Immuno-PET: A Navigator in Monoclonal Antibody Development and Applications

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Discuss the technical advances that have led to recent rapid developments in monoclonal antibody imaging techniques.

2. List the monoclonal antibodies that are currently available for cancer imaging and cancer therapy.

3. Identify potential roles for immuno-PET in cancer staging and treatment selection.

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ABSTRACT

Monoclonal antibodies (mAbs) have been approved for use as diagnostics and therapeutics in a broad range of medical indications, but especially in oncology. In addition, hundreds of new mAbs, engineered mAb fragments, and nontraditional antibody-like scaffolds directed against either validated or novel tumor targets are under development. Immuno-positron emission tomography (PET), the tracking and quantification of mAbs with PET in vivo, is an exciting novel option to improve diagnostic imaging and to guide mAb-based therapy. In this review, recent technical advances leading to a jump ahead in mAb imaging are discussed. The availability of proper positron emitters, sophisticated radiochemistry, and advanced PET and PET–computed tomography scanners is crucial in these developments. Immuno-PET might play an important future role in cancer staging, in the improvement and tailoring of therapy with existing mAbs, and in the efficient development of novel mAbs. An overview of the preclinical and first clinical immuno-PET studies is provided. The Oncologist 2007;12:1379–1389

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GENERAL INTRODUCTION
Recent advances in molecular and cellular biology have facilitated the discovery of novel molecular targets on tumor cells, for example, key molecules involved in proliferation, differentiation, cell death and apoptosis, angiogenesis, invasion, and metastasis or associated with cancer cell stemness [1]. This knowledge has boosted the design of targeted pharmaceuticals, with monoclonal antibodies (mAbs) forming the most rapidly expanding category. mAbs can be used as disease-specific contrast agents for diagnostic imaging. To date, the U.S. Food and Drug Administration (FDA) has approved five diagnostic mAbs, including four for the detection of cancer. In addition, mAbs are gaining momentum for use in disease-selective therapy. Presently, 21 mAbs (all intact immunoglobulins) have been approved by the FDA for therapy, most of them for systemic treatment of cancer. The yearly sales of mAbs were estimated to be 8–10 billion dollars in 2005, and are expected to rise spectacularly to 20–30 billion dollars in 2010 [2]. Hundreds of new mAbs are under development worldwide.

The introduction of immuno-positron emission tomography (PET), the combination of PET with mAbs, is an attractive novel option to improve diagnostic tumor characterization, because it combines the high sensitivity and resolution of a PET camera with the specificity of a mAb [3]. Immuno-PET is like performing “comprehensive immunohistochemical staining in vivo,” for which purpose the mAb has to be labeled with a positron emitter to enable visualization with a PET camera. In fact, each mAb targeting a specific tumor cell surface marker or extracellular matrix component is a candidate for use in immuno-PET. This allows for the development of a new generation of mAb-based imaging probes in addition to existing PET tracers, of which the non–tumor-specific metabolic tracer 18-fluoro-2-deoxy-D-glucose (18FDG) is currently used in >90% of all PET imaging procedures.

Apart from its imaging capabilities, PET also has the potential for quantification of molecular interactions, which is especially attractive when immuno-PET is used as a prelude to therapy with one of the approved mAbs. In a personalized therapeutic approach, immuno-PET enables the confirmation of tumor targeting and the quantification of mAb accumulation (in fact, radioactivity uptake). Thus, patients might be selected who have the best chance to benefit from expensive mAb-based therapy, while treatment schedules can be adapted to improve treatment efficacy and/or reduce toxicity. Moreover, immuno-PET might also play a role in the efficient selection, characterization, and optimization of novel high-potential mAbs or mAb conjugate candidates for diagnosis and therapy.

THE ANTIBODY REVOLUTION
A century ago, Paul Ehrlich postulated the notion that a “magic bullet” could be developed to selectively target disease. He envisioned that antibodies could act as such [4]. The introduction of hybridoma technology for mAb development by Köhler and Milstein in 1975 turned this magic bullet concept into a realistic option [5]. With this technology, an unlimited range of mAbs can be obtained against any particular cellular antigen. However, the first generations of mAbs had limitations for clinical use, because their murine origin made them immunogenic. Developments in recombinant DNA technology circumvented this, resulting in the production of chimeric (c-mAb), humanized (h-mAb), and complete human mAbs. Besides intact mAb molecules (molecular weight, ~150 kDa), mAb fragments and engineered variants are also used, like F(ab′)2, F(ab′), Fab, single chain Fv (scFv), and the covalent dimers scFv2, diabodies, and minibodies (molecular weights ranging from 25 to 100 kDa) [6], as well as several types of protein therapeutics based on nontraditional scaffolds, like, for example, domain antibodies, affibodies, nanobodies, and anticilins [7].

