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A ^{90}Y labeled phosphorodiamidate morpholino oligomer for pretargeting radiotherapy

Guozheng Liu*, Shuping Dou, Yuxia Liu, Yuzhen Wang, Mary Rusckowski, and Donald J Hnatowich

Department of Radiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Abstract

While ^{188}Re has been used successfully in mice for tumor radiotherapy by MORF/cMORF pretargeting, previous radiolabeling of the 18 mer amine-derivatized cMORF with ^{90}Y , a longer physical half life nuclide, was not very successful. After developing a method involving a pre-purification heating step during conjugation that increases labeling efficiency and label stability, the biodistribution of ^{90}Y -DOTA-Bn-SCN-cMORF was measured in normal mice and in MORF-CC49 pretargeted mice that bear LS174T tumors. Absorbed radiation doses were then estimated and compared to those estimated for ^{188}Re . The pharmacokinetics of the ^{90}Y -DOTA-cMORF in normal mice and in the pretargeted nude mice was similar to that observed previously with $^{99\text{m}}\text{Tc}$ - and ^{188}Re -MAG₃-cMORFs. While the ^{90}Y -DOTA-cMORF cleared rapidly from normal tissues, tumor clearance was very slow and tumor radioactivity accumulation was constant for at least 7 days such that the tumor/blood (T/B) ratio increased linearly from 6 to 25 over this period. Therefore by extrapolation, normal tissue toxicities following administration of therapeutic doses of ^{90}Y may be comparable to that observed for ^{188}Re in which the T/B increased from 5 to 20. In conclusion, radiolabeling of DOTA-cMORF with ^{90}Y was improved by introducing a prepurification heating step during conjugation. The ^{90}Y -DOTA-cMORF provided a similar T/B ratio and biodistribution to that of ^{188}Re -MAG₃-cMORF and retained well in the tumor pretargeted with MORF-CC49. Because of the longer physical half life, the T/NT absorbed radiation dose ratios were improved in most organs and especially in blood.

INTRODUCTION

The pretargeting using a pair of morpholino oligomers (MORF/cMORF) has been shown to be effective in preclinical studies.^{1, 2} A major advantage of the MORF/cMORF pretargeting approach is the ease with which the cMORF effector can be chemically modified, for example to change chelators or linkers for different radionuclides, without affecting the cMORF/MORF affinity critical to the pretargeting approach. Thus far, this effector has been radiolabeled with $^{99\text{m}}\text{Tc}$ and ^{111}In for imaging and ^{188}Re for radiotherapy.^{2–4} We now report on the radiolabeling of the cMORF with ^{90}Y , biodistribution of ^{90}Y -cMORF, and the estimated absorbed radiation dose improvements using ^{90}Y -cMORF/MORF pretargeting compared to ^{188}Re -cMORF/MORF pretargeting. Although ^{90}Y and ^{188}Re have similar average beta energies (0.935 vs 0.716 Mev respectively), under identical conditions (i.e. tumor retention and clearance from normal tissues) the longer physical half life of ^{90}Y (64

*Request for reprints: Guozheng Liu, Ph D, Division of Nuclear Medicine, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655-0243. Phone: 508-856-1958; Fax: 508-856-6363. guozheng.liu@umassmed.edu.

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h) compared to ^{188}Re (17 h) will provide higher tumor to organ absorbed radiation dose ratios because a larger portion of the ^{90}Y in circulation will be cleared without depositing its energy in the normal tissues.

Previous ^{90}Y labeling using para-isothiocyanate benzyl DOTA (i.e. p-SCN-Bn-DOTA) as a chelator was judged to be not very successful due to low labeling efficiencies.⁵ A possible explanation for these marginal results may have been identified in a subsequent ^{111}In labeling of cMORF using p-SCN-Bn-DTPA as a chelator, in which heat sensitive side conjugation products were observed.⁶ Because introduction of a heating step after conjugation reaction and before purification, after subsequent labeling, was found to greatly improve label stability in that study, a similar approach was applied in this investigation using p-SCN-Bn-DOTA.

