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Imaging Cardiac Stem Cell Therapy: Translations to Human Clinical Studies

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Abstract

Stem cell therapy promises to open exciting new options in the treatment of cardiovascular diseases. Although feasible and clinically safe, the *in vivo* behavior and integration of stem cell transplants still remain largely unknown. Thus, the development of innovative non-invasive imaging techniques capable of effectively tracking such therapy *in vivo* is vital for a more in-depth investigation into future clinical applications. Such imaging modalities will not only generate further insight into the mechanisms behind stem cell-based therapy, but also address some major concerns associated with translational cardiovascular stem cell therapy. In the present review, we summarize the principles underlying three major stem cell tracking methods: (1) radioactive labeling for positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging, (2) iron particle labeling for magnetic resonance imaging (MRI), and (3) reporter gene labeling for bioluminescence, fluorescence, MRI, SPECT, and PET imaging. We then discuss recent clinical studies that have utilized these modalities to gain biological insights into stem cell fate.

Keywords

cardiovascular diseases; stem cells; imaging; radionuclide; magnetic resonance; bioluminescence

Introduction

Coronary artery disease (CAD) is one of the leading causes of death in the Western world and remains a significant public health problem both in the United States and globally (1). During the course of CAD, ischemic insult to the myocardium causes cardiomyocyte death, leading to fibrous tissue deposition and adverse ventricular remodeling. Stem cell therapy has potential as a novel approach for treating CAD. Multiple studies have linked transplantation of stem cells to promotion of neovascularization, regeneration of infarcted myocardium, and enhancement of myocardial perfusion (2–8). As basic research transitions toward preclinical and clinical trials, *in vivo* noninvasive imaging is becoming an

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increasingly powerful and popular tool for assessing the efficacy of stem cell therapy as well as addressing concerns regarding their safety and long-term persistency (Figure 1). Here, we describe state-of-the-art stem cell imaging methods, their clinical applications, and current limitations.

Current methods for *in vivo* stem cell tracking

Radionuclide Imaging

PET and SPECT scans offer highly sensitive spatial visualization of cells *in vivo* through radioactive tracers. Direct labeling of stem cells for radionuclide imaging is thus primarily used to gather information on the homing and biodistribution of transplanted therapeutic cells. SPECT radiotracers emit gamma radiation that can be measured directly, whereas PET radiotracers emit 511-keV gamma rays following positron-electron annihilation (9). Presently, the most commonly used stem cell radioactive labels are 2-[F-18]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) for PET imaging and Indium-111-oxyquinoline (^{111}In-oxine) for SPECT imaging. Multiple studies addressing the *in vivo* applicability of these labeling techniques have been performed. These results have proven radionuclide imaging to be a feasible method for visualization of stem cell survival and migration following cardiac delivery (9–12).

Limitations—Although radionuclide imaging provides a highly sensitive visualization scan, drawbacks such as ionizing radiation, non-target signal leakage, and signal loss due to cell dilution are significant issues that need to be considered in the experimental study design. Another deficiency of radionuclide imaging is the inability to track stem cells long-term. This is largely due to the relatively short half-lives of [^{18}F]-FDG (110 min), ^{99}Tcm (6 h), and ^{111}In-oxine (67.9 h) radiotracers.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) detects the changes in electromagnetic flux caused by nuclear realignment of magnetic dipoles in the body (13). The main advantages of MRI are the capabilities to yield high anatomic resolution and the applicability in evaluating downstream functional recovery. Gadolinium and superparamagnetic iron-oxide (SPIO) are agents that can be used for direct stem cells labeling, the latter of which has been FDA-approved since 1996. Recently, however, one such FDA-approved SPIO contrast agent, Feridex, was pulled off the market due to poor sales performance (14). Nevertheless, SPIO labeling is more commonly used in both clinical and preclinical trials as its particles offer the highest sensitivity among the different MR contrast agents that are currently available (15). Although it is feasible to label stem cells with gadolinium (16), the major disadvantages of gadolinium are the large amounts of gadolinium chelates needed to produce a signal and its lower sensitivity compared to SPIO, rendering it a less preferred option as an MRI contrast agent (17).

