# Multimodality molecular imaging in cardiac regenerative therapy

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**Stem cell therapy holds great promise for the repair and regeneration of damaged myocardium. Disappointing results from recent large-scale randomized trials using adult stem cells, however, have led some to question the efficacy of this new therapeutic. Because most clinical stem cell trials have not incorporated molecular imaging to track cell fate, it may be premature to abandon this approach. Herein, we will review how multimodality imaging can be incorporated into cardiac regenerative therapy to facilitate the translation of stem cell therapy.**

**Key Words: Myocardial perfusion imaging: SPECT • PET/CT imaging • Diagnostic and prognostic application**

| Abbreviations | | | |
|---|---|---|---|
| BLI | Bioluminescence imaging | PET | Positron emission tomography (PET) |
| FLI | Fluorescence imaging | RFP | Red fluorescent protein |
| HSVttk | Herpes simplex virus truncated thymidine kinase | SPECT | Single-photon emission computed tomography |
| MRI | Magnetic resonance imaging | | |

## INTRODUCTION

Although one in nine deaths is attributed to heart failure in the United States,[1] available medical therapy only slows its progression and surgical options are limited to the placement of left ventricular assist devices or organ transplantation. Because therapeutic options are few, cardiac regenerative therapy has become a great interest to many researchers and clinicians. In an effort to bring this therapeutic strategy to the bedside, several large-scale randomized trials using adult stem cells have been conducted over the past decade to evaluate its safety and efficacy.[2] These studies, however, have only shown marginal benefit, highlighting that the hurdles in clinical translation identified in preclinical studies incorporating multimodality molecular imaging have not been adequately addressed. In this review, we will discuss the fundamentals of multimodality stem cell imaging, findings from a select number of recent pre-clinical molecular imaging stem cell trials that highlight the remaining challenges that need to be addressed, and strategies to integrate molecular imaging into clinical stem cell trials.

## FUNDAMENTALS OF STEM CELL IMAGING

Molecular imaging can noninvasively track cells in vivo after transplantation, enabling the visualization of stem cell behavior and fate. Cell tracking requires

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labeling cells with molecular imaging probes,[3] which emit signals that can be detected. Cell labeling is accomplished in one of two ways: (1) direct labeling and (2) indirect labeling with reporter genes. To achieve direct labeling, cells are exposed to molecular probes in vitro. Molecular probes either bind to the cell surface or travel intracellularly via diffusion, endocytosis, or active transport. Although it is easy to perform and relatively less expensive, direct labeling may not accurately reflect cell behavior because the tracer may leak over time or get diluted if the cell divides. Moreover, macrophages and other cell scavengers can engulf nonviable cells and emit signal, which reduces the specificity of this approach.

Reporter gene technology addresses some of these limitations. A reporter gene is a gene that incorporates into the cell genome through viral or nonviral integration. The reporter gene carries a promotor that induces overexpression and either a sequence that produces a protein that interacts with a probe (e.g., exogenous reporter) or a protein that acts as a signal element (e.g., endogenous reporter). Because of concerns for random integration of reporter genes and potential immunogenicity from exogenous reporters, clinical reporter gene imaging has been limited to isolated case reports in patients with cancer.[4] The introduction of novel genome editing techniques such as CRISPR-Cas technology and human reporter genes may enable the application of reporter gene imaging in stem cell tracking in humans.

In general, molecular probes consist of three components: (a) a ligand to recognize the molecular target (e.g., antibody or protein); (b) a signal element that emits signal (e.g., radionuclide, fluorophore, or iron particle); and (c) a linker that joins the ligand to the signal element (e.g., cell, nanoparticle, or polymer). The ideal imaging probe will generate signal only when the cell is viable (e.g., imaging specificity), emit adequate signal for detection (e.g., imaging sensitivity), and produce minimal toxicity to transplanted cells and the transplant recipient. These molecular probes transmit signal detected by their respective imaging system including optical probes for bioluminescence imaging (BLI) and fluorescence imaging (FLI), radionuclide probes for positron emission tomography (PET), and single-photon emission computed tomography (SPECT), and magnetic resonance imaging probes for magnetic resonance imaging (MRI). The advantages and disadvantages of these methods are summarized in Table 1 and have been detailed in other comprehensive reviews.[5,6] Of note, because of the limited imaging depth available for optical imaging, only nuclear and MRI-based imaging are feasible for in vivo cell tracking in large animals including humans.

