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Laboratory-Clinic Interface

Molecular imaging to enlighten cancer immunotherapies and underlying involved processes

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ABSTRACT

Cancer immunotherapy has led to impressive antitumor effects. However, not all patients respond to immunotherapy, serious toxicity can occur and combination therapy may be warranted. Strategies for rational early treatment choices are urgently required. In the absence of ideal accompanying biomarkers it remains challenging to capture the dynamic, heterogeneous and complex tumor behavior. Tumor immune response involves next to tumor cells, numerous other cells and molecules in the tumor microenvironment. We review research to identify potential novel imaging biomarkers by non-invasive whole body molecular imaging with positron emission tomography and single-photon emission computed tomography for cancer immunotherapy. Firstly, imaging with radiolabeled immune checkpoint targeting molecules. Secondly, imaging of immune cells with *ex vivo* or *in vivo* radiolabeled tracers and thirdly, imaging extracellular matrix components, including adhesion molecules, growth factors and cytokines. These molecular imaging strategies – used alone, in combination or serially – could potentially contribute to patient selection upfront or early during immunotherapy.

Introduction

Cancer immunotherapy is increasingly becoming an important treatment strategy across a broad spectrum of tumor types [1]. Over 2000 different immunotherapeutics are in the development pipeline and several monoclonal antibody (mAb) immune checkpoint inhibitors have already been approved for use in the clinic [2]. Moreover, combinations of these immune checkpoint inhibitors with chemotherapeutic drugs and targeted agents can enhance their antitumor effect, while radiotherapy can also induce immunomodulatory effects [3]. However, not all patients benefit from immunotherapy, serious toxicity can occur and most immunotherapeutic drugs are expensive. Moreover, the rapidly increasing number of immunotherapy combinations, which are currently evaluated in over 3000 ongoing clinical trials, require an unprecedented number of patients and major financial investments.

Therefore, strategies to improve patient selection and accelerate immuno-oncology clinical development are urgently needed [2]. In this respect the development and implementation of biomarkers is critical, but this has been slowed by the complexity and dynamics of the tumor immune response. Next to serum or peripheral blood biomarkers, which would be convenient for clinical use, analyses of tumor tissues have been expanded [4]. There are now two FDA approved biomarkers, the programmed death-ligand 1 (PD-L1) measured with immunohistochemistry (IHC), and microsatellite instability-high and mismatch repair deficient status measurement by IHC and polymerase-chain-reaction (PCR)-based assays. Interest in mutational tumor load as a predictive biomarker is also increasing, with growing evidence that a higher mutational load leads to a higher probability response chance [5,6]. However immune checkpoint inhibitors can sometimes induce responses in tumors without these biomarkers or fail to induce responses despite presence of these biomarkers [7]. A possible explanation is that a single biopsy may not capture the dynamics of the complex immune response and the heterogeneity across various tumor lesions in a patient and even within a single lesion [8,9]. Moreover, the available PD-L1 IHC assays show differences in PD-L1 detection, especially in immune cells [10,11].

Based on the abovementioned biomarkers, prediction of response to immunotherapy is obviously a challenge. Moreover during immunotherapy, routine anatomic tumor response measurement can be difficult due to pseudoprogression. Therefore special immune-related response criteria have been developed and finally, guidelines were defined for response (iRECIST) criteria to be used in trials testing

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immunotherapies [12–14]. These measurements, however, only provide information about tumor size, and therefore do not specify other characteristics of the tumor.

To overcome some of these issues, additional information can be obtained using whole body molecular imaging modalities, positron emission tomography (PET) and single-photon emission computed tomography (SPECT), with specific radiopharmaceuticals to capture a more detailed, dynamic picture of characteristics of all tumor lesions within the body of an individual patient. These techniques can provide non-invasive information about the biodistribution of immunomodulatory drugs in the body, heterogeneity of target expression, effects of immunotherapy on immune cells, and therapy effects on other cells in the tumor microenvironment (TME). Several tracers, earlier studied clinically for infectious inflammatory disease, can be used in oncology to provide information about the TME. Moreover, numerous novel tracers are being developed. We therefore performed a literature search (for search strategy see Appendix A).

This review summarizes current preclinical and clinical research and molecular imaging approaches under clinical development, to support immunotherapy decision making.

Cells involved in tumor immunology and immunotherapeutics

Besides tumor cells, multiple non-malignant cells are recruited to the tumor site where they settle in the TME [15,16]. These include tumor infiltrating lymphocytes (TILs), such as T-cells, B-cells and Natural Killer (NK) cells, as well as macrophages, dendritic cells (DCs) and granulocytes, and their precursors. These cells can create an inflammatory environment that enhances tumor growth [17–19]. Moreover the TME is characterized by the extracellular matrix (ECM), which contains components such as cell adhesion molecules, growth factors and cytokines. The three distinct tumor phenotypes relevant for response to immunotherapy are an inflamed phenotype, genomically unstable with a high presence of TILs, the immune-excluded phenotype with immune cells in the tumor surrounding stroma, and the immune-desert phenotype, genomically stable with fewer TILs, and containing highly proliferating tumor cells [20,21].

