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Perspective

Clinical trials of MRI-based immune cell imaging: challenges and perspectives

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Immune cell-based therapies, such as chimeric antigen receptor T cells (CAR T) therapies and dendritic cell (DC) vaccines are producing tremendous opportunities for treating previously incurable diseases. Cellular immunotherapy holds some unique advantages. Adoptively transferred immune cells can persist in the body and offers a long-term protection to patients. In addition, genetically engineered immune cells are usually designed to target specific cells, therefore cause fewer side effects as compared to traditional non-specific treatments. At present, the main challenges of cellular immunotherapy include (1) identifying patients who respond to the therapy, (2) early assessment of therapy effectiveness, (3) long-term effect and toxicity. Importantly, the persistence and survival of transferred immune cells is considered the primary factor that determines patients' response. Therefore, non-invasive tracking of therapeutic immune cells would provide essential information regarding above questions and be of great benefit to the development of more effective immunotherapies [1].

Various types of imaging technologies have been developed for *in vivo* cell tracking, including optical imaging that uses fluorescence/bioluminescence, magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT). Among these modalities, MRI provides the best soft tissue contrast and spatial resolution, therefore is most suitable for identifying transplanted cells in their anatomical context [2]. In addition, MRI contrast agents usually have longer half-lives compared to radiative probes (PET/SPECT), and allow the possibility of cell tracking over relatively-long periods of time. At present, MRI is the most employed modality for tracking stem cells and immune cells in multiple clinical trials.

The most commonly used MRI contrast agent in the clinic is gadolinium (Gd)-based paramagnetic chelates (Table 1). However, due to the concerns over its potential toxicity and relatively low sensitivity, it has never been approved for cell tracking purposes (Table 1). So far, almost all of the clinical trials on MRI-based immune cell imaging were performed by superparamagnetic iron oxide nanoparticles (SPIONs)-mediated ^{1}H MRI, due to its good imaging sensitivity and biocompatibility (Table 1) [3]. In addition, the use of perfluorocarbon (PFC) nanoemulsions paired with ^{19}F MRI is under active research and have been applied to the tracking of DCs (Table 1) [4]. At present, MRI-based immune cell imaging has not been applied in clinical practice, and all clinical trials are still at the poof of principle stage. There are still many questions need to be answered before its practical transition to the clinic. What are the optimal labelling procedures for different types of immune cells? Is the detection quantitative? Is it feasible for long-term tracking? How to evaluate the safety of the cell labelling procedure? In this perspective, we addressed these clinically relevant questions and provided perspectives for future clinical translation.

Imaging of immune cells: phagocytic vs. non-phagocytic. So far, all clinical trials of MRI-based immune cell imaging were performed on phagocytic cells (Table 1). Labelling of macrophages is usually achieved by intravenous administration of SPIONs (Table 1 and Fig. S1a online), which are spontaneously taken up by circulating and tissue macrophages. MRI-based macrophage imaging has been widely used to identify inflammation and tumour lymph node metastasis in multiple trials (Table 1). In addition, MRI-mediated DCs tracking was achieved by pre-incubating the cells with SPIONs or PFC (Table 1) [5]. As illustrated in Fig. S1b (online), after injection of SPIONs-labelled DCs, MRI successfully detected the signal at the injection site and distant LNs in melanoma patients (Fig. S1b online) [5].

Labelling of non-phagocytotic cells, such as T lymphocytes, B lymphocytes and NK cells, is more challenging as compared to phagocytic cells, since they normally do not internalize sufficient number of particles. Labelling of T cells usually requires additional surface conjugation work or transfection agents, such as cationic proteins, polyamines, cell-penetrating peptides (CPP), and nuclear localization sequences (NLS) (Fig. S1c online). However, most of the transfection agents and peptides are non-FDA approved. One particular agent, protamine sulphate (PS), was shown to significantly enhance the uptake of nanoparticles in T cells, and has been proven less toxic as compared to other chemical conjugation methods. More importantly, PS is an FDA-approved drug for heparin anticoagulation, therefore SPIONs-PS labelling of T cells can be easier to gain regulatory approval compared to other strategies.

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Table 1
Comparison of available methods for MRI-based immune cell imaging.

