Therapeutics, Targets, and Chemical Biology

Alpha- versus Beta-Particle Radiopeptide Therapy in a Human Prostate Cancer Model (\(^{213}\)Bi-DOTA-PESIN and \(^{213}\)Bi-AMBA versus \(^{177}\)Lu-DOTA-PESIN)

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Abstract

Recurrent prostate cancer presents a challenge to conventional treatment, particularly so to address micrometastatic and small-volume disease. Use of \(\alpha\)-radionuclide therapy is considered as a highly effective treatment in such applications due to the shorter range and exquisite cytotoxicity of \(\alpha\)-particles as compared with \(\beta\)-particles. \(^{213}\)Bi is considered an \(\alpha\)-emitter with high clinical potential, due to its short half-life (45.6 minutes) being well matched for use in peptide-receptor radionuclide \(\alpha\)-therapy; however, there is limited knowledge available within this context of use. In this study, two novel \(^{213}\)Bi-labeled peptides, DOTA-PEG\(_4\)-bombesin (DOTA-PESIN) and DO3A-CH\(_2\)CO-8-aminooctanoyl-Q-W-A-V-G-H-L-M-NH\(_2\) (AMBA), were compared with \(^{177}\)Lu (\(\beta\)-emitter)-labeled DOTA-PESIN in a human androgen-independent prostate carcinoma xenograft model (PC-3 tumor). Animals were injected with \(^{177}\)Lu-DOTA-PESIN, \(^{213}\)Bi-DOTA-PESIN, or \(^{213}\)Bi-AMBA to determine the maximum tolerated dose (MTD), biodistribution, and dosimetry of each agent; controls were left untreated or were given nonradioactive \(^{175}\)Lu-DOTA-PESIN. The MTD of \(^{213}\)Bi-DOTA-PESIN and \(^{213}\)Bi-AMBA was 25 MBq (0.68 mCi) whereas \(^{177}\)Lu-DOTA-PESIN showed an MTD of 112 MBq (3 mCi). At these dose levels, \(^{213}\)Bi-DOTA-PESIN and \(^{213}\)Bi-AMBA were significantly more effective than \(^{177}\)Lu-DOTA-PESIN. At the same time, \(^{177}\)Lu-DOTA-PESIN showed minimal, \(^{213}\)Bi-DOTA-PESIN slight, and \(^{213}\)Bi-AMBA marked kidney damage 20 to 30 weeks posttreatment. These preclinical data indicate that \(\alpha\)-therapy with \(^{213}\)Bi-DOTA-PESIN or \(^{213}\)Bi-AMBA is more efficacious than \(\beta\)-therapy. Furthermore, \(^{213}\)Bi-DOTA-PESIN has a better safety profile than \(^{213}\)Bi-AMBA, and represents a possible new approach for use in peptide-receptor radionuclide \(\alpha\)-therapy treating recurrent prostate cancer. Cancer Res; 71(3); 1009-18. ©2011 AACR.

Introduction

Approximately 30,000 men with a history of radical prostatectomy have recurrence of prostate cancer yearly in the United States, and for the vast majority, increasing levels of prostate-specific antigen is the sole evidence of disease (1). Salvage radiotherapy is the only potentially curative therapy in these patients, with a complete response rate of 67% (2).

An attractive alternative concept to deliver radiation doses to micrometastatic disease is radioimmunotherapy (RIT) and peptide-receptor radionuclide therapy (PRRT) (3, 4). \(^{90}\)Y- and \(^{177}\)Lu-labeled peptides are successfully being introduced in the treatment of metastasized neuroendocrine tumors (5), and RIT with monoclonal antibodies has already shown high response rates in the treatment of non-Hodgkin lymphoma (6, 7). A number of antibodies (e.g., CC49, CYT-356, and J591) labeled with \(\beta\)-emitting isotopes have been evaluated in prostate cancer patients, but they have yielded only a minor response in a small number of patients (8-10).

