A Pretherapy Biodistribution and Dosimetry Study of Indium-111-Radiolabeled Trastuzumab in Patients with Human Epidermal Growth Factor Receptor 2-Overexpressing Breast Cancer

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Abstract

Purpose: The purposes of this study were to evaluate the organ biodistribution, pharmacokinetics, immunogenicity, and tumor uptake of ¹¹¹Indium (¹¹¹In)-MxDTPA-trastuzumab in patients with human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancers and to determine whether ⁹⁰Y-MxDTPA-trastuzumab should be evaluated in subsequent clinical therapy trials.

Experimental Design: Patients with HER2-overexpressing breast cancers who were to undergo planned trastuzumab therapy first received unlabeled trastuzumab (4–8 mg/kg IV), followed 4 hours later by 5 mCi ¹¹¹In-MxDTPA-trastuzumab (10 mg antibody). Serial blood samples, 24-hour urine collections, and nuclear scans were performed at defined time points for 7 days.

Results: Eight (8) patients received ¹¹¹In-MxDTPA-trastuzumab, which was well tolerated with no adverse side-effects. Three (3) of 7 patients with known lesions demonstrated positive imaging on nuclear scans. No anti-antibody responses were observed for 2 months postinfusion. Organ doses (cGy/mCi) assuming radiolabeling with ⁹⁰Y were 19.9 for heart wall, 17.6 for liver, 4.6 for red marrow, and 2.8 for the whole body. Tumor doses ranged from 24 to 172 cGy/mCi.

Conclusions: In summary, results from this study indicate that ⁹⁰Y-MxDTPA-trastuzumab is an appropriate agent to evaluate in therapy trials. No evidence of an immune response to ¹¹¹In-MxDTPA-trastuzumab was detected, predicting for the ability to administer multiple cycles. With the exception of cardiac uptake, pharmacokinetics and organ biodistribution were comparable to other ⁹⁰Y-labeled monoclonal antibodies previously evaluated in the clinic. Cardiac uptake was comparable to hepatic uptake and therefore predicted to not be prohibitively high as to result in dose-limiting cardiotoxicity.

Key words: breast cancer, indium-111, radioimmunotherapy, radiolabeled antibody, trastuzumab

Introduction

Breast cancer is the most common malignancy among women, with more than 192,000 cases of invasive breast cancer diagnosed in the United States each year.¹ Growth factors and their receptors play a critical role in cancer cell

differentiation and development, and therefore, they are potential targets for novel tumor-directed therapies. Overexpression of human epidermal growth factor receptor 2 (HER2) is observed in ~25% of breast cancers and has been correlated with increased metastatic incidence and poorer survival in patients.^{2–5}

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Strategies to block the function of overexpressed HER2 may inhibit tumor growth, decrease metastatic potential, and improve clinical outcome. Antibodies directed against the extracellular domain of HER2 inhibit the growth of experimental tumors.⁶⁻⁹ One of the most studied antibodies is 4D5, a murine monoclonal antibody directed against the extracellular domain of HER2, which was found to inhibit the growth of human breast cancer cells overexpressing HER2. The antigen recognition regions of this murine monoclonal antibody were subsequently grafted on to a human antibody framework (IgG₁) resulting in trastuzumab (Herceptin®). The antitumor effects of trastuzumab are through at least three mechanisms, which include blocking or altering the growth signaling properties of HER2, activating antibody-dependent cellular cytotoxicity, and enhancing the cytotoxic effects of concomitant chemotherapy and radiotherapy in experimental systems.¹⁰⁻¹²

Trastuzumab has been evaluated as a single agent or combined with chemotherapy in Phase I, II, and III studies and is one of the first commercially available monoclonal antibodies for the treatment of cancer. Results from these trials demonstrated (1) the feasibility of administering therapeutic doses of trastuzumab alone or in combination with chemotherapy, (2) a response rate of ~15% with the antibody alone, and (3) in Phase III trials a significant improvement in response rates with trastuzumab combined with chemotherapy compared with chemotherapy alone.^{13,14} Immunogenicity was minimal, with no antibodies to trastuzumab detected in the initial five clinical studies evaluating the antibody.