In recent years, multimodality molecular imaging has been used to harness the power of each imaging system and minimize the limitations of single modality approaches. Hybrid-molecular imaging platforms often combine a system that produces excellent anatomic detail with a system that can detect molecular events with high sensitivity and specificity. Co-labeling cells with a PET reporter gene as well as an MRI probe, for example, can achieve in vivo cell tracking with excellent sensitivity and specificity while providing anatomical co-localization, respectively. This approach and other multimodality imaging strategies have been used to study stem cell behavior in small and large animal models.

## FINDINGS FROM PRE-CLINICAL MULTIMODALITY STEM CELL IMAGING TRIALS

A number of small and large animal studies have used multimodality imaging to study stem cell behavior in vivo. These tracking studies have shown that stem cells do not engraft, survive, or proliferate in adequate numbers to provide a robust improvement in efficacy. Importantly, stem cell transplantation appears to be safe at least when only a minority of cells survive. Additional evaluation of safety will be needed, however, once larger grafts are achieved.

### Monitoring efficacy

To be effective, stem cells must remain and survive in the injured myocardium long enough to improve cardiac function. Unfortunately, numerous preclinical molecular imaging studies in small and large animals that delivered isolated stem cells into the peri-infarct area have shown that regardless of stem cell type, delivery method, and delivery timing, most cells do not survive more than 4 weeks even in immunodeficient animals.[7] Although improved retention can be achieved by transplanting cells via intramyocardial injection compared to the intracoronary or intravenous approach, this approach does not produce the most consistent results.[8] Preliminary findings in small animals using molecular imaging have also shown that survival can be enhanced by co-delivering cells with pro-survival agents (e.g., immunosupressants[9] and miRNAs[10]) or embedding cells into scaffolds.[11,12]

Many of these multimodality imaging studies have employed a dual or triple fusion reporter gene system that incorporates an in vivo cell-tracking probe (e.g., BLI for small animals and/or PET reporter probe for large animals) with an optical probe (e.g., green fluorescent protein) for histological confirmation. In a proof of concept study that successfully tracked canine-induced pluripotent stem cells shortly after transplant, Lee et al co-labeled cells with a triple fusion reporter

**Table 1.** Summary of common imaging techniques used in cell therapies

| Imaging type | Imaging sensitivity (cell detection limit) | Acquisition time | Advantages | Disadvantages |
|---|---|---|---|---|
| Bioluminescence imaging | 1000 cells | Seconds | Cheap, simple, high sensitivity, high throughput | Restricted use in small animals, only produces 2D images, low resolution |
| Fluorescence imaging | 1,000,000 cells | Seconds to minutes | Cheap, simple | Cells need to be close to the surface, low resolution, expensive, complicated |
| Magnetic resonance imaging | 10,000 cells | Minutes to hours | 3D imaging, soft tissue contrast, high resolution, no radiation | |
| Positron emission tomography | 10,000 cells | Seconds to minutes | 3D imaging, high sensitivity | Anatomical reference and radiotracer required |
| Single-photon emission computed tomography | 10,000 cells | Minutes | 3D imaging | Anatomical reference and radiotracer required |

