Immuno-PET imaging of the tumor antigen HER2/neu allows for the noninvasive detection and monitoring of oncogene expression; such detection and monitoring are of prognostic value in patients with breast cancer. Compared with the full-size antibody trastuzumab, smaller protein tracers with more rapid blood clearance permit higher imaging contrast at earlier time points. Antigen-binding fragments (Fab) of antibodies with moderately prolonged circulation achieved through the genetic fusion with a long, conformationally disordered chain of the natural amino acids Pro, Ala, and Ser (PASylation)—a biologic alternative to chemical conjugation with polyethylene glycol, PEG—offer a promising tracer format with improved pharmacokinetics for in vivo imaging. Recently, the transition metal radionuclide $^{89}$Zr has attracted increasing interest for immuno-PET studies, complementing the conventional halogen radionuclide $^{124}$I.

**Methods:** To allow direct comparison of these 2 radioactive labels for the same protein tracer, the recombinant αHER2 Fab fused with 200 Pro, Ala, and Ser (PAS$_{200}$) residues was either conjugated with $^{124}$I via an iodination reagent or coupled with deferoxamine (Df) and complexed with $^{89}$Zr. After confirmation of the stability of both radioconjugates and quality control in vitro, immuno-PET and biodistribution studies were performed with CDF1-Foxn1nu mice bearing HER2-positive human tumor xenografts.

**Results:** $^{89}$Zr-Df-Fab-PAS$_{200}$ and $^{124}$I-Fab-PAS$_{200}$ showed specific tumor uptake of 11 and 2.3 percentage injected dose per gram 24 h after injection, respectively; both led to high tumor-to-blood (3.6 and 4.4, respectively) and tumor-to-muscle (20 and 43, respectively) ratios. With regard to off-target accumulation, overt $^{124}$I activity was seen in the thyroid, as expected, whereas high kidney uptake was evident for $^{89}$Zr; the latter was probably due to glomerular filtration and reabsorption of the protein tracer in proximal tubular cells. **Conclusion:** Both $^{89}$Zr- and $^{124}$I-labeled versions of αHER2 Fab-PAS$_{200}$ allowed PET tumor imaging with high contrast. With its residualizing radiometal, the tracer $^{89}$Zr-Df-Fab-PAS$_{200}$ showed better in vivo stability and higher tumor uptake.

**Key Words:** Fab; HER2; PASylation; plasma half-life; PET

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We previously applied PASylation to the trastuzumab Fab to improve its pharmacokinetic properties for PET imaging; the optimal tumor-to-blood ratio for the PAS tag was approximately 200 residues (PAS200) (12). Here, we report on a comparison between $^{89}$Zr labeling and $^{124}$I labeling of the recombinant αHER2 Fab-PAS200 protein tracer for immuno-PET imaging of HER2-positive human xenograft tumors in mice.

**MATERIALS AND METHODS**

**Preparation of αHER2 Fab**

Recombinant protein production in *Escherichia coli* was performed as previously described (12). The purity, disulfide bond formation, and hydrodynamic volume were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion chromatography (SEC) and, finally, electrospray ionization–mass spectrometry (ESI-MS).

**In Vitro Binding Studies of αHER2 Fab**

The target affinity of the Fab and its derivatives on the HER2-positive human breast adenocarcinoma cell line SK-BR-3 (13) was determined by cytofluorometric titration as previously described (12). The cells ($10^6$) were incubated with 100 μL of the purified Fab (or trastuzumab) at appropriate dilutions and then stained with a fluorescein-conjugated anti-human κ light-chain antibody (Invitrogen/Life Technologies). Counting was done with a FACSAria Cell-Sorting system (BD Biosciences).

