Biodistribution and Therapeutic Efficacy of $^{125/131}$I-, ^{186}Re-, $^{88/90}$Y-, or ^{177}Lu-Labeled Monoclonal Antibody MN-14 to Carcinoembryonic Antigen in Mice with Small Peritoneal Metastases of Colorectal Origin

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Therapeutic efficacy in radioimmunotherapy depends, among other things, on the choice of the radionuclide. The aim of the present study was to determine the most suitable radionuclide for radioimmunotherapy with monoclonal antibody MN-14 to carcinoembryonic antigen in an experimental model of small peritoneal metastases of colorectal origin. **Methods:** In nude mice with intraperitoneal LS174T tumors (diameter, 1–3 mm), the biodistributions of MN-14 labeled with ^{131}I (^{131}I-MN-14), ^{186}Re-mercaptoacetyltriglycine (^{186}Re-MN-14), and ^{88}Y-diethylenetriaminepentaacetic acid (DTPA) (^{88}Y-MN-14) after intravenous and intraperitoneal administration were determined. Subsequently, the therapeutic efficacies of equally toxic activity doses of ^{131}I-MN-14 (9.25 MBq per mouse), ^{186}Re-MN-14 (9.25 MBq per mouse), ^{90}Y-MN-14 (3.15 MBq per mouse), and MN-14 labeled with ^{177}Lu-DTPA (^{177}Lu-MN-14) (8.33 MBq per mouse) after intraperitoneal administration were determined. **Results:** Each of the radioimmunoconjugates preferentially accumulated in tumor nodules, both after intravenous administration and after intraperitoneal administration. Values for clearance from blood were similar for all radioimmunoconjugates. The uptake of ^{88}Y-MN-14 in the liver and spleen was significantly higher than the uptake of ^{131}I-MN-14 or ^{186}Re-MN-14. Maximal uptake values (mean ± SD) in tumors were 58 ± 7 percentage injected dose per gram of tissue (%ID/g) for ^{131}I-MN-14 (24 h after administration), 83 ± 19 %ID/g for ^{186}Re-MN-14 (72 h after administration), and 148 ± 89 %ID/g for ^{88}Y-MN-14 (192 h after administration). Dosimetric analysis of the biodistribution data estimated that the radiation doses guided to the tumor by intraperitoneally administered ^{131}I-MN-14, ^{186}Re-MN-14, ^{90}Y-MN-14, and ^{177}Lu-MN-14 were 150, 100, 45, and 200 Gy, respectively. The median survival time of control mice, treated with unlabeled MN-14, was 42 d, whereas the median survival times of mice treated with ^{131}I-MN-14, ^{186}Re-MN-14, ^{90}Y-MN-14, and ^{177}Lu-MN-14 were 100 d (range, 58–142; $P < 0.0001$), 72 d (range, 46–84; $P = 0.0002$), 82 d (range, 46–142; $P < 0.0001$), and 136 d (range, 56–142; $P < 0.0001$), respectively. At the completion of the experiment (142 d after tumor cell inoculation), no residual disease was found in 8 of 9 long-term survivors (^{131}I, $n = 3$; ^{90}Y, $n = 1$; and ^{177}Lu, $n = 4$). **Conclusion:** The uptake of ^{88}Y-MN-14 in small peritoneal LS174T xenografts was higher than the uptake of ^{131}I-MN-14 or ^{186}Re-MN-14. The present study indicates that ^{131}I and ^{177}Lu are the most suitable radionuclides for the radioimmunotherapy of small peritoneal metastases.

Key Words: ^{131}I; ^{186}Re; ^{90}Y; ^{177}Lu; radioimmunotherapy; peritoneal metastases

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Radioimmunotherapy—the use of radiolabeled monoclonal antibodies (mAbs) against tumor-associated antigens—has not fulfilled its promise for solid cancers as it has for hematologic malignancies. Besides the limited radiosensitivity of carcinomas compared with hematologic malignancies, solid tumors are generally characterized by a limited vascular supply, heterogeneous uptake of the antibodies in the tumors, and elevated interstitial pressure in combination with a relatively long transport distance in the interstitium (*1*). Radioimmunotherapy therefore is considered more suitable for the treatment of microscopic or minimal residual disease, allowing radiolabeled mAbs to achieve uptake in tumors high enough to result in tumoricidal radiation doses.

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An important issue in radioimmunotherapy is the selection of the radionuclide. β-Emitting isotopes, such as ^{131}I and ^{90}Y, are the most commonly used radionuclides in radioimmunotherapy. ^{186}Re and ^{177}Lu are β-emitting radionuclides that have been considered for radioimmunotherapy more recently. The physical characteristics of these 4 radionuclides, however, differ significantly with respect to half-life, the presence of γ-radiation, the energy of the β-emission, and consequently the maximum depth of penetration of the β-particles in tissue, as summarized in Table 1.

Koppe et al. previously characterized an experimental model of small peritoneal metastases with the human colon carcinoma cell line LS174T (*2*). In this model, radioimmunotherapy with mAb MN-14 labeled with ^{131}I was very effective in delaying the development of peritoneal carcinomatosis, even at relatively low activity doses. Therapeutic efficacy in this model might be improved by use of other radionuclides with more favorable characteristics for radioimmunotherapy.

In the present study, experiments were performed with the aim of selecting the most suitable radionuclide for the radioimmunotherapy of small peritoneal metastases of colorectal origin. For this purpose, a series of experiments first was performed to investigate the biodistribution of MN-14 labeled with $^{131/125}$I, ^{186}Re, or ^{88}Y in nude mice with small intraperitoneal xenografts of colon cancer. Then, the therapeutic efficacy of MN-14 labeled with ^{131}I, ^{186}Re, ^{90}Y, or ^{177}Lu was assessed and correlated with the results of the biodistribution experiments.

