Therapeutic Advantage of ^{90}Yttrium- *versus* ^{131}Iodine-labeled PAM4 Antibody in Experimental Pancreatic Cancer[1]

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ABSTRACT

Objectives: Radioimmunotherapy studies using ^{131}I-PAM4 have demonstrated significant antitumor effects in mice bearing human pancreatic cancer xenografts. For several reasons ^{90}Y has been proposed as a more effective radionuclide for radioimmunotherapy of pancreatic cancer. The present study examined whether one radionuclide was more efficacious than the other in tumor-bearing mice.

***Methods*: Athymic nude mice bearing CaPan1 xenograft tumors (~1.0 cm^3) were given increasing doses of either ^{90}Y-PAM4 or ^{131}I-PAM4 up to their respective maximal tolerated doses [MTDs (260 and 700 μCi, respectively)].**

***Results*: ^{90}Y-PAM4 provided significantly greater growth inhibition than the ^{131}I-PAM4 ($P < 0.035$). Median survival time for the untreated mice was 6 weeks, whereas median survival times for the ^{131}I-treated mice and ^{90}Y-treated mice at their respective MTDs were 17.5 weeks and >26 weeks (the end of the study period), respectively. Within the ^{131}I-PAM4-treated group, two of eight mice were responders (>50% decrease in tumor size) for a median of 14 weeks. At the end of the study (26 weeks), 1 mouse was alive with no sign of tumor. All of the ^{90}Y-PAM4-treated mice were responders with a median duration of response of 20 weeks. Six of the seven mice were alive at week 26, with four mice having no evidence of disease.**

***Conclusions*: These data demonstrate the advantage of ^{90}Y over ^{131}I as the radionuclide for PAM4-targeted radioimmunotherapy of xenografted pancreatic cancer. Furthermore, the duration and extent of the antitumor response suggests that multiple treatment cycles of ^{90}Y-PAM4 may provide an effective therapeutic for the control of pancreatic cancer.**

INTRODUCTION

Pancreatic cancer is one of the most insidious forms of cancer afflicting adults. The 1999 United States estimates of new cancer cases put pancreatic cancer at 9th and 10th among women and men, respectively. However, it is the fourth leading cause of all cancer deaths in both women and men, surpassed only by lung, colorectal, prostate, and breast cancers. Attributable largely to the location and silent growth of the tumor, most patients present at a time when it is too late to undergo any curative treatment. This has translated into a 5-year survival rate of less than 3% (1).

Our laboratory has developed MAb[3] PAM4 that by immunohistochemistry was found to be reactive with ~85% of pancreatic cancer specimens (2). Except for a weak reaction with the normal gastrointestinal tract, PAM4 was nonreactive with normal tissues, including the pancreas. In preclinical animal studies, radiolabeled PAM4 targeted to human pancreatic tumor xenografts at high concentrations, demonstrating high specificity (3, 4). Experimental RAIT using ^{131}I-labeled PAM4 was carried out in mice bearing s.c. and/or orthotopic human-derived pancreatic tumors. Tumor growth inhibition and long-term survival were significantly greater than for isotype-matched, nonspecific, control and untreated mice. In clinical Phase I trials using the ^{131}I- or ^{99m}Tc-labeled PAM4 antibody, scintigraphic evidence of tumors was found in 8 of 10 patients suspected of pancreatic cancer (5, 6). Surgery confirmed pancreatic cancer in the 8 patients. One of the two negative patients was found to have chronic pancreatitis and not pancreatic cancer, and the other negative patient had a poorly differentiated pancreatic cancer that was nonreactive with PAM4 by immunohistochemistry.

Currently, ^{131}I is the primary radionuclide chosen for RAIT. The major reasons for this are: (*a*) the labeling methods for ^{131}I are well defined; (*b*) it is abundant and inexpensive; and (*c*) its γ emissions allow for the tracing of *in vivo* distribution by external camera imaging, and its β emissions are good for therapy.

Certain physical properties have been proposed for an ideal radionuclide to be used in RAIT (7). These include a physical half-life ($t_{[1/2]}$) of 1–3 days, major energy deposition between 0.5 and 30 cell diameters, and single decay-to-ground state. ^{90}Y is a radiometal that fits these criteria and may, thus, be a better choice than ^{131}I for RAIT. ^{90}Y has a greater energy emission than ^{131}I (β, E_{max} = 2.27 MeV *versus* β, E_{max} = 0.81 MeV, respectively) and a shorter half-life ($t_{1/2}$ = 2.67 days *versus*

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[3] The abbreviations used are: MAb, monoclonal antibody; AUC, area under the curve; cPAM4, chimeric PAM4 antibody; MTD, maximal tolerated dose; RAIT, radioimmunotherapy; DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; ITC-Bz-DTPA, isothiocyanatobenzyl DTPA.

$t_{1/2}$ = 8.06 days, respectively). In addition, ^{90}Y will deposit its energy out to 1.81 cm *versus* only 0.55 cm for ^{131}I (8). Of particular note, when conjugated to an internalizing antibody, ^{90}Y will be retained within the tumor cell for longer periods of time than will ^{131}I (9–12). The residualizing property of radiometals such as ^{90}Y may allow for a higher radiation dose to the tumor than could be achieved by ^{131}I. Considering the potential advantages of using a residualizing radionuclide, we examined the antitumor efficacy of ^{90}Y-labeled DOTA-PAM4 for the treatment of pancreatic cancer.

MATERIALS AND METHODS

Experimental Animal Model. CaPan1, a human pancreatic carcinoma that was obtained as a cell culture from the American Type Culture Collection (Manassas, VA), was established as a solid tumor by s.c. injection of 10^7 cells into 5-week-old, female athymic *nu/nu* mice (Taconic, Germantown, NY). Once tumors had grown to ~1 cm^3, they were serially propagated by s.c. injection of 0.2 ml of a 20% (w/v) tumor suspension prepared by mincing the tumors in 0.9% saline with subsequent passage through a 40-mesh wire screen. CaPan1 cells produced moderate-to-well-differentiated tumors. Tumors used in this study were passaged <10 times. Animal studies were approved by the Garden State Cancer Center's Institutional Animal Care and Use Committee and were performed in accordance with the American Association of Laboratory Animal Care (AALAC), United States Department of Agriculture (USDA), and Department of Health and Human Services (DHHS) regulations.

