

Comparison of Pretargeted and Conventional CC49 Radioimmunotherapy Using ^{149}Pm , ^{166}Ho , and ^{177}Lu

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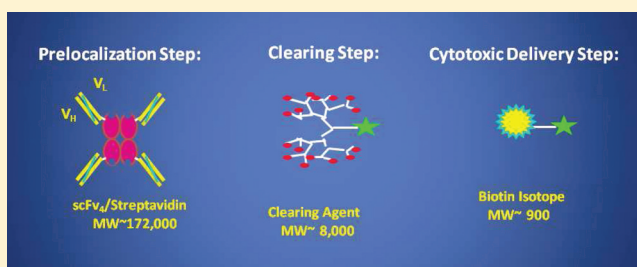
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ABSTRACT: The therapeutic efficacies of radiolabeled biotin, pretargeted by monoclonal antibody (mAb)–streptavidin fusion protein CC49 scFvSA, were compared to those of radiolabeled mAb CC49, using the three radiolanthanides in an animal model of human colon cancer. The purpose of the present study was to compare antibody pretargeting to conventional radioimmunotherapy using ^{149}Pm , ^{166}Ho , or ^{177}Lu . Nude mice bearing LS174T colon tumors were injected sequentially with CC49 scFvSA, the blood clearing agent biotin-GalNAC₁₆, and ^{149}Pm -, ^{166}Ho -, or ^{177}Lu -DOTA-biotin. Tumor-bearing mice were alternatively administered ^{149}Pm -, ^{166}Ho -, or ^{177}Lu -MeO-DOTA-CC49. Therapy with pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -DOTA-biotin increased the median time of progression to a 1 g tumor to 50, 41, and 50 days post-treatment, respectively. Therapy with ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -MeO-DOTA-CC49 increased the median time to progression to 53, 24, and 67 days post-treatment, respectively. In contrast, saline controls showed a median time to progression of 13 days postinjection. Treatment with pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -biotin or ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -CC49 increased tumor doubling time to 18–36 days, compared to 3 days for saline controls. Among treated mice, 23% survived >84 days post-therapy, and 11% survived 6 months, but controls survived <29 days. Long-term survivors showed tumor growth inhibition or partial regression, extensive necrosis in residual masses, and no evidence of nontarget tissue toxicity at necropsy. Both pretargeted and conventional RIT demonstrated considerable efficacy in an extremely aggressive animal model of cancer. Our results identified ^{177}Lu as an optimal radiolanthanide for future evaluation of these agents in toxicity and multiple-dose therapy studies.



INTRODUCTION

Radiolabeled monoclonal antibodies have been used extensively for radioimmunotherapy (RIT), most notably treatment of non-Hodgkin's lymphoma.¹ However, RIT is generally unsuccessful in solid tumor therapy due to insufficient tumor uptake without bone marrow toxicity. In addition, high tumor-to-background ratios generally cause radiolabeled mAbs to exhibit relatively poor imaging contrast.

The mAb CC49 is a pancarcinoma antibody² that targets the tumor-associated glycoprotein-72 (TAG-72).³ Immunohistochemical studies demonstrated that its first-generation mAb, B72.3, binds to the majority of human epithelial malignancies,^{4–9} including 82% of colon carcinomas,¹⁰ while being essentially nonreactive with most normal human tissues.¹¹ The mAb CC49 has the same range of immunoreactivity as B72.3.^{2,11} However, CC49 has a 6.4-fold higher binding affinity for TAG-72 than B72.3,² enabling it to react with a greater number of malignant cell types.¹¹

Compared to B72.3, ^{131}I -labeled CC49 showed greater tumor xenograft targeting¹² and improved therapeutic efficacy¹³ in nude mouse models. Clinical RIT trials of ^{131}I -CC49 demonstrated high tumor uptake, but did not produce any substantial responses.^{14–16} Therefore, a single-chain (scFv) construct of CC49 was developed and shown to have promise for tumor therapy in tumor-bearing mouse models.¹⁷ Additional improvements in RIT will likely require novel approaches, such as the use of different radionuclides, new antibody constructs, and novel delivery platforms.

First, the development of new therapeutic radionuclides, such as radiolanthanides, should be explored. The decay characteristics of three radiolanthanides for tumor therapy are compared to traditionally used ^{131}I and ^{90}Y in Table 1. All of these radiolanthanides have γ emissions suitable for tracking RIT

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Table 1. Decay Characteristics of Radionuclides for Tumor Therapy

radionuclide	$t_{1/2}$ (h)	β^- MeV (%)	γ keV (%)	range in tissue (mm)
^{149}Pm	53.1	0.78 (9) 1.06 (90)	286 (3.1)	5
^{166}Ho	26.9	1.76 (47) 1.84 (52)	81 (5.4)	9
^{177}Lu	159.6	0.497 (90)	208 (11)	2
^{131}I	192	0.606 (87)	364 (82)	3
^{90}Y	64.0	2.27 (100)	---	11

agents in living systems and estimating absorbed radiation doses to normal and malignant tissues. The radiolanthanides ^{149}Pm , ^{166}Ho , and ^{177}Lu have a range of half-lives and β^- energies for therapy. An advantage to using these radiolanthanides is that their chemistries are quite similar. DOTA, a macrocyclic chelating agent, complexes many radiometals, including lanthanides, with extremely high thermodynamic, kinetic, and *in vivo* stability.¹⁸ DOTA can be used for stable attachment of ^{149}Pm , ^{166}Ho , or ^{177}Lu to RIT constructs.

