

Treatment of Advanced Pancreatic Carcinoma with ⁹⁰Y-Clivatuzumab Tetraxetan: A Phase I Single-Dose Escalation Trial

Seza A. Gulec¹, Steven J. Cohen², Kenneth L. Pennington¹, Lionel S. Zuckier³, Ralph J. Hauke⁴, Heather Horne⁵, William A. Wegener⁵, Nick Teoh⁵, David V. Gold⁶, Robert M. Sharkey⁶, and David M. Goldenberg^{5,6}

Abstract

Purpose: Humanized antibody hPAM4 specifically binds a mucin glycoprotein expressed in pancreatic adenocarcinomas. This phase I study evaluated a single dose of ⁹⁰Y-clivatuzumab tetraxetan (⁹⁰Y-labeled hPAM4) in patients with advanced pancreatic cancer.

Experimental Design: Twenty-one patients (4 stage III; 17 stage IV) received ¹¹¹In-hPAM4 for imaging and serum sampling before ⁹⁰Y-hPAM4. Study procedures evaluated adverse events, safety laboratories, computed tomography (CT) scans, biomarkers, pharmacokinetics, radiation dosimetry, and immunogenicity (HAHA).

Results: ¹¹¹In-hPAM4 showed normal biodistribution with radiation dose estimates to red marrow and solid organs acceptable for radioimmunotherapy and with tumor targeting in 12 patients. One patient withdrew before ⁹⁰Y-hPAM4; otherwise, 20 patients received ⁹⁰Y doses of 15 ($n = 7$), 20 ($n = 9$), and 25 mCi/m² ($n = 4$). Treatment was well tolerated; the only significant drug-related toxicities were (NCI CTC v.3) grade 3 to 4 neutropenia and thrombocytopenia increasing with ⁹⁰Y dose. There were no bleeding events or serious infections, and most cytopenias recovered to grade 1 within 12 weeks. Three patients at 25 mCi/m² encountered dose-limiting toxicity with grade 4 cytopenias more than 7 days, establishing 20 mCi/m² as the maximal tolerated ⁹⁰Y dose. Two patients developed HAHA of uncertain clinical significance. Most patients progressed rapidly and with CA19-9 levels increasing within 1 month of therapy, but 7 remained progression-free by CT for 1.5 to 5.6 months, including 3 achieving transient partial responses (32%–52% tumor diameter shrinkage).

Conclusion: ⁹⁰Y-Clivatuzumab tetraxetan was well tolerated with manageable hematologic toxicity at the maximal tolerated ⁹⁰Y dose, and is a potential new therapeutic for advanced pancreatic cancer. *Clin Cancer Res*; 17(12); 4091–100. ©2011 AACR.

Introduction

PAM4 is a monoclonal antibody developed against a human pancreatic cancer cell line which binds to a mucin produced primarily in pancreatic adenocarcinomas (1–4). Immunohistologic studies have indicated that PAM4-reactive mucin is present in early transformation, such as PanIn (pancreatic intraepithelial neoplasia) and

IPMN (intraductal papillary mucinous neoplasm) lesions, but not in normal pancreatic ducts, pancreatitis, or other normal tissues (1). This antigen can also be detected in the serum, with evidence of a high specificity and sensitivity for pancreatic cancer detection (3), suggesting that it is a potentially important biomarker for malignant transformation of the pancreas.

The specificity of PAM4 also makes it an attractive agent for pancreatic cancer imaging and therapy (2). Pilot clinical studies using ¹³¹I-labeled murine PAM4 in 5 patients with suspected pancreatic cancer showed promising localization in lesions in 4 patients subsequently confirmed to have pancreatic cancer at surgery. No targeting was noted in a patient with pancreatitis (5). Preclinical studies in nude mice bearing human pancreatic carcinoma xenografts indicated that ¹³¹I- and ⁹⁰Y-labeled PAM4 could significantly control tumor growth, even in well-established subcutaneous implants, with the best responses occurring with ⁹⁰Y-PAM4 (6, 7). When combined with gemcitabine, further therapeutic improvements were observed (8–10).

These preclinical studies provided the impetus to humanize PAM4 (hPAM4) so that clinical studies could be conducted to assess ⁹⁰Y-hPAM4 for the treatment of

Authors' Affiliations: ¹Goshen Center for Cancer Care, Goshen, Indiana; ²Fox Chase Cancer Center, Philadelphia, Pennsylvania; ³New Jersey Medical School—UMDNJ, Newark, New Jersey; ⁴University of Nebraska Medical Center, Omaha, Nebraska; ⁵Immunomedics, Inc., Morris Plains, New Jersey; and ⁶Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, New Jersey

Note: Presented in part at the 54th Annual Meeting of the Society of Nuclear Medicine, Washington, DC, June 2–6, 2007.

Current address for S.A. Gulec: The Herbert Wertheim College of Medicine, Florida International University Miami, Florida.

Corresponding Author: David M. Goldenberg, Garden State Cancer Center, 520 Belleville Avenue, Belleville, NJ 07109. Phone: 973-844-7002; Fax: 973-543-0607; E-mail: dmg.gscancer@att.net

doi: 10.1158/1078-0432.CCR-10-2579

©2011 American Association for Cancer Research.

Translational Relevance

Pancreatic cancer continues to be one of the most difficult cancers to treat. In this study, we describe the initial clinical testing of a new antibody-targeted radiotherapeutic, ^{90}Y -labeled clivatuzumab tetraxetan. This agent, based on the humanized version of the PAM4 monoclonal antibody that binds a mucin produced very specifically by pancreatic carcinoma, was very effective as a single agent in arresting the growth, and even curing, human pancreatic carcinoma xenografts in animal models. Additionally, preclinical studies indicated that when combined with gemcitabine, therapeutic efficacy improved. Our ultimate goal is to evaluate ^{90}Y -clivatuzumab tetraxetan in combination with gemcitabine. The first step, reported herein, is an evaluation of the maximum tolerated dose of ^{90}Y -clivatuzumab tetraxetan when given once as a single agent. Hematologic toxicity was dose-limiting. Tumor targeting was observed in a majority of patients by using ^{111}In -labeled antibody, and even though the mucin antigen is present in the serum, it did not seem to hinder the biodistribution or clearance of the antibody. Three of 20 treated patients with advanced (stage III or IV) disease had transient partial responses, suggesting that additional studies with ^{90}Y -clivatuzumab tetraxetan combined with gemcitabine should be pursued.

advanced (stage III/IV) pancreatic carcinoma by using a DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) conjugate of hPAM4 (clivatuzumab tetraxetan), which had been developed in kit form for 15-minute ^{90}Y radiolabeling. Our ultimate goal was to test a fractionated dosing regimen in combination with gemcitabine given in an amount to enhance radiation sensitization. However, before initiating this protocol, an open-label, multicenter phase I clinical trial in patients with stage III/IV pancreatic adenocarcinoma was carried out. The primary objective was to determine dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) for ^{90}Y -hPAM4 given as a single infusion. Organ distribution, pharmacokinetics (PK), tumor targeting, and dosimetry were also obtained in all patients by a pretherapy imaging study by using ^{111}In -labeled hPAM4 as a surrogate to predict ^{90}Y -hPAM4 clearance and distribution. Because hPAM4 binds a mucin detected in the serum, patients also received 1 of 2 hPAM4 protein doses with their radiolabeled injections to determine whether the antigen's presence would alter PK and organ distribution.

