[\(^{177}\text{Lu}\)]Pertuzumab: Experimental Therapy of HER-2–Expressing Xenografts

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Abstract

Pertuzumab (Omnitarg) is a novel antibody against HER-2, domain II. HER-2 is a tyrosine kinase receptor that is overexpressed in several carcinomas, especially breast cancer. Pertuzumab, labeled with the low-energy \(^{177}\text{Lu}\) emitter \(^{177}\text{Lu}\), might be a candidate for targeted radiotherapy of disseminated HER-2–positive micrometastases. The radiolabeled antibody \(^{177}\text{Lu}\)pertuzumab showed favorable targeting properties in BALB/c (nu/nu) mice with HER-2–overexpressing xenografts. The absorbed dose in tumors was more than five times higher than the absorbed dose in blood and more than seven times the absorbed dose in any other normal organ. Experimental therapy showed that \(^{177}\text{Lu}\)pertuzumab delayed tumor progression compared with controls (no treatment, \(P < 0.0001\); nonlabeled pertuzumab antibody, \(P < 0.0001\); and \(^{177}\text{Lu}\)-labeled irrelevant antibody, \(P < 0.01\)). No adverse side effects of the treatment could be detected. Thus, the experimental results support the planning of clinical studies applying \(^{177}\text{Lu}\)pertuzumab for therapy. [Cancer Res 2007; 67(1):326–31]

Introduction

The basic characteristics of \(^{177}\text{Lu}\)pertuzumab, a radioactive labeled monoclonal antibody binding to HER-2, have recently been described (1). The high SKOV-3 xenograft radionuclide uptake compared with other organs encouraged us to do experimental therapy using the same animal model.

HER-2, or ErbB2, is a transmembrane receptor-like protein, which is part of the HER family of tyrosine kinase receptors (2, 3). HER-2 is unique in this family because it has no known ligand but seems to be constitutively open to dimerization (whereas other members of the receptor family have to be stimulated by a ligand; ref. 4). HER-2 has been found to be the preferred partner for heterodimerizations with the other HER family members, and this may be the main reason for the oncogenicity of HER-2 (5). Pertuzumab (rhuMab 2C4) is designed to bind to the dimerization domain (domain II) of HER-2 and sterically hinder heterodimerization or homodimerization (6, 7). The antibody has been found safe and clinically active in a recently published phase I clinical trial (8).

Materials and Methods

Cell line, chemicals, equipment, and abbreviations. All experiments were done using the SKOV-3 cell line (ATCC HTB 77). The cells were cultivated in 25-, 75-, or 175-cm\(^2\) flasks (Nunc, Wiesbaden, Germany) using HAM’s F-10 medium complemented with 10% (v/v) fetal bovine serumSigma, St. Louis, MO), 2 mmol/L L-glutamine, and 100 IU/mL penicillin/streptavidin (Biochrom, Berlin, Germany).

Omittarg was a kind gift from Genentech (San Francisco, CA). The antibody pertuzumab was purified by size-exclusion chromatography of the Omnitarg preparation using MilliQ-water as eluent on a PD-10 column (GE Healthcare, Uppsala, Sweden). The purified antibody was freeze-dried in a Heto FD 3 (Heto, Holten, Denmark) overnight. Isothiocyanate-benzyl-CHX-A^\(^{4}\)-diethylenetriaminepentaacetic acid (DTPA) was purchased from Macrocycles (Dallas, TX). Gamma counting was done using an automated well crystal scintillator (Wallac Wizard 1480 3”, Perkin-Elmer, Wellesley, MA) within an energy window of 30 to 340 keV. Instant TLC strips were purchased from Gelman Sciences (Ann Arbor, MI) and were analyzed using a cyclone phosphor-imaging system (Perkin-Elmer).

The chimeric monoclonal antibody cMAB U36 against CD44v6 was used as negative control because SKOV-3 does not express CD44v6 in detectable amounts. The selection, production, and purification of the antibody have been described previously (16–18). The antibody was a kind gift from Prof. van Dongen (Department of Otolaryngology/Head and Neck Surgery, Vrije University Medical Center, Amsterdam, the Netherlands).

Samples for histology were dehydrated in a Ventana vacuum-infiltrating processing (Dalan, Sweden) using an 11-step program with increasing concentrations of ethanol, followed by xylene, and paraffin treatment. The samples were embedded in paraffin using a Tissue-Tec TEC embedding machine (Sakura Finetek, Tokyo, Japan) and cut in a Leica RM2165 Microtome (Leica, Leipzig, Germany).

