Preclinical molecular imaging to study the biodistribution of antibody derivatives in oncology

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Chapter 1

General introduction

BACKGROUND

Being a major cause of death worldwide, cancer remains one of the key disease areas with the greatest unmet medical needs. Chemotherapy, the cornerstone of systemic treatment, damages DNA in cells affecting all dividing cells. Currently, drugs are being developed that target tumors more selectively. Much experience has been obtained with targeted antibodies, which are successfully used in daily clinical practice for the treatment of cancer. Among other mechanisms, antibodies can bind and subsequent inactivate pro-oncogenic proteins. Their successful use spurred interest in the development of antibody derivatives, including the variable domains of heavy chain only antibodies (VHHs or nanobodies; Fig. 1A). With their small size of 15 kDa, high stability and straightforward production[1], they can be used as potential antitumor agents by inactivating pro-oncogenic proteins and as targeted carriers of cytotoxic payloads[2]. Due to their specific binding to tumor-associated antigens, nanobodies may also serve as imaging agents.

In addition, antibodies and antibody derivatives can be produced so that they simultaneously target both tumor and host immune cells. By linking both cell types these proteins can stimulate the targeted host immune cells to kill the targeted tumor cells. The use of drugs that stimulate host immune cells to destroy cancer cells is known as cancer immunotherapy. Cancer immunotherapy is currently gaining much attention and many immune system stimulating bispecific antibody constructs are in development. However, the first bispecific antibody (blinatumomab) that activates immune cells (cytotoxic T-cells) to destroy tumor cells has just recently been approved for the treatment of cancer. Blinatumomab is approved for the treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia. Blinatumomab is a member of the novel class of BiTE antibodies that facilitate the linkage between tumor cells and cytotoxic T-cells. BiTE antibody constructs are engineered by combining two single-chain (sc)Fv domains of two different antibodies (Fig. 1B). One scFv domain is directed against the epsilon chain of cluster of differentiation (CD)3, a part of the T-cell receptor complex, and the other domain is directed against a tumor-associated antigen. After connecting CD3ε on T-cells with tumor-associated antigens on tumor cells, BiTE antibodies activate T-cells to destroy adjacent tumor cells.[3]

In addition to Blinatumomab that targets soluble tumors, other solid tumor targeting BiTE antibodies are in development, including AMG 110 (MT110; solitomab), AMG 211 (MEDI-565; MT111) and AMG 212 (BAY2010112). These BiTE antibodies target respectively anti-epithelial cell adhesion molecule (EpCAM), anti-carcinoembryonic antigen (CEA) and anti-prostate-specific membrane antigen (PSMA). Several phase I trials with these BiTE antibodies are ongoing or completed.[4,5]

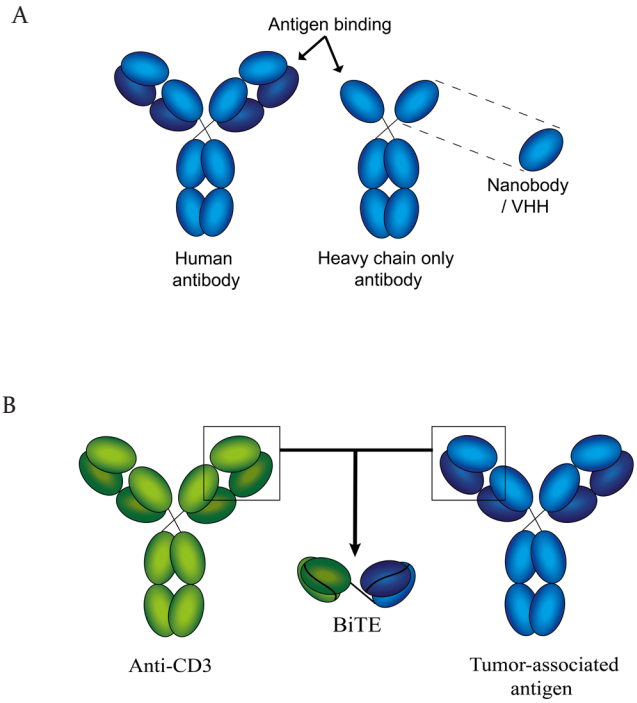

Figure 1: Antibody derivatives used in this thesis. (A) A nanobody or VHH is the variable domain of heavy chain only antibodies that are produced in camelids. (B) BiTE antibody constructs are engineered by combining two scFv domains, derived from an anti-CD3ε antibody and an antibody targeting a tumor-associated antigen. (C) The nanobody construct MSB0010853 is derived from two anti-HER3 nanobodies and an anti-albumin nanobody. MSB0010853 binds HER3 at domain 1 and at a second unknown domain of HER3 (defined as x).

Many anti-cancer drugs are currently in development, including the nanobody and BITE antibody constructs described in this thesis. However, only a minority will eventually be approved for clinical use. Two of the biggest challenges are the identification of the best drug candidates in early phase clinical studies and the search for an optimal design of late phase clinical studies. Early insight in tumor uptake, biodistribution and (organ) pharmacokinetics of drug candidates might facilitate to address these challenges. Insight in tumor uptake might enable optimization of patient selection, dose finding, dose scheduling and administration (e.g. bolus versus infusion), while insight in biodistribution and (organ) pharmacokinetics might help to anticipate toxicity in highly exposed organs.