Materials and Methods

Patients

Patients were required to be 18 years or older with histologically or cytologically confirmed stage III/IV pancreatic adenocarcinoma. Patients with stage III disease must

have progressed after initial therapy, whereas those with stage IV disease should not have received more than 1 prior chemotherapy regimen. All patients were required to be 4 weeks beyond chemotherapy, radiotherapy, major surgery, or other experimental treatments, and to have recovered from all acute toxicities (at study entry, \leq grade 1). Patients previously receiving nitrosoureas, actinomycin D, prior radioimmunotherapy, other antibody-based therapies, or prior radiation to more than 30% of the red marrow or to maximal tolerable level for any organ were excluded. Additional requirements included an adequate performance status [≥ 70 Karnofsky performance status (KPS) or Eastern Cooperative Oncology Group (ECOG) ≤ 1] with life expectancy 3 months or more, adequate hematologic parameters without ongoing transfusional support (hemoglobin ≥ 10 g/dL, ANC $\geq 1.5 \times 10^9/\text{L}$, platelets $\geq 150 \times 10^9/\text{L}$), no known history of active coronary artery disease, unstable angina, myocardial infarction, or congestive heart failure present within 6 months or cardiac arrhythmia requiring antiarrhythmia therapy, no known history of active chronic obstructive pulmonary disease or other moderate-to-severe respiratory illness present within 6 months, and adequate renal and hepatic function (creatinine and bilirubin $\leq 1.5 \times$ institutional upper limit of normal, AST and ALT $\leq 2.0 \times$ IULN). Patients with CNS metastatic disease, a single tumor mass more than 10 cm in its greatest diameter, anorexia ($>$ grade 2), nausea, vomiting or signs of intestinal obstruction, known HIV, hepatitis B, or hepatitis C positivity, known autoimmune disease or presence of autoimmune phenomena (except rheumatoid arthritis requiring only low-dose maintenance corticosteroids), infection requiring intravenous antibiotic use within 1 week, use of corticosteroids within 2 weeks, and other concurrent medical or psychiatric conditions that, in the investigator's opinion, could confound study interpretation or prevent completion of study procedures, were excluded. The Institutional Review Board approved the study at each participating site, and written informed consent was obtained from all patients.

Radiolabeling

The DOTA conjugate of hPAM4 was prepared by the sponsor (Immunomedics, Inc.). Quality control testing included using an anti-idiotypic antibody after radiolabeling the DOTA conjugate to ensure immunoreactivity of the final product was maintained. For patient use, radiolabeling of DOTA-conjugated hPAM4 with $^{111}\text{InCl}_3$ (sterile solution; Mallinckrodt/Covidien Imaging Solutions) or $^{90}\text{YCl}_3$ (MDS Nordion) was carried out by a local radiopharmacy by using 5 mg or less protein for ^{111}In or 2 mg/mCi or less for ^{90}Y , as reported previously (11). Instant thin layer chromatography testing was done and was required to show less than 10% unbound radionuclide to be released for patient use. Using a precalibrated dose calibrator, the prescribed radiolabeled doses were prepared as an 8-mL volume in a 10-mL syringe and delivered to the investigational sites for use within 2 hours of preparation.

Treatment

All patients received 3 to 5 mCi of ^{111}In -hPAM4 at least 1 week before ^{90}Y -hPAM4 to assess PK and ensure that biodistribution and radiation dosimetry estimates for therapy were acceptable. The prepared ^{111}In - and ^{90}Y -hPAM4 each contained less than 10 mg total antibody and both were administered intravenously by slow injection over 10 minutes. Initially, cohorts of 3 to 6 patients received a single dose of ^{90}Y -hPAM4 with ^{90}Y doses starting at 15 mCi/m² (555 MBq/m²) and escalated in 5-mCi/m² (185 MBq/m²) increments. DLT was defined as grade 4 hematologic toxicity lasting 7 days or more; a grade 3 nonhematologic toxicity of any duration; or failure of platelets, absolute neutrophil count (ANC), or hemoglobin to recover to grade 1 within 12 weeks, with platelet and red blood cell (RBC) transfusions, as well as growth factors, permitted to aid hematologic recovery. Escalation continued as long as 0 of 3 or 1 of 6 patients at a particular ^{90}Y dose encountered DLT, but ceased if there were 2 or more DLTs. The highest ^{90}Y dose with 6 patients and at most 1 DLT was determined to be the MTD, with several additional patients then treated at or below the MTD. Prior to each radiolabeled antibody injection, additional unconjugated hPAM4 was administered intravenously over 30 to 40 minutes (10% infused in 10 minutes and if vital signs were stable, remaining dose over 20–30 minutes) during dose escalation (100 mg total antibody doses), but not after the MTD was determined (<10 mg total antibody doses). All antibody administrations were given without steroids or any other premedication.

Study assessments

After ^{111}In -hPAM4, blood samples were collected at 0.5, 1, 2, and 4–6 hours and then on days 1, 2, 3, 4–5, and 6–7 for ^{111}In radioactivity serum levels which were measured at each site by using a precalibrated γ -scintillation counter. Whole-body anterior/posterior planar ^{111}In scintigraphy was also carried out repeatedly over 7 days by using a precalibrated γ -camera with an ^{111}In -calibration standard in the field of view. The initial 6 patients also had total urine collections over 3 days starting 4 hours after administration of ^{111}In -hPAM4. All images were evaluated for antibody biodistribution and tumor localization by the local nuclear medicine physician. To proceed to treatment, the biodistribution had to be acceptable with no abnormal pattern of uptake among the major organs that could not be explained by normal variability or incomplete radiolabeling, but demonstrable evidence of tumor targeting was not required. Antibody PK used SAAM II version 1.1.1 software (SAAM Institute, Inc.) to generate multiexponential fits to decay-corrected ^{111}In serum levels. The radiation dosimetry methodology has been described previously (12, 13). Briefly a Co-57 patient transmission scan allowed ^{111}In counts from organ regions of interest (ROI) on each whole-body image to be corrected for radiation attenuation losses, whereas a computed tomography (CT) image allowed for organ-specific subtraction of background counts from overlapping tissue. Using standard male

and female phantom organ masses, solid organ radiation dose estimates were determined by MIRDOSE3.1 software (14) from the geometric mean of corrected anterior and posterior ROI counts, whereas red marrow radiation dose estimates were generated according to the method of Sgouros (15). To proceed with treatment, the dose estimates were required to be below standard limits for external beam radiation therapy (16), but often used for nonmyeloablative radioimmunotherapy trials (<300 cGy for red marrow, <3,000 cGy for liver, and <2,000 cGy for kidney and lung).

