Antibody imaging as biomarker in early cancer drug development and treatment

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CHAPTER 2

Molecular imaging of tumors with radioactive labeled antibodies from laboratory to the clinic

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Background

Many novel molecular targets for anticancer treatment have been discovered, resulting in development of numerous targeted anticancer drugs, including therapeutic monoclonal antibodies (mAb). Molecular imaging using radiolabeled mAbs can identify noninvasively the presence of specific targets against which the antibody is raised. Moreover it provides whole-body information about tumor uptake and organ distribution of the antibodies. Depending on the radiation characteristics of radioisotope labeled to the antibody imaging can be performed with planar scintigraphy and single photon emission computed tomography (SPECT) or positron emission tomography (PET). This approach can potentially serve in the clinic for patient selection, tumor staging, drug development and as (early) predictive biomarker for tumor response.

In the late 1970s, first clinical studies showed that radiolabeled antibodies could visualize tumors. Currently fully human mAbs and a suitable radioisotope for PET-imaging is available, zirconium-89 ($^{89}$Zr). $^{89}$Zr has been labeled to various antibodies and tested successfully preclinically and clinically.

Discussion

Tracer development and preclinical validation

ImmunoPET is defined as the tracking and quantification of radiolabeled mAbs with PET in vivo. MAbs are large molecules penetrating slowly but constantly into solid tumor tissue. Given their low serum clearance, this leads slowly to an increasing tumor accumulation over days. For adequate visualization, the physical half-life (t½) of the radioisotope should be compatible with the relative long biological t½ of the antibody. Therefore indium-111 ($^{111}$In; t½ = 67.3 h) is of interest for imaging with gamma-camera (SPECT) and $^{89}$Zr (t½ = 78.4 h) for PET imaging. Imaging with gamma-emitting isotopes iodine-131 ($^{131}$I, t½ = 192.5 h), iodine-123 ($^{123}$I, t½ = 13.2 h), technetium-99m ($^{99m}$Tc, t½ = 6.0 h) and $^{111}$In showed the proof-of-principle of antibody imaging. For PET imaging the positron-emitting isotopes copper-64 ($^{64}$Cu, t½ = 12.7 h), iodine-124 ($^{124}$I, t½ = 100.2 h) and $^{89}$Zr have been used. $^{89}$Zr is coupled to the lysine residues of a mAb via a chelate, with a multistep procedure using a succinylated- derivative of desferrioxamine B (N-sucDf) as bifunctional chelate. In contrast to the directly labeled $^{124}$I, $^{64}$Cu and $^{89}$Zr are trapped inside the cell after internalization of the mAb (residualization).

ImmunoPET studies in human tumor bearing mice can show the efficiency of tumor targeting with a particular mAb. New antibody based radiopharmaceuticals are tested optimally in a dose-escalation study combined with imaging in different tumor models with variable antigen expression and compared to non-specific tumor uptake. Both soluble targets, such as vascular endothelial growth factor (VEGF) and transforming growth factor
ß (TGF-ß), as well as receptors such as HER2 and proteins expressed on the cell membrane can be visualized. Antibody based imaging cannot visualize intracellular targets. We developed \(^{89}\text{Zr}\)-bevacizumab (binds VEGF), \(^{89}\text{Zr}\)-fresolimumab (binds mammalian active TGF-ß isoforms), \(^{89}\text{Zr}\)-trastuzumab (binds HER2), and showed in human tumor bearing mice a quantitative indication of the distribution and specific tumor accumulation of these antibodies.\(^9\)–\(^{11}\) Moreover, immunoPET can evaluate changes in the target by drug treatment. We were thus able to visualize downregulation of HER2 and VEGF tumor expression by HSP90 inhibitor treatment.\(^{12}\)\(^{13}\) These studies showed that radiopharmaceuticals such as \(^{89}\text{Zr}\)-trastuzumab, \(^{89}\text{Zr}\)-bevacizumab and an anti-VEGF fragment \(^{89}\text{Zr}\)-ranibizumab have the potential to be used as an early read out of target effects of drugs (Fig. 1B).\(^{12}\)\(^{14}\)

**Figure 1.** Schematic overview of (A) radiopharmaceutical development for PET and (B) application as pharmacodynamic biomarker for tumor response assessment.

