Abstract. The purpose of this study was to determine therapeutic effects and systemic toxicity of $^{212}$Pb-trastuzumab in an orthotopic model of human prostate cancer cells in nude mice. TCMC-Trastuzumab was radiolabeled with $^{212}$Pb. The $^{212}$Pb-trastuzumab generated from the procedure was intact and had high binding affinity with a dissociation constant (of $3.9 \pm 0.99$ nM. PC-3MM2 cells, which expressed a lower level of HER2 both in culture and in tumors, were used in therapy studies. A single intravenous injection of $^{212}$Pb-trastuzumab reduced tumor growth by 60-80%, reduced aortic lymph node metastasis, and prolonged the survival of tumor-bearing mice. Treatment with $^{212}$Pb-trastuzumab did not cause significant changes in body weight, serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), hematological profiles, and histological morphology of several major organs of tumor-bearing mice. These findings suggest that a systemic delivery of $^{212}$Pb-trastuzumab could be an effective modality for management of advanced human prostate cancer.

Introduction

Prostate cancer is the most common cancer and the second most common cause of cancer death among men in the United States (1). As detection techniques improve, more patients are diagnosed with localized disease and can be cured by either surgery or radiation therapy. Metastasis in many patients, however, still occurs prior to the initial diagnosis. Because of natural resistance to most chemotherapeutic agents, hormonal therapy is the mainstay treatment for advanced diseases, however, this treatment is only palliative: delaying tumor progression to castration-refractory prostate cancer (CRPC) by an average of less than 18 months (2,3). Currently, there are limited effective therapies for management of advanced CRPC. Thus, there is a great need to develop novel and more effective therapies in this setting.

Numerous studies have clearly documented significant contribution of HER2/EGFR signaling in the progression of human prostate cancer and HER2 overexpression in prostate cancer occurs at relevant frequency without gene amplification. Increased HER2 expression correlates with an aggressive behavior of tumor cells through stimulation of tumor cell proliferation and was associated with Ki67 labeling index (4). HER2 is overexpressed in approximately 20-30% of prostate cancers and in 78% of androgen-independent cancers (5). It is, therefore, preferentially expressed in hormone-refractory and metastatic prostate cancers (6). The pretreatment serum HER2 and its extracellular domain (HER2 ECD) values were shown to be independent predictors of biochemical recurrence of prostate cancer (7,8) and were associated with prostate cancer progression after radical prostatectomy (9,10). Moreover, chronic treatment with bicalutamide induced overexpression of HER2 and a reduction of PTEN and EGFR/HER2 ratio, which was associated with an increase in Akt and Erk activity (11,12). In addition to stimulation of tumor cell proliferation through HER2/EGFR signaling, HER2 also contributes to prostate cancer progression through stabilizing AR protein in the absence of androgen (13) phosphorylating and trans-activating AR transactivation (14-16), as well as crosstalk with TrkA in a subset of prostate cancer cells (17).

Preclinical therapy studies showed that downregulation of HER2 expression and activation by BN/GRPR inhibitors AN-215 and RC-3095 inhibited growth of LNCaP and PC-3 human prostate cancer cells (18). Moreover, inhibitory effects of EGFR inhibitor erlotinib were much more potent on androgen-sensitive prostate cancer cells when compared to those on CRPC.
cells. Whereas the carnertinib efficacy may have therapeutic significance in HER2 overexpressing AR+ CRPC models in combination with hormone manipulation (19). However, preclinical studies in animal models of human prostate cancer using HER2-specific antibody trastuzumab alone showed limited therapeutic responses (20-22). Similarly, HER2 dimerization inhibitor pertuzumab as a single agent was found to be ineffective in patients with hormone-refractory prostate cancer (23). Two additional multicenter phase II trials using the EGFR-HER2 inhibitor lapatinib (24) and trastuzumab (25) confirmed that the treatment was well tolerated but demonstrated no significant antitumor activity even in a hormonal therapy-naive population of patients.