This thesis focuses on the use molecular imaging in the process of drug development of nanobodies and BiTE antibodies. By labeling drug candidates with radionuclides, these drugs can non-invasively be traced in vivo. As a result, tumor uptake, biodistribution and (organ) pharmacokinetics of drug candidates can be visualized and quantified non-invasively with molecular imaging.[6] The gained information can potentially be used to facilitate their clinical development. In addition, this thesis focuses on the use of near infrared fluorescent imaging in order to study the distribution of these drug candidates at a cellular level and to develop an imaging agent that could facilitate the visualization of tumor tissue in an intra-operative setting.[7,8]

The aim of this thesis is to obtain insight in the biodistribution and tumor uptake of novel tumor targeting antibody derivatives using molecular imaging, in order to support drug development.

OUTLINE OF THE THESIS

The clinical success of therapeutic antibodies has raised interest in the development of other types of protein drugs. These proteins are currently also modified to try to improve their efficacy, tumor targeting, dosing frequency and toxicology profile. Examples of frequently used protein modifications include humanization, glycosylation, polyethylene glycol (PEG)ylation and (non) covalent albumin binding. In order to support fast preclinical and clinical drug development of protein drugs, there is an increasing focus on obtaining information about their biodistribution, their tumor targeting potential and the presence of their targets in tumors. These properties can be assessed by radiolabeling these drugs and visualize them non-invasively with positron emission tomography (PET) imaging. However, radiolabeling and other above-mentioned protein modifications could unintentionally affect tumor uptake, biodistribution and other pharmacokinetics of protein drugs. In order to optimize the use of molecular imaging and to interpret the obtained data correctly, insight in protein characteristics that affect biodistribution and tumor uptake is essential. In **chapter 2** we reviewed literature, concentrating on often-applied protein modifications and their effect on biodistribution and tumor uptake of these proteins. We searched PubMed to identify data for this review. Full articles were obtained and references

were checked for additional material as appropriate. The different types of proteins that were included in this review are antibodies, antibody fragments and non-immunoglobulin protein scaffolds. In this chapter we first focused on how specific protein properties including size, target interaction, binding to neonatal Fc receptor, and charge can affect the biodistribution of proteins. Subsequently, we aimed to describe how protein modification such as glycosylation, humanization, albumin binding, PEGylation, radioactive labeling and drug conjugation affect the biodistribution and tumor uptake of these drugs.

Given the fact that optical imaging lacks ionizing radiation, optical imaging is recently gaining more attention.[9] Furthermore, molecular imaging using optical imaging allows image-guided tumor resection.[7] Due to their high and specific target affinity, fluorescently labeled (anti-HER2) nanobodies could potentially be used as a probe for optical molecular imaging of HER2 positive breast cancer.[1] In **chapter 3** we focused on the selection and evaluation of anti-HER2 nanobodies that are conjugated to the near infrared fluorophore IRDye 800CW. By using phage display, three anti-HER2 nanobodies (11A4, 18C3, 22G12) were selected. They were subsequently conjugated with IRDye 800CW to the C-terminal cysteine (site-specific conjugation) or to lysines (random conjugation). Binding affinities of these probes were tested in vitro using a HER2 positive human breast cancer cells line (SKBR3). To test the potential of these probes, serial optical imaging was performed in male nude BALB/c mice, xenografted with human breast cancer cell lines SKBR3 or MDA-MB-231 (HER2 negative). The performance was compared with trastuzumab-800CW and an IRDye 800CW labeled, non-HER2 binding, control nanobody (R2). To demonstrate the potential of image-guided surgery, we resected a SKBR3 tumor that was subcutaneously xenografted in mice, under the guidance of the fluorescent signal of the most potent IRDye 800CW labeled nanobody.

As a third member of the HER family, HER3 activation and subsequent dimerization with other members of the family can initiate pro-oncogenic signaling. Blocking HER3 signaling could therefore potentially inhibit cancer progression. Interestingly, a mixture of two anti-HER3 antibodies (A5 and F4) blocked ligand-induced and independent HER3 signaling, and inhibited tumor cell growth better than each antibody alone.[10] Blocking two different HER3 epitopes, with a biparatopic nanobody construct (MSB0010853), is therefore an interesting option. In contrast to the single VHH described in chapter 3, MSB0010853 (39.5 kDa) consists of two HER3 targeting nanobodies and an additional third that is able to bind albumin, extending its half-life (Fig. 1C). The aim of the study described in **chapter 4** is to gain insight in the pharmacological behavior of MSB0010853 by labeling the nanobody construct with 89-Zirconium (^{89}Zr). ^{89}Zr is a PET isotope with a half-life of 78.4 hours, matching the potential long circulation time of albumin binding constructs.[11] Dose- and time-dependent biodistribution and tumor uptake of ^{89}Zr-MSB0010853 were evaluated in male nude BALB/c mice bearing subcutaneous human tumors. In addition, tumor uptake was determined in three tumor models with different HER3 expression. Cross-reactivity of MSB0010853 for HER3 and albumin of mice origin contributed to a translational mouse model.