Adverse events were classified according to the MedDRA (Medical Dictionary for Regulatory Affairs, Chantilly, VA) and graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0. Serum chemistries, physical exams, vital signs, and urinalysis were conducted over 12 weeks after treatment, with weekly blood counts or more frequently for grade 3 or higher cytopenias. Blood samples were drawn at baseline and then at 4, 8, and 12 weeks posttreatment for determination of human anti-hPAM4 antibodies (HAHA) by ELISA, using an anti-idiotypic antibody as a standard. Values greater than 50 ng/mL were considered positive. Serum levels of CA19-9 were obtained at baseline and then at 1, 4, 8, and 12 weeks posttreatment. Serum levels of the PAM4-reactive mucin were evaluated at the same time points by ELISA assay according to Gold and colleagues (3, 4). CT scans were obtained at baseline and 4, 8 and 12 weeks after ^{90}Y -hPAM4, with the best treatment response at any of these time points classified by Response Evaluation Criteria in Solid Tumors (RECIST) criteria (17) as a complete response (CR), partial response (PR), stable, or progressive disease.

Statistical analyses

Safety analyses focused primarily on the treatment day and the 12-week posttreatment evaluation period, but included any potentially treatment-related toxicities occurring after 12 weeks. Response rates were summarized by descriptive statistics. Progression-free survival (PFS) was calculated from the ^{90}Y -hPAM4 administration to the earliest occurrence of progression, death, or last contact. Duration of response from the onset of an objective response (OR; i.e., CR, or PR) to the same events and overall survival (OS) from the ^{90}Y -hPAM4 administration to the earliest occurrence of death or last contact were also summarized.

Results

Twenty-one patients with pancreatic adenocarcinoma (17 with stage IV metastatic disease and 4 with stage III locally advanced disease) were enrolled. Three patients with metastatic disease were treatment-naïve, whereas all the other 18 patients had received prior chemotherapy (\pm external radiation \pm primary tumor resection). All patients had CT-measurable disease, primarily involving the pancreas and/or liver, and most had elevated baseline

serum levels of both CA19-9 and PAM4-reactive mucin. Demographics and patient characteristics are summarized in Table 1. The 21 patients received ^{111}In -hPAM4 and underwent pretherapy evaluations of antibody biodistribution and PK, tumor targeting, and radiation dosimetry. All patients had normal biodistribution, and radiation dosimetry estimates acceptable for treatment. One patient withdrew prior to ^{90}Y -hPAM4 after developing gastric outlet obstruction from disease progression. All the other 20 patients received their dose of ^{90}Y -hPAM4.

Table 1. Demographics/baseline data ($N = 21$)

Sex, male/female; n	9/12
Age, median (range); y	62 (34–77)
ECOG, 0/1	12/9
Disease stage at study entry	
III (locally advanced)	4
IV (metastatic)	17
Time from diagnosis, median (range), mo	9 (0.1–34)
Prior therapy, n	18
Modalities	
Chemotherapy ^a	18
External radiation therapy	8
Surgery ^b	6
Time from last therapy, median (range), mo	1.7 (0.9–27.6)
CT identified tumor locations, n	
Pancreas/pancreatic bed	16
Liver	11
Chest/lungs	4
Other	4
Largest target lesion, median (range); cm	4.5 (2.1–6.3)
Hematology, median (range)	
Hemoglobin, g/dL	12.4 (10.1–15)
Neutrophils, $\text{K}/\mu\text{L}$	4.6 (2.8–10)
Platelets, $\text{K}/\mu\text{L}$	261 (149–958)
CA19-9, median (range); U/mL	338 (5–33,359)
^{90}Y -hPAM4 dose level	
15 mCi/m ² (555 MBq/m ²)	8
20 mCi/m ² (740 MBq/m ²)	9
25 mCi/m ² (925 MBq/m ²)	4

^aEight patients received only gemcitabine, 1 patient received only fluorouracil, and 9 patients received gemcitabine plus one or more other agents including fluorouracil or capecitabine ($n = 5$), an epidermal growth factor receptor inhibitor ($n = 3$), docitaxel ($n = 2$), a platinum-based agent ($n = 2$), or irinotecan ($n = 1$).

^bSurgeries included pancreaticoduodenectomy ($n = 4$), distal pancreatectomy ($n = 1$), and palliative hepatojejunostomy/gastrojejunostomy ($n = 1$). All 6 patients subsequently received chemotherapy, and 4 received external radiation therapy, prior to study entry.

Treatment administration

All DOTA-hPAM4 radiolabeled products met release criteria (median unbound isotope: 0.7% ^{111}In , 1.1% ^{90}Y). During the initial dose escalation portion of the study, 15 patients received ^{90}Y -hPAM4 at ^{90}Y doses of 15 mCi/m² (555 MBq/m²; $n = 4$), 20 mCi/m² (740 MBq/m²; $n = 7$), and 25 mCi/m² (925 MBq/m²; $n = 4$). After the MTD was determined, 6 additional patients were entered to provide more experience (3 at 15 mCi/m², 2 at 20 mCi/m², 1 withdrawing after ^{111}In -hPAM4). All administrations of study drug occurred without interruption or premature termination, with ^{111}In - and ^{90}Y -hPAM4 both given at total antibody doses of 100 mg for the initial 15 patients and less than 10 mg for the final 6 patients.

