Clinical Tracking of Cell Transfer and Cell Transplantation: Trials and Tribulations

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Cell therapy has provided unprecedented opportunities for tissue repair and cancer therapy. Imaging tools for in vivo tracking of therapeutic cells have entered the clinic to evaluate therapeutic cell delivery and retention in patients. Thus far, clinical cell tracking studies have been a mere proof of principle of the feasibility of cell detection. This review centers around the main clinical queries associated with cell therapy: Have cells been delivered correctly at the targeted site of injection? Are cells still alive, and, if so, how many? Are cells being rejected by the host, and, if so, how severe is the immune response? For stem cell therapies, have cells differentiated into downstream cell lineages? Is there cell proliferation including tumor formation? At present, clinical cell tracking trials have only provided information on immediate cell delivery and short-term cell retention. The next big question is if these cell tracking tools can improve the clinical management of the patients and, if so, by how much, for how many, and for whom; in addition, it must be determined whether tracking therapeutic cells in every patient is needed. To become clinically relevant, it must now be demonstrated how cell tracking techniques can inform patient treatment and affect clinical outcomes.

Clinical cell therapy has the potential to revolutionize modern medicine by providing new opportunities for the treatment of previously incurable diseases and tissue injuries. Despite great promises from preclinical applications, there are many open questions regarding how imaging tools can increase the safety of immune and stem cell therapies in patients and facilitate the outcome of successful tissue regeneration or tumor eradication. We discuss herein several key questions that affect the clinical translation of cell tracking technologies in patients. First, previous studies showed our ability to track the distribution of therapeutic cells in the whole body. Once the biodistribution in patients is understood, it is debatable whether we need to track therapeutic cells in every patient. Second, information about therapeutic cell survival could be used to determine whether a patient needs repeat dosing or other rescue interventions. However, we have yet to prove that this can affect outcome. Third, the propagation of allogeneic “off-the-shelf” cell therapeutics adds an urgent need for clinically applicable diagnostic tools that can help detect the occurrence of a host immune response to the cell transplant. However, it is challenging to differentiate between immune responses that promote stem cell engraftment or rejection. Finally, especially for cell products derived from pluripotent cells, we need clinical imaging tools for in vivo detection of undesired cell proliferation, including tumor formation. In this review, we will discuss some of the trials and tribulations associated with clinical cell tracking.

Tribulations: MRI Cell Tracking Used Gadolinium-based Agents

Because gadolinium chelates are widely used as contrast agents for clinical MRI, it is not surprising that they have been used to label and track therapeutic cells in preclinical models (1,2). Because, to our knowledge, no clinical trials of tracking gadolinium-labeled cells have been performed, the question arises if this approach would be sensitive enough and safe. Since December 2006, the U.S. Food and Drug Administration has investigated reports of rare, but debilitating, cases of nephrogenic systemic fibrosis after gadolinium exposure, primarily in patients with decreased renal function (3,4). The origin of the disease is thought to be secondary to the deposition of free gadolinium ion into subcutaneous tissue with resultant inflammation and fibrosis. More recently, it has been discovered that repeated intravenous injections of linear gadolinium chelates can lead to gadolinium deposition in the brain in patients with normal renal function (5). Accordingly, although no clinical implications were noted, many practitioners and patient advocates requested that gadolinium deposition be kept as low as possible and that gadolinium-based contrast agents be used only when absolutely necessary, with preferential use of macrocyclic chelates as more stable alternatives (6).

If gadolinium-labeled cells were administered to humans, which has not been the case yet, the total quantity of gadolinium delivered with labeled cell transplants (up to 10 pg gadolinium per cell or 100 μg for 1 × 10^7 cells per}
individual) is three orders of magnitude lower than the gadolinium dose delivered with intravenous contrast agent injections for MRI (0.2 mmol gadolinium per kilogram body weight or 2.27 g for a 70-kg individual). However, although intravenously injected gadolinium chelates are eliminated by the kidneys by means of glomerular filtration, it is not clear if and how gadolinium chelates would be eliminated when administered directly into tissues with therapeutic cells. For the above reasons, it is unlikely that gadolinium chelates will be used clinically for cell tracking.

