Molecular imaging on the move
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Chapter 1

General introduction
BACKGROUND

During early cancer drug development, detailed information on drug pharmacokinetics, including normal organ distribution, target expression at baseline and target kinetics over time are of great interest to increase insight in the mechanism of action, and to potentially optimize treatment schedule and patient selection. Furthermore, in the current era of immunotherapy, also information on the immune system and its changes over time is of potential relevance in order to incorporate obtained knowledge for future drug development, as well as for design of combination therapies. However, whole body information of normal organ drug distribution and target expression in humans is usually not available, as current pharmacokinetic and pharmacodynamic analyses are based on blood and/or tumor sampling. Assumptions with regard to pharmacokinetics are mainly based on empirical models, which are a simplified approximations of reality. Moreover, knowledge from preclinical models are of limited value when studying whole body drug effects as animal models do not completely reflect the situation in men. Furthermore, pharmacodynamic analysis in humans can be hampered by the invasiveness of biopsy procedures or by the limited amount of available tumor material as lesions may not be accessible for a biopsy. As a consequence, target expression, as well as potential heterogeneity within and between metastatic sites, at baseline and over time are disregarded.

A well-known therapeutically relevant receptor in breast cancer is the human epidermal growth factor receptor 2 (HER2). Administration of trastuzumab, the anti-HER2 monoclonal antibody, improved overall survival in women with HER2-positive disease at various disease stages. Other members of the HER-family, e.g. HER3, and growth factors such as the transforming growth factor-β (TGF-β) have been evaluated as potential drug target in several tumor types. But the most successful novel approach in cancer treatment is the activation of the immune system by immune checkpoint inhibition, which is less dependent on tumor characteristics such as driver mutations. Molecules of the programmed death-1 (PD-1) receptor/programmed death-ligand 1 (PD-L1) axis promote attenuation of T-cell activation, which subsequently suppresses the immune response and enables the tumor to evade the host’s immune system. PD-1/PD-L1 checkpoint inhibitors, such as nivolumab, pembrolizumab and atezolizumab, overcome this functional unresponsiveness and can induce impressive and durable responses, which led to registration of these drugs across several tumor types. In certain settings, PD-L1 expression is currently being used as biomarker for patient selection. Identifying patients likely to benefit, however, remains challenging, as response is also observed in a substantial number of patients without high tumor PD-L1 expression. A macroscopic, non-invasive molecular imaging readout for an immune checkpoint like PD-L1 might provide new insights by assessing the expression status in normal tissues and in tumor lesions throughout
the whole body, potentially at multiple time points, capturing information about the tumor immune infiltrate and changes over time.

Molecular imaging with positron emission tomography (PET) is a non-invasive technique which can make use of monoclonal antibodies labeled with a radionuclide to assess their biodistribution and target expression at the whole body level. Depending on the characteristics of the molecule, a radionuclide with either a relatively short half-life (e.g. fluorine-18, $^{18}$F, with 110 minutes) or a longer half-life (e.g. zirconium-89, $^{89}$Zr, with 78.4 hours) can be chosen. $^{13,14}$ $^{89}$Zr, thereby, has increasingly been used for labeling of monoclonal antibodies due to its favorable characteristics for PET imaging: i) $^{89}$Zr remains in cells after internalization of the antibody-target complex leading to high tumor image contrast via accumulation, and ii) $^{89}$Zr’s half-life allows target binding over a longer period of time and therefore properly matches the long half-live of monoclonal antibodies. $^{14}$ Until now over 20 therapeutic antibodies have been labeled with $^{89}$Zr and tested in clinical trials to assess biodistribution and target expression. $^{13}$ Performing serial PET scans before and during treatment, furthermore, allows investigation of target accessibility during treatment and may therefore be used to determine whether target saturation has been achieved. Multiple imaging trials in cancer patients have delivered detailed information on target distribution and dynamics, but data is not comparable due to different analysis approaches. With analysis of new targets and comparative analysis of already gathered data, PET could support understanding of working mechanisms, as well as development of future therapies, and improve patient selection.

**AIM OF THIS THESIS**

The aim of this thesis is to investigate the role of molecular imaging with monoclonal antibodies to increase knowledge of whole body pharmacokinetics and pharmacodynamics, and to evaluate the contribution of molecular imaging to therapy decision making and to response prediction.

**OUTLINE OF THE THESIS**

Chapter 2 provides a literature overview of the potential role of molecular imaging in breast cancer. To this end, we performed a search of the current literature on molecular imaging of the two general tumor processes, proliferation and glucose metabolism, of the for breast cancer relevant receptors, the hormone receptors and the growth factor receptors, as well as molecular imaging of the tumor micro-environment. We thereby focused on the ability of molecular imaging to predict and monitor treatment response in this patient population.
Chapter 3 describes the study performed to determine tumor target expression before and during treatment, as well as the normal organ distribution of the anti-HER3 monoclonal antibody lumretuzumab. To this end, lumretuzumab was labeled with $^{89}$Zr and serial PET imaging in patients with HER3-positive solid tumors was performed, at baseline and after the first antibody dose. Patients enrolled in the phase I drug dose finding trial were also eligible for participation in the imaging trial: after administration of 37 MBq $^{89}$Zr-lumretuzumab, initially only at baseline and later also after the first pharmacodynamic-active dose, up to 3 PET scans (2, 4 and 7 days after injection) were performed. Blood samples were collected during the imaging series to determine $^{89}$Zr and lumretuzumab pharmacokinetics. Normal organ distribution and tumor tracer uptake at baseline and after the first dose were evaluated by calculating the standardized uptake value.