^{131}I- and ^{125}I-Antibody Radiolabeling. The purification and characterization of cPAM4 has been described previously (2). Radiolabeling of MAbs with ^{125}I and ^{131}I (NEN Life Science Products, Inc., Boston, MA) was performed by the chloramine-T method (13). The specific activity of the radiolabeled antibodies was in the range of 9.88–12.3 μCi/μg. Quality assurance was performed as described previously (3). All of the radiolabeled preparations contained <8% aggregated material and <5% unbound iodine.

^{90}Y- and ^{111}In-Antibody Radiolabeling. DOTA (Macrocyclics, Inc., Richardson, TX) was conjugated to cPAM4 IgG by a previously described method (14). ^{90}Yttrium chloride (NEN Life Science Products, Inc., Boston, MA) or ^{111}indium chloride (Iso-Tex Diagnostic, Inc., Friendswood, TX) was added to a reaction vial with DOTA-cPAM4 IgG at a ratio of 5 mCi per mg of cPAM4. The reaction was incubated at 45°C for 15 min and then quenched by removal from the heat source and the addition of 0.1 volume of 100 mM DTPA. This mixture was then incubated at room temperature for 5 min. A volume of 1% human serum albumin in PBS was added to give a final activity concentration of ~1 mCi/ml for ^{111}In-labeled and ~5 mCi/ml for ^{90}Y-labeled DOTA-cPAM4. The specific activity for the ^{111}In label was 5.41 μCi/μg with 1.7% unbound material and less than 1% aggregated material. The specific activity for the ^{90}Y label was 4.49 μCi/μg with 12.9% unbound material and <6% aggregated material. The final labeled antibody product was placed in a dose calibrator (Capintec CRC15R; Ramsey, NJ) and the time and activity recorded.

Biodistribution Studies. Tumor volumes were determined by caliper measurements in three dimensions and calculated by length × width × depth. Mice bearing tumors of similar size were placed into groups of at least eight animals per time point. Groups of mice received an i.v. injection of a mixture of ^{125}I-cPAM4 (10 μCi) and ^{111}In-DOTA-cPAM4 (25 μCi). The radiolabeled antibody was augmented with unlabeled antibody to ensure that each mouse received a total of 50 μg of antibody. At specific time points, the tumors, as well as various tissues (liver, spleen, kidney, lung, blood, pancreas, and bone), were removed, weighed, and the radioactivity determined in a 2-channel gamma scintillation counter. Data are expressed as %ID/g. Localization indices were calculated by the equation:

$$\frac{\%\text{ID/g of }^{111}\text{In in Tumor}}{\%\text{ID/g }^{111}\text{In in Blood}} \div \frac{\%\text{ID/g of }^{125}\text{I in Tumor}}{\%\text{ID/g }^{125}\text{I in Blood}}$$

Radiation dose estimates were calculated from the biodistribution data. The integral of the time-activity curves was computed trapezoidally with the assumption of no activity at zero time (15). Student's *t* test was used to assess significant differences.

Experimental RAIT. Mice bearing CaPan1 tumors of ~1 cm^3 at the start of the study were used throughout these studies. The MTD of ^{131}I-labeled and ^{90}Y-ITC-Bz-DTPA-labeled cPAM4 IgG were previously determined by i.v. administration of increasing doses of ^{131}I-cPAM4 or increasing doses of ^{90}Y-ITC-Bz-DTPA-labeled cPAM4 (16). The MTD was defined as that dose of radiolabeled antibody that resulted in all of the mice surviving at least 4 weeks without losing more than 20% of their starting body weight. In mice bearing 1 cm^3 CaPan1 tumors, the MTD was found to be 700 μCi for ^{131}I-labeled cPAM4 and 260 μCi for ^{90}Y-labeled cPAM4.

In those experiments comparing ^{131}I *versus* ^{90}Y RAIT, tumor-bearing mice received either increasing doses of ^{131}I-cPAM4 (350, 525, or 700 μCi) or increasing doses of ^{90}Y-DOTA-cPAM4 (130, 175, 220, or 260 μCi). One group of mice was left untreated as a control. The mice were weighed prior to being injected and then once a week to ensure that their body weight remained more than 80% of starting weight. Measurements for determination of tumor size were likewise performed on a weekly basis.

Data Analysis. Statistical analyses of tumor growth data were based on AUC and survival time. Profiles of individual tumor growth were obtained through linear and exponential curve modeling. The *t* test was used to assess the statistical significance between the two MTD treatment groups, as well as to perform other pair-wise comparisons. As a consequence of incompleteness on some of the growth curves (caused by deaths), statistical comparisons of AUC were performed only up to the time at which the first animal within a group was killed. AUC analyses were supported by statistical comparisons of survival data; in this case, survival was defined as time for a tumor to reach 5 cm^3. At the termination of the study (26 weeks), some of the animals had not yet experienced the end point, and their observations were considered as censored. The Mantel-Haenszel log-rank test was then used for comparison of treatment arms.