*2D*, two dimensional; *3D* three dimensional

**Table 2.** Selected stem cell clinical studies in cardiac regenerative therapy

| Author (year) | Disease (n) | Imaging system | Labeling technique | Cell retention/ survival (cell type delivery and imaging time) |
|---|---|---|---|---|
| Hofmann[20] | STEMI (9) | PET | 18F-FDG | 2% (BMC, ICA, 1.5 hour)<br>3.8% (BMC, ICA + ICV, 1.5 hour)<br>25% (CD34+ BMC, ICA, 1.5 hour) |
| Karpov[21] | Transmural MI (44) | SPECT | 99mTc-HMPAO | 6.8% (BMMNC, ICA, 2.5 hour)<br>3.2% (BMMNC, ICA, 24 hour) |
| Blocklet[22] | STEMI (6) | PET | 18F-FDG | 5.5% (CD34+ PBMNC, ICA, 1 hour) |
| Goussetis[23] | Chronic IHD | SPECT | 99mTc-HMPAO | 9.2% (BMC, ICA, 1 hour) |
| Kang[24] | STEMI (20) | PET | 18F-FDG | 0 (PBMNC, ICV, 2 hour)<br>1.5% (PBMNC, ICA, 2 hour) |
| Penicka[25] | STEMI and chronic IHD (10) | SPECT | 99mTc-HMPAO | <5% (BMMNC, ICA, 2 hour) |
| Schächinger[26] | Acute-chronic MI (19) | GC | 111In-Oxime | 6.9% (PBMNC, ICA, 1 hour)<br>2% (PBMNC, ICA, 3–4 days) |
| Silva[27] | STEMI (30) | SPECT | 99mTc-HMPAO | 3.1% (BMMNC, ICV, 24 hour)<br>10.3% (BMMNC, ICA, 24 hour) |
| Barbosa da Fonseca[28] | Ischemic stroke (6) | SPECT WB GC | 99mTc | 1.7% (BMMNC, MCA, 2 hour) |
| Barbosa da Fonseca[29] | Chagas cardiomyopathy (6) | GC | 99mTc | 5.4% (BMMNC, ICA, 1 hour)<br>4.3% (BMMNC, ICA, 3 hour)<br>2.3% (BMMNC, ICA, 24 hour) |
| Vrtovec[19] | DCM (40) | SPECT WB GC | 99mTc-HMPAO | 4.4% (CD34+ PBMNC, ICA, 18 hour)<br>19.2% (CD34+ PBMNC, IM, 18 hour) |

*STEMI*, ST elevation myocardial infarction; *PET*, Positron emission tomography; *FDG*, fludeoxyglucose; *BMC*, bone marrow cells; *ICA*, intra-coronary artery; *ICV*, intra-coronary vein; *Tc*, Technetium; *MI*, myocardial infarction; *SPECT*, Single-photon emission computed tomography; *In*, indium; *HMPAO*, hexamethylpropyleneamine oxime; *BMMNC*, bone marrow mononuclear cells; *PBMNC*, peripheral blood mononuclear cells; *IHD*, ischemic heart disease; *MCA*, middle cerebral artery; *IM*, intramuscular

gene driven by a ubiquitin promotor carrying the following reporters: (1) firefly luciferase (FLuc) for ex vivo BLI, (2) herpes simplex virus truncated thymidine kinase for in vivo PET, and (3) red fluorescent protein (RFP) for histology. Cells were also labeled with iron oxide for MRI localization.[13] Similarly, Parashurama et al[14] transplanted marrow stromal stem cells labeled with superparamagnetic iron oxide particles and a similar reporter gene system (except for the replacement of RFP with enhanced green fluorescent protein) and demonstrated that lower detection limits of $1.5 \times 10^7$ and $2.5 \times 10^8$ cells for the MRI probe and PET reporter probe, respectively. Although it is informative, these studies were not performed in animal models of myocardial infarction and the duration of imaging post transplant was limited. Little information is also available on whether transplanting cells embedded into bioengineering constructs rather than in suspension improves cell retention and survival. Findings from one study that transplanted cardiac tissue slices from mice that genetically expressed FLuc and green fluorescent protein did show 14% cell survival after one month.[11] The authors hypothesized that the cardiac tissue slices were able to form vascular network with the host myocardium, resulting in improved cell viability. Similarly, a second study from the same group demonstrated high engraftment, long-term survival, and maturation of cardiomyocytes derived from human embryonic stem cells when delivered in bioengineering construct.[12] Cell engraftment and survival, however, did not correlate with increased left ventricular function. Additional studies are, thus, needed to determine whether these strategies to enhance survival will result in significant increases in left ventricular function.[15]

