Studies to optimize selection for immunomodulatory cancer therapy with focus on immunoPET

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Chapter 2

Theranostics utilizing antibodies and antibody-related therapeutics

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ABSTRACT

Theranostics uses radiolabeled compounds to determine a treatment strategy by combining therapeutics and diagnostics in the same agent. Monoclonal antibodies (mAbs) and antibody-related therapeutics are a rapidly expanding group of cancer medicines. Theranostic approaches utilizing these drugs in oncology are particularly interesting since antibodies are designed against specific targets on the tumor cell membrane, on immune cells as well as targets in the tumor microenvironment. In addition, these drugs are relatively easy to radiolabel.

Non-invasive molecular imaging techniques, such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET), provide information on whole body distribution of radiolabeled mAbs and antibody-related therapeutics. Molecular antibody imaging can potentially elucidate drug target expression, tracer uptake in the tumor, tumor saturation as well as whether there is heterogeneity for these parameters within the tumor. These data can support drug development and might aid in patient stratification and monitoring of treatment response.

Selecting a radionuclide for theranostic purposes generally starts by matching mAb or antibody-related therapeutic and radionuclide half-life. Furthermore, PET imaging allows better quantification than the SPECT technique. This has raised interest for theranostics using PET radionuclides with a relatively long physical half-life, such as 89-zirconium (^{89}Zr). In this review we provide an overview of ongoing research on mAb and antibody-related theranostics in the preclinical and clinical oncological setting. We identified 24 antibodies or antibody-related therapeutics labeled with PET radionuclides used for theranostic purposes in patients. For this approach to become integrated in standard-care, further standardization is required with respect to the procedures involved.

INTRODUCTION

Theranostics is a treatment strategy that utilizes a single agent both for diagnostic and therapeutic purposes. Theranostic procedures are based on radiolabeling compounds of interest. In cancer patients, this potentially enables evaluation of drug target expression and actual presence of the drug at the tumor site in patients *in vivo* using imaging methods such as Single Photon Emission Computed Tomography (SPECT) or Positron Emission Tomography (PET). Particularly interesting are theranostic approaches using monoclonal antibodies (mAbs) and antibody-related therapeutics since they belong to a rapidly expanding group of effective anticancer drugs. Antibody-related therapeutics include bispecific antibodies (e.g., bispecific T-cell engagers (BiTEs)), engineered antibody structures (e.g., minibodies, diabodies, nanobodies), antibody-drug conjugates (ADCs), and radiolabeled antibodies for radioimmunotherapy (RIT). These drugs have ideal characteristics for theranostic approaches since they are designed against a specific target, often on the cell surface, and are relatively easy to radiolabel.

As of December 2016, 24 mAbs or antibody-related therapeutics have been approved by the U.S. Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA) for use in cancer patients. These drugs comprise 20 mAbs, one BiTE, and three mAbs with a payload of which two are ADCs and one RIT antibody. The approved mAbs and antibody-related therapeutics are directed against targets on the tumor cell membrane, immune cells as well as targets in the microenvironment.

MAbs are administered in the non-curative and curative setting. In the non-curative setting these drugs have proven effect on (disease free) survival.[1-3] In the adjuvant setting, the human epidermal growth factor receptor 2 (HER2) antibody trastuzumab and the cytotoxic T lymphocyte antigen-4 (CTLA-4) antibody ipilimumab increase overall survival in breast cancer and melanoma, respectively.[4-5]

In oncology, even when a drug has proven clinical benefit for a certain patient population, not all patients will benefit. This can potentially be related to heterogeneity in tumor target expression, vascularization of the tumor or the presence of an immunosuppressive tumor microenvironment. Treatment decisions, both in routine practice and in drug development, are frequently made using information obtained from a biopsy of a single tumor lesion. Furthermore, recommended dosing schedules are mostly determined on blood-based pharmacokinetic analyses. Differences in drug target expression and drug uptake between the various tumor lesions within a single patient are almost never taken into account. In this respect, a theranostic approach is of potential interest since it might provide insight into tumor target heterogeneity and inform on whether the drug reaches the tumor lesions. For this reason, molecular antibody imaging can also be a valuable tool in drug development, drug decision making and patient enrichment strategies.

In this review, we provide an overview of current research on mAbs and antibody-related therapeutics visualized using PET imaging both in the preclinical and clinical oncological setting.

