Cell Therapy

Subjects: Oncology

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Cell therapy is a rapidly evolving field involving a wide spectrum of therapeutic cells for personalised medicine in cancer. In vivo imaging and tracking of cells can provide useful information for improving the accuracy, efficacy, and safety of cell therapies.

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1. Introduction

Cell therapy is a rapidly evolving field and an important tool for personalised medicine in cancer. A wide spectrum of therapeutic cells coined as “living drugs” has been developed in recent years for the treatment of cancer, with many undergoing clinical trials, and some now licensed for clinical use. These include tumour-infiltrating lymphocytes (TILs), chimeric antigen receptor (CAR) T-cells, natural killer (NK) cells, dendritic cells, macrophage-based therapies, and drug-loaded neutrophils. CAR T-cells targeting CD19 (Kymriah^{TM} and Yescarta^{TM}) are the first cell-based therapies to be approved by the United States Food and Drug Administration (FDA) for the treatment of acute lymphoblastic leukemia and diffuse large B-cell lymphoma, respectively. More recently, Tecartus^{TM}, a CD20-directed CAR T-cell therapy was granted FDA approval for the treatment of adult patients with relapsed or refractory mantle cell lymphoma [1]. Novel technologies such as CRISPR-Cas9 gene-editing and bispecific CAR T-cell constructs have also been introduced in recent years to improve T-cell targeting and function within the tumour microenvironment [2][3].

As only a few are approved and clinically available, cell therapies largely remain at the research and translational phases, with safety and cost-benefit considerations representing some of the major challenges [4]. To successfully translate and clinically implement cell therapies, a better understanding of in vivo cellular behaviour is required, including biodistribution, tumour trafficking, tissue retention, and clearance. New tools for the optimisation of cell therapies are required to answer key questions, such as cellular localisation and accumulation at the target site, dynamic biodistribution, function, and viability of these cells over time in vivo, as well as the precise dosing, timing, and delivery of the administered cells to desired sites within the body.

In vivo imaging and tracking of cells can provide useful information for improving the accuracy and efficacy of cell therapies. Non-invasive imaging is ideal for the whole-body quantification and longitudinal monitoring of cellular and molecular processes. Cells can be labelled and tracked using a number of imaging modalities such as single-photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), and optical imaging [5]. White blood cell scintigraphy has been used for the imaging of infection and inflammation since the 1980s [6][7]. Autologous leukocytes are routinely labelled with lipophilic agents such as Technetium-99 m hexamethyl propylene amine oxime ([^{99m}Tc]Tc-HMPAO), [^{111}In]In-oxine, and [^{111}In]In-tropolone [8][9]. Magnetic nanoparticles and fluorine-19 perfluorocarbon labelling of cells offers the opportunity to track cells without the use of ionising radiation but is limited by the low sensitivity of MRI and MR spectroscopy (MRS), as well as significant concentration of contrast agents that are required for detection [10][11]. Although cell tracking using optical imaging can provide valuable insights on single cell behaviour and cell-cell interactions at a microscopic level [12], the poor tissue penetrance of light and the limited spatial resolution of these techniques at a whole-body level, has limited the clinical application of optical imaging [13].

Positron emission tomography (PET), as a non-invasive imaging tool, has been successfully applied to tracking the spatio-temporal dynamics of administered therapeutic cells. PET is usually combined with computed tomography (PET/CT) to allow anatomical co-registration and attenuation correction of the detected photons for improved detection. PET/CT is widely available in most large hospitals and by far the most frequently used tracer is the glucose analogue [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), used to probe increased tumour metabolism for image-based treatment response assessment [14][15]. PET offers a very high sensitivity for cell tracking: Only a trace amount of the radiopharmaceutical in the order of picomolar concentrations is needed for detection, pharmacokinetic modelling, and

determining the biodistribution of the administered activity [16][17]. Importantly, the measured PET signal on imaging is highly quantitative as individual counts can be directly related to the actual quantity of label, and simple reproducible metrics such as the standard uptake value (SUV), as well as tracer kinetics, can be used to provide quantitative measures of tracer uptake [18]. Recent development in total-body PET scanner technology to image the entire body has the potential to improve the sensitivity of detection by up to 40-fold for the whole body, and up to 5-fold for a single organ, and therefore offers a promising tool to quantifiably track a very small number of labelled therapeutic cells in vivo [19]. Furthermore, the use of PET to detect and track therapeutic cells has been facilitated by the development of new radiopharmaceuticals which offer a wide range of potential labels for cell labelling.

A number of different approaches can be used to label and image the spatial distribution of therapeutic cells in tissue: Direct labelling of cells ex vivo, indirect labelling of cells in vivo using gene reporters, as well as the detection of specific antigens expressed on the target cells using antibody-based radiopharmaceuticals (immuno-PET).

2. In Vivo Cell Tracking Using PET: Opportunities and Challenges for Clinical Translation in Oncology

Imaging of cell-based cancer immunotherapies including genetically engineered cells has found an important role in basic cancer research and is becoming a valuable tool for the translation of new cell therapies into clinical settings. The ability to follow the biodistribution of these cells in vivo provides important information on whether target engagement has been successful, the intratumoural and intermetastatic heterogeneity of therapeutic cell delivery, and how the cell uptake changes longitudinally. These data could help predict and stratify which patients will respond to therapy as part of a personalised treatment and could also be used to detect early response to therapy before changes in tumour size are apparent. In this way, labelling a small percentage of the injected therapeutic cell population could act as a companion biomarker for the larger proportion of cells used for the treatment. Cell labelling methods have a wide range of applications in addition to their use in oncology, and these approaches could be of great value for labelling stem cells or other cell therapies in neurological and autoimmune diseases, as well as for studying infectious diseases.

