Studies to optimize selection for immunomodulatory cancer therapy with focus on immunoPET

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Chapter 1

General introduction

BACKGROUND

Immune evasion is one of the hallmarks of cancer. The development of antibodies targeting immune checkpoints has resulted in major advances in the systemic treatment of patients with cancer. The use of immune checkpoint inhibitors has resulted in impressive tumor responses and improved overall survival across several tumor types. However, only a subset of patients benefits from immunotherapy. Therefore, there is a need for optimization of patient selection and development of novel immunotherapeutic treatment strategies.

Biomarkers that can predict which patients have an increased chance of responding to immunotherapy are scarce. This may, in part, be caused by heterogeneity in drug target expression in tumor lesions between patients and even within patients and dynamic target expression over time. Current tumor tissue-based analyses only provide a snapshot of the immune tumor microenvironment at the time and place of a tumor biopsy. The biopsy does not reflect the whole body target expression. Moreover, immune checkpoint inhibitors target the *interaction* between immune checkpoints on tumor cells and immune cells. Thus, whole-body target expression, including target expression in both tumor and lymphoid tissues, may play a crucial role in understanding the treatment efficacy of these drugs. It is largely unknown whether drugs reach their target, whether drugs accumulate in the tumor microenvironment or in lymphoid tissue and whether target saturation can be reached.

ImmunoPET imaging comprises a non-invasive technique in which an antibody or antibody-related molecule is chelated to a radionuclide and visualized using positron emission tomography (PET). An arsenal of radionuclides with different half-lives is available, allowing matching to the serum half-life of the antibody or antibody-related molecule. The most commonly used radionuclide for antibody-based imaging is 89-Zirkonium (^{89}Zr). Its physical half-life of 78.4 h matches the serum half-life of most antibodies. Combined with PET imaging, which has a high spatial resolution, a quantitative assessment of

the radiolabeled antibody in healthy tissue and the tumor can be performed. ImmunoPET imaging using ^{89}Zr-labeled antibodies is, therefore, increasingly utilized in studies with immune checkpoint inhibitors. This tool is a potential biomarker for the treatment effect and might enhance our knowledge on *in vivo* drug behavior of existing and novel immunotherapeutic drugs.

AIM OF THIS THESIS

This thesis focuses on imaging with PET and radiolabeled immune checkpoint inhibitors to increase the knowledge about these medicines, their whole body distribution, drug target expression, and factors determining patient response.

OUTLINE OF THE THESIS

In the theranostic approach, therapeutics are radiolabeled to inform treatment strategies. In **chapter 2,** an overview is given of previous and ongoing research into monoclonal antibodies and antibody-related medicines in the preclinical and clinical oncological settings. Available studies investigating such approaches with monoclonal antibodies and antibody-related medicines were identified. A PubMed search was performed using the search terms 'PET' AND 'Cancer' AND 'Antibody' OR 'ADC' OR 'Bispecific' in combination with the most commonly used PET radionuclides 64-copper (^{64}Cu), 68-gallium (^{68}Ga), 86-yttrium (^{86}Y), 89-zirconium (^{89}Zr) and 124-iodine (^{124}I). Also, by searching ClinicialTrials.gov, ongoing studies over the past 10 years with theranostic potential, captured with the search terms 'Cancer' AND 'PET' NOT 'FDG', were included in this review. All manuscripts and ongoing studies were manually screened for relevance.

The review in **chapter 3** focuses on current applications of molecular imaging in the drug development phase of small molecules, antibodies, and anti-hormonal anticancer drugs. English literature was searched in PubMed, the Dutch trial registry, the EudraCT, and ClinicalTrials.gov databases. The abstracts of annual meetings from 2015 until 2018

of the American Society of Clinical Oncology, American Association of Cancer Research, European Society of Medical Oncology, and San Antonio Breast Cancer Symposium were additionally screened. The search focused on molecular imaging in the context of target expression, pharmacokinetics, and pharmacodynamics in cancer. Reference lists of articles and citing articles were manually studied for relevance.