Trials: MRI Cell Tracking with Superparamagnetic Iron Oxide–based Agents

Hydrogen 1 (1H) MRI-based cell tracking can be used for MRI-guided cell injections in real time (7,8), preferably with a temporal resolution on the order of a few seconds (9). Using MRI guidance, one can make adjustments to the speed of injection, the volume being injected, or catheter positioning to ensure proper cell delivery (10). As of today, nine clinical trials have reported on the use of superparamagnetic iron oxide (SPIO)–based cell tracking. Partly due to stringent U.S. Food and Drug Administration regulations, all of these studies were conducted in accordance with Food and Drug Administration regulations (9) (Table 1). Most of these studies used first-generation clinical SPIO formulations (Feridex [Berlex Laboratories, Montville, NJ] or Endorem [Guerbet, Aulnay-sous-Bois, France]), which are taken up by cells through simple micropinocytosis and phagocytosis. In the first study (12), autologous dendritic cells were primed with synthetic melanoma antigens and injected under US guidance into regional lymph nodes in patients with stage IV melanoma. With use of 50:50 mixtures of Endorem-labeled dendritic cells and indium 111 (111In)-oxine–labeled dendritic cells, the sensitivity of detection for a 3.0-T clinical imager was determined to be 1.5 × 105 cells, that is, 1% of the total number of injected cells (1.5 × 106). This landmark study set the stage for subsequent studies and demonstrated the importance of anatomic information as it was observed that cells were injected in the wrong location in half the patients; such misinjection could not be seen on previous SPECT scans with use of 111In-oxine labeling only.

In the second study (13), patient-derived neural stem cells were labeled with Feridex and injected into the temporal lobe of a patient with brain trauma. The labeling procedure used an unapproved, nonclinical grade transfection agent, Effectene (Qiagen, Germantown, Md). The authors noted migration of the iron signal from the injection site to the brain trauma site, indicating the ability of MRI to monitor cell migration in vivo.

The third study (14) used autologous CD34+ bone marrow stem cells, which were injected into the spinal cord by means of a lumbar puncture. Remarkably, the investigators used nonclinical grade magnetic beads coated with polystyrene (Dynabead; ThermoFisher Scientific, Waltham, Mass), which have a large diameter of 1–5 μm. Three independent clinical studies have labeled pancreatic islet cells with SPIO and injected these labeled cells into the liver via the portal vein (15,16,22). There was a poor correlation between the number of hypointense spots and injected islets, which calls into question the value of this procedure as a surrogate marker for cell survival. Other clinical MRI cell tracking studies have demonstrated that SPIO-labeled therapeutic cells can be detected in the spinal cord and occipital horns of patients with multiple sclerosis, amyotrophic lateral sclerosis (17), and neonatal ischemic hypoxia (18). In the latter study, the hypointense signal was reportedly still seen 4 months after injection of SPIO-labeled stem cells into the lateral ventricles, which is indicative of slow clearance of the SPIO label from the cerebrospinal fluid (Fig 1) (19).

Is the administration of SPIO-labeled cells safe? To our knowledge, only two groups have reported inhibition of chondrogenic differentiation of mesenchymal stem cells after labeling with either Feridex or Resovist (Bayer-Scheri, Berlin, Germany) in a dose-dependent manner (24–26). However, cells were viable and proliferated normally but were not able to form an extracellular proteoglycan matrix through a hitherto unknown mechanism. In the most comprehensive study to date, involving 20 patients (20), systemic delivery of no less than 1 × 109 SPIO-labeled peripheral blood mononuclear cells was found to be safe, with no changes in cellular cytokine release profiles, migration, and homing to induced inflammation. As for the healthy recipients, no significant changes in cardiovascular physiology, blood chemistry, and white blood cell counts were noted. Unlike gadolinium, iron is a naturally occurring metal present in the human body, at a dose of approximately 4 g in the average adult. The total dose of iron introduced into the human body with SPIO-based MRI cell tracking is approximately 10 mg, or 0.25% of total body iron, even for such a high dose of 1 × 109 cells (assuming an upper end of 10 pg iron per cell). A detailed study investigating a potential up- or downregulation of 40,000 genes noted only temporary changes of gene expression profiles in SPIO-labeled neural stem cells, indicating that cells are well adapted to process fluctuations in cellular iron (27). It is theoretically possible that SPIO biodegradation can lead to the formation of redox-reactive Fe2+ species. However, there is no evidence to date that the low SPIO doses delivered with labeled cells would cause such an effect in patients.