## Monitoring safety

Arguably more important than robust efficacy, the administration of stem cell therapy needs to be safe. Preclinical studies using various delivery methods including intravenous, intracoronary, intramyocardial transplantation have been generally safe with minimal risk of embolization, perforation, tamponade, or perioperative arrhythmia. Post transplant complications

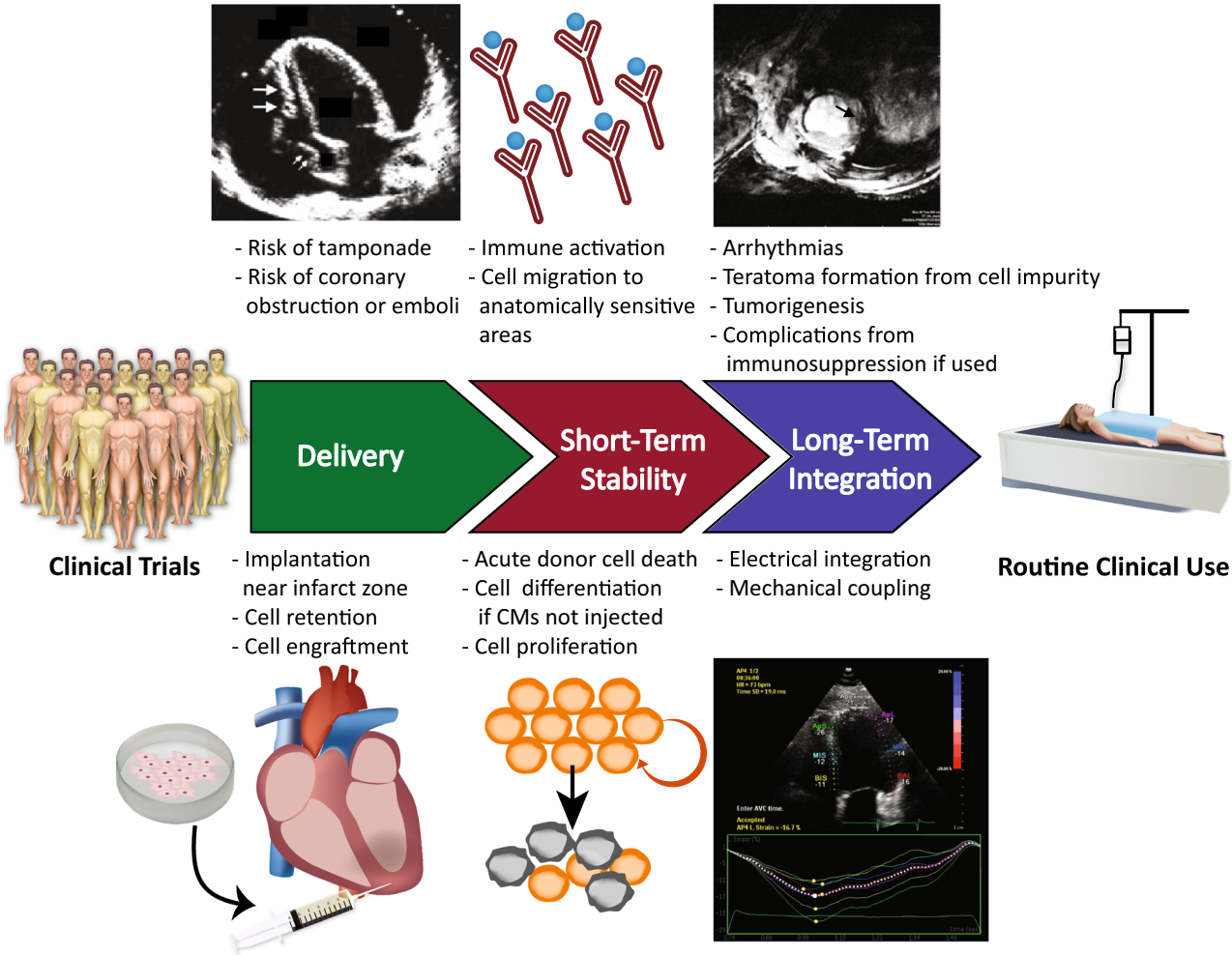


**Figure 1.** Incorporation of multimodality imaging in stem cell clinical trials to monitor the safety and efficacy at each phase of stem cell therapy. During transplantation, traditional imaging systems can assess the risk of tamponade associated with intramyocardial delivery or the risk of coronary thrombosis/emboli at the time of intracoronary delivery. Molecular imaging strategies can then determine if cells are transplanted near the infarct zone, if they remain at the target site, or if they survive. In the short-term, molecular imaging can also determine if cells migrate, proliferate, or differentiate. In the long-term, it will be important to determine whether tumors develop or arrhythmias are induced. Finally, it will be critical to show that cells mechanically and electronically couple to existing tissue for stem cell therapy to be a viable option for patients with end-stage heart failure.

including tumor formation and arrhythmias have also been uncommon. Of note, the risk of tumor formation is highest when transplanting derivatives of pluripotent stem cells because of potential contamination with undifferentiated cells, possibility of dedifferentiation, and risk of acquiring mutations during cultivation. To minimize these risks, it will be important to monitor cells with cardiac MRI. Based on findings from recent study by Riegler et al,[16] a combination of cardiac MRI including cine, *T*1 weighted, *T*2 weighted, *T*2* weighted, and late gadolinium enhancement sequences combined with serum biomarkers (e.g., CEA, AFP, HCG) outperformed echocardiography, detecting teratomas with a volume >17 $mm^3$ and a sensitivity of 87%. If identified, these teratomas can then be eliminated if the hPSC-derived cell products carry the PET reporter gene HSVttk, which encodes an enzyme that converts ganciclovir into a cytotoxic metabolite.