Intact mAbs have a long residence time in humans, ranging from a few days to weeks, which results in optimal tumor-to-nontumor ratios at 2–4 days postinjection (p.i.). In contrast, mAb fragments have a much faster blood clearance. This results in higher tumor-to-nontumor ratios at earlier time points p.i., but the absolute tumor uptake is often lower. These characteristics in general make intact mAbs the format of choice for therapy, while the optimal format for diagnosis is still under discussion. New strategies include the use of pretargeting approaches, which involve separating the targeting antibody from the subsequent delivery of an imaging or therapeutic agent that binds to the tumor-localized antibody [8].

For diagnostic purposes, mAbs have been labeled with γ-emitting radionuclides and imaged with a single photon emission computerized tomography (SPECT) camera. Until now, five technetium-99m (99mTc)- or indium-111 (111In)-labeled murine mAbs (m-mAbs) have been approved by the FDA for diagnostic imaging, among which four are for the imaging of cancer [9]. These comprise samumomab pendetide for imaging ovarian and colorectal cancer (OncoScint™; Cytogen, Princeton, NJ; 111In-labeled IgG binding to the tumor-associated glycoprotein 72 antigen), acritumomab for imaging of colorectal cancer (CEA-Scan™; ImmunoMedics, Morris Plains, NJ; 99mTc-labeled F(ab′)) to carcino-embryonic antigen (CEA), nolatumomab merpentan for imaging small cell lung cancer (Verluma™; Boehringer Ingelheim, Ingelheim, Germany; 99mTc-labeled Fab to epithelial cell adhesion molecule),...
and capromab pendetide for imaging prostate cancer (ProstaScint™; Cytogen; 111In-labeled IgG to prostate specific membrane antigen). These early-generation diagnostic mAbs are mainly used for staging disease in patients suspected of recurrent or metastatic disease, but their overall clinical impact has not been impressive thus far. The use of better mAb formats directed against more valuable targets (e.g., also suitable for therapy), in combination with sophisticated cameras for imaging, might change this landscape.

Currently, nine mAbs have been approved for cancer therapy, all being intact mAbs (Table 1). Five of the mAbs have been approved for the treatment of hematological malignancies: rituximab, gemtuzumab ozogamicin, alemtuzumab, ibritumomab tiuxetan, and tositumomab. Four mAbs have been approved for therapy of solid tumors: trastuzumab is used for the treatment of metastatic breast cancer; cetuximab, bevacizumab, and panitumomab have been approved for the treatment of metastatic colorectal cancer; while cetuximab and bevacizumab have also been approved for the treatment of head and neck cancer and non-small cell lung cancer, respectively. These “solid tumor mAbs” are, in general, most effective when combined with chemo- or radiotherapy. They interfere with signal transduction pathways by targeting growth factors or their receptors, the key drivers of tumor growth and survival. In addition, most of the naked therapeutic mAbs can also act by other effector mechanisms, like antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, or induction of apoptosis.

To enhance its therapeutic potency, gemtuzumab has been armed with the supertoxic drug ozogamicin, while ibritumomab tiuxetan (Zevalin®; Biogen Idec Inc., Cambridge, MA) and iodine I-131 tositumomab (Bexxar®; GlaxoSmithKline, Philadelphia) are radiolabeled mAbs containing the β-emitters yttrium-90 (90Y) and iodine-131 (131I), respectively.