Unfortunately, first-generation iron oxide nanoparticles such as ferumoxide (Feridex and/or Endorem) and ferucarbotran...
Figure 1: Hydrogen 1 MRI cell tracking with use of direct labeling. Superparamagnetic iron oxide (SPIO)--labeled autologous cord blood--derived cells were injected in patient with global cerebral ischemia. A, Volume rendering of MRI data of patient’s head obtained 24 hours after transplant. Semiautomatic pixel intensity segmentation shows ventricular system (green) and cell-derived SPIO signal within occipital horn of right ventricle (red). Needle shows route and trajectory of cell transplant via frontal horn. B, Posterosuperior view of patient’s head. C, T2*-weighted image with orthogonal view centered on cellular SPIO signal in occipital horn (arrowhead). D–I, Sagittal T2*-weighted MR images show time course of hypointense SPIO signal within occipital horn (arrowhead in E–H). Images were obtained, D, before and, E, 24 hours, F, 7 days, G, 2 months, H, 4 months, and, I, 33 months after transplant. [Reprinted, under a CC BY license, from reference 19.]
typically occurs during the first few days after cell transfer (38). However, cell apoptosis (cell death) or immune cell graft failure several months after therapeutic cells could help us understand which factors lead to lack of growth factors or cytokines, proinflammatory conditions, or immune rejection (39–41). In vivo SPIO labeling of macrophages can accelerate the diagnosis of failed cell transplants on the basis of an influx of iron-labeled macrophages into unlabeled cell transplants (42,43). In addition, SPIO-labeled viable and apoptotic stem cell transplants showed distinct MR signal differences in cartilage defects of arthritic joints (44–46). Preclinical investigations in animal models showed that a rapid decrease in the T2 signal of SPIO-labeled mesenchymal stem cells 2 weeks after implantation into cartilage defects correlated with lack of cartilage repair at 12 weeks (47). Apoptotic ferumoxytol-labeled mesenchymal stem cells (and not viable labeled mesenchymal stem cells) were cleared by macrophages (48,49). This is in accordance with previous investigations that showed that apoptosis induces macrophage phagocytosis (50).

However, the MR signal evolution of SPIO-labeled cells seems to vary in different target organs. In the heart, several investigators reported a prolonged hypointense signal of ferumoxides-labeled dead stem cell implants for up to 3 months (51), which corresponded to the SPIO nanoparticles being engulfed but apparently not biodegraded by cardiac macrophages (52). Berman et al (53) investigated long-term MR signal characteristics of live and dead SPIO-labeled immortalized neural stem cells after transplant into mouse brain parenchyma. They reported that viable cell proliferation and associated label dilution may dominate contrast agent clearance as compared with cell death and subsequent transfer and retention of iron within phagocytes and interstitium. Thus, the signal kinetics of viable and nonviable (apoptotic) SPIO-labeled cells may be different in different biologic contexts: (a) the labeled cells themselves may metabolize the SPIO particles at different rates; (b) the labeled cells may or may not proliferate (dilute SPIO particles); (c) the labeled cells may die at different rates due to differences in sensitivity to hypoxia, access to nutrients, and so on; and (d) local tissue macrophages may have different efficiencies in SPIO biodegradation depending on their quantity, tissue phenotype, and metabolic state.

**Tribulations: SPIO-based MRI Cell Tracking Is Not Inherently Quantitative**

Although some investigators reported a linear relationship between R2* values and labeled cell concentrations of 0.4–2.6 $\times$ 10^6 cells per milliliter in vitro (54), the in vivo situation is much more complicated. First, the degree of cellular iron oxide nanoparticle uptake shows major variations depending on the cell type (eg, significantly higher iron uptake by macrophages compared with undifferentiated stem cells and significantly higher iron uptake by stem cells compared with differentiated effector cells), nanoparticle size and/or chemical properties, and labeling technique, among other variables (31,48,49). Second, and more important, the SPIO-induced changes in T2 and/or T2* values depend on the formational state of the nanoparticles, that is, in an intracellular agglomerated and/or clustered state versus an extracellular nonclustered single-particle state. Thus, the R2 and R2* signal effects of iron oxide–labeled cells do not depend on cell quantities.