The high linear-energy transfer (LET) produced by an α-particle inside a cell can kill the cell with one to a few particles/cell in an oxygen tension- and dose rate-independent fashion (26-28). Radioimmunotherapy using α-particle-labeled agents exhibits minimal systemic toxic side effects and is potentially useful for tumors on which antigens are not significantly overexpressed. Indeed, promising therapeutic effects of α-particle emitters have been reported in multiple animal tumor models, including melanoma (29), colon cancer (30,31), leukemia (32), lung cancer (33), ovarian cancer (34), lymphoma (35), and prostate cancers (35). These reports prompted us to evaluate potential therapeutic effects of 212Pb-labeled trastuzumab against human prostate cancer using an orthotopic model in mice. Lead-212 is the parent radionuclide in a short-lived series of isotopes that include 210Po that emits an 8.784 MeV α-particle. Data presented here show that a single i.v. delivery of 212Pb-trastuzumab did not cause significant systemic toxicity, retarded primary tumor growth, reduced lymph node metastasis, and prolonged survival of tumor-bearing mice. These data suggest that 212Pb-trastuzumab could be an effective modality for therapy against advanced human prostate cancer.

Materials and methods

Mice. Specific pathogen-free male nude mice were purchased from Harlan Laboratories (Indianapolis, IN) and used in this study when they were 8-10 weeks of age. The mice were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the US Department of Agriculture, US Department of Health and Human Services, and the National Institute of Health. The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) and executed according to IACUC guidelines.

Radiolabeling of lead-212 (212Pb) to TCMC-trastuzumab. The bifunctional chelating agent TCMC [(1,4,7,10-tetra-(2-carbamoyl methyl)-cyclododecane] (provided by Dr Martin Brechbiel at the NCI-NIH) was conjugated to trastuzumab to derive TCMC-trastuzumab according to previously described procedures at a 20-fold molar excess ratio of chelate. The conjugation was performed by Goodwin biotechnology for AREVA Med. Characterization of TCMC-trastuzumab conjugate was performed by Brechbiel's group as previously reported (36). The radiolabeling of TCMC-trastuzumab to derive 212Pb-trastuzumab was carried out following procedures described in previous studies (30,31) with modifications. Briefly, 212Pb was eluted from a 224Ra generator (AREVA Med, LLC) using 4.5 ml of 2 N HCl. Potential organics were removed by acid digestion with boiling 8 N HNO3, and resuspended in 300 µl of 0.1 N HNO3. The amount of 212Pb was quantified with a high purity germanium detector. Chelation was performed by incubating with 1 mg of TCMC-trastuzumab per mCi of 212Pb for 1 h at 37˚C in the presence of 50 µl of 220 mg/ml sodium ascorbate and 30 µl of 5 M NH4OAc. The reaction was quenched with EDTA and the conjugated protein was passed through a PD-10 column (GE Healthcare, Piscataway, NJ) to remove unbound 212Pb.

Assessment of the integrity of 212Pb-trastuzumab. A standard radioimmunoassay (RIA) was performed to assess the affinity of the conjugated 212Pb-trastuzumab to HER2. In addition, an SDS-PAGE analysis of 212Pb-trastuzumab was performed. The radioactivity of 212Pb-trastuzumab was visualized in a Kodak Image Station IS4000MM Digital Imaging System (Eastman Kodak Co., Rochester, NY) and 212Pb-trastuzumab protein in the SDS-PAGE was visualized by staining with Coomassie blue to confirm the co-migration of the 212Pb with the protein.

Tumor cells and culture. PC-3MM2 cells (37) were generously provided by Dr Isaiah J. Fidler (The University of Texas M.D. Anderson Cancer Center, Houston, TX). The cells were maintained as a monolayer culture in MEM supplemented with 5% FBS, nonessential amino acids, sodium pyruvate, vitamin A, and glutamine. LNCaP (38,39), 22Rv1 (39,40), DU-145 (41), and MCF-7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). LNCaP, 22Rv1, and MCF-7 cells were maintained in RPMI-1640 supplemented with 10% FBS. DU-145 cells were cultured in MEM supplemented with 5% FBS. LAPC-4 cells (39,42) were obtained from Dr Karen Knudson (Kimmel Cancer Center, Thomas Jefferson Medical College, Philadelphia, PA) and maintained in IMDM medium supplemented with 10% FBS and 10 nM of DHT. Cells in exponential phase of growth were harvested by a 1:3-min treatment with a 0.25% trypsin - 0.02% EDTA solution. The flasks were tapped to detach the cells, a medium with 5% FBS was added, and the cell suspension was gently agitated to produce a single-cell suspension. Cell viability was ascertained by trypsin blue exclusion assay. Only cells with viability exceeding 95% were used.