Given its efficacy in the clinic, a number of groups have recently evaluated radiolabeled trastuzumab as a potential radioimmunodiagnosis or radioimmunotherapy (RIT) agent. Trastuzumab radiolabeled with the β -emitters ⁹⁰Y, ¹⁷⁷Lu, and ¹⁸⁸Re, the Auger electron emitter ¹¹¹Indium (¹¹¹In), and α -particle emitters ²¹²Pb, ²¹¹At, and ²²⁵Ac has been evaluated in *in vitro* and *in vivo* therapy studies with encouraging results.¹⁵⁻²⁴ Radiometals may be more suited for this antibody compared with radioactive iodine, given the internalization of cell surface-bound antibody, which results in metabolism and dehalogenation.²⁵

Recent preclinical studies demonstrate the tumor targeting capabilities of ¹¹¹In-anti-HER2 and the therapeutic potential of ⁹⁰Y-labeled anti-HER2. Tsai et al.²⁶ administered ¹¹¹In-DOTA-4D5 to nude mice bearing MCF-7 breast carcinomas transfected to overexpress HER2. Maximum tumor uptake was 32% injected dose/gram (ID/g) at 48 hours. Uptake to liver, kidneys, and lung ranged from 6% to 11% ID/g at 5 hours and decreased to <5% ID/g at 96 hours. Treatment of the nude mice bearing MCF7/HER2/neu xenografts with 100 μ Ci of ⁹⁰Y-labeled DOTA-4D5 resulted in a threefold reduction of tumor growth compared with untreated controls. Treatment of animals with 100 μ Ci of nonspecific antibody ⁹⁰Y-labeled DOTA-Leu16 showed no tumor growth inhibition. Treatment with unlabeled DOTA-4D5 demonstrated only a slight effect on tumor growth compared with untreated controls. ¹¹¹In-DOTA-trastuzumab has also been evaluated by the same group with tumor uptake of 25% ID/g observed, retention of activity at the tumor site, and tumor/organ biodistributions appropriate for therapy.²⁶ The same group also evaluated trastuzumab conjugated to MxDTTPA, which demonstrated superior radiolabeling of ¹¹¹In compared

with DOTA-trastuzumab. Biodistribution studies showed comparable tumor uptake and organ uptake between ¹¹¹In-DOTA-Herceptin versus ¹¹¹In-MxDTTPA-trastuzumab (unpublished data). *In vivo* ¹¹¹In-trastuzumab biodistribution studies have also been reported by other groups with comparable results.^{24,27}

Given the encouraging specific antitumor effects seen with radiolabeled trastuzumab in *in vivo* models, further clinical evaluation of radiolabeled trastuzumab as a potential radioimmunotherapeutic is warranted. The purpose of this pretherapy pilot study was to evaluate the organ biodistribution, organ absorbed dose estimates, pharmacokinetics, immunogenicity, tumor targeting, and tumor dose estimates of ¹¹¹In-MxDTTPA-trastuzumab in patients with HER2-overexpressing breast cancers. Results from this study would be used to determine whether ⁹⁰Y-MxDTTPA-trastuzumab should be evaluated as a radioimmunotherapeutic in subsequent clinical trials.

Materials and Methods

¹¹¹In-DOTA-trastuzumab

Trastuzumab (Herceptin) is a recombinant humanized monoclonal antibody that selectively binds with high affinity (K_d = 5 nM) to the extracellular domain of the HER2 protein. The antibody is an IgG₁ kappa that contains the human framework regions with the complementary-determining regions of a murine antibody (4D5) that binds to HER2. For this study, commercially available antibody was purchased from Genentech (South San Francisco, CA).

¹¹¹In is a radiometal with gamma-emission energies of 174 and 287 keV. Its half-life is 2.7 days. For this study, In-111 was supplied by Mallinckrodt Medical (St. Louis, MO) or Nycomed Amersham Imaging (Princeton, NJ).

Trastuzumab was first conjugated to the chelate MxDTTPA (kindly supplied by M. Brechbiel, NCI) and then radiolabeled with ¹¹¹In. Preparation of the radiolabeled dose involved incubation of ¹¹¹In at a ratio of 5 mCi to 10 mg, followed by size-exclusion HPLC purification. All administered doses demonstrated radiolabeling >90%, endotoxin levels <1 unit/mL, and immunoreactivity >95%. The final vial lot of purified conjugated antibody met standards set by the FDA. An investigational new drug application for ¹¹¹In-MxDTTPA-trastuzumab is currently on file with the FDA.