Alpha-emitters appear to be an attractive therapeutic alternative because \(\alpha\)-particles are short-range, high-energy particles (\(^{4}\)He\(^{2+}\)) with a much higher linear energy transfer (LET; 60–230 keV/\(\mu\)m) than \(\beta\)-particles (0.1–1 keV/\(\mu\)m). Alpha-emitters may offer additional advantages, including minimal dependence on tumor oxygenation (11) and the ability to break radio- and chemoresistance (12). Accordingly, several \(\alpha\)-emitters have been proposed as agents for radiotherapy (13). Although attractive due to its half-life (7.2 hours), \(^{211}\)At is burdened by its limited availability and the instability of \(^{211}\)At-labeled radioconjugates, especially at activity levels suitable for clinical use (14). The application of \(^{213}\)Bi for RIT and the
comparison with β-emitters has been investigated in a preclinical study (15) and has been translated into clinical studies (16, 17). 213Bi can be obtained from an in-house 225Ac/213Bi generator (18). However, the short half-life of 213Bi (45.6 minutes) can limit the efficiency of RIT as interstitial fluid pressure opposes the convective transport of large molecules such as antibodies (19, 20). The 131I-labeled antibody J591, for example, showed a maximum tumor uptake (11.4% IA/g) at 4 days after injection (21). PRRT is an alternative approach to RIT in the treatment of recurrent prostate cancer. Potential advantages of small radiopeptides over monoclonal antibodies are their fast diffusion, lack of immunogenicity, and fast blood clearance (3, 4). Therefore, 213Bi-labeled peptides are potentially more effective with less toxicity.

The gastrin-releasing peptide (GRP) was shown to stimulate cell proliferation in the androgen-independent human prostatic carcinoma cell line PC-3 (22). GRP mediates its action through the GRP receptor (GRP-R). Importantly, 100% of invasive prostatic carcinomas but not benign prostatic tissue express GRP receptors at a high density, making GRP-R an attractive target for imaging and therapy (23, 24). In recent years, several groups have synthesized and evaluated GRP analogues (25–27): Scopinaro and colleagues detected primary prostate cancer in all patients using 99mTc-labeled bombesin (28), and Lantry and colleagues found a complete response rate of 39% after treatment of PC-3 tumor-bearing mice with 56 MBq (1.5 mCi) 177Lu-AMBA (29). Despite these promising results, there are potentially limiting factors that have to be taken into account when considering therapeutic applications of GRP analogues. Preliminary studies in clinical studies have shown a high accumulation in the small/large intestine and relatively low uptake in tumor lesions when labeled with 99mTc (30). The low accumulation in tumor lesions may be due to a low metabolic stability of these radiopeptides, and excretion via the hepatobiliary tract is caused by high lipophilicity. Therefore, we set up a program to synthesize bombesin-based peptides with improved metabolic stability, reduced clearance from the hepatobiliary tract, and optimized tumor-to-kidney uptake ratios. From a series of peptides evaluated, radiolabeled DOTA-PESIN looked particularly promising (31).

The present study compares the efficiency and toxicity of 213Bi-DOTA-PESIN with those of 177Lu-DOTA-PESIN and 213Bi-AMBA in a human androgen-independent prostate carcinoma xenograft model.

Materials and Methods

Peptide synthesis and radiolabeling

Radiolabeling of 177Lu-DOTA-PESIN and synthesis of DOTA-PESIN and AMBA followed procedures described previously (29, 31). 213Bi was eluted from an 225Ac/213Bi-generator produced by the Institute for Transuranium Elements, Karlsruhe, Germany, using 600 μL 0.1 mol/L NaI/HCl solution (32, 33). Next, 50 μL of 20% ascorbic acid (radioprotectant) was added and the pH was adjusted to 8.5 to 8.7 using 2 mol/L Na2CO3. The buffered 213Bi eluate was incubated with 4.4 μmol/L DOTA-PESIN or 3.8 μmol/L AMBA (DO3A-CH2CO-8-aminoocanoyl-Q-W-A-V-G-H-L-M-NH2) for 5 minutes at 95°C in a microwave oven (Biotage Initiator). Afterward, 30 μL of 1 mmol/L Ca-DTPA solution was added to complex-free 213Bi. The labeling yield was analyzed by instant thin-layer chromatography (ITLC-SG; Pall Inc.) and high-performance liquid chromatography (HPLC) using a Chromolith Speed ROD RP-18 endcapped 50- to 4.6-mm column (MERCK) and an acetonitrile–water gradient.

Peptide stability in serum

To determine the in vitro stability of 177Lu-DOTA-PESIN, fresh human serum samples were spiked with 177Lu-DOTA-PESIN (30 pmol/1.5 mL serum) and incubated up to 192 hours at 37°C (5% CO2). Samples of 100 μL blood serum were then taken and added to 200 μL ethanol. The precipitated serum proteins (pellets) were separated by centrifugation for 10 minutes at 3,000 × g. The radioactivity in the pellets and in the supernatants was quantified in a COBRA II D5003 γ-counter (Canberra Packard). The supernatants were filtered by an RC-membrane filter (Minisart RC 15; Sartorius AG) and were analyzed by analytical HPLC. The half-life of the peptide in serum was calculated by fitting the data points to a first-order reaction.