Tumor cell inoculation. Mice were anesthetized and placed in the supine position. A lower midline incision was created to expose the prostate using a surgical procedure detailed in our previous study (42). A tumor cell suspension (2x106 cells in 20 µl PBS) was injected into the dorsal prostatic lobes using a 30-gauge needle through a 1-ml disposable syringe with a calibrated push button-controlled dispensing device (Hamilton Syringe Co., Reno, NV). The abdominal wound was closed in two layers using absorbable sutures and wound clips, respectively. The wound clips were removed 2 weeks after the surgery.

Therapy procedures. Tumor-bearing mice were randomized into groups and injected with 212Pb-trastuzumab on days 7 to 10 post tumor cell inoculation. Mice serving as controls were injected with PBS or trastuzumab at the same dose used in the highest dose of 212Pb-trastuzumab. Control and treated tumor-bearing
mice were monitored daily. Twice a week, mouse body weight was recorded for toxicity evaluation. Experiments were terminated 3 weeks after the therapy intervention. Tumor-bearing mice were sacrificed when they were moribund to evaluate effects of the therapy on survival. Blood samples were collected before sacrificing the mice for serum biochemistry evaluation and blood cell counting. Tumors were weighed and sampled for histology examination. Aortic lymph nodes were harvested and sampled for histology examination of metastatic lesions.

**Immunoblot analysis.** Tumor cell lysates were prepared in a lysis buffer containing a proteinase inhibitor cocktail (Sigma Chemicals). After a centrifugation at 12,000 rpm for 20 min, the supernatants were collected and subjected to immunoblotting analysis as described in our previous study (43) using antibodies against HER2, EGFR and β-actin. Immunoreactive signals were revealed after incubation with their respective secondary antibodies (Bio-Rad, Hercules, CA) using an ECL Western blot detection system (Millipore Co., Billerica, MA) and visualized in the Kodak Image Station IS4000MM Digital Imaging System.

**Immunohistochemical (IHC) analysis.** Expression of HER2 in tumor lesions was evaluated by IHC staining as described previously (44,45). Briefly, tumor tissues were rinsed with PBS, fixed in formalin, embedded in paraffin, and sectioned. Tissue sections (4 µm) were dewaxed and treated with 3% hydrogen peroxide (H₂O₂) in methanol (v/v). The treated slides were incubated in a blocking solution and then reacted for 18 h at 4°C in a humidified chamber with an anti-HER2 antibody. The sections were rinsed and incubated with peroxidase-conjugated secondary antibodies. A positive reaction was visualized by incubating the slides with the stable DAB and counterstaining with Mayer’s hematoxylin. The slides were dried and mounted with Universal mount, and images were digitized using an Olympus CCD camera (Olympus, Tokyo, Japan) and a personal computer equipped with Optimas Image Analysis Software (Optimas Corp., Bothell, WA).

**Blood sample analyses.** Blood for cell counting was collected into a vial coated with EDTA and cells were counted in the HEMAVET 950FS automatic cell counter (Drew Scientific Inc., Waterbury, CT). Blood was allowed to clot for 2 h at room temperature for serum biochemistry. Serum was collected after a centrifugation at 3,000 rpm for 15 min to remove the clotted material. Blood urea nitrogen (BUN) and serum glutamic pyruvic transaminase (SGPT) were measured using kits purchased from Teco Diagnostics (Anaheim, CA).

**Statistical analysis.** The therapy experiments were performed with 4-8 mice per group. Analysis of variance (ANOVA) was used to compare differences in tumor volumes and weights among study and control groups. An estimate of survival time used to compare differences in tumor volumes and weights with 4-8 mice per group. Analysis of variance (ANOVA) was performed to determine the integrity of the labeled protein. Human serum albumin (HSA) was added to purified ²¹²Pb-trastuzumab for stabilization.

