CHAPTER 1

General introduction

Background

Cancer is a major cause of death and its worldwide annual mortality rates are predicted to reach 11.5 million in 2030.[1] This indicates the urgent need for better drugs. With the recent advances in molecular and cellular biology, many molecules and key pathways involved in all hallmarks of cancer were identified.[2] This knowledge has shifted the anticancer drug development from DNA-damaging chemotherapy towards more personalized molecularly targeted drugs, which are increasingly part of standard care. Monoclonal antibodies (mAbs) are a relevant and rapidly expanding category of these specific molecular targeted agents as they are highly specific for one antigen and usually exhibit desirable safety profiles.[3–5] Also antibody-drug conjugates (ADC) have been developed, combining the antigen specificity of mAbs with the cell-killing capability of cytotoxins.[6–8] More than 50 mAbs are currently in advanced clinical development, including several ADCs.

Clinical drug development is a relatively slow and expensive process, limiting the number of drugs that can be advanced into late-stage trials. Moreover, for drugs in early development knowledge about whole-body target expression, drug biodistribution and organ pharmacokinetics is crucial, however often lacking in many phase I study designs. Also determination of a proper dose is challenging due to variation in kinetics between patients and lacking information about target saturation. Also, for all targeted drugs in oncology it is a challenge to predict which patients will benefit. To enrich the patient population, not only in patient care but also in early clinical trials, reliable predictive biomarkers are needed to assess tumor selective expression of the molecular target.[9] Currently immunohistochemistry (IHC) or quantitative polymerase chain reaction (qPCR) are performed on serum and/or tumor samples.[10] Limitations of this traditional biomarker analysis include invasiveness, procedural risks and accessibility of primary tumor and metastatic sites. Also tumor biopsies provide only static information in a small part of the tumor, while tumor heterogeneity is increasingly acknowledged to be present and to play an important role in efficacy of targeted therapy. [11–14]

Noninvasive molecular imaging, defined as the in vivo characterization and measurement of biological processes at the cellular and molecular level, is a potential complementary molecular analysis technique.[15] Depending on the characteristics of the labeled radioisotope, nuclear molecular imaging is performed with planar scintigraphy and single photon emission computed tomography (SPECT) or positron emission tomography (PET).

Antibodies themselves can be used as tracers for PET imaging, then called immunoPET.[3] This technique can visualize noninvasively the presence of specific targets, for which the antibody is selectively developed. Moreover, it can provide in vivo whole-body information about tumor uptake, engagement and modulation of the target and organ distribution of the antibodies. ImmunoPET may assist by improving patient selection, optimizing dose and schedule and rationalizing drug responses.

For patients with unresectable pancreatic cancer and platinum-resistant ovarian cancer, treatment options are very limited, causing these diseases to have disappointingly poor prognoses. Regretfully, for pancreatic and ovarian cancer, also no important 'drugable' targets (i.e. molecular drivers of tumor growth) are yet available. However, also overexpression of targets that do not have a distinct role in tumor growth can be used for several innovative drug types such as ADCs in which the antibody part serves as docking station for drug delivery. One of the many tumor specific molecules that is being investigated in this regard, is mesothelin (MSLN), a membrane bound protein of unknown function with limited expression in mesothelial cells lining pleural, pericardial and peritoneal surfaces.[16] Based on immunohistochemical (IHC) and genetic studies, MSLN is frequently overexpressed in pancreatic (almost 100%) and ovarian cancer (66-100%).[17–20] One ADC in development targeting MSLN is DMOT4039A, composed of the anti-MSLN mAb MMOT0530A and the potent mitotic agent monomethyl auristatin MMAE. In the early development of ADCs, the ability to safely and accurately predict the presence or absence of the target and binding of the ADC to the target would be extremely helpful. By using the unconjugated mAb of the ADC for PET imaging before treatment with the ADC in a phase I trial, important information concerning antibody tumor uptake, whole-body distribution, organ pharmacokinetics and relation between uptake, MSLN expression and response to ADC treatment can be gathered.

In 20-25% of primary breast cancers, the *HER2* gene is overexpressed. HER2-overexpression, when left untreated, is associated with aggressive growth and poor prognosis.[31–33] The anti-HER2 monoclonal antibody trastuzumab is part of treatment in the adjuvant as well as in the metastatic setting of HER2-positive breast cancer.[34,35] A novel approach in the treatment of metastatic of locally recurrent HER2-positive breast cancer is the ADC trastuzumab emtansine (T-DM1), composed of trastuzumab and the cytotoxic agent emtansine (DM1).[36] Until now, no biomarker beyond HER2 status based on IHC or in situ hybridization (ISH) on mainly primary tumors, has been validated to predict treatment efficacy of T-DM1. The negative predictive role of trastuzumab PET imaging for response to T-DM1 is assessed in the Zephir trial.

