Preclinical Development


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Abstract

Panitumumab (ABX-EGF or Vectibix), the first fully human monoclonal antibody targeting epidermal growth factor receptor (EGFR), was approved by the Food and Drug Administration for treatment of patients with metastatic colorectal cancer. Here, we report for the first time the radioimmunotherapy (RIT) of EGFR-positive human head and neck cancer in a nude mouse model using pure β− emitter 90Y-labeled panitumumab. Biodistribution and planar γ-imaging studies were carried out with 111In-DOTA-panitumumab. The RIT efficacy of 90Y-DOTA-panitumumab was evaluated in UM-SCC-22B tumor model. CD31, Ki67, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling, and H&E staining were done on UM-SCC-22B tumor sections after treatment. The tumor uptake of 111In-DOTA-panitumumab in UM-SCC-22B tumor-bearing nude mice was 26.10 ± 4.93, 59.11 ± 7.22, 44.57 ± 9.80, 40.38 ± 7.76, and 14.86 ± 7.23 % injected dose per gram of tissue at 4, 24, 72, 120, and 168 hours after injection, respectively. Immunotherapy with cold panitumumab (four doses of 10 mg/kg) did not cause significant antitumor effect. RIT with a single dose of 100 μCi 90Y-DOTA-panitumumab caused significant tumor growth delay and improved the survival in UM-SCC-22B tumor model. A single dose of 200 μCi 90Y-DOTA-panitumumab led to almost complete tumor regression (tumor volumes were 34.83 ± 11.11 mm3 and 56.02 ± 39.95 mm3 on days 0 and 46 after treatment, respectively). Histopathologic analysis of tumors and normal organs further validated the therapeutic efficacy and limited systemic toxicity of 90Y-DOTA-panitumumab. The high tumor uptake and prolonged tumor retention, as well as effective therapy, reveal that 90Y-DOTA-panitumumab may be a promising radioimmunotherapeutic agent to treat EGFR-positive solid tumors. Mol Cancer Ther; 9(8); 2297–308. ©2010 AACR.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is an epithelial cancer arising in the mucosa of the upper aerodigestive tract. Each year, more than half a million new cases of HNSCC are diagnosed in the world (1, 2). Although surgery or radiotherapy can generally lead to a good prognosis for early-stage (stage I or II) HNSCC, there is still a high failure rate for the advanced stage (stage III or IV; ref. 3). In contrast to many other cancers in which metastasis is the primary cause of death, local or regional recurrences are the most common causes of treatment failure or death in patients with HNSCC (4). About 50% of patients who develop recurrent disease have a very poor prognosis, with a median overall survival of only 6 to 9 months (5). Therefore, an effective systemic treatment for the local or regional recurrences of HNSCC is urgently needed to improve the survival rate of patients.

Radioimmunotherapy (RIT) using radiolabeled monoclonal antibodies (mAb) directed against tumor-associated antigens offers the opportunity to selectively irradiate tumor cells while sparing normal tissues. An important advantage of RIT compared with other treatment approaches is that radionuclides can kill adjacent tumor cells while sparing normal tissues. An important advantage of RIT compared with other treatment approaches is that radionuclides can kill adjacent tumor cells. As a consequence, not every tumor cell has to be targeted by antibodies but tumor cells still can be destroyed by the cross-fire effect. RIT has been implemented effectively for the treatment of non–Hodgkin’s lymphomas, and two Food and Drug Administration (FDA)–approved drugs, Zevalin and Bexxar (anti-CD20 mAbs labeled with 90Y and 131I, respectively), have been...
successfully used to treat relapsed or refractory non-Hodgkin’s lymphomas (6). However, the success of RIT in the therapy of bulky solid tumors has generally been limited possibly owing to the relative radioresistance of solid tumors and an inability to deliver sufficient dose to bulky tumor without substantial bone marrow toxicity (7). The intrinsic radiosensitivity of HNSCC (8–10) and the relative effectiveness of RIT for the treatment of small lesions or minimal residual diseases justify the development of RIT for the treatment of minimal residua and recurrence of HNSCC.

Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, is over-expressed in a variety of tumors, including head and neck (80–100%), renal (50–90%), lung (40–80%), breast (14–90%), colorectal (25–77%), ovarian (25–70%), prostate (39–47%), glioma (40–63%), pancreas (30–50%), and bladder (31–48%; ref. 11). The expression of EGFR is associated with a more aggressive malignant phenotype and poor prognosis. It has been reported that patients with high EGFR expression fared significantly worse for survival (12). For these reasons, EGFR has been chosen as an attractive candidate for targeted therapies of tumors, including head and neck carcinoma. Recent studies with cetuximab (Erbitux), a FDA-approved chimeric mAb inhibitor of EGFR, have shown clinical benefits across the range of treatment settings in advanced head and neck cancer (4). Panitumumab (also known as ABX-EGF or Vectibix), another anti-EGFR mAb, is the first fully human mAb approved by the FDA. Panitumumab (13). For these reasons, EGFR has been chosen as an attractive candidate for targeted therapies of tumors, including head and neck carcinoma. Recent studies with cetuximab (Erbitux), a FDA-approved chimeric mAb inhibitor of EGFR, have shown clinical benefits across the range of treatment settings in advanced head and neck cancer (4). Panitumumab (also known as ABX-EGF or Vectibix), another anti-EGFR mAb, is the first fully human mAb approved by the FDA. Panitumumab has been used to treat the EGFR-expressing metastatic colorectal cancer with disease progression (13). The clinical data indicate that panitumumab is well tolerated, does not require premedication, and is associated with a very low incidence of human anti-mouse antibodies.

