An α-Particle Emitting Antibody ([213Bi]J591) for Radioimmunotherapy of Prostate Cancer

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ABSTRACT

A novel α-particle emitting monoclonal antibody construct targeting the external domain of prostate-specific membrane antigen (PSMA) was prepared and evaluated in vitro and in vivo. The chelating agent, N-[2-amino-3-(p-isothiocyanatophenyl)propyl]-trans-cyclohexane-1,2-diamine-\(N^1, N^2, N^3, N^4\)-pentaacetic acid, was appended to J591 monoclonal antibody to stably bind the \(213^\text{Bi}\) radiometal ion. Bismuth-213 is a short-lived (\(t_{1/2} = 46\) min) radionuclide that emits high energy α-particles with an effective range of 0.07–0.10 mm that are ideally suited to treating single-celled neoplasms and micrometastatic carcinomas. The LCNa prostate cancer cell line had an estimated 180,000 molecules of PSMA per cell; J591 bound to PSMA with a 3-nM affinity. After binding, the radiolabeled construct-antigen complex was rapidly internalized into the cell, carrying the radiometal inside. \([213^\text{Bi}]\text{J591}\) was specifically cytotoxic to LNCaP spheroids, an in vitro model for micrometastatic cancer, also was investigated. \([213^\text{Bi}]\text{J591}\) effectively stopped growth of LNCaP spheroids relative to an equivalent dose of the irrelevant control \([213^\text{Bi}]\text{HuM195}\) or unlabeled J591. Cytotoxicity experiments in vivo were carried out in an athymic nude mouse model with an in vivo xenograft of LNCaP cells. \([213^\text{Bi}]\text{J591}\) was able to significantly improve \((P < 0.0031)\) median tumor-free survival (54 days) in these experiments relative to treatment with irrelevant control \([213^\text{Bi}]\text{HuM195}\) (33 days), or no treatment (31 days). Prostate-specific antigen (PSA) was also specifically reduced in treated animals. At day 51, mean PSA values were 104 ng/ml \(\pm/\mp 54\) ng/ml (mean = 4, untreated animals), 66 ng/ml \(\pm/\mp 16\) ng/ml (mean = 6, animals treated with \([213^\text{Bi}]\text{HuM195}\) and 28 ng/ml \(\pm/\mp 22\) ng/ml (mean = 6, animals treated with \([213^\text{Bi}]\text{J591}\)). The reduction of PSA levels in mice treated with \([213^\text{Bi}]\text{J591}\) relative to mice treated with \([213^\text{Bi}]\text{HuM195}\) and untreated control animals was significant with \(P < 0.007\) and \(P < 0.0136\), respectively. In conclusion, a novel \([213^\text{Bi}]\)-radiolabeled J591 has been constructed that selectively delivers α-particles to prostate cancer cells for potent and specific killing in vitro and in vivo.

INTRODUCTION

Metastatic prostate cancer that is resistant to hormone therapy has been treated with nonspecific, systemic bone-seeking agents such as \(^{32}\)P, \(^{89}\)Sr, and \(^{153}\)Sm (1–3), having the palliation of pain as the objective end point. Specifically targeted radiotherapy using prostate tumor-specific CC49 antibody labeled with \(^{131}\)I has been evaluated in two clinical trials with patients having metastatic prostate carcinoma. In one trial, antibody localization was seen in 11 of 14 patients along with significant myelosuppression, however, it was estimated that there were low radiation doses to tumor (~1000 cGy); there was no repeat dosing due to human antiumrine antibody responses, and no patients met the radiographic or PSA criteria for objective response (4). In the second trial, a high degree of tumor localization was observed in 13 of 15 patients, but human antimurine antibody response was seen in all patients; some degree of antitumor effect, as measured by pain relief, was seen in 6 of 10 symptomatic patients. However, once again, no patients met the radiographic or PSA criteria for objective response (5).

