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Whole-body imaging of adoptively transferred T cells using magnetic resonance imaging, single photon emission computed tomography and positron emission tomography techniques, with a focus on regulatory T cells

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Summary

Cell-based therapies using natural or genetically modified regulatory T cells (T_{regs}) have shown significant promise as immune-based therapies. One of the main difficulties facing the further advancement of these therapies is that the fate and localization of adoptively transferred T_{regs} is largely unknown. The ability to dissect the migratory pathway of these cells in a non-invasive manner is of vital importance for the further development of *in-vivo* cell-based immunotherapies, as this technology allows the fate of the therapeutically administered cell to be imaged in real time. In this review we will provide an overview of the current clinical imaging techniques used to track T cells and T_{regs} *in vivo*, including magnetic resonance imaging (MRI) and positron emission tomography (PET)/single photon emission computed tomography (SPECT). In addition, we will discuss how the finding of these studies can be used, in the context of transplantation, to define the most appropriate T_{reg} subset required for cellular therapy.

Keywords: regulatory T cells, whole-body imaging

Introduction

Survival of a transplanted allograft is dependent upon a delicate balance between effector T cells (T_{eff}) and regulatory T cells (T_{reg}) [1–4], whereby the number of T_{regs} must equal the number of T_{effs} to promote tolerance to alloantigens. In preclinical mouse transplant models this equates to a 1·1:1·2 ratio of T_{regs} to T_{eff} cells, or approximately 33–50% of T_{regs}, to be present at the transplant site to induce tolerance [5]. One way to achieve this number is by expanding T_{regs} *in vitro* and adoptively transferring them back into the patient [2]. We have demonstrated previously that adoptively transferring *ex-vivo* expanded murine and human T_{regs}, with specificity for the alloantigens expressed by the graft, results in graft prolongation in animal models [6,7]. These data suggest that T_{reg} cell therapy could be a beneficial therapy for transplant patients [8–12]. In fact, these cells have been used in the clinic to alleviate graft-*versus*-host disease in patients following bone marrow or human stem cell transplantation, resulting in clinical improvement [13]. With regard to organ transplantation, the ONE study (a multi-centre Phase I/II study) is investigating the efficacy of *ex-vivo*-expanded adoptive T_{regs} for the use in kidney transplant patients.

Although this cellular therapy has therapeutic potential, there are several questions regarding (i) lifespan: how long *in vivo* do T_{reg} cells survive following adoptive transfer; and (ii) localization: where do adoptively transferred T_{regs} go, do

they localize within the transplanted tissue and/or within the draining lymph node or does this alter during the lifespan of the transplant? (iii) do T_{reg} subsets function at defined locations, all remain unanswered. It is vital to understand each of these points so that the optimal T_{reg} for therapeutic purposes can be created.

Conventional imaging techniques have been utilized to address some of these questions. For example, using fluorescent microscopy in an islet allograft model, Zhang *et al.* observed that T_{regs} migrate from blood to the inflamed allograft, wherein they become activated, before migrating to the draining lymph nodes. This process was dependent upon multiple chemokine receptors including CCR2, CCR4 and CCR5 [14]. *In-vivo* imaging, using two-photon intravital laser-scanning microscopy of adoptively transferred T_{regs} in non-obese diabetic (NOD) mice with autoimmune diabetes, have indicated that T_{regs} are present in lymph nodes; these authors also noted that the contact time between T_{eff} and dendritic cells (DCs) is reduced when T_{regs} are present, suggesting that the latter cells prevent the clonal expansion of T_{eff} cells by limiting the DC–T_{eff} interaction time [15,16].

Although these studies have contributed towards understanding of the anatomical locations where T_{regs} reside, following adoptive transfer the techniques used do not allow the longitudinal study of cells within the same recipient. Unlike the aforementioned imaging techniques, whole-body imaging of T_{regs} has the potential to do precisely this.