Clinical trial design

Patients were eligible for this study if they had a histologic diagnosis of breast cancer with evidence of HER2 overexpression by at least 3+ (of a maximum of 4+) staining by immunohistochemistry or by a positive result on fluorescent *in situ* hybridization, had locally advanced or metastatic disease with at least one lesion evaluable by physical exam or radiologic study, and were about to undergo planned trastuzumab therapy. Patients with prior exposure to trastuzumab or other antibody therapy administrations were ineligible. This protocol was approved by the City of Hope Institutional Review Board and all patients gave voluntary, written informed consent.

The recommended dosing schedule for trastuzumab therapy is 4–8 mg/kg IV in the first week and 2 mg/kg IV

weekly thereafter. As part of their planned trastuzumab therapy, patients first received a loading dose of unlabeled trastuzumab of 4–8 mg/kg IV administered over ~90 minutes. Approximately 4 hours after initiation of loading dose, 5 mCi ^{111}In -MxDTA-trastuzumab (10 mg antibody) was administered over 30 minutes. Serial blood samples were collected just prior to infusion and at 30 minutes, 1 hour, 4 hours, 12–24 hours, 48 hours, 72–120 hours, and 168 hours after ^{111}In -MxDTA-trastuzumab infusion. Thus, there were blood samples available at seven time points for each patient for kinetic studies. Twenty-four (24)-hour urine collections were also collected for 5 consecutive days. Blood and urine samples were analyzed for total activity and by radiometric HPLC to acquire data on antibody metabolism and pharmacokinetics.

Radionuclide total-body and spot planar imaging were performed with a dual-headed gamma camera at ~1–3, 24, 48, and 72 hours and at a later time point between 96 and 168 hours after ^{111}In -MxDTA-trastuzumab infusion. A SPECT nuclear scan was also performed at ~48 hours and at a later time point between 72 and 96 hours.

After the initial loading dose of trastuzumab, patients received subsequent trastuzumab therapy infusions using either a 2 mg/kg IV dose weekly or 6 mg/kg IV dose every 3 weeks based on the preference of the treating physician. Administration of planned nonanthracycline chemotherapy was permitted on this trial.

Human antitrastuzumab antibody response

Serum immune responses to trastuzumab and MxDTA-trastuzumab were assayed before infusion and at 2 weeks, 1 month, and 2 months postinfusion using a double capture, solid-phase, quantitative radioimmunoassay, as described previously.²⁸ Assays for trastuzumab were performed separately from those for conjugated MxDTA trastuzumab, to differentiate antibodies against the native antibody as opposed to antibodies against conjugated trastuzumab. HER2 antigen used in the assay was provided by one of the authors (A.M.W.). Serum samples incubated with ^{111}In -MxDTA-trastuzumab were also examined using Superose 6 size-exclusion HPLC to detect possible immune responses not found by radioimmunoassay. Patients were found to have anti-idiotypic response if serum samples were positive by HPLC assay but were negative by radioimmunoassay.

Pharmacokinetic analysis and absorbed dose estimates

Blood and urine samples were counted for ^{111}In activity on a gamma counter and were processed on a HPLC size-exclusion Superose 6 column. For those organs seen in both anterior and posterior projections, ^{111}In activity was estimated using parallel-opposed nuclear images to construct the geometric mean uptake as a function of time. Otherwise, single-view images were acquired. All resultant curves demonstrating ^{111}In activity versus time were corrected for background and patient attenuation. Attenuation was estimated using each patient's computed tomography (CT) scans and attenuation coefficients were obtained from a separate series of experiments involving gamma camera efficiency in counting a planar ^{111}In phantom source as a function of tissue-equivalent absorber thickness. Given the

geometric mean or single-view uptake values and measured blood and urine activity, a five-compartment modeling analysis was performed to estimate residence times for ^{111}In activity in blood, urine, liver, and whole body. Details of this compartmental model have been published previously.²⁹ This five-compartment model was used throughout to provide a mathematical description of time-activity curves in tissues that are significant in the dose-estimation process. These included the blood (as a surrogate for red marrow), liver, and whole body. A multicompartment analysis provides a direct way to both interpolate and extrapolate data obtained by blood sampling and imaging and, thereby, enables a direct estimation of radiation dose.

^{90}Y radiation doses to normal organs based on biodistribution of ^{111}In -cT84.66 were estimated with the medical internal radiation dose method,³⁰ using S values obtained from the MIRD-DOSE3 program.³¹ Doses were calculated using male and female phantom organ sizes in these estimates. Red marrow radiation dose estimates were calculated using the American Association of Physicists in Medicine algorithm³² based on blood residence times determined from the five-compartment model.