MRI labelling drug	Detection method	Advantages	Limitations	Clinical trials of immune cell imaging		
Gd-based paramagnetic chelates	T1-weighted ^{1}H MRI Positive contrast	• Widely used as contrast agents for clinical MRI • Positive contrast is generally preferred • No transfection agent required	• Potential safety risk (nephrogenic systemic fibrosis) • Relatively low sensitivity • No quantification of cell number	No clinical trials available		
SPIONs	T2-weighted ^{1}H MRI Negative contrast	• FDA approved drug • Excellent sensitivity, allows single cell detection • Good biocompatibility, biodegradable • Cost-efficient • Can be applied in combination with reporter genes	• No quantification of cell number • Transfection agent is required for non-phagocytic cells • Negative signal can be similar to injury or hemorrhage • Cell mitosis causes signal decline • Cell death may cause non-specific host phagocytic cell labelling	Macrophages	*In vivo* (Intravenous administration)	• Prostate cancer lymph-node metastasis • Myocardial infraction inflammatory lesion • Multiple sclerosis (MS) inflammatory lesions • Carotid stenosis atherosclerotic lesions • Type 1 diabetes islet inflammation
				Dendritic cells	*Ex vivo* incubation	Melanoma (DCs vaccine)
				Peripheral Blood mononuclear cells (PBMC)	*Ex vivo* incubation	Healthy volunteer (reticuloendothelial system)
PFC nano-emulsions	^{19}F MRI Hot spot	• Quantification of cell number • Avoid confounding signals associated with injury and hemorrhage • No transfection agent required	• Very low sensitivity (minimum cell detection number is 10^3–10^5) • Dedicated hardware and software required	Dendritic cells	*Ex vivo* incubation	Colorectal adenocarcinoma (DCs vaccine)
MRI reporter genes	T2-weighted ^{1}H MRI or ^{1}H CEST MRI	• Enable long-term cell tracking • Avoid false positive signals from non-specific host phagocytic cell labelling • Provide cell survival, differentiation and signalling information	• Relatively low sensitivity • Safety issues regarding the use of genetic engineering	No clinical trials available		

Besides CAR T therapy, NK cell-based immunotherapy has drawn much attention as a potential treatment for both solid and blood cancers, therefore non-invasive imaging of NK cells is also at urgent need. Similar to T lymphocytes, NK labelling with SPIONs requires the assistance of transfection adjuvants. With the assistance of lipofection or electroporation, NK cells were successfully labelled with SPIONs and visualized *in vivo*, especially accumulated at the tumour site. In addition, ^{19}F emulsion can be effectively taken up by human NK cells by simple incubation method. However, *in vivo* ^{19}F signal could only be detected at the cell injection site, but not in tumour or distant organs, probably due to its lower sensitivity and quicker signal loss as compared to SPIONs. Overall, MRI-based NK cell imaging is still at pre-clinical stage, and SPIONs still hold the most potential for future clinical translation. In terms of B lymphocytes, SPIONs-mediated B cells imaging has been used to evaluate B-cell depletion efficacy after antibody (anti-CD79) administration in animal models. In general, there are limited reports on B cell imaging as compared to other immune cell types, probably due to their limited application in the clinical setting.

Qualitative or quantitative detection? Metal ion-based ^{1}H MRI cannot quantify the absolute number of cells for the following reasons. First, the imaging principle of ^{1}H MRI is through detecting the effect of proton relaxation in surrounding tissues, but not direct on the labelled cells. Second, the uptake of materials by individual cells is not uniform in a cell population [6]. Moreover, intracellular agglomeration and clustering of nanoparticles significantly affect SPIONs-induced changes in T2 and/or T2* values, resulting in uneven signal among induvial cells [6]. In consideration of above reasons, it is challenging to establish a reliable correlation between the ^{1}H MRI signal and absolute cell numbers.