Several mAbs that target prostate cancer were modified with the \(N-[2\text{-amino-3-(p-isothiocyanatophenyl)propyl}]-\text{trans-cyclohexane-1,2-diamine-N}^1, N^2, N^3, N^4\)-pentaacetic acid (SCN-CHXAmoieties), labeled with \(^{213}\)Bi, and their in vitro potency toward cancer cell lines was evaluated (6). This comparative study of potential therapeutic antiprostate mAbs resulted in the selection of J591 as the IgG to study further. J591 targets the external domain of PSA (7–9). PSA is a highly restricted prostate epithelial cell integral membrane glycoprotein, providing an immunogenic extracellular domain. PSA is expressed by a high proportion of prostate carcinomas, and its expression is further increased in higher-grade cancers, in metastatic disease and in hormone-refractory prostate cancers. There are several mAbs targeting the external domain of PSA that have been developed and described in the literature (7–9).

The construction of an α-particle emitting IgG radiotherapeutic moiety represents a “new class” of potent and specific radiopharmaceuticals used only recently in humans (10, 11). The radionuclide \(^{213}\)Bi is a short-lived (\(t_{1/2} = 46\) min) α-particle emitting metal-ion generated from the decay of \(^{225}\)Ac-225. Bismuth-labeled HuM195, an anti-CD33 antibody, demonstrated specific and potent cell killing ability when directed against a leukemia cell line (12). A robust, reliable \(^{225}\)Ac/\(^{213}\)Bi generator system was developed at Memorial Sloan-Kettering Cancer Center (13, 14) and used to treat 18 patients with 80 doses of \([213^\text{Bi}]\text{HuM195}\) in a Phase I clinical trial (11). Clinical conditions for reproducible radiolabeling of antibodies and quality assurance testing were developed (15). Feasibility for human use and therapeutic activity were demonstrated. The low abundance, low energy γ-emissions associated with the decay of \(^{213}\)Bi (440 KeV, frequency of 16% per disintegration) were used in evaluating the biodistribution patterns of \([213^\text{Bi}]\text{HuM195}\) in humans and in performing dosimetry calculations (16–18).

α-Particle therapy has been proposed for use in single-cell disorders, such as leukemias. The purpose of the current study was to evaluate the biochemistry, biology, and cytotoxicity of a potent \([213^\text{Bi}]\)-labeled J591 construct in a series of in vitro and in vivo models of prostate cancer to determine whether RIT with α-particles also would be suitable for clinical use against prostate cancer. Such an approach would be particularly appropriate after debulking radiation or surgery to reduce the size of metastatic deposits of cancer.

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**MATERIALS AND METHODS**

**J591.** The murine mAb J591, reactive with the external domain of PSMA, was obtained from the laboratory of Dr. Neil Bander (Department of Urology, New York Presbyterian Hospital-Weill Medical College of Cornell University; Refs. 7–9).

**Conjugation of Bifunctional Chelate to J591.** J591 was conjugated to N-[2-amino-3-(p-isothiocyanatophenyl)propyl]-trans-cyclohexane-1,2-diamine-N,N,N',N'-pentaaacetic acid (SCN-CHX-A-DTPA), a backbone substituted derivative of DTPA (19), using an Amicon, Inc. (Beverly, MA) continuous buffer exchange/dialysis apparatus (20). The average number of chelates per antibody ranged from one to three as determined by the yttrium arsenazo spectrophotometric method (21). Immunoreactivity of the construct was determined as described (12).

**Radioiodination Protocol, Radioiodination, and Purification of [**125I**]J591.** Bismuth-213 was produced (22–24) from an 225Ac-source generously supplied by the Institute for Transuranium Elements (Karlsruhe, Germany) or purchased from Oak Ridge National Laboratory (Oak Ridge, TN). The 225Ac was fashioned into a radionuclide generator that produced 213Bi using published procedures (13). Radiolabeling and purification of the mAb construct with 213Bi was carried out using published methods (12). The labeling efficiency of the reaction and purity of the final product was determined routinely using instant TLC with silica gel impregnated paper (Gelman Science Inc., Ann Arbor, MI; Ref. 12).