Adverse events and dose-limiting toxicity

There were no infusion reactions to administration of study drug. Other than events typically occurring in patients with pancreatic cancer and advanced disease (primarily gastrointestinal complications, pain, and constitutional complaints), the adverse event profile reflected cytopenias that were expected following radioimmunotherapy. The most frequent events (>10% of patients) were vomiting (35%); fatigue, anemia, nausea (30% each); abdominal pain, leukopenia (25% each), constipation, anorexia, thrombocytopenia, diarrhea (20% each), dehydration, back pain, ascites, asthenia, fever, and dyspnea (15% each). Eleven patients had 19 serious adverse events. Of these, 7 events were cytopenias attributed to radioimmunotherapy, 1 event of vomiting was considered possibly related to study drug but with multiple other likely explanations, whereas all other events (dehydration, pain, dyspnea, gastric outlet obstruction, asthenia, confusion) were attributed to complications of underlying disease and were unrelated to study drug. No bleeding episodes occurred, and other than 1 upper respiratory infection and 1 urinary tract infection, none of the patients developed infections. No abnormal pattern of changes occurred in standard serum chemistries, with no grade 3 to 4 events except for several isolated elevations of transaminase, alkaline phosphatase, or bilirubin consistent with hepatic involvement, biliary obstruction, and other abdominal complications expected in this population (data not shown). Grade 3 or 4 anemia was rare at any dose level and required few transfusions of RBCs, but the incidence of severe thrombocytopenia and neutropenia, as well as platelet transfusions and growth factor support, increased with total ^{90}Y dose (Table 2). For patients with grade 3 or 4 events, the median time from ^{90}Y -hPAM4 therapy to nadir value was 4.8 weeks (range, 3.8–6.6 weeks) for platelets and 4.8 weeks (range, 2.9–5.7 weeks) for neutrophils.

The MTD was declared to be 20 mCi/m², because during dose escalation there were no DLTs at 15 mCi/m², 1 DLT among 7 patients (6 evaluable for DLT assessment, 1 inevaluable after entering hospice 5 weeks posttreatment) at 20 mCi/m², and 3 patients at 25 mCi/m² with DLTs. No DLTs occurred in the 5 patients treated after dose escalation at or below the MTD. All DLTs were due to grade 4

Table 2. Hematologic toxicity stratified by ^{90}Y dose

	^{90}Y Dose level (mCi/m^2)		
	15 ($n = 7$)	20 ($n = 9$)	25 ($n = 4$)
<i>Patients with grade 3 or 4 nadirs (%)</i>			
Hemoglobin			
Grade 3	0 (0)	2 (22)	0 (0)
Grade 4	0 (0)	0 (0)	0 (0)
Platelets			
Grade 3	0 (0)	4 (44)	0 (0)
Grade 4	0 (0)	1 (11)	3 (75)
Neutrophils			
Grade 3	0 (0)	2 (22)	0 (0)
Grade 4	0 (0)	2 (22)	3 (75)
<i>Patients administered hematologic support (%)</i>			
RBC	0 (0)	4 (44)	2 (50)
Platelets	0 (0)	1 (11)	3 (75)
G-CSF	0 (0)	4 (44)	0 (0)
EPS	1 (14)	1 (11)	2 (50)

Abbreviations: G-CSF, granulocyte-colony stimulating factors; EPS, erythropoiesis-stimulating agents.

thrombocytopenia and/or neutropenia (thrombocytopenia was generally the more severe) lasting more than 7 days. There was no hematologic DLT due only to prolonged recovery beyond 12 weeks. This was unable to be assessed in 4 patients (3 patients entered hospice with early study terminations prior to recovery and 1 patient had platelet recovery to near grade 1 levels but then developed disseminated intravascular coagulation). Otherwise, the median recovery time from grade 3 or 4 nadirs to grade 1 levels was 2.7 weeks (range, 1.7–4.1 weeks) for platelets and 1.9 weeks (range, 1.0–2.8 weeks) for neutrophils.

Pharmacokinetics and biomarkers

Serum levels of ^{111}In -hPAM4, after correction for ^{111}In physical decay, generally decreased monoexponentially over 7 days, with a half-life (mean \pm SD) of 4.6 ± 0.9 days (range, 3.5–7.0) for the initial 15 patients (100 mg total antibody dose) and 3.8 ± 0.5 days (range, 3.2–4.6) for the final 6 patients (<10 mg total antibody dose). Serum antibody levels were also measured by ELISA assay over 7 days after ^{111}In -hPAM4. The median C_{max} was 21 $\mu\text{g}/\text{mL}$ (range, 10–41) with a half-life of 5.1 days (range, 3.9–13.2) for the initial 15 patients, and the median C_{max} was 4 $\mu\text{g}/\text{mL}$ (range, 2–7) for the final 6 patients who had measurable levels over 7 days, but too low for half-life determinations. CA19-9 serum levels were elevated in 18 of the 20 treated patients at study entry. By 4 weeks after ^{90}Y -hPAM4, CA19-9 levels had increased more than 25% from baseline in 15 patients and were either stable ($n = 3$) or had decreased more than 25% ($n = 2$) in the other 5 patients

(2 with no further evaluations, 2 with >25% increases at next evaluation, and 1 with levels still decreased at last evaluation at 12 weeks). In 18 treated patients with measured serum levels of hPAM4-reactive mucin, the median value at study entry was 21 units/mL (range, 1.5–1,398), including 14 patients (78%) with levels above upper limits established in patients without pancreatic cancer (>2.4 units/mL). Posttreatment mucin levels could not be reliably measured due to interference in the ELISA assay from administered hPAM4.

Biodistribution and tumor targeting

All ^{111}In -hPAM4 images were evaluated by the investigators as having normal biodistribution and with no evidence of fixed gastrointestinal or genitourinary uptake. ^{111}In -hPAM4 whole-body planar imaging is suboptimal for detecting pancreatic tumors or metastases in the liver. Nonetheless, the investigators reported visual evidence of tumor targeting in 12 patients, including pancreatic tumors or residual tumor in the pancreatic bed ($n = 9$) or other abdominal sites of confirmed involvement ($n = 3$). All the visualized pancreatic targeting occurred in patients with the higher serum levels of hPAM4-reactive mucin (28–332 U/mL), and involved 40% (6 of 15) of patients receiving 100 mg total antibody compared with 50% (3 of 6) receiving <10 mg. An internal review by the sponsor of all images found no qualitative differences in biodistribution or intensity of tumor uptake between patients receiving 100 mg or <10 mg total antibody. An example of antibody biodistribution and tumor targeting with ^{111}In -hPAM4 imaging is provided in Figure 1.