Similar to the HER receptor proteins, EpCAM is expressed on many tumor cells. EpCAM

is expressed on epithelial tumors and cancer stem cells. Therefore, it is an attractive target for BiTEs. An EpCAM targeting BiTE called solitomab (AMG 110) enables T-cell mediated killing of EpCAM positive cancer cells.[12] Preliminary results of a phase I study with AMG 110 demonstrated that doses up to up to 48 µg/day were tolerated.[4] The study also revealed signs of pharmacological activity. In addition to AMG 110, another BiTE antibody (AMG 211) has been developed that targets CEA. In the presence of T-cells, AMG 211 can trigger dose-dependent in vitro cell killing of CEA-expressing human colon, pancreatic, stomach, lung, breast, and prostate cancer cell lines.[13] For solid tumors of especially the gastrointestinal tract, AMG 211 is an interesting new BiTE antibody construct, as it in vitro lyses explants of metastatic colorectal cancer cells of patients who progressed on chemotherapy.[14] AMG 211 is currently tested in a phase 1 clinical trial in colorectal cancer patients.[15] In order to support drug development of AMG 110 and AMG 211, we preclinically studied the biodistribution and tumor uptake of radiolabeled/fluorescent labeled AMG 110 and AMG 211. These data can potentially be used to initiate clinical studies with radiolabeled BiTEs to determine their biodistribution and tumor uptake in early phase clinical trials.

In **Chapter 5** we studied the tumor targeting potential and tissue distribution of AMG 110 after labeling it with ^{89}Zr or IRDye 800CW. For ex vivo biodistribution studies, ^{89}Zr-AMG 110 at protein doses of 20-500 µg, was administered to nude BALB/c mice xenografted with EpCAM expressing HT-29 colorectal adenocarcinoma cells. Non-invasive microPET imaging and ex vivo biodistribution was performed up to 72 hours after ^{89}Zr-AMG 110 injection. Ex vivo biodistribution of ^{89}Zr-AMG 110 was studied up to 72 hours after injection. Non-specific distribution was determined using ^{89}Zr labeled Mec14, a non-specific control BiTE targeting a hapten named mecoprop and human CD3ε. With flow cytometry EpCAM expression has been determined on the tumor cell lines that were xenografted in nude BALB/c mice, being the HT-29 cell line, the human head and neck squamous cell cancer FaDu cell line and the EpCAM negative promyelocytic HL-60 cell line. Subsequently, ex vivo tumor uptake of ^{89}Zr-AMG 110 has been measured and correlated with EpCAM expression. Labeling of AMG 110 with IRDye 800CW allowed us to determine the intratumoral distribution of AMG 110. Non-specific distribution of Mec14 was determined in the same by co-injecting IRDye 680RD labeled Mec14.

Chapter 5 proved that molecular imaging with BiTEs was feasible. **Chapter 6** therefore describes the study in which a second BiTE antibody (AMG 211), which is in phase 1 development, was labeled with ^{89}Zr or IRDye 800CW in order to study its tumor targeting property, tissue distribution and in vivo integrity. Dose-dependent biodistribution was determined ex vivo after administration of ^{89}Zr-AMG211 in a dose range of 10-500 µg, in nude BALB/c mice xenografted with CEA-expressing LS174T colorectal adenocarcinoma cells. Tumor uptake and biodistribution of 10 µg ^{89}Zr-AMG211 was visualized with non-invasive microPET imaging in LS174T xenografted mice. To check for CEA-specific tumor uptake, biodistribution and tumor uptake ^{89}Zr-Mec14 was also studied in the same mice models. In order to study the influence of CEA expression on ^{89}Zr-AMG211 tumor uptake we determined ^{89}Zr-AMG211 tumor uptake in LS174T, CEA-positive human breast cancer BT474 and CEA-negative promyelocytic HL-60 cell lines. CEA expression

of these cell lines was determined flow cytometrically. The integrity of ^{89}Zr-AMG211 has been studied in LS174T xenografted mice, at 24 h after injection. Tumor, liver and kidneys were lysed and subsequently analyzed, together with serum, with gel electrophoresis combined with autoradiography image analysis. Co-injection of IRDye 800CW labeled AMG 211 and IRDye 680RD labeled Mec14 in LS174T xenografted mice enabled us to study CEA dependent distribution of AMG 211. If PET imaging with ^{89}Zr-AMG211 is feasible, it will be used to study the distribution and tumor uptake of AMG211 in cancer patients. Therefore, we tested if we could produce ^{89}Zr-AMG211 according to the Good Manufacturing Practice (GMP) guidelines.

The results of all the studies presented in this thesis are summarized in **chapter 7.** The future perspectives following the studies described in this thesis are discussed in **chapter 8**.

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