After the successful ^{90}Y labeling, the biodistribution of cMORF was measured and the absorbed radiation doses to normal organs and tumor pretargeted with MORF-CC49 were estimated and compared with those previously for ^{188}Re -cMORF in the identical tumor model.^{3, 4} The dosages and timing determined to be optimal with $^{99\text{m}}\text{Tc}$ -cMORF⁷ and used previously with ^{188}Re -cMORF⁴ were again used in this study, because the antibody is the same and the blood clearance among $^{99\text{m}}\text{Tc}$, ^{188}Re and ^{90}Y labeled cMORFs was found to be similar. For the same reason and as explained below, the relationship between tumor accumulation of ^{90}Y -cMORF and tumor size was assumed and also confirmed to be the same as that for ^{188}Re -cMORF and for $^{99\text{m}}\text{Tc}$ -cMORF.

MATERIALS AND METHODS

The MORF (5'-TCTTCTACTTCACAACATA) and cMORF (5'-TAGTTGTGAAGTAGAAGA) were obtained from Gene-Tools (Philomath, OR) with a primary amine attached to the 3' equivalent terminal via a 3-carbon linker. The p-SCN-Bn-DTPA or -DOTA was from Macrocyclics (Dallas, TX). The P4 resin (Bio-Gel P-4 Gel, medium) was from Bio-Rad Laboratories (Hercules, CA). The $^{111}\text{InCl}_3$ and $^{90}\text{YCl}_3$ was from Perkin Elmer Life Science Inc (Boston, MA). The CC49 antibody was conjugated with MORF as previously described.^{7, 8} All other chemicals were reagent grade and were used without purification.

The cMORF, MORF and antibody concentrations were determined by UV spectrophotometry. Size exclusion HPLC was used for the cMORF analysis. The HPLC system was equipped with a SuperdexTM 75 column (optimal separation range: 1×10^2 to 7×10^3 Da; Amersham Pharmacia Biotech, Piscataway, NJ) and with both UV and radioactivity in-line detectors. The eluant was 0.10 M pH 7.2 phosphate buffer at a flow rate of 0.60 mL/min. Radioactivity recovery was routinely measured and was always greater than 90%.

^{90}Y measurements

Because ^{90}Y is a pure beta emitter, accurate quantitation of radioactivity in organs can be difficult under certain circumstances.⁹ In this investigation, we confirmed that radioactivity of liquid ^{90}Y samples may be accurately measured in a NaI(Tl) well detector by using standards,⁹⁻¹¹ provided that the volume was the same, the samples were in the same type of counting tube, and especially that the radioactivity was homogeneously distributed. As such, tissue samples were digested in the SOLVABLE solution (PerkinElmer, Waltham, MA) in 5-mL polystyrene tubes and the volume was adjusted to 2.5 mL before counting. The ^{90}Y radioactivity value provided by the manufacturer was considered to be accurate and was used to calibrate the NaI(Tl) well counter.

DOTA conjugation and ^{90}Y labeling of cMORF

In a previous study,⁶ after conjugation with p-SCN-Bn-DTPA, the cMORF was radiolabeled efficiently with ^{111}In but the radiolabel was shown to be unstable to heating. Suspecting that the conjugation procedure had produced both stable and unstable conjugates, heating the reaction mixture before purification in the conjugation process yielded a product that provided both a high radiolabeling efficiency and a stable ^{111}In label. The same approach was applied in this investigation to the conjugation of cMORF with p-SCN-Bn-DOTA. Thus 2.5 mg of p-SCN-Bn-DOTA was dissolved in 0.25 mL of a 0.5 M $\text{Na}_2\text{CO}_3\text{-NaHCO}_3$ buffer at pH 9.8 and mixed with 2.3 mg of lyophilized 3'-amine derivatized cMORF. After an overnight incubation at room temperature, 1 mL of 0.25 M NH_4OAc buffer at pH 5.2 was added and the conjugation solution was heated at 100 °C for about 3 h before loading onto a 1 × 50 cm open P4 column. The column was eluted with the 0.25 M NH_4OAc buffer and three peak fractions of about 0.6 mL were pooled. The radiolabeling was achieved by adding 1–3 μL of $^{90}\text{YCl}_3$ solution in 50 mM HCl to 10–30 μL of the purified DOTA-cMORF solution followed by heating at 100 °C for 10 min. All animal studies were performed with ^{90}Y -DOTA-Bn-SCN-cMORF prepared in this fashion (^{90}Y -cMORF).