Search strategy

To identify available studies investigating theranostic approaches with mAbs and antibody-related therapeutics, a PubMed search was performed on November 21st, 2016. The search terms 'PET' AND 'Cancer' AND 'Antibody' OR 'ADC' OR 'Bispecific' were used in combination with the most commonly used PET radionuclides 64-copper (^{64}Cu), 68-gallium (^{68}Ga), 86-yttrium (^{86}Y), 89-zirconium (^{89}Zr) and 124-iodine (^{124}I). We focused on studies published during the last 5 years, to capture most recent developments, but included relevant studies published earlier. In addition, we searched ClinicialTrials.gov on November 17th, 2016 for ongoing studies over the past 10 years with the search terms 'Cancer' AND 'PET' NOT 'FDG'. Both searches were limited to manuscripts published in English. Case-reports, reviews, and books were excluded. In total 1,448 preclinical and clinical studies were found. All manuscripts and ongoing studies were

manually screened for relevance using the following inclusion criteria: First, a full-sized mAb, ADC, bispecific antibody, or fragment with theranostic potential was used. Second, in case of a study in which humans were included subjects were aged 18 years or older. Finally, we limited our search to the most commonly used PET radionuclides in order to provide a comprehensive overview of relevant agents with prime theranostic potential. Manuscripts were excluded if (potential) theranostic applications were not found with the same agent.

General aspects of molecular imaging using mAbs and antibody-related therapeutics

MAbs and antibody-related therapeutics can be efficiently labeled with a wide range of radionuclides. In general, the different labeling techniques can easily be applied to most mAbs and antibody-related therapeutics. These drugs can therefore be utilized in studies ranging from mouse to man.[6]

Chelation and radiolabeling for molecular antibody imaging

Technetium-99 (^{99m}Tc), Copper-64 (^{64}Cu), gallium-68 (^{68}Ga), yttrium-86 (^{86}Y), zirconium-89 (^{89}Zr), indium-111 (^{111}In), iodine-123 (^{123}I), iodine-124 (^{124}I), iodine-131 (^{131}I) and lutetium-177 (^{177}Lu) are the most commonly used radionuclides for molecular imaging using mAbs and antibody-related therapeutics in the field of oncology (Table 1). Selecting a suitable radionuclide generally starts by matching mAb or antibody-related therapeutic and radionuclide half-life. This is essential to ensure that radioactivity can be detected sufficiently long for the drug to reach its target while minimizing duration of exposure to harmful radiation.[6] Serum half-life mainly depends on the structure and size of the mAb or antibody-related therapeutic. Generally, serum half-life is shorter for a smaller mAb construct in comparison to a full-sized mAb because the molecular weight is often below the renal clearance threshold of ~70 kDa. For example, the serum half-life of cetuximab (± 150 kDa) is 3-4 days while the serum half-life of the BiTE antibody blinatumomab (± 60 kDa) is only several hours. In addition, serum half-life depends on the IgG subtype the mAb or antibody-related therapeutic is derived from and whether the (constructed) mAb is

fully humane, humanized murine or chimeric. The serum half-life of mAbs and antibody-related therapeutics can vary from 30 minutes to 30 days.

Table 1: Characteristics of radionuclides used in antibody or antibody-related theranostics in oncology

Isotope	Half-life	Residualizing
	PET	
^{68}Ga	67.7 min	+
^{64}Cu	12.7 h	+
^{86}Y	14.7 h	+
^{89}Zr	78.4 h	+
^{124}I	100.3 h	-
	SPECT	
^{99m}Tc	6.0 h	+
^{123}I	13.2 h	-
^{111}In	67.3 h	+
^{177}Lu	159.5 h	+
^{131}I	192.5 h	-

Furthermore a chelator is required in order to link metal-based radionuclides, e.g., ^{64}Cu, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{111}In and ^{177}Lu, to a mAb or antibody-related therapeutic. Deciding on a chelator for human use depends on the radionuclide, the most stable chemical link, and the clinical applicability in terms of validation.

Another important consideration when choosing a nuclide for radiolabeling is whether the mAb or antibody-related therapeutic becomes internalized after binding to its target. For example, when radiometal-labeled drugs are metabolized, the metal-based radionuclide is trapped intracellularly in lysosomes through residualization.[7] This results in higher absolute uptake of the tracer and leads eventually to higher tumor-to-blood ratios. Iodine-labeled drugs are characterized by rapid renal clearance of the radionuclide from the tumor cell, since iodinated mAbs do not residualize. However,

methods are available to increase the internalization of iodine-labeled drugs. For instance, a bivalent peptide consisting of 4 D-amino acids (D-a.a. peptide) increased the residence time of the ^{125}I radiolabel in RCC significantly when compared to ^{111}In-labeled control peptide.[8]