**Bismuth-213 Activity Determination.** Bismuth-213 activity was measured with a Squibb CRC-17 Radiolotope Calibrator (or equivalent model; E. R. Squibb and Sons, Inc., Princeton, NJ) set at 775 and multiplying the displayed activity value by 10. The activity value reported by the CRC calibrator was verified by counting a sample aliquot point source at a fixed geometry on a pulse height multichannel analyzer (Canberra Industries, Meriden, CT). The counting efficiency of the 213Bi 440 KeV γ-emission was determined using a plot of counting efficiency versus the γ-energy of standard radionuclides sources (13).

**Binding Affinity and Determination of the Number of Antigen-binding Sites.** A nonlinear regression curve fit of the data obtained for the specific binding of [**111In**]J591 was performed to determine the J591 equilibrium binding affinity with LNCaP cells and the number of J591-binding sites per cell. Briefly, the [**111In**]J591 construct was labeled, purified, and characterized in a similar fashion to the CHX-A-DTPA-HuM195 construct (12). Freshly prepared LNCaP cells at 4°C (5E4 cells/tube) were reacted with a 50-fold excess of unlabeled J591 and serial increments of [**111In**]J591 (in triplicate) or just the serial increments of [**111In**]J591 (in triplicate) to generate the equilibrium J591-binding curves. A nonlinear regression method was used to fit a curve to the plot of cpm per cell pellet versus radiolabeled antibody. B max was corrected for the immunoreactivity of the preparation and used to determine the number of antigen-binding sites per cell. An apparent KD was read from the plot at a value of 0.5 times the immunoreactivity corrected B max.

**Modulation of Cell Surface Antibody-Antigen Complexes.** Internalization of the cell surface antibody-antigen complex was determined by incubating 1.0 mg/ml [**111In**]J591 with 2E5 cells (×2) in a volume of 2.50 ml of RPMI media as a function of time at 37°C. The [**111In**]J591 construct was substituted for the [**210Bi**]-labeled construct in this assay because it has a longer half-life and as a radiometal behaves similarly to 213Bi in vitro; the [**111In**]J591 construct was labeled, purified, and characterized in a similar fashion to the CHX-A-DTPA-HuM195 construct (12). Exposed cells were pelleted at −0.5 min, 2 h, 4 h, and 24 h, and the pellets were washed twice in RPMI media; the surface-bound [**111In**]J591 was stripped with 1 ml of 50 mM glycine (Aldrich Chemical Co., Milwaukee, WI)/150 mM NaCl (pH 2.8; Aldrich Chemical Co.), at 24°C for 10 min. Total cell-associated radioactivity and internalized (acid-resistant) radioactivity were determined by counting the [**111In**]-labeled samples with a Packard Cobra Gamma Counter (Packard Instrument Co., Meriden, CT) with a 15–550 KeV energy window. To avoid nonspecific and Fc receptor binding by the cells, the assays were performed in the presence of 2% human serum.

**[**213Bi**]J591 Potency and Specificity against Single Cells in Vitro.** The potency and specificity of [**213Bi**]J591 for killing single prostate cancer cells was determined using SE4 LNCaP cells (PSMA +) or SKOV3 cells (PSMA −) in 0.200 ml in 96-well plates. In these experiments, serial dilutions of [**213Bi**]J591 were added to the cells to yield final activity in the wells ranging from 10–10,000 nCi/ml. The experiments were done with different specific activities of the bismuth-labeled constructs (0.06–6.4 mCi/µg). The plates were incubated 24 h at 37°C in 5% CO2. After incubation, cell viability was determined by [**3H**]thymidine (DuPont NEN, North Billerica, MA) incorporation (12).