Whole-body imaging techniques currently being used to track T cells and other immune cells include both nuclear and optical imaging. Nuclear imaging is composed of administration of radioactive probes that take part in a physiological or biological process by an organism. The uptake of the radiotracer then allows imaging, such that the biological processes *in vivo* and cell recruitment can be measured [17–19]. In comparison, optical imaging uses non-ionizing radiation and measures light generated from a probe within the cell. Although optical imaging (fluorescence and bioluminescence) has been used successfully in small animal models, this technology is not feasible, and has not yet been used in human whole-body scans. The imaging techniques relevant to cell tracking in a clinical setting are via nuclear imaging and magnetic resonance imaging (MRI), and these will be the focus of the next sections; however, for an overview on optical imaging please refer to Kircher *et al.* [20]. What whole-body nuclear imaging systems have been used to look at T cells *in vivo* so far?

MRI

MRI is a non-invasive imaging technique that provides three-dimensional images with high resolution [21]. Its popularity is due to high signal-to-noise ratios and soft tissue contrast as well as the availability of safe intracellular contrast agents. The principle of MRI is based on the hydrogen nuclei spin of organic compounds within an individual [22]. One of the main limitations of MRI imaging is low sensitivity; others are highlighted in Table 1 [23];

Table 1. Summary of preclinical imaging methods, their labels and advantages/disadvantages.

Imaging	Isotopes	Labelling	Advantages	Disadvantages	Ref
PET (high-energy gamma rays)	Carbon-11 Fluorine-18 Oxygen-15 Copper-64 Iodine-124 Nitrogen-13 Zirconium-89	Direct/indirect	Detects picomolar concentrations Non-invasive High sensitivity Shows organ function	Ionizing radiation High running cost Cyclotron required on site (limited availability) Resolution dependent on isotope used and its positron range Limited half-life of the isotopes used	[25,26,30, 33–35]
SPECT (lower-energy gamma rays)	Iodine-131 Iodine-125 Indium-111 Technetium-99 m	Direct/indirect	Detects picomolar concentrations Isotopes widely available Non-invasive High sensitivity Long half-life of isotopes used Simultaneous imaging of multiple radiolabels Ability to measure slow kinetic processes	Ionizing radiation High running cost Limited spatial resolution Quantitation	[25,26,30,31, 33–35,73]
MRI (radio waves)	Contrast agents: SPIO Gadolinium	Direct/indirect	No ionizing radiation High resolution High soft tissue contrast Non-invasive	Low sensitivity Require higher levels of labelling Long scan time Very expensive	[20,22]

PET: positron emission tomography; SPECT: single photon emission computed tomography; MRI: magnetic resonance imaging.

Table 2. Summary of spatial resolution, sensitivity and depth of preclinical imaging techniques in further detail.

Imaging technique	Spatial resolution	Sensitivity	Depth	Preclinical use	Clinical use	Cost
MRI	< 1 mm	Low	No limit	Yes	Yes	High
PET	1–2 mm	High	No limit	Yes	Yes	High
SPECT	< 1 mm	High	No limit	Yes	Yes	High

PET: positron emission tomography; SPECT: single photon emission computed tomography; MRI: magnetic resonance imaging.

however, the main benefit of this technique is that it does not require ionizing radiation.

Single photon emission computed tomography (SPECT) and positron emission tomography (PET)

SPECT and PET are the two main techniques used in nuclear imaging [24–26], in conjunction with computed tomography (CT). The sensitivity of PET is typically higher than SPECT, and both are more sensitive than the previously described MRI-based technology. They possess the ability to detect picomolar, or low concentrations, of radioisotopes, making them an attractive prospect for clinical and preclinical small-animal imaging [27,28]. SPECT functions by detecting a single gamma ray emitted from radioisotopes. Emitted gamma rays are collected by sensitive 'gamma camera' detectors, which rotate around the object. Single or multiple detectors can be used for SPECT to generate multiple single two-dimensional images from various angles which, once reconstructed, generate a three-dimensional tomographic image [26,29,30]. In contrast, PET imaging relies on the simultaneous detection of two photons (of 511 KeV) emitted in opposite directions during the annihilation of a positron colliding with an electron in tissue [31,32].