Immunotherapeutics target cells and components in the TME to improve the tumor immune response. Tumors can escape the immune response due to a dominance of inhibitory immune signaling pathways: the immune checkpoints. Inhibiting these pathways by mAbs leads to (re)activation of the immune response, enabling immune cells to attack cancer cells. Immune checkpoint inhibitors now have a prominent role in clinical practice, with several mAbs targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein (PD-1)/PD-L1 FDA/EMA approved. To enhance their immune-mediated effector functions advanced modifications are made, including changed amino acid sequence or glycoengineering, potentially leading to induced antibody-dependent cell-mediated cytotoxicity (ADCC) and cell-mediated cytotoxicity (CMC) [22]. In addition, numerous bispecific antibodies (BsAbs) are being developed that recognize two different epitopes, with one arm targeting host effector cells, such as CD3 on T-cells, and the other arm targeting cancer cells [23]. In this way effector cells are directed to cancer cells.

Cancer vaccines, preventive as well as therapeutically administered are another group of immunotherapeutics. Moreover, adoptive cell transfer (ACT) is performed; in this approach the patient's own ex vivo-activated effector immune cells are re-injected. These T-cells can be genetically engineered to produce receptors on their surface, called chimeric antigen receptors (CARs), which recognize specific tumor antigens. Especially in lymphoid malignancies treatment with CAR-T cells has antitumor effects [24]. Another strategy was tested preclinically with *in situ* vaccination. Here immune enhancing agents are injected locally into the tumor, thereby triggering a T-cell immune response [25]. Approved immunotherapeutics for clinical use and their indications are summarized in Table S1.

Molecular imaging and immunotherapy

Molecular imaging techniques, including SPECT and PET imaging, are widely used in the clinic. PET is increasingly performed given its better temporal and spatial resolution and the possibility for absolute quantification. Extensively used radioisotopes for SPECT are technetium-99 m (^{99m}Tc) and indium-111 (^{111}In), with half-lives of 6 h and 2.8 days, respectively, as well as iodine isotopes (^{123}I, ^{125}I, and ^{131}I). For PET imaging, shorter half-life radioisotopes can be used, such as fluor-18 (^{18}F), gallium-68 (^{68}Ga), and carbon-11 (^{11}C) with half-lives of 109.7, 67.7 and 20.3 min, respectively, while zirconium-89 (^{89}Zr) and copper-64 (^{64}Cu) have longer half-lives of 78.4 and 12.7 h.

The various radioisotopes require different labeling methods. Iodines can be labeled directly, whereas for radiometal ions, such as ^{89}Zr, the molecule is first conjugated to a chelator, followed by chelating the metal ion. These radiometals have residualizing properties, meaning that after internalization of the target by the tumor cells, the radioisotope is trapped inside cells, leading to an accumulation of PET signal over time [26]. In contrast, non-residualizing radioisotopes, such as the iodines, are rapidly detached from tumor cells. The most optimal imaging technique and tracer depends on the intended aim for tracer use, for instance on which cells have to be targeted, the properties of the drug targets, and the tumor type and localization.

Molecular imaging could provide a biomarker for immunotherapy (Fig. 1). Firstly, targeting tumor cells, for instance using drug-based tracers such as radiolabeled immune checkpoint inhibitors, might be a tool for evaluating drug biodistribution and target expression. Secondly, as the effect of immunotherapy is driven by activation of immune cells, serial imaging of immune cells might give information on immune cell migration and can detect specific immune cell populations. When used upfront and during immunotherapy, this might be a tool for response prediction. Thirdly, molecular imaging of components in the ECM could increase insight into their role in immunotherapy efficacy.

Targeting tumor cells for molecular imaging

Targeting immune checkpoint proteins for molecular imaging

Immune checkpoint receptors and their ligands are expressed by tumor and immune cells (Fig. S1). SPECT or PET imaging, using radiolabeled mAbs or smaller molecules targeting these immune checkpoints, can provide information on the biodistribution of these molecules and indicate whether they reach the tumor. Imaging studies with radiolabeled mAbs for other tumor targets have shown intra- and interpatient heterogeneity in tumor uptake. Moreover, the drug does not always reach the tumor, even though the target is present. Low tumor uptake was seen in 29% of the patients with ^{89}Zr-trastuzumab (anti-HER2) PET and in 37% of the patients with ^{111}In-labeled anti-human death receptor 5 antibody tigatuzumab SPECT, even though based on IHC the tracer target was considered to be positive [27,28].

Preclinical studies (Table 1) have shown the feasibility of visualizing biodistribution of immune checkpoint inhibitors and immune checkpoint targeting molecules. ^{64}Cu-DOTA-anti-CTLA PET has visualized CTLA-4 positive mouse tumors [29]. In vitro studies showed that T-cells were responsible for the CTLA-4 expression within these tumors. ^{64}Cu-DOTA-ipilimumab tumor uptake was demonstrated in mice xenografted with different human non-small cell lung cancer (NSCLC) cell lines [30]. For PD-L1, radiolabeled and fluorescently labeled PD-L1 targeting antibodies accumulated only in PD-L1-positive tumors. Moreover, high and low PD-L1 tumor expression could be discriminated (Fig. 2A) [31–37]. Modulation of PD-L1 expression was visualized, as interferon-γ treatment radiotherapy and paclitaxel increased uptake while doxorubicin treatment lowered uptake [36–38].

PD-L1 imaging has not only visualized PD-L1-positive tumors, but also normal lymphoid organs in immune competent mouse models. Substantial uptake of PD-L1 targeting mAbs was seen in spleen, thymus, lymph nodes and brown adipose tissue (BAT), which consists of brown