**Tribulations: SPIO-based MRI Cell Tracking Cannot Report Directly on Cell Viability**

Timely detection of an impending loss or death of therapeutic cells could help us understand which factors lead to unfavorable outcomes and enable rescue interventions, such as application of growth factors, immune suppression, repeated cell transplants, or alternative repair procedures (35,36). Current clinical MRI studies help diagnose stem or immune cell graft failure several months after therapeutic cell delivery on the basis of lack of tissue repair (37) or tumor regression (38). However, cell apoptosis (cell death) typically occurs during the first few days after cell transfer.
Tribulations: SPIO-based MRI Cell Tracking Cannot Be Used for Long-term Tracking of Rapidly Dividing Cells and in Areas of Hemorrhage and/or Traumatic Injury

If labeled cells proliferate, the intracellular SPIO particles divide among daughter cells. This causes dilution of the ini-
tial contrast agent concentration and can decrease signal effects of viable, dividing stem cells—but only if cells disperse from a single into multiple imaging voxels. The presence and rate of dilution and resultant MR signal decay depend on the initial iron concentration within the cell, the size of the cell, the metabolic activity of the cell, the proliferation rate, and, for stem cells with self-renewal, the degree of asymmetric cell division (56). The T2 signal effects with SPIO-labeled stem cells can be similar to those with hemorrhage and/or trauma, which complicates image interpretation. Other potential confounding factors are sequestered dead bone fragments or susceptibility artifacts from surgical procedures, which can cause hypointense signal intensity on T2-weighted MR images.

**Tribulations: SPIO-based MRI Cell Tracking Cannot Help Differentiate Labeled Cell Transplants from Invading Host Macrophages**

Long-term tracking of SPIO-labeled cells may be accompanied by the death of some of the transplanted cells, resulting in release of the iron oxide label and secondary phagocytosis by resident macrophages. Thus, the detected MR signal at the transplant site on long-term follow-up studies may result from a combination of SPIO in originally labeled stem cells and SPIO in resident macrophages (52,57,58).

An influx of macrophages into a stem cell graft can be a sign of pathologic conditions such as cell death or immune rejection and can be visualized by in situ labeling of macrophages in the reticuloendothelial system with intravenous injection of ferumoxytol before the implantation of unlabeled stem cells (Fig 2) (45). This approach has been used for noninvasive monitoring of macrophage influx into cell transplants undergoing an immune rejection (42,43). Future clinical trials must show whether this new imaging test is sensitive enough to detect macrophage migration after administration of clinical ferumoxytol doses in patients.

**Trials: MRI Cell Tracking with Fluorinated Tracers**

Soon after the invention of $^1$H or proton MRI, fluorine 19 ($^{19}$F) MRI was introduced (59). Holland et al (59) realized that because the $^{19}$F spin is detected directly, the fluorinated compound should be referred to as a “tracer,” rather than a contrast agent, as there is no contrast due to the absence of background signal (there is a negligible amount of fluorine in bone and teeth, but this $^{19}$F exists in solid form that cannot be detected with MRI). Since its introduction for cell tracking (60), $^{19}$F MRI has seen a renaissance in the field of molecular and cellular tracking. It has clinical translational potential as the perfluorocarbons that are most commonly used are inert (not able to undergo any biologic or chemical reaction) and thus inherently safe, leaving the body as an evaporated substance (61). $^{19}$F MRI is inherently quantitative as the $^{19}$F tracer in the cells is detected directly as a “hot spot” (62) (unlike in the case of SPIO, where cells are detected indirectly through changes in the $^1$H signal), so that one can perform “in vivo cytometry” (63) when performing spin-density–weighted imaging. Furthermore, the use of $^{19}$F-specific imaging avoids the confounding low-signal-intensity changes associated with injury and hemorrhage that can be encountered with $^1$H SPIO-based cell tracking.

