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

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Chapter 8

Summary

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 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 has spurred interest in the development of antibody derivatives, including the variable domains of heavy chain only antibodies (VHHs or nanobodies). Nanobodies are small with a molecular weight of 15 kDa, highly stable and straightforward to produce. Therefore these proteins are ideal building blocks to create a large variety of tumor targeting constructs. These tumor targeting nanobody constructs potentially have therapeutic properties as they can block pro-oncogenic receptors and target cytotoxic payloads. Due to their specific binding to tumor-associated antigens, nanobody constructs 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. Due to the successes in the field of cancer immunotherapy, it is gaining much attention as an innovative treatment to fight cancer. As a novel and relatively new type of anticancer drug Bispecific T-cell Engagers (BiTEs) can stimulate the host immune system to kill cancer cells. By linking cytotoxic T lymphocytes (CTLs) to tumor cells BiTEs stimulate CTLs to kill adjacent tumor cells. BiTEs bind the epsilon chain of cluster of differentiation 3 (CD3ε), a part of the T-cell receptor complex on CTLs and a tumor-associated antigen. As many tumor cells highly express epithelial cell adhesion molecule (EpCAM) or carcinoembryonic antigen (CEA), BiTE antibodies that connect EpCAM or CEA-positive tumor cells with CTLs have the potential to treat a large variety of solid cancers.

Many anti-cancer drugs are currently in development, including the nanobodies 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 drugs might help to address these challenges. These characteristics can be visualized and quantified non-invasively with positron emission tomography (PET) imaging. When incorporated in early phase clinical studies PET imaging can potentially support decision making with regard to clinical development of these drugs.

This thesis aimed to obtain insight in the biodistribution and tumor uptake of antibody derivatives using molecular imaging, in order to support their drug development.

Chapter 1 provides a concise background and introduction to this thesis. In **chapter 2** we

reviewed the literature on how protein modification can affect the biodistribution and tumor uptake of protein drugs. Newly developed protein drugs that target tumor-associated antigens are often modified to increase their therapeutic effect, tumor exposure and safety profile. During the development of protein drugs, molecular imaging is increasingly used to obtain additional information about their in vivo behavior. Although molecular imaging is increasingly being used in the process of protein drug development, much still remains unclear about how to interpret molecular imaging data correctly. Consequently, there is a need for gaining more insight in the correct way of interpreting (pre)clinical imaging data. Therefore, an overview has been presented of which protein properties and protein modifications can affect biodistribution and tumor uptake of protein drugs. Protein modifications discussed are: radiolabeling, drug conjugation, glycosylation, humanization, albumin binding and polyethylene glycol (PEG)ylation. Protein modifications often alter protein levels in blood, liver, spleen, kidneys and tumor. However, the impact of protein modification on the in vivo behavior of proteins is depending on the type of protein and should therefore be determined for each type of protein drug separately. Furthermore, the model in which the in vivo behavior is tested could affect the outcome. The overview of specific protein properties and modifications that affect biodistribution and tumor uptake of protein drugs, as presented in this chapter, may facilitate a correct interpretation of (pre)clinical imaging data with protein based radiopharmaceuticals and fluorescently labeled proteins.

Fluorescent imaging can potentially facilitate the visualization of tumors overexpressing specific tumor-associated antigens. Due to the fact that nanobodies show fast tumor accumulation, fluorescent nanobodies might enable tumor visualization shortly after injection. **Chapter 3** describes the selection and evaluation of high affinity anti-human epidermal growth factor receptor (HER)2 nanobodies, which were labeled with the near-infrared fluorophore (IRDye 800CW). By studying their biodistribution and tumor uptake we aimed to facilitate the development of a potential imaging drug that enables visualization of HER2 overexpressing tumors. Site-specific conjugation of IRDye 800CW to three anti-HER2 VHHs preserved high affinity binding with the following dissociation constants: 11A4 1.9 ± 0.03, 18C3 14.3 ± 1.8 and 22G12 3.2 ± 0.5 nM. Based upon different criteria such as binding, production yield and tumor accumulation, 11A4 was selected for further studies. Comparison of 11A4-IR with trastuzumab-IR showed ~20 times faster tumor accumulation of the anti-HER2 VHH, with a much higher contrast between tumor and background tissue (11A4-IR 2.5 ± 0.3, trastuzumab-IR 1.4 ± 0.4, 4 h post-injection). 11A4-IR demonstrated to be a potentially useful tool in image-guided surgery.

HER3, unlike other members of the HER family, has reduced kinase activity. However, HER3 activation and subsequent dimerization with other members of the HER family can initiate pro-oncogenic signaling. Blocking HER3 signaling can therefore potentially inhibit cancer progression. A recent study showed that blocking two HER3 epitopes with two anti-HER3 antibodies inhibited tumor cell growth more effectively than each antibody alone. Blocking two HER3 epitopes with MSB0010853, a biparatopic nanobody construct, is therefore an interesting option. **Chapter 4** describes the development and preclinical evaluation of an 89-zirconium

(^{89}Zr) labeled MSB0010853. In addition to HER3, MSB0010853 binds albumin to extend its circulation time, potentially enhancing tumor uptake. MSB0010853 binds HER3 at domain 1 and at a second unknown domain. All three Nanobodies target human and mouse antigen. Radiolabeling of MSB0010853 with ^{89}Zr was performed with a radiochemical purity of >95%. Ex vivo biodistribution showed protein dose- and time-dependent distribution of ^{89}Zr-MSB0010853 in all organs. Uptake of ^{89}Zr-MSB0010853 in H441 non-small cell lung cancer tumors was only time-dependent. Tumor could be visualized up to at least 96 h after injection. Highest mean standard uptake value of 0.6 ± 0.2 was observed at 24 h after injection of 25 μg ^{89}Zr-MSB0010853. ^{89}Zr-MSB0010853 organ distribution and tumor uptake in mice were time-dependent. Tumor uptake correlated with HER3 expression. In contrast to tumor uptake except for kidney uptake, organ distribution of ^{89}Zr-MSB0010853 was protein dose-dependent for the tested protein doses (5, 10, 25, 100 and 1000 μg). ^{89}Zr-MSB0010853 PET imaging gives insight in in vivo behavior of MSB0010853.