Many different SPECT and PET isotopes are used in nuclear medicine, as summarized in Table 1. Some PET radioisotopes require an on-site cyclotron for their production [25,30,33]. The half-lives of these isotopes are typically short, ranging from 2 to 110 min. Although most of the PET isotopes have a short half-life, isotopes such as Copper-64 ($t_{1/2} = 12{\cdot}7$ h) or Iodine-124 ($t_{1/2} = 4{\cdot}18$ days), which have a longer half-life, can be used for imaging over extended periods of time [34]. Unlike PET, SPECT radioisotopes are readily available, i.e. they do not require an on-site cyclotron. In a preclinical setting SPECT offers better spatial resolution than PET. Importantly, SPECT offers the unique advantage of imaging multiple isotopes simultaneously, based on the detection of the different energies of the emitted photons, and hence the potential for tracking two or more cell populations at the same time [35]. With appropriate image reconstruction techniques PET, and more recently SPECT, can offer quantitative data such as *in-vivo* concentration of the isotope in a particular region of interest. However, both are not without their limitations, such as sensitivity, specificity resolution and quantitation, as highlighted in Tables 1 and 2.

Combinational techniques (SPECT-computed tomography (CT), PET-MRI and PET-CT)

Combinational approaches are now furthering the advancement of imaging. Functional imaging with SPECT and PET is combined with X-ray CT to provide anatomical and functional information from a single study [23]. The main advantage associated with this hybrid imaging approach is the increased accuracy of anatomical localization of the radiotracer uptake, enhanced by the typically high-resolution structural images, which is not achieved using SPECT or PET alone. Additional advantages include the ability to use the structural images as a basis for attenuation correction of the emission data and the use of structural images for more accurate definition of regions of interest for quantification of radiotracer uptake in various organs. There are many advantages associated with this hybrid system, including shorter acquisition time, better attenuation correction, increased specificity and a more accurate image of cell activity. Hybrid imaging modalities such as PET-CT, SPECT-CT and PET-MRI can be used.

The recent introduction of systems that combine PET and MRI opens new horizons for multi-modality molecular imaging. These systems offer simultaneous morphological, functional and molecular information of a living system. In the near future, PET-MRI may emerge as a new powerful multi-modality technique offering considerable potential for imaging applications beyond correlation of functional and anatomical images.

New small-animal micro-CT instruments have the ability to generate high-resolution anatomical three-dimensional images. Advancements in micro-CT technology, such as smaller detector elements and more powerful X-ray tubes, allow improved spatial resolution and faster scan times of an entire mouse (0·8 s). Iodinated contrast agents have a short half-life (10 min) in circulation; however, because of faster scan times and the use of clinical contrast agents, perfusion studies may be performed. Gold nanoparticles have now been used as a better contrast agent. The higher the atomic number and electron density, the higher the attenuation coefficients leads to greater the differentiation between tissues for the CT imaging. The atomic number and electron density of gold (79 and 19·32 g/cm^3, respectively) are much higher than those of the currently used iodine (53 and 4·9 g/cm^3), making it a more effective contrast agent. The major disadvantage of the technique is exposure to ionizing radiation [36–38].