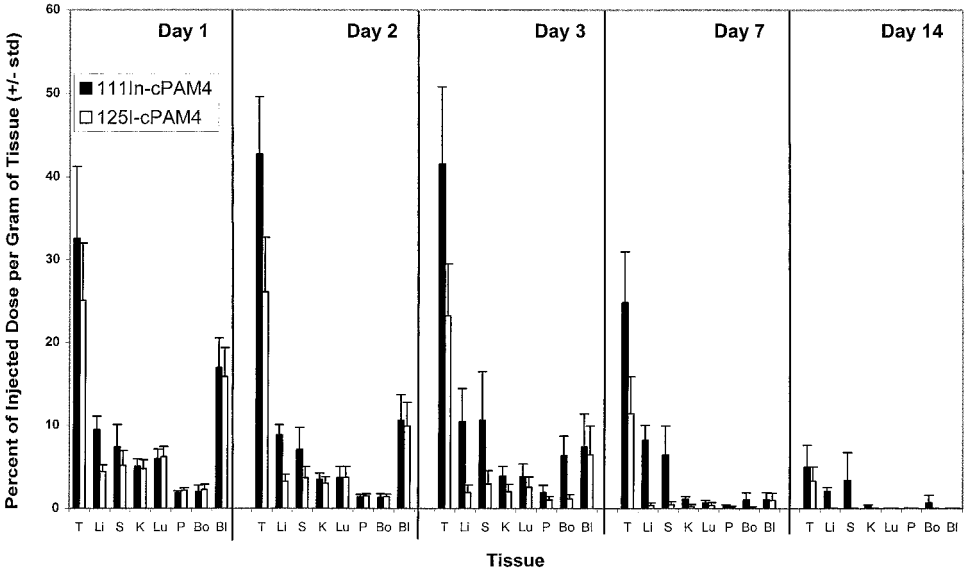

Fig. 1 The biodistribution of coinjected ^{111}In-labeled (25 μCi) and ^{125}I-labeled (10 μCi) cPAM4 in CaPan1 tumor-bearing nude mice. Mice were killed on days 1, 2, 3, 7, and 14, and tissues were collected and counted as described in "Materials and Methods." *Tissue*: *T*, tumor; *Li*, liver; *S*, spleen; *K*, kidney; *Lu*, lung; *P*, pancreas; *Bo*, bone; *Bl*, blood. *Bar*, SD.

Table 1 Radiation dose estimates for ^{90}Y-cPAM4 IgG absorbed dose (cGy) when administered at the MTD

	Tumor	Blood	Liver	Spleen	Kidney	Lung	Pancreas	Bone	Tumor:Blood Ratio
^{131}I-cPAM4 (700 μCi)	8559	2615	986	875	970	1221	475	494	3.27
^{90}Y-cPAM4 (260 μCi)	8068	1971	1660	1202	648	681	294	429	4.09

RESULTS

Biodistribution Studies. Athymic nude mice bearing the CaPan1 human pancreatic carcinoma (~1 cm^3 at the start of the study) were given both 25 μCi ^{111}In-DOTA-cPAM4 and 10 μCi ^{125}I-cPAM4. Groups of mice were killed on days 1, 2, 3, 7, and 14. Tissue uptake of radiolabeled antibodies are presented as the %ID/g of tissue (wet weight) in Fig. 1. Tumor uptake of ^{111}In-DOTA-cPAM4 was significantly greater than ^{125}I-cPAM4 at the 2, 3, and 7 day time points ($P < 0.0002$, 0.0004, and 0.0005, respectively). The highest levels of radiolabeled antibody within the tumor occurred on days 2 and 3, with a 1.67 ± 0.18- and 1.82 ± 0.15-fold higher level of ^{111}In-radiolabeled over ^{125}I-radiolabeled cPAM4, respectively. A steady decline of radioisotope was observed within the tumors after day 3 with an effective $t_{1/2}$ of 45 h for ^{111}In and 112 h for ^{125}I. Blood clearances were similar for the two isotopes ($t_{1/2}$ = 22 h and 32 h for ^{111}In and ^{125}I, respectively). Localization ratios (^{111}In:^{125}I) of 1.56 ± 0.19, 1.58 ± 0.29, and 1.81 ± 0.51 for days 2, 3, and 7, respectively, clearly demonstrated the increased tumor uptake and retention of ^{111}In-radiolabeled over ^{125}I-radiolabeled cPAM4. Nontumor tissue uptake and clearance of the radiolabeled antibodies were similar for most tissues except in the liver, spleen, and bone, for which the %ID/g ^{111}In was significantly higher than for ^{125}I. Radiation dose estimates were calculated from the time-activity curves of the biodistribution studies (Table 1). At their respective MTDs of 260 μCi for ^{90}Y-DOTA-cPAM4 and 700 μCi for ^{131}I-cPAM4 (16), approximately equal radiation doses would be delivered to the tumors. Tumor:blood radiation dose ratios were 3.27 for ^{131}I-cPAM4 and 4.09 for ^{90}Y-DOTA-cPAM4.

RAIT Studies. Groups of athymic nude mice bearing CaPan1 tumors of ~1 cm^3 were given a single cycle of either ^{90}Y-DOTA-cPAM4 (130, 175, 220, or 260 μCi) or ^{131}I-cPAM4 (350, 525, or 700 μCi). Each dose was augmented with unlabeled cPAM4 so that all of the mice received a total of 50 μg of antibody. Overall, the treatments were well tolerated. However, some early deaths did occur with both radionuclides. In the 700-μCi ^{131}I-cPAM4 group, one of nine mice was found dead at week 3. This mouse had a 19% drop in body weight at week 2 *versus* only 6 ± 6% for the group as a whole, which suggested a therapy-related cause. Although previous studies determined the MTD to be 700 μCi, we may be too close to the toxic level and may need to use a slightly lower dose in the future. Likewise one of eight mice within the 260-μCi ^{90}Y-DOTA-cPAM4 group was found dead at week 3. Although therapy itself cannot be ruled out as the cause of death, the mouse lost only 3% of its body weight at the time of death with a body weight loss of only 3 ± 3% for the group as a whole. The nadir for this treatment group (7 ± 4% weight loss) occurred at day 7. The two mice were excluded from data analyses. The nadir for the 700-μCi ^{131}I-cPAM4-treated group was also reached at day 7 with an 11 ± 4% weight loss. A comparison of weight loss between the two MTD-treated groups approached a significant difference ($P < 0.056$). Both groups of mice recovered by week 4.