The first bispecific antibody that activates cytotoxic T-cells to destroy tumor cells (blinatumomab) has just recently been approved for the treatment of cancer. Blinatumomab is a member of the novel class of BiTE antibodies that facilitate the linkage between tumor cells and cytotoxic T-cells. After connecting CD3ε on T-cells with tumor-associated antigens on tumor cells, BiTE antibodies activate T-cells to destroy adjacent tumor cells. We used molecular imaging as a non-invasive tool to study the biodistribution and tumor uptake of BiTEs, in order to gain insight in their in vivo behavior. In **chapter 5** we labeled an EpCAM recognizing BiTE antibody (AMG 110) with ^{89}Zr or IRDye 800CW. EpCAM is expressed on many epithelial tumors and cancer stem cells, it therefore is an interesting target for BiTEs. Specific binding to EpCAM positive HT-29 human colorectal adenocarcinoma cells was maintained after ^{89}Zr labeling of AMG 110. In mice, HT-29 tumors could clearly be visualized as soon as 3 h after ^{89}Zr-AMG 110 injection and up to at least 72 h. Increasing the protein dose from 20 μg to 500 μg did not affect biodistribution of ^{89}Zr-AMG 110. Maximum tumor uptake of ^{89}Zr-AMG 110 was obtained 6 h after tracer injection (5.35 ± 0.22 %ID/g). Non-specific distribution was determined using ^{89}Zr labeled Mec14, a non-specific control BiTE targeting a hapten named mecoprop and human CD3ε. At 24 h after injection, tumor uptake of ^{89}Zr-Mec14 was significantly lower than ^{89}Zr-AMG 110, respectively 0.7 ± 0.1 %ID/g and 5.3 ± 0.3 %ID/g. Tumor uptake of ^{89}Zr-AMG 110 depended on and correlated with EpCAM expression on tumor cells. Intratumoral distribution of 800CW-AMG 110 demonstrated that AMG 110 accumulated in vital EpCAM expressing tumor tissue, as determined ex vivo. In contrast, IRDye 680RD labeled Mec14 accumulated in necrotic areas of the tumor, confirming EpCAM specific tumor uptake of 800CW-AMG 110. The data in chapter 5 support using ^{89}Zr and IRDye 800CW to evaluate tumor and tissue uptake kinetics of bispecific T cell engager antibody constructs in preclinical and clinical settings.

Chapter 5 proved that molecular imaging with BiTEs was feasible. In **chapter 6** a second BiTE (AMG 211), currently studied in a phase one clinical study (NCT02291614), was radiolabeled with ^{89}Zr. Tumor and tissue uptake kinetics and in vivo integrity of ^{89}Zr-AGM211 was preclinically evaluated in mouse models in order determine the feasibility to study ^{89}Zr-AGM211 in vivo

behavior in the clinical setting. AMG 211 is a BiTE antibody that targets carcinoembryonic antigen (CEA) on tumor cells and CD3ε on T cells. For solid tumors of especially the gastrointestinal tract, AMG 211 is an interesting new BiTE antibody construct. Chapter 6 provides insight in tumor targeting and biodistribution of AMG 211 in preclinical models by non-invasive molecular imaging. ^{89}Zr-AMG211 tumor uptake is dose dependent as determined at 6 h after injection. Highest relative tumor uptake was observed with 2 μg (7.5 ± 1.5 %ID/g) and lowest tumor uptake with 500 μg (3.9 ± 0.1%ID/g). Ten μg ^{89}Zr-AMG211 resulted in a higher uptake in CEA-positive xenografts than CEA-negative xenografts. Although blood half-life of ^{89}Zr-AMG211 was 1 hour, tumor retention occurred up to 24 h. Ex vivo autoradiography showed intact ^{89}Zr-AMG211 only in CEA-positive xenograft lysates. Ex vivo autoradiography revealed a time-dependent disintegration of ^{89}Zr-AMG211. ^{89}Zr-Mec14 did not accumulate in CEA-positive tumors. 800CW-AMG211 was specifically localized in CEA-expressing vital tumor tissue. GMP-produced ^{89}Zr-AMG211 fulfilled production specifications. The results in chapter 6 already allowed to perform PET imaging with ^{89}Zr-AMG211 in a currently ongoing clinical trial (NCT02760199).

The results of the studies performed in this thesis are discussed in **chapter 7**, along with the future prospectives regarding the imaging modalities used in this thesis, half-life extention of therapeutic proteins and the clinical use of BiTEs. Both nuclear and optical imaging hold great promise to support drug development. Intensifying the collaboration between academia and industry and standardization of imaging protocols would help to spur the implementation of molecular imaging in clinical trials. The biodistribution and tumor uptake of ^{89}Zr-AMG211 is currently studied in a phase one clinical study.

In conclusion, this thesis described the development en preclinical evaluation of a fluorescent and/or radioactive labeled BiTEs and VHHs/nanobodies to assess biodistribution, tumor uptake and in vivo integrity of these drug candidates.