Cell line and animal model

Animals were maintained and treated in compliance with the guidelines of the Swiss Veterinary Office (approvals 2085 and 789). The animals were 5- to 8-week-old (20–24 g) athymic female nude mice (Harlan) xenografted with human androgen-independent prostate carcinoma cells (PC-3) at a density of 106 cells per mouse as described previously (31). PC-3 cells were obtained from the European Collection of Cell Cultures (ECACC) and have been cultured for less than 3 months before implantation. The cell line was authenticated by ECACC based on viability, isoenzyme analysis, DNA profiling, and PCR mycoplasma analysis. Before and after the experiment, the receptor expression of the PC-3 xenograft was assessed using in vitro autoradiography as described in the next section. After 10 days, tumors reached a size between 5.4 and 8.6 mm, which was measured with a caliper. Mice were then stratified according to the size of their tumor and randomly assigned to the different cohorts.

GRP-R expression in PC-3 tumors and recurrent PC-3 tumors

Ten PC-3 tumor–bearing mice were treated with 3 × 5 MBq (3 × 0.14 mCi) 213Bi-DOTA-PESIN. After development of recurrent tumors (7/10 mice; tumor diameter ~10 mm), the mice were divided into 2 groups: The first 3 mice were injected with 5 MBq (0.14 mCi) 213Bi-DOTA-PESIN and sacrificed 1 hour later. Organs, blood, and tumors were collected and the activity concentration [percentage of injected activity per gram (% IA/g)] was determined. The remaining 4 treated mice, as well as 4 untreated PC-3 tumor–bearing (control) mice, were sacrificed and prepared for GRP-R quantification studies using in vitro receptor autoradiography as described previously (24). Additional ex vivo autoradiography of tumor
samples was performed in 4 naïve mice after i.v. injection of 14 MBq (0.38 mCi) $^{177}$Lu-DOTA-PESIN (tumor diameter <10 mm).

**Biodistribution studies**

PC-3 tumor–bearing mice received an injection into the tail vein with the respective radiolabeled peptide at a protein amount of 200 pmol (320 ng). Afterward, the mice were sacrificed at 1, 4, 24, or 72 hours ($^{177}$Lu-DOTA-PESIN) and at 0.5, 1, 2, or 4 hours ($^{213}$Bi-labelled conjugates; n = 3–6 per cohort). Organs, blood, and tumors were collected and the activity concentration was measured (31). To determine the radiopeptide concentration-dependent uptake in organs and tumors, mice were injected with 10, 40, 200, or 400 pmol (0.25–37 MBq: 6.8–1,000 μCi) $^{177}$Lu-DOTA-PESIN as described above, and sacrificed 4 hours postinjection. For the determination of nonspecific tumor uptake, animals were coinjected with 200 pmol radiolabeled peptide and 20 pmol nonradioabeled DOTA-PESIN.

For renal uptake reduction studies, 150 mg/mL (3–15 kDa) l-polyglutamic acid (Sigma-Aldrich) and/or 40 mg/mL Gelofusine was dissolved in saline as described before (34). The animals were injected i.v. with 100 μL of one solution or 200 μL of both solutions just before i.v. administration of 200 pmol $^{177}$Lu-DOTA-PESIN. Mice were sacrificed 1 hour after injection and the radioactivity in organs and tumors was measured.

**Radiation dosimetry**

Determination of the absorbed dose in the tumor and normal tissue was performed as described in more detail previously (35). The mean absorbed dose, $D_{tissue}$, to a tissue with mass, $m_{tissue}$, was estimated for various tissues as $D_{tissue} = \frac{\text{AnE}_\phi}{m_{tissue}}$, where nE is the mean energy emitted per nuclear transformation and $\phi$ is the absorbed fraction. In these calculations, we considered the energy (nE) of electrons (including $\beta$-component of $^{213}$Bi) and $\alpha$-particles only because the energy deposition of $\gamma$-rays is negligible in small animals (36). The absorbed fraction $\phi = 1$ was set for all measured organs because of the short maximal path length of $^{213}$Bi and $^{177}$Lu (<100 μm and 2 mm) in tissues.