**[**213Bi**]J591 Potency and Specificity against Multicellular Spheroids in Vitro.** The spheroid cytotoxicity experiments used two fixed activity levels [50,000 nCi/ml (specific activity of 2.5 Ci/g)] and 16,500 nCi/ml (specific activity of 0.6 Ci/g]) of [**213Bi**]J591 with treatment of groups of 24 similarly sized spheroids. Controls were spheroids: (a) exposed to equivalent activity levels and protein concentrations of a [**213Bi**]-labeled irrelevant IgG (HuM195); (b) unlabeled J591; and (c) no treatment (each group had 24 spheroids). Spheroids of LNCaP cells were initiated using described methods (25, 26). Initial spheroid volumes were approximately 1E-12 m3, which was a spheroid with a diameter equal to 0.124 mm and comprising about 1000 cells. Spheroid volumes were measured every 3 days for the duration of the study (1–2 months) using a microscope (Axio phot 2; Carl Zeiss Ltd., Göttingen, Germany) with a digital camera (EOS-DCS 5; Kodak, Rochester, NY; Refs. 26 and 27).

**LNCaP Tumor Model in Mice.** LNCaP tumor cells in Matrigel (Becton Dickinson Labware, Bedford, MA) were xenografted into athymic nude mice (8 weeks of age). Mice received an i.m. injection of 6–7E6 LNCaP tumor cells mixed with Matrigel in the right hind leg at a volume of 0.25 ml. Tumor growth in vivo was assessed at several early time points by sacrificing mice and examining the morphology, size, vascularization, and encapsulation of the tumor cells in the leg histologically.

**[**213Bi**]J591 Potency and Specificity in Vivo.** Two cytotoxicity experiments were carried out in vivo using the mouse model described above. In the first experiment, xenografted mice were separated into two treatment groups (5 animals, 6 per group) and one control group (n = 4 and treated 2 days after tumor implantation as: (a) cumulative 0.08 mCi [**213Bi**]J591 (four approximately equal daily doses); (b) cumulative 0.12 mCi nonspecific [**213Bi**]HuM195 (four approximately daily equal daily doses); and (c) no treatment). All animals were retro-orbitally bled on days 1, 6, and 51 posttreatment, and PSA levels were assayed using the IMX PSA Assay (Abbott Laboratories, Abbott Park, IL). Animals were also monitored for the appearance of a blue-colored lesion beneath the skin at the site of implantation (indicating the onset of a well-vascularized tumor) and the rate of tumor growth. In a second study, 32 xenografted male athymic nude mice were separated into five treatment groups and one control group and treated 2 days after tumor implantation as: (a) single 0.09 mCi dose of [**213Bi**]J591 (n = 6); (b) cumulative 0.13 mCi [**213Bi**]J591 (four approximately equal daily doses; n = 6); (c) single 0.09 mCi non-specific of [**213Bi**]HuM195 (n = 5); (d) cumulative 0.12 mCi dose of nonspecific [**213Bi**]HuM195 (four approximately equal daily doses; n = 5); (e) single dose of 0.02 mg of native J591 (n = 5); and (f) control group receiving no treatment (n = 5). All animals were retro-orbitally bled on day posttreatment, and PSA levels were assessed using the IMX PSA Assay. Animals were also monitored for the appearance of a blue-colored lesion beneath the skin at the site of implantation and rate of tumor growth as a function of time. All mice receiving [**213Bi**]-labeled mAbs and unlabeled mAbs were given injections via the retro-orbital sinus while under anesthesia of xylazine (10 mg/kg) and ketamine*HCl (200 mg/kg), following the guidelines set forth in institutional protocols. PSA data were evaluated using an unpaired t test (two-tailed). The data for median tumor-free survival versus time was evaluated using a log-rank test and plotted as a Kaplan-Meier survival curve.

**RESULTS**

**Equilibrium Binding Affinity and the Number of Antigen-binding Sites.** A nonlinear regression curve fit analysis (2, 0.995) of the [**111In**]J591 equilibrium binding data yielded an immunoreactivity-corrected B max value of 13,744 cpm per 50,000 LNCaP cells (Fig. 1). Using this value of B max, it was estimated that there were 180,000 molecules of J591 bound per cell. The equilibrium binding affinity, KD, was taken from the fit curve at 0.5 times B max and estimated to be 3 nm.