MATERIALS AND METHODS

Animal Model for Small Peritoneal Metastases

Male nude BALB/c mice (Charles River Laboratories), 8–9 wk old and weighing 20–25 g, were used in the experiments. Mice were allowed to become accustomed to laboratory conditions for at least 1 wk before experimental use. Mice were housed under nonsterile standard conditions (temperature, 20°C–24°C; relative humidity, 50%–60%; 12 h of light and 12 h of dark) in filter-topped cages (5 mice per cage), with free access to animal chow (Snif Voer) and water. Peritoneal metastases were induced as described previously (*2*). In brief, mice were inoculated intraperitoneally with 10^6 LS174T cells (CCL 188; American Type Culture Collection) suspended in 500 μL of RPMI 1640 medium in a 2.5-mL syringe by use of a 23-gauge needle. In this model, the first macroscopic tumor nodules are seen 7–10 d later, whereas bulky peritoneal carcinomatosis develops 3–5 wk after tumor cell inoculation. All experiments were approved by the institutional animal welfare committee of the University Medical Center Nijmegen and were conducted in accordance with the principles set forth by the Revised Dutch Act on Animal Experimentation (1997).

TABLE 1
Physical Characteristics of β-Emitters Used Most Often in Radioimmunotherapy

Radionuclide	Half-life (d)	Maximum β-energy (MeV)	Maximum penetration depth (mm)	γ-Emission, in keV (%)
^{131}I	8.0	0.606	3.0	364 (82), 637 (6.5)
^{90}Y	2.7	2.28	12.0	None
^{186}Re	3.8	1.08	5.4	137 (9.5)
^{177}Lu	6.7	0.497	2.5	208 (11), 113 (7)

mAb

Murine mAb MN-14 is a high-affinity (association constant, 10^9 L/mol) class III anti–carcinoembryonic antigen (CEA) immunoglobulin G1 (IgG1) antibody produced by a hybridoma cell line culture kindly provided by Immunomedics, Inc. (*3*). The antibody was purified by protein A chromatography as described previously (*4*). Purity was checked by fast protein liquid chromatography on a Biosep 3000 column (Phenomenex); elution was done with phosphate-buffered saline (PBS; pH 7.2) at 1 mL/min.

Radioiodination

The antibody was radioiodinated with ^{125}I or with ^{131}I (Amersham Biosciences or MDS Nordion, respectively) by use of the IODO-GEN method (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril; Pierce Biotechnology, Inc.) (*5*). Briefly, the antibody and ^{125}I or ^{131}I were incubated at room temperature in 85 μL of PBS (0.10 mol/L; pH 7.4) in a glass vial coated with 50–100 μg of IODO-GEN. After 10 min, the reaction was stopped by the addition of 100 μL of a saturated tyrosine solution. The reaction mixture then was separated on a PD-10 column (Amersham Biosciences); elution was done with PBS and 0.5% bovine serum albumin (BSA). The labeling efficiency of all of the radioiodination reactions exceeded 90%. In the biodistribution experiments, the specific activities of ^{125}I-MN-14 and ^{131}I-MN-14 were 41 and 78 kBq/μg, respectively. The specific activity of ^{131}I-MN-14 in the therapy experiments was 0.96 MBq/μg.

^{186}Re Labeling

$^{186}ReO_4^-$ was obtained from Tyco Mallinckrodt Medical BV. The specific activities of the ReO_4^- batches used in the biodistribution and therapy experiments were 37 and 65 GBq/mg, respectively. The antibody was labeled with ^{186}Re by use of *S*-benzoyl-mercaptoacetyltriglycine (*S*-benzoyl-MAG3) as a chelator as described by Visser et al. (*6*). Briefly, 180 μg of MAG3 (1.0 mg/mL) was incubated with 150 μL of Na_2CO_3 (1.0 mol/L), 150 μL of Na_2SO_3 (100 mg/mL), and 696 MBq of $^{186}ReO_4^-$ (100 μL) in a boiling water bath (10 min). The solvent was evaporated, and the solid phase was incubated for another 15 min. After derivatization of ^{186}Re-MAG3 with 2,3,5,6-tetrafluorophenol (100 mg/mL in acetonitrile:H_2O, 9:1), the derivatized ^{186}Re-MAG3 ester was purified on a Sep-Pak C_{18} cartridge (Waters Corp.) and then reacted with 900 μg (biodistribution experiments) or 400 μg (therapy experiments) of a concentrated MN-14 solution at pH 9.5. The mean numbers of MAG3 groups per MN-14 IgG molecule in the biodistribution and therapy experiments were 2.6 and 4.1, respectively. The ^{186}Re-MAG3-MN-14 conjugate (hereafter referred to as ^{186}Re-MN-14) was purified on a PD-10 column. The overall labeling efficiencies of the ^{186}Re labeling procedures performed in the biodistribution and therapy experiments were 36% and 18%, respectively, resulting in specific activities of 0.12 and 0.33 MBq/μg, respectively.

^{88}Y, ^{90}Y, and ^{177}Lu Labeling

All conjugation and labeling procedures were performed under strict metal-free conditions. To allow labeling of the antibody with

^{88}Y, ^{90}Y, and ^{177}Lu, MN-14 was conjugated with isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (ITC-DTPA; Macrocyclics). Briefly, ITC-DTPA was conjugated to MN-14 in $NaHCO_3$ buffer (0.1 mol/L; pH 8.2) by use of a 100-fold molar excess of ITC-DTPA as described by Ruegg et al. (*7*) with minor modifications (conjugation period of 1 h at room temperature). The DTPA-MN-14 conjugate was purified by extensive dialysis against ammonium acetate buffer (0.1 mol/L; pH 5.0). The number of DTPA ligands per antibody molecule was determined by the method of Hnatowich et al. (*8*). The purified DTPA-MN-14 conjugate (ratio of DTPA to MN-14, 2.5:1; 0.8 mg/mL) was incubated with ^{88}Y (Isotope Products Europe Blaseg), ^{90}Y (The Perkin-Elmer Corp.), or ^{177}Lu (University of Missouri Research Reactor) in ammonium acetate buffer (0.1 mol/L; pH 5.4) at room temperature for 20 min. The specific activities of the ^{88}Y-DTPA-MN-14, ^{90}Y-DTPA-MN-14, and ^{177}Lu-DTPA-MN-14 preparations (hereafter referred to as ^{88}Y-MN-14, ^{90}Y-MN-14, and ^{177}Lu-MN-14, respectively) were 48.1 kBq/μg, 370 kBq/μg, and 1.48 MBq/μg, respectively.

Quality Control for Radiolabeled Preparations

All radiolabeled MN-14 preparations were purified by gel filtration on a PD-10 column; elution was done with PBS supplemented with 0.5% BSA. For all preparations, the amount of free radiolabel was determined by instant thin-layer chromatography with silica gel strips (Gelman Sciences, Inc.) and with citrate buffer (0.1 mol/L; pH 6.0) as the mobile phase. The radiochemical purity of all radiolabeled antibody preparations used in the experiments exceeded 96%.