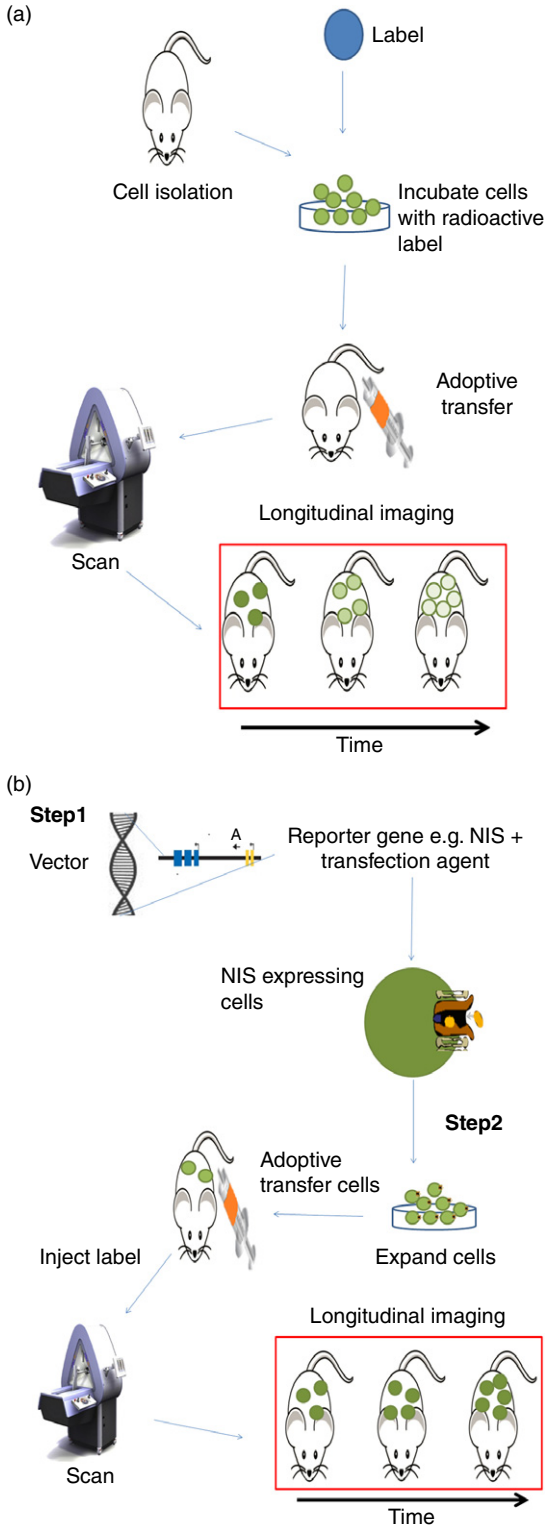

Fig. 1. Outline of direct and indirect cell labelling. (a) Direct cell labelling involves the introduction of a radioactive isotope into cells. Cells isolated from the donor mice can be incubated with the radiolabel. Once labelled, they can be transferred adoptively into the recipient before single photon emission computed tomography (SPECT) scanning. With direct labelling, as the cells divide and the label effluxes, the period of time in which the cells can be tracked is limited. (b) Indirect cell labelling involves genetic modification of the cell. Cells are transduced with a vector encoding a reporter gene, for example human sodium/iodide symporter (hNIS). Reporter genes can also encode for fluorescent proteins or enzymes. Transduced cells are incubated and expanded *in vitro* prior to adoptive transfer. With the exclusion of fluorescent reporter proteins imaging requires radiolabel injection. The transduced cells can be visualized longitudinally by direct *in-vivo* injection of the radioisotope. As the reporter gene is passed to newly divided cells, long-term imaging is possible.

PET-MRI is a relatively new hybrid technology offering both the sensitivity of PET alongside the anatomical and soft tissue information provided by MRI. This hybrid technology has been used to study cells in a cancer setting [17].

Functional and morphological information gathered using PET-MRI, in conjunction with the radiolabelled glucose analogue [2-flourine-18]-flouro-2-deoxy-D-glucose ([^{18}F]-FDG), has identified areas of cancerous growth and differentiates between benign and malignant tumours, depending on metabolic uptake [18,19,39].

Direct *versus* indirect cell labelling

To visualize cells by MRI/SPECT/PET they must first be labelled. This is achieved by either direct or indirect labelling. Direct labelling is a process in which the cells remain free of any genetic alteration or manipulation (Fig. 1a), and can be achieved simply by incubating cells with the radiolabel or contrast agent of choice *in vitro* [20,40].