**Modulation of Cell Surface Antibody-Antigen Complexes.** The [**111In**]J591-PSMA (cell surface antibody-antigen) complex was rap-
idly internalized immediately after binding to the cell. Approximately 66% of the complex was internalized after 2 h, and 79% was internalized after 4 h (Fig. 2). The total cell-associated counts and, therefore, the cell surface counts continued to increase as a function of time through 24 h.

**Cell Kill against Single Cells in Vitro.** LNCaP cell kill by $^{[213}\text{Bi}]$J591 proved to be both specific activity dependent and activity concentration dependent (Fig. 3). The $LD_{50}$ value of $^{[213}\text{Bi}]$J591 was 220 nCi/ml at a specific activity of 6.4 Ci/g, 315 nCi/ml at a specific activity of 3.8 Ci/g, and 4400 nCi/ml at a specific activity approaching 0.06 Ci/g. At the low specific activities represented by the last group, there is essentially no specific binding of the $\alpha$-particle emitting J591 to the targets. Thus, this level of cell kill represents the nonspecific cytotoxicity in the system used here. Additional experiments evaluated $^{[213}\text{Bi}]$J591 against a PSMA-negative tumor cell line, SKOV3. The $LD_{50}$ value derived was 13,000 nCi/ml at a specific activity level of 1.6 Ci/g (data not shown). Therefore, cytotoxicity was specific activity related, dose related, and antigen specificity dependent.

**Cell Kill against Multicellular Spheroids in Vitro.** We have also investigated the potency and specificity of $^{[213}\text{Bi}]$J591 directed against LNCaP spheroids by assessing the change in volume of the spheroid as a function of time following various treatments. The spheroids initially comprised ~1000 LNCaP cells. The volumes of all of the spheroids treated with a single dose of $^{[213}\text{Bi}]$J591 decreased markedly over a 2-month period (Fig. 4). Their size was more than 100-fold smaller than the untreated spheroid control group in this time period. The $^{[213}\text{Bi}]$HuM195-treated spheroids demonstrate a 1-week delay in growth, followed by an increase in volume. Two other control groups, untreated spheroids and spheroids exposed to unlabeled J591 (0.027 mg/ml), showed similar increases in volume to each other as a function of time with an almost 100-fold increase in spheroid volume after 1 month.

**LNCaP Tumor Model in Mice.** LNCaP tumor cells were mixed with Matrigel (Becton Dickinson Labware) and xenografted into athymic nude mice, 8 weeks of age. Mice received an i.m. injection of $6 \times 10^6$ LNCaP tumor cells mixed with Matrigel in the right hind leg at a volume of 0.25 ml. Tumor growth in vivo was assessed histologically at days 2, 3, 5, 7, and 10 (Table 1). At the time RIT was administered (day 2), the tumors were characterized histologically as disorganized cell clusters and nodules each comprised of several thousands of cells. The nodules were not vascularized and not encapsulated. On day 3, the tumors were more organized and were becoming vascularized, but still not encapsulated. By the 5th day, vascularization was more pronounced, and on day 7 the tumors were encapsulated. The purpose of the RIT studies (described below) was to examine the ability of the agent to treat the tumors before encapsulation and neovascularization.

**Cell Kill in Vivo.** A single course of the $^{[213}\text{Bi}]$J591 drug, administered in four daily doses, improved ($P < 0.0031$) median tumor-free survival (Fig. 5) of LNCaP xenografted mice relative to mice treated with $^{[213}\text{Bi}]$HuM195 or untreated controls. The median tumor-free survival times were 31 days ($n = 4$, untreated animals), 33 days ($n = 6$, $^{[213}\text{Bi}]$HuM195-treated animals), and 54 days ($n = 6$, $^{[213}\text{Bi}]$J591-treated animals).

PSA is an important surrogate marker for prostate cancer burden in humans (28). It can also be used in mice with prostate cancer cell lines expressing PSMA.