The immunoreactive fraction (IRF) of the radiolabeled MN-14 preparations, except for ^{90}Y-MN-14, at an infinite antigen excess was determined with freshly trypsinized LS174T cells essentially as described by Lindmo et al. (*9*) with minor modifications. Briefly, a fixed amount of labeled antibody (10,000 cpm) was incubated with increasing concentrations of LS174T tumor cells (1.2×10^6–20×10^6 cells per milliliter) in 0.5 mL of binding buffer (RPMI 1640 medium containing 0.5% BSA and 0.05% NaN_3). A duplicate of the lowest cell concentration was incubated in the presence of an excess of unlabeled antibody to correct for nonspecific binding. After 6 h of incubation at 37°C, the cells were washed, and activity in the pellet was determined by use of a well-type γ-counter. The inverse of the tumor cell–bound fraction was plotted against the inverse of the cell concentration, and the IRF was calculated from the *y*-axis intercept. The IRFs (mean ± SD) of the radiolabeled preparations used in the biodistribution and therapy experiments were 89.3% ± 6.5% and 77% ± 6.1%, respectively. Labeled antibody preparations were administered within 2 h after radiolabeling.

Biodistribution After Intraperitoneal or Intravenous Administration

To assess the effects of both the route of administration and the radiolabel on the biodistribution of radiolabeled MN-14, mice were inoculated intraperitoneally with 10^6 LS174T tumor cells suspended in RPMI 1640 medium (500 μL). Ten days later, mice received 1.22 MBq of ^{186}Re-MN-14 intraperitoneally and 0.481 MBq of ^{88}Y-MN-14 intravenously or vice versa (5 mice per group). Mice were killed by O_2:CO_2 asphyxiation and dissected at 24, 48, 72, 96, or 192 h after the administration of the radiolabeled antibody preparations (5 mice per group). At dissection, tumors, blood, liver, spleen, kidneys, intestine, lungs, muscle, and the right femur were sampled, blotted dry, and weighed. Activity was measured in a shielded well-type γ-counter (Wizard; Pharmacia-LKB). To correct for physical decay and to calculate the uptake of the radiolabeled antibody in each sample as a fraction of the injected dose, counts in aliquots of the injected doses were determined simultaneously. The results were expressed as the percentage injected dose per gram of tissue (%ID/g). Because it was shown in a previous study that at protein doses exceeding 25 μg, the uptake of radiolabeled antibody MN-14 in tumors tended to be lower (*2*), the total protein dose of each preparation was adjusted to 10 μg per mouse (total, 20 μg per mouse) by adding unlabeled MN-14 when necessary. The results were compared with the previously reported data on the biodistribution of radioiodinated MN-14 after intravenous or intraperitoneal administration in the same model (*2*).

Estimation of Radiation Dose Administered to Tumors

The biodistribution data were used to calculate the areas under the curve (AUCs), corrected for physical decay. Subsequently, the data were processed by use of the MIRDOSE3 software program (Oak Ridge Associated Universities) (*10*) to estimate the radiation dose absorbed by the tumor (tumor absorbed radiation dose) for ^{131}I-MN-14, ^{186}Re-MN-14, ^{90}Y-MN-14, and ^{177}Lu-MN-14 at 50% their maximal tolerated activity doses (MTDs). For this purpose, it was assumed that the biodistributions of both ^{90}Y-MN-14 and ^{177}Lu-MN-14 would have been similar to that of ^{88}Y-MN-14.

Radioimmunotherapy Studies

Ten days after intraperitoneal tumor cell inoculation, groups of 10 mice received intraperitoneal injections of ^{131}I-MN-14 (9.25 MBq per mouse), ^{186}Re-MN-14 (9.25 MBq per mouse), ^{90}Y-MN-14 (3.15 MBq per mouse), ^{177}Lu-MN-14 (8.33 MBq per mouse), and unlabeled MN-14 (control). These doses represented equally toxic activity doses of the respective radionuclides, that is, equal to 50% their respective MTDs. The MTD of each antibody-bound radionuclide after intraperitoneal administration was defined as the activity dose below the lowest dose that resulted in either the death of any animal in groups of 5 animals or a body weight loss of more than 20% and was empirically determined as described previously (*11*). The MTDs of ^{131}I-MN-14, ^{186}Re-MN-14, ^{90}Y-MN-14, and ^{177}Lu-MN-14 after intraperitoneal administration were 18.5, 18.5, 6.29, and 16.65 MBq, respectively. Preparations were administered in 400 μL of PBS supplemented with 0.5% BSA. The protein dose was adjusted to 20 μg by adding unlabeled MN-14 to a fraction of the primary radioimmunoconjugate preparations when necessary. Because the labeling efficiency was lower than anticipated, the protein dose of the ^{186}Re-MN-14 preparation was 28 μg.

Mice were monitored daily, and body weight and abdominal circumference were measured twice per week as described previously (*2*). Besides death, a humane endpoint was defined as a decrease in body weight of 20% or more or an increase in the abdominal circumference of 10% or more attributable to intraperitoneal tumor growth, compared with the body weight or abdominal circumference measured on the day of tumor inoculation. When either one of these criteria was met, mice were killed by O_2:CO_2 asphyxiation and cervical dislocation. All intraperitoneal tumor deposits were meticulously dissected and weighed. The experiment was terminated at 142 d after tumor cell inoculation, when the remaining mice were euthanized and dissected. The abdominal cavity was conscientiously inspected. The liver, spleen, lungs, pancreas, greater omentum, and diaphragm were removed for routine histopathologic hematoxylin–eosin staining and immuno-

histochemical staining with a rabbit anti–human CEA polyclonal antibody (A0115; Dakocytomation) (*12*).

Statistical Analysis

Statistical analysis was performed by use of GraphPad InStat 3.00 software (GraphPad Software). Single comparisons were analyzed by use of the 2-tailed, Welch-corrected, unpaired *t* test or the nonparametric, 2-tailed Mann–Whitney U test. For the biodistribution experiments, uptake in tissues and levels in blood were compared by use of a 1-way ANOVA. Bonferroni correction for multiple testing was applied. For the therapy experiments, survival curves were compared by use of the log–rank test. All tests were 2 sided; the level of statistical significance was set at a P value of <0.05.