Currently, the radiometal isotopes 90Y (maximum β− energy, 2.27 MeV; half-life, 2.67 days) attracts increasing interest in the field of RIT. 90Y is a pure high-energy β− emitter and is considered to be retained intracellularly after antibody internalization, thereby increasing the retention time of the isotope within the tumor (6). In this study, we labeled the fully human anti-EGFR mAb panitumumab with 90Y and investigated the therapeutic potential of 90Y-panitumumab in UM-SCC-22B human head and neck cancer xenografted mice.

Materials and Methods

All commercially available chemical reagents were of analytic grade and used without further purification. The bifunctional chelator 1,4,7,10-tetraazadodecane-N,N,N′,N″-tetraacetic acid (DOTA) was purchased from Macrocyclics, Inc. 1-Ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC), N-hydroxysulfosuccinimide (SNHS), and Chelex 100 resin (50–100 mesh) were purchased from Sigma-Aldrich. Water and all buffers were passed through a Chelex 100 column (1 × 15 cm) before use in DOTA conjugation and radiolabeling procedures to ensure that aqueous buffers were metal-free. PD-10 desalting columns were purchased from GE Healthcare. Female BALB/c nude mice (4–5 weeks of age) were obtained from the Department of Experimental Animal, Peking University Health Science Center (Beijing, China). 90YCl₃ solution (in 0.05 mol/L HCl) was obtained from Perkin-Elmer. Panitumumab was obtained from Amgen, Inc.

Cell culture and animal models

UM-SCC-22B human head and neck squamous carcinoma cell line was obtained from the University of Michigan (14). MDA-MB-435 human melanoma (15, 16) cell line was purchased from the American Type Culture Collection. UM-SCC-22B cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS) at 37°C in humidified atmosphere containing 5% CO₂. MDA-MB-435 cells were grown in L-15 medium supplemented with 10% FBS at 37°C in humidified atmosphere containing 100% air.

For UM-SCC-22B tumor and MDA-MB-435 tumor-bearing animal models, the UM-SCC-22B cells (3 × 10⁴) or MDA-MB-435 cells (3 × 10⁴) at a volume of 100 μL were inoculated s.c. into the right thigh (for RIT studies) or right upper flanks (for biodistribution and γ-imaging studies) of female BALB/c nude mice. The animals were used for therapy studies when the tumor size reached ∼50 mm², whereas the animals were used for biodistribution and imaging studies when the tumor size reached ∼200 mm². All animal experiments were done in accordance with the Guidelines of Peking University Health Science Center Animal Care and Use Committee.

Immunofluorescence staining

UM-SCC-22B cell staining study was done as previously described with some modifications (17). Briefly, 70% to 85% confluent tumor cells grown in 35-mm MatTek glass-bottomed culture dishes were fixed using 4% paraformaldehyde for 10 minutes. After blocking with 10% FBS in PBS for 30 minutes, the cells were incubated with panitumumab (5 μg/mL) for 1 hour. The cells were then washed with PBS and stained with FITC-coupled mouse anti-human IgG (hIgG; 1:100; Sigma) for 30 minutes. After the final wash with PBS, the cells were visualized using a Leica TCS-NT confocal microscope (Wetzlar).

Five-micrometer-thick UM-SCC-22B tumor tissue sections were cut from the frozen blocks, allowed to dry in the air, and stored at −80°C. For immunofluorescence staining, the frozen UM-SCC-22B tumor sections were warmed to room temperature, fixed with ice-cold 4% paraformaldehyde, washed with PBS, permeabilized with 0.2% Triton X-100 for 10 minutes, and blocked with 10% FBS for 1 hour at room temperature. After a brief wash with PBS, the sections were incubated with mAb panitumumab (10 μg/mL) for 1 hour at 37°C and then visualized with FITC-coupled mouse anti-hIgG (1:100; Sigma) under a Leica TCS-NT confocal microscope.
EGFR-binding affinity of panitumumab