[17] Administration of ganciclovir would effectively turn the reporter gene into a suicide gene that can be used to treat teratomas. Finally, the risk of arrhythmia may require more intense monitoring if large grafts are attained. Chong et al, for example, noted significant ventricular ectopy in monkeys who achieved grafts measuring 0.7-5.3% of the left ventricle.[18] Because the risks of tumorigenicity and arrhythmogenicity will only

rise once larger grafts are achieved, continued efforts to develop better imaging tools to monitor patient safety are still critical for clinical translation.

## STEM CELL CLINICAL IMAGING STUDIES

Unlike the many studies in animals that have helped define the clinical hurdles for translation, only a handful of human studies to date have incorporated some form of cell tracking (Table 2). Because of safety concerns with the use of reporter gene imaging as discussed above, these studies have mainly used radionuclide probes that directly label cells for short-term tracking (up to 4 days, depending on the probe half-life). Based on these studies, it appears that the majority of cells do not reach the injured myocardium, with most migrating into the lung and liver. Of the tested delivery routes, intramyocardial delivery appears to achieve better initial retention than intracoronary or intravenous delivery.[19] The majority of cells that are retained in the myocardium, however, do not survive longer than 24 hours. These findings are reminiscent of results from preclinical studies that delivered cell suspensions devoid of adjuvant agents, as discussed above. Future clinical molecular imaging studies should be conducted to determine whether these adjuvant agents are effective in improving survival and graft function in humans.