RESULTS

Biodistribution of Radioiodinated MN-14

The biodistribution of radioiodinated MN-14 in this model of small peritoneal metastases after intraperitoneal or intravenous administration was described previously (*2*). In brief, from 24 h on, the levels in blood of intraperitoneally injected ^{131}I-MN-14 and intravenously injected ^{125}I-MN-14 were similar. In the first 24 h after administration, the intraperitoneal route resulted in higher uptake in tumors than the intravenous route. From 48 h after administration on, tumor uptake with the intraperitoneal route was similar to that with the intravenous route (approximately 50 %ID/g). When corrected for the physical decay of ^{131}I, the AUCs for the levels in blood were similar after both routes of administration (0.56 and 0.58 h × MBq/g), whereas the AUC for uptake in tumors after intraperitoneal administration was somewhat higher (2.46 vs. 1.96 h × MBq/g).

Comparison of Biodistributions of Radioiodinated MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 After Intraperitoneal Administration and After Intravenous Administration

The mean tumor weight for the mice that received radioiodinated MN-14 was comparable to that for the mice that received ^{186}Re-MN-14 or ^{88}Y-MN-14 (19.5 ± 24.2 and 16.1 ± 16.6 mg, respectively; $P = 0.50$). The levels in blood of radioiodinated MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 after intraperitoneal and intravenous administration are shown in Figure 1. From 24 h after administration on, the levels in blood of all of the radioimmunoconjugates were similar after both routes of administration. The tissue distributions of ^{186}Re-MN-14 and ^{88}Y-MN-14 after both routes of administration are summarized in Tables 2 and 3, respectively. At all time points, the uptake in the liver and spleen of ^{88}Y-MN-14 was higher than that of radioiodinated MN-14 and ^{186}Re-MN-14 after both routes of administration. The uptake in the liver of ^{88}Y-MN-14 remained higher after intravenous administration than after intraperitoneal administration throughout the experiment, whereas the values for uptake in the spleen were similar at all time points. From 48 h after administration on, the uptake of ^{88}Y-MN-14 in bone (represented by the femur) was significantly higher than that of ^{186}Re-MN-14. However, the maximum uptake of ^{88}Y-MN-14 in bone was very low (2.6 ± 0.3 %ID/g at 72 h after intravenous administration). Values for uptake in other normal tissues were similar for all of the radioimmunoconjugates after both routes of administration.

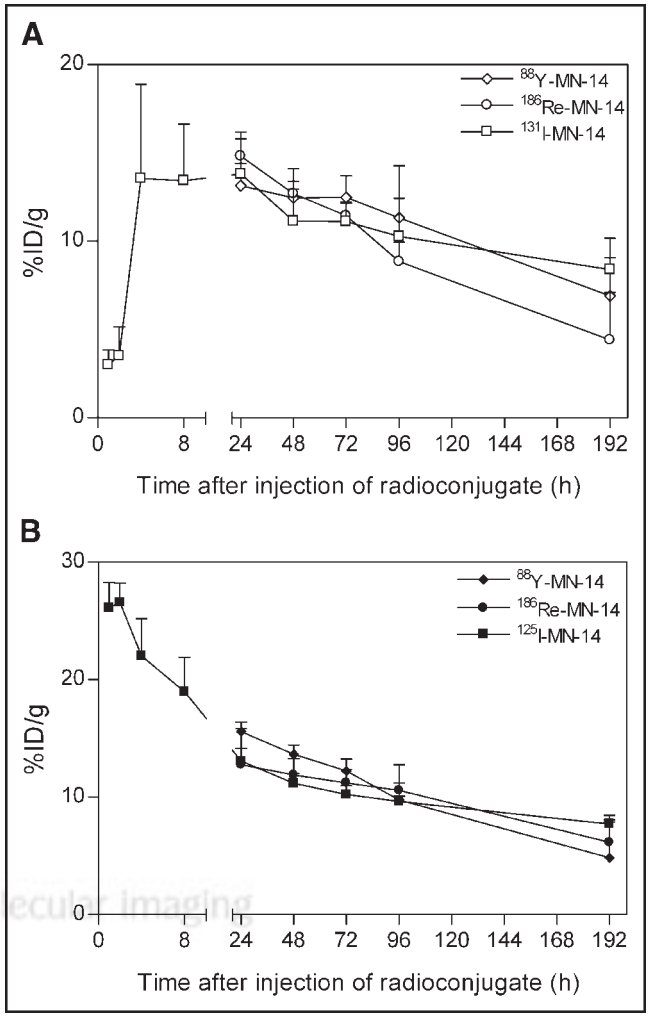

FIGURE 1. (A) Levels in blood after intraperitoneal administration of ^{131}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14. Values are given as mean ± SD (5 mice per group). (B) Levels in blood after intravenous administration of ^{125}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14. Values are given as mean ± SD (5 mice per group).

Figure 2 shows the uptake in tumors of ^{131}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 after intraperitoneal and intravenous administration. The uptake in tumors of ^{88}Y-MN-14 was higher than that of ^{186}Re-MN-14 or radioiodinated MN-14 at all time points, except for 24 h after intravenous administration. The maximum values for uptake in tumors after intraperitoneal administration of ^{131}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 were 58.5 ± 6.8 %ID/g (24 h after administration), 83.4 ± 18.5 %ID/g (72 h after administration), and 148.1 ± 89.4 %ID/g (192 h after administration), respectively.