The EGFR-binding affinity of panitumumab was determined by saturation binding assay using previously described method with some modifications (18). Briefly, 125I-panitumumab was prepared using the Iodogen method (19) and used as the radioligand. UM-SCC-22B cells were seeded in a Millicell 96-well filter plate (10^4 per well) and then incubated with increasing concentrations (0–17 nmol/L) of 125I-panitumumab. The total volume for each well was adjusted to 200 μL with 1% bovine serum albumin (in PBS). The plate was incubated at 4°C for 2 hours. At the end of the incubation period, the cells were filtered by the Millicell Multiscreen vacuum manifold and washed five times with cold PBS (pH 7.4). The hydrophilic polystyrene diiodonaphthalene filters were then collected, and the radioactivity was determined using a gamma counter (Wallac 1470-002, Perkin-Elmer). Nonspecific binding was determined in the presence of an excess of cold panitumumab (>100-fold). A saturation binding curve and Scatchard transformation were obtained by nonlinear regression analysis, and the K_d and b_max (maximum number of binding sites) values of 125I-panitumumab were calculated using GraphPad Prism 4.0 software (GraphPad Software, Inc.).

DOTA conjugation and radiolabeling

DOTA was activated by EDC and SNHS for 30 minutes with a molar ratio of DOTA/EDC/SNHS = 10:5:4 as previously described (20). The DOTA-OSSu (10 μmol, calculated on the basis of SNHS) was added to panitumumab (50 nmol) in 0.1 N NaHCO_3 solution (pH 9.0). After stirring at 4°C overnight, DOTA-panitumumab was purified by the PD-10 column. The concentration of DOTA-panitumumab was determined based on UV absorbance at 280 nm using unconjugated panitumumab with known concentrations as the standards. hIgG (as a control) was also conjugated with DOTA using the same protocol. The number of DOTA per panitumumab was determined using previously reported method (20), and it was calculated to be ~9. To evaluate the effect of DOTA conjugation on the bioactivity of panitumumab, competitive cell-binding assay was done using 125I-panitumumab as the EGFR-specific radioligand. Experiments were done on UM-SCC-22B cells. Briefly, 200,000 counts per minute (cpm) of 125I-panitumumab in triplicate were added to UM-SCC-22B cells in the presence of increasing concentrations of unmodified panitumumab or DOTA-panitumumab. After washing the unbound radiotracer, the specific binding of 125I-panitumumab to UM-SCC-22B cells was analyzed by measuring the radioactivity associated with the cells using a gamma counter. The best-fit IC_{50} values were calculated by fitting the data with nonlinear regression using GraphPad Prism 4.0 software.

90YCl_3 in 0.05 N HCl was added to DOTA-panitumumab conjugate at a reaction ratio of 0.68 μg of DOTA-panitumumab per MBq of 90Y. The reaction mixture was incubated in 0.2 N sodium acetate buffer for 30 minutes at 37°C with constant shaking. 90Y-DOTA-panitumumab was purified by the PD-10 column using PBS as the eluent. The radioactive fractions containing 90Y-DOTA-panitumumab were collected and passed through a 0.2-μm syringe filter for further in vitro and in vivo experiments. A similar procedure was used for 111In-DOTA-panitumumab and 90Y-DOTA-hIgG preparations.

The radioimmunoreactive fraction of 90Y-DOTA-panitumumab was determined by incubating with EGFR-positive UM-SCC-22B cells in suspension culture under the condition of antigen excess (n = 3). The radioimmunoreactive fraction was calculated as follows: radioimmunoreactive fraction = bound activity on cells/total added activity × 100%.

Biodistribution of 111In-DOTA-panitumumab

UM-SCC-22B tumor-bearing mice (20–25 g) were randomly divided into five groups (n = 4 per group). Animals were anesthetized by i.p. injection of sodium pentobarbital at a dose of 45.0 mg/kg. Each mouse was injected with 185 kBq of 111In-DOTA-panitumumab via tail vein. The mice were sacrificed at 4, 24, 72, 120, and 168 hours after injection. Blood, heart, liver, spleen, lung, kidney, stomach, intestine, bone, muscle, and tumor were harvested, weighed, and measured for radioactivity in a gamma counter. Organ uptake was calculated as percentage of injected dose per gram of tissue (% ID/g). Values were expressed as mean ± SD (n = 4 per group).

Planar γ-imaging

γ-Imaging was done on two UM-SCC-22B tumor-bearing mice. Animals were anesthetized with an i.p. injection of sodium pentobarbital at a dose of 45.0 mg/kg. Each animal was administered ~13 MBq of 111In-DOTA-panitumumab in 0.2 mL of saline. Animals were placed prone on a two-head γ-camera (GE Infinia HawkEye) equipped with a parallel-hole, middle-energy, and high-resolution collimator. Anterior images were acquired at 24, 72, and 96 hours after injection and stored digitally in a 128 × 128 matrix. The acquisition count limits were set at 200 k.