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**Translation of preclinical developed labeled antibodies to clinical applicable tracers**

To implement immunoPET from preclinical investigations to clinical trials a cross-disciplinary team of chemists, pharmacists, biologists, physicists and MDs is required to take care of tracer production, image quantification, clinical validation, standardization, trial design and execution.
The radiopharmaceutical/investigational medicinal product (IMP) has to be produced under current good manufacturing practice (cGMP) guidelines. $^{89}$Zr-oxalate is nowadays commercially available. Quality control, to be executed after radiolabeling, includes tests to assess the chelation ratio, appearance, (radiochemical) purity, mAb integrity, immunoreactivity, sterility and apyrogenicity. All data needs to be collected in the investigational medicinal product dossier (IMPD), which is required for approval of clinical trials by the internal and competent authorities in the EU. The IMPD includes information related to the quality, manufacture and control of the Investigational Medicinal Product, data from non-clinical studies and from its clinical use. In the US an investigational new drug (IND) study can be performed with radiolabeled antibodies. The IND, including the same information as the IMPD, as well as the clinical trial should be submitted to the FDA after approval by all internal committees. The IND does not necessarily have to include dosimetry but should preferably contain a plan for collection and analysis of dosimetry data. Limited toxicity studies are required as the radiolabeled antibodies can be administered at doses that are already known to have no pharmacologic effect in humans.

**Clinical applications**

The feasibility of radiolabeled antibodies for clinical tumor imaging was demonstrated for $^{111}$In-trastuzumab with SPECT imaging in metastatic breast cancer patients with HER2 positive disease, in which 45% of lesions visible on conventional imaging was detected, as well as newly discovered lesions in 13 of 15 patients.\(^\text{15}\) This experience obtained with gamma-camera imaging was translated to PET imaging when the PET isotope $^{89}$Zr became available. $^{89}$Zr-U36,\(^\text{16}\) $^{89}$Zr-rituximab, $^{89}$Zr-trastuzumab,\(^\text{17}\) $^{89}$Zr-cetuximab, $^{89}$Zr-bevacizumab\(^\text{18}\) and $^{89}$Zr-ibritumomab tiuxetan\(^\text{19}\) were produced and administered to patients. After intravenous bolus injection of the radiolabeled antibody, patients should be observed the time required for acute adverse or allergic reactions to the antibody. Then 4-5 days after the $^{89}$Zr-antibody tracer injection the PET scan is performed, which was found to be the optimal scanning moment to assess $^{89}$Zr-labeled antibody uptake in patients.\(^\text{17}\) This is based on the relatively slow accumulation of the antibody and the radioactive decay of $^{89}$Zr in conjunction with a relative small radioactivity dose. Serial $^{89}$Zr-immunoPET-scans can be performed, for example to assess effects of antibody therapy on the tumor target saturation. Based on antibody characteristics, radioactive decay and dose of $^{89}$Zr, the second tracer injection should be ~14 days after the former $^{89}$Zr injection.