Tumor growth curves were generated with statistical analysis of AUC performed up to the time at which an animal within a group was lost because of disease activity. The 350-, 525-, and 700-μCi ^{131}I-cPAM4-treated groups all had tumors that were significantly smaller than tumors within the untreated group ($P < 0.003$, 0.015, and 0.003, respectively) at week 3, the last

Table 2 Comparisons of tumor growth inhibition and survival between the various ^{131}I- and ^{90}Y-labeled cPAM4 treatment groups

Treatment	*n*	Mean size of tumors at nadir (± SD)	Time when nadir reached (wk)	Median survival time (wk)	No. of survivors at wk 26 (disease-free)[a]
Untreated	10	N/A[b]	N/A	6	1 (1)
350 μCi ^{131}I	10	N/A	N/A	13	2 (2)
525 μCi ^{131}I	8	N/A	N/A	12	0
700 μCi ^{131}I	8	0.61 cm^3 (0.24)	7	17.5	1 (1)
130 μCi ^{90}Y	8	0.78 cm^3 (0.61)	6	16	2 (1)
175 μCi ^{90}Y	8	0.33 cm^3 (0.40)	7	>26	4 (4)
220 μCi ^{90}Y	7	0.10 cm^3 (0.07)	9	>26	5 (2)
260 μCi ^{90}Y	7	0.19 cm^3 (0.13)	10	>26	6 (4)

[a] At week 26, any surviving mice were humanely sacrificed and examined by necropsy for macroscopic signs of internal metastasis. Any solid mass at the tumor transplant site was removed and examined histologically to confirm the lack of tumor cells.

[b] N/A, the mean tumor sizes for these mice never regressed below the initial tumor sizes.

week that data from the untreated group could be compared with the treated groups. There were no significant differences in tumor growth between the different dose levels of ^{131}I-cPAM4 at any of the time points examined. Survival analyses also did not demonstrate any statistically significant differences between the different ^{131}I-treatment groups. However, the 700-μCi group had a longer median survival (17.5 weeks) compared with the 350-μCi (13 weeks)- and 525-μCi (12 weeks)-treated groups (Table 2).

Like the ^{131}I-cPAM4-treated mice, at week 3 all of the ^{90}Y-DOTA-cPAM4-treated mice (130-, 175-, 220-, and 260-μCi groups) had significantly smaller tumors than tumors within the untreated group ($P < 0.016$, 0.006, 0.006, and 0.004, respectively). Comparisons between ^{90}Y treatment groups showed that administration of 175, 220, or 260 μCi gave significantly greater antitumor activity than did 130 μCi at week 10 (at which time, deaths started to occur in the 130-μCi-treated group; Table 2). Of particular note, there were no significant differences in tumor growth inhibition between the 175-μCi treatment group and the 220- and 260-μCi treatment groups up to the last assessable time point, week 12, or between the 220- and 260-μCi treatment groups up to week 17. Median survival times for the 175-, 220-, and 260-μCi treatment groups were ≥26 weeks (the end of the study).

The tumor growth curves for individual mice within the untreated, the 700-μCi ^{131}I-cPAM4, and the 260-μCi ^{90}Y-DOTA-cPAM4 groups are presented in Fig. 2. At these equitoxic doses, mice treated with ^{90}Y-DOTA-cPAM4 exhibited a significantly greater degree of tumor growth inhibition than mice treated with ^{131}I-cPAM4. At week 11, the last assessable time point for the ^{131}I-cPAM4-treated group, mice had a mean tumor size of 1.64 ± 1.72 cm^3 compared with a mean tumor size of 0.20 ± 0.13 cm^3 for the mice treated with ^{90}Y-DOTA-cPAM4. This was a significant 8.2-fold difference in tumor size ($P < 0.035$). Even at the lower ^{90}Y dose of 220 μCi, the tumors were significantly smaller (0.17 ± 0.21 cm^3, $P < 0.035$) than those mice that received the MTD of ^{131}I-cPAM4.

Survival curves for the untreated, the 700-μCi ^{131}I-cPAM4, and 260-μCi ^{90}Y-DOTA-cPAM4 groups are presented in Fig. 3. The MTD of ^{90}Y-DOTA-cPAM4 provided a significantly longer survival than both the untreated and 700-μCi ^{131}I-cPAM4-treated groups (Table 3). The relative risk values of the untreated and ^{131}I-cPAM4-treated groups were 17.1 and 15.4, respectively. At the end of the study period (26 weeks), 1 of 10 untreated mice was alive and disease-free. Within the 700-μCi ^{131}I-cPAM4-treated group, two of eight mice were responders (tumor size, ≤50% of starting size) for a median of 14 weeks. The remaining six mice had stable disease (tumor size between 50 and 125% of starting size) for a median of 10 weeks. At the end of the study period, one mouse was still alive with no sign of disease by gross and microscopic observation. All seven of the 260-μCi ^{90}Y-DOTA-cPAM4-treated mice were responders, with a median response time of 20 weeks. Six of the seven mice were still alive at week 26 with four mice having no evidence of residual disease. The 220-μCi ^{90}Y-DOTA-cPAM4-treated group was also more affected than the 700-μCi ^{131}I-cPAM4-treated group in terms of survival ($P < 0.011$); however, the 175-μCi ^{90}Y-DOTA-cPAM4-treated group was not ($P < 0.078$). There were no significant differences between the survival times for the 175-, 220-, and 260-μCi ^{90}Y-DOTA-cPAM4-treated groups.

DISCUSSION

PAM4 is an IgG1 MAb that recognizes the MUC1 mucin expressed by human pancreatic carcinomas (2). In preclinical studies, ^{125}I-labeled PAM4 targeted pancreatic tumors with high specificity and could achieve high concentrations of radioisotope within these tumors while minimizing radiation dose delivered to nontumor tissues (3, 4). Similar targeting specificity was observed in initial clinical trials (5, 6). In experimental RAIT studies, ^{131}I-labeled PAM4 was shown to have significant antitumor effects against both s.c. and orthotopic human pancreatic tumor xenografts (4, 17).

