Chapter 8

Summary and future perspective
SUMMARY

Over the last decades, drug development has improved survival in multiple cancer types. Especially with the arrival of agents targeting the immune system like immune checkpoint inhibitors, a new era of cancer treatment has begun in which even in case of metastatic disease complete responses over several years have been observed. Despite all achievements in the recent years, however, cancer remains a leading cause of death worldwide. Multiple cancer types do not or not sufficiently respond to currently available treatment. Non-response, thereby, is a multilayer problem including inherent resistance and tumor heterogeneity, as well as development of resistance originating from either the tumor cells themselves or the microenvironment during the course of disease. Furthermore, multiple drugs and/or combinations cannot be used broadly in the clinic despite potential potency due to major side effects. Obtaining detailed information on drug pharmacokinetics and pharmacodynamics already early in drug development, providing information on target heterogeneity and early response prediction in a patient friendly way, are major challenges in clinical research. One possible technique that could address these challenges might be molecular positron emission tomography (PET) imaging with radioactively labeled antibodies.

The aim of the research described in this thesis was to investigate the role of molecular imaging with different monoclonal antibodies in increasing knowledge of whole body pharmacokinetics and pharmacodynamics. Furthermore, we evaluated the contribution of molecular imaging to therapy decision making and to response prediction.

Chapter 1 provides a general introduction of the topic and outlines the thesis. In chapter 2, we reviewed the literature on the potential role of molecular PET imaging in breast cancer as response evaluation of bone metastases, the most common site of metastases in this cancer type, is hampered since these lesions are not measurable according to the generally applied Response Evaluation Criteria in Solid Tumours (RECIST). Molecular imaging, in contrast, is not dependent on measurement of anatomical size changes, but can be used to assess status and changes of general tumor processes, such as glucose metabolism and DNA synthesis, and also more specific targets, like hormone receptors, growth factor receptors and targets in the tumor microenvironment before and during therapy. In breast cancer, especially $^{89}$Zr-trastuzumab PET, as measure for the human epidermal growth factor receptor 2 (HER2) and $^{18}$F-fluoroestradiol ($^{18}$F-FES) PET for evaluation of the estrogen receptor status seem to have the potential to aid treatment selection for the individual patient. Furthermore, serial imaging of general tumor processes with tracers such as $^{18}$F-fluorodeoxyglucose (FDG) may provide early prediction of anti-tumor efficacy. Future trials will need to specify uptake characteristics for different breast cancer subtypes, as well as for different chemotherapy and/or targeted therapy regiments, the optimal moment of scanning, the quantification method and validation of PET with conventional imaging.
Next to the HER2 protein, which is firmly established as important drugable target in breast cancer, the more recently identified HER3 protein is a potential target in multiple cancer types. In chapter 3 we describe biodistribution and tumor uptake by serial imaging with the $^{89}$Zr-labeled therapeutic anti-HER3 antibody lumretuzumab before and during treatment in patients with advanced or metastatic HER3-positive solid tumors. Optimal PET conditions were found to be 4 and 7 days after administration of $^{89}$Zr-lumretuzumab with 100 mg unlabeled lumretuzumab. The highest tracer uptake was seen in the liver (mean standardized uptake value (SUVmean) 6.4 ± 1.1 on day 4 after injection), followed by the circulation, the kidneys, spleen and intestine. Tracer uptake was much lower in brain, muscle, bone, abdominal cavity and lung (SUVmean of 0.2 to 0.9). Tumor SUVmax 4 days after tracer injection was 3.4 (± 1.9), ranging from 0.5 up to 8.9 with an up to 6-fold difference in mean tumor tracer uptake between patients. About 33% of tumor lesions with a diameter ≥ 10 mm were $^{89}$Zr-lumretuzumab PET-negative. Saturation analysis assessed in seven patients showed that 4 days after lumretuzumab administration, tumor uptake decreased by 11.9% (± 8.2), 10.0% (± 16.5) and 24.6% (± 20.9) at pharmacodynamic-active doses of 400, 800 and 1600 mg, respectively, when compared to baseline. Membranous HER3 was completely downregulated in paired skin biopsies already at and above 400 mg lumretuzumab. In contrast to the phase I pharmacodynamic data, there was no clear evidence of tumor saturation by PET imaging as tumor uptake did not plateau with increasing doses. The latter might be explained by multiple factors: the signal visualized with PET is 1) a combination of membrane-bound activity and the intracellular fraction as the tracer is being internalized, 2) a function of time as after internalization the relatively long-living radionuclide $^{89}$Zr remains in tumor cells and PET images were performed serially over several days in contrast to a biopsy which reflects a single moment in time and 3) also influenced by factors such as enhanced permeability and retention effects in tumor lesions, and potentially unspecific tracer uptake e.g. due to the effect of Fc gamma receptor engagement within the tumor environment.