**Radiolabeling of Fab**

A 5-fold molar excess of ρ-SCN-deferoxamine (Macrocyclics) was dissolved in dimethyl sulfoxide, and the mixture was added to a 30 μM (2 mg/mL) solution of the purified Fab in 0.1 M NaHCO$_3$ (pH 8.5) and incubated overnight at 25°C. Unconjugated chelator was removed by gel filtration on a PD-10 column (GE Healthcare) with 20 mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid) (HEPES)–NaOH (pH 7.0) as an eluent. Radiolabeling of the purified Df-Fab-PAS200 was performed in accordance with a published procedure (14). Typically, $^{89}$Zr-oxalate (111 MBq [3 mCi]; specific activity, 150 MBq/nmol; IBA Molecular) dissolved in 1 M oxalic acid was mixed with 150 μL of water and 50 μL of 2 M Na$_2$CO$_3$, and the mixture was incubated for 3 min at room temperature (RT). After CO$_2$ liberation, 200 μL of 0.5 M HEPES–NaOH (pH 7.0), 75 μL of 1 M gentisic acid–NaOH (pH 7.0), and 9 nmol of the Fab in a volume of approximately 100 μL were added, and the mixture was incubated for 60 min at RT. Finally, the radiolabeled Fab was separated from free $^{89}$Zr on a PD-10 column. Radiolabeling efficiency and radiochemical purity were analyzed by thin-layer chromatography (TLC) on glass microfiber chromatography paper with 20 mM sodium citrate (pH 5) as the mobile phase. For SDS-PAGE analysis and in vitro antigen-binding studies, the Fab was also labeled with nonradioactive Zr(IV) chloride (Sigma-Aldrich) dissolved in 1 M oxalic acid.

**Radioiodination of Fab**

Radioiodination of Fab-PAS200 was performed by the IODO-GEN (Pierce) method as previously described (12). Generally, 0.44 nmol of the purified Fab and 37 MBq of Na$^{124}$I (−1.1 MBq/nmol; IBA Molecular) or Na$^{125}$I (specific activity, 74 MBq/nmol; Hartman Analytic) were incubated for 15 min at RT and the reagents were removed on a PD-10 column. Radiochemical purity was analyzed by TLC with 0.9% (w/v) NaCl as the mobile phase. For SDS-PAGE analysis and tested for antigen-binding activity. The in vitro stability of $^{89}$Zr-Df-Fab-PAS200 and $^{125}$I-Fab-PAS200 in phosphate-buffered saline (PBS) at various time points up to 72 h through quantification of protein-associated radioactivity by SEC and TLC. (E) Analysis of serum samples from tumor-bearing mice by SDS-PAGE and then phosphorimaging 24 h after injection with $^{89}$Zr-Df-Fab-PAS200 or $^{125}$I-Fab.

**In Vivo Studies**

For biodistribution analyses and PET imaging, female CD1–Foxn1null mice (Charles River Laboratories) were injected subcutaneously with $5 \times 10^6$ SK-BR-3 cells, leading to tumor sizes of 300–800 mm$^3$. Small-animal PET studies were performed by tail vein injection of 7 MBq of $^{89}$Zr- or $^{124}$I-labeled Fab-PAS200 (n = 2) with a specific activity of 11 or 80 MBq/nmol, respectively. Static maximum-intensity-projection images...
were acquired under isoflurane anesthesia with an Inveon PET/CT small-animal scanner (Siemens Medical Solutions). Quantitation of appropriate PET images (n = 1 or 2) was performed by drawing specific regions of interest in tumor and muscle tissues with Inveon software.

For biodistribution studies, mice (n = 5 or 6) were injected intravenously with 0.37–0.74 MBq each of 89Zr- or 124I-labeled Fab-PAS200. PET scans were performed for 89Zr-Df-Fab-PAS200 (A) and 124I-Fab-PAS200 (B) (the latter data taken from Menderl et al. (12)).

**RESULTS**

**Preparation of αHER2 Fab-PAS200**

For in vivo imaging of HER2-positive tumors, we applied the recombinant αHER2 Fab hu4D5v8 carrying a PAS tag of 200 residues (PAS200) at the C terminus of the light chain to achieve a moderately extended plasma half-life (11,12). The Fab was produced as a functional protein through periplasmic secretion in E. coli and was purified in a functional state, including quantitation of disulfide bridge formation between heavy and light chains, as confirmed by SDS-PAGE analysis (Fig. 1). Furthermore, SEC revealed a homogeneous peak with a decreased elution volume, indicating an expanded molecular size of the PASylated Fab, as intended.