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*Fig. 1. Equilibrium $^{[111}\text{In}]$J591 binding plot with LNCaP cells. The cpm of specific bound $^{[111}\text{In}]$J591 are plotted versus the concentration of $^{[111}\text{In}]$J591 added after subtracting the contribution of nonspecifically bound $^{[111}\text{In}]$J591. Specific bound radiolabeled J591 (•) and nonlinear regression curve fit (solid line) data are presented. Experiments were conducted as described in “Materials and Methods.”*

*Fig. 2. Internalization of radiometal conjugated J591. cpm versus time for total (●), internalized (□), and surface bound (▲) $^{[111}\text{In}]$J591 as shown for LNCaP cells. Experiments were conducted as described in “Materials and Methods.”*

*Fig. 3. Cytotoxicity of $^{[213}\text{Bi}]$J591 on LNCaP target cells. The percentage of viable cells versus varying activity concentrations of $^{[213}\text{Bi}]$J591 at three different drug-specific activities are shown (○, 0.06 Ci/g; ▲, 3.8 Ci/g; •, 6.4 Ci/g). Experiments were conducted as described in “Materials and Methods.”*
DISCUSSION

α-Particle emitting radiolabeled antibody constructs have been proposed as potent, selective agents to kill single cells (30, 31). We have shown this in vitro and in humans using an α-particle emitting anti-CD33 mAb for treatment of leukemia (10–12, 15, 16). Tens of billions to hundreds of billions of individual leukemia cells in the blood and marrow were killed safely. The kinetics and geometry of single-cell killing, however, might not be predictive of killing micrometastatic clusters of tumor cells as would be expected with the early spread of carcinomas. Therefore, we now ask whether an α-particle emitting agent would be useful in a model for solid tumors, such as prostate cancer, that form micrometastatic disease in the bone marrow. In this study, we describe for the first time the construction of a prostate-specific, α-particle emitting agent capable of: (a) binding and internalizing into target cells; (b) selectively killing both individual cells and 1000-cell spheroid clusters; and (c) prolonging tumor-free survival and reducing PSA in mice bearing prostate cancer xenografts.

Table 1  Histopathological characterization of LNCaP tumor xenografts implanted i.m. in athymic nude mice, 8 weeks of age

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Morphology</th>
<th>Nodule size (mm)</th>
<th>Vascularized</th>
<th>Encapsulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Nodules and scattered cells</td>
<td>0.1 × 0.2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Small nodules</td>
<td>0.2 × 1.0</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Nodules</td>
<td>0.5 × 1.3</td>
<td>++</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Nodules</td>
<td>1.0 × 2.0</td>
<td>++</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Nodules</td>
<td>1.0 × 2.0</td>
<td>++</td>
<td>Yes</td>
</tr>
</tbody>
</table>

xenografts (29). PSA levels in tumor-bearing mice also responded to the treatment (Table 2). Mean PSA values, 51 days after treatment, were 104 ng/ml +/- 54 ng/ml (n = 4, untreated animals), 66 ng/ml +/- 16 ng/ml (n = 6, [213Bi]HuM195-treated animals), and 28 ng/ml +/- 22 (n = 6, [213Bi]J591-treated animals). The reduction of PSA levels in mice treated with [213Bi]J591 and untreated control animals was significant with P < 0.007 and P < 0.0136, respectively. In another similar experiment where unlabeled J591 was also examined as an additional control, the mean PSA values 30 days after treatment were 31 ng/ml +/- 10, [213Bi]HuM195-treated animals), and 26 ng/ml +/- 11 ng/ml (n = 10, [213Bi]HuM195-treated animals), and 12 ng/ml +/- 8 ng/ml (n = 12, [213Bi]J591-treated animals) (Fig. 6). In this experiment, animals received either one single 213Bi drug administration or four consecutive daily administrations of a smaller dose of drug. There were no statistically significant differences in responses (measured PSA levels) observed between the 1× daily and the 4× daily treatment regimens for the [213Bi]J591 and the [213Bi]HuM195 treatments, respectively, nor between the unlabeled J591 and untreated controls. Reduction of PSA levels, however, in all mice (n = 12) treated with [213Bi]J591 (1 × daily and the 4× daily treatment regimens pooled) relative to all mice (n = 10) treated with [213Bi]HuM195 (1 × daily and the 4× daily treatment regimens pooled) and all control animals (groups untreated and treated with unlabeled J591 pooled; n = 10) was significant with P < 0.0443 and P < 0.0192, respectively.