Limitations—MRI offers high 3-D spatial resolution, simultaneous detection of functional, molecular and anatomical data, and direct clinical translation. In addition, unlike radionuclide imaging, MRI operates intrinsically without ionizing radiation and hence may have fewer adverse effects to the donor cells and recipient patients. However, the sensitivity of MRI and its compatibility with many cardiac devices still need to be improved. Furthermore, inaccurate signal readings can arise from the presence of residual labeling particles and the leakage of labels to non-stem cells, such as macrophages (18).

Reporter Gene Tracking

Reporter gene tracking requires the use of cells transduced with reporter gene constructs such as green fluorescent protein (fluorescence), firely luciferase (bioluminescence), sodium iodide symporter (SPECT), herpes simplex virus thymidine kinase (PET), and transferrin receptors (MRI), to name a few (19). The most commonly used imaging techniques for tracking stem cells in preclinical models are bioluminescence imaging (BLI) and fluorescence imaging (FLI). BLI relies on the chemical reaction between firefly or renilla luciferase enzymes and their respective optical probes, D-Luciferin and coelenterazine. Once injected, these probes are oxidized by the stably expressed enzymes, which results in the emission of light at 480–560 nm wavelength. FLI, on the other hand, does not require the administration of a secondary substrate but rather an excitatory light source. Employing the long-term and sensitive characteristics of reporter gene tracking, many studies were able to perform time-lapse trials over the course of multiple months. For example, BLI has been utilized to track the effects of immunosuppressive drugs on inducing immunotolerance to human embryonic stem cell (ESC) transplantation (20). Other studies have compared survival among different types of stem cells, including bone marrow cells (BMC), mesenchymal stem cells (MSC), ESC-derived endothelial cells, and ESC-derived cardiac cells (21–25). Recent studies have also investigated the use of PET reporter gene imaging such as herpes simplex virus thymidine kinase (HSV-tk) to track stem cell viability in large animal models (26–27). These studies not only introduce but also provide solid evidence for the potentials this method has in permitting long-term characterization of cell survival, proliferation, and migration

Limitations—The use of lentiviral or retroviral vectors to incorporate transgenes into stem cells may cause random genomic integration (28), and hence poses considerable risk to the translation of this methodology into clinical therapy. Novel non-viral methods capable of site-specific integration of reporter genes are now being investigated which may prove to be safer than traditional viral strategies (29).

Clinical studies utilizing stem cell therapy imaging

Radionuclide Imaging

Multiple studies have utilized radioactive labeling of therapeutic cells as a means for monitoring cell fate acutely following delivery (30). In these studies, labeling techniques such as ^{18}F-FDG, Tc-99m-HMPAO, and ^{111}In-oxine have been used to track either bone marrow-derived stem cells (BMCs) or circulating progenitor cells (CPCs) following myocardial infarction in patients (31–38) (Table 1). One particular study employed radioactive labeling of ^{18}F-FDG with PET imaging to monitor bone marrow cell homing and biodistribution in nine male patients post acute myocardial infarction (Figure 2A–D). The study demonstrated a significant increase in myocardial homing of fractionated CD34^{+} cells (14–39%) compared to unfractionated BMCs (1.3–2.6%) (31).

Another study reports the use of ^{111}In-oxine with SPECT imaging to characterize homing of circulating progenitor cells (CPCs) (37). After tracking intracoronary infused proangiogenic progenitor cells in 17 patients post-infarction, the investigators found three critical factors that likely contributed to the uptake of labeled CPCs: the length of time between infarction and intracoronary cell infusion, the extent of tissue damage, and the condition of the patient's coronary circulatory function (37). These new insights will significantly contribute to the development of better stem cell therapy designs as well as the optimization of their therapeutic efficacy.

Magnetic Resonance Imaging

To date, a total of five clinical studies have been published using MRI to track cells *in vivo*. Interestingly, all have been in fields outside of cardiovascular stem cell therapy (39–43) (Table 2). In the first study, launched in 2004 in the Netherlands, researchers intranodally injected SPIO and ^{111}In-oxide (in an equal ratio) labeled autologous dendritic cells into eight stage-III melanoma patients using ultrasound guidance. Prior to implantation, the researchers had demonstrated that that SPIO labeling did not affect the cells' phenotype or function (39). Following implantation, MRI was used to track the migration of the cells. MRI demonstrated a valuable ability to detect low numbers of labeled dendritic cells, effectively track cell delivery, follow inter- and intra-nodal migration patterns, and provide detailed anatomical information (39). In four of the eight patients, MRI revealed accidental mis-injection of the cells into subcutaneous fat rather than the targeted lymph node bed. Such mis-injections were largely attributed to the poor resolution of ultrasound guidance, thus highlighting the superior application of MRI for guiding and tracking cell delivery.