MRI

Cells are labelled directly with various magnetic resonance (MR) contrast agents derived from paramagnetic metal cations, e.g. gadolinium, or supermagnetic nanoparticles (ranging from 50 nm to greater than 1 μM in size) such as (supermagnetic iron oxide particles (SPIOs) and ultra-small supermagnetic iron oxide particles (USPIOs) [41]. In general, non-phagocytic cells such as T cells have a low labelling efficiency and poor contrast agent incorporation [42–44]; however, many methods have been used to improve this, including the use of transfection agents (e.g. poly-L-lysine, lipofectamine, SuperFect and protamine sulphate), electroporation, use of the HIV-TAT peptide, which enables cross-linked USPIOs to translocate across the cell membrane and into the nucleus [45–47], micrometer-sized paramagnetic iron-oxide particles and antibodies [48]. The use of the aforementioned reagents is not without problems, including toxicity and immune modulation. Recently, however, Liu *et al.* developed MRI-fluorescent cellular

imaging agents, nano-sized iron-oxide particles coated with polyethylene glycol and conjugated to fluorescent dyes (IOPC-NH_2), which labelled more than 90% of both rat and human T cells (Jurkat T cells) *in vitro* without the requirement of transfection, electroporation, antibodies or the HIV-TAT peptide. No toxic effect, or loss of T cell function, was reported using these particles [49].

SPECT/PET

To date, and as mentioned earlier, only a few imaging agents have been approved clinically for tracking cells by SPECT and PET, including [^{111}In] oxiquinolon ([^{111}In]) [20] and [^{99m}]Tc-hexamethylpropyleneamine oxime ([^{99m}Tc]-HMPAO) [20,30,50]. [^{99m}Tc]-HMPAO is thought to enter the cell via passive diffusion across the plasma membrane, followed by the intracellular conversion of a lipophilic complex to a hydrophilic complex by reducing agents [51]. Several disadvantages are associated with the use of direct labelling. These include loss of the radiolabel: the short half-life of the radiolabel, 6 h for [^{99m}Tc]-HMPAO, make longitudinal studies difficult using this method [40,51]. The viability of the cells that are being imaged, live cells cannot be distinguished from dead cells and, lastly, phagocytic cells in the body, may engulf the administered labelled dead cells, leading to images that are difficult to interpret [21,52].

An alternative method to investigate the long-term migration of viable cells is to label cells indirectly. Indirect cell labelling avoids the previously described limitations of direct labeling, as the cells to be imaged are modified genetically to express a reporter gene, which is translated into either an enzyme or cell surface transporter [20,53]. Within the cells, stable clonal expression of the reporter gene allows the labelled cell to be observed longitudinally over their entire lifetime (Fig. 1b). Additionally, the reporter genes are incorporated into progeny daughter cells following division [40]. Expression of these reporter genes is limited to living cells, thus eliminating background signals from dead cells. In spite of these advantages, this labelling method may result in epigenetic gene silencing, due to DNA methylation [20], however, this can be prevented by treating cells *in vitro* in a dose- and time-dependent manner with a DNA methyltransferase inhibitor [54].

Delivery of reporter genes to cells can be accomplished by either viral or non-viral methods. Viral methods, e.g. lentiviral and retroviral vectors, integrate the gene of choice into the genome [20], while non-viral gene transfer includes the use of polymer, nanoparticles or chemical vectors [55]. The herpes simplex virus thymidine kinase type 1 (HSV1-tk) and its variants are commonly used reporter genes which mediate the uptake of radiolabels, including [^{18}F] [56], and is one of the most common reporter genes for PET imaging. This receptor functions by phosphorylating exogenously administered substrates, which are then retained in the cell due to a negative charge. Despite being used in patients, HSV1-tk and its derivatives are not expressed endogenously in humans, presenting a major immunological concern in that their use risks generating an immune response against cells and tissues transduced with this gene [57]. However, the use of the human mitochondrial thymidine kinase 2 (hTK2) gene may help to resolve this issue.

Several other human reporter genes have been developed. The human noradrenaline transporter gene (hNET) encodes a transmembrane protein that mediates the transport of noradrenaline analogues across the cell membrane [56]. Its exclusive localization to the central and peripheral sympathetic nervous systems and clinically approved radiolabelled probe make this gene an attractive reporter. [^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG) is a clinically approved radiolabel probe for imaging tissues expressing high levels of hNET. MIBG can be radiolabelled with ^{123}I or ^{131}I for SPECT and γ-camera imaging and with ^{124}I for PET imaging [58].