### Table 1. Therapeutic monoclonal antibodies approved for cancer treatment

<table>
<thead>
<tr>
<th>FDA approval year</th>
<th>Generic name (trade name)</th>
<th>Target</th>
<th>Type</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Rituximab (Rituxan®; Genentech, Inc., South San Francisco, CA)</td>
<td>CD20</td>
<td>Chimeric IgG1</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>1998</td>
<td>Trastuzumab (Herceptin®; F. Hoffmann-La Roche, Basel, Switzerland)</td>
<td>HER-2/neu</td>
<td>Humanized IgG1</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>2000</td>
<td>Gemtuzumab ozogamicin (Myelotarg®; Wyeth Pharmaceuticals, Inc., Madison, NJ)</td>
<td>CD33</td>
<td>Humanized IgG1 conjugated to calicheamicin</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>2001</td>
<td>Alemtuzumab (Campath®; Berlex Inc., Wayne, NJ)</td>
<td>CD52</td>
<td>Humanized IgG1</td>
<td>Chronic lymphatic leukemia</td>
</tr>
<tr>
<td>2002</td>
<td>90Y-ibritumomab tiuxetan (Zevalin®; Biogen Idec Inc., Cambridge, MA)</td>
<td>CD20</td>
<td>90Y-radiolabeled murine IgG1</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>2003</td>
<td>131I-Tositumomab (Bexxar®; GlaxoSmithKline, Philadelphia)</td>
<td>CD20</td>
<td>131I-radiolabeled murine IgG2a</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>2004</td>
<td>Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA)</td>
<td>VEGF</td>
<td>Humanized IgG1</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>2006</td>
<td>Cetuximab (Erbitux®; ImClone Systems, Inc., New York)</td>
<td>EGFR</td>
<td>Chimeric IgG1</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>2006</td>
<td>Panitumumab (Vectibix®; Amgen Inc., Thousand Oaks, CA)</td>
<td>EGFR</td>
<td>Human IgG1</td>
<td>Colorectal cancer</td>
</tr>
</tbody>
</table>

*aConjugated antibodies.

Abbreviations: Y, yttrium; I, iodine; CD, cluster of differentiation; EGFR, epidermal growth factor receptor; FDA, U.S. Food and Drug Administration; HER-2/neu, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor.

Because of a ‘cross-fire’ effect, radionuclides are especially attractive as warheads to be used in radioimmunotherapy (RIT), because, in order to be effective, not all tumor cells have to be targeted by radiolabeled mAbs.

The therapeutic value of the aforementioned mAbs has been outlined in several excellent reviews [10, 11]. Clinical success with the aforementioned therapeutic mAbs has boosted research and development on new mAbs enormously [12].

**ADDED VALUE OF ANTIBODY IMAGING**

Despite clinical optimism, it is fair to state that the efficacy of current mAbs is still quite limited, with benefit for just a portion of patients. Moreover, the costs of mAb therapy are excessive, and this item became the subject of national discussions about right to cancer care [13, 14]. The question is how to improve the efficacy of mAb-based therapy and how to identify patients with the highest chance of benefit. In other words: when, how, and for whom should antibody-based therapy be reserved? Stakeholders in these discussions are physicians, pharmaceutical and insurance companies, health care authorities, and first of all patient groups.

We foresee that quantitative imaging of mAbs can be a valuable tool at several stages of mAb development and application. From first-in-man clinical trials with new mAbs, it is important to learn about the ideal mAb dosing for optimal tumor targeting (e.g., saturation of receptors), the uptake in critical normal organs to anticipate toxicity, and the interpatient variations in pharmacokinetics and tumor targeting. mAb imaging might provide this information in an efficient and safe way, with fewer patients treated at suboptimal doses. This approach is especially attractive when the mAb of interest is directed against a novel tumor target that has not been validated in clinical trials before. Just to exemplify, mAbs directed against new promising tumor cell and tumor stroma targets, like c-Met, vascular endothelial growth factor receptor (VEGFRs), insulin-like growth factor receptors, and death receptors, belong to this category.

Quantitative mAb imaging might also be of value to guide the optimal use of FDA-approved mAbs. In current practice, tissue analyses are often performed to confirm target expression and to select patients for mAb therapy. For example, patients with metastatic breast cancer are only eligible for therapy with the anti–human epidermal growth factor receptor (HER)-2 mAb trastuzumab when protein expression and to select patients for mAb therapy. For example, patients with metastatic breast cancer are only eligible for therapy with the anti–human epidermal growth factor receptor (HER)-2 mAb trastuzumab when protein overexpression or gene amplification has been confirmed on a biopsy of the tumor by immunohistochemistry or fluorescence in situ hybridization (in 20%–30% of patients). It is questionable, however, whether a representative overview of in vivo HER-2 expression status can be obtained by analysis of just one single biopsy. It is possible that HER-2 expression in the primary tumor and metastatic lesions differ, or does not remain stable during the course of the disease, for example, upon chemotherapy and/or hormonal therapy [15–18]. Taking multiple or repeated biopsies is not a solution, especially because lesions are often heterogeneous (resulting in nonrepresentative biopsies) and not easily accessible. It is of note that HER-2 also has a functional role in normal tissues like the heart. This is probably the reason for the cardiotoxicity induced by trastuzumab, especially when combined with anthracyclines. Interestingly, shortly after completion of anthracycline treatment, myocardial HER-2 overexpression was demonstrated in 50% of patients [19]. Therefore, it seems worthwhile to evaluate the value of trastuzumab imaging for the prediction of cardiotoxicity [19, 20].