In the second study, a 34 yr old man with brain trauma in the left temporal lobe underwent experimental treatment in China using neural stem cells labeled with Feridex I.V., an FDA approved ferumoxide (40). Feridex I.V. and effectene, a currently unapproved lipfection agent, were incubated with the cultured stem cells one day prior to implantation. Subsequent MRI tracking revealed a change in hypointensity along with an increase in periphery signal two to three weeks following transplantation, indicating cellular movement towards the lesion border (Figure 2E–L). However, after 7 weeks, hypointense signal was no longer present due to signal dilution. These results were compared to a control patient, a 42 yr man with brain trauma in the right temporal lobe who received unlabeled neural stem cells. No significant changes in signal around the control patient's lesion post-implantation were observed.

A third study performed in Brazil assessed 10 patients with chronic spinal cord injury who received $CD34^+$ bone marrow stem cells labeled with magnetic beads (41). In the fourth clinical study, Swiss researchers intraportally transplanted human cadaver islet cells labeled with ferucarbotran, a type of clinically approved SPIO, into four patients with type 1 diabetes. Following transplantation, the researchers observed insulin independence in all treated patients and saw no correlation between number of islet cells delivered and hypointense spots in the liver (42). In the final and most recent study, autologous MSCs were injected intravenously into 15 patients in Israel with Multiple Sclerosis (MS) and 19 with Amyotrophic Lateral Sclerosis (ALS). Using SPIO labeling for MRI, researchers in this study were able to assess both the safety and feasibility of stem cell transplantation in MS and ALS patients. In fact, within the 6-to 25-month observation period following transplantation, there were no incidences of significant adverse effects and new activity of the disease reported (43). These results, along with others mentioned in the study, established that MRI monitoring of SPIO-labeled cells was indeed clinically feasible. Overall, these five studies highlight the prominent role MRI can play in achieving a more in-depth understanding of clinical cell treatment.

Reporter Gene Imaging

Although commonly used in murine models, reporter gene tracking for stem cell therapy has been reported in only one human study so far (Table 2). As mentioned above, this is in large part due to concerns associated with random chromosomal integration. In a study by Yaghoubi et al., a 57 yr old male diagnosed with grade IV glioblastoma multiforme received direct infusion of genetically engineered $CD8^+$ cytolytic T cells (CTLs) as a part of an adoptive cellular gene immunotherapy clinical trial (44). These cells were transfected with plasmids to express the interleuin 13 zetakine gene (a tumor targeting protein) as well as

HSVtk (a PET reporter gene and a suicide gene). PET reporter probe 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine (^{18}F-FHBG) was used to track the fate of theses CTLs infused intracranially, and the transduced cells were subsequently observed to localize at the tumor site (Figure 2M). Compared with control patients, ^{18}F-FHBG accumulation in the CTL-infused patients was more than 2.5× higher in both the resection and tumor sites (44). This study demonstrated that reporter gene based imaging is a feasible method for tracking of therapeutic cell treatments in humans, which will prove vital in understanding the long-term efficacy of any stem cell therapy. Additionally, another key advantage of this imaging method is in the direct relationship between image signal and cell viability. Because signal expression depends on reporter gene expression, only viable cells transduced with the reporter gene can contribute to the signal, thus eliminating the problem of false signals that often plague direct labeling approaches (see Figure 1). Lastly, in the event of cellular misbehavior such as tumor formation (45), it is possible to use the HSV-tk reporter and suicide gene technique to image the ablation of these cells *in vivo* (46).