The human sodium/iodide symporter (hNIS) is a transmembrane glycoprotein ion channel that allows the receptor-mediated uptake of a broad range of radiolabels, including radioiodine ([^{123}I], [^{124}I] and [^{131}I]) and [^{99m}Tc]-pertechnetate ([$^{99m}TcO_4^-$]), into cells. This receptor is expressed naturally in a few organs, including thyroid gland, stomach and salivary gland, and under physiological conditions, transports iodine into the cells for sodium exchange [56]. It has the advantage that several radiotracers, including [$^{99m}TcO_4$], have been clinically approved for PET and SPECT imaging. Like hNET, hNIS is a human gene (with the same sequence as NIS expressed in the human thyroid), therefore it is thought that it will not elicit an immune response if applied clinically [24,56,59,60].

Whole-body imaging of T cell therapies *in vivo*

MRI

Both MRI and SPECT/PET-CT whole-body imaging of $CD4^+$ and $CD8^+$ murine and human T cells have been reported for cancer, autoimmune and transplant studies [61]. For example, Srinivas *et al*. investigated the migration of antigen-specific T cells using Flourine-19 MRI contrast agents in a murine model of inflammation. These authors observed that over a period of 21 days antigen-specific T cells migrated to the draining lymph node [62]. In a mouse ovalbumin (OVA)-specific melanoma model, this technique was used to track tumour-specific cytotoxic T cells (CTLs) labelled with highly derivatized cross-linked iron oxide nanoparticles *in vivo* [21]. These authors observed not only the recruitment of cells to the tumour, but also reported that serial injections of tumour-specific CTLs resulted in recruitment to different tumour anatomical locations with each administration. Although this study reported that as

few as three cells/voxel within the tumour could be detected, Smirnov *et al.* were able to image individual anionic maghemite nanoparticle-labelled tumour-specific T cells in a similar model by modifying the signal-to-noise ratio by reducing the noise of the detection magnetic coil [63].

Liu *et al.* tracked adoptively transferred IOPC-NH_2 nanoparticle-labelled rat T cells in a rat and lung transplant model. These authors showed that localized infiltration of IOPC-NH_2-labelled T cells into the allograft myocardium as well as the lungs within 24 h using MRI [49]. MRI has also been used to track murine $CD8^+$ T cells infiltrating the pancreas. This was achieved by co-culturing $CD8^+$ T cells from non-obese diabetic (NOD) mice or by injecting NOD mice with supermagnetic nanoparticles coated with multiple copies of a high-affinity β cell peptide/major histocompatibility complex (MHC) ligand. This leads to labelling through endocytosis of these beads, of 90% of $CD8^+$ T cells in/from diabetic NOD animals and 2% in/from prediabetic NOD mice. MRI *in-vivo* tracking of adoptively transferred labelled $CD8^+$ T cells from diabetic animals was performed on the day of transfer and up to 16 days later. T cells were found in the pancreatic tissue and increased in numbers over time, reaching maximum numbers by day 9 and decreasing by day 16. Infiltration was confirmed histologically.

SPECT/PET-CT

SPECT/PET-CT technology is now used preclinically, employing both indirect and direct methods of radiolabelling to visualize the localization of T cells *in vivo* in animal models. $CD4^+$ T cells labelled directly; using anti-CD4 antibodies coupled to [^{111}In] before injection and SPECT imaging, were visualized in the colon of mice with colitis [64]. Direct labelling of *in-vitro*-expanded influenza haemagglutinin (HA)-specific $CD8^+$ T cells with [^{111}In]-oxine was used to assess the efficacy of these cells as an adoptive therapy for cancer. Using SPECT-CT to track these antigen-specific cells 2, 24, 48 and 120 h post-injection into mice expressing HA^+ and HA^- tumours, Pittet *et al.* observed that HA-specific T cells localized centrally in the HA^+ tumours as early as 2 h after transfer, but remained in the periphery of HA^- tumours [52]. They also correlated tumour growth with increased density of CTLs at the tumour site [52]. Direct labelling has also been extended to human T cells. Tracking of [^{111}In] directly labelled human T cells, engineered to express a chimeric antigen receptor recognizing a breast cancer antigen in a preclinical breast cancer model, Parente-Pereira *et al.* observed that human T cells migrate to the lungs after intravenous injections, then to the liver, spleen and lymph nodes, although not to tumour mass located within the peritoneal cavity, after 3 h. This was achieved only when the human T cells were administered at the tumour site via intraperitoneal injection [65].