Commonly used radionuclides in molecular antibody imaging

Although radionuclides with different physical half-life are available for radiolabeling, the clinical use of many nuclides is hampered by the requirement of a cyclotron either on-site or about one physical half-life of transport time away from the site. An alternative is using a generator for which a radionuclide laboratory suffices. Then the long-lived 'mother' radionuclide allows for instant/constant availability of the 'daughter' radionuclide. For example, the ^{68}Ga radionuclide is produced using a generator – containing the cyclotron produced 'mother' radionuclide ^{68}Ge – allowing radiolabeling of mAbs or antibody-related therapeutics at the site of administration. Unfortunately, this radionuclide has a relatively short physical half-life of 68 min, limiting its use for imaging full-sized antibodies that need several days to achieve sufficient tumor-to-blood ratios.

During the past years, molecular imaging using the positron emitter ^{89}Zr for antibody labeling has been increasingly used. This radionuclide has suitable characteristics for molecular antibody imaging, since its physical half-life of 78.4 h generally matches the serum half-life of most mAbs and antibody-related therapeutics *in vivo* and is compatible with the time needed for residualization, generally allowing high tumor-to-background ratios. Furthermore, procedures have been developed for production of ^{89}Zr at large scale, and mAbs and antibody-related therapeutics can be stably labeled with this radionuclide.[9]

Pharmacokinetics and target visualization of radiolabeled mAbs

Most radiolabeled full-sized antibodies have a relatively long effective half-life of 14-21 days. After administration, the drug is distributed throughout the body and taken up by the tumor and other tissues that express its target. Over time, generally tumor-to-background ratio will increase due to tracer binding to the tumor and residualization of the

radiolabel in tumor tissue and clearance of the non-bound tracer from circulation and background organs/tissues.

When tumor accumulation of the radiolabeled drug takes place, this is the consequence of target location, target expression levels, target saturation and internalization of the drug. In addition, several kinetic aspects such as perfusion and vascularization may influence tumor visualization. For example, tumor uptake of ^{111}In-labeled death receptor 5 (DR5) targeting antibody CS-1008 was observed in only 63% of 19 patients with metastatic colorectal cancer even though all patients were considered to have DR5 positive lesions.[10]

Interestingly, also tracer uptake data in normal tissue can help explain observed side-effects. ^{111}In-trastuzumab scintigraphy revealed an increase in myocardial uptake shortly after anthracycline treatment in a subgroup of patients.[11] This observation might explain why trastuzumab related cardiotoxicity can occur when this drug is combined with anthracycline-based chemotherapy.

Clinical imaging studies generally start with a protein dose finding and time point finding phase to explore tumor-to-background ratios and image quality at different time-points.[12] Especially in case of dose-dependent pharmacokinetics the optimal protein dose may have to be higher. A radioactive dose of 37 MBq with a scan time of 45-60 minutes allows adequate visualization at day 4 – 7 in case of a ^{89}Zr-labeled, full-sized mAb.[13,14] The mAb or antibody-related therapeutic is linked to a certain amount of radioactivity per mg, so called specific activity expressed in MBq/mg. Specific activity for most mAbs and antibody-related therapeutics is generally limited to 750-1000 MBq/mg due to radiolysis. Unlabeled (naked) antibody is then added to the radiolabeled mAb in order to allow higher tumor uptake of the tracer for adequate tumor visualization. When the total protein dose that can be safely administered to the patient is relatively low, e.g., in the µg range, reaching sufficient radioactive dose for successful imaging is difficult. In case of T-cell engaging drugs, protein dose is generally

low to avoid side effects, which makes the use of these drugs as theranostics challenging.[15,]