Clinical trials of RAIT have met with some success in recent years. Whereas many positive clinical responses have been achieved against lymphomas (18–21), solid tumors have had only mixed results (22–25). Besides the antibody itself, a major factor influencing the efficacy of RAIT is the choice of radionuclide. Many investigators have reported that the use of residualizing radionuclides, such as ^{90}Y, yields higher concentrations of radioisotope within the tumor, with consequent greater therapeutic effect than can be achieved by use of ^{131}I (9–12, 26–28). However, others have found that for certain

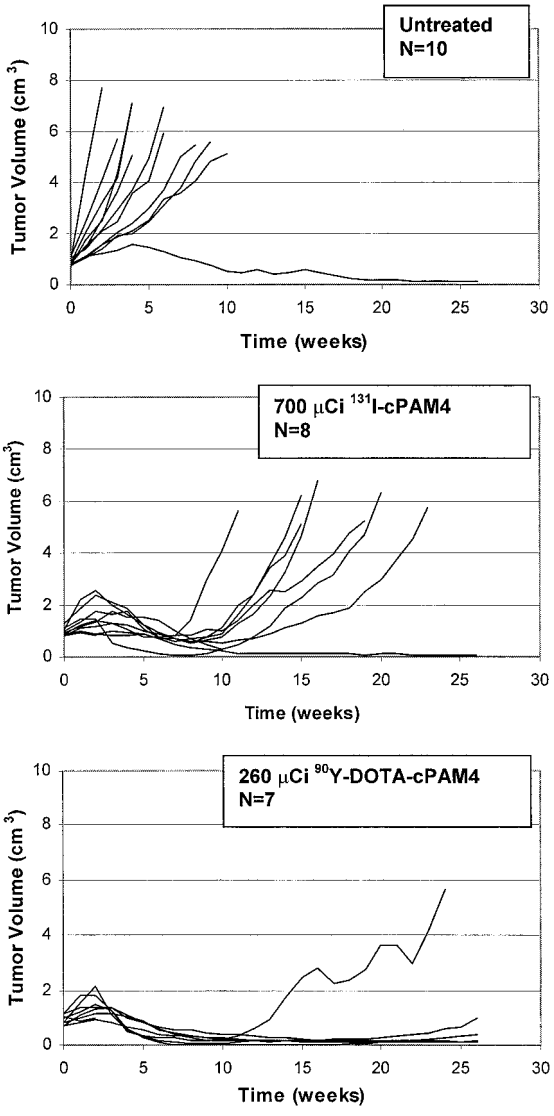

Fig. 2 Tumor growth curves from individual animals from the untreated and the 700-μCi ^{131}I- and 260-μCi ^{90}Y-cPAM4 treatment groups. The initial tumor sizes were 0.89 ± 13 cm^3 (untreated), 0.95 ± 0.15 cm^3 (^{131}I), and 0.93 ± 0.17 cm^3 (^{90}Y). Tumors were measured weekly as described in "Materials and Methods." Mice were humanely sacrificed when tumor volume exceeded 5.0 cm^3. *n* = number of mice in each group.

MAbs and tumor types, ^{131}I- could be superior to ^{90}Y-labeled antibodies (29, 30).

We examined the biodistribution of cPAM4 labeled with either ^{111}In or ^{125}I in mice bearing s.c. CaPan1 tumors. Similar to what had been reported by others (11, 26–28), we found that, in our tumor model system, there was an ~2-fold increase in %ID/g ^{111}In in the tumor as compared with ^{125}I. However, there was also greater uptake in the liver, spleen, and bone. The uptake in the liver and spleen was not an unexpected outcome, because both these organs are the most active in IgG catabolism (31) and as a residualizing radionuclide, ^{111}In would be retained longer in these tissues than would be the ^{125}I. It should be noted

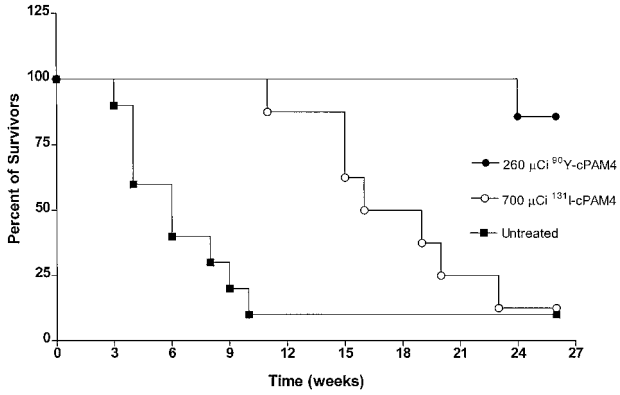

Fig. 3 The survival curves generated from the untreated, the 700-μCi ^{131}I-, and the 260-μCi ^{90}Y-cPAM4 treatment groups. The graph represents the percentage of survivors from each group to 26 weeks post-therapy, when the study was ended.

that at the MTD for ^{90}Y-DOTA-cPAM4, the absorbed radiation dose for the liver (1660 cGy) would still be well below the reported toxic level of 3000 cGy in mice (32). The other tissue of interest, in which free ^{111}In and ^{90}Y can accumulate, is the bone (33). This is an important consideration, because it adds to the overall radiation dose delivered to the bone marrow, which has been shown to be the dose-limiting tissue of RAIT (22, 34–37). Whereas we did observe a greater %ID/g ^{111}In in the bone, our dosimetry estimates at the MTD of ^{90}Y-DOTA-cPAM4 show that the amount of absorbed radiation dose would be similar to the amount that would be delivered at the MTD of ^{131}I-cPAM4 (429 cGy *versus* 494 cGy, respectively).

Calculations on theoretical tumor models have suggested that for large tumors (as is the usual clinical presentation of pancreatic cancer), ^{90}Y-labeled MAbs would achieve a higher therapeutic effect in comparison with ^{131}I-labeled MAbs (38). Supporting this, we found that when administered at their respective MTDs, the ^{90}Y-DOTA-cPAM4-treated mice exhibited a longer period and greater degree of antitumor response than mice that had received ^{131}I-cPAM4. The increased antitumor effects provided by ^{90}Y-DOTA-cPAM4 translated into a significantly longer survival time and cure for approximately one-half of the mice. Similar results were also noted at the lower doses of ^{90}Y-labeled cPAM4 (representing as little as 67% of the MTD).