Besides tumor-specific molecular targets present on the cell membrane such as MSLN and HER2, also soluble targets in the micro environment are suitable for molecular imaging. The vascular endothelial growth factor A (VEGF-A), involved in tumor angiogenesis, is overexpressed in many cancer types among which is breast cancer.[26,27] As breast cancer is still the most frequent cancer type in women, focus of much research is on improving current breast cancer detection, characterization and management. Imaging using the anti-VEGF-A antibody bevacizumab may aid in this need. Previously, a (micro) dose of bevacizumab was labeled for PET imaging with Zirkonium-89 (^{89}Zr) in renal cell, neuroendocrine tumor and breast cancer patients.[28–30] The combination of imaging and antibodies can also be applied using fluorescent labels for optical imaging. In this way, fluorescent labeled bevacizumab is of interest for tumor visualization and characterization not only for diagnostic purposes, treatment monitoring and drug development, but also for surgical guidance in primary breast cancer patients.

Aim of the thesis

The aim of this thesis is to investigate the role of molecular imaging with both nuclear as well as optical imaging with monoclonal antibodies as biomarker in the treatment of different cancer types, to predict treatment efficacy and to guide decision making in early clinical drug development and during primary surgery.

Outline of the thesis

By reviewing existing literature and writing from our own experience, in **chapter 2**, an overview of the translational process of tracer development is presented: from determination of an interesting target and targeting antibody, to preclinical validation *in vitro* and *in vivo*, to producing the investigational medicinal product dossier to finally implement all knowledge to clinical trials.

In **chapter 3**, a review discusses the potential of antibody PET imaging to improve the process of early anticancer drug development. The literature is reviewed by searching PubMed for relevant articles which are summarized and discussed in tables and text. Also ongoing clinical trials with radiolabeled antibodies are summarized.

Mesothelin (MSLN) is a highly specific tumor marker that is currently exploited as target for antibody-drug conjugates (ADCs). To determine the overexpression of MSLN in different tumor types, in **chapter 4** we compared historical immunohistochemistry data with functional genomic mRNA profiling of a set of 16,172 patient derived tumor samples.

As is known from immunohistochemical and recent genetic data (see chapter 4), MSLN is frequently overexpressed in pancreatic and ovarian cancer. In **chapter 5** we assessed the tumor targeting characteristics and biodistribution of the anti-MSLN antibody AMA labeled to ^{89}Zr and IRDye800CW in mice bearing human pancreatic tumor xenografts. MicroPET was performed at 24, 72, and 144 hours after tracer injection in mice bearing HPAC or Capan-2 tumors.

Thereafter we report in **chapter 6** a clinical immunoPET study using the same anti-MSLN antibody (^{89}Zr-MMOT0530A) that is part of an ADC (DMOT4039A) in phase I development. Our aim was to determine antibody tumor uptake and biodistribution and to investigate the relation between immunoPET uptake and MSLN expression on archival tumor samples and response to DMOT4039A treatment. Patients eligible for the phase I study were also asked for participation in this imaging sub study and received 37 MBq ^{89}Zr-MMOT0530A followed by PET/CT scans at 2, 4, and 7 days after injection. Standardized uptake values (SUV) were calculated for tumor lesions and organs and were compared within and between patients.

Subsequently in **chapter 7** the phase I study with DMOT0439A is described, in which the maximum tolerated dose, recommended phase II dose, pharmacokinetics and

preliminary signs of clinical activity are determined in a total of 71 patients. Both the every-3-week and the weekly schedule were assessed.

Trastuzumab-emtansine (T-DM1) is a relatively novel ADC recently approved for the treatment of patients with HER2-positive metastatic breast cancer (progressive after a prior trastuzumab-based therapy). We explored in **chapter 8** the role of baseline ^{89}Zr-trastuzumab PET and early ^{18}F-FDG PET scan performed after one cycle T-DM1 to identify patients unlikely to benefit from T-DM1.

In **chapter 9** we report a feasibility study in primary breast cancer patients using the near-infrared fluorescent (NIRF) VEGF-A targeting tracer bevacizumab-IRDye800CW. The aims of the study were to assess safety and to determine and quantify *in vivo* as well as *ex vivo* tracer tumor uptake. A microdose of 4.5 mg bevacizumab-IRDye800CW was administered to patients 3 days before surgery. At surgery *in vivo* images were made using a prototype intraoperative camera for real-time images. Thereafter additional *ex vivo* analyses for evaluation of tumor specific uptake in tissue was executed by fluorescence microscopy, VEGF-A immunohistochemistry and enzyme-linked immunosorbent assay (ELISA).

Finally, a summary of the obtained results of this thesis is described in **chapter 9** and in **chapter 10** these new findings and future perspectives of antibody imaging as biomarker in cancer drug development and treatment are discussed.

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