Using theranostics in clinical decision making

We identified six different antibody structures that are currently used as theranostic agents in patients. In Fig. 1, we illustrate how these compounds are directed against a specific target located on the tumor cell or in the tumor microenvironment, e.g., macrophages, dendritic cells and T cells. In addition, this figure provides a simplified illustration of the therapeutics in their radiolabeled form for theranostic purposes. Until now, most molecular imaging clinical trials have been performed using radiolabeled FDA and/or EMA approved drugs such as trastuzumab in breast cancer, cetuximab in colorectal cancer and bevacizumab across several indications (Table 2). An example of ^{89}Zr-trastuzumab-PET in breast cancer is shown in Fig. 2. We identified 14 clinical imaging studies with trastuzumab, which makes this the most frequently investigated therapeutic mAb in molecular imaging (Fig. 1A). Several lessons can be learned from these studies. First, ^{111}In-trastuzumab-SPECT imaging showed new HER2-positive tumor lesions in 13 out of 15 metastatic breast cancer patients that were not detected using conventional imaging.[16] This shows that molecular antibody imaging can help identify tumor lesions that are missed on conventional imaging techniques. Second, serial SPECT imaging with ^{111}In-trastuzumab before and after 12 weeks of trastuzumab treatment showed persistent uptake in all tumor lesions, with only a 20% decrease in tumor tracer uptake.[17] This indicates that HER2 is constantly available at the tumor cell surface to bind to trastuzumab and that the tumor is not completely saturated by trastuzumab treatment. Third, a study with ^{89}Zr-trastuzumab PET in metastatic breast cancer compared tumor uptake between 10 mg and 50 mg naked trastuzumab in addition to the tracer dose. In trastuzumab-naïve patients, 50 mg naked trastuzumab was needed for adequate imaging.[13] This is likely due to the dose dependent pharmacokinetics of trastuzumab. This study showed the relevance of adequate naked antibody dose for sufficient accumulation of radiolabeled antibody in the tumor.

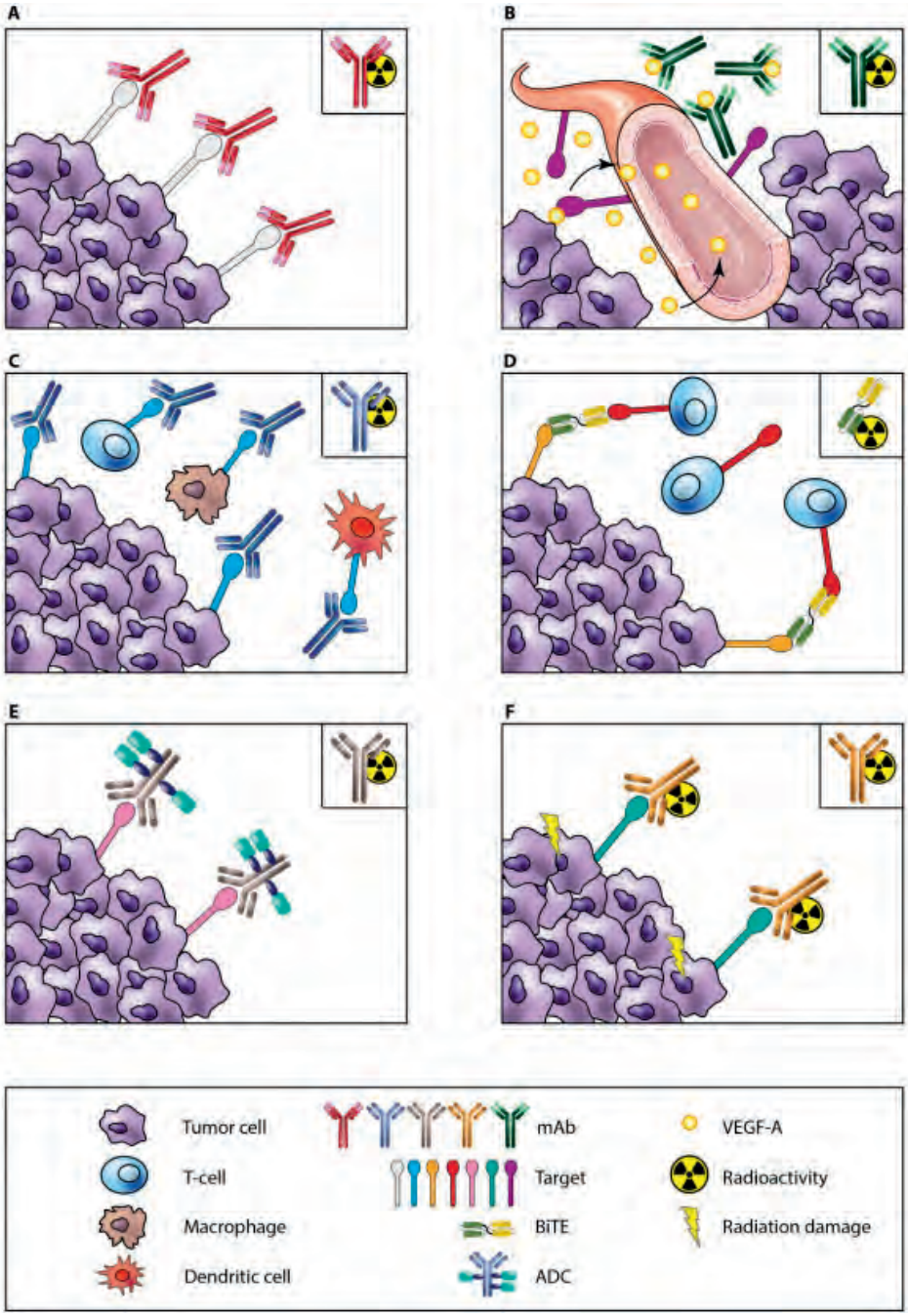

Figure 1: Antibodies and antibody-related theranostics
Six different antibody structures which are clinically used. Additionally, in each right corner we illustrate the radiolabeled compound used for theranostics. Here we present six examples of theranostics **A)** using mAbs,