RIT and immunotherapy

To assess the therapeutic efficacy of 90Y-DOTA-panitumumab and cold panitumumab, UM-SCC-22B tumor-bearing nude mice with a uniform tumor size of ~50 mm³ (9 days after inoculation) were chosen for this study. For the 90Y-DOTA-panitumumab–based RIT study, the mice were segregated into five groups and injected via tail vein with a single dose of 7.4 MBq (containing ~6 μg DOTA-panitumumab; n = 6) or 3.7 MBq (containing ~6 μg DOTA-panitumumab; n = 5) of 90Y-DOTA-panitumumab, as well as 3.7 MBq (containing ~6 μg DOTA-hIgG) of 90Y-DOTA-hIgG (n = 5), panitumumab (~6 μg; n = 5), and saline (n = 5), respectively. For the cold panitumumab–based immunotherapy study, one group of six mice was injected via tail vein with four doses of panitumumab (10 mg/kg)
every 3 days. The tumor size and body weight were measured daily. The tumor volume was calculated using the following formula: volume = 4/3π (1/2 length × 1/2 width × 1/2 height). Mice were euthanized when the tumor size exceeded the volume of 1,500 mm³ or the body weight lost >20% of its original weight. Tumors, livers, and kidneys were harvested after the therapeutic studies and subjected to histopathologic examinations. Survival curve plots and Kaplan-Meier analysis were carried out using Prism 4.0 software.

**EGFR specificity of RIT**

To evaluate the EGFR-specific therapy of ⁹⁰Y-DOTA-panitumumab, we did the RIT study in MDA-MB-435 tumor-bearing nude mice, which express very low level of EGFR (21). Tumor-bearing animals were separated into two groups (n = 6 per group) and received either 3.7 MBq of ⁹⁰Y-DOTA-panitumumab or saline. These animals were then subjected to tumor size and body weight measurement as described above.

**Histologic analysis**

After RIT studies, the UM-SCC-22B tumors and major organs (livers and kidneys) of mice were harvested. The tumor samples were immediately frozen in OCT medium (Sakura Finetek USA, Inc.) and then cut into 5-μm-thick slices for immunofluorescence staining. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), CD31, and Ki67 staining were carried out on UM-SCC-22B tumor sections to investigate the status of tumor apoptosis, tumor vasculature, and tumor cell proliferation, respectively. Fluorescent TUNEL assay was conducted by following the Instruction Manual of In Situ Cell Death Detection kit (Roche) as previously described (22). CD31 and Ki67 staining were done as previously described (22, 23). Briefly, frozen UM-SCC-22B tumor sections were fixed with cold acetone, blocked with 10% donkey serum, and incubated with rat anti-mouse CD31 antibody (1:100; BD Biosciences) and rabbit anti-human Ki67 (1:100, NeoMarkers), respectively. After washing with PBS, the tumor sections were then incubated with FITC-conjugated goat anti-rat or Cy3-conjugated donkey anti-rabbit secondary antibodies (1:200; Jackson ImmunoResearch Laboratories), respectively. The slides were mounted with 4′,6-diamidino-2-phenylindole and visualized under a Leica TCS-NT confocal microscope.

To investigate the toxicity of RIT, liver and kidney samples were fixed in 10% formalin/PBS and embedded in paraffin. Histologic sections of 5- to 8-μm thickness were prepared and stained with H&E for routine histopathologic examination.

**Statistical analysis**

Quantitative data are expressed as means ± SD. Means were compared using one-way ANOVA and Student’s t test. P values of <0.05 were considered statistically significant.

**Results**

**In vitro EGFR targeting of panitumumab**

UM-SCC-22B cells and tumor tissues were incubated with panitumumab and then visualized with FITC-coupled secondary antibody. It was found that panitumumab bound strongly to both UM-SCC-22B cells and tumor tissues (Fig. 1A). The staining was mostly on the cell membrane, as EGFR is mainly expressed on the cell surface.

To determine the binding affinity (Kₐ) and the number of binding sites per UM-SCC-22B cell (Bₘₐₓ) for ¹¹¹In-panitumumab, saturation binding assay was done. As shown in Fig. 1B, panitumumab bound to EGFR on cell surface with a high affinity (Kₐ of 1.33 ± 0.29 nmol/L). Scatchard analysis revealed an average of 1.83 ± 0.01 × 10⁵ available binding sites per UM-SCC-22B cell.

**DOTA conjugation and radiolabeling**

The competitive cell-binding assay revealed that DOTA-panitumumab inhibited the binding of ¹¹¹In-panitumumab to UM-SCC-22B cells in a dose-dependent manner (Fig. 1C). The IC₅₀ values for DOTA-panitumumab and panitumumab were 0.11 ± 0.02 and 0.10 ± 0.01 nmol/L, respectively, showing that DOTA-panitumumab possessed similar EGFR-binding avidity with panitumumab. ⁹⁰Y-DOTA-panitumumab was generated at an average yield of ~80%, and the radiochemical purity was >99% after PD-10 column purification. Using UM-SCC-22B cells, the immunoreactive fraction of ⁹⁰Y-DOTA-panitumumab was measured to be 74.4 ± 2.9%.