In vitro stability of ^{90}Y -cMORF

The ^{90}Y -cMORF was tested for radiolabel stability in phosphate buffer, in saline, and in fresh mouse serum. Specifically, 60 μL of the ^{90}Y -cMORF solution was added to 250 μL of each medium before incubation either at room temperature (phosphate buffer and saline) or 37 °C (mouse serum). An aliquot was removed for HPLC analysis at different times over 48 h. In addition, the ^{90}Y -cMORF was radiolabeled at different specific activities up to 3.5 mCi/37 μg of cMORF in the final volume of 80 μL . The radiolabel stability was examined after 20 h at room temperature by HPLC for evidence of radiolysis.

Pharmacokinetics of ^{90}Y -cMORF

Optimization of a pretargeting protocol requires information on pharmacokinetics.^{7, 12–15} Accordingly, the in vivo behavior of the ^{90}Y labeled cMORF was again measured. Normal CD-1 mice (Charles River Labs, MA) in 5 groups (N=4) each received 1 μg (45 μCi) of ^{90}Y -cMORF by a tail vein injection and were euthanized at 10 min, 0.5, 1, 3 and 6 h. Organs were harvested, digested, and counted as described above. The %ID and %ID/g were calculated against a standard of the injectate. All animal studies were performed with the approval of the UMMS Institutional Animal Care and Use Committee.

Subsequently, the dosage and timing parameters for pretargeting were selected following the guide lines described previously.¹⁵ Any convenient dosage of the pretargeting antibody may be selected that does not saturate the accessible tumor antigens and any pretargeting interval may be used that provides an acceptable antibody tumor/normal tissue ratio. Because the antibody and the mouse tumor model were the same, the antibody dosage and the pretargeting interval used in this study were identical to those previously used for $^{99\text{m}}\text{Tc}$ -cMORF and ^{188}Re -cMORF.^{4, 7} The optimal dosage of the cMORF effector is related to antibody dosage, pretargeting interval and its own blood clearance curve. Because the blood clearance of ^{90}Y -cMORF was shown to be sufficiently similar to those of $^{99\text{m}}\text{Tc}$ -cMORF and ^{188}Re -cMORF, the optimal cMORF dosage determined previously was used again.

Twenty NIH Swiss Nude mice (Taconic Farms, Germantown, NY) each received 10^6 LS174T colon cancer cells in the left thigh. After 11 days, when the tumors were about 0.3 g, each mouse received via a tail vein 30 μg MORF-CC49 (MORFs per antibody = 0.68) and 48 h later 2.5 μg of ^{90}Y -MORF (50 μCi). Thus the cMORF/MORF molar ratio was close to the optimal 3.1.^{4, 7} The biodistribution of the ^{90}Y -cMORF was followed for 7 days. The target thigh containing the tumor and the contralateral thigh were excised at the same

anatomical position. The skin on both thighs was removed, and the tumor weight was calculated by subtracting the weight of the normal thigh from that of the tumored thigh. After the bone and as much of the muscle tissues as possible were removed, the tumor was cut into pieces of 0.3–0.4 g, digested, and the radioactivity was measured in a NaI(Tl) well counter along with other organs as described above. Because the dosages and timing in this study are assumed to be optimal based on the above discussion, the tumor accumulations of the effector may therefore be assumed to be at their MPTAs (Maximum Percent Tumor Accumulations, defined as the percent tumor accumulation under the condition when the MORFs in tumor are just saturated by the cMORF).^{14, 15}

Absorbed radiation dose estimates

The estimated absorbed radiation doses to normal organs and to tumor by pretargeting with ⁹⁰Y-cMORF were compared with those by pretargeting with ¹⁸⁸Re-cMORF. The AUCs (area under the radioactivity-time curve, also known as cumulated radioactivity concentration) were calculated from the best fits to the %ID/g data with decay corrections, to estimate the absorbed radiation doses for the normal organs of interest. Using the self-absorbed model^{16, 17} as in our previous ¹⁸⁸Re studies,^{3, 4} the absorbed radiation dose in Rads was calculated by the expression $2.143 \times 0.935 \text{ (MeV)} \times \text{AUC } (\mu\text{Ci} \cdot \text{h/g})$, where 2.143 is a unit conversion factor and 0.935 MeV is the average energy of the ⁹⁰Y beta particles.