**Tribulations: $^{19}$F MRI Cell Tracking Needs Large Numbers of Labeled Cells**

So far, only the results of one clinical $^{19}$F MRI cell tracking trial have been published (Fig 3) (21), with one being in progress (23). In the former study, patient-derived autologous dendritic
cells were labeled with CS-1000 (CelSense, Pittsburgh, Pa), a commercial perfluorocarbon formulation, and $10^6$ cells were injected intradermally in three patients with adenocarcinoma. The cell injection site was clearly visualized, with a 50% loss of signal after 24 hours due to cell death followed by perfluorocarbon clearance or cell migration away from the injection site. However, no cells were detected in the lymph nodes within the same field of view. The $^{19}$F MRI signal at the injection site for two patients receiving a lower dose of $10^6$ dendritic cells, that is, within a comparable range assuming the same cell injection depth. Although it is not possible to generalize the sensitivity of clinical $^{19}$F versus $^1$H MRI cell tracking because the sensitivity using $^{19}$F surface coils decreases exponentially with increasing tissue depth, when comparing the two clinical dendritic cell tracking studies performed at 3.0 T (12,21), the detection threshold of $^1$H MRI is greater than 10-fold than that with $^{19}$F MRI (1.5 vs $10^6$ vs $10^4$ dendritic cells). This is in stark contrast to the sensitivity reported at higher field strengths in animal studies, which demonstrated a sensitivity of $^{19}$F MRI detection of approximately $5 \times 10^4$ cells in vivo (64). Hence, the low sensitivity of fluorinated cell detection at clinical field strengths is an important limitation and may not be applicable to every cell therapy scenario.

**Tribulations: $^{19}$F-based MRI Cell Tracking Requires Dedicated Hardware and Software**

Nonproton multinuclear MRI includes carbon 13, oxygen 17, $^{19}$F, sodium 23, and phosphorus 31, necessitating coils tunable to the resonance frequency, proper interfaces, and suitable software. For $^{19}$F MRI, it is desirable to have coils optimized for the $^{19}$F resonance frequency (65), but frequently coils are designed for optimal $^1$H MRI with the capability to tune to $^{19}$F at the cost of sensitivity. Custom-built clinical coils easily run between $50\,000 and $100\,000. Many clinical systems have image acquisition limitations in place to prevent equipment damage, overheating, and patient injuries. Master passwords from the vendor may be required to bypass these limitations. Often, this can lead to long administrative processes with research agreements that protect the intellectual property right of the parties involved. In addition, there is no current procedural terminology, or CPT, code for $^{19}$F MRI; that is, these studies cannot be covered by the patient’s insurance anytime soon. All these factors must be taken into account when considering performing clinical $^{19}$F MRI cell tracking studies.

**Trials: PET/SPECT Cell Tracking with Direct Radioactive Labeling**

Imaging cells labeled with radioisotopes represent historically the first example of noninvasive cell tracking in humans. For diagnostic imaging of occult inflammation or infection, $^{111}$In-oxquinoln labeling of autologous white blood cells has been the only cell tracking technique approved so far (in 1985) by the U.S. Food and Drug Administration. Nuclear imaging is especially suited for whole-body distribution studies, as the use of tracer agents that are detected directly enable "hot spot" imaging without background signal intensity, similar to $^{19}$F MRI. However, the sensitivity of nuclear imaging is far greater than that of $^{19}$F MRI and one can perform whole-body imaging, unlike MRI. Radionuclides used as cell tracking labels have different physical half-lives (eg, $^{19}$F: 109 minutes; technetium 99m [99mTc]: 6 hours; copper 64: 12 hours; iodine 123: 13.2 hours; $^{111}$In: 2.8 days; zirconium 89 [89Zr]: 3.3 days; iodine 124: 4.2 days; and iodine 125: 59.5 days) that determine the amount of time that cells can be monitored noninvasively after cell labeling. With a physical
REPORTER GENES WERE INTRODUCED FOR PET IMAGING ON THE BASIS OF RADIOACTIVE FLUORINATION OF THE SUBSTRATE (71). UNLIKE THE CELLULAR TK, HSV-TK HAS A BROAD SUBSTRATE SPECIFICITY, INCLUDING PYRIMIDINES AND PYRIMIDINE AND PURINE ANALOGS. THE ENZYME PHOSPHORYLATES ITS SUBSTRATE, LEADING TO ITS PROLONGED RETENTION WITHIN THE CELL. UPON ADMINISTRATION OF POSITRON-EMITTING SUBSTRATES, SUCH AS 1-(2’-DEOXY-2’-[18F]-B-D-ARABINOFRANOSYL)-5-IODORACIL, OR 18FIAU, OR 9-[4-[18F]FLUORO-3-(HYDROXYMETHYL)BUTYL]GUANINE, OR 18FHBG, THE TRANSUCED ENZYME IS RESPONSIBLE FOR ACCUMULATION OF THE RADIOACTIVE PROBE ONLY IN CELLS THAT EXPRESS THE REPORTER GENE. HSV-TK–BASED REPORTER GENE CELL TRACKING HAS BEEN USED TO MONITOR T CELL TRAFFICKING (72,73) INCLUDING PET VISUALIZATION OF T CELL HOMEING IN PATIENTS WITH GLIOMA (Fig 5) (74,75).