Pretherapy imaging might have added value for patient selection, because it can be used to assess target expression and mAb accumulation in all tumor lesions and normal tissues, noninvasively, quantitatively, and even over time (four-dimensional). This information might be particularly relevant when mAb therapy is combined with other treatment modalities like chemotherapies and radiotherapy, to find routes to maximum synergism. Ideally, topographic information on tumor extension is obtained to enable assessment of homogeneity of mAb tumor accumulation.

Until now, pretherapy antibody imaging has mostly been applied as a prelude to RIT for assessment of dosimetry [3]. In this setting, quantitative imaging is particularly attractive because of the small therapeutic window of RIT, with bone marrow toxicity being dose limiting.

**ANTIBODY IMAGING: FROM SPECT TO PET**

Before PET technology became broadly available, extensive experience was gained with SPECT camera imaging of mAbs (radioimmunoscntigraphy [RIS]). For this purpose, a gamma-emitting radionuclide has to be coupled to the mAb, for example, $^{99m}$Tc, $^{111}$In, $^{131}$I, or rhenium-186 ($^{186}$Re). The type of information obtained with this technology is illustrated by images from our own archives (Figs. 1 and 2) using mAbs that were promising in those days on the basis of preclinical data. Figure 1 shows a planar image obtained with the $^{186}$Re-labeled c-mAb U36 in a patient with a tumor of the oropharynx. The c-mAb U36 is an antibody directed against CD44v6, a target associated with tumor metastasis and cancer cell stemness. c-mAb U36 showed selective tumor targeting, and even 14 days p.i. there were still high tumor levels of c-mAb U36 [21]. These images justified further development of c-mAb U36 for therapeutic purposes. This was not the case for a second c-mAb called SF-25. The c-mAb SF-25 had shown a very promising reactivity profile in a large panel of normal and tumor tissues, and had demonstrated antimetastatic potential in...
tumor-bearing nude mice, but had never been evaluated in patients before [22]. Figure 2 shows an image with 99mTc-labeled c-mAb SF-25 of the first head and neck cancer patient in the study [23]. Instead of selective tumor targeting, extensive uptake was observed in the liver, spleen, skeleton, and brain. The latter appeared to be a result of mAb crossreactivity with endothelium of the blood vessels in the brain, which had not been noticed in previous immunohistochemistry analyses. The massive accumulation in the liver, spleen, and bone marrow can be explained by the reactivity of SF-25 with Kupffer cells in the liver, the red pulpa and follicle centra in the spleen, and cellular components in the sediment of bone marrow aspirates, as was observed in immunohistochemical evaluation of SF-25. This one and only image let the sponsor of this study decide to discontinue clinical development of this particular mAb.

Although these SPECT camera images were very informative, there was still room for improvement, as illustrated by Figure 1. For example, the images did not provide much detail of nontarget organs. The fact that the tumor looked much larger than in reality also has to do with the limited resolution. No anatomical details are visible. Most importantly, a need for more accurate quantification was a reason to explore the potential of PET for mAb imaging.

**IMMUNO-PET: PRINCIPLES AND TECHNICAL DEVELOPMENTS**

Immun-PET is based on the coincidental detection of a mAb labeled with a positron-emitting radionuclide. The emitted positron will travel a distance of a few millimeters, depending on the initial positron energy and the density of the surroundings (Table 2). After having lost its kinetic energy, combining with an electron leads to the so-called annihilation process, yielding two photons, each with an energy of 511 keV emitted simultaneously in opposite directions. The distribution of a PET conjugate in a patient can be monitored by detection of the annihilation photon pairs with a PET camera. A PET camera consists of a ring of detectors placed around the body of the patient. If two photons are registered by detectors on opposite sides of the body within a very short
time interval (typically 5–15 nanoseconds), it is assumed that somewhere along the line between the two detectors an annihilation event has taken place. By calculating the crossing of all lines, the location of the radiation source (radiolabeled mAb) can be determined. For quantification, PET can provide reliable information when appropriate corrections are performed [3].

In 1975, the first real PET scanner containing thallium-doped sodium iodide detectors was developed [24]. Since then, the PET scanner has undergone several improvements with respect to scintillation crystals and system design. Scintillation materials have been developed with much better efficiency for detecting 511-keV photons. One of the most recent achievements is the revival of time-of-flight scanners [25], after their introduction in the beginning of the 1980s [26]. It is clear that these technical advances will further improve PET sensitivity (less radioactivity needed) and resolution.