One of the main antitumor effects of RAIT is at the tumor vascular level (39–41). In a previous study, ^{131}I-PAM4 RAIT was found to cause significant suppression of tumor vascular permeability in 1.0-g CaPan1 tumors at radiation doses of 3000 cGy (41). Even at the lowest doses administered in the current study (130 μCi for ^{90}Y and 350 μCi for ^{131}I), we delivered in excess of 4000 cGy to the tumors. To date, the exact mechanism(s) by which RAIT perturbs the intratumoral vasculature has not been fully elucidated. There is some evidence that the damage caused to the blood vessel is an indirect result via a tumoricidal response rather than direct radiation damage to the vessel itself (40). It may be that a higher percentage of tumor cells are killed with the ^{90}Y-cPAM4 with a net result of causing a greater degree of damage to existing blood vessels (suppres-

Table 3 Statistical analysis comparing the effect of the MTDs of ^{90}Y- and ^{131}I-labeled cPAM4 on survival and tumor growth

		Median survival time[a]		Normalized tumor growth (wk 11)	
Treatment 1 *vs.*	Treatment 2	Ratio[b]	*P*	Ratio[c]	*P*
260 μCi ^{90}Y	Untreated	>4.33[d]	0.0008	N/A[e]	N/A
	700 μCi ^{131}I	>1.49[d]	0.0018	0.13	0.0351
700 μCi ^{131}I	Untreated	2.91	0.0233	N/A	N/A

[a] Survival is defined as the time for a tumor to become greater than 5.0 cm^3.
[b] Ratio of median survival times. Values >1.00 indicate that the first treatment group had longer survival.
[c] Ratio of average normalized tumor size at week 11. Values <1.00 indicate that the first treatment group had less tumor growth.
[d] These ratios have a (>) sign because the experimental end point was 26 weeks, and there were still mice alive in the 260-μCi ^{90}Y-treatment group at this time point. Therefore, these ratios reflect the fact that the median for these mice was actually >26 weeks.
[e] N/A, 9 of 10 untreated mice did not survive to week 11 and, therefore, could not be analyzed.

sion of vascular permeability) as well as having an antiangiogenic effect. Additional studies need to be done to better understand how ^{90}Y-delivered radiation effect these and other aspects of tumor physiology.

On the basis of the biodistribution data, we estimated that the CaPan1 tumor in both cases would receive approximately the same absorbed radiation dose (~8000 cGy). The clear superiority of ^{90}Y over ^{131}I for PAM4-directed RAIT suggested that factors other then total absorbed tumor dose need to be considered when assessing the efficacy of RAIT. In this respect an important issue affecting the outcome of RAIT may be the heterogeneous distribution of intratumoral radiation dose. The choice of radioisotope may, thus, be critical. For large, bulky tumors, the relatively short pathlength for ^{131}I β emissions results in a heterogeneous dose distribution attributable to the nonuniform distribution of the antibody within solid tumors. On the other hand, ^{90}Y, having a longer pathlength of radiation emission, can provide for a more uniform dose distribution. That we could obtain similar antitumor effects at lower doses of ^{90}Y-DOTA-cPAM4 further suggests that dose distribution within the tumor may be as important, if not more important, than absolute absorbed dose.

In summary, we found that, at their respective MTDs, ^{90}Y-labeled cPAM4 had a significantly greater antitumor effect than ^{131}I-labeled cPAM4. One of the problems associated with RAIT of solid tumors is the relatively low tumor uptake of administered radiolabeled antibody in the human in comparison with that observed in mice (reviewed in Ref. 42). By using ^{90}Y-labeled cPAM4, we were able to demonstrate that this high-energy, residualizing radionuclide, further enhanced the antitumor effect of PAM4-directed RAIT in our tumor-model system, although both isotopes delivered a similar radiation dose to the tumor as a whole. This may allow for a similar enhancement of antitumor effects in patients, even when the relative uptake of antibody remains unchanged. Several approaches are being considered to further increase the antitumor efficacy of ^{90}Y-DOTA-cPAM4. First, the duration and extent of the antitumor response, even at the lower doses, suggests that multiple treatment cycles of ^{90}Y-labeled cPAM4 may provide an effective therapeutic for control of pancreatic cancer. Akin to this approach, others have shown that fractionated doses of RAIT can be more effective than single larger doses (43–46). This may be especially appropriate for ^{90}Y, because the bone marrow appears to rebound faster after ^{90}Y administration than after ^{131}I (30). Finally, we may be able to further increase tumor uptake of ^{90}Y by increasing the total PAM4 protein dose that is administered. We have previously demonstrated that increasing the PAM4 protein dose significantly increases tumor uptake of radiolabeled PAM4, as well as providing a more uniform distribution of the antibody within the tumor (3). Studies are now underway to determine whether these procedures will further enhance the therapeutic effect of ^{90}Y-DOTA-cPAM4 RAIT for pancreatic cancer.

REFERENCES

1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer Statistics. CA-Cancer J. Clin., *49:* 8–31, 1999.
2. Gold, D. V., Lew, K., Maliniak, R., Hernandez, M., and Cardillo, T. Characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin. Int. J. Cancer, *57:* 204–210, 1994.
3. Gold, D. V., Alisauskas, R., and Sharkey, R. M. Targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4. Cancer Res., *55:* 1105–1110, 1995.
4. Alisauskus, R., Wong, G. Y. C., and Gold, D. V. Initial studies of monoclonal antibody PAM4 targeting to xenografted orthotopic pancreatic cancer. Cancer Res., *55:* 5743s–5748s, 1995.
5. Mariani, G., Molea, N., Bacciardi, D., Boggi, U., Fornaciari, G., Campani, D., Salvadori, P. A., Giulianotti, P. C., Mosca, F., Gold, D. V., Sharkey, R. M., and Goldenberg, D. M. Initial tumor targeting, biodistribution, and pharmacokinetic evaluation of the monoclonal antibody PAM4 in patients with pancreatic cancer. Cancer Res., *55*: 5911s–5915s, 1995.
6. Gold, D. V., Juweid, M., Cardillo, T., Goldenberg, D. M., and Sharkey, R. M. Localization of pancreatic cancer with monoclonal antibody PAM4. Crit. Rev. Oncol. Hematol, *39:* 147–154, 2001.
7. DeNardo, S. J., and DeNardo, G. L. New Approaches in Biology, Diagnosis, and Treatment. *In*: P. J. Moloy and G. L. Nicholson (eds.), Cellular Oncology, pp. 282–292. New York: Praeger Pub., 1983.
8. Bureau of Radiological Health. Radiological Health Handbook, Revised Edition. Rockville, MD: United States Department of Health, Education, and Welfare, 1970.
9. Shih, L. B., Thorpe, S. R., Griffiths, G. L., Diril, H., Ong, G. L., Hansen, H. J., Goldenberg, D. M., and Mattes, M. J. The processing and fate of antibodies and their radiolabels bound to the surface of tumor cells *in vitro*: a comparison of nine radiolabels. J. Nucl. Med., *35*: 899–908, 1994.
10. Mattes, M. J., Griffiths, G. L., Diril, H., Goldenberg, D. M., Ong, G. L., and Shih, L. B. Processing of antibody-radioisotope conjugates after binding to the surface of tumor cells. Cancer (Phila.), *73:* 787–793, 1994.
11. Stein, R., Goldenberg, D. M., Thorpe, S. R., Basu, A., and Mattes, M. J. Effects of radiolabeling monoclonal antibodies with a residualizing iodine radiolabel on the accretion of radioisotope in tumors. Cancer Res., *55:* 3132–3139, 1995.
12. Press, O. W., Shan, D., Howell-Clark, J., Eary, J., Appelbaum, F. R., Matthews, D., King, D. J., Haines, A. M. R., Hamann, P., Hinman, L., Shochat, D., and Bernstein, I. D. Comparative metabolism and