When the HSV-TK reporter gene is used, its substrate 18FHBG or 18FIAU may be administered repeatedly because the radioactivity decays so fast. In theory, cells can then be tracked as long as they are alive and express the enzyme. This scenario does not exist for cells that are directly radiolabeled before administration. The PET reporter gene strategy offers a unique advantage over any other direct means of labeling cells, be it with radioactive tracers or with MRI, US, or CT agents. Only live cells are imaged because their molecular machinery is needed not only for enzyme expression but also for accumulation of the substrate by active phosphorylation. It is also highly unlikely that the transfected HSV-TK gene is transferred to other cells upon cell death. Hence, it is much more

**Figure 5:** PET cell tracking with reporter gene. MRI/PET of herpes simplex virus–derived enzyme thymidine kinase transduced cytotoxic T cells (CTL) in patient with glioma after injection of 9-[4-[fluorine 18]fluoro-3-(hydroxymethyl)butyl]guanine [18FHBG], A, before and, B, 1 week after cell infusion. Allogeneic cytotoxic T cells and interleukin-2 were injected intratumorally (arrows). Top row shows T1-weighted (T1W) MR images. Bottom row shows MRI/PET overlay images. Three-dimensional volumes of interest were drawn with a 50% 18FHBG maximum standardized uptake value (SUV) threshold, outlined in yellow. C, Voxel-wise analysis of 18FHBG standardized uptake value (SUV) before and after infusion of cytotoxic T cells. (Reprinted, under a CC BY license, from reference 75.)

**Trials: PET/SPECT Cell Tracking with Reporter Genes**

In the early 1990s, the herpes simplex virus (HSV)–derived enzyme thymidine kinase (TK) was transfected in brain cells by using a retroviral vector as a novel means to treat glioma after the administration of the antiviral drug ganciclovir (70). This smart approach exploits a previously considered disadvantage of retroviral vectors: their inability to transfer genes into nondividing normal brain cells. Instead, retroviral transfection results in gene delivery only to dividing tumor cells, saving the neural tissue. Soon thereafter, reporter genes were introduced for PET imaging on the basis of radioactive fluorination of the substrate (71). Unlike the cellular TK, HSV-TK has a broad substrate specificity, including pyrimidines and pyrimidine and purine analogs. The enzyme phosphorylates its substrate, leading to its prolonged retention within the cell. Upon administration of positron-emitting substrates, such as 1-(2’-DEOXY-2’-[18F]-β-D-ARABINOFRANOSYL)-5-iodoracil, or 18FIAU, or 9-[4-[18F]fluoro-3-(hydroxymethyl)butyl]guanine, or 18FHBG, the transduced enzyme is responsible for accumulation of the radioactive probe only in cells that express the reporter gene. HSV-TK–based reporter gene cell tracking has been used to monitor T cell trafficking (72,73) including PET visualization of T cell homing in patients with glioma (Fig 5) (74,75).

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it has been calculated that approximately a whole gram of gold will be needed for in vivo cell tracking in patients (76), which is more than 4000 times higher than the total amount of gold in the body. Repeated CT scanning for dynamic cell tracking also poses a serious risk, as the cumulative radiation exposure increases the probability of developing cancer.

The premise of US cell tracking is a very attractive one as US machines are ubiquitous, are relatively cheap, are fast, and come in a portable form. In addition, US is routinely used for imaging-guided cell injection procedures, including therapeutic cells (12). Conversely, US is not a whole-body imaging technique, and the contrast provided by a US contrast agent is far from specific, with low sensitivity. The gold and SPIO nanoparticles discussed earlier can also provide US contrast and, hence, have been used for US cell tracking purposes, often combined with CT (77,78). Clinically, however, the only class of US contrast agents consists of gas-filled microbubbles. Because it is possible, due to their large size, to image a single microbubble, this innocuous material appears to be the best candidate for future clinical US cell tracking (Fig 6) (79). A radically novel approach is to exploit the genetic machinery of floating aquatic bacteria to produce air-filled protein nanostructures that can be used as acoustic (80) and MRI reporters (81), where ultrasound pressure waves can be used to make the MRI contrast a specific one (82).