A newly developed immunotherapeutic strategy uses bispecific antibodies, including bispecific T-cell engager (BiTE) antibody constructs. A BiTE is a recombinant bispecific antibody composed of two linked scFvs from two different antibodies. One targets a cell-surface protein on T cells (for example, CD3ε) and the other targeting an antigen on the tumor cell, linked by a short flexible linker. By binding to the tumor antigen and T cell simultaneously, a BiTE can potentially mediate a T-cell response to kill tumor cells. BiTEs are increasingly being investigated in a variety of tumor types. Since BiTe antibody constructs potentially have a different binding affinity for the target of each arm, knowledge about drug biodistribution might support drug dosing schedules, predict toxicity, and aid drug trial design. Therefore, as described in **chapter 4**, we performed a two-center first-in-human study in patients with advanced gastrointestinal adenocarcinomas. The aim was to explore the biodistribution of AMG 211 (also known as MEDI-565). This is a BiTE directed against carcinoembryonic antigen (CEA) on tumor cells and cluster of differentiation 3 (CD3) on T-cells. The patients received 37 mBq ^{89}Zr-AMG211 with varying unlabeled AMG 211 doses until optimal imaging results were achieved. PET scans were initially performed 6, 24, and 48 hours after tracer injection. This was changed into earlier time points, namely 3, 6, and 24 hours from the second patient onwards, after the review of the data from the first patient showed rapid ^{89}Zr-AMG 211 clearance from the circulation. Patients received ^{89}Zr-AMG211 followed by PET imaging prior to AMG211 treatment and/or immediately after the end of the second AMG 211 treatment period of 28 days within the phase 1 study. In this phase 1 study 6,400-µg/day or 12,800-µg/day AMG211 was given. ^{89}Zr-AMG 211 PET analysis was quantified by drawing spherical volumes

of interest (VOI) in healthy tissues and tumor lesions, of which standardized uptake values (SUV) were calculated. A comparison was made between patients, different unlabeled AMG 211 doses, and the moment of PET scanning.

Immune checkpoint inhibitors targeting the programmed cell death protein-1/ligand-1 (PD-1/PD-L1) axis are of great interest since long-term disease control can be achieved in subsets of patients across several tumor types. However, currently approved predictive biomarkers are far from perfect. Atezolizumab is a PD-L1 antibody currently EMA-approved for urothelial cancer, non-small cell lung cancer, triple-negative breast cancer, and hepatocellular carcinoma. We aimed to gain insight into whole-body biodistribution and PD-L1 target expression, tumor tracer uptake. In addition, the PD-L1 expression measured immunohistochemically assessed in a single tumor biopsy was correlated with lesion response to atezolizumab. Therefore, in **chapter 5,** we performed PET imaging with the ^{89}Zr labeled atezolizumab in patients with bladder cancer, non-small cell lung cancer, or triple-negative breast cancer before atezolizumab treatment. In this first-in-human study, patients received 37 mBq ^{89}Zr-atezolizumab plus 10 mg unlabeled atezolizumab followed by PET imaging 1 hour and days 2, 4, and 7 post tracer injection. A tumor biopsy was obtained after the last PET scan, and was assessed immunohistochemically for tumor cells. to relate to tumor SUVmax in an exploratory manner. Patients received atezolizumab treatment every 3 weeks until (i)RECIST disease progression. Treatment response was assessed every 6 weeks with contrast-enhanced CT. ^{89}Zr-atezolizumab PET analysis was quantified by drawing a VOI in healthy tissues and tumor lesions, of which SUVs were calculated. Lymphoid tissues and sites of inflammation were assessed visually. Lesion specific ^{89}Zr-atezolizumab tracer uptake was compared to the treatment response of these lesions and treatment response to atezolizumab per (i)RECIST.