Radiation dosimetry and total body clearance

Table 3 provides estimates of the radiation dose that each mCi of ^{90}Y -hPAM4 delivers to the whole body and organs. There are no substantial differences between patients receiving 100 mg or less than 10 mg total antibody, although the liver radiation dose seems modestly decreased in patients receiving additional antibody. Combining the 2 groups, the estimated radiation doses received from treatment are summarized in Table 4. The median dose to the whole body and each individual organ increases with each ^{90}Y dose level, as expected. However, even at 25 mCi/m^2 , which exceeds the MTD of 20 mCi/m^2 , the highest radiation dose received by any patient remains well below the defined organ radiation dose limits (red marrow <300 cGy; liver <3,000 cGy, lungs and kidneys <2,000 cGy). Pooled urine was collected in 6 patients at intervals over 3 days after ^{111}In -hPAM4 and the counts were corrected for ^{111}In physical decay. The median estimated percent of the administered antibody eliminated in the urine was 3.7% (range, 2.5%–7.5%), with a median of 1.2% excreted by 4 hours, 0.8% from 4 to 24 hours, 0.7% from 24 to 48 hours, and 0.9% over 48 to 72 hours. From this, the median half-life for whole-body clearance of the antibody by urinary excretion was 63 days (range, 33–94 days) compared with 60 days (range, 39–91 days) determined by whole-body scintigraphy for these 6 patients.

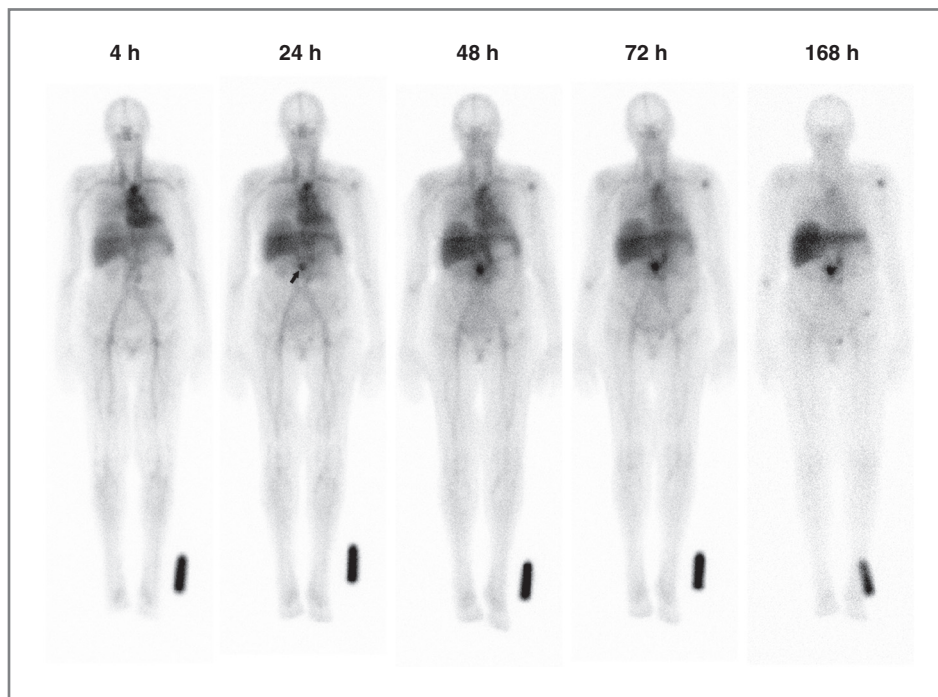


Figure 1. Serial anterior whole-body planar scintigraphic images showing normal antibody biodistribution and tumor targeting in a patient with a recurrent mass in the pancreatic bed after undergoing a Whipple procedure. CT imaging had revealed a 1.5×2.1 -cm mass surrounding the distal superior mesenteric artery. The images were acquired sequentially over 1 week, beginning 4 hours after administration of ^{111}In -hPAM4. Image intensities were normalized by using an ^{111}In standard near the left foot to correct for ^{111}In physical decay and different acquisition times over this period. Uptake at the pancreatic site is clearly seen at 24 hours (arrow), and becomes progressively more prominent on subsequent images. Mild decreased intensity across the upper aspect of the liver is due to attenuation from breast tissue. Mild focal uptake in the left shoulder and pelvic area is of uncertain significance.

Of the 12 patients with visual tumor targeting by ^{111}In -hPAM4 scintigraphy, the radiation doses delivered to the primary tumor could be estimated in 2 patients who had discrete focal uptake seen on both anterior and posterior images. The patient shown in Figure 1 was treated at 20 mCi/m^2 , with a 1.5×2.1 -cm pancreatic mass receiving $5,035 \text{ cGy}$, and remaining stable in size when evaluated 4 weeks later, although new lesions were seen at that time. The other patient was treated at 15 mCi/m^2 , with a much larger 4.3×3.4 -cm mass in the head of the pancreas receiving a more modest radiation dose of 457 cGy and increasing in size 4 weeks later.

Immunogenicity

Baseline levels of anti-hPAM4 antibodies (HAHA) were all at or below the 50 ng/mL cutoff of the assay. Posttreatment serum samples evaluated for at least 8 weeks in most patients were negative, except for 2 patients with positive levels (one $1,460 \text{ ng/mL}$ at 8 weeks and one $1,193 \text{ ng/mL}$ at 4 weeks) of uncertain significance, because there were no associated clinical sequelae.

Clinical efficacy

Of the 20 patients, 13 had progressed by the first CT-based evaluation at 4 weeks following therapy. Although none of the other 7 patients achieved CRs, 3 patients (1 treated at 15 mCi/m^2 and 2 at 20 mCi/m^2) met RECIST

Table 3. Radiation dose estimates (mGy/mCi) stratified by antibody dose

	Total antibody administered	
	100 mg (n = 15) ^a	<10 mg (n = 5)
Whole body	2.7 ± 0.4	2.5 ± 0.4
Red marrow	4.1 ± 1.2	3.8 ± 0.9
Liver	15.0 ± 5.0	20.8 ± 1.9
Spleen	20.6 ± 12.9	22 ± 13.8
Lungs	14.8 ± 5.7	11.7 ± 4.6
Kidneys	16.0 ± 5.0	18.7 ± 4.8
Testes ^b	6.9 ± 5.2	8.2 ± 3.3
Ovaries ^c	2.1 ± 0.2	1.9 ± 0.3
Lens of eye	2.0 ± 0.2	1.6 ± 0.4
Urinary bladder	1.4 ± 0.4	1.7 ± 0.3

NOTE: Mean \pm SD of the estimated radiation dose to whole body and organs from ^{90}Y -hPAM4 per mCi of ^{90}Y administered.

^aPatients receiving additional hPAM4 with both ^{111}In -hPAM4 and ^{90}Y -hPAM4.

^bCalculated in males only.

^cCalculated in females only.