Other reporter genes are now being explored in animal studies. This includes the use of the HSV-TK reporter gene and a thymidine substrate analog containing an imino proton that can be visualized with chemical exchange saturation transfer MRI (83) as well as other chemical exchange saturation transfer MRI reporter genes (84,85), and the sodium iodide symporter, a thyroid protein that transports iodine into cells in exchange for sodium (86,87).

Finally, MPI has emerged as the latest cell tracking technology. MPI, which was developed nearly 15 years ago, is based on the principle that SPIO nanoparticles can be magnetized by an external magnetic field with a nonlinear response at zero field (which can be moved around in three-dimensional space) (88). With SPIO particles being widely used for MRI cell tracking and the existence of robust cellular SPIO-labeling methods, it seemed natural to pursue MPI cell tracking and compare that to MRI (89). The first MPI/MRI cell tracking study revealed a far greater sensitivity of MRI over MPI (Fig 7) (90), despite theoretical calculations that favor MPI (91). Unlike SPIO-based MRI cell tracking, MPI is inherently quantitatively similar to 19F MRI in that SPIO is not a contrast agent here, affecting 1H relaxation indirectly, but a tracer that is detected directly creating “hot spot” images that are easy to interpret (90). Another major advantage is the linear relationship between MPI signal intensity and SPIO concentration, regardless of whether the SPIO is aggregated inside cells or present as single entities in solution. However, the same limitation encountered in MRI applies to MPI, in that SPIOs are not taken up in identical quantities by different cells (thereby limiting exact cell quantifications) and the signal does not report on cell viability and may not be cell-specific over time, as SPIOs from dying cells could be taken up by surrounding host macrophages. Other studies have used CT for anatomic localization of the MPI signal (92).

specific than the direct labeling approaches, where the label still persists after cell death and can even be transferred to other live cells (including macrophages) as discussed earlier.

Preclinical Works in Progress

Optical imaging and conventional CT have been successfully applied in preclinical cell tracking. For both modalities, there are severe subject size and dose limitations such that it is highly unlikely these modalities will be used any time soon for clinical cell tracking, if ever. Because light photons from a wide range of frequencies cannot penetrate into and out of deeper tissues, optical imaging studies performed in mice cannot be simply translated to humans. CT, on the other hand, has whole-body imaging capability, but the low contrast agent sensitivity presents a major problem for clinical translation because high doses of radiopaque agents will be required. For instance, for gold nanoparticles, an average 70-kg human individual contains 0.229 mg of gold versus 4000 mg of iron. For gold, when scaling up from the mouse,
tracking techniques is now available (Table 2), but it is not yet clear which of these will be integrated into clinical workflows. New opportunities may present themselves for interventional radiologists performing real-time imaging-guided cell injections. At the present time, 1H MRI is the only clinical imaging modality that allows real-time imaging of prelabeled cells within their anatomic context. Follow-up of 1H MRI could be performed with either SPECT or PET with reporter genes. With a variety of clinically translatable cellular imaging techniques available or in development, now is the time to show if and how these can affect clinical outcomes.

however, because many cell therapy applications are performed in soft tissues, MRI takes preference. Hence, efforts are under way to develop a hybrid MPI/MRI unit (93). Unlike any of the above discussed imaging modalities, a clinical MPI unit does not exist—although there are no theoretical limits toward building a clinical MPI instrument (94).

**Conclusion**

As cell therapy is coming of age, as with any emerging form of medical treatment, our medical imaging community is likely to become an integral part of it. A plethora of noninvasive cell tracking techniques is now available (Table 2), but it is not yet clear which of these will be integrated into clinical workflows. New opportunities may present themselves for interventional radiologists performing real-time imaging-guided cell injections. At the present time, 1H MRI is the only clinical imaging modality that allows real-time imaging of prelabeled cells within their anatomic context. Follow-up of 1H MRI could be performed with either SPECT or PET with reporter genes. With a variety of clinically translatable cellular imaging techniques available or in development, now is the time to show if and how these can affect clinical outcomes.
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