To which degree therapeutic levels of atezolizumab saturate PD-L1 in healthy tissues and the tumor is unknown. Gaining insight into the

level of target engagement is relevant because no clear dose-response relationship for atezolizumab has been found, and the maximum tolerated dose was not reached in drug development studies. Moreover, understanding whether the target of immune checkpoint inhibitors can be saturated enables discussion of alternative dosing regimens for these expensive drugs, such as 'minimal required active drug dose.Therefore, in **chapter 6,** we aimed to assess on-treatment atezolizumab biodistribution and degree of PD-L1 target saturation by performing imaging with ^{89}Zr-atezolizumab during atezolizumab treatment. In this chapter, a preliminary report of the ongoing study is provided. Patients with a tumor type likely to benefit from treatment with an immune checkpoint inhibitor were eligible. In the first patient cohort, 37 mBq ^{89}Zr-atezolizumab was administered together with the cycle 1 therapeutic dose of atezolizumab, followed by PET scans 4 and 7 days post tracer injection. If ^{89}Zr-atezolizumab uptake in the tumor was still present, a second patient cohort would be opened. In this second patient cohort, ^{89}Zr-atezolizumab was administered with cycles 1 and 2, followed again by PET scans on days 4 and 7 post tracer injection. A tumor biopsy was obtained after the PET scan on day 7 if feasible, which was assessed immunohistochemically for PD-L1 expression on tumor cells and immune cells. Patients received atezolizumab treatment every 3 weeks until (i)RECIST disease progression. Treatment response was determined every 6 weeks with contrast-enhanced CT. ^{89}Zr-atezolizuab PET analysis was quantified by drawing a VOI in healthy tissues and tumor lesions, of which SUVs were calculated. ^{89}Zr-atezolizumab uptake was expressed as geometric mean SUVmax for tumors and SUVmean for normal tissues estimated using linear mixed effect models, averaging days 4 and 7. Both cycles were compared, taking within-patient clustering into account. The median ^{89}Zr-atezolizumab tracer uptake per patient was compared to treatment response to atezolizumab. Lymphoid tissues and sites of inflammation were visually assessed.

Pembrolizumab is a PD-1 antibody that is EMA-approved for several tumor types, including melanoma and non-small cell lung cancer. Increasing knowledge of whole body PD-1 expression and migration

of PD-1 expressing immune cells to the tumor might optimize patient selection. Therefore, in **chapter 7**, we performed a first-in-human study with ^{89}Zr-pembrolizumab in locally advanced or metastatic melanoma and non-small cell lung cancer before treatment with pembrolizumab. Patients received 37 mBq ^{89}Zr-pembrolizumab plus unlabeled pembrolizumab before their first treatment cycle per standard care, followed by up to 3 PET scans days 2, 4 and 7 post tracer injection. In part A, optimal unlabeled pembrolizumab dose and timing of PET/CT imaging were assessed and implemented in part B. After the last PET scan, a tumor biopsy was obtained if feasible. Tumor biopsies were stained for PD-1, PD-L1, and CD8. ^{89}Zr-pembrolizumab PET analysis was quantified by drawing a VOI in healthy tissues and tumor lesions, of which SUVs were calculated. ^{89}Zr-pembrolizumab uptake was expressed as geometric mean SUVmax for tumors and SUVmean for healthy tissues. Lymphoid tissues and sites of inflammation were visually assessed. Treatment response was determined according to RECIST v1.1.

Increasingly, combinations of immune checkpoint inhibitors with other medicines, including chemotherapy, are being explored. Patients with head and neck cancer can benefit from immune checkpoint inhibitors, and these are often given post-chemotherapy. To facilitate future studies, determined the preferred chemotherapy regimen is important. In **chapter 8** we describe the comparison of carboplatin with 5-fluorouracil versus cisplatin as concomitant chemoradiotherapy for locally advanced head and neck squamous cell cancer. We performed a retrospective cohort analysis of patients with locally advanced head and neck squamous cell cancer treated with concomitant chemoradiotherapy in two Dutch cancer centers between 2007 and 2016. One center routinely administered carboplatin 300-350 mg/m^2 at days 1, 22, and 43, followed by 5-fluorouracil 600 mg/m^2/day for 96 hours. In the other center, cisplatin 100 mg/m^2 on days 1, 22, and 43 was given. The primary endpoint was chemotherapy completion rate, and secondary endpoints were overall survival, disease-free survival, locoregional control, and distant free interval. Associations between clinicopathological parameters and overall

survival were determined with univariate and multivariate Cox regression analyses.

Finally, a summary of the obtained results of this thesis and future perspectives are provided in **chapter 9.**

Chapter 10 contains the thesis summary in Dutch.