e.g., trastuzumab targeting HER2 on tumor cells, **B)** in angiogenesis, e.g., bevacizumab targeting VEGF-A, **C)** using immune checkpoint inhibitors, e.g., PD-L1 antibody targeting PD-L1 on tumor cells and immune cells, **D)** using BiTEs, e.g., AMG 211 targeting CEA on tumor cells and CD3ε on T-cells, **E)** using ADCs, e.g., trastuzumab emtansine targeting HER2 on tumor cells using the radiolabeled naked trastuzumab, **F)** using RITs, e.g., ^{90}Y-ibritumomab tiuxetan.
Abbreviations: ADC, antibody-drug conjugate; BiTE, bispecific T-cell engager; CD, cluster of differentiation; CEA, carcinoembryonic antigen; HER2, human epidermal growth factor receptor 2; mAb, monoclonal antibody; PD-L1, programmed death receptor 1 ligand; RIT, radioimmunotherapy; VEGF-A, vascular endothelial growth factor A.

Fourthly another study showed the additive value of ^{89}Zr-trastuzumab PET imaging to biopsies to assess intra-patient tumor heterogeneity and to predict treatment outcome in HER2-positive breast cancer patients treated with T-DM1. One third of the patients with HER2-positive breast cancer showed little or no ^{89}Zr-trastuzumab uptake across their metastases and experienced a shorter median time-to-treatment failure compared to patients with a more homogenously positive HER2 PET scan.[18] This illustrates a successful theranostic approach to assess tumor heterogeneity and predict treatment outcome. Finally, ^{89}Zr-trastuzumab PET imaging can serve in patients as functional read out for therapeutics which affect HER2 expression, such as the heat shock protein (HSP)90 inhibitor AUY922.[19] The ongoing IMPACT-breast study is evaluating the clinical utility of ^{89}Zr-trastuzumab PET and ^{18}F-fluoroestradiol PET imaging in 200 newly diagnosed metastasized breast cancer patients (ClinicalTrials.gov identifier NCT01957332).

Another well-known drugable target is the epidermal growth factor receptor 1 (EGFR), which is targeted by antibodies such as cetuximab and panitumumab. One study demonstrated large differences in tumor ^{89}Zr-cetuximab tracer uptake between intrahepatic and extrahepatic tumors in K-RAS wildtype metastatic colorectal cancer patients. Extrahepatic tumor uptake of ^{89}Zr-cetuximab was demonstrated, while liver metastases appeared as 'cold spots'. Four of six patients with ^{89}Zr-cetuximab uptake in tumor lesions had clinical

benefit while progressive disease was observed in three of four patients without ^{89}Zr-cetuximab uptake.[14] Another study with ^{89}Zr-cetuximab was performed in head and neck squamous cell cancer (HNSCC).[20] Both studies used a therapeutic dose of naked cetuximab followed by ^{89}Zr-cetuximab for imaging which might have at least partly saturated the tumor and therefore might have reduced ^{89}Zr-cetuximab tumor uptake.

Angiogenesis is a hallmark of cancer and is stimulated by vascular endothelial growth factor (VEGF)-A. Several studies have been performed with the VEGF-A antibody ^{89}Zr-bevacizumab (Fig. 1B).[21-24] They clearly illustrate that a drug targeting a growth factor in the microenvironment can be visualized using protein tracer doses as low as 5 mg. In renal cell cancer (RCC) ^{89}Zr-bevacizumab PET showed heterogeneous tracer accumulation in tumor lesions. Serial ^{89}Zr-bevacizumab PET showed that a therapeutic dose of bevacizumab and interferon-α reduced the tracer uptake.[21] This suggested that one therapeutic dose reduced access by this angiogenesis inhibitor to the tumor of the antibody. A ^{89}Zr-bevacizumab study in advanced non-small cell lung cancer (NSCLC) demonstrated a fourfold higher tracer uptake in tumor versus non-tumor tissue.[22] In children with diffuse intrinsic pontine glioma treated with radiotherapy, heterogeneity of ^{89}Zr-bevacizumab tumor uptake was shown.[23] ^{89}Zr-bevacizumab tracer uptake is not limited to malignant disease. In the presence of VEGF-A benign lesions can also be visualized, as exemplified in patients with von Hippel-Lindau disease.[24]