Estimating the absorbed radiation dose to tumor is more complicated, since tumor accumulation is strongly related to tumor size^{18–20} and the tumor size is an unpredictable variable among these studies. Therefore a comparison of tumor dose for different effectors requires normalizing the tumor accumulation to a certain tumor size using its accumulation vs. size relationship under optimal dosage and timing (i.e. at the MPTA). The relationship for ^{99m}Tc-cMORF was previously measured accurately for a substantial number of animals and tumor sizes.¹⁸ Because the in vivo behavior of cMORF was found to be sufficiently independent of the radiolabels of ^{99m}Tc, ¹⁸⁸Re and ⁹⁰Y, the MPTA-tumor size relationship for ^{99m}Tc-cMORF was assumed to be the same for ¹⁸⁸Re-cMORF and ⁹⁰Y-cMORF. This assumption was confirmed by comparing the predicted and observed tumor accumulations (see below). In the absorbed radiation dose comparison between ⁹⁰Y-cMORF and ¹⁸⁸Re-cMORF, a tumor size of 0.5 g was selected, since most tumors in this and our previous studies fall roughly in the range between 0.25 to 1.0 g.

RESULTS

DOTA conjugation and ⁹⁰Y labeling of cMORF

Similar to the conjugation with p-SCN-Bn-DTPA,⁶ conjugation of cMORF with p-SCN-Bn-DOTA without pre-purification heating yields a conjugate that provides a high ¹¹¹In labeling efficiency (Fig 1A, a) but with label instability leading to partial decomposition after heating at 100°C for 1 h (Fig 1A, b). The equivalent results with ⁹⁰Y are essentially identical (Fig 1A, c and d). However, after introducing a heating step after conjugation but before purification, the conjugation product can radiolabeled with either ¹¹¹In or ⁹⁰Y at high efficiency and the label becomes stable against heating (Fig 1A, e and g, respectively). The hybridization ability of the cMORF after conjugation and radiolabeling with either ¹¹¹In or ⁹⁰Y is preserved, as shown by the shift to higher molecular weight of the labeled cMORF peaks upon the addition of its MORF complement (Fig 1A, f and h, respectively).

In vitro stability of ⁹⁰Y-cMORF

As shown in Fig 1B, b and c, after introducing the heating step into the conjugation procedure, the ⁹⁰Y radiolabel on cMORF is also stable in phosphate buffer, saline, and serum at least for 48 h (the small peak at 21 min in c is due to self-association of cMORF at

room temperature). Fig 1 B, d–f presents the radiochromatograms of ^{90}Y -cMORF after 20 h of incubation at room temperature in the 80 μL labeling solution at values of specific radioactivity of 63, 1430, and 3440 $\mu\text{Ci}/37\text{ }\mu\text{g}$. The increasing decomposition products are most probably the result of radiolysis. To avoid complications due to radiolysis, samples of ^{90}Y -cMORF were used immediately after preparation.

Pharmacokinetics of ^{90}Y -cMORF

Table 1 presents the biodistributions at various times of ^{90}Y -cMORF (prepared by the modified conjugation that includes the pre-purification heating) in normal mice and in tumor-bearing mice pretargeted with MORF-CC49. As shown by the data in normal mice, the ^{90}Y -cMORF clears rapidly, similar to the $^{99\text{m}}\text{Tc}$ -cMORF and ^{188}Re -cMORF.^{21, 22} The blood and normal organ clearance is essentially completed by 3 h, although with slightly higher normal tissue levels.

Because of the longer half life of ^{90}Y compared to ^{188}Re , tumor retention and normal organ clearance could be followed for longer periods. As shown in Table 1, the tumor accumulation in %ID is fairly constant with time, while the tumor accumulation in %ID/g is decreasing due to tumor growth. As for normal organs, except in kidney, the background radioactivity is much lower than that in tumor and clears rapidly with time, similar to our earlier results with ^{188}Re -cMORF.^{3, 4} Fig 2(a–h) reproduces the normal organ ^{90}Y levels from 3 h to 7 days from the table. The solid lines represent the best fits and the dotted lines reproduced from the values for ^{188}Re in our earlier study.⁴

