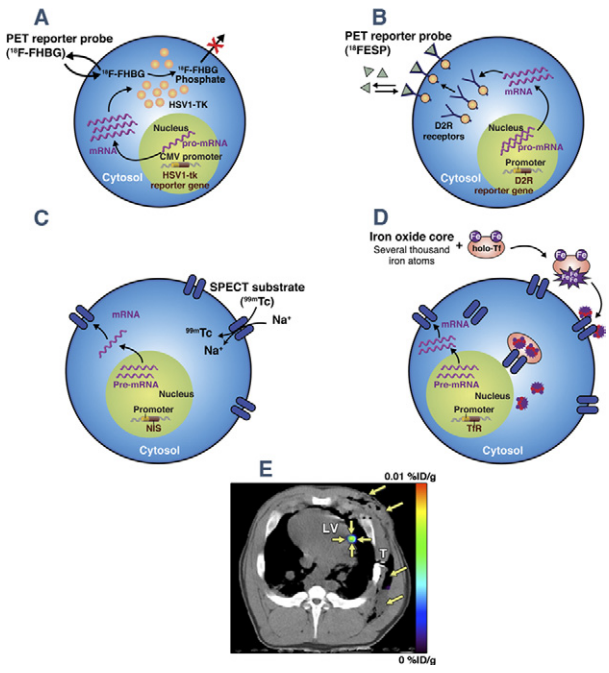

Figure 2. Reporter Gene Imaging Strategies

(A) Enzyme-based PET imaging. ^{18}F-FHBG is a substrate molecular probe that is phosphorylated by the HSV1-tk enzyme resulting in intracellular trapping of the probe in cells expressing the *HSV1-tk* gene. **(B)** Receptor-based PET imaging. ^{18}F-FESP is a ligand molecular probe interacting with the D2R to result in trapping of the probe in cells expressing the *D2R* gene. **(C)** Symporter-based SPECT imaging. ^{99m}Tc is taken up by the progenitor cell expressing the NIS reporter gene in exchange for Na^+. **(D)** Receptor-based MRI. Iron enters the cell through transferrin receptors. The signal detection by MRI is based on the T2* effect (as in direct labeling). **(E)** Representative PET-CT image of 3×10^7 mesenchymal stems cells, transduced with Ad-CMV-HSV1-sr39tk and transplanted to the myocardium of swine. ^{18}F-FHBG was administered intravenously and transverse nonenhanced PET-CT imaging was performed after 4 h. **Small arrows** depict the signal at the intramyocardial injection site, whereas **large arrows** point to the postoperative changes after delivery. Ad-CMV-HSV1-sr39tk = mutant version of the herpes symplex virus type 1 driven by the cytomegalovirus in adenoviral capside; CMV = cytomegalovirus; D2R = dopamine-2 receptor; ^{18}F-FESP = 3-*N*-(2-[^{18}F]fluoroethyl)spiperone; ^{18}F-FHBG = 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine; HSV1-sr39tk = mutant herpes simplex virus type 1 thymidine kinase; NIS = sodium iodide symporter; TfR = transferrin receptor; other abbreviations as in Figure 1. Adapted, with permission, from Willmann et al. (19) and Wu et al. (33).

homing to the infarct border zone compared with unselected populations. Also noted was the signal from noncardiac sites such as the liver and spleen, which could represent free ^{18}F- or actual labeled cells.

Another consideration is that the radionuclide's biological half-life or the amount of time that the radionuclide stays in the intracellular compartment may vary depending on the radionuclide and may differ between cell types and cell characteristics (e.g., senescence, phenotype). Furthermore, all radionuclides emit a certain level of ionizing radiation, with its potential toxicity to both the cell and host. Previous studies used an average of 100 MBq to label 1×10^8 BMCs and did not observe significant cell toxicity (22,34). The potentially harmful risk of ionizing radiation from medical procedures is a hypothetical one and stems from studies of the radiation exposure experienced by survivors from the atomic bombs in Hiroshima and Nagasaki. However, there are no definitive studies on the effects of ionizing radiation from medical procedures (35). Further studies are needed to precisely and accurately determine the consequence that this level of low radiation may or may not have on the host. Therefore, the use of amounts of radionuclides as low as reasonably appropriate appears to be a reasonable strategy.

In summary, direct-labeling methods are good strategies to confirm successful cell delivery and short-term retention of transplanted cells. Furthermore, their implementation is relatively straightforward and has already been used in clinical studies (Fig. 1C) (22). However, these imaging modalities are less suitable for providing answers on the long-term viability and biology of transplanted cells.

Long-term assessment of cell therapy. To address issues such as cell functionality and/or long-term viability, imaging modalities that are dependent on the viability of the cell should be used. Recent advances in noninvasive imaging and reporter gene technology have provided novel tools with which to study transgene expression noninvasively (13,33,36). Reporter gene constructs produce proteins that interact with an exogenously given probe, producing a signal that can be monitored noninvasively (13,33,36,37).

The most common use of reporter genes in vivo is for the longitudinal study of cell viability (11,38–40), and this strategy can be used to investigate the activity of a specific biological pathway when a reporter gene is driven by a cell-specific promoter (41). Commonly used reporter gene systems are either based on an intracellular enzyme (e.g., herpes simplex virus type 1 thymidine kinase [HSV1-tk], an enzyme that phosphorylates an exogenously administered substrate, which in turn is retained inside the cell and imaged with PET (Fig. 2A); a cell membrane receptor, such as mutant dopamine receptor D_2R, imaged with PET (Fig. 2B) (42); or the cell membrane sodium-iodine symporter NIS (Fig. 2C), whose activity can be imaged with PET or SPECT (41,43). Recently, efforts have been devoted to developing MRI reporter genes (44), based on the production of different proteins, mostly intracellular metalloproteins (transferrin, ferritin, tyrosinase) (Fig. 2D) (45), that accumulate iron intracellularly, creating a paramagnetic effect that can be detected on T2*-weighted images.