\(^{177}\text{Lu}\) was purchased from NRG (Petten, The Netherlands). Samples for histology were dehydrated in a Ventana vacuum-infiltrating processing (Dalan, Sweden) using an 11-step program with increasing concentrations of ethanol, followed by xylene, and paraffin treatment. The samples were embedded in paraffin using a Tissue-Tec TEC embedding machine (Sakura Finetek, Tokyo, Japan) and cut in a Leica RM2165 Microtome (Leica, Leipzig, Germany).

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were incubated overnight with isothiocyanate-benzyl-CHX-A°-DTPA (130 nmol/L) in 0.07 mol/L borate buffer (pH 9.2). The conjugated antibodies were purified on a NAP-5 column (GE Healthcare), and the buffer was changed to metal-free 1 mol/L sodium acetate buffer with 5 mg/mL ascorbic acid (pH 5.6). Chelation to \(^{177}\text{Lu}\) was done for 30 min to 1 h, and the eluted products were purified from free \(^{177}\text{Lu}\) on NAP-5 columns with PBS (pH 7.2) as running buffer. Radiochemical purity after purification was >99% according to instant TLC (SG plates) with 0.2 mol/L citric acid buffer. The specific activity after labeling was 22 MBq/mg for the biodistribution study with \(^{177}\text{Lu}\) pertuzumab. For the tumor treatment study, the specific activities were 200 MBq/mg for \(^{177}\text{Lu}\) pertuzumab and 300 MBq/mg for \(^{177}\text{Lu}\) pertuzumab.

### Table 1. Biodistribution of \(^{177}\text{Lu}\) in BALB/c (nu/nu) mice carrying SKOV-3 xenografts with an approximate diameter of 0.5 cm and injected with \(^{177}\text{Lu}\) pertuzumab

<table>
<thead>
<tr>
<th>Time after injection (d)</th>
<th>0.33</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>4.50 ± 0.96</td>
<td>3.39 ± 1.31</td>
<td>2.59 ± 0.46</td>
<td>1.86 ± 1.02</td>
<td>1.30 ± 0.43</td>
<td>0.41 ± 0.21</td>
</tr>
<tr>
<td>Heart</td>
<td>1.25 ± 0.21</td>
<td>1.11 ± 0.53</td>
<td>0.72 ± 0.17</td>
<td>0.54 ± 0.26</td>
<td>0.40 ± 0.17</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.71 ± 0.43</td>
<td>1.69 ± 0.66</td>
<td>1.22 ± 0.21</td>
<td>1.04 ± 0.45</td>
<td>0.83 ± 0.24</td>
<td>0.38 ± 0.13</td>
</tr>
<tr>
<td>Tumor</td>
<td>6.11 ± 0.64</td>
<td>5.47 ± 2.76</td>
<td>12.87 ± 3.10</td>
<td>13.75 ± 3.88</td>
<td>12.51 ± 1.40</td>
<td>6.67 ± 2.02</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.41 ± 0.13</td>
<td>0.81 ± 0.68</td>
<td>0.45 ± 0.16</td>
<td>0.44 ± 0.16</td>
<td>0.47 ± 0.28</td>
<td>0.42 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td>1.65 ± 0.31</td>
<td>1.29 ± 0.42</td>
<td>1.08 ± 0.37</td>
<td>0.89 ± 0.30</td>
<td>0.74 ± 0.45</td>
<td>0.80 ± 0.06</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.34 ± 0.11</td>
<td>0.52 ± 0.15</td>
<td>0.31 ± 0.06</td>
<td>0.24 ± 0.11</td>
<td>0.19 ± 0.07</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.18 ± 0.28</td>
<td>1.17 ± 0.35</td>
<td>0.87 ± 0.19</td>
<td>0.95 ± 0.28</td>
<td>0.75 ± 0.18</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.81 ± 0.39</td>
<td>1.52 ± 0.51</td>
<td>1.18 ± 0.13</td>
<td>1.04 ± 0.27</td>
<td>0.76 ± 0.28</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.43 ± 0.10</td>
<td>0.43 ± 0.20</td>
<td>0.30 ± 0.05</td>
<td>0.23 ± 0.10</td>
<td>0.18 ± 0.04</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.47 ± 0.21</td>
<td>0.43 ± 0.15</td>
<td>0.31 ± 0.07</td>
<td>0.23 ± 0.11</td>
<td>0.18 ± 0.09</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Colon</td>
<td>0.39 ± 0.11</td>
<td>0.40 ± 0.16</td>
<td>0.26 ± 0.09</td>
<td>0.19 ± 0.11</td>
<td>0.16 ± 0.07</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Feces</td>
<td>0.24 ± 0.06</td>
<td>0.41 ± 0.23</td>
<td>0.22 ± 0.05</td>
<td>0.25 ± 0.13</td>
<td>0.32 ± 0.11</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Skin</td>
<td>0.86 ± 0.22</td>
<td>1.09 ± 0.53</td>
<td>0.99 ± 0.24</td>
<td>0.81 ± 0.39</td>
<td>0.58 ± 0.41</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.18 ± 0.06</td>
<td>0.25 ± 0.13</td>
<td>0.24 ± 0.04</td>
<td>0.16 ± 0.07</td>
<td>0.12 ± 0.04</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Bone</td>
<td>0.13 ± 0.05</td>
<td>0.42 ± 0.12</td>
<td>0.14 ± 0.07</td>
<td>0.12 ± 0.04</td>
<td>0.16 ± 0.06</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.52 ± 0.08</td>
<td>1.46 ± 0.55</td>
<td>0.47 ± 0.30</td>
<td>0.25 ± 0.13</td>
<td>0.26 ± 0.14</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>0.64 ± 0.18</td>
<td>0.97 ± 0.36</td>
<td>0.61 ± 0.20</td>
<td>0.94 ± 0.95</td>
<td>0.45 ± 0.14</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>Brain</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.06</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
</tbody>
</table>