Table 4. Radiation dose estimates (mGy) stratified by ^{90}Y dose

	^{90}Y Dose Level (mCi/m ²)		
	15 (n = 7)	20 (n = 9)	25 (n = 4)
Whole body	67 (56–75)	96 (86–109)	129 (119–141)
Red marrow	108 (88–190)	139 (118–163)	176 (124–202)
Liver	418 (304–698)	546 (339–853)	803 (669–914)
Spleen	319 (89–862)	762 (68–1,890)	1,112 (631–1,444)
Lungs	334 (181–475)	483 (400–1,036)	534 (461–572)
Kidneys	409 (287–772)	526 (306–916)	635 (461–779)
Testes ^a	182 (80–356)	151 (45–176)	630 (492–769)
Ovaries ^b	49 (33–54)	68 (60–78)	94 (94–94)
Lens of eye	48 (33–54)	68 (60–78)	94 (83–113)
Urinary bladder	41 (34–52)	48 (24–97)	70 (59–85)

NOTE: Median (range) estimated radiation dose to whole body and organs from ^{90}Y -hPAM4 for patients receiving ^{90}Y at dose levels of 15, 20, or 25 mCi/m².

^aCalculated in males only.

^bCalculated in females only.

criteria for unconfirmed PRs (imaging examples are provided in Fig. 2). They achieved 32% to 52% shrinkage of the sum of the longest diameters of their target lesions at 4-week evaluations (1 patient with a 4.5-cm pancreatic mass decreasing to 3.3 cm and a 2.3-cm liver mass no longer visible, the others with locally advanced disease decreasing from 6.3 cm to 3.0 cm and 3.7 cm to 2.5 cm), but subsequently progressed with new lesions at their 8-week evaluations. The remaining 4 patients had stable disease at 4-week evaluations (2 patients with ~5-cm masses both progressing by 8 weeks, the others with 2- to 3-cm masses progressing at 12- and 24-week evaluations). The median PFS for all 20 patients was only 4.3 weeks after treatment, whereas the 7 patients who had not progressed by the first CT-based evaluation at 4 weeks following therapy remained progression-free for 1.5 to 5.6 months.

All 20 treated patients have now died. The median OS was 4.3 months (range, 1.5–22.2), with 9 patients expiring 1.5 to 2.9 months after therapy, 5 patients surviving 4.1 to 5.6 months, and 6 patients surviving 9.3 to 22.2 months. Exploratory survival analyses were inconclusive regarding ^{90}Y dose dependency, due to small patient numbers at each dose level. There was an apparent trend toward improved survival for patients at study entry with smaller-sized tumors [median OS = 7.5 months for maximum lesions ≤ 4.5 cm ($n = 10$) vs. 2.2 months for larger lesions ($n = 10$)] and better performance status [median OS = 5.6 months for ECOG 0 ($n = 11$) vs. 2.9 months for ECOG 1 ($n = 9$)], and for those without progression 4 weeks after treatment [median OS = 5.6 months with PR or stable disease ($n = 7$) vs. 2.4 months with progression ($n = 13$)].

Discussion

The outlook for patients with advanced pancreatic cancer remains dismal, with the 2 approved systemic therapies

(gemcitabine and erlotinib) offering only a modest survival benefit (18, 19). More recently, a 3-drug combination of fluorouracil (5-FU), oxaliplatin, and irinotecan showed improved survival compared with gemcitabine monotherapy (20). However, patients with metastatic disease are still not cured with conventional chemotherapy, and there remains an urgent need for new approaches, such as the antibody-targeted radiotherapeutic, ^{90}Y -hPAM4 evaluated in this initial single-dose phase 1 trial. This agent is based on the humanized version of the PAM4 antibody that targets a mucin glycoprotein specifically expressed in pancreatic adenocarcinoma. Preclinical studies with ^{90}Y -hPAM4 not only showed efficacy as a single agent in treating human pancreatic carcinoma xenografts, but also showed an enhanced response when combined with gemcitabine (8–10), which is a well-known radiosensitizing agent (21). Additionally, other radioimmunotherapies have indicated that patients were able to tolerate a higher total cumulative dose of ^{90}Y when given in weekly fractions (22). However, before proceeding to clinical trials in combination with gemcitabine and taking advantage of a fractionated dosing regimen to increase the radiation dose, this study was undertaken to evaluate a single dose of ^{90}Y -hPAM4 administered as a single agent to patients with advanced pancreatic cancer.

This trial showed that ^{90}Y -hPAM4 was well tolerated and that up to 100 mg of the hPAM4 antibody could be given without infusion-type reactions. As expected with radioimmunotherapy, the only significant toxicity was transient hematologic suppression, with grades 3 and 4 neutropenia and thrombocytopenia increasing as the ^{90}Y dose was escalated. In spite of the fact that most patients had received gemcitabine or other prior chemotherapy that can damage the bone marrow, there were no bleeding events or serious infections even at the highest ^{90}Y dose tested, and most cytopenias recovered to grade 1 levels within 12 weeks.