The ratios of the uptake of the radiolabeled mAbs in tumor and the blood levels of the radiolabeled mAbs (tumor-to-blood ratios) after intraperitoneal and intravenous

TABLE 2
Tissue Distributions of Intraperitoneally and Intravenously Administered ^{186}Re-MN-14 in Nude Mice Bearing Intraperitoneal LS174T Colon Cancer Xenografts

Administration	Organ	Mean ± SD (n = 5) %ID/g at following hour after injection of radioconjugate:				
		24	48	72	96	192
Intraperitoneal	Tumor	29.9 ± 7.3	77.8 ± 31.4	83.4 ± 18.5	66.0 ± 5.4	50.3 ± 29.2
	Blood	14.8 ± 1.0	12.7 ± 0.7	11.4 ± 0.8	8.9 ± 1.1	4.4 ± 2.7
	Muscle	1.24 ± 0.4	1.2 ± 0.1	1.2 ± 0.1	1.6 ± 1.4	0.5 ± 0.2
	Lungs	8.3 ± 1.2	7.0 ± 0.8	8.4 ± 1.5	6.6 ± 1.1	3.8 ± 2.2
	Spleen	3.8 ± 0.7	3.5 ± 0.4	3.2 ± 0.4	2.3 ± 0.5	1.6 ± 0.6
	Kidneys	4.3 ± 0.2	3.7 ± 0.2	3.4 ± 0.1	3.2 ± 0.4	1.7 ± 0.7
	Liver	5.2 ± 0.7	4.8 ± 0.3	4.6 ± 0.5	3.9 ± 0.5	2.0 ± 0.7
	Intestine	3.5 ± 0.8	1.8 ± 0.3	2.1 ± 0.4	1.5 ± 0.5	0.7 ± 0.3
	Femur	0.8 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
Intravenous	Tumor	58.1 ± 18.4	50.3 ± 21.1	68.2 ± 10.7	65.1 ± 14.2	67.8 ± 42.3
	Blood	12.8 ± 3.0	11.9 ± 1.36	11.2 ± 1.1	10.6 ± 2.2	6.14 ± 1.9
	Muscle	0.9 ± 0.2	1.2 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.7 ± 0.4
	Lungs	7.8 ± 2.4	10.5 ± 1.1	8.5 ± 0.8	9.6 ± 1.3	5.8 ± 1.8
	Spleen	3.8 ± 1.1	3.2 ± 0.3	2.8 ± 0.3	2.5 ± 0.2	1.9 ± 0.4
	Kidneys	3.8 ± 0.8	3.8 ± 0.3	3.3 ± 0.4	2.9 ± 0.3	1.5 ± 1.0
	Liver	5.4 ± 1.2	4.7 ± 0.3	4.5 ± 0.8	3.5 ± 0.5	1.6 ± 1.1
	Intestine	2.8 ± 0.9	2.2 ± 0.3	1.7 ± 0.1	1.4 ± 0.1	1.2 ± 0.2
	Femur	0.9 ± 0.4	0.8 ± 0.4	1.0 ± 0.2	0.5 ± 0.1	0.5 ± 0.1

administration are shown in Figure 3. From 24 h after administration on, the tumor-to-blood ratio of radioiodinated MN-14 remained relatively stable (between 4.0 and 6.0), whereas the tumor-to-blood ratio of ^{186}Re-MN-14 steadily increased to 12.0 at 192 h after administration. The tumor-to-blood ratio of ^{88}Y-MN-14 was higher than those of both radioiodinated MN-14 and ^{186}Re-MN-14 at every time point. The maximum tumor-to-blood ratio of ^{88}Y-MN-14 was reached at 192 h after intravenous administration (mean ± SD, 24.7 ± 11.0).

Tumor Absorbed Radiation Dose

Dosimetric analysis of the biodistribution data (summarized in Fig. 2 and Tables 2 and 3) by use of MIRDOSE3 methodology indicated that intraperitoneal administration of

TABLE 3
Tissue Distributions of Intraperitoneally and Intravenously Administered ^{88}Y-MN-14 in Nude Mice Bearing Intraperitoneal LS174T Colon Cancer Xenografts

Administration	Organ	Mean ± SD (n = 5) %ID/g at following hour after injection of radioconjugate:				
		24	48	72	96	192
Intraperitoneal	Tumor	90.8 ± 13.4	83.5 ± 29.0	139.5 ± 22.9	128.5 ± 26.5	148.1 ± 89.4
	Blood	13.2 ± 1.3	12.4 ± 1.7	12.5 ± 1.2	11.3 ± 3.0	6.9 ± 2.2
	Muscle	0.9 ± 0.1	1.4 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.5
	Lungs	7.9 ± 1.2	11.3 ± 1.5	10.0 ± 1.0	10.5 ± 2.0	7.2 ± 2.3
	Spleen	4.9 ± 0.8	5.2 ± 0.5	5.8 ± 0.8	5.9 ± 0.9	7.9 ± 1.3
	Kidneys	4.0 ± 0.3	4.2 ± 0.4	3.9 ± 0.4	3.3 ± 0.6	1.6 ± 1.5
	Liver	6.6 ± 0.6	6.7 ± 1.1	7.4 ± 1.3	6.8 ± 1.3	4.8 ± 3.1
	Intestine	2.9 ± 0.7	2.5 ± 0.5	2.0 ± 0.1	1.7 ± 0.3	1.7 ± 0.3
	Femur	0.9 ± 0.2	1.3 ± 0.5	2.0 ± 0.4	1.3 ± 0.2	2.4 ± 0.7
Intravenous	Tumor	27.4 ± 9.4	105.3 ± 50.5	130.5 ± 49.9	115.1 ± 5.4	104.3 ± 55.9
	Blood	15.6 ± 0.8	13.6 ± 0.8	12.2 ± 1.0	9.8 ± 1.5	4.9 ± 3.0
	Muscle	1.0 ± 0.2	1.2 ± 0.1	1.3 ± 0.2	2.3 ± 2.6	0.6 ± 0.3
	Lungs	8.8 ± 1.2	8.0 ± 0.9	9.4 ± 1.9	8.0 ± 1.4	4.7 ± 2.4
	Spleen	5.1 ± 0.9	6.8 ± 0.4	6.4 ± 0.9	5.8 ± 0.6	7.1 ± 0.8
	Kidneys	4.4 ± 0.2	4.1 ± 0.2	3.9 ± 0.3	3.8 ± 0.5	2.3 ± 0.9
	Liver	9.5 ± 0.8	9.8 ± 0.6	9.4 ± 0.9	10.1 ± 1.9	8.9 ± 2.2
	Intestine	3.6 ± 0.8	2.0 ± 0.4	2.5 ± 0.4	1.9 ± 0.7	0.9 ± 0.3
	Femur	0.6 ± 0.3	2.0 ± 0.5	2.6 ± 0.3	1.5 ± 0.2	2.1 ± 0.8