**Radiopeptide therapy**

All therapy studies were performed at the maximum tolerated dose (MTD). The MTD was defined as the highest possible dose that did not result in any animal deaths or a weight loss of greater than 15%, with the next higher dose level resulting in 10% to 20% of the animals dying (15). The MTD was determined for $^{177}$Lu-DOTA-PESIN, $^{213}$Bi-DOTA-PESIN, and $^{213}$Bi-AMBA using a total of 125 PC-3 tumor–bearing mice. Animals were observed until their death or a loss of greater than 30% of their initial weight, at which time they were removed from the group and sacrificed. Kidneys and pancreas were prepared for histologic investigation. The tumor size of s.c. tumors was measured before therapy and at 2- to 4-day intervals thereafter. Tumor volumes were calculated assuming an elliptic shape. Animals were either left untreated (control) or injected with a single dose of 1.7 nmol (3 μg) nonradioactive $^{175}$Lu-DOTA-PESIN. Long-term 3- and 5-dose efficacy studies (3 doses: 0, 2, and 4 days; 5 doses: 0, 2, 4, 21, and 23 days) were conducted in PC-3 tumor–bearing mice with 3 × 5 and 5 × 5 MBq (3 × 0.14 and 5 × 0.14 mCi) $^{213}$Bi-DOTA-PESIN/$^{213}$Bi-AMBA. Long-term $^{177}$Lu-DOTA-PESIN treatment efficacy studies were also performed in PC-3 tumor–bearing mice with a cumulative dose of 56 and 112 MBq (1.5 and 3 mCi; 2 doses: 0 and 1 day; 4 doses: 0, 1, 14, and 15 days). In each group, 10 animals were studied up to 30 weeks after injection. Kidney toxicity was evaluated in all surviving animals as described below under the heading of “Histology and bone marrow toxicity.”

**Determination of blood counts**

A total of 32 PC-3 tumor–bearing mice were either left untreated (controls) or injected with 10 MBq (0.27 mCi), 2 × 7 MBq (2 × 0.19 mCi), 3 × 7 MBq (3 × 0.19 mCi), or 4 × 7 MBq (4 × 0.19 mCi) $^{213}$Bi-DOTA-PESIN. Total and differential leukocyte counts, red blood cells, and platelet counts were determined in the same animal 10 days before treatment and 10, 30, and 75 days thereafter. Maximal 150 μL heparinized specimens were collected by sublingual blood sampling (37). The samples were counted on an ADVIA 120 (Bayer).

**Histology and bone marrow toxicity**

For organ histology and bone marrow toxicity (BMT) studies, mice with tumors and naïve nude mice were treated with 4 × 28 MBq (4 × 0.77 mCi) $^{177}$Lu-DOTA-PESIN or 5 × 5 MBq (5 × 0.14 mCi) $^{213}$Bi-DOTA-PESIN or 5 × 5 MBq (5 × 0.14 mCi) $^{213}$Bi-AMBA as described above under the heading of “Radiopeptide therapy” (n ≥ 10 animals per group). At 20 or 30 weeks posttreatment, mice were sacrificed under isoflurane and CO2 anesthesia. Organs (kidneys, pancreas, liver, spleen, lungs, heart, sternum, muscle, stomach, small and large intestine) were fixed in 10% formalin, embedded in paraffin, cut at nominally 4-μm sections, and stained with hematoxylin and eosin. Modified Wright staining was performed for bone marrow smears. Findings were compared with sections from untreated nude mice.

In a subgroup of 4 mice, tumors were collected and histologically analyzed 8 days after injection of 3 × 5 MBq (3 × 0.14 mCi) $^{213}$Bi-DOTA-PESIN. The histopathologic and bone marrow evaluations were performed by board-certified veterinary pathologists (J. Boisclair and A. Provencher-Bolliger).

**Statistical analysis**

Differences in the tissue uptake values were statistically analyzed with one-way ANOVA, including Tukey’s posttest for pairwise comparison. Blood counts before and after treatment were compared by Student’s t test for paired data. Differences in the therapeutic efficiency between the treatment modalities in s.c. tumors were analyzed by assuming an exponential tumor growth pattern; nonlinear regression analysis based on asymptotic approximation was used (38). Survival analysis was based on the Kaplan–Meier product limit, and groups were compared using the log-rank test.
Results