Tumor absorbed radiation doses were estimated using ^{111}In uptake versus time curves determined from serial nuclear imaging data. Regions of interest were drawn around each tumor lesion, and the conjugate view method³³ was used to estimate activity. Trapezoidal interpolation was used to integrate the time-activity curve and estimate residence time. CT scans were used to define tumor volume as well as the effective attenuation factor for the conjugate view method. For lesions not clearly defined by CT scans, nuclear medicine region of interest (length and width) was used to estimate the tumor volume, assuming an ellipse with the third dimension defined by the geometric mean of the length and width. Absorbed fraction was a function of tumor size and determined via separate Monte Carlo simulation. Edge effects were thus taken into account.³⁴ Uniform uptake was assumed within the tumor. This methodology still uses the medical internal radiation dose strategy but requires that the effective β loss caused by the finite range of ^{90}Y β radiation³⁵ is computed using the following formula:

$$\beta \text{ dose} = 2.12E_{\beta} \text{ AUC}(\text{tumor}) \times \text{absorbed fraction}/(\text{tumor mass}),$$

where E_{β} is the mean β energy of ^{90}Y or 0.93 MeV, area under the curve (AUC) (residence time) is in hours, and tumor mass is in grams.

Results

Eight (8) patients received ^{111}In -MxDTA-trastuzumab and the data are summarized in Table 1. Seven (7) patients had known radiologic metastatic lesions prior to administration. For patient 1, an initial positive bone scan was later read as negative and, therefore, had no known metastatic disease. Six (6) patients began planned chemotherapy during the week of ^{111}In -MxDTA-trastuzumab administration and imaging, whereas 2 patients began planned chemotherapy the week after completion of all imaging and pharmacokinetic studies.

^{111}In -MxDTA-trastuzumab was well tolerated with no adverse side-effects observed. Of the 7 patients with known lesions, 3 demonstrated positive imaging on nuclear scans

TABLE 1. PATIENT SUMMARY

Patient no.	Age (years)	Chemotherapy prescribed with trastuzumab	Concurrent with ^{111}In -trastuzumab	Lesions	Months of chemotherapy and trastuzumab	Months of additional trastuzumab	Anti- ^{111}In -trastuzumab response
1	34	Docetaxel Carboplatin	No	None	5	13	Negative
2	52	Docetaxel Gefitinib	Yes	Malignant pleural effusion	25	5	Negative
3	65	Docetaxel	No	Bone metastases		56+	Negative
4	56	Docetaxel Carboplatin	Yes	Malignant pleural effusion Pretracheal node	9	36	Negative
5	36	Docetaxel Gefitinib	Yes	Left breast mass Left axillary nodes	4	28	Negative
6	48	Docetaxel Gefitinib	Yes	Left breast mass Left axillary nodes Liver metastases	6	12	Negative
7	41	Docetaxel Gefitinib	Yes	Pulmonary metastases Bone metastases	36	48+	Negative
8	36	<i>Nab</i> -paclitaxel Carboplatin	Yes	Left breast mass	3	3	Negative

Imaged lesions are shown in bold type.

FIG. 1. Fifty-two-year-old woman who presented with a left breast primary and recurred 4 years later with a malignant left pleural effusion (patient 2). Planar images at 120 hours demonstrate intense and prolonged uptake on ^{111}In -MxDTPA-trastuzumab to the left pleural cavity.

(Table 1 and Figs. 1–3). All patients received an additional 3 to >56 months of trastuzumab therapy. Although the number of patients is small, there was no obvious correlation between positive imaging with ^{111}In -MxDTPA-trastuzumab and tumor size, location, and duration or response to trastuzumab therapy. Antiantibody response to ^{111}In -MxDTPA-trastuzumab was monitored for 2 months after administration in all 8 patients, with none demonstrating an immune response to the agent.