In chapter 4, we used $^{89}$Zr-fresolimumab to visualize the transforming growth factor-β in recurrent high-grade glioma before treatment with the monoclonal antibody fresolimumab. On day 4 after tracer injection, normal brain tissue tracer uptake was low with an SUVmean of 0.3, ranging from 0.2 to 0.5, and mean tumor tracer uptake calculated as SUVmax was 4.6, ranging from 1.5 up to 13.9. Treatment with fresolimumab was generally well tolerated without infusion-related reactions and mainly low grade adverse events. Despite the good penetration capacity of the monoclonal antibody into the recurrent high-grade glioma lesions as visualized by PET imaging, no clinical benefit of single agent fresolimumab treatment was observed in this small and often extensively pretreated patient group in which only one dose of fresolimumab was tested.

Until now, imaging trials mainly focused on biodistribution analysis of single agents, disregarding comparison across different antibodies, which, however, might be of great value during drug development. Therefore, in chapter 5, we performed a comparative biodistribution
analysis of four $^{89}$Zr-labeled monoclonal antibodies previously explored in clinical studies based on the $^{89}$Zr-harmonization protocol and conducted according to our standardized delineation protocol for $^{89}$Zr-tracers. $^{89}$Zr-lumretuzumab (anti-HER3), $^{89}$Zr-MMOT0530A (anti-mesothelin), $^{89}$Zr-bevacizumab (anti-vascular endothelial growth factor) and $^{89}$Zr-trastuzumab (anti-HER2) showed a similar distribution pattern in healthy tissue. On day 4 after tracer injection, about one-third of the injected tracer dose was found in the circulation, up to 15% in the liver and only 4% in the spleen and kidney. Lower tracer concentrations were seen in bone marrow, lung, compact bone, muscle, fat and the brain. Despite low tracer accumulation per gram of tissue, large-volume tissues, especially fat, can influence overall distribution: On average, 5-7% of the injected tracer dose accumulated in fat, with a peak of 19% in a patient with morbid obesity. The similar biodistribution of the four antibodies is probably based on the similar molecular structure, binding characteristics and metabolic pathways. With this first comparative analysis we aimed to create a basis for a prospectively growing imaging data warehouse of antibody-based tracers. In the future, this warehouse will need to grow to increase its impact: especially with addition of tracers imaging the immune system, new molecules belonging to another IgG subclass and with different molecule size or structure such as bispecific antibodies.

In chapter 6, we describe the results from first-in-human imaging with $^{89}$Zr-labeled atezolizumab, an anti-programmed death-ligand 1 (PD-L1) antibody, and correlated uptake data with immunohistochemistry and RNA results from the tumor tissue samples and with treatment response. With 10 mg unlabeled antibody added, blood pool, and liver and kidney $^{89}$Zr-atezolizumab uptake on day 4 were comparable to day 4 results of earlier described $^{89}$Zr-antibody tracers. In contrast to earlier studied monoclonal antibody based tracers, we observed high and variable $^{89}$Zr-atezolizumab uptake in the spleen, non-malignant lymph nodes and sites of inflammation, which corresponds with local PD-L1 expression by CD8-positive T-cells and antigen presenting cells such as dendritic cells and macrophages. Tumor uptake was generally high, with a SUVmax up to 46, but heterogeneous within and between patients and tumor types. In our exploratory study, which did not include a large patient population and no immunohistochemically PD-L1 highly positive tumor biopsies, we observed that higher tracer uptake prior to atezolizumab treatment was related to better response, progression free and overall survival; more so than immunohistochemistry and RNA sequencing. Further development of this imaging biomarker for PD-L1 status, including expansion of the study population needs to take place to confirm these first results.