α-PARTICLE EMITTING ANTIBODY TARGETING PSMA FOR RIT

Fig. 4. Cytotoxicity of [213Bi]J591 on LNCaP spheroids. LNCaP spheroid volume versus time following treatment is shown: ●, [213Bi]J591; ●, [213Bi]HuM195; ●, J591; ▲, no treatment growth control. Experiments were conducted as described in “Materials and Methods.”

Table 2  P for the observed PSA values between the [213Bi]J591-treated mice and the [213Bi]HuM195-treated mice and controls

<table>
<thead>
<tr>
<th>Time</th>
<th>[213Bi]J591 (n = 12)</th>
<th>PSA (ng/ml)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 30</td>
<td>P &lt; 0.0043 (n = 10)</td>
<td>104 ± 54 ng/ml</td>
<td>P &lt; 0.0192 (n = 10)</td>
</tr>
<tr>
<td>Day 51</td>
<td>P &lt; 0.0007 (n = 6)</td>
<td>66 ± 16 ng/ml</td>
<td>P &lt; 0.0136 (n = 4)</td>
</tr>
</tbody>
</table>
LNCaP cell kill in vitro using $^{213}$BiJ591 proved to be both specific activity dependent and activity concentration dependent. There was a 20-fold difference in the LD$_{50}$ values in vitro, spanning a 100-fold range in specific activities, indicating the specificity of the drug for cells expressing PSMA. Additional data demonstrated that $^{213}$BiJ591 did not specifically kill a PSMA-negative tumor cell line, SKOV3, supporting the fact that the drug does not target or destroy tissue that does not express PSMA. Therefore, $^{213}$BiJ591 was an effective and specific radiolabeled agent for ablation of individual prostate cancer cells in vitro. This is an advantage of using α-particle emitting radionuclides because the particles transfer their energy in a region that is in very close proximity to the target.

We have also investigated the potency and specificity of $^{213}$BiJ591 directed against LNCaP spheroids initially comprising ~1000 cells in vitro. Spheroids of this size approximate the micrometastatic disease condition and can serve as a therapeutic test for investigating the potency and specificity of α-particle emitting IgG directed against multicellular targets. The $^{213}$BiJ591 must be efficacious against the spheroids if it is expected to be clinically effective against metastatic prostate cancer. A single dose of the $^{213}$BiJ591 drug was effective in arresting the growth of LNCaP spheroids relative to an equivalent dose of $^{213}$BiHuM195, an irrelevant control mAb that has a minimal effect against the LNCaP spheroids, a similar dose of unlabeled J591, and untreated spheroids. Control experiments exhibited similar spheroid growth kinetics, with the average spheroid volume increasing almost 100-fold over a period of 1 month. The $^{213}$BiHuM195-treated spheroids exhibited a delay in growth of approximately 1 week, presumably due to nonspecific irradiation effects. It was estimated that random hits from α particles emitted from nonspecific IgG dispersed in the media could result in delivery of about 1% of the radiation dose as compared with a $^{213}$Bi-labeled specific mAb (27). Other experiments have shown that anti-PSMA IgG penetrates one to two cell layers into spheroids during the time period (45–90 min) that $^{213}$Bi would be expected to be most active. The $^{213}$BiJ591-treated spheroids decreased in volume by about 10% over a 3-week period with no further growth during the 2-month duration of the experiment. These data were repeated in a second experiment with similar results. Because the spheroid model may resemble the micrometastatic disease situation, it was apparent that $^{213}$BiJ591, despite incomplete penetration into the spheroid mass, was able to specifically target large cell clusters, destroy targeted cells, and interrupt further growth of the unirradiated spheroid core. Additional studies ongoing in this area are focusing on the use of a multiple dosing schedule that would effectively peel away the remaining cell layers that might still be viable. A multiple dosing schedule may eradicate the disease point source in its entirety while targeting any other remaining or new disease sites; however, the schedule and dose details need to be better understood.