NOTE: Mice were sacrificed at 8 h, 1, 3, 5, 7, or 14 d after injection, and a panel of organs, including blood drawn from the heart, was collected, weighed, and analyzed for radioactive content. Mean, decay corrected, SUV, and SE from four mice are presented.

The organ uptake values, non-corrected for physical half-life, were time integrated to obtain the residence time per gram tissue for dosimetry calculations. Integration between time 0 and 14 days was made by the trapezoid method. The two last time points were fitted to a single exponential function, which was used to estimate the residence time from 14 days to infinity. The extrapolated area was in all organs <18% of the total calculated area.

The mean uptake values and their SDs given in Table 1 were used to randomly generate new statistically normally distributed uptake values. These new sets of uptake values were time integrated in the same way as described above. This produced a set of 30 randomly distributed residence time values. The relative error (1 relative SD) in this data set was used as the relative error of the calculated absorbed dose in the organ.

The radioactive decay of \(^{177}\text{Lu}\) produces mainly low energy β particles. S values for \(^{177}\text{Lu}\) were obtained from RADAR phantoms (Unit Density Spheres) published on the Internet. The S value for a 1 g sphere (0.0233 mGy/MBq s) was used to calculate the organ doses by multiplying it to the organ residence values.

### Tumor treatment

The agents for tumor treatment were given i.v. in tail vein 7 days after xenograft inoculation. The study was a semi-randomized blind setup. Groups of mice were randomly selected for one of five treatments: 7 MBq \(^{177}\text{Lu}\) pertuzumab (n = 8 mice), 5 MBq \(^{177}\text{Lu}\) pertuzumab (n = 8 mice), 7 MBq \(^{177}\text{Lu}\) pertuzumab (n = 8 mice), native pertuzumab (non-labeled antibody control, n = 9 mice), and PBS (vehicle, n = 8 mice). After injection, the key was sealed in an envelope, keeping the identity of the groups secret until after the data analysis.

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The mice were weighed, and tumor sizes were measured two to three times per week. The tumor size measurement was done using an electronic slide caliper. The tumor volume was calculated using an ellipsoid estimation of tumor form. The mice were followed until their tumors reached a volume of \( \text{f} \text{1cm}^3 \) before they were euthanized and dissected. Dissection consisted of removal of tumor for final volume measurement (length, breadth, and depth) and removal of kidneys for analysis of possible renal damage. When mice had to be euthanized due to tumor size, their tumor size was at all later times considered to be \( \text{1 cm}^3 \).

Survival curve plots and Kaplan-Meier analysis were done using Prism 4 (GraphPad Software, San Diego, CA). The end point was a tumor volume reaching \( \text{0.5 cm}^3 \). Mice removed from the study due to other reasons than having reached the end point were censored. The study was, for the remaining animals, finished after 102 days at which time they were censored. Euthanasia was done with an overdose of anesthesia (ketamin and xylazin) followed by cervical dislocation.

**Histology.** Tumors and kidneys were fixed in 4% phosphate-buffered formalin, processed, and embedded in paraffin. From selected blocks, 4-\( \mu \text{m} \)-thick sections were cut, put on coated slides (Superfrost plus, Menzel-Gläser, Germany), and dried for 12 h at 37°C. The sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol to distilled water.

