CHAPTER 1

General introduction

BACKGROUND

Cancer is a major global disease burden, and cancer incidence and mortality are rising (1). Conventional treatment strategies include surgery, radiotherapy, and systemic therapy. The arsenal of cancer treatment has evolved with the arrival of monoclonal antibodies (2). The group of monoclonal antibodies acting as immune checkpoint inhibitors plays an increasing role across several tumor types. Immune checkpoint inhibitors, including antibodies targeting program death 1 (PD-1) or its ligand (PD-L1), aim to elevate tumor immune evasion, harnessing a patient's immune system to attack tumor cells (3-5). These antibodies can induce tumor response and increase disease free-, progression-free-, and overall survival. A second fast-growing group of monoclonal antibodies that demonstrated prolonged survival in patients with certain cancer types is the group of antibody-drug conjugates (ADCs). ADCs are composed of an antibody, specific for a tumor-associated antigen, coupled to an anti-cancer drug or toxin via a conjugated linker. Upon target binding, the payload destructs the tumor cell (6). Moreover, targeted radionuclide therapy has emerged as another drug class to treat cancer. Beta particle emitting radionuclide lutetium-177 radiolabeled to tumor targeting moieties shows antitumor activity in patients with neuroendocrine tumors and prostate cancer (7). More potent, less far-traveling alpha particle emitting radionuclides, such as thorium-227 (^{227}Th) and actinium-225 (^{225}Ac), can be labeled to tumor-targeting monoclonal antibodies. Several trials with targeted alpha conjugates as anticancer treatment are ongoing in patients with solid tumors (NCT04147819, NCT03724747, NCT04597411, NCT04644770, NCT05219500, NCT03746431). Theranostics refer to molecules coupled to one or two distinct radionuclides that serve both therapeutic- and diagnostic purposes. Knowledge of the biodistribution of monoclonal antibodies might provide insight into the antibody's behavior and support further development and use of the antibody. Target abundance between and within lesions can differ among patients. Positron emission tomography (PET) enables the visualization of antibodies when labeled with positron-emitting isotopes. This tool, referred to as ImmunoPET, provides whole-body information regarding antibody distribution in a non-invasive manner. Zirconium-89 (^{89}Zr), with a physical half-life of 3 days, is compatible with antibody whole-body distribution over 3-7 days. Monoclonal antibodies are complex macromolecules and expensive drugs for clinical use. Biosimilars are highly similar copies of already-approved off-patent antibodies, called originators. Their availability has the potential to reduce drug costs. For cancer indications, there are now a few biosimilars available and more expected in the coming years (9).The research in this thesis focusses on monoclonal antibodies and aims to identify challenges for monoclonal antibody biosimilars in cancer and explore preclinical ^{89}Zr PET imaging of antibody therapies to evaluate their in vivo behavior, e.g., biodistribution, tumor uptake, and pharmacodynamics (Fig. 1).