The two therapeutic experiments carried out in vivo demonstrated the ability of $^{213}$BiJ591 to improve the duration of tumor-free survival and to suppress PSA relative to controls in an animal model. Following the trends observed in the spheroid cytotoxicity experiments, unlabeled J591 was ineffective in treatment and was comparable with no treatment. In addition, the $^{213}$BiHuM195 demonstrated a minimal effect in delaying tumor growth (2–3 days) and in decreasing PSA values relative to controls. Table 2 compares the Ps for the observed PSA values between the $^{213}$BiJ591-treated mice and the $^{213}$BiHuM195-treated mice and controls. The tumor model used in this study was not optimal for examining the efficacy of an α-particle emitting radioimmunotherapeutic agent because an α-particle has a pathlength of two to four cell diameters. However, in the absence of reliable metastatic models for prostate cancer in vivo, we used an i.m. tumor model and treated at a relatively early time point following tumor inoculation; this allowed us to observe an effect due to the $^{213}$BiJ591 agent that resulted in tumor growth delay and a lower PSA value relative to controls. The specific $^{213}$BiJ591 was directed against clusters of cells that at the time of RIT were approximately the size of the spheroids that were studied in vitro. As with the spheroids, the tumors in vivo comprised nodules containing 1000–2000 LNCaP cells that were not vascularized and not encapsulated.

We have been successful in a Phase I clinical trial using $^{213}$BiHuM195 (10, 11) to treat 18 patients with acute myelogenous leukemia. Ten of 12 evaluable patients had reductions in peripheral blood leukemia cells, and 12 of 18 patients had decreases in bone marrow blasts. Doses up to 1 mCi/kg were used safely, and there was no acute toxicity seen. In the treatment of leukemia there may be ~1 kg of tumor present (1E12 cells) with ~20,000 HuM195 molecules bound by individual HL60 leukemia cells (32).

The clinical situation in prostate cancer may favor the use of a $^{213}$Bi-labeled antibody based on: (a) low tumor burden; (b) anti-PSMA-IgG-PSMA internalization; and (c) the relatively large number of PSMA-binding sites per cell. In comparison with leukemia, there should be at least 10–100 times less prostate cancer tumor burden at the time of treatment, even with widespread micrometastatic lesions. Furthermore, J591 results in the internalization of the J591-PSMA complex into LNCaP cells (8), which favors the therapeutic use of $^{213}$Bi-labeled IgG constructs because internalized radiometal ions (a) will tend to remain with the cell and not be released and carried away from the target and (b) decays within the cell will necessarily deposit energy within the cell. We undertook the measurement of the radiometal-labeled construct-antigen complex internalization to determine the amount of drug internalized initially and as a function of time. Cellular internalization of α-particle emitting radionuclides ensures that the $^{213}$Bi particulate decay traverses the cell, enhancing the cytotoxicity relative to surface-bound species. We anticipate the ability to internalize much of the targeted, cell bound $^{213}$BiJ591 activity within a 4-h period allowing the $^{213}$Bi to decay and deliver its dose efficiently within the target cell. Additionally, it has been observed that the LNCaP cells continue to bind J591 and internalize it following exposure to the construct, presumably by expression of new (recycled) antigen-binding sites (Ref. 8 and this study). From a therapeutic standpoint, this modulation phenomenon works to therapeutic advantage because $^{213}$BiJ591 in circulation can continue to be bound even after initial saturation of the cell binding sites. The LNCaP cells bind...
result in a lethal dose of clusters to half of these cells. This has been demonstrated that several 213Bi-atoms targeted per cell may be required to eradicate individual prostate cancer cells and 1000 cell spheroid clusters in animal models. These features support the investigation of such an approach in human clinical setting to treat metastatic prostate cancer.

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