The use of molecular antibody imaging for tumor detection was explored in a large multicenter phase 3 trial in which 14 centers in the United States participated. Pre-surgical ^{124}I-girentuximab PET was compared to CT and histopathologic diagnoses in 195 patients with unclassified renal lesions. Girentuximab targets the membrane protein carbonic anhydrase IX (CA9), which is expressed in more than 95% of clear cell (cc) RCC. ^{124}I-girentuximab PET had both superior sensitivity and specificity to CT in identifying ccRCC from other renal masses, both benign and malignant.[25] This study showed the

possibility of performing a novel molecular imaging study across 14 centers. In a multicenter trial in patients with metastatic RCC with good or intermediate prognosis the value of ^{89}Zr-girentuximab PET combined with ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG)-PET is being tested to see whether it can help in selecting patients with relatively indolent disease for whom start of treatment can be postponed (ClinicalTrials.gov identifier NCT02228954).

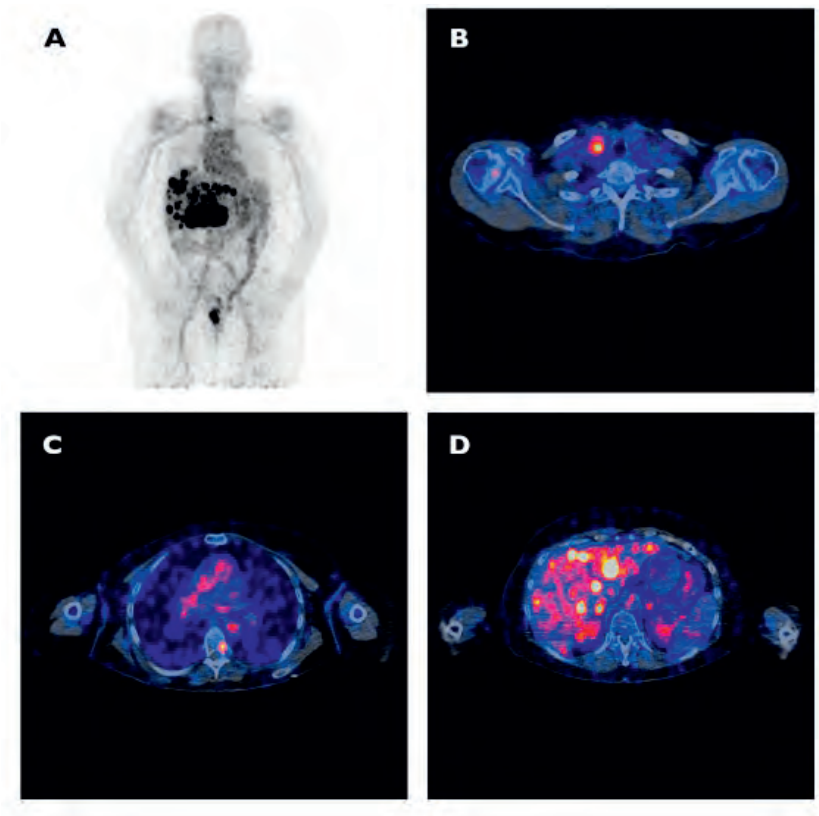

Figure 2: ^{89}Zr-trastuzumab-PET imaging
Patient with HER2-positive metastatic breast cancer. Imaged 4 days after injection with 37 MBq ^{89}Zr-trastuzumab and total protein dose of 50 mg. **A)** Maximum intensity projection (MIP) image of the ^{89}Zr-trastuzumab PET/CT-scan showing tracer presence in the circulation, uptake in intra-hepatic metastases, and intestinal excretion. **B)** Transverse plane of fused PET/ low dose CT of the chest with tracer uptake in cervical lymph node. **C)** Transverse plane with tracer uptake in metastasis left-sided in Th7. **D)** Transverse plane showing tracer uptake in liver metastases.

Molecular imaging in immunotherapy

Immune checkpoint inhibitors are immunomodulatory mAbs that block immune checkpoints by targeting CTLA-4, PD-1, or PD-L1 (Fig. 1C). These drugs show activity across multiple tumor types. The four immune checkpoint inhibitors ipilimumab, nivolumab, pembrolizumab (anti-PD-1) and atezolizumab (anti-PD-L1) are FDA and EMA approved to treat specific tumor types. However, not all patients benefit from these drugs and patients may experience major immune-related toxicities. Moreover, these drugs are extremely expensive. Molecular antibody imaging may provide insight into the immune response and might therefore support better patient and treatment selection.