Conclusion

The rapidly growing field of clinical stem cell therapy remains an emerging area of research, and although it holds great potential for the treatment of cardiac disease, much more development is needed before it can provide full-fledged treatment options. Despite being in its infancy with a limited body of published evidence for some modalities, the use of non-invasive imaging technologies for visualizing stem cell fate post-transplantation is an intriguing new approach that has the potential to greatly enhance our knowledge of cell fate and improve clinical trial designs. Here, we reviewed three basic methodologies which were initially used in animal research and eventually translated into human studies. Radionuclide imaging is expedient because of its clinical safety, wide availability, and high detection sensitivity, allowing researchers to monitor their biodistribution and homing. MRI has the ability to track cells with high anatomic resolution while also evaluating downstream functional recovery. Reporter gene imaging permits long-term characterization of cell survival, proliferation, and migration. Despite these advantages, there are significant barriers yet to be overcome for each technique. For example, the radioactive labeling technique is hampered by the short half-lives of most radioactive tracers, the iron particle labeling technique is limited by signals that may not necessarily reflect cell viability, and the reporter gene technique is constrained by potential chromosomal mutagenesis under the traditional lentiviral transduction method. Hence at present, no one imaging modality has thus far proven to be singularly superior to all others in the tracking of stem cell therapy. However, through continuing improvement of innovative imaging modalities, more promising possibilities for stem cell therapy will undoubtedly emerge. Advances in imaging technologies may play an increasingly critical role in eventually unleashing the full potential of clinical stem cell therapy for treatment of cardiovascular diseases and other intractable conditions.

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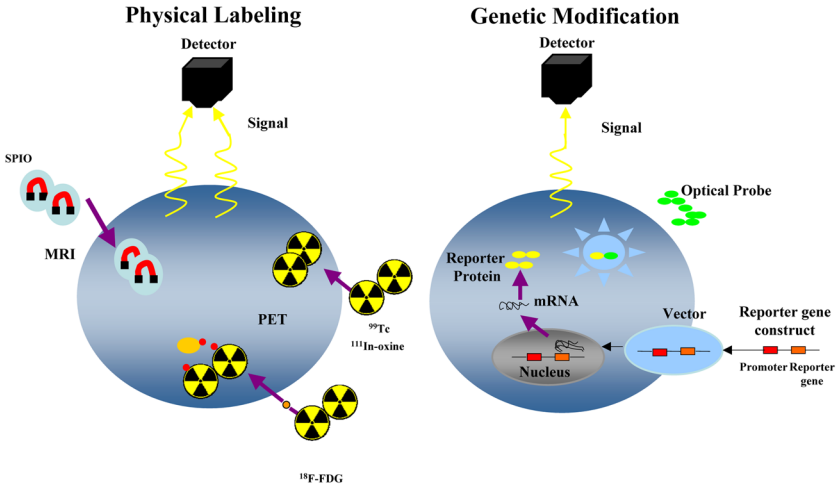

Figure 1. Schematic of common imaging approaches for tracking stem cells *in vivo*

Genetic modification of therapeutic cells utilizes reporter gene integration. Physical labeling of therapeutic cell requires the use of *ex vivo* labeling prior to implantation or injection. ^{18}F-FDG = F18-fluorodeoxyglucose; SPIO = super-paramagnetic iron oxide; Tc = technetium; In = indium.