The kidney slices were then stained with H&E. The tumor slices were treated with hydrogen peroxide doped PBS for 10 min to quench endogenous peroxidase. Retrieval was preformed by pressure boiling the samples in citrate buffer for 7 min. The samples were stained for HER-2 with the A0485 antibody (DAKO, Glostrup, Denmark). Antibody dilutions 1:300 and 1:900 were applied, and the samples were incubated in 4°C overnight. After rinsing, the samples are treated with one drop EnVision antibody (DAKO) per sample and incubated for 45 min in room temperature. The samples were rinsed again and then developed using Fexin plus (DAKO) for 15 min.

**Results**

**Biodistribution**

The uptake of \([177\text{Lu}]\)pertuzumab was low in most organs as can be seen in Table 1 and Fig. 1A to C. The radioactivity concentration, expressed as percent injected activity per gram organ, was always below 8% for all normal organs, excluding blood. The tumor uptake values were at all times higher than the values in normal organs, including blood. The SUV were below 1.8 for all normal organs, excluding blood. This concentration decreased with time in all organs, excluding the tumor xenografts, which continued to accumulate radioactivity until 5 days after injection. The accumulation of radioactivity in the tumor combined with decreasing activity concentration in other organs lead to a nuclide half-life corrected tumor-to-blood ratio starting at 1.4 at 8 hours after injection and continually increasing to a maximum of 19 after 2 weeks.

**Gamma Camera Imaging**

The gamma camera images collected 3 days after injection are shown in Fig. 2. The tumors accumulated large amounts of \( ^{177}\text{Lu} \). Small amounts of radioactivity could also be found in the liver area. A region of interest analysis of the tumor and corresponding lateral region gave a tumor to background ratio of 29 for the left mouse and 24 for the right mouse.

**Dosimetry**

The dosimetry calculations indicated that the absorbed dose to the tumor would, in our case, be at least five times the dose given to blood. For other normal organs, the situation was even better. Calculated absorbed doses to selected organs are presented in Table 2.
One mouse in the native pertuzumab group had to be removed from the study after 28 days due to weight loss. This mouse did not receive radioactivity, and the weight loss was probably due to stress or illness. One mouse from the 5 MBq $^{177}$Lu pertuzumab group was killed at day 72 due to tumor necrosis that spread to the dermis, causing open wounds. Both of these mice were censored. Open wounds were also observed in a few other mice but only after they reached the end point tumor volume of 0.5 cm$^3$.

The obtained tumor take values corresponded well to previous experiences with this model system where the tumor takes have been ~90% to 95% (data not shown).

Analysis of survival with Kaplan-Meier analysis showed that the group that received 7 MBq $^{177}$Lu pertuzumab had a significantly improved time to tumor progression versus mice that received PBS, native pertuzumab, or 7 MBq $^{177}$Lu U36 ($P < 0.0001$, $P < 0.0001$, and $P < 0.01$, respectively). Mice receiving 5 MBq $^{177}$Lu pertuzumab had a significantly longer time to tumor progression than mice that received PBS or native pertuzumab ($P < 0.0001$ and $P < 0.0005$, respectively).

**Histology**

No morphologic damage was detectable in sections of either irradiated or nonirradiated kidneys, neither in a blind or open examination. The tumor cells, analyzed in tissue sections, were found to express HER-2 whether treated or not. Treated tumors seemed, morphologically, somewhat more chaotic than untreated, showing large heterogeneity with partial degradation, fibrotic regions, and lymphocyte infiltration.

**Discussion**

The biodistribution study was designed to give information on both early and late kinetics (uptake, retention, and clearance) to allow for dosimetry calculations. The results from the biodistribution study showed that the tumor uptake of $^{177}$Lu was higher than the uptake in all analyzed normal organs and in the blood. Furthermore, the low radioactivity concentration in bone indicated that the lutetium was not lost from the chelator allowing for dosimetry calculations. The results from the biodistribution of $^{177}$Lu pertuzumab presented in Fig. 1 and Table 1.

**Table 2. Doses to organs in different treatment groups**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Dose from 7.3 MBq $^{177}$Lu pertuzumab (Gy)</th>
<th>Dose from 4.6 MBq $^{177}$Lu pertuzumab (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>50.86 ± 5.57</td>
<td>32.03 ± 4.19</td>
</tr>
<tr>
<td>Blood</td>
<td>9.79 ± 1.53</td>
<td>6.16 ± 1.06</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.81 ± 0.23</td>
<td>3.03 ± 0.26</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.70 ± 0.52</td>
<td>2.96 ± 0.39</td>
</tr>
<tr>
<td>Skin</td>
<td>3.72 ± 0.68</td>
<td>2.34 ± 0.46</td>
</tr>
<tr>
<td>Liver</td>
<td>5.41 ± 0.94</td>
<td>3.40 ± 0.64</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.96 ± 0.85</td>
<td>3.12 ± 0.58</td>
</tr>
<tr>
<td>Heart</td>
<td>2.77 ± 0.50</td>
<td>1.74 ± 0.34</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2.25 ± 0.55</td>
<td>1.42 ± 0.36</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>2.94 ± 0.69</td>
<td>1.85 ± 0.45</td>
</tr>
<tr>
<td>Brain</td>
<td>0.28 ± 0.05</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Bone</td>
<td>0.82 ± 0.19</td>
<td>0.52 ± 0.12</td>
</tr>
</tbody>
</table>