**Biodistribution of ¹¹¹In-DOTA-panitumumab**

Biodistribution studies were done using female BALB/c nude mice bearing UM-SCC-22B human head and neck cancer xenografts. The biodistribution data for ⁹⁰Y-DOTA-panitumumab are summarized in Fig. 2A. ¹¹¹In-DOTA-panitumumab was cleared rapidly from blood with 30.97 ± 1.76, 10.54 ± 0.66, 2.00 ± 1.49, 1.62 ± 1.24, and 0.20 ± 0.20 % ID/g at 4, 24, 72, 120, and 168 hours after injection, respectively. At these time points, the tumor uptake of ¹¹¹In-DOTA-panitumumab was 26.10 ± 4.93, 59.11 ± 7.22, 44.57 ± 9.80, 40.38 ± 7.76, and 14.86 ± 7.36 % ID/g, respectively. The maximum tumor uptake of ¹¹¹In-DOTA-panitumumab occurred at 24 hours after injection and then decreased slowly. Compared with other normal organs, ¹¹¹In-DOTA-panitumumab showed rapid and high uptake in liver, with 15.71 ± 1.36 % ID/g at 4 hours after injection, and then decreased to 7.05 ± 3.37 % ID/g at 168 hours after injection. Spleen uptake of ¹¹¹In-DOTA-panitumumab was relatively constant, ranging from 6.01 ± 0.50 to 6.46 ± 3.26 % ID/g from 4 to 168 hours after injection. The area under curve of ¹¹¹In-DOTA-panitumumab uptake in tumors was significantly more than that in other normal organs (Fig. 2B), indicating a much greater radiation exposure to the tumor than to normal organs.
γ-Imaging

The imaging studies of 111In-DOTA-panitumumab were done in BALB/c nude mice bearing UM-SCC-22B human head and neck cancer xenografts. Figure 3 illustrates the representative γ-images of tumor-bearing mice administered with ∼13 MBq of 111In-DOTA-panitumumab. The planar γ-images showed a similar trend with the biodistribution data. At 24 hours after injection, UM-SCC-22B tumors could be clearly visualized and the activity accumulated in the liver was also obvious due to the hepatic clearance of 111In-DOTA-panitumumab. With the clearance of radioactivity from normal organs, the much better tumor contrast was observed at 72 and 96 hours after injection.

RIT and immunotherapy

The therapeutic efficacy of 90Y-DOTA-panitumumab was investigated in EGFR-positive UM-SCC-22B tumor-bearing nude mice. As shown in Fig. 4A, single injection of low-dose panitumumab (∼6 μg) did not induce significant tumor growth inhibition compared with saline. The tumors in the 3.7 MBq 90Y-DOTA-IgG group grew somewhat slower than those in saline and cold panitumumab groups during the first 10 days, and then grew more quickly and reached an average volume of 1,496.4 ± 91.18 mm³ on day 15 after treatment. The tumors in the 3.7 MBq 90Y-DOTA-panitumumab group displayed significant growth inhibition compared with the control groups from day 4 to day 17 (P < 0.01), but after 17 days, an obvious tumor relapse was observed. In the 7.4 MBq 90Y-DOTA-panitumumab group, the tumor growth was almost completely inhibited up to day 46 after treatment, and only one mouse in this group showed tumor relapse on day 48. Almost no UM-SCC-22B tumor inhibition effect was observed in the panitumumab-based immunotherapy group (10 mg/kg × four doses). The findings suggest that 90Y-DOTA-panitumumab exerted a strong therapeutic effect against UM-SCC-22B tumor xenografts compared with cold panitumumab-based immunotherapy. All mice in the therapy studies showed no significant body weight loss (<20% of original weight) after treatment (Fig. 5A), showing no significant acute toxicity for 90Y-DOTA-panitumumab.

Kaplan-Meier analysis was done and is shown in Fig. 4B. The mice were sacrificed when the tumor size reached ≥1,500 mm³ or body weight lost ≥20% of their original values. From the Kaplan-Meier curves, it was
found that mice administrated with 7.4 MBq 90Y-DOTA-panitumumab (median survival time, >80 days) or 3.7 MBq 90Y-DOTA-panitumumab (median survival time, 35 days) had a significantly longer survival time than that administrated saline (median survival, 17 days), single dose of panitumumab (∼6 μg; median survival, 14 days), four doses of panitumumab (10 mg/kg; median survival, 14 days), or 3.7 MBq 90Y-DOTA-IgG (median survival, 17 days; \( P < 0.01 \)). The mice that received 7.4 MBq 90Y-DOTA-panitumumab also had a significantly prolonged survival time compared with those that received 3.7 MBq 90Y-DOTA-panitumumab (\( P < 0.01 \)).