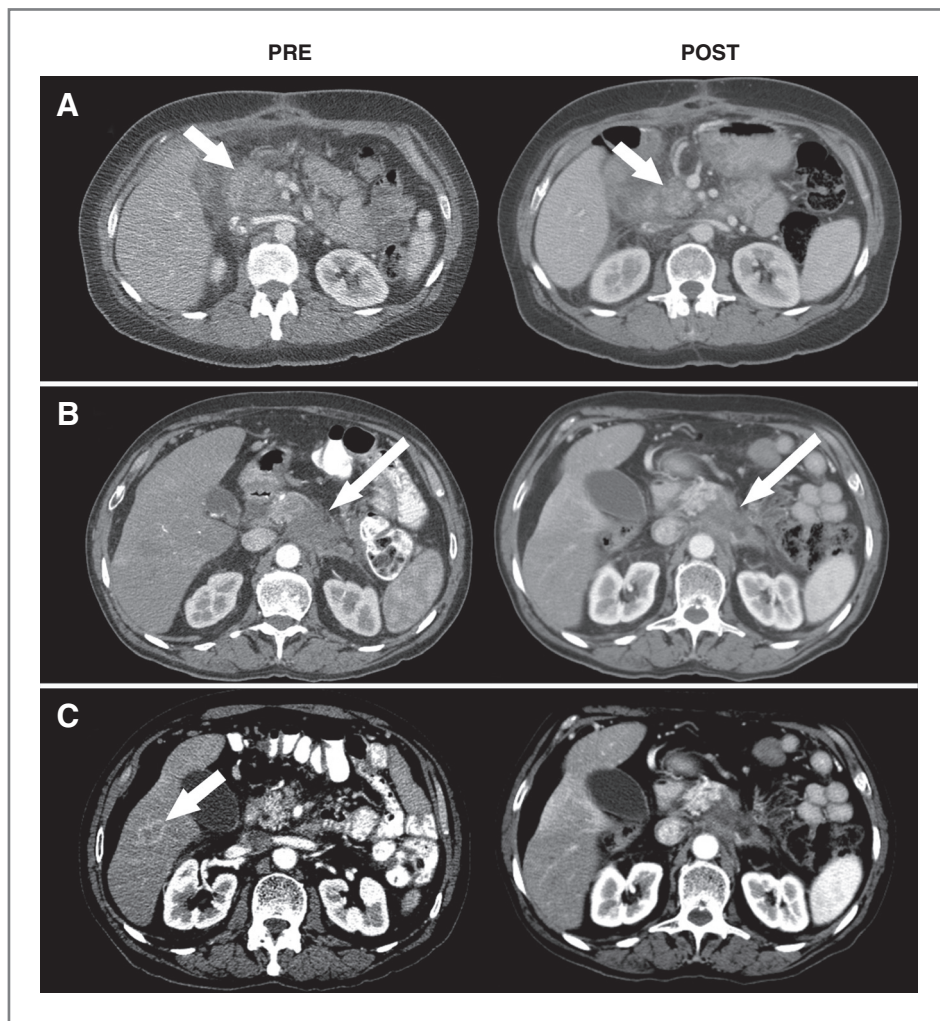


Figure 2. CT image examples before (PRE) and after treatment with ^{90}Y -hPAM4 (POST). A, a 63-year-old female diagnosed 5 months earlier with locally advanced pancreatic cancer was previously treated with gemcitabine and external radiation therapy. At study entry, the patient had a 6.3-cm mass in the head of the pancreas which reduced to 3.0 cm by 4 weeks after ^{90}Y -hPAM4 (arrows). B and C, a 70-year-old male diagnosed 4 months earlier with locally advanced disease received 5-FU and external radiation therapy initially and then gemcitabine. At study entry, the patient had a 4.5-cm mass in the body of the pancreas which reduced to 3.3 cm after treatment with ^{90}Y -hPAM4 (long arrows) and a 2.3-cm lesion in the liver (short arrow) that was no longer measurable.

Although dosimetry was evaluated in each patient prior to ^{90}Y -hPAM4 administration, the limitation of red marrow dosimetry to predict hematologic toxicity is well known with other ^{90}Y -labeled antibodies, likely reflecting damage from prior chemotherapy or other causes of interpatient variability in bone marrow reserve (23). Thus, even though the radiation dose estimates to the red marrow remained below standard limits (300 cGy), dose escalation stopped after 3 patients at 25 mCi/m^2 encountered protocol-defined DLT with grade 4 cytopenias more than 7 days. As only 1 of 6 evaluable patients already treated at the next lower dose level of 20 mCi/m^2 had a DLT with grade 4 thrombocytopenia lasting more than 7 days, 20 mCi/m^2 was declared as the MTD for single administration of ^{90}Y -hPAM4 in this population in which most patients had received at least one prior treatment. Of note, this last patient with DLT had been heavily pretreated with 4 prior chemotherapeutic agents, yet the platelet counts were still able to recover to grade 1 levels posttreatment, allowing the patient to initiate gemcitabine when disease progression subsequently occurred. Further, after the MTD was determined, 2 additional patients were treated at 20 mCi/m^2 and

neither encountered DLT. Thus, although limited by the relatively few number of patients treated in this study, a single administration of ^{90}Y -hPAM4 had manageable hematologic toxicity at the MTD, and on this basis, a starting cumulative dose of 20 mCi/m^2 was selected for initiating subsequent studies in which ^{90}Y -hPAM4 is given in fractionated doses in combination with gemcitabine (24).

Most patients entered this study with elevated serum levels of PAM4-reactive mucin. There was no evidence that tumor targeting was substantially hindered, because ^{111}In -hPAM4 visualized pancreatic tumors in patients at the higher serum levels, and all patients showed a normal antibody biodistribution pattern over 7 days with the expected retention in the liver for a radiometal-labeled antibody and with no unusual splenic uptake. As a precaution to avoid potential antibody sequestration by serum mucin or by low-level nonspecific antigen expression on some tissues, patients initially entered during dose escalation had their radiolabeled antibodies given with additional hPAM4 (100 mg total antibody dose), whereas for comparison, patients entered after the MTD was determined received their radiolabeled antibodies

without any additional hPAM4 (<10 mg total antibody dose). There seems to be no advantage to adding additional antibody with this therapy, because no differences were noted between PK, biodistribution, or normal organ dosimetry estimates for the 2 groups, except for the radiation dose estimate to the liver, which appeared modestly increased in patients without additional antibody, but remained well within acceptable limits for radioimmunotherapy at all ^{90}Y dose levels in this study. Although only a small number of patients in this study did not receive additional antibody, it did appear that tumor targeting was at least comparable in this group, and in a preliminary report from an ongoing trial of ^{90}Y -hPAM4 administered without additional antibody to a larger group of patients, neither blood clearance nor liver uptake seemed appreciably influenced by serum mucin levels (24). Furthermore, targeting studies in animal models instead reported that uptake in human pancreatic xenografts was reduced at higher antibody doses (25), presumably a result of competitive binding at tumor sites between the unlabeled and radiolabeled antibodies, further supporting the decision to continue ^{90}Y -hPAM4 therapy in future studies without requiring administration of additional antibody.

Although animal models have been highly supportive of the antitumor activity of radiolabeled antibodies in solid tumors, ORs have been infrequently reported clinically (26). Thus, we are encouraged by the antitumor activity observed in this initial small study, with 3 transient PRs and 4 patients achieving at least temporary disease stabilization by CT-based criteria, and 2 patients having decreases in

serum levels of CA19-9, albeit short-lived. These results, obtained with a single dose of ^{90}Y -hPAM4 alone in this difficult-to-treat population, support a more promising treatment regimen now underway in which ^{90}Y -hPAM4 is fractionated into several weekly doses to enhance the total radiation dose delivered to tumors and also administered in combination with radiosensitizing doses of gemcitabine to increase the potency of the radiation delivered (24).

In conclusion, radioimmunotherapy with a single dose of ^{90}Y -clivatuzumab tetraxetan is well tolerated without the need for premedication or additional antibody, and with manageable hematologic toxicity at the maximal tolerated ^{90}Y dose. Further study of this promising treatment strategy for pancreatic cancer is ongoing.