Imaging allows longitudinal tracking of therapeutic cells within a patient to be undertaken non-invasively, as well as the detection of tumour heterogeneity, which is more difficult with competing approaches such as tissue biopsy or liquid biomarkers. PET affords a very high sensitivity for the detection of radiolabelled cells, and can report on cell tracking at high spatial and temporal resolution. The spatial resolution of the radiolabel within the tumour is limited by the fundamental PET resolution determined by the mean distance travelled by a positron before annihilation, which varies with positron energy and is isotope-specific, e.g., 0.6 mm for fluorine-18, 1.2 mm for zirconium-89, and 2.9 mm for gallium-68 [20]. In practice, the achievable spatial resolution is lower and usually of the order of several millimetres for most clinical PET applications. Temporal resolution is limited by the number of counts acquired within a given time window to ensure that the signal from the tumour or organ can be discriminated from background or noise. The required temporal resolution for monitoring cell influx and efflux is of the order of hours to days and is therefore not limited by the temporal resolution of the scanner, but rather by the loss of signal due to either the isotope half-life or from label dilution due to cellular proliferation for the direct cell labelling approaches. In practice, this is limited to 7–10 days for long half-live radionuclides but could be extended in the future with the increased sensitivity that will be afforded by total body PET systems [19].

The approaches to cell labelling described in this review provide complementary information: Some assess the resident tumour immune populations, while others report on the trafficking of cells in or out of the tumour. Direct ex vivo cell labelling specifically shows the distribution of the injected labelled population and how it is taken up into the tumour or organ of interest, with little or no background signal to complicate the analysis. A potential complication of all cell labelling approaches, including direct cell labelling, is that some of the labels could be released and may subsequently accumulate in adjacent cellular subpopulations. Antibody or antibody fragment labelling is also highly specific, albeit for a target rather than a cell population, so may label more than one resident cell population and will demonstrate some non-specific background accumulation which may reduce the sensitivity for detection. Antibody labelling also has the benefit of providing functional information in addition to spatial localization and can inform on cellular activation status and cell-cell interactions. Reporter genes are the most attractive approach given their potential for a very high level of cellular specificity and since the target is not diluted with cellular proliferation or tumour metastasis. The choice of reporter gene/target is based on multiple factors: (a) The availability of specific tracers which ideally are suitable for PET and clinically approved; (b) the background expression in tumours or normal organs, which ideally is as low as possible; (c) favourable dosimetry to minimise concerns over radiation exposure, and allow for measurement at multiple time points after administration of the tagged cells; (d) limited or no biological effect deriving from expression of the transgene; (e) alternatively, a transgene may be chosen to serve as a therapeutic effector or target for its application. The potential for

non-human reporter genes to be immunogenic must also be taken into consideration when addressing ideal system design, since this may affect functionality and survival of transduced cells once injected into humans.

Two or more of these approaches could be combined using isotopes with different half-lives to provide a multiparametric readout of both the resident immune cells, as well as influx of cells from the circulation, i.e., a dual-isotope imaging approach. Alternatively, a PET label could be incorporated into an experimental bifunctional probe, using MRI-based approaches, for example, to probe more than one cellular population simultaneously or to provide complementary simultaneous readouts as part of hybrid imaging with PET/MRI.

For clinical translation of PET cell labelling to be more widely used, significant technical and regulatory hurdles need to be overcome. SPECT cell labelling is already part of clinical routine, and therefore good manufacturing practice (GMP) approaches and the required infrastructure required for radiolabelling of cells already exists in many larger institutions. The radiochemistry synthesis involved may have to be upscaled so that it can be stably reproduced for routine large scale clinical use. CAR T-cell manufacturing processes are well established and therefore the addition of PET labelling as a companion biomarker for these therapies which already have obtained regulatory approval, would require a change in practice and new approvals. As the PET label is found in trace quantities, most of the labelling approaches described here do not require additional toxicology assessment when an established PET label is conjugated to human cells. However, if there is a possibility of probe-target interaction resulting in deleterious effects on cell function and viability, specific toxicology studies may be required in some instances.

The introduction of a transgene into live cells for human administration can raise safety concerns and requires extensive regulatory scrutiny and validation before it can be considered for clinical use to ensure long term stability and safety. In addition, preparation of transduced cells for human injection is significantly more complicated and expensive than the requirements for direct cell labelling procedures. Although reporter genes pose significant challenges before being used routinely in a clinical setting, they present many benefits when this can be achieved.

A key element in the translation of these techniques is clinical acceptance and evidence of utility in a clinical setting. The imaging of cell therapies is a relatively new area and a regulatory framework for more routine imaging studies remains to be defined. Most studies to date have involved small numbers of patients from a single institution. Future larger multisite studies are required to provide the evidence for both regulators and clinicians, and it will be important to engage early with the pharmaceutical industry when designing these studies. Repeatability and reproducibility are also key steps in the technical validation stage of these studies. In the longer term, if cell labelling can be shown to better stratify expensive cell therapies, then the imaging costs can be defrayed by reducing the use of ineffective treatments or replacing them with more effective therapies at earlier timepoints. This will provide evidence to deliver a change in clinical practice, and education and training of both the imaging and oncological communities will facilitate this. In conclusion, in vivo cell labelling using PET is a promising area for improving the understanding of tumour biology, as well as addressing important clinical questions in the emerging field of cell therapies.

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