In chapter 7, we describe results of a trial in metastatic breast cancer patients presenting with a clinical dilemma defined as failure of the standard work-up to evaluate the present HER2 status. In this specific patient population we assessed the contribution of $^{89}$Zr-trastuzumab PET to disease understanding and clinical decision making. In 90% of the cases the physicians' disease understanding was increased by $^{89}$Zr-trastuzumab PET, in 50% of the patients the physicians'
confidence for the pre-planned treatment choice was increased without affecting the choice, and in 40% of the patients treatment was altered based on the $^{89}$Zr-trastuzumab PET result. As central pathology revision with renewed HER2 staining delivered new insights in three of 20 patients, implementation of this step in the standard setting might be worth considering. HER2 status of circulating tumor cells, detected in 50% of the study population, was not correlated to $^{89}$Zr-trastuzumab PET result or treatment decision.

**DISCUSSION AND FUTURE PERSPECTIVES**

**Assessment of clinical utility of molecular imaging**

To be able to implement molecular imaging in clinical practice, amongst others information on the predictive value of the tracer in comparison and/or in addition to the standard work-up, evaluation of efficacy and cost-effectiveness is needed. For molecular imaging in breast cancer, this information is becoming more and more available. In addition to already published molecular imaging trials in this cancer type, in **chapters 2 and 7** of this thesis we describe the potential role of molecular PET imaging in general, and the role of HER2 imaging in clinical decision making in a specific patient population presenting with a clinical dilemma with regard to their disease's HER2 status. Clinical utility is currently further assessed in the Dutch, prospective, multicenter IMPACT-metastatic breast cancer trial (ClinicalTrials.gov identifier NCT01957332) in which next to $^{18}$F-FDG, also estrogen receptor and HER2 imaging is investigated in a larger population of newly diagnosed metastatic breast cancer patients. In the future, preliminary findings of other targets evaluated in first-in-human imaging trials and (additional) information on clinical utility also in a variety of tumor types is necessary. Furthermore, molecular imaging findings will need to be (further) cross validated, on the one hand with current golden standard tumor biopsy based techniques such as immunohistochemistry, fluorescent in situ hybridization, in combination with RNA and/or DNA sequencing. On the other hand, molecular imaging should be compared with molecular techniques which also have the potential to capture whole body target status, such as circulating tumor cells or circulating tumor DNA. Finally, the use of molecular imaging as biomarker should be confirmed in larger patient populations, ideally as part of randomized controlled trials, which can only be realized when reliable automated delineation algorithms become available.

**Increase knowledge of target biodistribution by means of comparative analysis**

In **chapters 3-6** we describe the biodistribution of newly developed tracers and results of the first comparative biodistribution analysis of four $^{89}$Zr-labeled monoclonal antibody tracers including establishment of a basis for a molecular imaging warehouse. Upscaling of imaging
trials in general is hampered by amongst others cost factors and radiation burden. In this light, collection of data in a warehouse, similar to meta-analyses in clinical intervention trials, could contribute to more firm evidence to support utility of molecular imaging in clinical practice. Also, knowledge of drug distribution, and establishment and fine tuning of standardized protocols could be increasingly being facilitated this way. An example of the usefulness of data sharing is provided by the RECIST criteria, which have been verified with a warehouse containing imaging and outcome data from numerous trials. As the scientific community in the recent years is more and more recognizing the strength of data sharing, this might be a hopeful approach also for molecular imaging. The impact of the findings based on the established molecular imaging warehouse can increase in the coming years, especially with expansion of the warehouse by adding tracers visualizing the immune system and new drug constructs with unknown biodistribution such as bispecific antibodies.