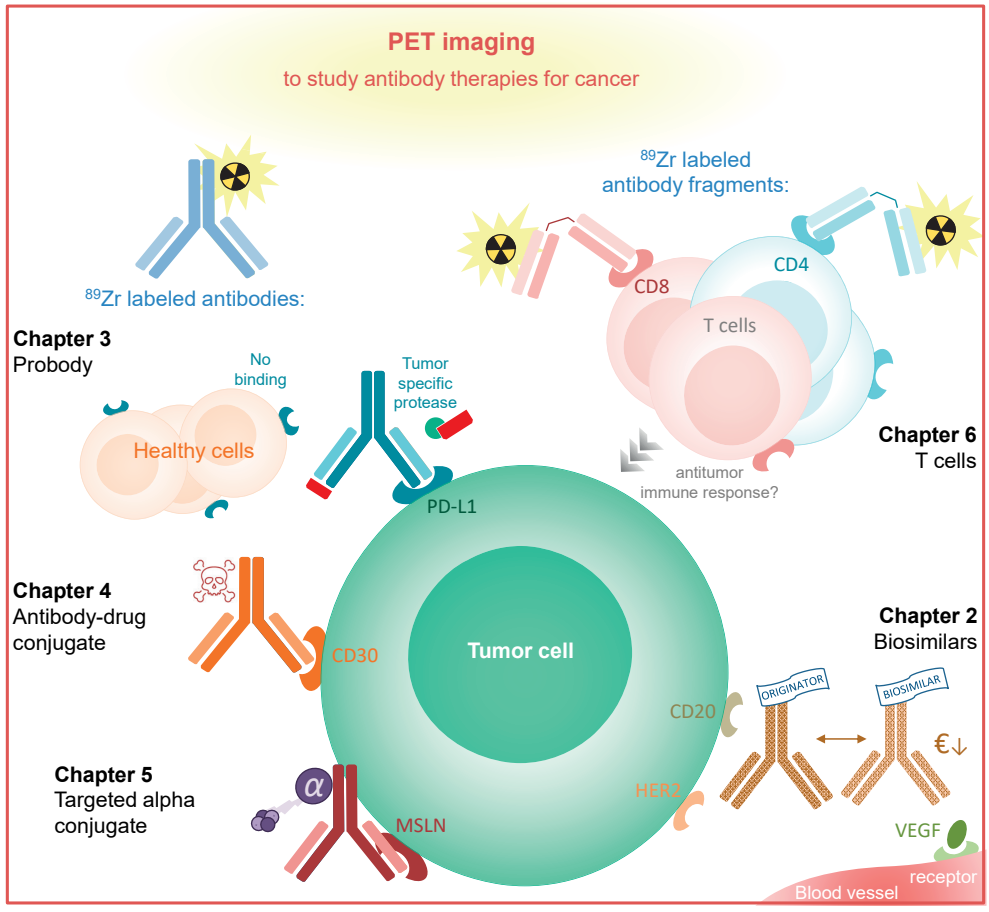

Figure 1. Aim of this thesis. The research in this thesis aims to get insight into monoclonal antibody biosimilars for cancer treatment (chapter 2); and explore preclinical ^{89}Zr PET imaging to evaluate biodistribution and tumor uptake of a Probody (chapter 3), an antibody-drug conjuate (chapter 4) a targeted alpha conjugate (chapter 5), and $CD4^+$ and $CD8^+$ T cell targeting antibody fragments. CD, cluster of differentiation; HER, human epidermal growth factor receptor; MSLN, mesothelin; PD-L1, programmed death ligand-1; VEGF, vascular endothelial growth factor.

OUTLINE THESIS

Use of monoclonal antibodies in cancer therapy is rapidly increasing, and the entry of more new antibodies is expected to accelerate even further (2). Development and uptake of biosimilars may at least partly retain rising therapy costs. The goal of the review in **Chapter 2** is get insight into the reasons behind the modest biosimilar market. In order to fully understand monoclonal antibody biosimilars for cancer, we first summarize structural-, functional-, and product-related properties, sources of variability and their potential effect on pharmacology. Thereafter EMA-approved bevacizumab biosimilars were evaluated to see in which aspects and to what extent variability occurs in practice. Investigational and approved cancer monoclonal antibody biosimilars are mapped to capture the global landscape. Lastly, the challenges for access to monoclonal antibody biosimilars for cancer treatment are identified. Relevant English-written articles published until November 2022 concerning cancer monoclonal antibodies and variability were searched in PubMed using the terms 'antibody', 'pharmacology', 'critical quality attribute', 'variability', or synonyms. The terms 'biosimilar' and 'cancer' were used to extract ongoing themes that could be identified as challenges for cancer monoclonal antibody biosimilars. From European Public Assessments Reports (EPARs), data regarding the analytical assessment of different approved bevacizumab biosimilars was summarized. Websites www.clinicialtrials.gov and www.gabionline.net were used to identify approved and investigational biosimilars for cancer indications. Additional biosimilars were found on PubMed, websites of pharmaceutical industries, and summaries of global market reports. The websites www.antibodysociety.org, and www.iqvia.com were used to retrieve further relevant information regarding (biosimilar) monoclonal antibodies. Regulatory information was searched on www.who.int, www.ema.eu, and www.fda.gov.

Immunotherapies can cause immune-related toxicities (10). To potentially reduce peripheral anti-PD-L1-mediated toxicities, a Probody therapeutic CX-072 has been developed. CX-072 is an antibody cross-reactive with murine and human PD-L1, activated in vivo by tumor-specific proteases. In **chapter 3,** we aimed to determine biodistribution and tumor targeting of CX-072 and to prove if the Probody design prevents accumulation in healthy PD-L1 expressing tissues. Therefore, CX-072 was radiolabeled with 89Zr and PD-L1 targeting of ^{89}Zr-CX-072 was studied with PET imaging in BALB/c nude mice bearing human MDA-MB-231 tumors and in C57BL/6J mice bearing syngeneic MC38 tumors, compared to a nonspecific Probody ^{89}Zr-PbCtrl and the parental antibody ^{89}Zr-CX-075. PET imaging days 1, 3, and 6 post-injection were followed by *ex vivo* biodistribution. Tumors were studied by autoradiography for ^{89}Zr-CX-072 distribution and stained for PD-L1 expression by immunohistochemistry. Activated CX-072 species in tissue lysates were detected.