Five preclinical studies with radiolabeled anti-PD-L1 antibodies showed antibody uptake in PD-L1 overexpressing tumors. These studies provided data on drug biodistribution and on the influence of dose escalation on target saturation in mice. In addition to tumor uptake, high tracer uptake was also observed in organs such as the spleen, thymus and lymph nodes.[26-30] This might reflect expression of PD-L1 by immune cells, including T cells, dendritic cells and macrophages.

Three preclinical molecular antibody imaging trials with radiolabeled anti-PD-1 antibodies to visualize T-cells in mice and one in non-human primates have been published. All studies showed tracer uptake patterns to be comparable to those of PD-L1 antibody in healthy mice, with uptake in tumor and secondary lymphoid organs such as spleen and lymph nodes.[29,31,32]

The first molecular antibody imaging clinical trials with immune checkpoint inhibitors are ongoing. One study is investigating the ^{89}Zr-labeled PD-L1 antibody atezolizumab in patients with bladder cancer, NSCLC and triple negative breast cancer (TNBC) (ClinicalTrials.gov identifier NCT02453984) and another is investigating ^{89}Zr-labeled PD-1 antibody pembrolizumab in melanoma and NSCLC patients (ClinicalTrials.gov identifier NCT02760225).

BiTEs are a relatively novel approach in immunotherapy (Fig. 1D). These bispecific antibodies consist of two linked, single-chain variable fragments directed against a surface target antigen on cancer cells and the cluster of differentiation 3ε (CD3ε) on T-cells. Simultaneous binding of tumor and T-cells mediates tumor directed T-cell cytotoxicity and cytokine production without the need for co-stimulatory molecules.[33] Blinatumomab, a CD19/CD3ε BiTE, is approved for the treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia. Two BiTEs have been radiolabeled with ^{89}Zr and studied in mice.[34,35] The epithelial cell adhesion molecule (EpCAM) targeting BiTE AMG 110 labeled with ^{89}Zr at a 20 μg dose was studied in nude BALB/c mice bearing EpCAM expressing colorectal cancer xenografts. Highest ^{89}Zr-AMG 110 uptake was found in kidneys, followed by liver and tumor.[34] AMG 211, a CEA/CD3ε directed BiTE radiolabeled with ^{89}Zr showed protein dose-dependent CEA-specific targeting of ^{89}Zr-AMG211 in mouse tumor xenograft models.[35] An ongoing clinical study is exploring the biodistribution of ^{89}Zr-AMG 211 in patients with gastrointestinal adenocarcinomas (ClinicalTrials.gov identifier NCT02760199).

Molecular imaging to study antibodies with a payload

ADCs are a subclass of antibody-related therapeutics (Fig. 1E). These drugs consist of a tumor specific mAb conjugated to a cytotoxic payload via a linker. ADCs are designed to improve the potency of chemotherapy by increasing the accumulation of the cytotoxic drug within neoplastic cells thereby reducing systemic toxic effects. The antibody part of the ADC does not need to exert a therapeutic effect as it serves as an anchor to deliver cytotoxins directly to cancer cells. Brentuximab vedotin and trastuzumab emtansine (T-DM1) are standard of care in respectively patients with CD30 positive Hodgkin's lymphoma or anaplastic large cell lymphoma and patients with HER2 overexpressing metastatic breast cancer. Currently, more than 80 ADCs are in clinical development.

The only molecular imaging study performed with a radiolabeled ADC involved brentuximab vedotin. In mice bearing xenografted tumors with varying levels of CD30 expression a tumor-to-blood ratio of 15.05 was seen for ^{89}Zr-brentuximab vedotin compared to 0.78 for ^{124}I-brentuximab vedotin 144 hours after administration, suggesting that ^{89}Zr was a more suitable radionuclide for this ADC.[36]