Antibody pretargeting is a RIT approach in which an antibody conjugate or fusion protein is attached to an artificial “receptor”. Such an agent is administered first and allowed to accumulate in tumors. Then, radionuclide therapy is given in the form of a small molecule that binds rapidly with high affinity to the mAb-receptor construct prelocalized to tumor cells. This process can cause immediate accumulation of therapeutic radiation in tumors, allowing for considerable improvements in tumor-to-background ratios, tumor absorbed dose, therapeutic efficacy, and normal organ toxicity.

Strategies for pretargeting include mAb/hapten,^{19–25} biotin/avidin or streptavidin,^{26–39} and oligonucleotide/antisense oligonucleotide analogue^{40–43} approaches. The high-affinity binding of biotin to streptavidin ($\sim 10^{13} \text{ M}^{-1}$) makes this system especially attractive for pretargeted RIT. Both mAb–biotin and mAb–streptavidin conjugates have been investigated for pretargeting of radiolabeled streptavidin and biotin, respectively. However, radiolabeled streptavidin has been shown to result in high kidney uptake and high liver uptake resulting from cross-linking of circulating biotinylated mAb, compromising tumor targeting.³⁶ Clearance of biotinylated mAb with cold streptavidin or avidin have only slightly increased tumor-to-blood ratios.^{44,45} Furthermore, radiolabeled streptavidin diffuses slowly into tumors over a 24 h period.³⁸ Thus, radiolabeled streptavidin can show considerable retardation in its tumor penetration. Pharmacokinetic modeling studies⁴⁶ indicated that a protocol involving radiolabeled biotin and mAb–streptavidin conjugates produces the highest tumor-to-blood ratio and residence time of radioactivity in tumors. Small, hydrophilic, and rapidly diffusible pretargeting agents undergoing rapid renal elimination, with minimal uptake in normal tissues, are ideal.⁴⁷

A chemical streptavidin conjugate⁴⁸ of CC49 was labeled *ex vivo* with ^{111}In -DTPA-biocytyl. This conjugate showed very similar tumor uptake and biodistribution as ^{111}In -DTPA-CC49. These studies suggested that streptavidin-CC49 constructs have potential for pretargeting applications.

As opposed to a whole antibody chemical conjugate, the use of a fusion protein based on the murine CC49 scFv construct and streptavidin (CC49 scFvSA)^{49,50} was evaluated. CC49 scFvSA forms a 176 kDa tetramer with high immunoreactivity

and high biotin binding efficiency and stability.^{49,51} Evidence of *in vivo* stability of the fusion protein was suggested by specific tumor uptake, as well as intended uptake in the liver following administration of a synthetic clearing agent.⁴⁹ The biodistribution of CC49 scFvSA-pretargeted ^{111}In -DOTA-biotin in nude mice bearing LS174T human colon cancer xenografts demonstrated very similar tumor uptake as a chemical conjugate, but with an increase in tumor-to-blood ratio.⁵²

CC49 scFvSA has also been used to pretarget ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -DOTA-biotin to LS174T colorectal tumors in nude mice.³⁵ Maximum tumor uptakes of ^{149}Pm (22.9% ID/g), ^{166}Ho (30.2% ID/g), and ^{177}Lu (35.4% ID/g) were achieved at 1 to 4 h postinjection. Extremely rapid blood clearance was accompanied by urinary excretion of 59–66% ID within 1 h. In LS174T-bearing nude mice, the biodistributions of CC49 scFvSA-pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -DOTA-biotin were very similar. Most normal tissue uptakes were nearly identical for all three radiometals at all time points. Thus, this pretargeting system provided a highly uniform delivery platform for the evaluation of new therapeutic lanthanide radionuclides.

However, the three radiolanthanides showed some significant differences in maximum LS174T xenograft uptake and washout of radioactivity from the tumor with time. Areas under the time–activity curves showed that ^{177}Lu had the highest tumor-to-blood ratio, an indication that it might provide the best “therapeutic index” for pretargeted RIT. Yet, preliminary dosimetry estimates suggested that washout reduced the mouse tumor absorbed dose from ^{177}Lu by 4- to 5-fold, compared to ^{149}Pm and ^{166}Ho . Therefore, differences in tumor uptake and retention, physical half-lives, and β^- particle path lengths may play important roles in determining the effectiveness of pretargeted RIT with these radiolanthanides. In the present studies, the therapeutic efficacies of radiolabeled biotin, pretargeted by CC49 scFvSA, were compared to those of intact radiolabeled mAb CC49, using the three radiolanthanides, ^{149}Pm , ^{166}Ho , and ^{177}Lu , in nude mice bearing human colon cancer xenografts.