In chapter 4 we aimed to investigate $^{89}$Zr-fresolimumab uptake, an antibody against TGF-β, in patients with recurrent high-grade glioma and to evaluate tumor response to fresolimumab treatment. Before fresolimumab treatment, patients received 37 MBq $^{89}$Zr-fresolimumab intravenously and underwent a PET scan of the brain 4 days after tracer injection. A second scan already 2 days after injection was additionally performed in some patients to assess the tumor tracer accumulation over time. Moreover, to assess normal organ distribution of $^{89}$Zr-fresolimumab a whole body PET instead of a brain only scan was performed in part of the enrolled patient population. Tumor tracer uptake was assessed by calculation of the standardized uptake value and treatment response was evaluated by magnetic resonance imaging of the brain. Blood samples were collected to assess fresolimumab and $^{89}$Zr-fresolimumab pharmacokinetics, next to amount of TGF-β1 in plasma. In addition to standard of care immunohistochemistry, p-SMAD2 was analyzed in archival paraffin embedded primary tumor tissue as readout for TGF-β signalling.

In the past, multiple imaging trials with different $^{89}$Zr-labeled antibodies have been performed. Data comparison, however, was hampered by the lack of a harmonization protocol with regard to the performance of the PET scan including the reconstruction method and a standard delineation protocol. In chapter 5, the first comparative biodistribution analysis of four of our $^{89}$Zr-labeled monoclonal antibodies was performed. PET scans of nine patients per tracer were selected when the administered tracer activity was 37 MBq ($\pm$ 10%), the PET scan was performed 4 days after tracer injection together with a low-dose computed tomography (CT) and the tracer was complemented with the previously determined optimal unlabeled imaging protein dose. The scans were reconstructed based on the recently published $^{89}$Zr-harmonization protocol and analyzed according to our standardized delineation protocol for $^{89}$Zr-tracers using the software A Medical Imaging Data Examiner (AMIDE version 0.9.1; Stanford University). Normal tissue distribution of all four tracers, calculated as percentage injected dose per kilogram tissue normalized to the calibrated dose of the $^{89}$Zr-tracer and corrected for decay at the time of scanning, was compared and influence of tumor load, body weight and fat percentage were assessed.
The clinical trial, which is described in chapter 6, aimed to study the uptake of the $^{89}$Zr-labeled PD-L1 antibody atezolizumab in primary and metastatic tumor lesions and normal organ drug distribution in patients with non-small cell lung cancer, triple negative breast cancer or bladder cancer prior to treatment with atezolizumab. At baseline, eligible patients received $^{89}$Zr-atezolizumab including 10 mg unlabeled antibody followed by up to four PET scans 1 hour, 2, 4 and 7 days after tracer injection. During the PET imaging series, blood samples were collected for determination of tracer amount in the peripheral blood, peripheral blood mononuclear cell fraction and atezolizumab serum concentration. After the last PET scan a tumor biopsy was obtained for immunohistochemistry and RNA sequencing, and patients received atezolizumab monotherapy until disease progression. Response to treatment was monitored every 6 weeks by a diagnostic CT scan. PET image analysis was performed with the Accurate tool for volume-of-interest-based lesion and background analysis, and correlated to PD-L1 immunohistochemistry and RNA expression data from the tumor biopsies, as well as treatment response. Additionally, we studied PD-L1 and CD8 immunohistochemistry in normal lymph node and spleen tissue, and internalization of $^{89}$Zr-atezolizumab in vitro in the human lung mucoepidermoid pulmonary H292 and the bronchioalveolar H358 tumor cell line, as well as in peripheral blood mononuclear cells pooled from healthy volunteers.

In metastatic breast cancer management, up-to-date information of HER2 status is essential, due to variable expression during the course of the disease. This information, however, cannot always be obtained as lesions might not be accessible, due to patient- or tumor related factors, resulting in a dilemma with regard to treatment decisions. In the trial described in chapter 7 we aimed to assess the clinical value of a $^{89}$Zr-trastuzumab PET in patients with metastatic breast cancer and a known history of HER2-positive disease, in whom standard work-up, including a bone scan, a $^{18}$F-fluorodeoxyglucose PET, a CT and if feasible a biopsy, failed to clarify HER2 status of their disease. We performed a $^{89}$Zr-trastuzumab PET scan in 20 patients presenting with such a clinical dilemma, next to central pathology revision of archival tumor material, and assessed HER2 status of circulating tumor cells. The referring clinicians completed three questionnaires to rate the clinical value of the additional PET scan in terms of diagnostic understanding and treatment decision. HER2 status of circulating tumor cells was correlated to treatment decision and $^{89}$Zr-trastuzumab PET result.

Finally, results of this thesis are summarized and future perspectives are given in chapter 8. The summaries in Dutch and German are provided in chapters 9 and 10.
REFERENCES