Imaging of the immune system

Imaging of the checkpoint inhibitor atezolizumab and its favorable predictive capacity is described in chapter 6. In the extension phase of this imaging trial (ClinicalTrial.gov identifier NCT02453984) patients are imaged during atezolizumab treatment to assess influence of the treatment dose on healthy tissue and tumor tracer uptake. In the coming years, molecular imaging will further be used as a tool to assess the immune system and its various components. Already now, several trials are recruiting to evaluate uptake characteristics of other checkpoints and/or their inhibitors (\(^{89}\)Zr-CX-072 and \(^{18}\)F-BMS-986192 both targeting PD-L1; \(^{89}\)Zr-pembrolizumab and \(^{89}\)Zr-nivolumab both targeting PD-1). Also first tracers to visualize activated T-cells (e.g. \(^{99m}\)Tc-IL2 or \(^{18}\)F-IL2) or specific subgroups of T-cells (e.g. CD8 imaging) are in development; And for other immune cells like B-cells, macrophages and Natural Killer cells, and even components of the extracellular matrix, tracers are becoming available, too. In the evolving field of immunotherapy with new targets arising (e.g. OX40, LAG 3, TIM 3, GITR, CD137) and an increasing number of combination regimens for several cancer types, knowledge of dynamics of the players of the immune system and their interaction with the microenvironment, especially over time and in response to the various treatment options, will be a major issue. Not only combinations with other immune cell activating or checkpoint targeting drugs will be a main research focus, but also combination strategies thought to induce expression of a certain immune response related target, to act synergistically with immunotherapy or to support immune cell function, will further be assessed. Candidate targets of interest, thereby, are for example the transforming growth factor-β, which can be produced by aggressive tumors in large amounts leading to local immune suppression via regulatory T cells and the vascular endothelial growth factor, which – when overproduced – leads to fast development
of leaky vessels in tumors possibly hampering immune cell trafficking to the tumor. Both, when blocked, could aid the mechanism of action of immunotherapies. Also, combination with certain chemotherapies or radiotherapy could support immunotherapy when administered concurrently or sequentially, as they increase presentation of tumor-associated antigens on antigen-presenting cells and stimulate T cell diversification. Molecular imaging by PET might play a role in this development with its capacity to obtain whole body information over time in a non-invasive manner. Thereby, next to understanding mechanisms of action, the ultimate research goal will remain on patient selection to further improve overall survival. Additionally, fluorescent molecular imaging might add to this by delivering more detailed information on the microscopic level. And finally, as the latter technique is not using radioactive probes but fluorescence, it could be used more repeatedly and not only in the field of oncology but also in immune mediated inflammatory diseases.

Assessment of heterogeneity and its clinical relevance

As visualized in all imaging trials and described in performed molecular analyses within and outside of this thesis, target heterogeneity within tumor lesions and also over time has been recognized as important phenomenon in oncology. Heterogeneity exists across tumor types and subtypes; there is heterogeneity of tumor cell characteristics, as well as in the tumor (immune) microenvironment. Thereby, heterogeneity can be found at all levels of the cell machinery, e.g. at the protein level, the RNA or DNA level, and it can also be measured in the blood pool as circulating tumor cells or circulating cell free tumor DNA. In the coming years, we will continue describing this heterogeneity in various tumor types and changes throughout treatment by means of tissue-based molecular analyses methods including computational pathology, blood-based methods and non-invasive (molecular) imaging. We will, furthermore, need to continue adding clinical outcome data to assess which markers are relevant for clinical decision making and prognosis. Thereby, it will be of interest to assess whether single characteristics serve satisfactorily as biomarkers or whether – and if so, which clusters of characteristics are most informative, next to the question of tumor specificity. Depending on the prevalence of the targetable characteristic(s) per tumor type, it will not be possible to gather necessary clinical data in large randomized controlled trials. Smart basket trial designs will increasingly be necessary to define the clinical value of targeted therapies in various tumor types. Finally, to deal with all the data which can be obtained per patient and/or tumor (site) in combination with the already obtained knowledge about clinical outcome, we will need to develop software tools to help interpreting, combining and updating these data and support clinical decision making.

In conclusion, in this thesis we describe the role of molecular imaging to obtain deeper knowledge of pharmacokinetics and -dynamics of several monoclonal antibodies that are
in clinical development or already approved drugs for patient use. Furthermore, we show in modest size studies that molecular imaging may aid decision making and response prediction in specific patient populations, which, in the future might support patient selection to improve treatment outcome and survival.