To facilitate accurate interpretation of PET images and quantification, PET can be combined with computed tomography (CT) or magnetic resonance imaging (MRI) to allow simultaneous registration of both biologic function and anatomy. Nowadays, about 90% of all PET sales comprise PET/CT scanners. Although combining PET with MRI is technologically more challenging because of the strong magnetic fields restricting the use of certain electronic components, the first MRI-compatible PET scanners have been developed [27].

### POSITRON EMITTERS FOR IMMUNO-PET

For a positron emitter to be appropriate for immuno-PET, it has to fulfill several requirements. The positron emitter should have appropriate decay characteristics for optimal resolution and quantitative accuracy, its production should be easy and cheap, and it should allow facile, efficient, and stable coupling to mAbs. Maintenance of the antibody’s in vivo binding and biodistribution characteristics is imperative, while the physical half-life ($t_{1/2}$) of the positron emitter should be compatible with the time needed for a mAb or mAb fragment to achieve optimal tumor-to-nontumor ratios (typically 2–4 days and 2–6 hours, respectively).

Given these considerations, the following positron emitters for immuno-PET are under investigation at this moment: gallium-68 ($^{68}$Ga; $t_{1/2}$, 1.13 hours), fluorine-18 ($^{18}$F; $t_{1/2}$, 1.83 hours), copper-64 ($^{64}$Cu; $t_{1/2}$, 12.7 hours), yttrium-86 ($^{86}$Y; $t_{1/2}$, 14.7 hours), bromine-76 ($^{76}$Br; $t_{1/2}$, 16.2 hours), zirconium-89 ($^{89}$Zr; $t_{1/2}$, 78.4 hours), and iodine-124 ($^{124}$I; $t_{1/2}$, 100.3 hours) (Table 2). While the very short-lived positron emitters $^{68}$Ga and $^{18}$F can only be used in combination with mAb fragments or in pretargeting approaches, $^{89}$Zr and $^{124}$I are particularly suitable in combination with intact mAbs, because their long half-lives allow

<table>
<thead>
<tr>
<th>Positron emitter</th>
<th>Production</th>
<th>Half-life (hours)</th>
<th>Main $\beta^+$ energies (keV) (%)</th>
<th>Intrinsic spatial resolution loss (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{68}$Ga</td>
<td>$^{68}$Ge/$^{68}$Ga generator</td>
<td>1.13</td>
<td>1,899</td>
<td>87.9</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>$^{18}$O(p,n)</td>
<td>1.83</td>
<td>634</td>
<td>100.0</td>
</tr>
<tr>
<td>$^{64}$Cu</td>
<td>$^{64}$Ni(d,2n)</td>
<td>12.7</td>
<td>653</td>
<td>17.4</td>
</tr>
<tr>
<td>$^{86}$Y</td>
<td>$^{86}$Sr(p,n)</td>
<td>14.7</td>
<td>1,221</td>
<td>11.9</td>
</tr>
</tbody>
</table>
| $^{76}$Br | $^{75}$As(3He,2n)  
$^{76}$Se(p,n) | 16.2 | 871 | 6.3 | 5.3 |
| $^{89}$Zr | $^{89}$Y(p,n)  
$^{124}$Te(p,n) | 78.4 | 897 | 22.7 | 1.0 |
| $^{124}$I | $^{124}$Te(d,2n) 
$^{128}$I(p,n) | 100.3 | 1,535 | 11.8 | 2.3 |

Abbreviations: As, arsenic; Br, bromine; Cu, copper; F, fluorine; Ga, gallium-68; Ge, germanium; He, helium; I, iodine; Ne, neon; Ni, nickel; O, oxygen; Se, selenium; Sr, strontium; Te, tellurium; Y, yttrium; Zr, zirconium.

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Table 2. The main characteristics of positron emitters used in preclinical and clinical radioimmunoscintigraphy studies

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imaging at late time points for obtaining maximum information. The long half-life also offers an advantage for logistics related to transportation. A possible disadvantage is a greater radiation burden to patients, estimated at 20–40 mSv when using a 75-MBq radioactivity dose, but this aspect will become less critical when scanners become more sensitive. While the positron emitters 76Br and 124I can be coupled directly to mAbs, the others require indirect labeling methods, using bifunctional chelates or other prosthetic groups.