However, for longitudinal analysis of viable cells *in-vivo*, indirect labelling is more preferable. This approach has been used to visualize human T cells *in vivo* in a cancer setting. Using Epstein–Barr virus (EBV)-specific human T cells transduced with the *HSV1-tk* gene, Koehne *et al.* [66] observed, following administration of [131] and [124] 2′-fluoro-2′-deoxy-1-b-D-arabinofuransyl-5-iodouracil (FIAU), via microPET scanning, that adoptive transferred HSV1-EBV-specific cells locate to a human EBV tumour xenograft in SCID mice. These cells accumulated 1, 8 and 15 days after adoptive transfer at the tumour site. Radiolabelled transduced cells retained cytolytic capacities following exposure to the isotope, suggesting that indirect labelling did not affect T cell function. Yaghoubi *et al.* expanded this study and published the first human study looking at reporter gene-based imaging of therapeutic T cells expressing the interleukin-13 zetakine gene (which encodes a receptor protein that targets T cells to tumour cells) and the PET imaging reporter gene herpes simplex virus 1 thymidine kinase (*HSV1 tk*) suicide gene [67]. These authors detected genetically engineered CTLs non-invasively, using PET, in a patient with a grade IV glioblastoma with the PET reporter probe, [^{18}F]-radiolabelled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine ([^{18}F]-FHBG). A PET scan to detect engineered CTLs was performed 3 days after the patient had completed a 5-week course of CTL infusions. These authors showed [^{18}F]-FHBG accumulation in the intact tumour of the CTL-infused patient, suggesting that the engineered CTLs were present at the tumour site; this was confirmed by biopsy.

In addition to the HSV-tk reporter, hNET expressing human EBV-specific T cells have been imaged *in vivo* using SPECT and PET in NOD/severe combined immunodeficiency (SCID) mice expressing an EBV tumour [56]. As few as 10^4 hNET-expressing antigen-specific T cells could be imaged using either SPECT or PET following direct intratumoral injection, highlighting the sensitivity of these imaging modalities. A selective and progressive accumulation over 28 days of EBV-restricted $CD8^+$ T cells within an EBV-expressing tumour was observed using SPECT and PET, with image intensity correlating closely with the number of cells accumulating at the antigen site [56].

Whole-body imaging of T_{regs} *in vivo*

Although whole-body imaging has been used to track $CD8^+$ T cells in tumour and autoimmune disease models and $CD4^+$ T cells in colitis, very little visualization of adoptively transferred T_{regs} in real time has been reported. In one study Feng *et al.* observed, using SPECT, that prior to transplantation directly labelled ($^{99m}TcO_4^-$) adoptively transferred T_{reg} cells are localized mainly in the spleen, liver and lungs, but following skin transplantation they were found primarily in the allograft and spleen [68]. Unfortunately, because the cells were labelled directly, imaging was performed at only

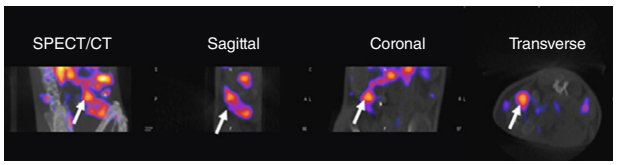

Fig. 2. Sodium/iodide symporter NIS-expressing T_{regs} can be imaged *in vivo* by nano single photon emission-computed tomography-computed tomography (NanoSPECT-CT) through *in-vivo* uptake of [$^{99m}TcO_4^-$]. C57BL/6 mice were adoptively transferred with 1×10^6 NIS-expressing T_{regs} via intravenous injection; 24 h later mice received 20 MBq of [$^{99m}TcO_4^-$] before being imaged using NanoSPECT-CT under general anaesthesia for 1 h. The white arrow indicates the spleen in different views of the scan [69].