retention of iodine-125, yttrium-90, and indium-111 radioimmunoconjugates by cancer cells. Cancer Res., *56:* 2123–2129, 1996.

13. McConahey, P. J., and Dixon, F. J. A method of trace iodination of proteins for immunological studies. Int. Arch. Allergy Appl. Immunol., *29:* 185–192, 1966.

14. Govindan, S. V., Shih, L. B., Goldenberg, D. M., Sharkey, R. M., Karacay, H., Donnelly, J. E., Losman, M. J., Hansen, H. J., and Griffiths, G. L. ^{90}Yttrium-labeled complementarity-determining-region-grafted monoclonal antibodies for radioimmunotherapy: radiolabeling and animal biodistribution studies. Bioconjug. Chem., *9:* 773–782, 1998.

15. Blumenthal, R. D., Sharkey, R. M., Kashi, R., and Goldenberg, D. M. Comparison of therapeutic efficacy and host toxicity of two different I-131-labeled antibodies and their fragments in the GW-39 colonic cancer xenograft model. Int. J. Cancer, *44:* 292–300, 1989.

16. Cardillo, T., Blumenthal. R., and Gold, D. V. Comparison of ^{90}Y- and ^{131}I-radiolabeled Mab-PAM4 for radioimmunotherapy of experimental human pancreatic cancer. Proc. Am. Assoc. Cancer Res., *38:* 404, 1997.

17. Gold, D. V., Cardillo, T., Vardi, Y., and Blumenthal, R. Radioimmunotherapy of experimental pancreatic cancer with ^{131}I-labeled monoclonal antibody PAM4. Int. J. Cancer, *71:* 660–667, 1997.

18. Behr, T. M., Wormann, B., Gramatzki, M., Riggert, J., Gratz, S., Behe, M., Griesinger, F., Sharkey, R. M., Kolb, H-J., Hiddemann, W., Goldenberg, D. M., and Becker, W. Low- *versus* high-dose radioimmunotherapy with humanized anti-CD22 or chimeric anti-CD20 antibodies in a broad spectrum of B cell-associated malignancies. Clin. Cancer Res., *5* (Suppl.)*:* 3304s–3314s, 1999.

19. O'Donnell, R. T., DeNardo, G. L., Kukis, D. L., Lamborn, K. R., Shen, S., Yuan, A., Goldstein, D. S., Carr, C. E., Mirick, G. R., and DeNardo, S. J. A clinical trial of radioimmunotherapy with ^{67}Cu-2 IT-BAT-Lym-1 for non-Hodgkin's lymphoma. J. Nucl. Med., *40:* 2014–2020, 1999.

20. O'Donnell, R. T., DeNardo, G. L., Kukis, D. L., Lamborn, K. R., Shen, S., Yuan, A., Goldstein, D. S., Mirick, G. R., and DeNardo, S. J. ^{67}Copper-2-iminothiolane-6-[*p*-(bromoacetamido)benzyl]-TETA-Lym-1 for radioimmunotherapy on non-Hodgkin's lymphoma. Clin. Cancer Res., *5* (Suppl.)*:* 3330s–3336s, 1999.

21. Wiseman, G. A., White, C. A., Witzig, T. E., Gordon, L. I., Emmanouilides, C., Raubitschek, A., Janakiraman, N., Gutheil, J., Schilder, R. J., Spies, S., Silverman, D. H., and Grillo-Lopez, A. J. Radioimmunotherapy of relapsed non-Hodgkin's lymphoma with zevalin, a ^{90}Y-labeled anti-CD20 monoclonal antibody. Clin. Cancer Res., *5* (Suppl.)*:* 3281s–3286s, 1999.

22. Behr, T. M., Salib, A. L., Liersch, T., Behe, M., Angerstein, C., Blumenthal, R. D., Fayyazi, A., Sharkey, R. M., Ringe, B., Becker, H., Wormann, B., Hiddemann, W., Goldenberg, D. M., and Becker, W. Radioimmunotherapy of small volume disease of colorectal cancer metastatic to the liver: preclinical evaluation in comparison to standard chemotherapy and initial results of a Phase I clinical study. Clin. Cancer Res., *5* (Suppl.)*:* 3232s–3242s, 1999.

23. Juweid, M. E., Hajjar, G., Stein, R., Sharkey, R. M., Herskovic, T., Swayne, L. C., Suleiman, S., Pereira, M., Ribin, A. D., and Goldenberg, D. M. Initial experience with high-dose radioimmunotherapy of metastatic medullary thyroid cancer using ^{131}I-MN-14 F(ab)$_2$ anti-carcinoembryonic antigen Mab and AHSCR. J. Nucl. Med., *41:* 93–103, 2000.

24. Rosenblum, M. G., Verschraegen, C. F., Murray, J. L., Kudelka, A. P., Gano, J., Cheung, L., and Kavenagh, J. J. Phase I study of ^{90}Y-labeled B72.3 intraperitoneal administration in patients with ovarian cancer: effect of dose and EDTA coadministration on pharmacokinetics and toxicity. Clin. Cancer Res., *5:* 953–961, 1999.