**Figure 1.** Characterization of ²¹²Pb-trastuzumab. (A and B) After the radio-labeling of ²¹²Pb to trastuzumab, the reaction was quenched with EDTA and the free ²¹²Pb was removed using a PD-10 column. The ²¹²Pb-trastuzumab was sampled before (lane 1) and after purification (lane 2) for SDS-PAGE analysis with unlabeled trastuzumab (lane 3) to determine the integrity of the labeled protein. Human serum albumin (HSA) was added to purified ²¹²Pb-trastuzumab for stabilization. (C) The radioimmunoassay (RIA) was performed to determine affinity of ²¹²Pb-trastuzumab to HER2.

**Results**

**Integrity and immunoreactivity of ²¹²Pb-trastuzumab.** The radiograph of the SDS-PAGE analysis shows that the vast majority of the ²¹²Pb signal was associated with protein migrated at 140 kDa (Fig. 1A), which strongly suggested that ²¹²Pb was associated with intact trastuzumab. Coomassie-blue staining of the same gel confirmed that ²¹²Pb-trastuzumab migrated the same distance as unlabeled trastuzumab (Fig. 1B) and demonstrates that the ²¹²Pb-conjugated trastuzumab was pure and intact. A RIA analysis shows that the binding of ²¹²Pb-conjugated to ErbB2 in recombinant ErbB2/Fc chimera protein-coated wells was completely blocked by the presence of free form ErbB2/Fc and demonstrates a highly specific binding of ²¹²Pb-trastuzumab.

Curve fitting analysis showed that the equilibrium dissociation constant (Kd) of the binding was 2.86±0.25 nM (Fig. 1C). The Kd for analysis of ²¹²Pb-trastuzumab from three independent conjugations was 3.9±0.99 nM, further indicating the reproducibility of the procedure. Taken together, these data indicated that we have successfully radiolabeled ²¹²Pb eluted from the generator to intact and functional trastuzumab.

**Identification of tumor model for therapy studies.** Expression of HER2 and EGFR was analyzed in several lines of human prostate cancer cells used most commonly in preclinical studies. As shown in Fig. 2A, PC-3-MM2 cells expressed the lowest level of HER2 among all cell lines examined. Whereas HER2 expression was readily stained by IHC in tumors formed by LNCaP and LAPC-4 cells, it was almost undetectable in tumors formed by PC-3-MM2 cells (Fig. 2B). To investigate the effectiveness of ²¹²Pb-trastuzumab therapy on tumors expressing relatively lower
level of HER2, the orthotopic model of PC-3MM2 tumor was chosen to perform the preclinical therapy studies.

Significant therapeutic effects of $^{212}$Pb-trastuzumab. PC-3MM2 cells were inoculated into the prostates of nude mice. After one week, when the prostatic tumors reached ~50-100 mg (45), tumor-bearing mice were randomized and treated with a single i.v. injection of 10 or 20 µCi $^{212}$Pb-trastuzumab. Tumor-bearing control mice were left untreated or i.v. injected with 45 µg/mouse, a dose equivalent to trastuzumab present in 20 µCi $^{212}$Pb-trastuzumab. Mice were sacrificed 21 days after the treatment to evaluate tumor volumes and metastasis. As shown in Fig. 3A, a single injection of unconjugated trastuzumab did not affect the growth of PC-3MM2 tumors. In contrast, growth of PC-3MM2 tumors was significantly suppressed in mice treated with 10 and 20 µCi $^{212}$Pb-trastuzumab. Tumor weights in $^{212}$Pb-trastuzumab treated mice were 36 and 60% (p<0.05) of the weight of tumors in untreated or trastuzumab-treated mice. Tumor growth retardation was 74% (p<0.05) using 20 µCi $^{212}$Pb-trastuzumab when the experiment was repeated. Aortic lymph node metastases were observed in all control mice but in only 40% of the mice treated with 20 µCi $^{212}$Pb-trastuzumab.

The efficacy of $^{212}$Pb-trastuzumab therapy on survival was evaluated using tumor-bearing mice treated with 20 µCi $^{212}$Pb-trastuzumab. Effects on survival of tumor-bearing mice show that treatment prolonged survival (p=0.0046) with a median (50%) survival rate of 47 and 61 days for untreated and

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Figure 2. Determination of HER2 expression in human prostate cancer cells. (A) Cell lysates were prepared from several lines of human prostate cancer cells and analyzed by immunoblotting. (A) Lysate from MCF-7 human breast cancer cells was analyzed in the same gel as a positive control. (B) Paraffin-embedded sections from tumors formed by PC-3MM2, LNCaP, and LAPC-4 cells in nude mice were stained by IHC using antibody against HER2.