Another important consideration in the choice of positron emitter is whether the mAb becomes internalized after binding to the target antigen. Degradation of 76Br- and 124I-labeled mAbs upon internalization results in rapid clearance of these radionuclides from the target cells, and therefore the PET image shows less tumor contrast and does not reflect the actual mAb distribution. In contrast, when 68Ga-, 64Cu-, 86Y-, and 89Zr-labeled mAbs are processed, the positron emitters are trapped intracellularly in lysosomes. This phenomenon of residualization should be taken into account when selecting a positron emitter for immuno-PET applications. For example, imaging of trastuzumab, cetuximab, and bevacizumab can best be performed using a residualizing positron emitter.

Another option is the use of immuno-PET as a scouting procedure for the selection of RIT candidate patients on the basis of dosimetry. For this purpose, the radioimmunoconjugates used for immuno-PET and RIT should demonstrate similar biodistributions, and therefore, radionuclides (and, if required, chelates) with comparable chemical properties have to be chosen. The most commonly used beta emitters in RIT studies are copper-67 (67Cu; t1/2, 62 hours), lutetium-177 (177Lu; t1/2, 161 hours), 90Y (t1/2, 64 hours), and 131I (t1/2, 192 hours). Examples of PET/RIT radionuclide pairs are 64Cu/67Cu, 124I/131I, 86Y/89Y, 89Zr/177Lu, and 89Zr/89Y.

During the past few years, appropriate positron emitters and chelates have become commercially available, as well as standardized procedures for the production of optimal quality antibody conjugates (Good Manufacturing Practice). To the best of our knowledge, 18F, 68Ga, 64Cu, 86Y, and to a lesser extent 124I are commercially available from a few different vendors at reasonable costs, while 89Zr will become commercially available shortly. These achievements will open avenues to routine clinical application of immuno-PET.

**IMMUNO-PET: STATE OF THE ART**

Essential tools for immuno-PET became available very recently. Most immuno-PET studies have been performed in animal models, while the number of clinical evaluations is rising.

The most widely available positron emitter is 18F, because of its use in 18FDG-PET. With a half-life of 110 minutes, 18F-immuno-PET is restricted to mAb fragments. Efforts have been made in the development of 18F-mAb conjugates since 1992 [28], but inefficient labeling has hampered routine clinical application until now. Very recently anti-CEA diabodies were labeled with 18F, and yielded promising high-contrast small animal PET images within a few hours p.i [29, 30].

Another interesting short-lived positron emitter is 68Ga, because it is easily eluted from a germanium-68 generator, and therefore is always readily available. This positron emitter has been applied, in particular, in pretargeting strategies [31, 32] and for labeling of small mAb fragments. Pretargeted PET with 68Ga was evaluated in patients with primary breast cancer. Patients received an anti-Muc1/anti-Ga chelate bispecific mAb, followed 18 hours later by the 68Ga chelate [31]. Fourteen of 17 biopsy-proven lesions, averaging 25 ± 16 mm in size, were clearly visualized. No false-positive readings were obtained. Albeit with a small number of patients, it was concluded that PET offered better sensitivity for tumor detection than conventional RIS. PET imaging with 68Ga-trastuzumab F(ab)2 was used to monitor HER-2 expression in animal tumors during treatment with 7-allylaminogeldanamycin, a heat shock protein-90 inhibitor causing HER-2 degradation [33]. That study confirmed the potential of quantitative immuno-PET imaging: a linear correlation (R2 = 0.972) was found between tracer uptake quantified by radioactivity measurement of excised tumors and tracer uptake estimated noninvasively by PET.

In 1995, the anti–colorectal carcinoma m-mAb 1A3 was labeled with the longer-lived positron emitter 64Cu using a 1,4,8,11-tetraazacyclotetradecanetetraacetic acid chelate and evaluated in 36 patients with suspected advanced primary or metastatic colorectal cancer [34]. Immuno-PET was performed 4–36 hours p.i. All patients underwent CT or MRI. In 29 patients, all 17 primary and recurrent sites were clearly visualized, and 23 of 39 metastases were detected. Absence of tumor was confirmed in five patients, while tumor status was not confirmed in two patients. Interestingly, 11 new occult tumor sites were detected by immuno-PET. The sensitivity of immuno-PET was best in the abdomen and pelvis. Detection of metastases in the liver and lung, the most important sites of this disease, was difficult because of high blood-pool activity at the early imaging time points, which were chosen because of the short half-life of 64Cu (12.7 hours). Detection of liver metastases was also hampered by accumulation of 64Cu chelate con-
plexes in the liver. These results prompted the search for other $^{64}$Cu chelates and suggest a preference for even longer-living positron emitters when using intact mAbs to enable imaging at late time points.