Radiolabeling ADCs themselves is considered to increase the risk of instability of the molecule. Therefore, radiolabeling the naked antibody that is part of an ADC for PET imaging is a safe alternative. The naked antibody uptake is assumed to reflect ADC uptake and thus might predict whether the patient will respond to ADC therapy. Three preclinical trials in mice and one study in both mice and non-human primates used this approach. Organ biodistribution and tracer tumor uptake was assessed.[37-40] One study explored three doses of ^{89}Zr-labeled naked antibody as part of an ADC targeting mesothelin in mice bearing human pancreatic tumor xenografts. Tumor uptake decreased with increasing doses of the naked mAb, indicating dose-dependent and saturable tracer distribution at doses of 25 and 100 µg in mice.[38] Biodistribution and tumor uptake were also investigated with ^{89}Zr-labeled anti-mesothelin naked antibody in patients subsequently treated with a mesothelin directed ADC. Results showed uptake of the radiolabeled naked antibody in pancreatic and ovarian tumors.[41] Others administered a CEACAM6 directed ADC to monkeys and assessed biodistribution with the naked ^{64}Cu-anti-CEACAM6 mAb. Highest tracer uptake was seen in the bone marrow. Neutropenia and anemia occurred in all animals treated with this ADC, suggesting tissue-specific toxicity can be predicted by antibody tracer uptake.[38]

Two clinical studies explore radiolabeled trastuzumab as a biomarker in predicting response to T-DM1 treatment in HER2 positive metastatic breast cancer. The ZEPHIR trial is designed to prospectively investigate the role of pretreatment ^{89}Zr-trastuzumab PET combined with early response assessment using FDG-PET to select patients with metastatic HER2-positive tumors unlikely to benefit from T-DM1 treatment. An analysis of the first 56 patients showed that a negative ^{89}Zr-

trastuzumab PET and absence of response on early FDG-PET resulted in a negative predictive value of 100% for response according to RECIST 1.1 criteria. Substantial inter- and intrapatient heterogeneity of tracer uptake was observed. Sixteen out of 56 HER2-positive patients (29%) had a negative ^{89}Zr-trastuzumab PET result and intra-patient heterogeneity was detected in 46% of patients.[18] The same approach is ongoing for ^{64}Cu-labeled trastuzumab in predicting response to T-DM1 therapy (ClinicalTrials.gov identifier NCT02226276).

Antibodies can also function as targeted delivery vehicles for radionuclides as part of RIT to selectively kill tumor cells (Fig. 1F). Currently, ^{90}Y-ibritumomab tiuxetan is approved for treatment of B-cell non-Hodgkin lymphoma. An example of RIT that is being investigated in mice is the ^{177}Lu-labeled CD37 directed antibody targeting B lymphocytes for the treatment of B-cell non-Hodgkin lymphoma.[42]

Translation of molecular antibody imaging to clinical practice

There are a number of challenges in translating (pre)clinical antibody imaging studies using theranostics to standardized and ultimately daily-routine patient-care. Knowledge from preclinical models can often not be extrapolated to humans unconditionally since most antibodies are specific for human targets. In addition, until now, most clinical trials with mAb or antibody-related therapeutics have been performed in relatively small groups of patients, precluding firm conclusions regarding clinical relevance. Performing larger studies will require harmonization and standardization of the radiolabeling and imaging procedures across centers as well as proper access to the required radionuclide. Larger studies using ^{89}Zr are easily feasible since transport of this nuclide or ^{89}Zr-labeled drugs can be well organized because of the relatively long physical half-life. The availability of ^{64}Cu is more limited by its relatively fast decay.

When multicenter studies are performed, evidence that the final radiolabeled drug products and manufacturing processes are comparable should be provided for all steps in the manufacturing

process that are conducted at more than one center. Fortunately, it is increasingly possible to access templates for routine documentation such as Investigational Medicinal Product Dossiers (IMPDs) for mAb or antibody-related tracers.[43,44]

We identified 46 medical centers, including 24 in the US, 18 in Europe, 3 in Asia and 1 in Australia, which recently participated in clinical trials with antibody or antibody-based PET theranostics. ^{89}Zr is by far the most used positron-emitting nuclide for antibody labeling. It is encouraging that of the 24 antibodies or antibody-related therapeutics labeled with several PET-radionuclides that have been investigated as theranostics in patients, 11 were investigated in the multi-center setting.

Finally, the integration of antibody PET imaging in clinical practice is costly. For instance, mAb labeling and a series of PET scans in one patient costs several thousand US Dollars. However, when proven valuable for making clinical decisions based on whole-body information obtained with molecular antibody imaging, a theranostic approach might in the end prevent expensive treatment of patients that do not benefit from therapy due to lack of target expression or drug uptake and might therefore lead to fewer side effects and better outcomes.

CONCLUSION

Theranostics with antibodies and antibody-related therapeutics can provide meaningful in vivo insight in biodistribution and tumor uptake of radiolabeled drugs. This approach is currently being investigated extensively across numerous centers. Properly powered studies are required to prove that theranostics can play an important role in drug development and become a valuable tool in patient selection for antibody based therapies.

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