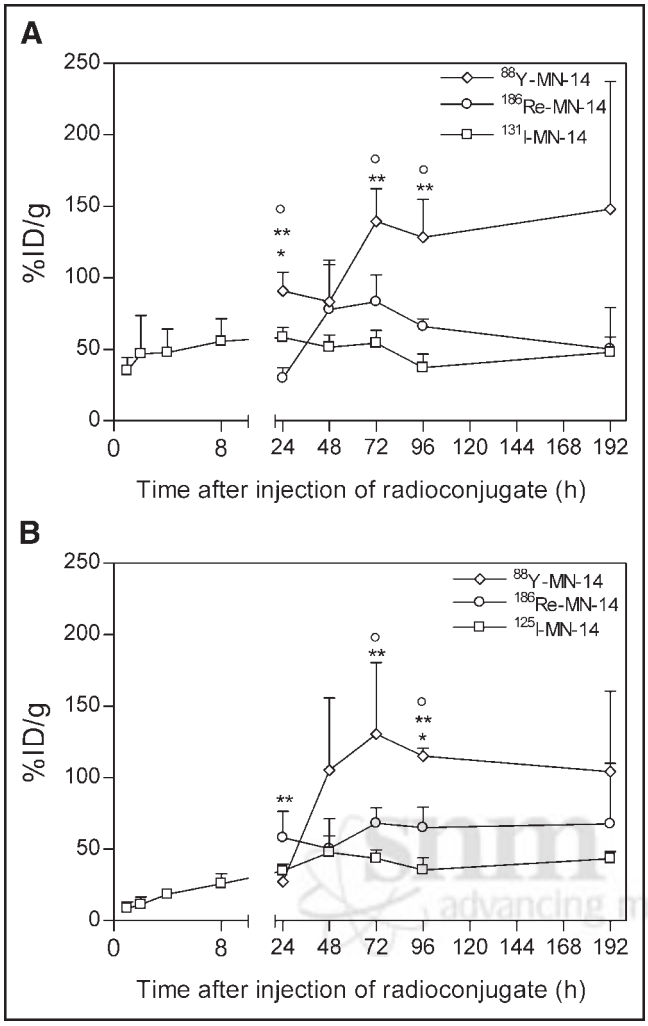

FIGURE 2. (A) Uptake of ^{131}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-1 in peritoneal LS174T tumor xenografts after intraperitoneal administration. Values are given as mean ± SD (5 mice per group). Double asterisks indicate significant differences between ^{88}Y and ^{186}Re, single asterisks indicate significant differences between ^{186}Re and ^{131}I, and circles indicate significant differences between ^{88}Y and ^{131}I (1-way ANOVA with Bonferroni correction). (B) Uptake of ^{125}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 in peritoneal LS174T tumor xenografts after intravenous administration. Values are given as mean ± SD (5 mice per group). Double asterisks indicate significant differences between ^{88}Y and ^{186}Re, single asterisks indicate significant differences between ^{186}Re and ^{125}I, and circles indicate significant differences between ^{88}Y and ^{125}I (1-way ANOVA with Bonferroni correction).

equally toxic activity doses (50% the MTDs) of ^{131}I-MN-14 (9.25 MBq per mouse), ^{186}Re-MN-14 (9.25 MBq per mouse), ^{90}Y-MN-14 (3.15 MBq per mouse), and ^{177}Lu-MN-14 (8.33 MBq per mouse) would result in tumor absorbed radiation doses of 150, 100, 45, and 200 Gy, respectively. For these calculations, it was assumed that the weight of the tumor nodules was 10 mg, corresponding to a diameter of 2.6 mm.

Radioimmunotherapy

Three mice developed subcutaneous tumors at the site of tumor cell inoculation and therefore were excluded from the analysis. Thirty-three mice were killed when their abdominal circumference had increased by 10% because of intraperitoneal tumor growth. The tumor weight (mean ± SD) in these mice was 2.23 ± 1.10 g. Furthermore, 5 mice were killed because of 20% loss of body weight, and 3 additional mice were killed because of poor clinical condition without weight loss reaching 20% or abdominal circumference increase reaching 10%. The tumor weights in these mice were 1.52 ± 1.16 and 1.79 ± 0.41 g, respectively; these values were not statistically significantly different from the tumor loads found in the 33 mice mentioned above ($P = 0.35$).

The survival curves for the different treatment groups are shown in Figure 4. The median survival time for the control

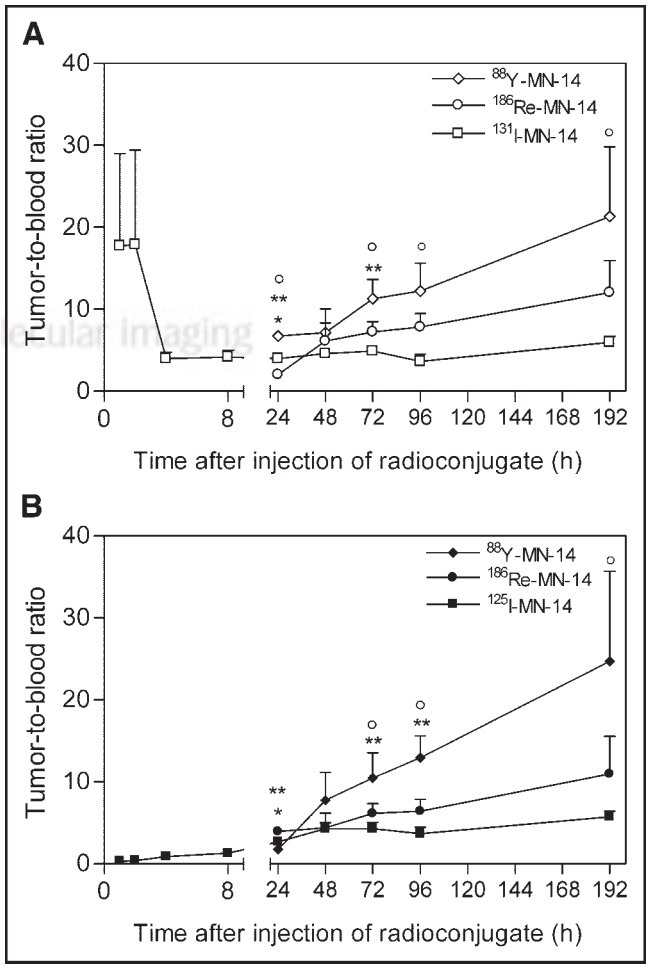

FIGURE 3. (A) Tumor-to-blood ratios after intraperitoneal administration of ^{131}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-1 in mice bearing peritoneal LS174T tumor xenografts. Values are given as mean ± SD (5 mice per group). Symbols are as described in the legend to Figure 2A. (B) Tumor-to-blood ratios after intravenous administration of ^{125}I-MN-14, ^{186}Re-MN-14, and ^{88}Y-MN-14 in mice bearing peritoneal LS174T tumor xenografts. Values are given as mean ± SD (5 mice per group). Symbols are as described in the legend to Figure 2B.