The genetically engineered anti-CEA minibody T84.66/GS18 was labeled with $^{64}$Cu via the macrocyclic chelate $\{\text{1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA)}\}$, and PET studies were performed in human colon carcinoma-bearing mice [35]. Two to 24 hours p.i., tumors in the range of 27–395 mg could be readily detected. With this chelate, however, significant nonspecific uptake was seen in the kidneys and liver, hampering the detection of hepatic lesions. High liver uptake is a result of extensive Cu(II) dissociation from the chelator, followed by binding of the Cu(II) to natural ligands. Recently, new chelates for coupling of $^{64}$Cu have been reported, which lead to less liver uptake, but these chelates are awaiting clinical evaluation [36].

In another study, the value of $^{64}$Cu-immuno-PET imaging for noninvasive quantification of receptor expression was demonstrated. To this end, $^{64}$Cu-DOTA-cetuximab was used for targeting of seven xenograft models with different levels of epidermal growth factor receptor (EGFR) expression [37]. Good correlation ($R^2 = 0.80$) was found between tracer uptake measured by PET and EGFR expression as measured by Western blotting. The same group reported selective tumor targeting with $^{64}$Cu-DOTA-MEDI-522, a humanized mAb directed against the human integrin $\alpha_v\beta_3$ [38].

Immuono-PET with $^{86}$Y-labeled mAbs has been studied, in particular, in the context of RIT, for the prediction of biodistribution and dosimetry of therapeutic $^{90}$Y-labeled mAb conjugates [39, 40]. A problem of $^{86}$Y, however, is its half-life of 14.7 hours, which is relatively short for optimal $^{90}$Y ($t_{1/2}$, 64 hours) dosimetry prediction.

The first studies with $^{76}$Br-labeled mAbs were performed >10 years ago [41]. More recently, the $^{76}$Br-human recombinant antibody fragment L19-SIP, directed against extra domain B of fibronectin, was shown to be an attractive probe for immuno-PET imaging of tumor angiogenesis [42]. Since L19-SIP is also being explored in several therapeutic approaches for the destruction of tumor vasculature [43–45], such an imaging probe might be of value for patient-tailored therapy.

The long-lived positron emitters $^{124}$I and $^{89}$Zr are particularly suitable for immuno-PET when used in combination with intact mAbs [46]. $^{124}$I-labeled mAbs were already used for clinical immuno-PET about 15 years ago, but the number of patients included in those studies was small [47, 48]. Diagnostic results were far from optimal, because of, among other reasons, the poor quality of the m-mAbs used, which were lacking the specificity of today’s mAbs. Currently, interest in $^{124}$I-labeled mAbs has been renewed, partly as a result of superior methods for the production of $^{124}$I and better control over coupling $^{124}$I to mAbs [49]. Excellent visualization and quantification results were obtained with several $^{124}$I-labeled mAb constructs in a variety of xenograft models [50–53]. PET imaging of $^{124}$I-labeled mAbs may also form an attractive option as a scouting procedure prior to $^{131}$I RIT. For this purpose, $^{124}$I- and $^{131}$I-labeled mAbs should show congruent biodistributions, which can be achieved with well-standardized iodination methods [49].

Two clinical applications have attracted attention. Jayson et al. [54] used various doses of $^{124}$I-HuMV833, a mAb binding to VEGF$_{121}$ and VEGF$_{165}$, to perform PET imaging studies in 12 patients with various progressive solid tumors. Antibody distribution and clearance were markedly heterogeneous between and within patients and between and within individual tumors. These differences may represent variation in the available targets for the mAb, which could have implications for anti-VEGF therapy. Whether or not the mAb dose and type of radionuclide (residualizing or not) play a role in imaging results will be a topic of future studies.

In the other clinical application, $^{124}$I-immuno-PET was used for in vivo profiling of renal cancer. Divgi et al. [55] used the $^{124}$I-labeled c-mAb G250 to predict the presence of clear cell renal carcinoma in 25 patients scheduled for surgical tumor resection. G250 is directed against carbonic anhydrase-IX and is overexpressed in clear cell renal carcinoma. It might be informative to know which renal cancer patients have this aggressive tumor type because of treatment decisions, although opinions on this point differ [56]. Fifteen of 16 clear cell carcinomas were identified accurately by immuno-PET, and all nine non–clear cell renal masses were negative for the tracer. This study illustrates how molecular imaging with specific probes can contribute to personalized medicine.