Radiolabeling and biodistribution

The labeling yield of $^{177}$Lu- and $^{213}$Bi-DOTA-PESIN was 99.1% ± 0.5% and 97.8% ± 2.4% at a specific activity of 57.8 and 83.6 GBq/μmol, respectively ($^{213}$Bi-AMBA 95.3% ± 3.1%, 86 GBq/μmol). These data correspond also to the radiochemical purity as no impurity beside free $^{213}$Bi and $^{177}$Lu were detected (Supplementary Fig. S1). The uptake of radiolabeled DOTA-PESIN in GRP-R–positive organs was peptide amount dependent, with the best tumor-to-organ uptake ratio at a peptide concentration of 200 pmol (Supplementary Fig. S2). Therefore, biodistribution, treatment efficiency, and toxicity studies were performed with a peptide amount of 200 pmol. Figure 1 and Supplementary Table S1 show the concentrations (% IA/g) of $^{177}$Lu-DOTA-PESIN and $^{213}$Bi-DOTA-PESIN versus $^{213}$Bi-AMBA in tumor and organs of PC-3 tumor–bearing mice. Because of the short physical half-life of $^{213}$Bi, biodistribution studies were performed up to 4 hours postinjection, in contrast to 72 hours with $^{177}$Lu-DOTA-PESIN. No significant differences were found between the biodistribution of $^{213}$Bi- and $^{177}$Lu-labeled DOTA-PESIN. In contrast, $^{213}$Bi-AMBA showed significantly higher uptake in most organs tested but not in the tumor. The blood clearance was biexponential, with almost the same half-lives for $^{213}$Bi-DOTA-PESIN and $^{213}$Bi-AMBA ($t_{1/2a} = 5$ minutes, $t_{1/2b} = 23$ minutes). The enzymatic stability of $^{177}$Lu-DOTA-PESIN was studied in human blood serum, resulting in a serum stability half-life of 8.4 ± 3.2 hours. The tumor in the mouse was rapid, reaching its apogee earlier than 30 minutes postinjection (~12% IA/g). One hour after injection, the uptake in the PC-3 tumor was high, with 11.6% ± 1.4% IA/g for $^{177}$Lu-DOTA-PESIN, 9.0% ± 2.0% IA/g for $^{213}$Bi-DOTA-PESIN, and 9.4% ± 1.9% IA/g for $^{213}$Bi-AMBA ($P = 0.19$). In vivo competition experiments with a 100-fold excess of cold peptide reduced the tumor uptake of $^{177}$Lu-DOTA-PESIN by more than 95% (Supplementary Table S2). Both peptides were excreted predominantly through the kidneys, with maximum kidney uptake values of 6.5% ± 1.8% IA/g ($^{213}$Bi-DOTA-PESIN) and 13.7% ± 2.2% IA/g ($^{213}$Bi-AMBA; $P < 0.001$). However, Gelofusine and polyglutamic acid did not have any effect on the kidney uptake of DOTA-PESIN (Supplementary Table S2). The highest activity accumulation was found in the mouse pancreas, with a maximum uptake of 24.9% ± 1.6% IA/g ($^{213}$Bi-DOTA-PESIN) and 31.6% ± 3.8% IA/g ($^{213}$Bi-AMBA) ($P < 0.003$). Other GRP-R–positive organs showed much lower radiopeptide accumulation (maximal uptake <5% IA/g).

GRP-R expression in recurrent PC-3 tumors

Seven of ten mice developed a recurrent tumor 19 to 28 days after treatment with 3 × 5 MBq $^{213}$Bi-DOTA-PESIN. In 3 of these 7 mice, biodistribution studies were performed 1 hour after injection of 5 MBq $^{213}$Bi-DOTA-PESIN. The other 4 mice with recurrent tumor were used for in vitro autoradiography studies to quantify the GRP-R density. There was no significant difference in tumor uptake between untreated controls and $^{213}$Bi-DOTA-PESIN–pretreated animals (9.0% ± 2.0% and 7.1% ± 1.1% IA/g, respectively).
The GRP-R density in the recurrent tumor was 7,801 ± 480 dpm/mg tissue (Supplementary Table S3). This was not significantly different from the receptor density of untreated animals. The in vitro and ex vivo autoradiogram shows mostly homogeneous distribution of the tracer in all untreated and pretreated tumors (Supplementary Figs. S3 and S4).

**Acute dose-limiting toxicity**

For MTD finding, varying amounts of 177Lu-213Bi-DOTA-PESIN and 213Bi-AMBA were injected, starting at 10 MBq and increasing in 10% to 30% steps (5 animals per group) using a fractionated scheme (5–7 MBq per injection). Weight loss (>15%) and treatment-related death were defined as acute toxicity when occurring within 8 weeks after the beginning of receptor-targeted radiopeptide therapy, whereas later weight loss and deaths were regarded as chronic toxicity, unless they were assignable to tumor growth. All animals tolerated 2 × 28 MBq 177Lu-DOTA-PESIN and 3 × 5 MBq 213Bi-DOTA-PESIN/AMBA without acute treatment-related weight loss or lethality. A 10% increase in these activities resulted in a weight loss of greater than 15% and an activity increase of 25% resulted in a 10% to 20% lethality.