## STRATEGIES FOR INCORPORATING MULTIMODALITY IMAGING INTO CARDIAC REGENERATION

Multimodality molecular imaging can be incorporated into every step of stem cell clinical trial from delivery to the evaluation of short-term stability and long-term integration (Figure 1). Information on cell fate in large clinical trials incorporating imaging can help answer lingering questions that still remain even after a decade of cardiac regenerative research. Without this information, for example, it will be hard to decipher results from dose finding studies because even though millions of cells are initially injected, efficacy will likely depend on the number of cells that survive and are retained at the injured site. Stem cell tracking can also determine which cell source may be most effective as well as whether co-delivery with pro-survival agent(s) or biomaterial(s) can extend cell survival and retention. While the evaluation of short-term stability can be now be incorporated, assessing the long-term integration of these cells will require reporter gene imaging, which is currently not FDA approved in humans, but may gain greater acceptance with safer genome editing techniques and less immunogenic probes. Nevertheless, traditional clinical strategies such as MRI and event monitoring can help identify the risk of tumor development and arrhythmias to ensure safe clinical translation. Efficacy can continue to be monitored indirectly by the assessment of regional and global function, perfusion, and viability using standard echocardiography, cardiac magnetic resonance imaging and PET.

## SUMMARY

While exciting progress has been made in cardiac regenerative therapy within the last decade, stem cell therapy has a long road ahead before it can become a viable and robust therapeutic option. To continue to improve the efficacy of stem cells, it requires visualization of cell fate using multimodality imaging. While preclinical research using cell tracking has helped define current obstacles for the clinical application of cellular therapy, it will be important to incorporate these strategies into clinical trials so that we can overcome these hurdles and bring cardiac regenerative therapy to the bedside.

## Disclosure

*None.*

## References

1. Writing Group M, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ et al. Heart disease and stroke statistics-2016 update: A report from the American Heart Association. *Circulation*. 2016;133:e38-360.
2. Nguyen PK, Rhee JW, Wu JC. Adult stem cell therapy and heart failure, 2000 to 2016: A systematic review. JAMA Cardiol. 2016;1:831-41.
3. Liang G, Nguyen PK. Molecular probes for cardiovascular imaging. J Nucl Cardiol. 2016;23:783-9.
4. Yaghoubi SS, Campbell DO, Radu CG, Czernin J. Positron emission tomography reporter genes and reporter probes: Gene and cell therapy applications. Theranostics. 2012;2:374-91.
5. Nguyen PK, Riegler J, Wu JC. Stem cell imaging: From bench to bedside. Cell Stem Cell. 2014;14:431-44.
6. Chen IY, Wu JC. Molecular imaging: The key to advancing cardiac stem cell therapy. Trends Cardiovasc Med. 2013;23:201-10.
7. Nguyen PK, Lan F, Wang Y, Wu JC. Imaging: Guiding the clinical translation of cardiac stem cell therapy. Circ Res. 2011;109:962-79.
8. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation. 2005;112:I150-6.
9. Pearl JI, Lee AS, Leveson-Gower DB, Sun N, Ghosh Z, Lan F et al. Short-term immunosuppression promotes engraftment of embryonic and induced pluripotent stem cells. Cell Stem Cell. 2011;8:309-17.