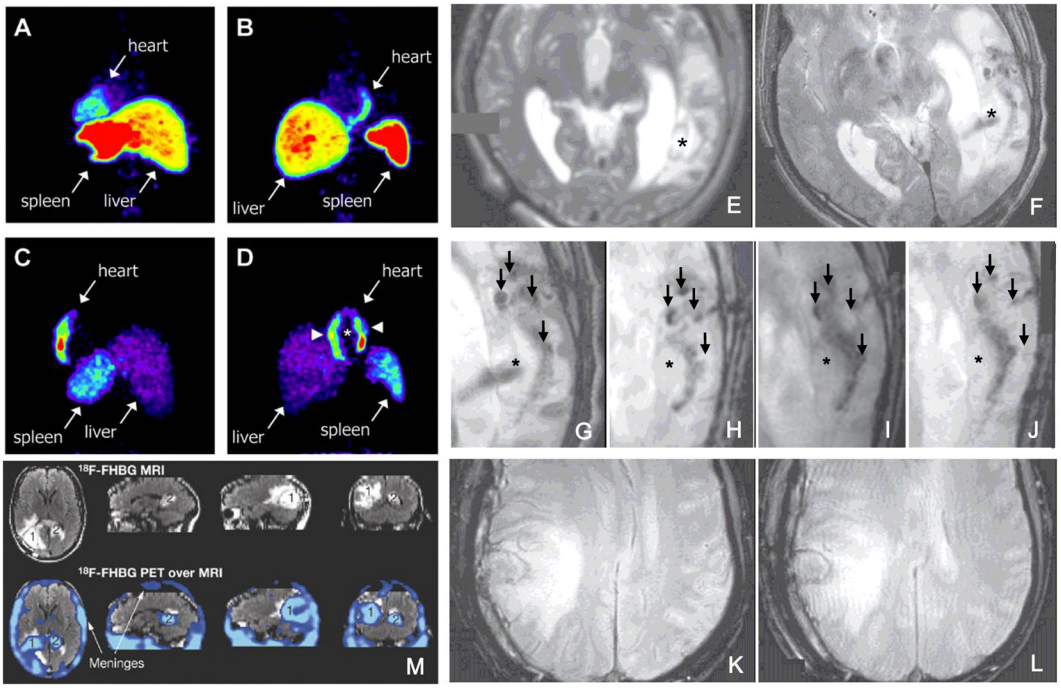

Figure 2. Different imaging modalities for tracking stem cells in patients. (A–D) Myocardial homing and biodistribution of ^{18}F-FDG-labeled BMCs. Left posterior oblique (A) and left anterior oblique (B) views of chest and upper abdomen of patient 2 taken 65 minutes after transfer of ^{18}F-FDG–labeled, unselected BMCs into left circumflex coronary artery. (C–D) Views of ^{18}F-FDG–labeled, CD34-enriched BMCs into left anterior descending coronary artery. CD34+ cell homing is most prominent in infarct border zone (arrowheads) but not infarct center (*). (Reprinted with permission from [31]). **(E–L)** MRI scans from a patient who received neural stem cells labeled with iron oxide nanoparticles (E–J) and another patient who received unlabeled cells (K–L). Four hypointense signals (black arrows) were observed at injection sites around the lesion from day 1 (Panel G) to day 21 (Panel J). In these panels, the black arrows indicate the hypointense signal and the asterisk indicates the lesion. (Reprinted with permission from [40]). **(M)** MRI and PET superimposed brain images of a patient who received infusions of autologous cytolytic T cells that expressed interleukin-13 zetakine (therapeutic gene) and HSV-tk (PET reporter gene). Top images: MRI and bottom images: MRI-PET. (Reprinted with permission from [44]).

Table 1

Summary of radionuclide imaging clinical studies

Authors / Year	N	Cell Type	Delivery Method	Total Cells Delivered (10^6)	Cell Imaging Modality	Cell Labeling Technique	Labeling Efficiency / Viability (%/ %)	Notable Study Findings
Hofmann et al. (2005) Ref. 31	9	US BMNC / BM $CD34^+$	IC, IV	2486±654 (US; 5% LB); 1754±835 (100% $CD34^+$ + 40% $CD34^-$)	PET	^{18}F-FDG	99 / 92–96	$CD34^+$ enrichment ↑ homing, more to infarct border zone
Karpov et al. (2005) Ref. 32	44	BMNC	IC	89±49	SPECT	^{99m}Tc-HMPAO	NQ / 96±4	No ↑ in LVEF at 6 mo; ↓ TNF-α (d2), ↓ IL- 1β (d5), ↑ IGF (d12)
Kang et al. (2006) Ref. 33	20	CPC (+ G-CSF)	IC, IV	450	PET	^{18}F-FDG	73±17 / NQ	% cell retention not different between acute and old infarcts
Goussetis et al. (2006) Ref. 34	8	BMNC $CD133^+$ & BMNC $CD133^-$ $CD34^+$	IC	16±5	GCI	^{99m}Tc-HMPAO	29±5 / >95	3% ↑ LVEF at 10 mo
Blocklet et al. (2006) Ref. 35	6	CPC $CD34^+$	IC	15±9 (27–54% LB)	PET WB GCI	^{111}In-Oxine & ^{18}F- FDG	65±8 & 6±1 / NQ	No cells recirculated to heart by 43 hr; limited homing with $CD34^+$ enrichment
Penicka et al. (2007) Ref. 36	10	BMNC	IC	3934±954 (20% LB)	SPECT WB GCI	^{99m}Tc-HMPAO	90 / 94–99	5% ↑ LVEF at 4 mo for AMI ; no cell engraftment at 20 hr or ↑ LVEF at 4 mo for CMI
Schachinger et al. (2008) Ref. 37	19	CPC	IC	15±6 (10% LB)	WB GCI SPECT	^{111}In-Oxine	29±12 / 90±61	↑ cell homing to areas of low viability, reduced CFR; cell retention at 24 hr ↓ with infarct age
Dedobbeleer et al. (2009) Ref. 38	12	CPC $CD34^+$	IC	23±7 (9% LB)	PET	^{18}F-FDG	5±1 / 96±1	No ↑ LVEF, endothelial function, or neointimal thickening at 3 mo