Figure 2 shows the platelet and leukocyte counts before and after treatment with different activities of 213Bi-DOTA-PESIN. A significant decrease in platelet and leukocyte counts was found 10 days after injection of 3 × 7 and 4 × 7 MBq 213Bi-DOTA-PESIN. No significant decrease in red blood count was found after administration of 213Bi-DOTA-PESIN.

Additional MTD-finding trials were performed with a more fractionated scheme. Again, 3 × 5 MBq 213Bi-DOTA-PESIN or 213Bi-AMBA were injected but 21 days later additional 213Bi-DOTA-PESIN or 213Bi-AMBA injections were performed with increasing amounts of activity as described above. The same was done with 177Lu-DOTA-PESIN, starting with 2 × 28 MBq and continuing 14 days later. In doing so, 5 × 5 MBq 213Bi-DOTA-PESIN or 213Bi-AMBA (0, 2, 4, 21, and 23 days) and 4 × 28 MBq 177Lu-DOTA-PESIN (0, 1, 14, and 15 days) were tolerated by all animals without weight loss of greater than 15% or treatment-related lethality.

**Antitumor efficiency**

Figure 3 shows the therapeutic effect of 177Lu-DOTA-PESIN, 213Bi-DOTA-PESIN and 213Bi-AMBA at their respective MTDs in PC-3 tumor-bearing mice. 213Bi-labeled peptides led to a significant (P < 0.0002) growth retardation as compared with untreated controls or nonradioactive 177Lu-DOTA-PESIN–treated animals. Antitumor effects improved with dose intensification. 177Lu-labeled DOTA-PESIN was significantly less effective than 213Bi-labeled peptides at the MTD (>P = 0.02), whereas no significant difference was observed between control and 3 μg nonradioactive 177Lu-DOTA-PESIN treatment. Table 1 provides the efficiency of the therapeutic approach, medium survival times, and kidney toxicity after treatment with 4 × 28 MBq 177Lu-DOTA-PESIN, 5 × 5 MBq 213Bi-DOTA-PESIN or 5 × 5 MBq 213Bi-AMBA, and Fig. 4 shows the Kaplan–Meier plot.

**Dosimetric considerations and correlation with toxic effects**

The radiation dosimetry of 177Lu- versus 213Bi-labeled DOTA-PESIN and the comparison with 213Bi-AMBA are summarized in Table 1. The highest radiation dose was calculated for the pancreas although histopathologic evaluation revealed only minimal pancreatic fibrosis. The next highest dose was calculated for the PC-3 tumor resulting in tumor necrosis 8 days after injection of 3 × 5 MBq 213Bi-DOTA-PESIN. At the MTD, the calculated dose to the kidneys was highest for 213Bi-AMBA, resulting in marked to massive infarction in 50% of animals. The most relevant histopathologic findings are summarized in Table 1 and Fig. 4. In contrast, 4 × 28 MBq 177Lu-DOTA-PESIN treatment did not result in any kidney infarction and 5 × 5 MBq 213Bi-DOTA-PESIN treatment produced relevant kidney infarction in 25% of animals. Twenty weeks after injection of 213Bi-DOTA-PESIN and 213Bi-AMBA, minimal to marked tubular degeneration/karyomegaly was observed in all animals. However, tubular degeneration/karyomegaly was fully reversible at 30 weeks. On the other hand, the severity of
infarction resulting in end-stage kidneys increased with time. The survival rate of 213Bi-AMBA-treated animals was only 40% despite the high rate of complete remissions (80%). In these animals, the high mortality was partially attributable to severe kidney toxicity, with histologically proven end-stage kidneys in 2 cases (14.6 and 26.9 weeks after injection). No relevant histopathologic findings were noted in the other organs examined, including bone marrow.
Discussion

The combination of short-lived $^{213}$Bi with fast-diffusing, small molecules, such as peptides, seems advantageous: The peptides tested in this study (molecular weight $\sim 1.7$ kDa) showed high and fast tumor uptake with a maximum of greater than 11% IA/g tissue less than 30 minutes after injection. Because of the rapid background clearance, both radiopeptides provide much better target-to-nontarget ratios than antibodies at early times after injection. As a result, $^{213}$Bi-DOTA-PESIN and $^{213}$Bi-AMBA have ideal properties for targeted $\alpha$-particle therapy.