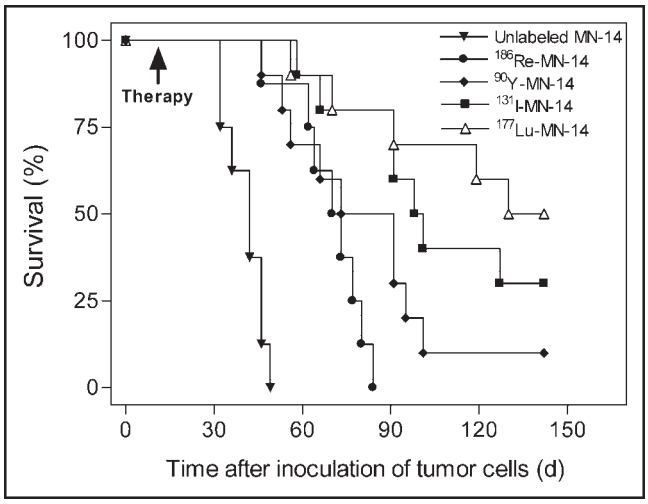

FIGURE 4. Survival curves for mice bearing peritoneal LS174T tumor xenografts after intraperitoneal administration of ^{131}I-MN-14 (9.25 MBq per mouse), ^{186}Re-MN-14 (9.25 MBq per mouse), ^{90}Y-MN-14 (3.15 MBq per mouse), ^{177}Lu-MN-14 (8.33 MBq per mouse), and unlabeled MN-14 (8–10 mice per group). *P* values for comparisons of survival curves for various treatment groups are given in Table 4.

mice, which received unlabeled MN-14, was 42 d (range, 32–49). The median survival times for the mice treated with equally toxic activity doses of ^{131}I-MN-14 (9.25 MBq), ^{186}Re-MN-14 (9.25 MBq), ^{90}Y-MN-14 (3.15 MBq), and ^{177}Lu-MN-14 (8.33 MBq) were 100 d (range, 58–142; $P < 0.0001$), 72 d (range, 46–77; $P = 0.0002$), 82 d (range, 46–142; $P = 0.0001$), and 136 d (range, 56–142; $P < 0.0001$), respectively (*P* values, determined by use of the log-rank test, were for comparisons with mice receiving unlabeled MN-14 [control mice]). The *P* values for the differences among the survival curves for the various treatment groups are shown in Table 4.

At the end of the experiment (142 d after tumor cell inoculation), 9 mice (5 treated with ^{177}Lu-MN-14, 3 treated with ^{131}I-MN-14, and 1 treated with ^{90}Y-MN-14) had no signs of intraperitoneal tumor growth. At dissection, 1 mouse treated with ^{177}Lu-MN-14 had some tumor growth (total intraperitoneal tumor load, 0.65 g), whereas in the remaining 8 mice, there was no evidence of disease. On histopathologic examination of the diaphragm, greater omentum, pancreas, liver, spleen, and lungs, no residual disease was found in any of these mice.

DISCUSSION

The primary aim of the present study was to select the most suitable radionuclide for radioimmunotherapy of small peritoneal metastases of colorectal origin. Radioimmunotherapy with ^{177}Lu-MN-14 resulted in the best median survival time, 136 d; this survival time was significantly better than that seen after treatment with ^{186}Re-MN-14 (72 d) or ^{90}Y-MN-14 (82 d) but did not differ significantly from that seen after treatment with ^{131}I-MN-14 (100 d). No residual tumor was found by histopathologic examination 142 d after tumor cell inoculation in 4 of 10 mice treated with ^{177}Lu-MN-14, 3 of 10 mice treated with ^{131}I-MN-14, and 1 of 10 mice treated with ^{90}Y-MN-14; these mice were considered cured at the end of the study.

In the biodistribution experiments, ^{88}Y-MN-14 resulted in much higher uptake in tumors than did either $^{125/131}$I-MN-14 or ^{186}Re-MN-14. The higher uptake of ^{88}Y-MN-14 in the tumor nodules probably reflects the longer tumor residence time of ^{88}Y than of $^{125/131}$I or ^{186}Re, which ensues from differences in intratumoral catabolism among the various radiolabels (*13*). Although anti-CEA mAbs, such as MN-14, that bind to CEA epitopes on the tumor cell surface are internalized only slowly and to a limited extent (*14,15*), intratumoral catabolism of antibodies has been shown to be significant not only for rapidly internalized antibodies but also for antibodies that bind to the cell surface (*16*). After internalization by cancer cells, radiolabeled antibodies are enzymatically degraded and metabolized in lysosomes (*17,18*). After intralysosomal metabolism of mAbs that are radioiodinated by conventional methods, the radioiodinated tyrosine residues are excreted, thereby reducing the residence time of the radioiodine label in tumors (*13*). After catabolism of mAbs labeled with $^{88/90}$Y-DTPA or ^{177}Lu-DTPA, the catabolic products are the radiolabeled chelators bound to amino acids such as lysine (e.g., ^{88}Y-DTPA-lysine) (*19,20*). Whereas radioiodinated tyrosine is excreted

TABLE 4
P Values for Comparisons of Survival Curves for Mice Treated with Unlabeled MN-14 or MN-14 Labeled with Equally Toxic Activity Doses of ^{186}Re, ^{131}I, ^{90}Y, and ^{177}Lu

Radionuclide (median survival time, in d)	*P* value for:				
	Unlabeled MN-14	^{186}Re-MN-14	^{90}Y-MN-14	^{131}I-MN-14	^{177}Lu-MN-14
Unlabeled MN-14 (42)		0.0002	<0.0001	<0.0001	<0.0001
^{186}Re-MN-14 (72)	0.0002		0.11	0.0014	0.0012
^{90}Y-MN-14 (82)	<0.0001	0.11		0.10	0.02
^{131}I-MN-14 (100)	<0.0001	0.0014	0.10		0.36
^{177}Lu-MN-14 (136)	<0.0001	0.0012	0.02	0.36	