For radio labeling with 89Zr(IV), the recombinant Fab was functionalized with the chelator reagent Df thiocyanate via Lys side chains, and ESI-MS measurements revealed a chelator-to-Fab ratio of approximately 2:1. The biochemical integrity of the Fab after conjugation was confirmed by SDS-PAGE and SEC (data not shown). The binding activity of αHER2 Fab-PAS200 was verified by cytofluorimetric (FACS) titration analysis of the HER2-positive human tumor cell line SK-BR-3 (13). A dissociation constant of 2.2 nM was measured for the unmodified recombinant Fab, which is comparable to the dissociation constant of 1 nM determined for the parental antibody trastuzumab (data not shown). Generally, only a moderate influence on target affinity was seen in this assay for PASylation, Zr-Df conjugation, or iodination: 4.7 nM for Fab-PAS200, 9.6 nM for na2Zr-Df-Fab-PAS200, and 1.8 nM for 123I-Fab (Fig. 1B).

**Radioisotope Labeling**

For in vivo PET imaging, αHER2 Fab-PAS200 was separately radiolabeled with each of the positron emitters, 89Zr (by Df coupling) and 124I (by IODO-GEN labeling). For biodistribution experiments, 89Zr- and 124I-labeled proteins were applied in combination (dual tracer analysis). Radiolabeling of Df-Fab-PAS200 with 89Zr(IV)-oxalate (14) resulted in a labeling yield of 90%–95% after 10 min of incubation at RT, whereas iodination by the IODO-GEN method resulted in a labeling yield of 80% after 15 min of incubation at RT. With both isotopes, a radiochemical purity of greater than 98% was determined by TLC after purification on a desalting column. Tracer stability was further confirmed by SDS-PAGE analysis of the 89Zr-labeled and radioiodinated Fabs (Fig. 1C).

The in vitro stability of 89Zr-Df-Fab-PAS200 (55.5 MBq/μL [1.5 mCi/μL]) in phosphate-buffered saline (PBS) at RT was investigated by SEC; after 24 and 72 h, only 2.2% and 8.9% of the bound 89Zr was dissociated from the Fab, respectively (Fig. 1D). Similarly, high tracer stability of this radioiodine chelate was described in other publications (15). In addition, tracer stability in vivo was assessed by analyzing serum collected from CD1-Foxn1nu mice 24 h after injection of 89Zr-Df-Fab-PAS200 (described later). No signs of tracer degradation or loss of 89Zr-Df were observed, and an intact disulfide bridge connecting heavy and light chains of the Fab was verified (Fig. 1E).

**In Vivo PET Tumor Imaging**

89Zr-labeled αHER2 Fab and 124I-labeled αHER2 Fab with enhanced plasma half-lives were compared for in vivo PET imaging of CD1-Foxn1nu mice bearing subcutaneous HER2-positive human tumor xenografts. Static maximum-intensity-projection PET images were collected at appropriate time points after tracer injection (Fig. 2). Tracer specificity was confirmed in blocking experiments with an excess of unlabeled trastuzumab. For both PASylated αHER2 Fab tracers, optimal imaging contrast was observed at just 24 h after injection, with high tumor-to-normal tissue contrast and stable tumor uptake for up to several days.

However, despite similar levels of tumor staining, notable differences in the metabolism of the 89Zr- and 124I-labeled protein tracers became apparent. The 89Zr-labeled Fab had high kidney uptake, most likely due to renal filtration of the tracer followed by reabsorption in proximal tubular cells (16). Although
89Zr- and 125I-labeled αHER2 Fab-PAS200 showed total tumor uptake 24 h after injection that was 4.8-fold higher than that of the 125I-labeled Fab, whereas similar tumor-to-blood ratios were observed with both radioisotopes (Fig. 3). These findings are in line with the residualizing character of this radioisotope. Finally, tracer specificity was verified in blocking experiments by injection of a 1,000-fold molar excess of trastuzumab, which resulted in 3.3-fold (89Zr-Df-Fab-PAS200) and 6.3-fold (125I-Fab-PAS200) reductions in tumor uptake (P < 0.001) 24 h after injection, respectively (Table 1).

Remarkably, when the 2 tracers were compared, the 89Zr-labeled Fab showed total tumor uptake 24 h after injection that was 4.8-fold higher than that of the 125I-labeled Fab, whereas similar tumor-to-blood ratios were observed with both radioisotopes (Fig. 3). These findings are in line with the residualizing character of 89Zr(IV), which stays trapped inside tumor cells after receptor-mediated endocytosis and lysosomal tracer degradation (21,22). Notably, however, the 2 protein tracers showed different levels of nonspecific accumulation, either in the thyroid (125I) or in the kidney (89Zr), leading to high organ-to-tumor ratios there.