Figure 3. Therapeutic effects of $^{212}$Pb-trastuzumab. (A) PC-3MM2 cells were orthotopically implanted into nude mice. One week later, the tumor-bearing mice were left untreated (control) or i.v. injected unlabeled trastuzumab (control), 10 or 20 µCi $^{212}$Pb-trastuzumab. Three weeks later, the experiment was terminated and tumors were removed and weighed. (B) One week after tumor cell implantation, the tumor-bearing mice were left untreated (control) or i.v. injected with 20 µCi $^{212}$Pb-trastuzumab. Mice were sacrificed when they were moribund to estimate survival of tumor-bearing mice. (C) Upon sacrificing mice from the survival study, tumors were removed and weighed. (D) The body weights of tumor-bearing mice in control and treated groups in (A) were recorded for evaluation of systemic toxicity of the treatments.
treated mice, respectively. Whereas all mice in control group became moribund by day 50, 5 out of 7 treated mice survived to day 60, 2/7 survived to day 83, and 1/7 mice survived to day 97. Furthermore, final tumor weights were greater in controls than in treated mice (Fig. 3C, p=0.0168), suggesting that a long-term retention of urine, a drawback of this orthotopic model, contributes at least partially to moribundity in treated mice.

The treatment with $^{212}$Pb-trastuzumab did not cause significant systemic toxic effects. To evaluate potential systemic toxicity of i.v. injection of $^{212}$Pb-trastuzumab in this PC-3MM2 orthotopic tumor model, we determined body weight, serum biochemistry, and histological changes in several organs.

The body weights were not significantly different among any of the groups of tumor-bearing mice regardless of treatment during the 3-week study (Fig. 3D). Given the differences of tumor weight among the treatment groups (Fig. 3A), the actual reduction of body weight in mice treated with $^{212}$Pb-trastuzumab, especially those treated with 20 µCi, was much less than in the control mice, suggesting a protective response due to treatment with $^{212}$Pb-trastuzumab.

Potential acute (3 weeks after therapy) toxic effects of $^{212}$Pb-trastuzumab on the liver and kidneys were investigated by analysis of serum SGPT and BUN in tumor-bearing mice in the first 72 h after an i.v. injection of 30 µCi $^{212}$Pb-trastuzumab and at end of the therapy studies described above. Results in Tables I and II demonstrate that treatment did not cause significant changes in the levels of serum SGPT and BUN and indicate that the therapy caused no significant liver and kidney toxicity in the tumor-bearing mice.

Next, we determined potential hematological toxicity of $^{212}$Pb-trastuzumab therapy. Ten days after an i.v. injection of 20 µCi $^{212}$Pb-trastuzumab, blood was harvested for cell counting using cells from untreated mice as a control. Data in Table III show the hematology profile of control and treated mice. Clearly, the treatment with $^{212}$Pb-trastuzumab did not cause significant alterations to the profiles of leukocytes, erythrocytes, and thrombocytes.

Finally, upon termination of the therapy study, several internal organs were removed and prepared for paraffin sectioning and H&E-staining. No significant discernable changes in the morphology of the organs due to $^{212}$Pb-trastuzumab were found (Fig. 4).

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**Table I. Serum SGPT and BUN after i.v. injection of $^{212}$Pb-Trastuzumab.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>SGPT (IU/l) Mean SD</th>
<th>BUN (mg/dl) Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>36</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>48</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>60</td>
<td>34</td>
<td>14</td>
</tr>
</tbody>
</table>

PC-3MM2 cells were inoculated into the prostate of nude mice. Two weeks later, the mice were i.v. injected with 30 µCi/mouse $^{212}$Pb-trastuzumab. Mice were sacrificed at various times and blood sampled for serum biochemistry analysis.