Nevertheless, SPIONs-mediated live cell imaging is usually regarded as "semi-quantitative" that the signal intensities can be compared between regions and among subjects. In practice, due to the heterogeneous environment of different tissues, especially in tumours with necrosis or edema, analysis of signal intensities must ensure that the true signal was not present in the baseline images. In one pre-clinical tracking of SPIONs-labeled T cells, voxels within lymph nodes having signal intensities less than 50% were used for quantitation, while in tumours those voxels having the lowest 10% of signal intensities were analyzed [7]. Moreover, the anatomic information obtained from MRI, although not quantitative, is essentially valuable in clinical settings. For example, the detection of transferred DCs in distant lymph nodes could predict a successful vaccine delivery in those patients [5].

Moreover, the non-quantitative issue of ^{1}H MRI can be overcome by the use of ^{19}F MRI, which directly detect the number of fluorine atoms and is inherently quantitative. Preclinical study has demonstrated the linear correlation between ^{19}F MRI signal and ^{19}F-atom concentration of pre-labelled T cells *in vivo*, allowing quantification of apparent T cell homing following cell administration. Unfortunately, the relatively low sensitivity is a major hurdle for the translation and clinical application of fluorine-based MRI. As reported by the clinical trial, the detection threshold of ^{19}F MRI for DCs imaging is above 1×10^6 cells, which is 10 times higher than the results from the ^{1}H MRI-based DCs tracking trial [5]. In order to improve the sensitivity of fluorine-based MRI, the development of highly sensitive detector and specialized data acquisition platform is urgently needed. In addition, the newly developed magnetic particle imaging (MPI) directly detects the presence of SPIONs particles in labelled cells, which successfully solved the non-quantitative problem of conventional SPIONs-mediated MRI [8]. Preclinical studies using MPI for tracking stem cells have demonstrated a linear quantification of both cell number and iron content *in vivo*. In addition, animal studies have reported a 200-cells detection limit *in vivo* by using MPI, which is remarkably

more sensitive than other imaging modalities. As commercial SPIONs were initially designed for MRI and may not be optimized for MPI detection, it is particularly interesting to develop MPI-tailored SPIONs to further improve its detection sensitivity.

In order to increase the imaging sensitivity and accuracy of MRI-based cell tracking, the use of multimodal imaging that combines MRI with one or more imaging modalities would maximize the strength of individual techniques. For the construction of multi-mode imaging agents, MRI contrast agent could be designed to combine the T1-T2 elements or conjugated with optical and radioactive reporters. The T1-T2 dual mode imaging enables self-confirmation of signals from labelled cells and minimize the background noise from heterogeneous environment, especially preferable for tracking the cells that accumulate in tumours, such as T cells and NK cells. Addition of optical probes such as fluorescent dyes and quantum dots (QD) on SPIONs enables sensitive imaging at nanomolar range (>50 cells), and are suitable for the tracking of small cell clusters. New nanofluorophores such as black phosphorus (BP) nanodots are also promising probe candidate for live cell imaging due to its unique optical properties and bright fluorescent nature. In addition, radioisotope-based modalities such as PET and SPECT can provide highly sensitive and quantitative imaging, however with relatively poor spatial resolution. MRI is advantageous over PET/SPECT as it processes superior spatial resolution, and does not involve any radiation. Therefore, the combination of MRI with PET/SPECT could reduce patients' radiation exposure to about 50%, and generate excellent anatomical and quantitative biological information.

Short- or long-term tracking? The choose of optimal detection time for *in vivo* cell imaging is quite variable depend on the types of target cells, the feature of nanoparticles, and disease models. For *in vivo* labelling of macrophages, imaging should be performed when the agent is mostly cleared from the blood to avoid the background interference from free SPIONs particles. In addition, the blood half-lives should have a steady stage to ensure a sufficient exposure time for macrophages phagocytosis. Previous clinical trials of macrophage imaging were performed at 24, 36, or 48 h after SPIONs administration, and no long-term tracking has been reported. The purpose of macrophage imaging is to capture a one-time image for the diagnosis of inflammation or tumour lymph node metastasis, therefore long-term tracking is not necessary at this circumstance.

