Molecular imaging of immunotherapy biodistribution and the tumor immune environment

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1

General introduction

Background

The recent advent of cancer immunotherapy has been reshaping the field of oncology. Immunotherapies have shown durable complete responses and increased overall survival in patients with various cancer types.[1] Immune checkpoint inhibitors that release the brakes on T-cells by targeting the inhibitory molecules cytotoxic T-lymphocyte-associated 4, programmed death 1 and programmed death ligand 1 are now registered drugs.[2] These immune checkpoint inhibitors harness the body's own immune system against the tumor. Although patients treated with immune checkpoint inhibitors can have durable responses in advanced disease settings, not all patients respond.[3] Whether these non-responders benefit from other immunotherapies, and whether patients can be identified that will respond are key questions to be answered in oncology.[4-6]

A novel form of cancer immunotherapy is the bispecific T-cell engager (BiTE) molecule.[7] BiTE molecules are recombinant proteins of around 50 kDa and have two binding arms, one with affinity for the tumor and one with affinity for a T-cell. Forcing the T-cell to engage to the tumor by the BiTE molecule can induce T-cell mediated tumor cell killing, independent of a preexisting T-cell receptor specificity against the tumor. One BiTE molecule, blinatumomab, is registered for the treatment of patients with B-cell acute lymphoblastic leukemia. Next to BiTE molecules, other T-cell bispecific antibody constructs are being developed.[8] Understanding their biodistribution, especially in a solid tumor setting, will provide insight for their application in the clinic.

Patients with an inflamed tumor immune microenvironment may have a better chance to respond to immunotherapy.[4, 9-11] Inflammation in the tumor can be described by the presence or absence of many players, including T-cells[11], macrophages[12] or cytokines such as interleukin-2 (IL-2).[13] Information of the composition of the tumor including its immune microenvironment can be studied and potentially be used to guide clinical decision. Tumor tissue from a biopsy can be stained for different characteristics with immunohistochemistry. However, a biopsy is invasive and will only provide insight in a small part of the tumor lesion.

Positron emission tomography (PET) imaging is a molecular imaging tool to visualize the location of a positron emitter in the body. Hence, it can reveal the *in vivo* behavior of molecules labeled with a positron emitter and provide tailored information depending on the characteristics of the labeled molecule. Preferably, the molecule is labeled with a positron emitter with a radioactive half-life matching the biological half-life of the molecule. For antibodies, this is Zirconium-89 (^{89}Zr) with a half-life of 3.27 days. PET imaging with labeled molecules can reveal parameters such as the biodistribution of a drug and whether their target is expressed, or the presence of immune cells in the tumor immune microenvironment, depending on the labeled molecule used.

Aim of the thesis

The aim of this thesis is by using molecular imaging, to evaluate the biodistribution of novel immunotherapies, with a focus on bispecific T-cell engagers, and to explore and characterize the tumor immune microenvironment.

Outline of the thesis

In **chapter 2**, the literature is reviewed about bispecific antibodies and bispecific antibody constructs in oncology. Articles published in English until September 5 2018 were searched using PubMed. The search strategy was based on the terms bispecific antibody, T cell engager, immune cell engager, antibody constructs, targeted delivery and variations of these terms. Moreover, the ClinicalTrials.gov database was searched until September 5 2018 for trials evaluating bispecific antibodies. Bispecific antibodies were considered to be approaching the clinic if their clinical trials were not all terminated, withdrawn or completed before 2014 without reporting results. Additionally, bispecific antibodies were also excluded when press releases stated that their development had ceased. The differences between the bispecific antibodies that are approaching the clinic are described, along with their current status in clinical development and the clinical findings with these drugs. Moreover, current hurdles in the clinical development of bispecific antibodies are identified.

For treating solid tumors, no BiTE molecules have been approved yet. To better understand the biodistribution of BiTE molecules targeting solid tumors, we used a murine BiTE molecule targeting T-cells via CD3 and the tumor via epithelial cell adhesion molecule (EpCAM). This murine BiTE, called muS110, allowed us to study the biodistribution and the influence of both targeting arms on its biodistribution in an immunocompetent mouse model. In **chapter 3** we describe the study in which muS110 was labeled with ^{89}Zr to study its biodistribution and immune system uptake by using PET imaging and *ex vivo* biodistribution. The distribution of muS110 was compared to two control ^{89}Zr-labeled BiTE molecules, one targeting only murine CD3 and one human-specific BiTE molecule. This was done in a tumor-bearing mouse model with and without T-cells to further elucidate the influence of each targeting arm on the biodistribution. Autoradiography and immunohistochemistry were performed on tissues of interest to evaluate whether tracer uptake was specific and CD3 or EpCAM mediated.