PET findings can be fused with computed tomography (CT) or magnetic resonance imaging (MRI) imaging. Increasingly imaging platforms are available that allow to perform simultaneously PET and CT and more recently also PET and MRI. PET and SPECT imaging provides information of biological processes at the molecular and cellular levels whereas CT and MRI produce detailed anatomical images at high spatial resolution. The resolution of current PET/CT scanners, together with a 37 MBq $^{89}$Zr-mAb dose, allows the detection of lesions $\geq 1$ cm. Using radiolabeled antibodies involves exposure to ionizing radiation. Dosimetry studies have estimated the radiation dose for 37 MBq of $^{89}$Zr labeled antibodies to be 20 mSv.\(^\text{20}\)
The first clinical trial with a $^{89}$Zr labeled antibody was performed with U36, a chimeric monoclonal antibody, in head and neck squamous cell carcinoma patients. All primary tumors and lymph node metastases in 18 of 25 positive neck levels as present during surgery were detected. The 7 non-detected tumor-involved lymph node levels were small lymph nodes with only a limited tumor involvement. $^{89}$Zr-trastuzumab in metastatic breast cancer patients with HER2 positive disease showed excellent tumor uptake and visualization of known tumor lesions in the liver, lung and bone. Moreover unknown brain metastases were detected. $^{89}$Zr-bevacizumab-PET in metastatic renal cell cancer patients visualized tumor lesions with high tumor to background ratios. $^{89}$Zr-bevacizumab PET studies in primary breast, renal cell and neuroendocrine tumor patients are currently ongoing.

Finally radiolabeled antibodies can be used for clinical dilemmas such as in a patient with a HER2+ and a HER2- breast cancer who develops metastases. $^{89}$Zr-trastuzumab-PET scan showed mediastinal metastasis, leading to initiation of anti-HER2 therapy.

Future

Soon much information will become available from recently finalized and ongoing clinical studies with $^{89}$Zr-antibody imaging. ImmunoPET is expected to be able to support drug development in early clinical trials of new antibodies by determining the relationship between target expression as measured with $^{89}$Zr-antibody tumor uptake and response to therapy. Also visualization of organ distribution and target accumulation of $^{89}$Zr labeled antibody might be useful to determine the optimal drug dose. Besides drug development, this dose finding might also be implemented in individual patients.

Using molecular imaging with radiolabeled antibodies permits serial characterization of the tumor as pharmacodynamic biomarker for treatment follow up at the whole-body level. It is well known that different tumor lesions have variable expression of targets over time and even heterogeneity exists within one lesion. Furthermore during treatment it may be helpful to evaluate whether the drug is achieving the desired effect on its target. In an ongoing study breast cancer patients receive the HSP90 inhibitor NVP-AUY922 and get a $^{89}$Zr-trastuzumab or $^{89}$Zr-bevacizumab PET (depending on HER2 expression) before treatment as well as early during treatment. By using the obtained PET images as pharmacodynamic biomarker for early prediction of response it becomes potentially possible to timely change the treatment plan and spare patients from side effects.

Robust trials need to be performed comparing presence of the target in tumor lesions with immunohistochemical staining of a biopsy with the molecular whole-body tumor profile using a $^{68}$Zr-immunoPET. When proven relevant for optimizing the right moment and dose for targeted therapy the next challenge is to make these novel PET-tracers more widely available. The t1/2 and stability of the $^{89}$Zr radiopharmaceutical allows to produce it at a significant distance of the tracer administration and scanning.

Antibody-drug conjugates (ADCs) such as trastuzumab-DM1, deliver toxins specifically
to the tumor and are a promising new class of targeted therapies. Numerous ADCs are currently in clinical development. To determine the amount of toxin delivered to the tumor, immunoPET might be used to calculate the targeting of the compound to the tumor by labeling the naked antibody with $^{89}$Zr. To determine radiation doses of radioimmunotherapy, $^{89}$Zr-mAbs can also be used to scout the uptake of therapeutic $^{90}$Y- and $^{177}$Lu-mAb conjugates.\(^\text{19}\)

Moreover, the knowledge obtained with $^{89}$Zr-labeled antibodies can be translated to optical imaging. Recently we developed IRDye 800CW-labeled bevacizumab and trastuzumab, and verified the results with $^{89}$Zr-bevacizumab and $^{89}$Zr-trastuzumab-PET. The tumor-targeted near-infrared fluorescence–labeled therapeutic antibodies showed specific tumor detection in vivo in a preclinical setting, using the real-time intraoperative clinical prototype camera system.\(^\text{25}\) Clinical testing with the fluorescent labeled antibodies has started based on a similar procedure as used for the radiolabeled antibodies.
References