■ EXPERIMENTAL PROCEDURES

General. $^{149}\text{PmCl}_3$ (50 mCi in 25–50 μL of 0.05 M HCl), $^{166}\text{HoCl}_3$ (80 mCi in 50–80 μL of 0.05 M HCl), and $^{177}\text{LuCl}_3$ (25 mCi in 10–20 μL of 0.05 M HCl) were obtained from the University of Missouri (MU) Research Reactor (MURR; Columbia, MO). The mAb–streptavidin fusion protein CC49 scFvSA,⁵¹ clearing agent biotin–GalNAc₁₆,⁵¹ and DOTA–biotin were obtained from NeoRx Corporation (Seattle, WA). Methoxy-DOTA (MeO–DOTA)⁵³ was provided by the DOW Chemical Company (Freeport, TX). CC49 was produced from the hybridoma cell line provided by Dr. Jeffrey Schlom at the National Cancer Institute (Bethesda, MD). Reversed-phase thin-layer chromatography (TLC) was performed on Whatman (Clifton, NJ) MKC₁₈F plates. The TLC plates were developed in 10% (w/v) ammonium acetate/methanol (1:1). Radio-TLC detection was accomplished using a Bioscan (Washington, DC) AR-2000 imaging scanner. The purity of the conventional radioimmunoconjugates was confirmed by gel filtration HPLC (GF-HPLC), using a Waters Delta 600 (Waters, Milford, MA) chromatograph equipped with a manual Rheodyne injector, a Waters 2487 dual-wavelength UV detector, a Packard 500TR Flow Scintillation Analyzer (Packard, Downers Grove, IL) with a LQ flow cell for ^{149}Pm , ^{166}Ho , and ^{177}Lu , a Waters busSAT/

IN analog–digital interface, and the Waters Millennium 32 software package. A Superose 12 HR 10/30 (Amersham Pharmacia, Torrance, CA) column (300 × 10 mm), an isocratic mobile phase of 100 mM NaH₂PO₄/0.05% NaN₃, pH 6.8, and a flow rate of 0.50 mL/min were used. All solutions were prepared using ultrapure water (18 MΩ-cm resistivity). All other reagents were purchased Fischer Scientific (Pittsburgh, PA). Outbred female nu/nu mice (4–6 weeks of age) were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Tumors were measured using Vernier calipers (Scienceware, D-2922/B/KWB).

Cell Line. The LS174T cell line was obtained from the American Type Culture Collection (Manassas, VA). Immediately prior to implantation into nude mice, the cells were tested for mycoplasma and screened for a panel of 13 murine pathogens by PCR. All test results were negative, and all sentinel mice in the facility housing the nude mice tested negative for these pathogens during the course of the studies.

Radiolabeling of Pretargeting RIT Conjugates. The labeling procedure used was previously described by Lewis et al.³⁵ Briefly, to 32.0 mCi of ¹⁴⁹Pm, 10.0 mCi of ¹⁶⁶Ho, or 25.0 mCi of ¹⁷⁷Lu in 80 μL of 0.20 M ammonium acetate, pH 5.0, containing approximately 1 mg/mL of gentisic acid, was added 32 μg of DOTA–biotin, 10 μg of DOTA–biotin, and 25 μg of DOTA–biotin, respectively. The reaction mixtures were incubated at 80 °C for 1 h, after which 1 mM DTPA, pH 6.0, was added. The reaction mixtures were incubated at room temperature for 10 min, after which radiochemical purity was assessed by reversed-phase radio-TLC. Typical labeling efficiencies were 99–100%.

Radiolabeling of Conventional RIT Conjugates. A modified method previously described by Mohsin et al. was used to label MeO–DOTA–CC49.⁵³ MeO–DOTA has been shown to bind ¹⁴⁹Pm, ¹⁶⁶Ho, and ¹⁷⁷Lu with high kinetic stability, as demonstrated by an *in vitro* hydroxyapatite assay.⁵³ An initial specific activity of 5 μCi/μg was used for ¹⁴⁹Pm-, ¹⁶⁶Ho-, and ¹⁷⁷Lu labeling reactions. In the labeling reactions, 320–365 μL of 1.0 M ammonium acetate, pH 4.5, was added to 6.5 mCi of ¹⁴⁹PmCl₃ in 24.0 μL of 0.05 M HCl, 3.0 mCi of ¹⁶⁶HoCl₃ in 2.0 μL of 0.05 M HCl, or 7.0 mCi of ¹⁷⁷LuCl₃ in 3.0 μL of 0.05 M HCl, followed by 1.04–1.42 mg of MeO–DOTA–CC49 in 320–370 μL of 0.25 M ammonium acetate, pH 7.0. The reaction mixture was incubated at 43 °C for 1 h, and DTPA, pH 6.0, was added to a final concentration of 1 mM. The reaction mixture was allowed to stand for 15 min at room temperature and then purified using a Bio-Spin 6 column (Bio-Rad, Hercules, CA) equilibrated with 10 mM Na₂HPO₄/150 mM NaCl. Labeling efficiencies ranged from 65% to 80%. The specific activity was adjusted to 1 μCi/μg after purification, by dilution with unmodified CC49. Radiochemical purity was determined after purification by GF-HPLC.