P values were determined by use of log-rank test.

by cells, $^{88/90}$Y-DTPA-lysine or ^{177}Lu-DTPA-lysine metabolites are trapped within lysosomes. Furthermore, because antibodies are metabolized by the liver and spleen, intracellular entrapment of the ^{88}Y radiolabel may also explain the higher uptake in these organs of this radiolabel than of $^{125/131}$I or ^{186}Re. To date, the fate and processing of ^{186}Re-MAG3-labeled antibodies bound to the surface of tumor cells have not been fully elucidated. Various studies, however, have shown that ^{186}Re is not retained in cells after intracellular catabolism (*17*,*21*). The higher uptake of ^{186}Re-MN-14 in tumors and the higher tumor-to-blood ratios of ^{186}Re-MN-14 than of radioiodinated MN-14 in our experiments, however, suggest that the catabolic product of ^{186}Re-MN-14 (presumably ^{186}Re-MAG3-lysine) may be released from cells at a lower rate than radioiodotyrosine.

In the therapy experiments, the administered activity doses of the different radioimmunoconjugates represented 50% the MTDs of the various radionuclides. The therapeutic efficacy of ^{90}Y-MN-14 was much lower than that of ^{177}Lu-MN-14 but did not differ significantly from that of ^{131}I-MN-14 or from that of ^{186}Re-MN-14. Because of a high mean β-emission, 935 keV, and consequently a relatively high tissue penetration depth (maximum, 12 mm), irradiation of small peritoneal metastases of only a few millimeters with ^{90}Y-MN-14 in this model is inefficient, because approximately 70% of the radiation energy is deposited outside the tumor xenografts. Furthermore, because of high-energy β-emission, the MTD of ^{90}Y-labeled IgG is lower than those of antibodies labeled with ^{131}I, ^{186}Re, and ^{177}Lu, which are similar. Indeed, dosimetric analysis of the biodistribution data indicated that the tumor absorbed radiation dose for ^{90}Y-MN-14 was much lower than those for the other radiolabels. Esteban et al. (*22*,*23*) studied the effects of ^{90}Y-based radioimmunotherapy with anti-CEA mAb ZCE025 in a similar model of small-volume peritoneal LS174T carcinomatosis. In that study, a clear dose–response effect was observed, although residual viable tumor growth was still found on histologic examination of mice 5 wk after administration of 4.44 MBq. Furthermore, Sharkey et al. (*24*) showed that in a mouse model of micrometastatic colon carcinoma in the lungs, radioimmunotherapy with ^{131}I was more effective than that with ^{90}Y. Therefore, ^{90}Y seems to be more appropriate in radioimmunotherapeutic applications for larger tumors, a conclusion in keeping with the findings of other investigations (*15*,*25*).

Although the dosimetric analysis correctly predicted that ^{177}Lu would be more efficacious than ^{186}Re and ^{90}Y, the apparently higher tumor absorbed radiation dose for ^{186}Re than for ^{90}Y did not result in improved survival. This discrepancy may be explained by several factors. First, the suboptimal protein dose of the ^{186}Re-MN-14 preparation (28 μg) could have had a negative impact on therapeutic efficacy, because it was previously demonstrated that tumor uptake is optimal up to antibody protein doses of 25 μg. Second, this discrepancy could have been attributable to inaccuracies in the dosimetric analysis, because blood, bone marrow, liver, and spleen were not included as source organs in this analysis. It is possible that the relatively high uptake of ^{90}Y-MN-14 in the liver and spleen (approximately 7 %ID/g after intraperitoneal administration) in combination with the high-energy β-emission (maximum tissue penetration depth, 12 mm) contributed to the sterilization of the small peritoneal metastases in the upper abdomen. Another explanation for the observed discrepancy between the survival time and the tumor absorbed radiation dose may be the failure of the animal model to reveal small differences in therapeutic efficacy.

Treatment of mice with ^{177}Lu-MN-14 resulted in the highest median survival time, 136 d; however, this survival time did not differ significantly from that obtained after treatment with ^{131}I-MN-14 (100 d) ($P = 0.36$). Given the longer intratumoral residence time of ^{177}Lu than of ^{131}I or ^{186}Re, the former radionuclide, with a medium-energy β-emission (maximum tissue penetration depth, 2.5 mm) and a half-life of almost 1 wk, seems to be very well suited for the treatment of microscopic or small-volume disease. From a clinical point of view, the radiophysical characteristics of ^{177}Lu may be more favorable than those of ^{131}I or ^{90}Y. First, because the half-life of ^{177}Lu (6.7 d) is longer than that of ^{90}Y (2.3 d), bone marrow toxicity may be lower because less of the decay occurs in the early time period after administration, when levels in blood are relatively high and uptake in the tumor is still low. Simultaneously, after intratumoral accumulation of the radiolabeled antibody, the tumor is irradiated over a prolonged period of time. Finally, the low-abundance, moderate-energy γ-rays emitted by ^{177}Lu pose fewer radiation safety issues than do those of ^{131}I for the patient's family and for health care personnel. In fact, promising results were reported by Alvarez et al. (*26*), who treated 27 patients with chemotherapy-refractory ovarian cancer by using intraperitoneal radioimmunotherapy with ^{177}Lu-labeled IgG antibody CC-49. Antitumor effects were noted even at lower dose levels, whereas patients with microscopic disease showed longer disease-free survival than did historic controls.

CONCLUSION

The uptake of ^{88}Y-MN-14 in small peritoneal LS174T xenografts was higher than that of ^{186}Re-MN-14 or ^{131}I-MN-14. At equally toxic activity doses, the therapeutic efficacy of ^{177}Lu-MN-14 was better than that of ^{186}Re-MN-14 or ^{90}Y-MN-14 but did not differ significantly from that of ^{131}I-MN-14, in keeping with the results of the dosimetric analysis. The results of these studies indicate that ^{177}Lu and ^{131}I are the most suitable radionuclides for the radioimmunotherapy of small peritoneal metastases and may be the best candidates for adjuvant treatment of patients at high risk for the development of intraperitoneal relapse of colorectal cancer.

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