The small size of BiTE molecules results in fast renal clearance, which complicates achieving steady drug levels in the blood pool. Therefore, the half-life extended BiTE (HLE BiTE) molecule, with an Fc-domain, was developed.[14] The increase in size should prolong their elimination half-life due to the slower hepatic clearance of larger proteins. In **chapter 4**, we describe the study in which we aim to investigate the *in vivo* behavior of the novel HLE BiTE molecules. We used an HLE BiTE targeting murine CD3 and murine mesothelin (MSLN HLE BiTE). To determine the specificity of uptake, MSLN HLE BiTE biodistribution was

compared to a non-specific control HLE BiTE in immunocompetent tumor-bearing mice via PET imaging. To evaluate dose-dependent kinetics, different protein doses of ^{89}Zr-labeled MSLN HLE BiTE were imaged at multiple time points. Organs of interest were the tumor and lymphoid tissues. Moreover, processed tissues were analyzed by autoradiography and immunohistochemistry to further investigate whether observed uptake was specific.

The biodistribution of bispecific antibody constructs, including BiTE molecules, is largely unknown in patients. Therefore, the first-in-human PET imaging study with a BiTE molecule, namely AMG 211, is performed and described in **chapter 5**. AMG 211 targets CD3 and CEA and was labeled with ^{89}Zr. PET imaging with ^{89}Zr-labeled AMG 211 in patients with gastrointestinal adenocarcinomas was performed to reveal the biodistribution in healthy tissues and tumor lesions. Patients underwent PET-scans before and during AMG 211 treatment and at 3, 6 and 24 hours after tracer injection. Standardized uptake values were calculated for healthy tissues and tumor lesions. Blood samples and urine were collected to supplement the imaging data and to evaluate the tracer integrity.

BiTE molecules, as most immunotherapies, rely on T-cells to eradicate the tumor. T-cells can secrete IL-2 upon activation. IL-2 binds to the IL-2 receptor, which is expressed on activated T-cells, among others.[15] Molecular imaging of the expression of the IL-2 receptor might provide insight in immune responses. Therefore, previously a ^{18}F-FB-IL2 IL-2 PET-tracer was developed, but its synthesis is complex and laborious.[16] In **chapter 6**, we describe how IL-2 is labeled through different labeling methods with different positron emitters to develop a facile synthesized IL-2 tracer to monitor immune responses for use in the clinic. First, we compared the synthesis of the three different IL-2 tracers, namely ^{18}F-AlF-RESCA-IL2, ^{68}Ga-Ga-NODAGA-IL2 and ^{18}F-FB-IL2. Next, we evaluated their *in vitro* characteristics. Finally, the pharmacokinetics and uptake of the IL-2 tracers in mouse models with and without human activated peripheral blood mononuclear cells were studied by PET imaging and *ex vivo* biodistribution analyses.

Macrophages play an important role in the tumor immune microenvironment, and efforts are underway to target or repolarize them.[17,18] Colony stimulating factor 1 receptor (CSF1R) is expressed on tumor-promoting macrophages in the tumor immune microenvironment. CSF1R is therefore evaluated as drug target.[19] To facilitate drug development, information about the physiological and tumor uptake of CSF1R targeting antibodies is warranted. In **chapter 7**, the study of the biodistribution of an anti-CSF1R antibody in mice is described. The biodistribution over time and dose-dependent kinetics of the ^{89}Zr-labeled anti-CSF1R antibody were evaluated by PET imaging in a mouse model of spontaneous breast cancer. To see whether the uptake was specific, the biodistribution was compared to an isotype control antibody.

Cathepsins are proteases that are often overexpressed in the tumor immune microenvironment of breast cancer (20). They promote tumor cell invasion and metastasis by degrading extracellular matrix and cleave cell-cell adhesion molecules. In **chapter 8**, we

use a cathepsin-targeted, quenched fluorescent activity-based probe, VGT-309. This probe is only activated when cleaved by cathepsins. Thereby activation outside of the tumor is prevented. When activated, the fluorescent signal can be detected in real-time. In this chapter we describe the evaluation of this probe and whether it potentially can be used to visualize residual tumor tissue during surgery, so-called image-guided surgery. We studied the biodistribution over time of VGT-309 in tumor-bearing immunocompetent mice. Next, we performed image-guided surgery on mice at various time points to assess whether two clinical imaging systems could detect the tumor and evaluate the translational potential of this probe.

In **chapter 9**, a summary of this thesis supplemented with future perspectives is presented. A Dutch summary can be found in **chapter 10**.

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