NOTE: Mean dose and SD are presented under the assumption that the distribution of activity in the therapy study follows the biodistribution of $^{177}$Lu pertuzumab presented in Fig. 1 and Table 1.
The tumors were high, as could be expected from our previously published retention studies (1). The tumor-to-blood ratio increased from about 1.4, 8 h after injection, up to about 19 after 2 weeks, which is a promising result.

The simplified dosimetry calculation used in this article, which does not include crossfire and photon contribution, is motivated by the local absorption of the low-energy particles and the low abundance of photons and other penetrating radiations in the \(^{177}\)Lu decay. Thus, the main uncertainty in the dose calculations is not due to this simplified model but rather due to the variation in kinetic data. The data show that the absorbed dose to the tumor is dominant to all normal organs.

For many radiolabeled antibodies used in targeted therapy, the critical organ usually is the red bone marrow due to crossfire from radioactivity in the blood. Because blood in our study is the organ that is obtaining the highest absorbed dose besides the tumor, one may suspect that bone marrow, also in targeting therapy with \([^{177}\text{Lu}]\text{pertuzumab}\), is the critical organ. However, the generally accepted coupling factor (red marrow-to-blood activity concentration ratio) is 0.36 (21). Because the absorbed dose tumor-to-blood ratio is \(\sim 5\), the expected tumor-to-bone marrow ratio would be 14, which is still an advantageous factor and indicate a possibility to obtain good therapy results.

The goal of clinical treatment with \([^{177}\text{Lu}]\text{pertuzumab}\) is primarily to eliminate micrometastases not visible at the time of surgery. With this in mind, \([^{177}\text{Lu}]\text{pertuzumab}\) was, in the experimental therapy experiments, given 7 days after xenograft inoculation. The xenografts had time to establish but not to grow into a detectable solid tumor.

The tumor growth curves were most retarded in the groups that received radioactivity targeted with pertuzumab, which of course is promising for the planning for clinical therapy trials on HER-2-overexpressing tumors. Furthermore, no groups of mice showed any significant weight loss after injection, indicating no major acute toxicity from the antibodies and/or the radiation.

To facilitate statistical analysis of the treatments, a survival curve analysis of Kaplan-Meier type was done. This analysis showed that the group that received 7 MBq \([^{177}\text{Lu}]\text{pertuzumab}\) had a significantly increased time to tumor progression in relation to the controls. Mice receiving 5 MBq \([^{177}\text{Lu}]\text{pertuzumab}\) also had a significantly longer time to tumor progression than mice that received PBS or native pertuzumab. Thus, the HER-2-specific radionuclide therapy proved more efficient than not HER-2-specific treatments.

However, although the time to tumor progression was significantly improved using HER-2–targeted radiotherapy, only two of all HER-2–targeted mice did not resume tumor progression. Immunohistology of sections from these two tumors showed presence of HER-2 in the remaining tissue, but because no tumor growth was observed in these cases, the HER-2–positive tumor cells seemed unable to divide.

We believe that the therapy could be improved further by using higher amounts of \([^{177}\text{Lu}]\text{pertuzumab}\). It is probable that the maximum tolerated doses have not been reached in this study. This is also indicated because no adverse effects could be found by the treatment. The obtained therapy results are encouraging, especially considering that the transplanted SKOV-3 cells have been reported to be radioresistant (22).

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The results showed that \(^{177}\text{Lu}\) pertuzumab can be used as an agent for HER-2–targeted radionuclide therapy in vivo. We believe that the optimal use for \(^{177}\text{Lu}\) pertuzumab would be in an adjuvant setting when there are strong indications of metastatic spread of tumor cells. We are currently planning studies of radionuclide uptake, when applying \(^{177}\text{Lu}\) pertuzumab in clinical phase I/II studies on breast and urinary bladder cancers overexpressing HER-2.

**References**

[\(^{177}\text{Lu}]\)Pertuzumab: Experimental Therapy of HER-2–Expressing Xenografts


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