25. Pai-Scherf, L. H., Carrasquillo, J. A., Paik, C., Gansow, O., Whatley, M., Pearson, D., Webber, K., Hamilton, M., Allegra, C., Brechbiel, M., Willingham, M. C., and Pastan, I. Imaging and Phase I study of ^{111}In- and ^{90}Y-labeled anti-Lewis^{y} monoclonal antibody B3. Clin. Cancer Res., *6:* 1720–1730, 2000.

26. Stein, R., Chen, S., Haim, S., and Goldenberg, D. M. Advantage of yttrium-90-labeled over iodine-131-labeled monoclonal antibodies in the treatment of a human lung carcinoma xenograft. Cancer (Phila.), *80:* 2636–2641, 1997.

27. Sharkey, R. M., Behr, T. M., Mattes, M. J., Stein, R., Griffiths, G. L., Shih, L. B., Hansen, H. J., Blumenthal, R. D., Dunn, R. M., Juweid, M. E., and Goldenberg, D. M. Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. Cancer Immunol. Immunother., *44:* 179–188, 1997.

28. Stein, R., Juweid, M., Mattes, M. J., and Goldenberg, D. M. Carcinoembryonic antigen as a target for radioimmunotherapy of human medullary thyroid carcinoma: antibody processing, targeting, and experimental therapy with ^{131}I and ^{90}Y labeled MAbs. Cancer Biother. Radiopharm., *14:* 37–47, 1999.

29. Sharkey, R. M., Motta-Hennessy, C., Pawlyk, D., Siegel, J. A., and Goldenberg, D. M. Biodistribution and radiation dose estimates for yttrium- and iodine-labeled monoclonal antibody IgG and fragments in nude mice bearing human colonic tumor xenografts. Cancer Res., *50:* 2330–2336, 1990.

30. Sharkey, R. M., Blumenthal, R. D., Behr, T. M., Wong, G. Y., Haywood, L., Forman, D., Griffiths, G. L., and Goldenberg, D. M. Selection of radioimmunoconjugates for the therapy of well-established or micrometastic colon carcinoma. Int. J. Cancer, *72:* 477–485, 1997.

31. Henderson, L. A., Baynes, J. W., and Thorpe, S. R. Identification of the sites of IgG catabolism in the rat. Arch. Biochem. Biophys., *215:* 1–11, 1982.

32. Rubin, P., and Casarett, G. W. Major digestive glands. *In:* Clinical Radiation Pathology, Vol. 1, pp. 241–292. Philadelphia: WB Saunders, 1968.

33. Catsch, A. Radioactive Metal Mobilization in Medicine. Springfield, IL: Charles C. Thomas, 1964.

34. Stein, R., Sharkey, R. M., and Goldenberg, D. M. Haematological effects of radioimmunotherapy in cancer patients. Br. J. Haematol., *80:* 69–76, 1992.

35. Vriesendorp, H. M., Quadri, S. M., Andersson, B. S., and Dicke, K. A. Hematological side effects of radiolabeled immunoglobulin therapy. Exp. Hematol., *24:* 1183–1190, 1996.

36. Blumenthal, R. D., Sharkey, R. M., Haywood, L., Behr, T., and Goldenberg, D. M. Application of cytokine intervention for improved radio-antibody dose delivery. Int. J. Cancer, *72:* 166–173, 1997.

37. Blumenthal, R. D., Alisauskas, R., Lew, W., Sharkey, R. M., and Goldenberg, D. M. Myelosuppressive changes from single or repeated doses of radioantibody therapy: effect of bone marrow transplantation, cytokines, and hematopoietic suppression. Exp. Hematol., *26:* 859–868, 1998.

38. Attard, A. R., Chappell, M. J., and Bradwell, A. R. Model-based calculation for effective cancer radioimmunotherapy. Br. J. Radiol., *68:* 636–645, 1995.

39. Blumenthal, R. D., Sharkey, R. M., Kashi, R., and Goldenberg, D. M. Suppression of tumor vascular activity by radioantibody therapy: implications for multiple cycle treatments. Sel. Cancer Ther., *7*: 9–16, 1991.

40. Blumenthal, R. D., Kashi, R., Sharkey, R. M., and Goldenberg, D. M. Quantitative and qualitative effects of experimental radioimmunotherapy on tumor vascular permeability. Int. J. Cancer, *61*: 557–566, 1995.

41. Blumenthal, R. D., Sharkey, R. M., Kashi, R., Sides, K., Stein, R., and Goldenberg, D. M. Changes in tumor vascular permeability in response to experimental radioimmunotherapy: A comparative study of 11 xenografts. Tumor Biol., *18*: 367–377, 1997.

42. Behr, T. M., Goldenberg, D. M., and Becker W. S. Radioimmunotherapy of solid tumors: a review "of mice and men." Hybridoma, *16*: 101–107, 1997.

43. Buchsbaum, D., Khazaeli, M. B., Liu, T., Bright, S., Richardson, K., Jones, M., and Meredith, R. Fractionated radioimmunotherapy of human colon carcinoma xenografts with ^{131}I-labeled monoclonal antibody CC49. Cancer Res., *55:* 5881s–5887s, 1995.

44. DeNardo, G. L., DeNardo, S. J., Lamborn, K. R., Goldstein, D. S., Levy, N. B., Lewis, J. P., O'Grady, L. F., Raventos, A., Kroger, L. A., Macey, D. J., McGahan, J. P., Mills, S. L., and Shen, S. Low-dose, fractionated radioimmunotherapy for B-cell malignancies using ^{131}I-Lym-1 antibody. Cancer Biother. Radiopharm., *13:* 239–254, 1998.

45. Borchardt, P. E., Quadri, S. M., Freedman, R. S., and Vriesendorp, H. M. Intraperitoneal radioimmunotherapy with human monoclonal IgM in nude mice with peritoneal carcinomatosis. Cancer Biother. Radiopharm., *15:* 53–64, 2000.

46. O'Donoghue, J. A., Sqouros, G., Divgi, C. R., and Humm, J. L. Single-dose versus fractionated radioimmunotherapy: model comparisons for uniform tumor dosimetry. J. Nucl. Med., *41:* 538–547, 2000.