Therapy Studies. All therapy studies were conducted in compliance with the guidelines established by the Animal Care and Use Committee of the University of Missouri–Columbia Animal Care Quality Assurance Office. Female athymic nude mice (20–25 g) were implanted subcutaneously (s.c.) in the hind flank with 2 × 10⁶ LS174T human colon cancer cell suspensions (0.15 mL) in Hank's Balanced Salt Solution. The tumor xenografts grew to 100–400 mg by day 15. All mice were fed a biotin-deficient diet (Purina Biotin Deficient Diet 5836, Purina Mills, Richmond, IN) for 6 days prior to radiopharmaceutical administration and up to 7 days postinjection.

For pretargeted RIT, groups of 8–10 mice were given a single injection of CC49 scFvSA (600 μg, 3.4 nmol), i.v. via the tail vein (*t* = 0). After 20–24 h, the synthetic clearing agent, biotin–GalNAc₁₆ (100 μg, 12.5 nmol), was given i.v. via the tail vein. Four hours after the clearing agent was administered, the mice received a tail vein injection of ¹⁴⁹Pm-, ¹⁶⁶Ho-, or ¹⁷⁷Lu–DOTA–biotin, at doses of 1.25 mCi/1.25 μg, 1.0 mCi/1.0 μg, and 1.5 mCi/1.5 μg, respectively. For conventional RIT, groups of 8–10 mice were given a single tail vein injection of ¹⁴⁹Pm-, ¹⁶⁶Ho-, or ¹⁷⁷Lu–MeO–DOTA–CC49, at doses of 250 μCi/250 μg, 200 μCi/200 μg, and 300 μCi/300 μg, respectively. Tumor growth was assessed by taking three-dimensional measurements of tumor volume using Vernier calipers. Tumor volume was calculated using the formula: $V = (\text{length} \times \text{width} \times \text{depth}) \times \pi/6$.

Tumor Dosimetry. Mouse xenograft absorbed doses were calculated as described previously⁵⁴ using a modification of the method of Hui et al.⁵⁵ The model of Hui et al. is based on the dimensions and masses of organs in a nude mouse of approximately 25 g body weight. All organs were modeled as ellipsoids, with the exception of bone and bone marrow. Whole femur was selected to represent the bone and bone marrow, which were modeled as concentric cylinders. Radioactivity was assumed to be uniformly distributed within each of the organs, the carcass, and the tumor. Self-organ tumor absorbed radiation energy was determined as the amount of absorbed energy remaining in the tumor per radioactive decay. Cross-organ tumor absorbed radiation energy was determined using the approximation that the energy of β⁻ particles that escaped the source organ was deposited in adjacent organs.⁵⁵ The ratios of energy deposited in the tumor from adjacent organs were assumed to be approximately proportional to the ratios of the surface areas that overlapped with the tumor. To better simulate the geometry of a flank xenograft, it was assumed that the tumor was a sphere, with half the volume in contact with the remainder of body and half, covered with 0.5 mm of skin, protruding above the surface.

The Monte Carlo radiation transport code MCNP-4C⁵⁶ was used to obtain absorbed fractions for monoenergetic β⁻ particles. MCNP-4C was run in photon electron mode, in order to track both β⁻ particles and Bremsstrahlung radiation produced by the particles. For each monoenergetic β energy, 10⁴ histories were run on a standard desktop personal computer, resulting in absorbed energy uncertainties on the order of 1%. When the absorbed fractions are averaged over the 25 to 50 energy bins representing a β spectrum, the final uncertainties are smaller than 1%. Standard energy cutoffs (0.001 MeV) and weight cutoffs for both β⁻ particles and photons were used. To calculate absorbed fractions for the polyenergetic β⁻ spectra of ¹⁴⁹Pm, ¹⁶⁶Ho, and ¹⁷⁷Lu, absorbed fractions were first determined at 51 energies from 0.025 to 2.5 MeV. β spectra were then calculated using the NUCDECAY code⁵⁷ and these monoenergetic responses were numerically integrated over the respective radiolanthanide spectra. An *S*-value was calculated for an average tumor weight of 200 mg, assuming radioactivity to be uniformly distributed within a sphere of unit density. This approach assumes that tumors of similar size in different animals have similar uptake characteristics, and the resulting absorbed dose is an average of that absorbed by each tumor.

Statistical Analysis. Response to therapy was assessed based on the number of days required for tumor volume to reach 1 g, using one-way analysis of variance (ANOVA) using

statistical software SPSS 12.0.1 (Chicago, IL). The survival fraction of each treatment group was evaluated by Kaplan–Meier density analysis using SPSS 12.0.1, with a confidence interval of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

An optimized pretargeting protocol,⁴⁹ based on the use of CC49 scFvSA, was employed for these studies. Injection of 600 μg (3.4 nmol) of CC49 scFvSA was sufficient to saturate tumors, as previously shown by the biodistribution of ¹²⁵I-labeled fusion protein.⁴⁹ After maximum tumor uptake at approximately 24 h, a molar excess of 100 μg (12.5 nmol) of the synthetic clearing agent biotin-GalNAc₁₆ was administered. This dose allowed for maximum clearance of circulating fusion protein to the liver for metabolic degradation, while having no effect on tumor retention.⁵¹ After a sufficient time for clearance (4 h later), radiolanthanide-labeled DOTA–biotin was injected. DOTA–biotin was labeled to the same specific activity with all three radiometals, so different amounts of DOTA–biotin were administered using ¹⁴⁹Pm, ¹⁶⁶Ho, and ¹⁷⁷Lu. However, in each case the dose of fusion protein was in molar excess, allowing efficient binding of differing amounts of radiolabeled DOTA–biotin.