10. Hu S, Huang M, Nguyen PK, Gong Y, Li Z, Jia F et al. Novel microRNA prosurvival cocktail for improving engraftment and function of cardiac progenitor cell transplantation. Circulation. 2011;124:S27-34.
11. Riegler J, Gillich A, Shen Q, Gold JD, Wu JC. Cardiac tissue slice transplantation as a model to assess tissue-engineered graft thickness, survival, and function. Circulation. 2014;130:S77-86.
12. Riegler J, Tiburcy M, Ebert A, Tzatzalos E, Raaz U, Abilez OJ et al. Human engineered heart muscles engraft and survive long term in a rodent myocardial infarction model. Circ Res. 2015;117:720-30.
13. Lee AS, Xu D, Plews JR, Nguyen PK, Nag D, Lyons JK et al. Preclinical derivation and imaging of autologously transplanted canine induced pluripotent stem cells. J Biol Chem. 2011;286:32697-704.
14. Parashurama N, Ahn BC, Ziv K, Ito K, Paulmurugan R, Willmann JK et al. Multimodality molecular imaging of cardiac cell transplantation: Part II. In vivo imaging of bone marrow stromal cells in swine with PET/CT and MR imaging. Radiology. 2016;280: 826-36.
15. Nguyen PK, Neofytou E, Rhee JW, Wu JC. Potential strategies to address the major clinical barriers facing stem cell regenerative therapy for cardiovascular disease: A review. JAMA Cardiol. 2016;1(8):953-62.
16. Riegler J, Ebert A, Qin X, Shen Q, Wang M, Ameen M et al. Comparison of magnetic resonance imaging and serum biomarkers for detection of human pluripotent stem cell-derived teratomas. Stem Cell Rep. 2016;6:176-87.
17. Kotini AG, de Stanchina E, Themeli M, Sadelain M, Papapetrou EP. Escape mutations, ganciclovir resistance, and teratoma formation in human iPSCs expressing an HSVtk suicide gene. Mol Ther Nucleic Acids. 2016;5:e284.
18. Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014;510:273-7.
19. Vrtovec B, Poglajen G, Lezaic L, Sever M, Socan A, Domanovic D et al. Comparison of transendocardial and intracoronary CD34+ cell transplantation in patients with nonischemic dilated cardiomyopathy. Circulation. 2013;128:S42-9.
20. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation. 2005;111:2198-202.
21. Karpov RS, Popov SV, Markov VA, Suslova TE, Ryabov VV, Poponina YS et al. Autologous mononuclear bone marrow cells during reparative regeneration after acute myocardial infarction. Bull Exp Biol Med. 2005;140:640-3.
22. Blocklet D, Toungouz M, Berkenboom G, Lambermont M, Unger P, Preumont N et al. Myocardial homing of nonmobilized peripheral-blood CD34+ cells after intracoronary injection. Stem Cells. 2006;24:333-6.
23. Goussetis E, Manginas A, Koutelou M, Peristeri I, Theodosaki M, Kollaros N et al. Intracoronary infusion of CD133+ and CD133-CD34+ selected autologous bone marrow progenitor cells in patients with chronic ischemic cardiomyopathy: Cell isolation, adherence to the infarcted area, and body distribution. Stem Cells. 2006;24:2279-83.
24. Kang WJ, Kang HJ, Kim HS, Chung JK, Lee MC, Lee DS. Tissue distribution of 18F-FDG-labeled peripheral hematopoietic stem cells after intracoronary administration in patients with myocardial infarction. J Nucl Med. 2006;47:1295-301.
25. Penicka M, Lang O, Widimsky P, Kobylka P, Kozak T, Vanek T et al. One-day kinetics of myocardial engraftment after intracoronary injection of bone marrow mononuclear cells in patients with acute and chronic myocardial infarction. Heart. 2007;93:837-41.
26. Schachinger V, Aicher A, Dobert N, Rover R, Diener J, Fichtlscherer S et al. Pilot trial on determinants of progenitor cell recruitment to the infarcted human myocardium. Circulation. 2008;118:1425-32.
27. Silva SA, Sousa AL, Haddad AF, Azevedo JC, Soares VE, Peixoto CM et al. Autologous bone-marrow mononuclear cell transplantation after acute myocardial infarction: Comparison of two delivery techniques. Cell Transpl. 2009;18:343-52.
28. Barbosa da Fonseca LM, Gutfilen B, Rosado de Castro PH, Battistella V, Goldenberg RC, Kasai-Brunswick T et al. Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection. Exp Neurol 2010;221:122-8.
29. Barbosa da Fonseca LM, Xavier SS, Rosado de Castro PH, Lima RS, Gutfilen B, Goldenberg RC et al. Biodistribution of bone marrow mononuclear cells in chronic chagasic cardiomyopathy after intracoronary injection. Int J Cardiol 2011;149:310-4.