**EGFR specificity of RIT**

To substantiate the EGFR-specific therapy of 90Y-DOTA-panitumumab, the RIT was also tested in the MDA-MB-435 tumor-bearing nude mice, which served as a tumor model with negligible EGFR expression (21). As shown in Fig. 5B, compared with saline group, the tumors in the 3.7-MBq 90Y-DOTA-panitumumab group exhibited a slight growth delay between day 12 and day 18 after treatment. Thereafter, however, the tumors in the two groups both grew rapidly and there was no significant difference on the tumor size after day 22 after treatment. The slight MDA-MB-435 tumor growth inhibition at early time points was most likely due to the nonspecific tumor accumulation because of the enhanced permeability and retention effect, subsequently leading to the radiation-mediated cell killing of 90Y-DOTA-panitumumab.

**Histologic analysis**

After RIT, UM-SCC-22B tumors were harvested, frozen, and cut into sections and then subjected to CD31, Ki67, and TUNEL staining (Fig. 6). We carried out the CD31 staining to study the effect of 90Y-DOTA-panitumumab (3.7 MBq) treatment on vascular damage. The tumor vessels...
in the 3.7 MBq $^{90}$Y-DOTA-panitumumab treatment group tend to have increased vascular fragmentation, dysmorphic vessel structure, and reduced endothelial presence compared with the saline group. There was no significantly decreased vasculature density in the 3.7 MBq $^{90}$Y-DOTA-IgG treatment group, but slightly increased vascular fragmentation can be observed. Treating with unlabeled panitumumab can induce an obvious decrease in the vasculature density, but no significant vascular fragmentation is found. Ki67 analysis shows reduced cell proliferation in the $^{90}$Y-DOTA-panitumumab-treated mice, whereas the mice in $^{90}$Y-DOTA-IgG, panitumumab, and saline groups have significantly higher cell proliferation index, respectively. To evaluate whether cell apoptosis was involved in the $^{90}$Y-DOTA-panitumumab-enhanced regression on UM-SCC-22B tumors, the TUNEL assay was used to quantify cell apoptosis in tumor sections from different groups. As shown in Fig. 6, $^{90}$Y-DOTA-panitumumab induced significantly higher percentage of TUNEL-positive cells than $^{90}$Y-DOTA-IgG, panitumumab, and saline.

We also did histopathologic examination to determine the toxicity of therapeutic doses of $^{90}$Y-DOTA-panitumumab. We tested the H&E staining of the kidney and liver slices after RIT. No obvious histologic damage is observed in kidneys and livers from mice administrated with 3.7 or 7.4 MBq of $^{90}$Y-DOTA-panitumumab (Supplementary Fig. S1), indicating the limited toxicity of the activities used in these therapeutic studies.

**Discussion**

Despite the initial introduction of RIT for tumor therapy some 30 years ago, this technology seems to reattract an increasing interest most recently (22, 24–27). The renewed interest may be attributed, in part, to the successful application of $^{90}$Y- and $^{131}$I-labeled anti-CD20 mAbs in the management of hematopoietic tumors, especially non–Hodgkin’s lymphoma. The promising antitumor efficacy of intact antibody-based RIT agents is most likely due to their much higher and more retentive tumor accumulation compared with other small-molecule-based agents. In this study, we successfully prepared the $^{111}$In- and $^{90}$Y-labeled panitumumab, a fully human anti-EGFR mAb. $^{111}$In-panitumumab exhibited high and retentive tumor accumulation in UM-SCC-22B human head and neck cancer xenografts, and $^{90}$Y-panitumumab showed high tumor suppression efficacy in the mouse model.

Given the apparent role of EGFR in tumor aggression and poor prognosis, as well as the high degree to which it seems to be overexpressed in almost all types of head and neck cancers, blockade of EGFR pathway has been well investigated as a rational strategy for targeted therapy of head and neck cancers. The chimeric anti-EGFR mAb Erbitux (cetuximab) has been reported to inhibit the growth of EGFR-expressing carcinoma cells and inhibit the invasion and metastasis of tumor cells in preclinical studies (4, 28). In a preliminary clinical trial, the combination of cetuximab with radiotherapy has led to a partial or complete response in patients with locoregional advanced HNSCC (29). With the successful applications, the FDA granted regulatory approval for the use of cetuximab in combination with external radiation to treat locoregional advanced head and neck cancer in 2006. However, as cetuximab is a chimeric antibody, it is still immunogenic and can produce allergic reaction in some patients (30, 31). Consequently, other new fully human anti-EGFR antibodies are currently undergoing preclinical evaluations. Because RIT can significantly reduce the mAb dose and destroy the tumor cells with low or without antigen expression, herein, we tested the RIT efficacy for head and neck cancer in xenografted animal model using radiolabeled panitumumab, the first fully human mAb against EGFR.