In the case of intact CC49,⁵³ we found that radiolanthanide labeling of the antibody at 5 $\mu\text{Ci}/\mu\text{g}$, followed by dilution to 1 $\mu\text{Ci}/\mu\text{g}$, gave superior radiolabeling and biodistribution results. Therefore, all CC49 doses were prepared according to this procedure.⁵³ While the amounts of radiolabeled CC49 administered differed among the three lanthanides, for each radiometal the tumor targeting would occur under conditions of antigen excess, allowing efficient uptake of the radiolabeled mAb.

The present studies compared directly, versus saline controls, the therapeutic efficacies of ¹⁴⁹Pm, ¹⁶⁶Ho, and ¹⁷⁷Lu for both pretargeted and conventional RIT in the same animal model of cancer. Initial single-dose therapy studies of CC49 scFvSA-pretargeted ¹⁴⁹Pm-, ¹⁶⁶Ho-, and ¹⁷⁷Lu-DOTA-biotin (Figure 1), as well as ¹⁴⁹Pm-, ¹⁶⁶Ho-, and ¹⁷⁷Lu-MeO-DOTA-CC49 (Figure 2), were performed in nude mice bearing LS174T human colon carcinoma xenografts. Animals were injected with radiopharmaceuticals or control vehicle on day 15 of tumor growth. After CC49 scFvSA pretargeting, mice were injected with 1.25 mCi of ¹⁴⁹Pm-DOTA-biotin, 1.0 mCi of ¹⁶⁶Ho-DOTA-biotin, or 1.5 mCi of ¹⁷⁷Lu-DOTA-biotin. For conventional RIT, mice received 0.25 mCi of ¹⁴⁹Pm-MeO-DOTA-CC49, 0.2 mCi of ¹⁶⁶Ho-MeO-DOTA-CC49, or 0.3 mCi of ¹⁷⁷Lu-MeO-DOTA-CC49.

Because these studies were not taken to toxicity, the relative injected doses of each of the radiopharmaceuticals had to be estimated from previously obtained biodistribution data.^{35,53} A major consideration was blood uptake, which would result in whole body irradiation and potential toxicity. A second consideration was tumor uptake, taking into account the half-life of the radionuclide and the maximum time of tumor localization. Due to its slow blood clearance, these factors were especially important for the intact antibody. For example, ¹⁶⁶Ho has a half-life of 26.9 h and a maximum tumor uptake at 96 h, meaning that most radioactive decays will occur in the blood and result in whole body irradiation. Thus, blood-to-tumor ratios were used to estimate injected doses of radiolanthanide-labeled CC49. Using 0.2 mCi of ¹⁶⁶Ho-, 0.25 mCi of ¹⁴⁹Pm-, and 0.3 mCi of ¹⁷⁷Lu-MeO-DOTA-CC49, the area under the curve (AUC) blood-to-tumor ratios⁵³ were each approximately