For tracking transferred immune cells, the ideal imaging scheme should be long-term. So far, the longest time reported for immune cell tracking in human is 7 das post cell administration from a PBMC tracking study [9]. The major hurdle for long-term tacking of SPIONs-labelled cells is the decline of signal. The speed of signal decline comes from three aspects: (1) cell proliferation, (2) cell exocytosis, and (3) lysosomal degradation of nanoparticles. In consideration of these factors, long-term tracking of DCs is more feasible as compared to T cells, since DC vaccines are usually not actively proliferating *in vivo*. On the contrary, CAR T cells proliferate exponentially in the body especially in patients who respond to the treatment, resulting in a large dilution of the signal. Ideally, the tracking of T cells requires the cells to be highly confined to a region, for example CAR T cells targeting solid tumours, and may be less reliable for the cells distributed widely in the body, such as CAR T cells targeting blood cancers.

Long-term tracking of SPIONs-labelled cells may be accompanied with false positive signal interference. When pre-labelled cells gradually die in the body, nanoparticles may be released from dead cells and spontaneously taken up by host phagocytic cells. When a large number of these passively labelled phagocytes accumulates, false positive signals may occur. It is believed that the MRI signal is most accurate for tracking initial cell distribution within a short time post cell injection. More detailed animal studies are required to reveal the dynamic signal changes *in vivo* and distinguish the true signals from the phagocyte's interference.

To avoid above problems, the use of MRI reporter genes is an alternative method for long-term cell tracking (Table 1). When integrated into the genome DNA, MRI reporter genes would not be diluted upon cell dividing, which is essentially important for the tracking of extensively proliferating CAR T cells [10]. Moreover, MRI reporter genes not only allow the tracking of cell distribution, but also provide extra information regarding cell survival, differentiation and specific signaling pathways. Generally, there are three types of MRI reporter genes, cell surface receptors that bind to metal-based contrast agent, enzymes, and iron-binding or iron-storage proteins. Although holds strong translational potential, MRI reporter genes suffer the problem of low sensitivity which limits its wide application. To increase the sensitivity and accuracy for reporter genes-mediated MRI, the development of more efficient gene delivery system and the employment of multiple reporter genes driven by different promoters is possible to complement the limitations of individual reporters. In addition, the use of optimized pulse sequences and sophisticated imaging processing platforms for specific reporters is likely to further improve their sensitivity. Looking forward, the reporter genes could be co-delivered in the same system with therapeutic genes, such as the CAR, and gain quicker regulatory approval.

Safety assessment. From the clinical trials of view, safety is the primary issue. Although cellular immunotherapy has fewer side effects as compared to conventional chemotherapy and radiotherapy, the combination of nanomaterials and immune cells may cause unexpected risks. Therefore, a comprehensive risk analysis should be performed to ensure the labelling procedure has minimal effect on cells' viability, immunological phenotype and function, and do not increase the incidence of adverse effect in patients. *In vitro*, the viability of SPIONs labelled immune cells could been assessed by Annexin-V/PI flow cytometry analysis, colorimetric lactate dehydrogenase (LDH), and cell counting kit-8 (CCK-8) assays. Immune cell phenotype is usually determined by flow cytometry analysis of specific cell surface markers. For instance, HLA class I, HLA-DR/DP, CD80, CD83, CD86, and CCR7 are common markers for phenotypic evaluation of DCs vaccines. *In vitro* cell function determination, such as cytokines release measurement, DC antigen uptake and presentation assays, and tumour lysis assays should be performed. In patients, incidence of all grades of adverse effect and the mortality rate should be compared with non-labelled cell therapies and other conventional strategies. In general, SPIONs are well tolerated in patients and showed a good safety profile as reported by multiple clinical studies [5,9].

In spite of the great promises of MRI-based immune cell imaging from preclinical and clinical studies, there are still challenges yet to be overcome regarding the labelling efficiency, quantitative issue, long-term monitoring, and safety assessment. Besides these scientific challenges, the cost-effectiveness of the technique also determines whether it can be applied as a routine practice for every patient. We believe in the near future, the wide application of MRI-based immune cell imaging would largely improve our understanding of cell behavior and cell fate in immunotherapy and immune cell-mediated disease, ultimately leading to novel therapies with higher efficacy.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2020.10.016.

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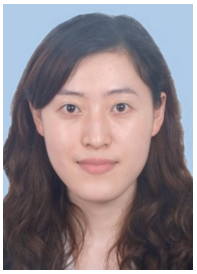

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