The tumor accumulation vs. size relationship for $^{99\text{m}}\text{Tc}$ -cMORF determined earlier in the identical animal model was: $\text{MPTA (\%ID/g)} = 4.51 \times \text{tumor weight (g)}^{-0.66}$.¹⁸ This relationship was assumed to hold true also for ^{90}Y -cMORF and ^{188}Re -cMORF, because the MPTA is proportional to the area under the blood clearance curve^{13–15} and, as Table 2 presents, the blood clearance of the three effectors are sufficiently similar. This assumption is supported by the results presented in Table 1. From the Table, the average tumor size at 3 h was $0.46 \pm 0.30\text{ g}$ and, based on the above relationship, the MPTA for ^{90}Y -cMORF is calculated to be 7.53 %ID/g, therefore in close agreement with the measured $7.15 \pm 2.19\text{ %ID/g}$ in Table 1. Previously, in the case of ^{188}Re -cMORF, the tumor accumulation was $8.66 \pm 1.18\text{ %ID/g}$ for a tumor size of 0.36 g,³ also in close agreement with the predicted value of 8.76 %ID/g. These agreements provide confidence in the following calculations of absorbed radiation doses in tumor using normalized tumor accumulation.

Absorbed radiation dose estimate

The AUCs for organs were calculated from the best fits to the biodistribution data with decay correction. The AUC for tumor was based on a predicted tumor accumulation of 7.13 %ID/g for a 0.5 g LS174T tumor obtained from the MPTA-tumor size relationship, assuming no tumor growth during the study. Both AUCs and the calculated absorbed radiation doses are listed in Table 3. Because of the higher accumulation and longer retention, the absorbed radiation dose to tumor by ^{90}Y pretargeting is at least 5-fold higher than those for other organs. The tumor dose would be even higher in a cohort of animals with smaller tumors than 0.5 g. For comparison, historical data for ^{188}Re pretargeting are also listed but again with the AUC and absorbed radiation dose for tumor size normalized to 0.5 g and calculated assuming no growth during therapy.

Fig 3 presents the T/NT AUC ratios (i.e. the absorbed radiation dose ratios or the therapeutic indexes²³) for ^{90}Y pretargeting and two previous ^{188}Re pretargeting studies in the same mouse model.^{3, 4} Although the normal tissue backgrounds are slightly higher for ^{90}Y (Fig 2), the T/NT AUC ratios are not lower but higher due to its longer half life.

DISCUSSION

Yttrium-90 is an attractive therapeutic radionuclide that has seen use in animal and patient studies while conjugated to antitumor antibodies. Of particular interest, ^{90}Y has been the radionuclide of choice in several pretargeting studies using avidin/biotin or the bispecific antibody/hapten recognition system.^{17, 24–38} In the development of tumor radiotherapy approaches by MORF/cMORF pretargeting, we have previously employed ^{188}Re as the therapeutic radionuclide.^{3, 4} We now consider ^{90}Y as an alternative of longer physical half life that can potentially improve radiotherapy effectiveness. Accordingly, in this investigation a method was developed to label this nuclide to the cMORF effector and to evaluate the properties of the ^{90}Y -cMORF in LS174T tumored NIH Swiss nude mice pretargeted with the MORF-CC49 antibody.

Although the radiolabeling of cMORF with ^{90}Y via p-SCN-Bz-DOTA was expected to present few difficulties, we observed instabilities of the ^{90}Y -cMORF, reminiscent of that observed previously when radiolabeling cMORF with ^{111}In via p-SCN-Bz-DTPA.⁶ A search of the literature provided no mention of similar instabilities when these bifunctional chelators were used to label antibodies. In our earlier study of ^{111}In -cMORF, we were able to resolve the label instability by including a prepurification heating step after conjugation. The same approach was applied in this investigation in connection with ^{90}Y -cMORF labeling and proved to be successful.

Change in the effector or pretargeting antibody would require the dosage and timing of the pretargeting protocol to be optimized. However, as we have now demonstrated, the pharmacokinetic and pretargeting behavior of cMORF is generally not influenced by the nature of the chelators and radiolabels at least among $^{99\text{m}}\text{Tc}$ -MAG₃-cMORF, ^{188}Re -MAG₃-cMORF and ^{90}Y -DOTA-Bn-SCN-cMORF. Accordingly, optimization of the dosage and timing parameters for ^{90}Y pretargeting became unnecessary since the values for $^{99\text{m}}\text{Tc}$ had been previously determined⁷ and can be used again with confidence. For the same reason, the tumor accumulation-size relationship useful for comparing potential therapeutic effect was assumed and confirmed to be the same as that for $^{99\text{m}}\text{Tc}$ established previously.