Brentuximab-vedotin is an ADC targeting CD30, used to treat Hodgkin's lymphoma, cutaneous T-cell lymphoma, and systemic anaplastic large T-cell lymphoma. So far, predictive markers for the tumor response have been absent since CD30 expression in tumor biopsies does not relate to treatment outcome (11-14). Heterogeneity of CD30 expression within and between lesions and tumor accessibility of the ADC might determine tumor response. The naked antibody brentuximab is radiolabeled with ^{89}Zr (^{89}Zr-brentuximab), and biodistribution and tumor uptake of ^{89}Zr-brentuximab will be evaluated in a PET imaging study in patients with a CD30 positive lymphoma eligible for brentuximab-vedotin therapy. Tumor uptake and pharmacokinetic and pharmacodynamic properties will be assessed. **Chapter 4** entails [^{89}Zr]-brentuximab development and all data on the production, quality, and stability of nSuc-Df-brentuximab and ^{89}Zr-brentuximab generated for the investigational medicinal product dossier (IMPD). In this chapter the different phases of clinical ^{89}Zr-brentuximab tracer development are briefly summarized, and the final IMPD is included. This IMPD includes all pharmacological and pharmaceutical information available regarding ^{89}Zr-brentuximab and is an essential document for the use of ^{89}Zr-brentuximab in the intended clinicalstudy.

Mesothelin-targeted ^{227}Th conjugate (^{227}Th-MSLN) is an investigational targeted alpha conjugate, developed to be evaluated as a treatment of mesothelin overexpressing cancers such as mesothelioma and ovarian cancer (15). In the study described in **chapter 5,** we aimed to assess if ^{89}Zr PET imaging could be a theranostic to guide the development of targeted alpha therapies. The same antibody-chelator conjugate is labeled with either ^{89}Zr (^{89}Zr-MSLN) or ^{227}Th (^{227}Th-MSLN) to investigate whether PET imaging with ^{89}Zr-MSLN matches ^{227}Th-MSLN tumor uptake biodistribution and antitumor activity. PET imaging with protein doses of 4, 20, and 40 µg ^{89}Zr-MSLN and ^{89}Zr-control were performed up to 168 h post-injection in high (HT29-MSLN) and low (BxPc3) mesothelin expressing human tumor-bearing nude mice. Next, ^{89}Zr-MSLN and ^{227}Th-MSLN ex vivo tumor uptake and biodistribution were compared at 6 time points in HT29-MSLN and medium mesothelin expressing (OVCAR-3) tumor-bearing mice. Finally, ^{89}Zr-MSLN PET imaging was performed before ^{227}Th-MSLN treatment in HT29-MSLN and BxPc3 tumor-bearing mice. With these experiments we aim to study if ^{89}Zr-MSLN PET enables specific mesothelin targeting, if it could predict ^{227}Th-MSLN tumor uptake and biodistribution, and whether ^{89}Zr-MSLN tumor uptake matches with ^{227}Th-MSLN antitumor activity, to guide clinical development.

Insight into whole-body immune cell status may identify individuals with a better chance to respond to immune checkpoint inhibitors and study therapy-induced antitumor immune response (16-18). In **chapter 6,** we evaluated $CD4^+$ and $CD8^+$ T-cell imaging with ^{89}Zr PET and its reproducibility in healthy mice. To do so, on day 0, 30 µg $F(ab')_2$ tracer ^{89}Zr-CD4, ^{89}Zr-CD8, or nonspecific ^{89}Zr-control was injected in naïve DBA/2 mice, who underwent PET 24 h post-injection (pi). On day 7, the same mice received a second tracer injection,

and PET was repeated for 24 h pi. *Ex vivo* biodistribution was studied. Furthermore, in mice bearing syngeneic subcutaneous KLN205, lung tumors received targeted alpha therapy with 250 kBq/kg, 0.75 mg/kg ^{225}Ac-mAb or vehicle at day 0. On day 4, ^{89}Zr-CD4 was injected, followed by PET at 24 h, to study if this actinium-225 labeled antibody (^{225}Ac-mAb) affected ^{89}Zr-CD4 tracer tumor uptake.

Chapter 7 summarizes this thesis and gives an outlook on future perspectives. **Chapter 8** provides a Dutch summary.

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