Previously published experience with receptor-targeted radiopeptide $\alpha$-therapy is limited (39–42). In this study, we present the preclinical evaluation of 2 peptides labeled with $^{213}$Bi for future receptor-targeted radiopetide therapy in prostate cancer patients.

In vitro investigations revealed that primary invasive prostate cancer shows upregulation of GRP-R (5,241 dpm/mg tissue), whereas normal prostate tissue displays a low or nonexistent level of GRP-R expression (23, 24). In our study, we used a human androgen-independent prostate cancer xenograft model characterized by a slightly higher GRP-R density than the one measured in human primary prostate cancer tissue sections.

Among peptides with affinity to GRP-R, DOTA-PESIN and AMBA have excellent properties for clinical use (30). Both peptides are stable (serum stability half-life: $> 8$ hours) and hydrophilic, resulting in the preferred renal-urinary mode of excretion. They show high affinity to GRP receptors (IC$_{50}$ values are 4.75 nmol/L for AMBA (ref. 29) and 9.5 nmol/L for DOTA-PESIN (ref. 31)) with fast and specific tumor uptake as well as rapid clearance from blood ($t_{1/2}$ values are 5 minutes, $t_{1/2b} = 23$ minutes).

At the MTD of $^{177}$Lu- and $^{213}$Bi-DOTA-PESIN (56 MBq and 15 MBq, respectively) no myelotoxicity was observed, whereas the injection of 28 MBq $^{213}$Bi-DOTA-PESIN proved to be toxic with a high mortality rate (75% of animals). These animals developed massive thrombo- and leukocytopenia. Therefore, BMT is limiting the MTD and is responsible for acute toxicity. However, the more fractionated approach with 5 × 5 MBq $^{213}$Bi-DOTA-PESIN and $^{213}$Bi-AMBA increased the MTD from 15 MBq to 25 MBq. This is in accordance with earlier observations that a fractionated dose regimen is less toxic than a single high-dose treatment (43). The activity of $^{225}$Ac/$^{213}$Bi generators is in the range of 1 to 2 GBq, which may require a fractionated approach in patients anyway (16).

Dose-limiting chronic toxicity in this trial, as in peptide receptor radiotherapy in general, was due to nephrotoxicity. It

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<th>Table 1. Summary of radiation dose estimation, treatment efficiency, toxicity, and survival after treatment with $^{177}$Lu-DOTA-PESIN, $^{213}$Bi-DOTA-PESIN, and $^{213}$Bi-AMBA</th>
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$^a$Microscopic findings are classified into 6 grades: grade 0, normal, no pathology; grade 1, minimal, very few, very small; grade 2, slight, few, small; grade 3, moderate, moderate number, moderate size; grade 4, marked, many, large; grade 5, massive, extensive number, extensive size, and end-stage kidney. Toxicity studies were carried out in non-tumor-bearing nude mice.
is assumed that following glomerular filtration, radiolabeled peptides bind to endocytic receptors at the luminal surface of proximal tubular cells and are internalized and trapped in the tubular cell lysosomes (34). Therefore, it is not surprising to observe tubular degeneration/karyomegaly 20 weeks post-treatment with $5 \times 5 \text{MBq}^{213}\text{Bi-AMBA}$. Interestingly, 30 weeks posttreatment, tubular degeneration/karyomegaly had completely recovered, showing the high regeneration potential of the renal tubular epithelium.

177Lu-DOTA-PESIN treatment by contrast did not induce any tubular degeneration but rather mesangial glomerulopathy. Alpha- and $\beta$-particle therapies affect different renal substructures because short-range, high-LET $\alpha$-particles mainly irradiate tubuli whereas long-range, low-LET $\beta$-particles reach the radiosensitive glomeruli. Although the calculated renal radiation dose was similar for $^{177}\text{Lu-DOTA-PESIN}$ (6.7 Gy/112 MBq) and $^{213}\text{Bi-DOTA-PESIN}$ (6 Gy/25 MBq) at the MTD, nephrotoxicity was slightly more pronounced after $^{213}\text{Bi-DOTA-PESIN}$ treatment. This is expected because high-LET $\alpha$-particles have a higher relative biological effectiveness than low-LET $\beta$-particles (44). Compared with $^{213}\text{Bi-DOTA-PESIN}$, the $^{213}\text{Bi-AMBA}$ renal dose was almost doubled, resulting in more pronounced nephrotoxicity. It has been shown previously that the renal uptake of $^{67}\text{Ga-DOTA-PESIN}$ cannot be reduced when overloading the kidneys with lysine (31). In contrast, $^{111}\text{In-BOM}$, DOTA-Sar5-[D-Tyr5, betaAla11, Thi13, Nle14]bombesin(6-14), can be significantly decreased when pretreated with L-polyglutamic acid or Gelofusine (34). However, in the present study, no significant decrease in renal uptake was noted after either of these pretreatments because $^{213}\text{Bi-DOTA-PESIN}$ renal uptake is relatively low (highest tumor-to-kidney ratios among bombesin analogues; ref. 30).