With the tracer level pretargeting data, potential therapeutic improvement of ^{90}Y as an alternative to ^{188}Re was evaluated. This evaluation was facilitated by using identical MORF and cMORF sequences, identical CC49 antibody and identical LS174T tumor model to that used previously.⁷ Since the average beta energies of ^{90}Y and ^{188}Re and the pharmacokinetics of the labeled cMORFs are similar, the T/NT dose ratio improvement is mainly due to the longer physical half life.

CONCLUSION

Radiolabeling of DOTA-cMORF with ^{90}Y was improved by introducing a prepurification heating step into the conjugation process. The T/B ratio and biodistribution of ^{90}Y -DOTA-cMORF labeled in this fashion were found to be similar to that observed previously for ^{188}Re -MAG₃-cMORF. Nevertheless, because of the longer physical half life of ^{90}Y , an improvement in therapeutic efficacy may be expected in future radiotherapy studies.

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Fig 1.

A: The HPLC radiochromatograms of ^{111}In and ^{90}Y labeled cMORFs after conjugating with p-SCN-Bn-DOTA without heating treatment (i.e. conventional) and radiolabeling with mild heating (a and c respectively) or heating at 100°C for 1 h (b and d respectively). Also presenting are the ^{111}In and ^{90}Y HPLC radiochromatograms of cMORF after conjugating with p-SCN-Bn-DOTA but after inclusion of the prepurification heating step during conjugation (i.e. modified) (e and g, respectively) as evidence of increased stability, and the corresponding radiochromatograms after addition of excess MORF (f and h respectively) as evidence of preserved hybridization affinity.

B: HPLC radiochromatograms of ^{90}Y -cMORF (a) immediately before and (b) 48 h after incubation in serum at 37°C and (c) 48 h after incubation in phosphate buffer at room temperature. Also presented are HPLC radiochromatograms of ^{90}Y -cMORF after 20 h of incubation at room temperature in the $80\ \mu\text{L}$ of labeling solution at specific radioactivities of 63, 1430, and $3440\ \mu\text{Ci}/37\ \mu\text{g}$ cMORF (d–f), showing decomposition most probably due to radiolysis.

Fig 2. Pharmacokinetics of ^{90}Y -cMORF in normal organs (a–h) of tumored mice pretargeted with MORF-CC49. The solid lines represent the best fits and the dashed lines represent the clearance curves of ^{188}Re -cMORF from a previous pretargeting study (reference 4). Error bars represent one standard deviation.

Fig 3. Histograms presenting the T/NT dose ratios (i.e. the tumor therapeutic indexes) for ^{90}Y and two previous ^{188}Re pretargeting studies in the same mouse model. a: Ref 4; b: Ref 3.

Biodistribution (%ID/g and %ID/organ) of ⁹⁰Y-cMORF in normal CD-1 mice (left) and in NIH Swiss nude mice bearing LS174T tumor xenograft and pretargeted with MORF-CC49 48 h earlier (right). Mean ± SD.