The biodistribution data were essentially in accordance with the PET experiments, in which similar uptake values in tumor and muscle for both radiolabeled αHER2 Fabs were found by quantitative image analysis (Table 2). In the liver and kidney, uptake values determined from the PET measurements approximately matched those determined from the biodistribution study. Apparent muscle activities were generally higher in PET quantification, leading to slightly decreased tumor-to-muscle ratios there.

**DISCUSSION**

Compared with conventional Fabs, which often show premature kidney clearance, Fabs with moderately prolonged plasma half-life enable increased tumor uptake as well as tumor-to-blood ratios (12). PASylation (i.e., the fusion of a protein with a conformationally disordered amino acid polymer having PEG-like properties (11)) can lead to improved tumor accumulation both because of the longer persistence in plasma and contact time and because of the enhanced permeability and retention effect (23). In contrast to PEG, the genetically encoded and biodegradable PAS tag requires no chemical modification and allows exact tuning of the desired plasma half-life. Using radiiodinated protein tracers, we previously demonstrated that a plasma half-life prolonged by approximately 4-fold (to 5.2 h) via fusion with a PAS polypeptide of 200 amino acid residues (12)—compared with the plasma half-life of unmodified αHER2 Fab (1.3 h)—led to favorable imaging contrast as well as tumor uptake (12).

**Biodistribution Studies**

The in vivo imaging data were complemented by dual tracer biodistribution experiments with the PASylated αHER2 Fab labeled with either 89Zr ($\beta^+$/γ) or the iodine isotope 125I (γ); these tracers were injected simultaneously to avoid variations in tracer administration between animals. 89Zr-Df-Fab-PAS200 showed high tumor accumulation, with a maximum percentage injected dose per gram (%ID/g) of 11, as well as favorable tumor-to-organ ratios 24 h after injection (Fig. 3). Tracer uptake lower than the activity in blood was observed in all analyzed organs with the exception of the kidney (81 %ID/g), the adrenal gland (2.7 %ID/g), the liver (3.7 %ID/g), and the spleen (1.9 %ID/g) at later time points (48 h after injection). The fast metabolism of mice most likely was responsible for the degradation or demetalation of 89Zr-Df-Fab-PAS200 in the liver (18). Interestingly, no overt accumulation in bones was detected here, although this was previously observed with 89Zr-Df-trastuzumab in mice (19,20).

When labeled with 125I, Fab-PAS200 showed prominent tumor accumulation, too, with a maximum %ID/g of 2.3 at 24 h after injection; these data revealed a high tumor-to-blood ratio of 4.4 and high tumor-to-normal tissue ratios (Table 1). With the exception of the thyroid, almost no nonspecific tracer uptake was seen in any other analyzed organ (Fig. 3B), probably because of the nonresidualizing character of this radioisotope. Furthermore, iodide was also released from blood into the acidic gastric lumen, as indicated by enhanced radioactivity in the stomach for 124I-Fab-PAS200 (Fig. 2). Acidic gastric lumen, as indicated by enhanced radioactivity in the stomach for 124I-Fab-PAS200 (Fig. 2).
TABLE 1
Biodistribution of $^{125}$I- and $^{89}$Zr-Labeled αHER2 Fab-PAS$_{200}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>89Zr-Df-Fab-PAS$_{200}$</th>
<th>125I-Fab-PAS$_{200}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Tumor</td>
<td>6.89 ± 2.02</td>
<td>11.1 ± 1.7</td>
</tr>
<tr>
<td>Blood</td>
<td>11.8 ± 2.7</td>
<td>3.05 ± 0.19</td>
</tr>
<tr>
<td>Heart</td>
<td>3.79 ± 0.97</td>
<td>1.55 ± 0.15</td>
</tr>
<tr>
<td>Lung</td>
<td>5.50 ± 1.41</td>
<td>1.90 ± 0.20</td>
</tr>
<tr>
<td>Liver</td>
<td>4.11 ± 1.03</td>
<td>3.45 ± 0.29</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.48 ± 0.41</td>
<td>2.10 ± 0.30</td>
</tr>
<tr>
<td>Kidney</td>
<td>49.4 ± 6.6</td>
<td>88.3 ± 12.8</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>3.76 ± 1.18</td>
<td>3.77 ± 1.12</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.06 ± 0.32</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.87 ± 0.95</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.61 ± 0.32</td>
<td>0.62 ± 0.08</td>
</tr>
<tr>
<td>Large intestine</td>
<td>2.28 ± 0.38</td>
<td>0.91 ± 0.18</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.72 ± 0.16</td>
<td>0.55 ± 0.23</td>
</tr>
<tr>
<td>Bone</td>
<td>1.52 ± 0.38</td>
<td>1.01 ± 0.27</td>
</tr>
<tr>
<td>Brain</td>
<td>0.30 ± 0.05</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Thyroid Ratio</td>
<td>2.88 ± 0.55</td>
<td>1.52 ± 0.14</td>
</tr>
<tr>
<td>Tumor-to-blood</td>
<td>0.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Tumor-to-muscle</td>
<td>9.6</td>
<td>20</td>
</tr>
</tbody>
</table>

Specific binding of this tailored Fab, labeled with either $^{124/125}$I or $^{89}$Zr, to HER2-positive xenograft tumors was verified in vivo by blocking experiments with the full-size antibody trastuzumab. Both PET and biodistribution analyses showed no unfavorable accumulation in most normal tissues, indicating stable radiolabeling and confirming that modification with the biochemically inert PAS tag is compatible with in vivo imaging. In fact, remarkably clear tumor imaging contrast was seen with PET 24 h after injection. Furthermore, excellent tumor-to-blood and tumor-to-normal tissue ratios were observed in dual tracer biodistribution experiments.

Non–tumor-related tracer accumulation was detected for the $^{89}$Zr-labeled Fab in the kidney, liver, and spleen and for the $^{124/125}$I-labeled Fab in the thyroid as well as stomach. This distinct radiolabel-specific behavior can be explained by the different mechanisms of in vivo demetallation and degradation, respectively. Moreover, the kidney uptake of $^{89}$Zr-Df-Fab-PAS$_{200}$ indicated that, despite an apparent molecular size above the threshold of the glomerular pores (11), the PASylated Fab is still excreted through renal filtration. Most likely, the protein tracer subsequently is reabsorbed in proximal tubular cells, possibly through the endocytic, ligand-specific receptors megalin or cubulin or by means of general pinocytosis (24). After internalization, the labeled Fab is lysosomally catalyzed into amino acids, which are released into the cytoplasm and returned to the circulation, whereas Zr-Df-lysine–peptide conjugates (or the naked metal ion) remain trapped intracellularly. Conversely, iodotyrosine, the degradation product of a radiodinated protein, is quickly released from cells after endocytosis (by tumor cells, in the kidneys, or in other tissues) (25), allowing redistribution in the body.

Notably, pronounced kidney accumulation has not been observed for $^{89}$Zr-labeled full-size antibodies, probably because of their extremely large molecular size and preferential clearance through the liver (7,10), but has been described for the $^{111}$In-DOTA-conjugated αHER2 Fab (26). Compared with the $^{89}$Zr-labeled Fab, other small protein tracers labeled with radionuclides, for example, the $^{111}$In-labeled αHER2 Affibody DOTA-ZHER2:342-pep2, have shown even higher kidney uptake (>200 %ID/g) 24 h after injection—even though the participation of megalin or cubulin receptors in specific cellular uptake was excluded (27). On the other hand, the kidney uptake of (in terms of molecular size) the more closely related bivalent protein tracer $^{111}$In-DOTA-(Fab’)$_2$-Herceptin (~65 %ID/g) 24 h after injection (28) was in the same range as that seen for our monovalent PASylated $^{89}$Zr-labeled Fab.

To offer guidance for choosing a suitable radiolabel in the preparation of protein tracers for clinical applications, we compared the 2 different radionuclides for the labeling of αHER2 Fab-PAS$_{200}$. Direct modification of the Fab with $^{124/125}$I, a fast and easy process, did not affect target affinity and benefited from the fact that Fabs contain several accessible tyrosine residues remote from the antigen-binding site. Similar functional results were obtained for the radiometal $^{89}$Zr, although conjugation with the chelator derivative Df tyiocyanate to free lysine side chains of the Fab was necessary before the actual radiolabeling. Nevertheless, this modification could be performed under mild conditions as well, preserving protein integrity and target affinity. As the protein portions of both (PASylated) $^{89}$Zr- and $^{124/125}$I-labeled Fabs were identical, similar behavior in vivo could be expected. Hence,
TABLE 2
Quantitation of PET Images (n = 1 or 2) for 124I- and 89Zr-Labeled αHER2 Fab-PAS200

<table>
<thead>
<tr>
<th>Parameter</th>
<th>89Zr-Df-Fab-PAS200</th>
<th>124I-Fab-PAS200</th>
</tr>
</thead>
<tbody>
<tr>
<td>%ID/g in tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Liver</td>
<td>9.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Tumor-to-muscle ratio</td>
<td>6.2</td>
<td>17</td>
</tr>
</tbody>
</table>

Differences in the relative signals for tumor and organs, such as the kidney and liver, should be caused by different routes of metabolism for the 2 radionuclides. We purposely did not apply reagents that would block thyroid or kidney uptake (e.g., Lugol’s solution, Irenat [Bayer Schering Pharma], or Gelofusine [B. Braun] (24)) as might be done in clinical investigations, thus allowing an unbiased comparison of tracer biodistribution.

In mouse PET experiments, the 124I-labeled Fab showed relatively fast blood clearance and high tumor-to-normal tissue ratios 24 h after injection, in line with the nature of 124I as a nonresidualizing radionuclide. Free iodide, which occurs after intra- or extracellular dehalogenation in blood plasma, rapidly accumulates in the thyroid because of specific import through the sodium iodide symporter (17). In comparison, 89Zr-Di-Fab-PAS200 had a slightly longer apparent half-time in circulation; this result could indicate higher tracer stability. Remarkably, tumor uptake that was almost 5-fold higher was observed for this label in the biodistribution analysis 24 h after injection, probably because 89Zr-Di was trapped inside tumor cells after antigen binding and receptor-mediated internalization. However, despite the slightly different pharmacokinetic behavior of the 2 tracers, both essentially showed equally high tumor-to-blood ratios and high PET imaging contrast 24 h after injection.

Our observations are in line with the results of previous investigations on the differences between residualizing and nonresidualizing radiolabels. For example, a study in which different single-chain Fv-Fc fusion proteins directed against carcinoembryonic antigen (CEA) were labeled with either 117In or 125I revealed higher tumor uptake of the radiometal (29). Furthermore, experiments with the head and neck squamous cell carcinoma–selective chimeric antibody U36, labeled with either 89Zr or 124I, resulted in significantly higher uptake in tumor and liver for 89Zr (30). In a similar comparison of the 89Zr- and 124I-labeled versions of the anti–prostate stem cell antigen minibody A11 in mice carrying prostate stem cell antigen–positive tumors, higher tumor uptake was again found for the residualizing radiometal, whereas better imaging contrast was seen for the 124I-labeled minibody (31).

Besides biodistribution studies in mice (20), 89Zr-labeled trastuzumab has already been tested in human patients with metastatic breast cancer (10), thus demonstrating potential for the noninvasive monitoring of HER2 status during cancer progression. Consequently, the clinical application of a protein tracer based on the corresponding Fab fragment should not be too difficult to achieve.

Compared with 89Zr-labeled full-size monoclonal antibodies, our PASylated Fab, with its optimized plasma half-life, offers the advantage of high contrast in immuno-PET imaging at just 24 h after injection. Notably, maximal contrast for 89Zr-Di-BzSCN-trastuzumab in mice with HER2-positive BT474M1 xenografts was reached only 96–144 h after injection (20), in accordance with its long-lasting circulation in blood.

CONCLUSION

Depending on the chemistries of the radioisotopes, distinct in vivo distribution profiles were observed for 2 differently labeled αHER2 Fab tracers with optimized plasma half-life. 89Zr-labeled Fab-PAS200 and 124I-labeled Fab-PAS200 showed equally high tumor-to-blood ratios and high PET imaging contrast 24 h after injection. However, on the basis of the superior in vivo stability of the 89Zr label and significantly higher tumor uptake overall, 89Zr-Di-Fab-PAS200 appears to be the preferred tracer for translation to clinical applications.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734. This work was supported by ERC Grant MUMI from the European Union and Collaborative Research Centre SFB-824 of the Deutsche Forschungsgemeinschaft. Arne Skerra is a managing director and shareholder of XL-protein GmbH. No other potential conflict of interest relevant to this article was reported.

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89Zr- VS. 124I-LABELED αHER2 FAB TRACER • Mendler et al.


High-Contrast Tumor Imaging In Vivo

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