All radiopeptides evaluated in this study showed the highest uptake and radiation dose in the pancreas. In the human...
pancreas, in contrast, only weak $^{213}$Bi-DOTA-PESIN/-AMBA uptake is expected because the normal human pancreatic acini do not express GRP receptors at a measurable density (45). Organs other than the kidneys and the pancreas, including bone marrow, did not show signs of radiotoxicity at the MTD.

At the MTD, $\alpha$-therapy with $^{213}$Bi-DOTA-PESIN was clearly superior to the treatment with $^{177}$Lu-DOTA-PESIN, although the tumor uptake was not significantly different between the 2 tracers. This finding is in accordance with an earlier study, which compared $\alpha$- and $\beta$-emitters in RIT (15). As expected, the 4- and 5-dose regimens were more efficient than the 2- and 3-dose regimens, with a marked shift toward complete response. One important prerequisite for effective multiple dose treatment is a fast recovery from receptor downregulation after treatment. Lantry and colleagues (29) found a fast GRP-R recovery within 1 hour after injection, and in the present study we found no decrease in GRP-R density in recurrent tumor disease. Twenty-one days after initial treatment when only small or micrometastatic disease (0–4.3 mm) was apparent, additional $^{213}$Bi-DOTA-PESIN or $^{213}$Bi-AMBA injections reduced the risk of recurrent disease by 57%. At the same time, the median survival time increased by greater than 15 weeks, which is 5 times more compared with the $^{177}$Lu-DOTA-PESIN treatment. The 4-cycle $^{177}$Lu-DOTA-PESIN treatment, however, did not reduce the risk of developing recurrent disease. This observation is in accordance with earlier observations that both individual cells and 1,000-cell spheroid clusters can be efficiently killed with short-range, high-LET $\alpha$-emitters but not with long-range, low-LET $\beta$-emitters (46, 47). Interesting, however, is the observation that short-range $\alpha$-therapy with a maximal range of 10 cell diameters is not only effective in the treatment of small residual disease but also in the treatment of solid tumors with a diameter between 5.4 and 8.6 mm. This can be explained by the receptor-mediated homogeneous distribution of the tracer in the tumor as shown by in vitro and ex vivo autoradiography as well as the high tumor dose of 13 Gy resulting in severe tumor necrosis 8 days after injection of 3 $\times$ 5 MBq $^{213}$Bi-DOTA-PESIN. The long-range $\beta$ component of the $^{213}$Bi decay is less likely to significantly augment the $\alpha$ effects in solid tumors because less than 10% of the total tumor dose is delivered by the $\beta$-decay of $^{213}$Bi (48) and 5 $\times$ 5 MBq $^{213}$Bi-DOTA-PESIN was significantly more effective than 4 $\times$ 28 MBq $^{177}$Lu-DOTA-PESIN despite having similar tumor dose. We explain the higher effect of $^{213}$Bi with its higher LET. Other factors, such as the bystander effect, may be of some importance but we are not aware that $\alpha$-particles have higher bystander effect than $\beta$-particles. In addition, the bystander effect becomes saturated at much lower doses than the ones we calculated for our tumors (49).

In summary, $\alpha$-therapy with $^{213}$Bi-DOTA-PESIN or $^{213}$Bi-AMBA was shown to be more effective than $\beta$-therapy in a prostate cancer animal model. Of equal importance, $^{213}$Bi-DOTA-PESIN has a better safety profile than $^{213}$Bi-AMBA and represents an important new approach for treating recurrent prostate cancer. Because of its short physical half-life, $^{213}$Bi appears to be especially suitable for use in conjunction with fast-clearing peptides; its 440-keV $\gamma$-emission can also be used for quantification and imaging.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Alpha- versus Beta-Particle Radiopeptide Therapy in a Human Prostate Cancer Model (177Lu-DOTA-PESIN and 213Bi-DOTA-PESIN and 213Bi-AMBA versus 177Lu-DOTA-PESIN)

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