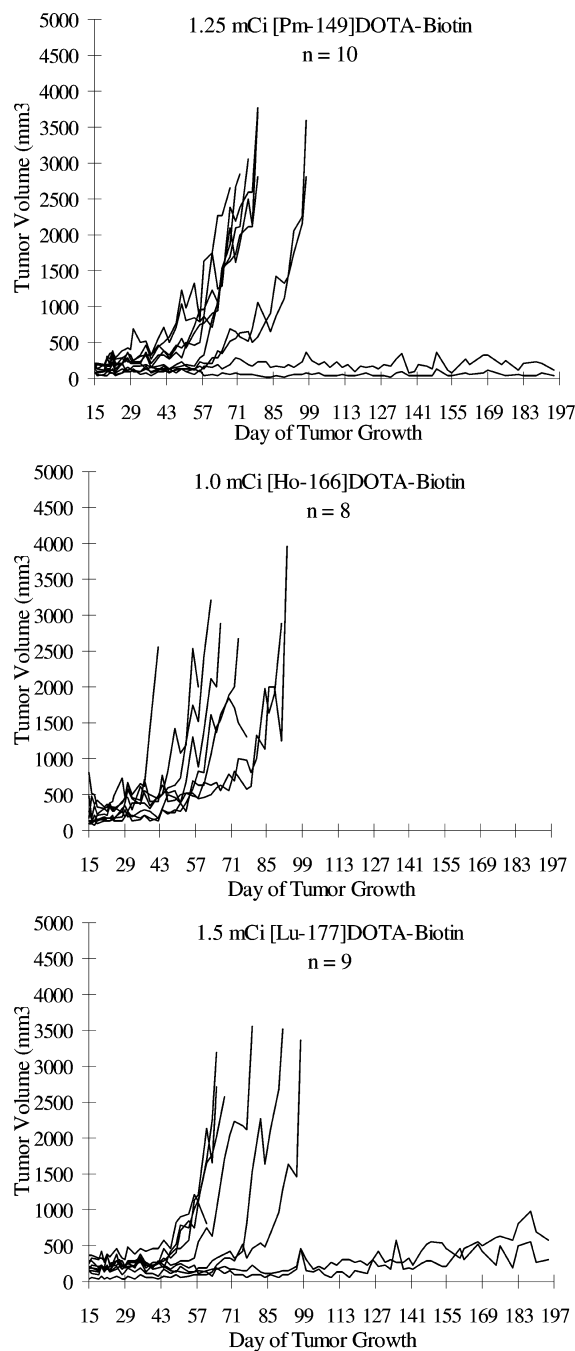


Figure 1. Tumor growth curves for LS174T-bearing nude mice treated with CC49 scFvSA-pretargeted ¹⁴⁹Pm-, ¹⁶⁶Ho-, and ¹⁷⁷Lu-DOTA-biotin.

0.1. The respective doses of ¹⁶⁶Ho-, ¹⁴⁹Pm-, and ¹⁷⁷Lu-DOTA-biotin were five times higher than those administered using the intact antibody, based on approximate whole body clearance of the pretargeting protocol. In the conventional and pretargeted RIT studies, no animals showed any signs of overt systemic toxicity, such as weight loss >20%, lethargy, or diarrhea.

We previously determined that the most statistically relevant end point criterion in this model is time to progression to a tumor burden of 1 g.⁵⁸ Figures 3 and 4 show the Kaplan–Meier plot of time to progression to a 1 g tumor for pretargeted and conventional RIT groups, respectively. Therapy with pretargeted ¹⁴⁹Pm-, ¹⁶⁶Ho-, and ¹⁷⁷Lu-DOTA-biotin increased the median time to progression to 50 ± 10 , 41 ± 5 , and 50 ± 10

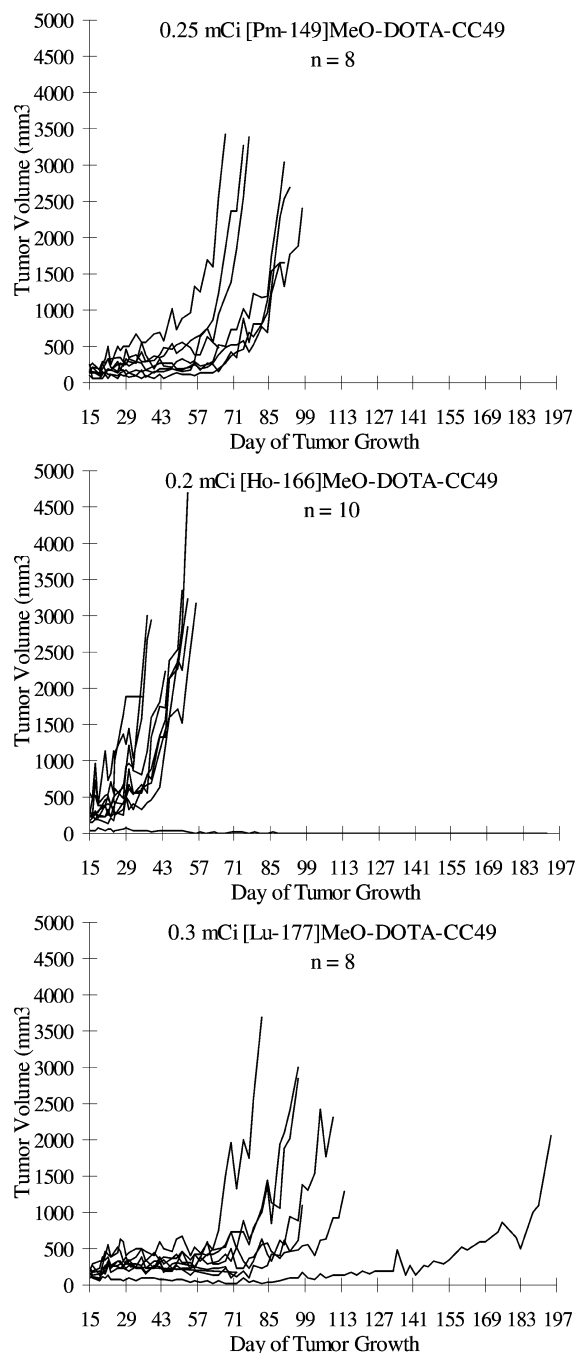


Figure 2. Tumor growth curves for LS174T-bearing nude mice treated with ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -MeO-DOTA-CC49.

days post-treatment, respectively. Therapy with ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -MeO-DOTA-CC49 increased the median time to progression to 53 ± 7 , 24 ± 2 , and 67 ± 9 days post-treatment, respectively. In contrast, saline controls showed a median time to progression of 13 ± 1 days postinjection and a mean tumor volume doubling time of only 3 days. Treatment with pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -biotin increased the average tumor volume doubling time to 27, 38, and 36 days, respectively. Therapy with ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -CC49 increased mean tumor volume doubling time to 18, 10, and 36 days, respectively.