Abbreviations – BMNC: bone marrow-derived mononuclear cells; US: unselected; CPC: circulating progenitor cells; IC: intracoronary; IV: intravenous; PCI: percutaneous coronary intervention; MI: myocardial infarction; LB: labeled; WB GCI: whole-body gamma camera imaging; ^{18}F-FDG: ^{18}F-fluorodeoxyglucose; ^{99m}Tc-HMPAO: ^{99m}Tc-hexamethylpropyl-eneamine; NQ: not quantified; UD: undetectable; IGF: insulin-like growth factor; IL: interleukin; TNF: tumor necrosis factor; LVEF: left ventricular ejection fraction; AMI: acute myocardial infarction; CMI: chronic myocardial infarction; CFR: coronary flow reserve; min: minute; hr: hour; d: day; mo: month; yr: year; ↑: increased or improved. All original values have been rounded to full digits. Reprinted with permission from [30].

Table 2

Summary of MRI and reporter gene clinical studies

Authors / Year	N	Location of Study	Patient Diagnosis	Cell Type	Delivery Method	Total Cells	Cell Imaging Modality	Labeling Technique (Contrast Agent)	Notable Study Findings
De Vries et al. (2005) Ref. 39	8	Nijmegen, Netherlands	Stage-III melanoma patients	Autologous dendritic cells	Intra- nodally	7.5×10^6 per labeling method	3.0-T MRI	SPIO and 111in-oxide	Use of iron oxides safe + appropriate for monitoring human cell therapy.
Zhu et al. (2006) Ref. 40	2	Shanghai, China	Brain trauma in left temporal lobe	Autologous neural stem cells	Implanted around brain damage	N/A	3.0-T MRI	Feridex I.V. (SPIO)	MRI is useful in the detection of stem-cell engraftment and migration.
Callera et al. (2007) Ref. 41	16	São Paulo, Brazil	Chronic spinal cord injury	Autologous CD34+ bone marrow stem cells	Lumbar puncture	Median: 0.7×10^6 Range: $0.45 – 1.22\times 10^6$	MRI	Magnetic nanoparticles (microspheres)	First study to show that MRI can track the migration of BMCs into the injured site in patients with chronic SCI.
Toso et al. (2008) Ref. 42	4	Geneva, Switzerland	Type 1 diabetes	Human cadaver islet cells	Intra- portally	Mean: 3.71×10^5 SD: 1.01×10^5	3.0-T MRI	Ferucarbotran (type of SPIO)	MRI imaging for SPIO-labeled-islet grafts is feasible. One limitation of MRI is iron overload.
Yaghoubi et al. (2009) Ref. 44	4	Stanford, California, United States of America	Grade IV glioblastoma multiforme	Autologous cytolytic CD8+ T cells	Infusion into site of tumor	1×10^9	PET MRI	HSV1 TK 18F-FHBG	First reporter gene-based imaging of cell therapy in humans. 18F-FHBG can accumulate within glioma tumors.
Karussis, et al. (2010) Ref. 43	34	Jerusalem, Israel	MS 2.5×106 ALS	Mesenchymal stem cells	Standard lumbar puncture	MS 2.5×10^6 ALS 17.4×10^6	1.5-T MRI	SPIO (Feridex)	MRI proves to be an effective means to track the effects of transplanted MSCs in MS and ALS patients.

Abbreviations – SPIO: superparamagnetic iron oxide; HSV1tk: herpes simplex virus 1 thymidine kinase; SCI: Spinal cord injury; MS: Multiple Sclerosis; ALS: Amyotrophic Lateral Sclerosis; 18F-FHBG: 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine.