A Novel Bispecific, Trivalent Antibody Construct for Targeting Pancreatic Carcinoma

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Abstract

Preclinical and clinical studies have demonstrated the application of radiolabeled mAb-PAM4 for nuclear imaging and radioimmunotherapy of pancreatic carcinoma. We have now examined the ability of a novel PAM4-based, bispecific monoclonal antibody (mAb) construct, TF10, to pretarget a radiolabeled peptide for improved imaging and therapy. TF10 is a humanized, bispecific mAb, divalent for mAb-PAM4 and monovalent for mAb-679, reactive against the histamine-succinyl-glycine hapten. Biodistribution studies and nuclear imaging of the radiolabeled TF10 and/or TF10-pretargeted hapten-peptide (IMP-288) were conducted in nude mice bearing CaPan1 human pancreatic cancer xenografts. 125I-TF10 cleared rapidly from the blood, with levels decreasing to <1% injected dose per gram (ID/g) by 16 hours. Tumor uptake of the TF10-pretargeted111In-IMP-288 was 24.3 ± 1.7% ID/g at this time point with no accumulation in any normal tissue. To show the utility of the pretargeting approach, 111In-IMP-288 was administered 16 hours after TF10. At 3 hours postadministration of radiolabeled peptide, imaging showed intense uptake within the tumors and no evidence of accretion in any normal tissue. No targeting was observed in animals given only the 111In- peptide. Tumor uptake of the TF10-pretargeted111In-IMP-288 was 24.3 ± 1.7% ID/g, whereas for 111In-IMP-288 alone it was only 0.12 ± 0.002% ID/g at 16 hours. Tumor/blood ratios were significantly greater for the pretargeting group (~1,000:1 at 3 hours) compared with 111In-PAM4-IgG (~5:1 at 24 hours; P < 0.0003). Radiation dose estimates suggested that TF10/90Y-peptide pretargeting would provide a greater antitumor effect than 90Y-PAM4-IgG. Thus, the results suggest that TF10 pretargeting may provide improved imaging for early detection, diagnosis, and treatment of pancreatic cancer as compared with directly radiolabeled PAM4-IgG. [Cancer Res 2008;68(12):4819–26]

Introduction

Pancreatic cancer is an insidious disease with a particularly high mortality rate. In large measure, this is due to the location of the pancreas in the retroperitoneum, where the tumor can grow in a silent fashion. Symptoms that might suggest the patient seek medical assistance are usually not evident until an advanced stage of tumor growth, and even at this time, the clinical presentation can be vague and representative of varying pathologies. Combined with the fact that at present, there are no effective means for treatment of this disease, patient outcomes are generally poor; 5-year survival is <5%. However, if detected early, when the cancer is still restricted to the pancreas, treatment by surgical resection, with or without chemotherapy and radiation therapy, can improve 5-year survival to roughly 25%; yet even this statistic is not very encouraging (1, 2). Consequently, considerable effort has been undertaken to find new methods to detect pancreatic cancer at an early stage of tumor growth (pancreatic intraepithelial neoplasia 3) before it becomes invasive, and to improve survival through earlier and more effective treatments.

We have identified a unique biomarker present on mucin expressed by >85% of invasive pancreatic adenocarcinomas, including early stage I disease and the precursor lesions, pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasia (3). The specific epitope, as detected by mAb-PAM4 (4), is absent from normal and inflamed pancreatic tissues, as well as most other malignant tissues. Thus, detection of the epitope provides a high diagnostic likelihood for the presence of pancreatic neoplasia. Early clinical studies using 131I- and 99mTc-labeled, murine PAM4 IgG or Fab’, respectively, showed specific targeting in 8 of 10 patients with invasive pancreatic adenocarcinoma (5, 6). One of the negative studies, one had a poorly differentiated pancreatic carcinoma that did not express the PAM4-epitope, whereas the other patient was later found to have pancreatitis rather than a malignant lesion. Accordingly, we believe that the high specificity of PAM4 for pancreatic cancer could be helpful in the detection and diagnosis of early disease. In addition to improved detection, 90Y-PAM4 IgG was found to be effective in treating large human pancreatic cancer xenografts in nude mice (7), and when combined with gemcitabine, further improvements in therapeutic response were observed (8, 9). A Phase I therapy trial in patients who failed gemcitabine treatment was recently completed, finding the maximum tolerated dose of 90Y-humanized PAM4 IgG to be 20 mCi/m² (10). Although all patients showed disease progression at or after week 8, initial shrinkage of tumor was observed in several cases. Clinical studies are now underway to evaluate a fractionated dosing regimen of 90Y-hPAM4 IgG in combination with a radiosensitizing dose of gemcitabine.

Preclinical studies in xenograft models of colorectal cancer and lymphoma have suggested that delivery of radionuclides with pretargeting procedures can provide a significant therapeutic advantage over directly radiolabeled antibodies (11–16). In addition, we have shown that pretargeting procedures can be used in combination with 99mTc or 121I for superior imaging capability over directly radiolabeled antibody fragments (17, 18). Thus, a pretargeting system based on the specificity of PAM4 for pancreatic
cancer could have an appreciable advantage over the directly radiolabeled conjugate. Previously, we reported initial pretargeting studies with a chemically conjugated bispecific antibody prepared by coupling the Fab’ fragment of PAM4 to an anti–diethylene-triaminepentaacetic acid (DTPA) Fab’, showing improved visualization of tumors with an 111In-DTPA-peptide, as compared with triaminepentaacetic acid (DTPA) Fab’.

The hPam4-Fab-DDD2 vector (30 μg) was linearized by digestion with SalI restriction enzyme and stably transfected into Sp/ESF myeloma cells (2.8 \times 10^8) by electroporation. After clonal selection in 0.2 μmol/L methotrexate (Calbiochem), terminal batch cultures of a hPam4-Fab-DDD2 expressing clone were grown in roller bottles in Hybridoma SFM (Invitrogen) supplemented with 0.8 μmol/L methotrexate. The culture broth was clarified by centrifugation, filtered (0.2 μm), and concentrated 10-fold by dialfiltration with exchange into 1 mmol/L EDTA, 0.04 mol/L PBS (pH 7.4).

TF10 was then prepared by combining 195 mg of hPam4-Fab-DDD2 processed supernatant fluid and 85 mg of purified h679-Fab-AD2, a fusion construct prepared from the h679-Fd and the anchoring domain (AD) from A-kinase anchor protein. The mixture was reduced overnight at room temperature under mild conditions with 2 mmol/L reduced glutathione before the addition of 4 mmol/L oxidized glutathione and an additional overnight incubation. The conjugate was purified by HSG-peptide, affinity column chromatography, followed by Q Sepharose ion-exchange chromatography. The process resulted in a yield of 180 mg of highly purified TF10 at 3.2 mg/mL in 0.02% polysorbate-20, 0.04 mol/L PBS (pH 7.4).

In vitro and in vivo characteristics of the peptide IMP-288 [7,10-tetraazacyclododecane-N,N,N’,N”-tetraacetic acid (DOTA)-d-Tyr-d-Lys(HSG)-d-Glu-d-Lys(HSG)-NH2], for use with 111In, have been reported (15). The hapten-peptide is divalent with respect to reactivity with mAb-679 (anti-HSG). Production of DOTA-conjugated hPAM4, whole-IgG, has been described (7). In vitro characterization of the TF10, along with PAM4-IgG and other PAM4-based constructs, was conducted by enzyme immunoassay (4), SDS-PAGE (17), high-performance liquid chromatography (19), and low-pressure size-exclusion column chromatography (19).

Radiolabeling. Sodium iodide (125I) and indium chloride (111In) were obtained from Perkin-Elmer. TF10 was routinely labeled with 125I by the iodogen method, with purification by use of size-exclusion spin columns. Radiolabeling of DOTA-peptide and DOTA-PAM4-IgG with 111InCl was done as previously described (17, 18). Purity of the radiolabeled products was examined by size-exclusion high-performance liquid chromatography with the amount of free, unbound isotope determined by instant TLC.

Bispecific PAM4 biodistribution studies. Female athymic nude mice (20 g (Taconic Farms), bearing s.c. CaPan1 human pancreatic cancer

Materials and Methods

Construction of TF10, bispecific PAM4 antibody, and peptide hapten. TF10 and IMP-288, hapten antibodies, were supplied by IBC Pharmaceuticals, Inc. TF10 was prepared as previously described for a similar bispecific mAb, TF2, an anti–carcinoembryonic antigen construct (17). Briefly, two engineered constructs were prepared based on the humanized forms of either mAb-PAM4 or mAb-679 (anti-HSG) haptens. The first, hPAM4-Fab-DDD2, was created as a fusion product of the hPAM4-Fd fragment with the dimerization and docking domain (DDD) of human cyclic AMP (cAMP)–dependent protein kinase A. A SacII/AgeI restriction fragment containing the coding sequence for the heavy chain constant domains (C\textsubscript{H}1-C\textsubscript{H}3), which was excised from a pdHL2 vector for hPam4 IgG expression, was replaced with a SacII/AgeI fragment that was excised from the plasmid vector CH1-DDD2-Fab-hMN-14-pdHL2, and contains the coding sequence for C\textsubscript{H}1 fused to a DDD2 peptide via a flexible linker.

The hPam4-Fab-DDD-pdHL2 vector (30 μg) was linearized by digestion with SalI restriction enzyme and stably transfected into Sp/ESF myeloma cells (2.8 \times 10^8) by electroporation. After clonal selection in 0.2 μmol/L methotrexate (Calbiochem), terminal batch cultures of a hPam4-Fab-DDD2-expressing clone were grown in roller bottles in Hybridoma SFM (Invitrogen) supplemented with 0.8 μmol/L methotrexate. The culture broth was clarified by centrifugation, filtered (0.2 μm), and concentrated 10-fold by dialfiltration with exchange into 1 mmol/L EDTA, 0.04 mol/L PBS (pH 7.4).

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Bispecific PAM4 biodistribution studies. Female athymic nude mice (20 g (Taconic Farms), bearing s.c. CaPan1 human pancreatic cancer
Bispecific mAb Targeting Pancreatic Cancer

Table 1. Biodistribution of $^{125}$I-TF10 in CaPan1 tumor–bearing nude mice

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>6 h</th>
<th>16 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>4.16 ± 0.42</td>
<td>7.16 ± 1.10</td>
<td>3.47 ± 0.66</td>
<td>1.65 ± 0.57</td>
<td>1.34 ± 0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>10.8 ± 1.36</td>
<td>2.12 ± 0.22</td>
<td>0.15 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.8 ± 1.90</td>
<td>3.52 ± 0.93</td>
<td>0.17 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Kidneys</td>
<td>9.60 ± 1.45</td>
<td>2.35 ± 0.39</td>
<td>0.10 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>6.17 ± 0.67</td>
<td>2.53 ± 0.13</td>
<td>0.17 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Blood</td>
<td>21.0 ± 1.93</td>
<td>4.33 ± 0.51</td>
<td>0.13 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Stomach</td>
<td>9.84 ± 2.22</td>
<td>2.83 ± 5.37</td>
<td>0.62 ± 0.19</td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.59 ± 0.25</td>
<td>2.10 ± 0.21</td>
<td>0.18 ± 0.05</td>
<td>0.04 ± 0.00</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.05 ± 0.17</td>
<td>2.15 ± 0.28</td>
<td>0.21 ± 0.06</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.77 ± 0.52</td>
<td>1.78 ± 0.28</td>
<td>0.06 ± 0.01</td>
<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Tumor weight (g)</td>
<td>0.36 ± 0.14</td>
<td>0.35 ± 0.14</td>
<td>0.34 ± 0.06</td>
<td>0.47 ± 0.14</td>
<td>0.47 ± 0.13</td>
</tr>
</tbody>
</table>

P > 0.05 for all, whereas the monovalent bsPAM4 chemical conjugate (PAM4 Fab−anti-DTPA-Fab') had a significantly lower avidity (half-maximal binding, 30.61 ± 2.05 nmol/L; P = 0.0379, compared with TF10), suggesting that TF10 binds in a divalent manner. The immunoreactive fraction of $^{125}$I-TF10 bound to the mucin was 87%, with 9% found as unbound TF10 and 3% as free iodide (Supplementary Fig. S2A). Ninety percent of the $^{125}$I-IMP-288 bound to TF10 (note that two peaks can be discerned that represent both monovalently and divalently bound products; Supplementary Fig. S2B). Of the total $^{125}$I-IMP-288 bound to TF10, 92% eluted at higher molecular weight when excess mucin (200 µg) was added, with only 3% eluting with the non–mucin-reactive TF10 fraction. An additional 5% of the radiolabeled peptide eluted in the free peptide volume. None of the radiolabeled peptide bound to the mucin antigen in the absence of TF10 (not shown).

Distribution of $^{125}$I-TF10 in CaPan1 tumor–bearing nude mice. TF10 showed a rapid clearance from the blood, starting with 21.03 ± 1.93 %ID/g at 1 hour and decreasing to just 0.13 ± 0.02 %ID/g at 16 hours (Table 1). The biological half-life was calculated to be 2.19 hours [95% confidence interval (95% CI), 2.11–2.27 hours]. Tissue uptake revealed enhanced activity in the liver, spleen, and kidneys at 1 hour, which cleared just as quickly by 16 hours ($T_{1/2} = 2.09$ hours (95% CI, 2.08–2.10), 2.84 hours (95% CI, 2.49–3.29), and 2.44 hours (95% CI, 2.28–2.63) for liver, spleen, and kidney, respectively. Activity in the stomach most likely reflects the accretion and excretion of radioiodine, suggesting that the radioiodinated TF10 was actively catabolized, presumably in the liver and spleen, thereby explaining its rapid clearance from the blood. Nevertheless, by 16 hours, the concentration of radioiodine within the stomach was below 1% ID/g. A group of five non–tumor-bearing nude mice given $^{125}$I-TF10 and necropsied at 16 hours showed similar tissue distribution, suggesting that the tumor had not affected the bispecific mAb distribution and clearance from normal tissues (data not shown). Of course, it is possible that differences occurred before the initial time point examined. Tumor uptake of TF10 peaked at 6 hours postinjection (7.16 ± 1.10 %ID/g) and had decreased to half maximum binding (3.47 ± 0.66 %ID/g) at 16 hours. Tumor uptake again decreased nearly 2-fold over the next 32 hours, but then was stable over the following 24 hours.

Results

In vitro characterization of the bispecific mAb TF10. Figure 1 presents a model of the bispecific, trivalent TF10 fragment created by the “dock-and-lock” method (17). The divalent hPAM4-Fab-D2D2 structure is dependent on the self-dimerization and docking domain (DDD) of human cAMP-dependent protein kinase A. The other arm of the bispecific construct, the monovalent h679-Fab-AD (anti-HSG) with anchoring domain (AD) from A-kinase anchor protein, naturally binds to the DDD2 domain, whereupon stable covalent disulfide bridges are formed. High-pressure size-exclusion chromatography showed a single peak eluting at approximately the same time as PAM4-IgG. The binding of TF10 to the target mucin antigen was analyzed by ELISA (Supplementary Fig. S1). The results showed nearly identical binding curves for the divalent TF10, PAM4-IgG, and PAM4-F(ab')2, (half-maximal binding was calculated as 1.42 ± 0.10, 1.31 ± 0.12, and 1.83 ± 0.16 nmol/L, respectively;
Biodistribution of TF10-pretargeted, 111In-labeled peptide. Although maximum tumor uptake of TF10 occurred at 6 hours, previous experience indicated that the radiolabeled peptide would need to be given at a time point when blood levels of TF10 had cleared to <1% ID/g (i.e., 16 hours; ref. 19). Higher levels of TF10 in the blood would lead to unacceptably high binding of the radiolabeled peptide within the blood (i.e., low tumor/blood ratios), whereas administering the peptide at a later time would mean the concentration of TF10 in the tumor would be decreased with consequently reduced concentration of radiolabeled peptide within the tumor. Thus, an initial pretargeting study was done using a 16-hour interval. With the amount of the 111In-IMP-288 held constant (30 μCi, 5.07 x 10^{-11} mol), increasing amounts of TF10 were given so that the administered dose of TF10 and IMP-288 expressed as mole ratio varied from 5:1 to 20:1. As shown in Table 2, at 3 hours the amount of 111In-IMP-288 in the blood was barely detectable (0.01%). Tumor uptake increased from 19.0 ± 3.49% ID/g to 28.55 ± 0.73% ID/g as the amount of bispecific mAb administered was increased 4-fold (statistically significant differences were observed for comparison of each TF10/peptide ratio, one group to another; P < 0.03 or better), but without any appreciable increase in normal tissue uptake. Tumor uptake in the animals given TF10 was >100-fold higher than when 111In-IMP-288 was given alone. Comparison of 131I activity in the normal tissues of the animals that either received or did not receive prior administration of TF10 indicated similar absolute values, which in most instances were not significantly different. This suggests that the bispecific mAb had cleared sufficiently from all tissues by 16 hours to avoid appreciable peptide uptake in these tissues. Tumor/blood ratios were >2,000:1, with other tissue ratios exceeding 100:1. Even tumor/kidney ratios exceeded 10:1. The highest tumor uptake of radioisotope with minimal targeting to nontumor tissues resulted from the 20:1 ratio; however, either of the TF10/peptide ratios could be used to achieve exceptional targeting to tumor, both in terms of signal intensity and contrast ratios. The 10:1 ratio was chosen for further study because the absolute difference in tumor uptake of radiolabeled peptide was not substantially different between the 10:1 (24.3 ± 1.71% ID/g) and 20:1 (28.6 ± 0.73% ID/g) ratios.

Images of the animals given TF10-pretargeted 111In-IMP-288 at a bispecific mAb/peptide ratio of 10:1, or the 111In-IMP-288 peptide alone, are shown in Fig. 2. The majority of these tumors were ≤0.5 cm in diameter, weighing ~0.25 g. The images show highly intense uptake in the tumor of the TF10-pretargeted animals (Fig. 2A, top). The intensity of the image background for the TF10-pretargeted animals was increased to match the intensity of the image taken of the animals given the 111In-IMP-288 alone (Fig. 2B, middle). However, when the images were optimized for the TF10-pretargeted mice, the signal intensity and contrast were so high that no additional activity was observed in the body. No tumor localization was seen in the animals given the 111In-IMP-288 alone, even when image intensity was enhanced.

An additional experiment was done to assess the kinetics of targeting 111In-hPAM4 whole-IgG compared with that of the TF10-pretargeted 111In-IMP-288 peptide (Table 3). Tumor uptake of the 111In-peptide was highest at the initial time point examined, 3 hours (15.99 ± 4.11% ID/g), whereas the blood concentration of radiolabeled peptide was only 0.02 ± 0.01% ID/g, providing a mean tumor/blood ratio of 946.3 ± 383.0. Over time, radiolabeled peptide cleared from the tumor with a biological half-life of 76.04 hours. Among nontumor tissues, uptake was highest in the kidneys, averaging 1.89 ± 0.42% ID/g at 3 hours with a steady decrease over time (biological half-life, 33.6 hours). Liver uptake started at 0.15 ± 0.06% ID/g and remained essentially unchanged over time. In contrast to the TF10-pretargeted 111In-IMP-288, the 111In-hPAM4-IgG had a slower clearance from the blood, albeit there was a substantial clearance within the first 24 hours, decreasing from 30.1% ID/g at 3 hours to just 11.5 ± 1.7% ID/g at 24 hours. Variable elevated uptake in the spleen suggested that the antibody was likely being removed from the blood by a similar mechanism as previously described (i.e., targeting of secreted mucin that had become entrapped within the spleen; ref. 20). Tumor uptake peaked at 48 hours with 80.4 ± 6.1% ID/g, and remained at an elevated level over the duration of the monitoring period. The high tumor uptake, paired with a more rapid than expected blood clearance for an IgG, produced tumor/blood ratios of 5.2 ± 1.0 within 24 hours. Figure 2C (bottom) shows the images of the animals at 24 hours postadministration of 111In-PAM4-IgG, illustrating that tumors could be visualized at this early time, but there was still considerable activity within the abdomen. Tumor/nontumor ratios were mostly higher for TF10-pretargeted 111In-labeled hapten-peptide as compared with 111In-hPAM4-IgG, except for the kidneys, where tumor/kidney ratios with the 111In-IMP-288 and 111In-hPAM4-IgG were similar at later times. However, tumor/kidney

Table 2. Biodistribution of 111In-IMP-288 alone (no TF10) or pretargeted with varying amounts of TF10

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Lungs</th>
<th>Blood</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>Pancreas</th>
<th>Tumor weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td>20:1</td>
<td>10:1</td>
<td>No TF10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>19.0 ± 3.49</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.04</td>
<td>1.59 ± 0.11</td>
<td>0.19 ± 0.06</td>
<td>0.01 ± 0.00</td>
<td>0.03 ± 0.02</td>
<td>0.12 ± 0.08</td>
<td>0.23 ± 0.10</td>
<td>0.02 ± 0.00</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>24.3 ± 1.71</td>
<td>0.21 ± 0.12</td>
<td>0.16 ± 0.07</td>
<td>1.72 ± 0.24</td>
<td>0.26 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.08 ± 0.03</td>
<td>0.39 ± 0.08</td>
<td>0.02 ± 0.01</td>
<td>0.32 ± 0.09</td>
</tr>
<tr>
<td>28.6 ± 0.73</td>
<td>0.17 ± 0.01</td>
<td>0.26 ± 0.10</td>
<td>1.53 ± 0.14</td>
<td>0.29 ± 0.04</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>0.25 ± 0.08</td>
<td>0.02 ± 0.00</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>0.12 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>1.71 ± 0.22</td>
<td>0.03 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.02 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.02 ± 0.00</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

%ID/g at 3 h (mean ± SD)
ratios for the TF10-pretargeted $^{111}$In-IMP-288 were high enough (e.g., $\sim 7:1$) at 3 hours to easily discern tumor from normal tissue. Figure 3 illustrates the potential therapeutic capability of the direct and pretargeted methods to deliver radionuclide ($^{90}$Y). Although the concentration (%ID/g) of radioisotope within the tumor seems to be much greater when delivered by PAM4-IgG than by pretargeted TF10 at their respective maximum tolerated dose (0.15 mCi for $^{90}$Y-PAM4-IgG and 0.9 mCi for TF10-pretargeted $^{90}$Y-IMP-288), the radiation dose to tumor would be similar (10,080 and 9,229 cGy for $^{90}$Y-PAM4-IgG and TF10-pretargeted $^{90}$Y-IMP-288, respectively). The advantage for the pretargeting method would be the exceptionally low activity in blood (9 cGy), almost 200-fold less than with the $^{90}$Y-PAM4 IgG (1,623 cGy). It is also important to note that the radiation dose to liver, as well as other nontumor organs, would be much lower with the TF10-pretargeted $^{90}$Y-IMP-288. The exception would be the kidneys, where the radiation dose would be similar for both protocols at their respective maximum dose (612 and 784 cGy for $^{90}$Y-PAM4-IgG and TF10-pretargeted $^{90}$Y-IMP-288, respectively). The data suggest that for $^{90}$Y-PAM4-IgG, as with most other radiolabeled whole-IgG mAbs, the dose-limiting toxicity would be hematologic; however, for the TF10 pretargeting protocol, the dose-limiting toxicity would be the kidneys.

Discussion

Current imaging procedures provide the critical information necessary for detection, diagnosis, and management of invasive pancreatic adenocarcinoma. Ultrasound, computerized tomography (CT), and magnetic resonance imaging (MRI) technologies, which provide anatomic images, along with PET imaging of the metabolic environment, have routinely been found to provide high sensitivity in the detection of pancreatic masses; however, these data are, for the most part, based on detection of lesions $>2$ cm in a population that is already presenting clinical symptoms (21–24). At this time in the progression of the pancreatic carcinoma, the prognosis is rather dismal. To improve patient outcomes, detection of small, early pancreatic neoplasms in the asymptomatic patient will be necessary.

In the past few years, several institutions have undertaken a renewed effort at discovery of small pancreatic masses in asymptomatic, high-risk patient groups undergoing targeted screening protocols (25, 26). Canto and colleagues (27), using CT and endoscopic ultrasonography, screened a series of 78 patients considered at high-risk for pancreatic cancer. If endoscopic ultrasonography was abnormal, endoscopic ultrasonography-fine needle aspiration and endoscopic retrograde cholangiopancreatography were done. By use of this protocol, a significant number of early, potentially curable, neoplastic masses, as well as precursor lesions, were discovered in asymptomatic patients (10.2% diagnostic yield). However, the majority of patients examined presented with moderate to severe pancreatitis, a potentially confounding environment for accurate detection and diagnosis, especially of small neoplastic lesions.

Winter and colleagues (28), in a retrospective analysis of patients undergoing an abdominal imaging study for nonpancreatic disease ($n=1,944$), reported discovery of “incidentalomas” (a peripancreatic
or pancreatic mass) in 86 patients (4.4% of patients examined). Others have recognized the challenge of managing the patient with incidentalomas or what seems to be cystic disease of the pancreas (i.e., to treat a potentially malignant disease by surgical resection, or wait and observe; ref. 29). Considering these incidentalomas can be identified retrospectively in almost 1% of all abdominal CT or MRI scans, this represents a large group of individuals from the general population for whom a significant diagnostic problem exists.

Imaging with a mAb-targeted approach, such as is described herein with mAb-PAM4, may provide the data necessary for diagnosis of these small, early cancers. Of prime importance is the specificity of the mAb. We have presented considerable data, including immunohistochemical studies of tissue specimens (3, 4) and immunossay of patient sera (30), to show that mAb-PAM4 is highly reactive with a biomarker, the presence of which provides high diagnostic likelihood of pancreatic neoplasia. Furthermore, we determined that PAM4, although not reactive with normal adult pancreatic tissues nor active pancreatitis, is reactive with the earliest stages of neoplastic progression within the pancreas (pancreatic intraepithelial neoplasia 1 and intraductal papillary mucinous neoplasia) and that the biomarker remains at high levels of expression throughout the progression to invasive pancreatic adenocarcinoma (3). Preclinical studies with athymic nude mice bearing human pancreatic tumor xenografts have shown specific targeting of radiolabeled murine, chimeric, and humanized versions of PAM4 (7, 20).

Others have also examined the application of mAbs to target and provide in vivo imaging of pancreatic cancers, mostly as xenografts in athymic nude mice, although a few have been studied in clinical trials as well (31–35). While it is difficult to compare all of these previous studies, for the most part radiolabeled whole-IgG has been used, with the result that the highest tumor/blood ratios occurred between 72 and 96 hours postinjection and were in the range of 2:1 to 10:1; in other words, similar to the results we obtained with radiolabeled PAM4 whole-IgG. However, to the best of our knowledge, none of the other mAbs used, with the result that the highest tumor/blood ratios occurred between 72 and 96 hours postinjection and were in the range of 2:1 to 10:1; in other words, similar to the results we obtained with radiolabeled PAM4 whole-IgG. However, to the best of our knowledge, none of the other mAbs used in vivo in pancreatic cancer models or patients has shown the specificity for pancreatic cancer that we have reported for PAM4.

We appreciate that high-quality antibody-targeted tumor imaging in mice bearing human xenografts is quite different from what may be achieved clinically. In general, the difference in pharmacokinetics between the species provides for a substantially lower tumor uptake of radiolabeled antibody and lower tumor/nontumor ratios in the human than in the mouse. However, we have reported successful, specific imaging of invasive pancreatic adenocarcinoma with murine PAM4 whole-IgG, radiolabeled with $^{131}$I or $^{99m}$Tc (5, 6), and more recently as part of a phase I imaging and therapy trial using $^{111}$In-DOTA humanized PAM4 (10).

Whereas imaging with radiolabeled antibodies (whole-IgG and/or their fragments and engineered constructs) has produced several Food and Drug Administration–approved imaging agents for several indications (e.g., arcitumomab, capromab, and others), the images usually suffer from relatively low signal strength (%ID/g) and insufficient contrast (tumor/nontumor ratio) for detection of small tumors. For detection of early, primary pancreatic cancer, which is tucked between several major organs and is more centrally located in the abdomen surrounded by major blood vessels, the sensitivity of the detection method will need to improve significantly.

In the current studies, we have examined a next-generation, recombinant, bispecific PAM4-based construct, TF10, which is divergent for the PAM4 arm and monovalent for the anti-HSG hapten arm. There are several important characteristics of this pretargeting system’s constructs, named dock-and-lock, including its general applicability and ease of synthesis (17, 36, 37). However, for the present consideration, the major differences from the previously reported chemical construct are the valency, which provides improved binding to tumor antigen, and, importantly, its pharmacokinetics. TF10 clearance from nontumor tissues is much more rapid than was observed for the chemical conjugate. Time for blood levels of the bispecific constructs to reach less than 1% ID/g was 40 hours postinjection for the chemical construct versus 16 hours for TF10. A more rapid clearance of the pretargeting agent has provided a vast improvement of the tumor/blood ratio, while maintaining high signal strength at the tumor site (%ID/g).

In addition to providing a means for early detection and diagnosis, the possibility to make use of the TF10 pretargeting system for therapy should not be overlooked. Consideration of the effective radiation dose to tumor and nontumor tissues favors the pretargeting method over directly radiolabeled PAM4-IgG. The dose estimates suggest that the two delivery systems have different dose-limiting toxicities: myelotoxicity for the directly radiolabeled

| Table 3. Tumor/nontumor ratios for the biodistribution of TF10-pretargeted peptide or IgG |
|---------------------------------|---------------------------------|
|                                | $^{111}$In-hapten-peptide at 3 h | $^{111}$In-DOTA-PAM4 IgG at 24 h |
| Tumor                           | 1.0 ± 0.0                       | 1.0 ± 0.0                       |
| Liver                           | 128.8 ± 70.6                    | 6.8 ± 1.4                      |
| Spleen                          | 50.9 ± 31.1                     | 5.6 ± 1.3                      |
| Kidneys                         | 9.1 ± 3.4                       | 13.7 ± 2.4                     |
| Lungs                           | 54.9 ± 20.4                     | 14.1 ± 2.0                     |
| Blood                           | 915.2 ± 404.3                   | 5.2 ± 1.0                      |
| Stomach                         | 508.0 ± 271.5                   | 6.10 ± 14.2                    |
| Small intestine                 | 233.7 ± 100.0                   | 18.9 ± 2.4                     |
| Large intestine                 | 98.4 ± 47.1                     | 35.1 ± 3.9                     |
| Pancreas                        | 894.0 ± 855.0                   | 35.4 ± 3.0                     |
| Tumor weight (g)                | 0.28 ± 0.21                     | 0.10 ± 0.06                    |
PAM4 versus the kidney for the TF10 pretargeting system. This is of significance for the future clinical development of radiolabeled PAM4 as a therapeutic agent. Gemcitabine, the frontline drug of choice for pancreatic cancer, can provide significant radiosensitization of tumor cells. In previous studies, we showed that combinations of gemcitabine and directly radiolabeled PAM4-IgG provided synergistic antitumor effects compared with either arm alone (8, 9). The dose-limiting factor with this combination was overlapping hematologic toxicity. However, because the dose-limiting organ for TF10 pretargeting seems to be the kidney rather than hematologic tissues, combinations with gemcitabine should be less toxic, thus allowing increased administration of radioisotope with consequently greater antitumor efficacy.

The superior imaging achieved with TF10 pretargeting in preclinical models, as compared with directly radiolabeled DOTA-PAM4-IgG, provides a compelling rationale to proceed to clinical trials with this imaging system. The specificity of the tumor-targeting mAb for pancreatic neoplasms, coupled with the bispecific antibody platform technology providing the ability to conjugate various imaging compounds to the HSG-hapten-peptide for SPECT (111In), PET (68Ga), ultrasound (Au), or other contrast agents, or for that matter 90Y or other radionuclides for therapy, provides high potential to improve overall patient outcomes (38).

In particular, we believe that a TF10-based ImmunoPET procedure will have major clinical value to screen individuals at high-risk for development of pancreatic cancer (e.g., genetic predisposition, chronic pancreatitis, smokers, etc.), as well as a means for follow-up of patients with suspicious abdominal images from conventional technologies and/or with indications due to the presence of specific biomarker(s) or abnormal biochemical findings. When used as part of an ongoing medical plan for following these patients, early detection of pancreatic cancer may be achieved. Finally, in combination with gemcitabine, TF10 pretargeting may provide a better opportunity for control of tumor growth than directly radiolabeled PAM4-IgG.

Disclosure of Potential Conflicts of Interest


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References


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