Disclosure of Potential Conflicts of Interest

H. Horne, W.A. Wegener, N. Teoh, and D.M. Goldenberg are employed by or have financial interests with Immunomedics, Inc. S.A. Gulec, S.J. Cohen, K.L. Pennington, L.S. Zuckier, and R.J. Hauke received research support from Immunomedics, Inc. for this study.

Acknowledgments

We thank Henry Founds, Preeti Trisal, and Kiril Gordeyev for contributions to the pharmacokinetic and immunoassay analyses; Dion Yeldell for performing radiation dosimetry assessments; and Susan Garl for clinical trial coordination.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 25, 2010; revised March 24, 2011; accepted March 28, 2011; published OnlineFirst April 28, 2011.

References

- Gold DV, Karanjawala Z, Modrak DE, Goldenberg DM, Hruban RH. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. *Clin Cancer Res* 2007;13:7380–7.
- Gold DV, Lew K, Maliniak R, Hernandez M, Cardillo T. Characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin. *Int J Cancer* 1994;57:204–10.
- Gold DV, Modrak DE, Ying Z, Cardillo TM, Sharkey RM, Goldenberg DM. New MUC1 serum immunoassay differentiates pancreatic cancer from pancreatitis. *J Clin Oncol* 2006;24:252–8.
- Gold DV, Goggins M, Modrak DE, Newsome G, Liu M, Shi C, et al. Detection of early-stage pancreatic adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2010;19:2786–94.
- Mariani G, Molea N, Bacciardi D, Boggi U, Fornaciari G, Campani D, et al. Initial tumor targeting, biodistribution, and pharmacokinetic evaluation of the monoclonal antibody PAM4 in patients with pancreatic cancer. *Cancer Res* 1995;55:5911s–5s.
- Cardillo TM, Ying Z, Gold DV. Therapeutic advantage of ^{90}Y trium-versus ^{131}I iodine-labeled PAM4 antibody in experimental pancreatic cancer. *Clin Cancer Res* 2001;7:3186–92.
- Gold DV, Cardillo T, Vardi Y, Blumenthal R. Radioimmunotherapy of experimental pancreatic cancer with ^{131}I -labeled monoclonal antibody PAM4. *Int J Cancer* 1997;71:660–7.
- Cardillo TM, Blumenthal R, Ying Z, Gold DV. Combined gemcitabine and radioimmunotherapy for the treatment of pancreatic cancer. *Int J Cancer* 2002;97:386–92.
- Gold DV, Modrak DE, Schutsky K, Cardillo TM. Combined ^{90}Y trium-DOTA-labeled PAM4 antibody radioimmunotherapy and gemcitabine radiosensitization for the treatment of a human pancreatic cancer xenograft. *Int J Cancer* 2004;109:618–26.
- Gold DV, Schutsky K, Modrak D, Cardillo TM. Low-dose radioimmunotherapy (^{90}Y -PAM4) combined with gemcitabine for the treatment of experimental pancreatic cancer. *Clin Cancer Res* 2003;9:3929S–37S.
- Griffiths GL, Govindan SV, Sharkey RM, Fisher DR, Goldenberg DM. ^{90}Y -DOTA-hLL2: an agent for radioimmunotherapy of non-Hodgkin's lymphoma. *J Nucl Med* 2003;44:77–84.
- Augensen FO, Wegener WA, Siegel J, Yeldell D, Sharkey RM, Goldenberg DM. Radiation dosimetry in ^{90}Y radioimmunotherapy (RAIT): development of a method suitable for clinical use with pretherapy ^{111}In imaging. *J Nucl Med* 2004;45:436P.
- Augensen FO, Wegener WA, Hamacher KA, Siegel JA, Yeldell D, Sharkey RM, et al. Radiation dosimetry in ^{90}Y radioimmunotherapy (RAIT): phantom testing of a method suitable for clinical use with pretherapy ^{111}In imaging. *J Nucl Med* 2005;46:340P–1P.
- Stabin MG. MIRDose: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 1996;37:538–46.
- Sgouros G. Bone marrow dosimetry for radioimmunotherapy: theoretical considerations. *J Nucl Med* 1993;34:689–94.
- Weichselbaum RR, Chen G, Hallahan DE. Biological and physical basis of radiation oncology. In: Holland JF, Frei E III, Bast RC Jr, Kufe DW, Morton DL, Weichselbaum RR, editors. *Cancer medicine*. 4th ed. Baltimore, MD: Williams & Wilkins; 1997. p.697–726.
- Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–16.
- Burris HA III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer: a randomized trial. *J Clin Oncol* 1997;15:2403–13.

19. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007;25:1960–6.
20. Conroy T, Desseigne F, Ychou M, Ducreux M, Bouche O, Guimbaud R, et al. Randomized phase III trial comparing FOLFIRINOX (F: 5FU/leucovorin [LV], irinotecan [I], and oxaliplatin [O]) versus gemcitabine (G) as first-line treatment for metastatic pancreatic adenocarcinoma (MPA): preplanned interim analysis results of thePRODIGE 4/ACCORD 11 trial. *J Clin Oncol* 2010;28:15s.
21. Morgan MA, Parsels LA, Maybaum J, Lawrence TS. Improving gemcitabine-mediated radiosensitization using molecularly targeted therapy: a review. *Clin Cancer Res* 2008;14:6744–50.
22. Morschhauser F, Kraeber-Bodéré F, Wegener WA, Harousseau JL, Petillon MO, Huglo D, et al. High rates of durable responses with anti-CD22 fractionated radioimmunotherapy: Results of a multicenter, phase I/II study in non-Hodgkin's lymphoma. *J Clin Oncol* 2010;28:3709–16.
23. Wiseman GA, Kormmehl E, Leigh B, Erwin WD, Podoloff DA, Spies S, et al. Radiation dosimetry results and safety correlations from 90Y-ibritumomab tiuxetan radioimmunotherapy for relapsed or refractory non-Hodgkin's lymphoma: combined data from 4 clinical trials. *J Nucl Med* 2003;44:465–74.
24. Ocean AJ, Guarino MJ, Pennington KL, O'Neil BH, Rocha Lima CS, Bekaii-Saab TS, et al. Therapeutic effects of fractionated radioimmunotherapy (RAIT) with clivatuzumab tetraxetan combined with low-dose gemcitabine (Gem) in advanced pancreatic cancer (APC). *J Clin Oncol* 2010;28:7S.
25. Gold DV, Alisaukas R, Sharkey RM. Targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4. *Cancer Res* 1995;55:1105–10.
26. Sharkey RM, Goldenberg DM. Perspectives on cancer therapy with radiolabeled monoclonal antibodies. *J Nucl Med* 2005;46:115S–27S.