Since the introduction of the long-lived positron emitter $^{89}$Zr as a residualizing radionuclide for immuno-PET, procedures have been developed for large-scale production of $^{89}$Zr and its stable coupling to mAbs [57–59]. In the meantime, several preclinical immuno-PET studies were performed with $^{89}$Zr-labeled mAbs as a prelude to clinical trials, for example, with the c-mAb U36 [58], DN30 (anti-c-Met), G250 [60], cetuximab [61], ibrutinumomab tiuxetan [62], rituximab, bevacizumab [63], and trastuzumab [64]. Also, for $^{89}$Zr-labeled mAbs, sensitive tumor detection and accurate quantification with PET were demonstrated [65], while PET with $^{89}$Zr-labeled mAbs appeared to be able to predict the biodistribution of $^{90}$Y- or $^{177}$Lu-labeled mAbs for RIT [61, 65]. Remarkably, the anti-VEGF mAb $^{89}$Zr-bevacizumab also showed selective tumor uptake, probably because of the fact that a proportion of
human VEGF is associated with extracellular matrix components [63]. Numerous clinical applications can be envisioned for this VEGF-specific tracer. Several other VEGF [54, 66] and VEGFR [67] PET imaging probes became available very recently.

Because of the promising results obtained with the c-mAb U36 in previous clinical RIS studies (Fig. 1), and to allow comparison of imaging modalities, a clinical immuno-PET feasibility trial was conducted with 89Zr-labeled c-mAb U36 [68, 69]. The aim was to determine the diagnostic value of immuno-PET with 89Zr-labeled c-mAb U36 in patients with head and neck squamous cell carcinoma (HNSCC) who were at high risk for having neck lymph node metastases. Twenty patients were scheduled to undergo resection of the primary tumor and uni- or bilateral neck dissection, and underwent CT and/or MRI and 89Zr-labeled c-mAb U36 immuno-PET prior to surgery. Immuno-PET detected all primary tumors (n = 17) as well as lymph node metastases in 18 of 25 positive neck levels. Missed lymph nodes were relatively small and contained just a small proportion of tumor tissue. Representative images are shown in Figure 3 and Figure 4. Note the level of detail (e.g., compare the heart regions in Fig. 1 and Fig. 3).

It was concluded that immuno-PET with 89Zr-labeled c-mAb U36 performs at least as well, for the detection of HNSCC lymph node metastases (and probably distant metastases), as CT/MRI, and that the use of PET-CT might further support image interpretation.

On the basis of these encouraging findings, several clinical trials with 89Zr-labeled mAbs were recently started, among which is a study with 89Zr-trastuzumab for the detection of HER-2–positive tumor lesions in breast cancer patients and for the quantification of HER-2 expression levels. Preliminary results from the first patients in that study [64] showed excellent tumor tracer uptake and a resolution that was much better than that observed in previous SPECT studies with 111In-trastuzumab [70].

For the clinically relevant tumor target HER-2, several other mAb formats are available for immuno-PET imaging, including a diabody [53], affibody [71], and F(âb')2 fragment [33]. The next step will be to fully exploit the clinical options. The fast kinetic mAb fragments may be most favorable for diagnosis, while for selection of mAb therapy candidates, it seems more attractive to use one single intact mAb format for pretherapy imaging as well as for therapy [64].

Figure 3. Immuno–positron emission tomography image with the zirconium-89-labeled chimeric monoclonal antibody U36 in a patient with a tumor of the right tonsil. Images were obtained within 1 hour (A), at 24 hours (B), at 72 hours (C), and at 120 hours postinjection (D). Slices are anterior (left) to posterior (right). Early images show mainly blood-pool activity with visualization of the nose, heart, lungs, and liver. In later images, the primary tumor is clearly visualized (arrow).

Figure 4. Fusion (C) of computed tomography (A) and coronal immuno–positron emission tomography (B) images with the zirconium-89-labeled chimeric monoclonal antibody U36 of a head and neck cancer patient with a tumor in the left tonsil and lymph node metastases (small arrows) at the left (level II and III) and right (level II) side of the neck. Images were obtained 72 hours postinjection. In these slices, only the lymph node metastases are visible.
CONCLUSION
Appropriate PET cameras, positron emitters, and chelates and optimal quality radioimmunoconjugates have become available. Consequently, immuno-PET might become a powerful tool in cancer diagnosis, for the efficient selection and development of therapeutic antibodies, and for the selection of patients most likely to benefit from antibody therapy.

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