The mean times to progression to a 1 g tumor were lowest for ^{166}Ho pretargeted and conventional RIT, compared to the

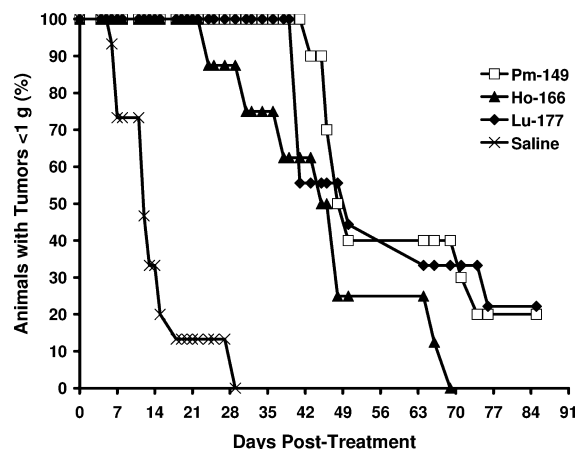


Figure 3. Time to progression to a tumor burden of 1 g in LS174T-bearing nude mice ($n = 8$ – 10 per group) treated with CC49 scFvSA-pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -DOTA-biotin, compared to saline controls.

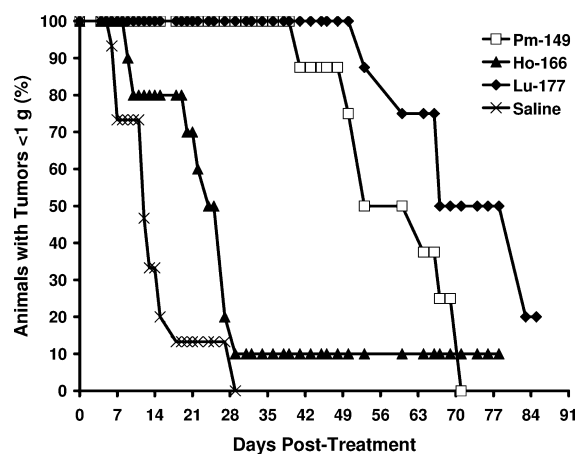


Figure 4. Time to progression to a tumor burden of 1 g in LS174T-bearing nude mice ($n = 8$ – 10 per group) treated with ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -MeO-DOTA-CC49, compared to saline controls.

other two radiolanthanides. For conventional RIT, a possible explanation for this result could be that it takes 96 h to reach maximum tumor uptake, and the half-life of ^{166}Ho is only 26.9 h. Most of the ^{166}Ho decay would then occur in the blood and not at the tumor site. In the case of pretargeting, maximum tumor uptake occurs within 4 h.³⁵ However, the 1.76 and 1.84 MeV β^- particles of ^{166}Ho have a maximum range of approximately 9 mm in soft tissue, meaning that proportionally less radiative energy, compared to ^{149}Pm and ^{177}Lu , would be deposited in the LS174T xenograft.

For pretargeted RIT using ^{149}Pm and ^{177}Lu , the mean times to progression to a tumor burden of 1 g were statistically identical. However, a significant difference was observed between ^{149}Pm and ^{177}Lu in the case of conventional RIT, with ^{177}Lu -MeO-DOTA-CC49 showing superior tumor growth inhibition. With these two radiolanthanides, both pretargeted and conventional RIT effected highly durable therapeutic responses in an extremely aggressive animal model of human colon cancer. Because ^{177}Lu and ^{149}Pm were statistically equivalent for pretargeting, but ^{177}Lu was superior for conventional RIT, ^{177}Lu was selected as an optimal radiolanthanide for future evaluation of both delivery platforms in toxicity and multiple-dose therapy studies.

Among mice treated with CC49 scFvSA-pretargeted ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -DOTA-biotin and ^{149}Pm -, ^{166}Ho -, and ^{177}Lu -MeO-DOTA-CC49, 23% survived >84 days post-therapy, and 11% survived 6 months, at which time the studies were terminated. All saline controls had to be sacrificed within 29 days of injection, with a median survival of only 18 days postinjection, demonstrating the highly aggressive nature of the LS174T model. Median survival in both the pretargeted ^{149}Pm and ^{177}Lu treatment groups was 82 days post-therapy, while in the conventional ^{149}Pm and ^{177}Lu RIT groups, median survival was 89 and 98 days, respectively. A summary of the therapy results is given in Table 2.

Table 2. Summary of Therapy Results

radiopharmaceutical	median survival (days)	survival at 84 days (%)	survival at 6 months (%)
^{149}Pm -DOTA-biotin	82	40	20
^{166}Ho -DOTA-biotin	70	25	0
^{177}Lu -DOTA-biotin	82	40	20
^{149}Pm -CC49	89	50	0
^{166}Ho -CC49	53	10 ^a	10 ^a
^{177}Lu -CC49	98	75	12.5

^aRepresents one animal with a 38 mg tumor that survived 6 months.

Some of the best previously reported outcomes of any therapeutic interventions in nude mice bearing established (>100 mg) LS174T xenografts have been a 63.2 day mean survival⁵⁹ following administration of ^{90}Y -labeled anti-CEA mAb ZCE025 and bone marrow transplantation and a 95.5 day median survival⁶⁰ after multiple bolus injections of ^{131}I -CC49, both of which were optimized treatment regimens. A more direct comparison of the CC49scFvSA pretargeting system can be found in an evaluation of the intraperitoneal model of LS174T colon cancer. Buchsbaum et al.⁵¹ used the same CC49scFvSA protocol, injected intraperitoneally, to obtain very similar biodistributions of ^{177}Lu , compared to our intravenous administration of ^{149}Pm , ^{166}Ho , and ^{177}Lu in the flank xenograft model.³⁵ In pretargeted ^{177}Lu therapy studies, Buchsbaum and co-workers achieved 57 and 60 day median survivals, following single injections of 600 or 800 μCi , respectively. These results were superior to those obtained with ^{90}Y . The ^{177}Lu doses they used were also considerably lower than the dose employed in our experiments, and death was used as the end point for their studies. In contrast, we injected 1.5 mCi of pretargeted ^{177}Lu -DOTA-biotin and measured time to progression to a 1 g tumor, as frequent ulcerations complicated further evaluation of the flank xenograft model. Regardless of these differences, the results of our initial single-dose therapy studies of pretargeted and conventional RIT with radiolanthanides compare favorably with the best results in the literature. In addition to studies in tumor-bearing mice, a CC49scFvSA/ ^{90}Y -DOTA-biotin pretargeting protocol has also been evaluated in patients with metastatic colon cancer, demonstrating the potential clinical utility of pretargeting with this fusion protein.⁶¹

All 6-month survivors showed no macroscopic or microscopic evidence of nontarget tissue toxicity at necropsy. These animals experienced tumor growth inhibition, partial regression, and, in one instance, complete regression. However, the mouse microscopically free of disease after 6 months initially had a 38 mg tumor treated with ^{166}Ho -MeO-DOTA-CC49, which was

an anomalous outcome. Histopathology of the residual masses from all other 6-month survivors (Figure 5) revealed extensive

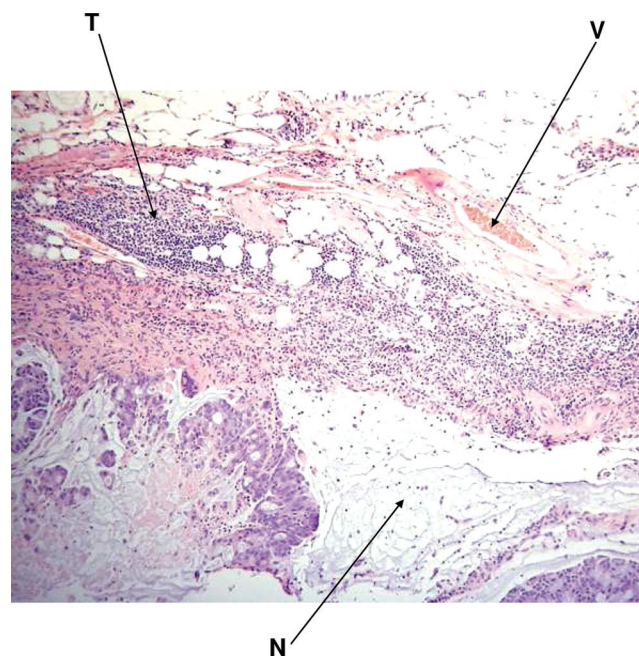


Figure 5. Hematoxylin- and eosin-stained section of the residual mass of a mouse 6 months after treatment with 1.5 mCi of CC49 scFvSA-pretargeted ^{177}Lu -DOTA-biotin, showing extensive necrosis (N), viable tumor tissue (T), and blood vessels (V).

(80–90%) tumor necrosis, with multifocal areas of pyogranulomatous inflammatory infiltrates. Conversely, 10–20% of each mass also consisted of viable and, with one exception, well-vascularized tumor cells exhibiting mitotic rates of 8–10 or 10–12 per 400 power field. However, these long-term survivors all had complete tumor growth inhibition or partial regression at 6 months post-therapy, suggesting that, in spite of the relatively high mitotic rates, the tumor cell-loss factor substantially outweighed the degree of proliferation.

CONCLUSIONS

These studies provide impetus to investigate why these experimental therapies demonstrate such considerable efficacy and how they can be improved further in an adjuvant setting. All animals with residual malignancy remained healthy with stable disease or in partial remission for a period corresponding to at least 25% of their life expectancy. This remarkable finding raises the possibility that radiolanthanide agents for pretargeted and conventional RIT may one day allow colorectal cancer patients to experience relatively normal longevity and quality of life. The future implementation of optimized radiolanthanide treatment regimens, such as multiple-dose therapy, has the potential to be even more efficacious for cancer treatment.

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■ ABBREVIATIONS:

CC49, pancarcinoma monoclonal antibody; B72.3, pancarcinoma monoclonal antibody; scFv, single-chain antibody; SA, streptavidin; LS174T, human colon cancer cell line; GalNAc₁₆, N-acetyl galactosamine hexadecamer; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; MeO-DOTA, methoxy-DOTA; RIT, radioimmunotherapy; TAG-72, tumor-associated glycoprotein-72; DTPA, diethylenetriaminepentaacetic acid; % ID, percent injected dose; % ID/g, percent injected dose per gram of tissue; TLC, thin-layer chromatography; GF-HPLC, gel filtration high performance liquid chromatography; AUC, area under the curve

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