Panitumumab possessed high affinity for EGFR on UM-SCC-22B cancer cells ($K_d$ of 1.33 ± 0.29 nmol/L; Fig. 1B). The in vitro analysis confirmed that DOTA conjugation of panitumumab did not impair the receptor affinity of panitumumab (Fig. 1C), and $^{90}$Y-DOTA-panitumumab possessed high radioimmunoreactivity (radioimmunoreactive fraction = 74.4 ± 2.9%) with EGFR-expressing UM-SCC-22B cells. We used $^{111}$In-DOTA-panitumumab as a biodistribution and imaging surrogate to predict the in vivo behaviors and radiation dosimetry of $^{90}$Y-DOTA-panitumumab because it has been reported that $^{111}$In- and $^{90}$Y-labeled antibodies, proteins, and peptides are biologically equivalent with respect to their uptake in tumors and other major organs (32). The results from the biodistribution studies showed that the uptake of $^{111}$In-DOTA-panitumumab in EGFR-positive UM-SCC-22B tumors was much higher than that in normal organs and blood. The tumor uptake of radiotracer reached a maximum of 59.11 ± 7.22 % ID/g at 24 hours after injection and remained high with time (14.86 ± 7.23 % ID/g at 7 days after injection).
injection). Furthermore, the low radioactivity accumulation in the bone suggested high stability of the $^{111}$In-DOTA chelate and confirmed that the chelator was suitable for $^{90}$Y-based therapy. Compared with some other EGFR-targeted antibodies (33, 34), $^{111}$In-DOTA-panitumumab in this study showed much higher tumor uptake and lower liver radioactivity accumulation, which was likely due to the different EGFR recognition properties and varied in vivo pharmacokinetics of different antibodies. It should be noted that the biodistribution of $^{111}$In-DOTA-panitumumab in the present study may not be a true mimicry of the clinical situation because panitumumab may not bind murine EGFRs that expressed in normal organs of mice. This may also be partially responsible for the faster clearance of $^{111}$In-DOTA-panitumumab. However, the high uptake of $^{111}$In-DOTA-panitumumab in UM-SCC-22B tumors still showed the excellent targeting property of $^{111}$In-DOTA-panitumumab to EGFRs of human origin. $^{90}$Y is a useful radioisotope for the targeted RIT of solid tumors due to its favorable physical characteristics. Recently, we determined the maximum tolerated dose of nude mice for $^{90}$Y-labeled mAb Abegrin, and it was found that BALB/c nude mice could tolerate up to 200 $\mu$Ci of $^{90}$Y-Abegrin (22). As panitumumab is also an intact mAb similar to Abegrin, we used a maximum radioactivity dose of 200 $\mu$Ci $^{90}$Y-DOTA-panitumumab for the RIT in this study. No evident weight loss was found after $^{90}$Y-DOTA-panitumumab treatment (Fig. 5A), indicating that 200 $\mu$Ci $^{90}$Y-DOTA-panitumumab was under the maximum tolerated dose of tumor-bearing nude mice. RIT with $^{90}$Y-DOTA-panitumumab caused a significant
tumor growth delay and improved the survival in EGFR-positive UM-SCC-22B tumor model (Fig. 4), and importantly, there was no significant radiation damage in the dose-limiting organs such as liver and kidneys (Supplementary Fig. S1). Achieving tumor treatment efficacy by $^{90}$Y-DOTA-panitumumab with limited whole-body toxicity is due to the much higher tumor uptake of panitumumab as determined by biodistribution study (Fig. 2A). As reported in the RIT studies, a clear activity-dependent relationship in the efficacy of a single administration of $^{90}$Y-DOTA-panitumumab was apparent in the established tumor model. Treatment of tumor-bearing nude mice with 3.7 MBq $^{90}$Y-DOTA-panitumumab caused $\sim$20 days of tumor growth delay, but at day 34 after treatment, almost all tumor reached a larger size ($\sim$1,500 mm$^3$). When using 7.4 MBq $^{90}$Y-DOTA-panitumumab for single-dose therapy, the growth of all tumors was inhibited up to $\sim$50 days, and only one tumor showed recurrence at day 48 after treatment. The lack of therapeutic efficacy of $^{90}$Y-DOTA-IgG compared with the saline control group confirmed the EGFR-specific RIT of $^{90}$Y-DOTA-panitumumab. To evaluate the influence of EGFR expression level on the therapy outcome, we selected MDA-MB-435 tumor model as a control. As there was a limited EGFR expression in MDA-MB-435 tumors (21), it was assumed that the tumor uptake of $^{90}$Y-DOTA-panitumumab would be very low, resulting in no tumor therapeutic efficacy. In fact, the tumor growth curves revealed no statistical significance between $^{90}$Y-DOTA-panitumumab and control groups (Fig. 5B), indicating the limited therapeutic efficacy of $^{90}$Y-DOTA-panitumumab in EGFR-negative tumors, which validated again that the antitumor effect of $^{90}$Y-DOTA-panitumumab was EGFR specific.