one time-point (5 h after transplantation) and not longitudinally. The fate of these cells was not examined over the lifespan of the transplanted tissue and no correlation with graft outcome was assessed. Recently, we also utilized SPECT-CT technology to image T_{reg} cells *in vivo* in a non-invasive manner [69]; however, unlike Feng *et al.* we used an indirect labelling method. Murine T_{reg} lines were transduced retrovirally with a construct expressing the hNIS glycoprotein ion channel gene [69]. We confirmed that NIS-expressing T_{reg} cells maintained their phenotype and suppressive ability following [$^{99m}TcO_4^-$] radiolabel exposure both *in vitro* and *in vivo* [69]. This was an important observation, given that longitudinal studies will require several injections of [$^{99m}TcO_4^-$] into the same recipient. Using SPECT-CT, we observed that adoptively transferred NIS-expressing T_{regs} localized within the lung and spleen of the recipient mouse 24 h after adoptive transfer and [$^{99m}TcO_4^-$] injection in the absence of any graft (Fig. 2). We have now extended our earlier study to image antigen-specific T_{regs} expressing NIS within a murine skin transplant model, and our preliminary data highlight the promising nature of these imaging modalities for assessing the location of transferred cells and graft outcome (unpublished data). Although we have not discussed the use of bioluminescence for T_{regs} tracking, two recent papers have been published which the reader might find interesting [70,71].

Summary and the future for transplantation studies

In conclusion, whole-body imaging techniques such as MRI and SPET/PET-CT have helped to answer important T cell therapy questions, including the localization of antigen-specific T cells following adoptive transfer *in vivo*. As all cell therapy needs to be tested for both efficacy and toxicity, preclinical *in-vivo* studies are important. The finding that, following adoptive transfer into immunodeficient mice, both unmodified and gene-modified human cells migrate to sites of antigen provides validation of these models for preclinical studies of adoptive T cell immunotherapy. This is good news for the transplant field, where clinical trials of human T_{reg} therapy is under way for graft-*versus*-host disease and solid organ transplants [13]. Now that long-term SPECT-CT imaging of modified murine T_{regs} *in vivo* is possible, the next step is to achieve this using human T_{regs}. We have shown recently that adoptive transfer of a human T_{reg} line with direct allospecificity protects against immune-mediated skin allograft injury in a humanized mouse model of xenotransplantation *in vivo* [7]. Extending this study to image human alloantigen-specific T_{regs} using SPECT-CT through the expression of human reporter genes [69], or using nano-sized iron-oxide particles coated with polyethylene glycol and conjugated to fluorescent dyes and MRI [72], in a humanized mouse model of skin/islet/vessel would help to bring T_{reg} therapy one step closer to clinical use. Using these preclinical models, the effective targeting of cellular therapy to the transplant site as well as the location of multiple administered T_{regs} should also be addressed. This is important, as tumour antigen-specific T cells given intravenously did not reach a peritoneal grown tumour while T cells administered via the intraperitoneal route did [65]. Whether regional administration is essential for T_{reg} function within transplant tolerance has yet to be assessed. In addition, whether or not serially administered T_{regs} locate to different transplant locations would also be of interest. Because SPECT-CT imaging allows the possibility of tracking two different cell types simultaneously, future studies looking at the localization of defined T_{reg} subsets or the interaction between T_{regs} and T_{effs} will be possible.

In conclusion, whole-body imaging will help significantly in assessing the efficacy of T_{reg}-based immunotherapy, helping to map the location and accumulation of adoptively transferred cells within the body during the life of the transplanted tissue.

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Disclosure

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