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**Table II. Serum SGPT and BUN at the end of the tumor reduction therapy.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGPT (IU/l) Mean SD</th>
<th>BUN (mg/dl) Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>Herceptin (45 µg)</td>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>10 µCi $^{212}$Pb-trastuzumab</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>20 µCi $^{212}$Pb-trastuzumab</td>
<td>31</td>
<td>16</td>
</tr>
</tbody>
</table>

PC-3MM2 cells were orthotopically inoculated into the prostate of nude mice. One week later, tumor-bearing mice were i.v. treated with 10 or 20 µCi $^{212}$Pb-trastuzumab. Untreated mice and mice i.v. injected with 45 µg non-radioactive trastuzumab (equivalent the amount of herceptin in 20 µCi $^{212}$Pb-conjugated counterpart) were used as controls. The experiment was terminated 3 weeks later and blood was sampled for serum biochemistry analysis.

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**Table III. Hematology profile of tumor-bearing mice.**

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>$^{212}$Pb-trastuzumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (x10³/µl)</td>
<td>4.4</td>
<td>8.9</td>
</tr>
<tr>
<td>NE (x10³/µl)</td>
<td>1.4</td>
<td>7.0</td>
</tr>
<tr>
<td>LY (x10³/µl)</td>
<td>2.6</td>
<td>1.4</td>
</tr>
<tr>
<td>MO (x10³/µl)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>EO (x10³/µl)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>BA (x10³/µl)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (x10⁶/µl)</td>
<td>8.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.2</td>
<td>13.8</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>48.2</td>
<td>49.8</td>
</tr>
<tr>
<td>MCH (g/dl)</td>
<td>54.9</td>
<td>51.9</td>
</tr>
<tr>
<td>MCHC (fl)</td>
<td>15.1</td>
<td>14.4</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>27.5</td>
<td>27.7</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT (x10³/µl)</td>
<td>751.0</td>
<td>663.7</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

WBC, white blood cells; NE, neutrophils; LY, lymphocytes; MO, monocytes; EO, eosinophil; BA, basophil; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume.

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Discussion

Trastuzumab, used alone or in combination with other drugs, is approved by the Food and Drug Administration for treatment of HER2-positive breast cancer. When combined with other drugs, it is also approved by FDA for therapies against metastatic gastric or gastroesophageal junction adenocarcinoma. Whereas rarely amplified, HER2 is expressed in most prostate cancers and overexpressed in advanced diseases (5), suggesting it could serve as a promising target for management of advanced prostate cancer. However, several clinical trials have failed to show any benefits using either antibody that inhibits HER2 dimerization (23) or small molecule inhibitor of EGFR-HER2 (24). Similarly, a clinical trial with trastuzumab as a single agent demonstrated poor efficacy in treating hormonal refractory human prostate cancer (46). \(^{212}\)Pb-trastuzumab has been shown to delay tumor growth and increase survival when injected i.p. in mice bearing i.p. tumor burdens (30,31) and is currently in phase I clinical trials for HER2\(^+\) abdominal cancers using i.p. administration (http://clinicaltrials.gov/ct2/show/NCT01384253). To explore effectiveness of systemic \(\alpha\)-particle-mediated radioimmunotherapy targeting HER2, we determined therapeutic effects of \(^{212}\)Pb-trastuzumab in a xenograft model of human prostate cancer and showed that a single i.v. injection of...


212Pb-trastuzumab significantly retards growth and progression of androgen-independent PC-3MM2 tumors in nude mice and prolonged survival of tumor-bearing mice.

Several considerations were adopted in this study to generate preclinical data more relevant to clinical translation, particularly for advanced prostate cancer where HER2 gene is rarely amplified. First, we chose to study efficacy of 212Pb-trastuzumab therapy using the PC-3MM2 model, which expresses a relatively lower level of HER2 both in culture and in tumors among several lines of human prostate cancer cell most commonly used in preclinical studies. Second, we determined the therapeutic efficacy in an orthotopic model, which, compared to other ectopic ones, mimics more closely the physiological microenvironment of human prostate cancer. Third, we determined potential systemic toxicity of the therapy by evaluating the body weight, serum biochemistry parameters, hematology profiles, and histological examination. The results presented in this report demonstrate that the 212Pb-trastuzumab therapy caused no significant systemic toxicity and was very effective in retarding tumor growth and prolonging survival of mice bearing the tumors that express very low level of HER2. These findings suggest that 212Pb-trastuzumab, used alone or in combination with other means, could be an effective modality for management of advanced prostate cancer.

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References