Many of the MRI reporter genes are based on the intracellular accumulation of iron for signal production, thus necessitating a critical steady intracellular iron level and having also potentially experiencing a dilution effect of ferritin iron when cells divide (45). Novel MRI reporter genes are targeted to produce amino acids with specific diamagnetic characteristics (chemical exchange saturation transfer) (46). Currently, MRI-based reporter genes have not yet become widely available (45).

Different from direct labeling, reporter gene systems have the advantage that the signal emitted is based on the viability and biology of the cell. The introduction of reporter genes into progenitor cells is mostly done using technologies of random reporter gene integration into the genome. Although there are risks of insertional mutagenesis, the risk may be low (47,48). Novel developments in site-specific integration technology may even circumvent this issue (49).

Currently, there is a larger number of reporter genes for PET (compared with SPECT) that have been used for cell imaging, which gives PET-based reporter gene imaging more flexibility in the number of biological events that can be studied in a single subject, albeit not simultaneously. However, PET probe production is more complex, needing advanced radiochemistry, and in many cases, it requires an on-site or nearby cyclotron. SPECT, on the other hand, can detect simultaneous signals of different energies by varying the detection windows, allowing the monitoring of cell therapies together with tissue perfusion with ^{201}Tl or ^{99}Tc, or even the concomitant monitoring of multiple cell types. SPECT tracer labeling is less complex but more limited and, for the most part, can be performed in a radionuclide pharmacy.

Reporter gene systems have been used in small animal studies under different pathophysiological conditions. In 2003, Wu et al. (39) demonstrated the feasibility of PET reporter genes to monitor the survival of murine cardiomyoblasts transfected with a mutant of the HSV1-tk after transplantation to the myocardium. Since then, a number of studies have used reporter genes to monitor the survival and biology of cells after transplantation to the myocardium (39–41,50–52), also combined with studies of myocardial perfusion (39,41). However, due to the complexity of the system and the need for a multidisciplinary approach, there is limited experience in large animals on the monitoring of transgene expression (53,54), the assessment of cell viability (Fig. 2E) (19,55), and only 1 reported experience (in oncology) in the use of reporter genes to monitor cell survival of immune T cells expressing HSV1-tk in patients by PET (56). In summary, reporter genes offer a promising alternative for long-term assessment of cellular viability and functionality.

A multimodality imaging approach may prove useful to better characterize the success of cardiac cell delivery. The success of delivery might be assessed by direct labeling using SPIO MRI or ^{18}F-fluorodeoxyglucose PET, whereas viability might be assessed using reporter gene techniques (e.g., HSV1-tk–PET). This information can be complemented with the evaluation of myocardial perfusion and the assessment of cardiac structure and function.

Regulatory Issues

It is important to ensure that any imaging strategy does not alter the survival, viability, and phenotype of the transplanted cells, the host organ, or the patient. For direct imaging approaches, most of the labeling compounds that will be useful clinically have been previously used. For example, ^{111}In (57) and ^{18}F-fluorodeoxyglucose (22,34) have been used for labeling of leukocytes and for studies of myocardial viability, respectively. Although previous experience relating to the safety of these compounds may be reassuring, we anticipate that each strategy will need to be tested in the specific cell of interest because not all cells may behave similarly. Thus, if direct-labeling agents (for SPECT, PET, or MRI) are to be used, it seems reasonable to test each cell type for toxicity before clinical implementation. Focus should be placed on cell viability, survival, and/or phenotype, including the assessment of the functions that are expected from the transplanted cells. Preclinical studies of these labeling compounds will be an important aspect of any Investigational New Drug application to the U.S. Food and Drug Administration.

Reporter gene strategies also present some regulatory issues that need to be addressed. In addition to the concepts related to the radionuclide probes described here, it is important to evaluate the potential effect of the introduction of reporter genes into the cell of interest. Preclinical studies have shown that the introduction of reporter genes did not significantly alter the phenotype of embryonic stem cells (47), but caution should be exercised when using different reporter genes and different vectors and different cell types. Successful use of these strategies in other patient populations (e.g., oncological) may pave the road for cardiac applica-

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tions. A possible approach will be that, after defining the cell and the reporter gene vector to be used, studies be performed to test the safety of the strategy. Genetic manipulation of cells will also necessitate the review by the Recombinant DNA Advisory Committee of the National Institutes of Health, a step that can take place in parallel with review by the U.S. Food and Drug Administration but must be complete before initiation of the study.

Conclusions

Cell therapy has great potential for the treatment of cardiovascular diseases, but many questions remain about the efficacy of cell delivery and the fate of delivered cells. Direct labeling and reporter gene strategies may be used to begin to define and track cell fate and should be strongly considered in early-phase clinical trials of cardiovascular cell delivery.

Reprint requests and correspondence: Dr. Lemuel A. Moyé, University of Texas–Houston School of Public Health, 1200 Herman Pressler, E-815, Houston, Texas 77030. *E-mail: lemmoye@msn.com*

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