Table 1

	In normal CD-1 mice					In pretargeted nude mice				
	10min	30 min	1 h	3 h	6 h	3 h	26 h	71 h	119 h	166 h
%ID/g										
Liver	0.92±0.07	0.68±0.14	0.58±0.04	0.56±0.10	0.58±0.07	0.85±0.07	0.98±0.05	0.77±0.02	1.03±0.22	0.86±0.14
Heart	1.12±0.11	0.25±0.05	0.16±0.05	0.14±0.07	0.07±0.01	0.29±0.01	0.19±0.06	0.07±0.03	0.09±0.03	0.03±0.03
Kidney	9.88±1.59	4.38±0.19	3.58±0.40	3.56±0.25	3.84±0.37	4.49±1.99	1.86±0.40	0.89±0.14	0.88±0.13	0.57±0.10
Lung	1.87±0.49	0.94±0.36	1.11±0.98	0.89±0.87	0.59±0.21	0.60±0.11	0.30±0.06	0.19±0.06	0.16±0.06	0.09±0.02
Spleen	0.57±0.06	0.24±0.02	0.18±0.02	0.24±0.07	0.19±0.02	0.36±0.03	0.40±0.13	0.32±0.05	0.58±0.04	0.43±0.10
Muscle	0.89±0.10	0.19±0.03	0.05±0.01	0.04±0.00	0.04±0.01	0.20±0.05	0.10±0.01	0.04±0.01	0.03±0.01	0.02±0.01
Pancreas	0.92±0.22	0.31±0.07	0.13±0.01	0.09±0.01	0.06±0.01	0.25±0.03	0.13±0.02	0.07±0.02	0.10±0.05	0.05±0.02
Salivary	1.09±0.38	0.26±0.04	0.12±0.02	0.08±0.01	0.10±0.02	0.29±0.04	0.18±0.05	0.12±0.03	0.16±0.04	0.09±0.04
Blood	4.27±0.46	0.94±0.13	0.41±0.03	0.16±0.01	0.10±0.01	1.47±0.28	0.79±0.17	0.23±0.02	0.21±0.09	0.10±0.03
Tumor	---	---	---	---	---	7.15±2.19	5.21±1.98	2.89±1.07	3.95±2.07	2.43±1.08
%ID/organ										
Stomach	0.29±0.05	0.07±0.01	0.04±0.01	0.05±0.05	0.02±0.00	0.10±0.05	0.11±0.04	0.03±0.01	0.03±0.01	0.02±0.00
Sm. Int.	1.03±0.10	0.51±0.09	0.50±0.04	0.15±0.04	0.07±0.00	0.64±0.08	0.36±0.11	0.33±0.51	0.06±0.01	0.05±0.01
Lg. Int.	0.50±0.06	0.11±0.02	0.06±0.00	0.40±0.06	0.13±0.03	0.19±0.05	0.49±0.10	0.07±0.01	0.07±0.02	0.04±0.01
Tumor	---	---	---	---	---	2.77±0.79	2.70±0.40	2.78±0.94	3.16±0.49	2.67±0.51
Tumor weight (g)	---	---	---	---	---	0.46±0.30	0.56±0.19	1.00±0.26	1.01±0.56	0.90±0.34

Table 2

Blood levels (%ID/g) of ^{99m}Tc -cMORF, ^{188}Re -cMORF, and ^{90}Y -cMORF in normal CD-1 mice at different times post injection. Mean $\pm$ SD.

Time point	10 min	30 min	1 h	3 h
^{99m}Tc -cMORF	4.48 ^a	1.89 ^a	0.73 ^a	0.06 $\pm$ 0.01 ^b ; 0.04 $\pm$ 0.01 ^c
^{188}Re -cMORF	---	---	---	0.06 $\pm$ 0.01 ^c
^{90}Y -cMORF	4.27 $\pm$ 0.46 ^d	0.94 $\pm$ 0.13 ^d	0.41 $\pm$ 0.03 ^d	0.16 $\pm$ 0.01 ^d

^aRef 13;

^bRef 21;

^cRef 22;

^dthis study

Table 3

The AUCs (uCi*^h/g) and absorbed radiation doses (rads) for the LS174T tumor and organs by cMORF/MORF pretargeting for the administration of 1 μ Ci of ⁹⁰Y- or ¹⁸⁸Re-cMORF.

	Tumor*	Blood	Kidney	Liver	Spleen	Lung	Heart	Muscle	Salivary gland	Ref
AUC(uCi* ^h /g)										
⁹⁰ Y	6.53	0.46	1.36	0.81	0.37	0.22	0.11	0.06	0.12	This study
¹⁸⁸ Re	1.75	0.47	0.58	0.31	0.18	0.23	0.12	0.05	0.20	Ref 3
¹⁸⁸ Re	1.75	0.15	0.40	0.15	0.08	0.08	0.04	0.02	0.05	Ref 4
Dose (Rad)										
⁹⁰ Y	13.1	0.92	2.72	1.62	0.73	0.44	0.22	0.13	0.25	This study
¹⁸⁸ Re	2.89	0.77	0.95	0.51	0.30	0.38	0.20	0.09	0.32	Ref 3
¹⁸⁸ Re	2.89	0.24	0.65	0.24	0.13	0.13	0.06	0.04	0.08	Ref 4

* The AUC and absorbed radiation dose for tumor are calculated based on the predicted accumulation of 7.13 %ID/g for a tumor of 0.5 g under the assumption of no growth.