Although panitumumab showed high accumulation in UM-SCC-22B tumors (Fig. 2A), the immunotherapy study using high doses of panitumumab did not show any therapeutic effect (Fig. 4A). Our results are consistent

![Figure 5](https://example.com/figure5.png)

**Figure 5.** A, mean body weight over time of the BALB/c nude mice administrated with 7.4 MBq $^{90}$Y-DOTA-panitumumab, 3.7 MBq $^{90}$Y-DOTA-panitumumab, 3.7 MBq $^{90}$Y-hIgG, panitumumab, or saline. Points, mean ($n$ = 5–6 per group); bars, SD. B, growth curves of the low EGFR-expressing MDA-MB-435 tumors in BALB/c nude mice after i.v. injection of 3.7 MBq $^{90}$Y-DOTA-panitumumab or saline. Points, mean ($n$ = 6 per group); bars, SD.
with a recent report showing that another anti-EGFR antibody, cetuximab (Erbitux), did not inhibit the growth of the UM-SCC-22B tumors as well (35). Several previous studies have also shown that some EGFR-positive tumors did not benefit from the treatment of anti-EGFR antibodies (36–38). Our biodistribution study has shown the high UM-SCC-22B tumor uptake of panitumumab (Fig. 2A), but the high tumor uptake may not directly lead to effective antitumor activity with cold panitumumab due to the disparity between the tumor EGFR expression and the antibody treatment outcome, although the detailed mechanism of UM-SCC-22B tumor resistance to panitumumab still needs further investigation. In contrast to immunotherapy, RIT relies on the cell-killing effect of radioisotopes (i.e., the higher tumor accumulation of the radioactivities would certainly lead to the more significant therapeutic efficacy because the tumor cells are usually sensitive to radiation). The high UM-SCC-22B tumor uptake of panitumumab as determined by biodistribution study guaranteed the high antitumor effect of 90Y-panitumumab.

The antiangiogenic effect of 90Y-panitumumab was observed by the ex vivo immunofluorescence staining result of significantly reduced CD31+ vascular endothelial cells (Fig. 6). Ki67 and TUNEL histopathologic analyses revealed the reduced proliferation and the increased apoptosis index of tumors in treatment animals, which further confirmed the therapeutic efficacy of 90Y-panitumumab (Fig. 6).

Increasing clinical evidence has shown the disparity between the EGFR expression level and the treatment outcome of anti-EGFR mAb-based immunotherapeutic agents (37–39). Besides the EGFR expression level, some additional factors such as tumor vasculature density and permeability, tumor interstitial pressure and binding site barrier, as well as the pharmacokinetics and tumor penetration ability of the bulky immunoglobulins would possibly influence the effective tumor delivery of anti-EGFR mAb (14, 40). Differing from panitumumab itself as the immunotherapeutic agent, 90Y-DOTA-panitumumab exerts its antitumor effect by localizing continuous irradiation to the area of malignancy, thereby destroying not only the antibody-binding EGFR-positive tumor cells but also the unbound tumor cells and the EGFR-negative tumor cells by the cross-fire effect.

The easy availability, high-receptor binding affinity, and excellent tumor therapeutic efficacy of 90Y-DOTA-panitumumab as determined in this study warrant its further possible clinical translation. In the meantime, it is also worth investigating the combination of 90Y-DOTA-panitumumab with other therapeutic approaches in the future. It is well documented that the inhibition of EGFR signaling can enhance the antitumor effect of radiation therapy (41, 42). Therefore, it would be interesting to investigate the synergistic effect of combining 90Y-DOTA-panitumumab–based RIT with other EGFR inhibition approaches such as tyrosine kinase inhibitor and other blocking antibodies. In addition, in comparison with the high-energy \( \beta^- \) emitter 90Y, 177Lu is a low-energy \( \beta^- \) emitter with three \( \beta^- \)-emissions [\( E_{\text{max}} = 0.176 \text{ MeV (12%)}, 0.384 \text{ MeV (9%)}, \) and 0.497 MeV (79%)\] and two \( \gamma \)-emissions \[113 \text{ keV (6.4%) and 208 keV (11%)\]}. Because of its low \( \beta^- \)-energy, 177Lu-labeled compounds might be useful for treatment of small and metastatic tumors (43). The presence of these two \( \gamma \)-emissions will allow scintigraphic imaging for quantification of biodistribution and determination of radiation dosimetry in clinical settings. Therefore, 177Lu-labeled panitumumab may be more suitable for the image-guided RIT of the local recurrence or micrometastasis of EGFR-positive tumors. Moreover, treatment of EGFR-positive tumors with 177Lu-labeled panitumumab
after pretreatment with $^{90}$Y-panitumumab may lead to the effective therapy of both large tumors and minimal residual tumors as a whole.

**Conclusion**

The *in vivo* studies showed the high tumor uptake and prolonged tumor retention of $^{111}$In-DOTA-panitumumab in UM-SCC-22B tumor-bearing nude mice. The RIT studies of $^{90}$Y-DOTA-panitumumab showed the effective growth inhibition of EGFR-positive tumors. From these results, we believe that $^{90}$Y-DOTA-panitumumab is a promising RIT agent for EGFR-positive tumor therapy. To the best of our knowledge, this is the first preclinical investigation of $^{90}$Y-labeled panitumumab for EGFR-targeted RIT of head and neck carcinoma.

**References**


**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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