Pharmacokinetic analysis and organ dose estimates were performed for all 8 patients. The terminal serum $T_{1/2\beta}$ was 255 hours (Table 2). Absorbed organ and tumor dose estimates assuming radiolabeling with ^{90}Y are summarized in Table 3. Absorbed dose estimates (cGy/mCi) for ^{90}Y -MxDTPA-trastuzumab were 19.9 ± 1.9 (mean ± 1 standard deviation) for heart wall, 17.6 ± 1.4 for liver, 4.6 ± 0.3 for red marrow, and 2.8 ± 0.1 for the whole body. For all 8 patients, normal organ biodistribution was comparable to that seen with ^{111}In and ^{90}Y anti-CEA cT84.66 evaluated at this institution,^{28,36–38} with the exception of heart uptake, which was higher and comparable to that of liver uptake. Four tumor lesions in 3 patients were evaluated for ^{90}Y absorbed dose estimates using tumor volumes estimated from nuclear images or CT scans (Table 4). Tumor doses were 24.0, 21.6, 28.8, and 46.8 cGy/mCi. Also, in patient 8, tumor dose was

FIG. 2. Thirty-six-year-old woman with a palpable left breast mass (patient 8). Planar images at 48 hours demonstrate uptake of ^{111}In -MxDTA-trastuzumab to the lesion (arrow).

estimated using a CT-derived tumor volume, resulting in a tumor dose estimate of 172 cGy/mCi.

Discussion

Trastuzumab (Herceptin) is one the first monoclonal antibodies to be approved for use in the treatment of non-hematologic malignancies and recognizes epidermal growth factor receptor 2 (HER2/neu), which is overexpressed by 20%–30% of breast cancers. Trastuzumab radiolabeled with the β -emitters ^{90}Y , ^{177}Lu , and ^{188}Re , the Auger electron emitter ^{111}In , and α -particle emitters ^{212}Pb , ^{211}At , and ^{225}Ac has been evaluated in *in vitro* and *in vivo* therapy studies with encouraging results.^{15–24} This concept is attractive because trastuzumab has known antitumor effects, which can be potentially additive or supraadditive when combined with the radiation dose delivered by RIT. In addition, immunogenicity of the antibody has been minimal in clinical trials, allowing for repeat administrations.

Radiolabeled trastuzumab has recently been evaluated in patients as primarily a potential imaging agent. ^{111}In -MxDTA-trastuzumab was administered to 10 patients with metastatic breast cancer receiving trastuzumab therapy in a pilot imaging and biodistribution trial.³⁹ Optimal tumor targeting was observed at antibody protein doses of 2–4 mg/kg body weight, which is the recommended dose for trastuzumab. Lower trastuzumab protein doses resulted in rapid

FIG. 3. Thirty-six-year-old woman (patient 5) with diffuse involvement of the left breast seen on axial computed tomography (A) and demonstrating diffuse uptake of ^{111}In -MxDTA-trastuzumab to the left breast (B) on 48 hour axial images.

hepatic clearance of the radiolabeled antibody. Responses to trastuzumab were only seen in the 6 patients who demonstrated tumor targeting of ^{111}In -labeled trastuzumab. The 4 nonresponders demonstrated poor tumor uptake of the radioimmunoconjugate. In addition, cardiomyopathy was

TABLE 2. SERUM CLEARANCE KINETICS

Patient no.	$T_{1/2\alpha}$	$T_{1/2\beta}$
1	12.26	209.41
2	17.11	256.72
3	22.61	693.15
4	12.06	174.60
5	11.99	217.29
6	2.23	94.95
7	0.81	203.87
8	4.17	188.87
Mean	10.41	254.86
Standard deviation	7.56	183.05

TABLE 3. PREDICTED ORGAN ABSORBED DOSE ESTIMATES FOR ^{90}Y -TRASTUZUMAB BASED ON ^{111}In -TRASTUZUMAB BIODISTRIBUTION DATA

	Mean $\pm$ SD (cGy/mCi) ^{90}Y -trastuzumab)	Range (cGy/mCi) ^{90}Y -trastuzumab)
Red marrow	4.6 $\pm$ 0.32	4.0–5.0
Liver	17.6 $\pm$ 1.4	15.4–18.9
Kidneys	12.9 $\pm$ 3.1	8.9–18.1
Heart	19.9 $\pm$ 1.9	16.8–21.7
Whole body	2.8 $\pm$ 0.1	2.7–2.9
Tumor ($n = 5$)	58.7 $\pm$ 64.1	21.6–172

SD, standard deviation.

observed in the 2 patients with cardiac uptake of the radiolabeled antibody. In the 8 patients without cardiac uptake, no cardiotoxicity was seen with trastuzumab therapy. A subsequent update¹⁶ reported on 20 patients. Of the 7 patients who demonstrated cardiac uptake of the radiolabeled antibody, 6 experienced significant cardiac toxicity. All 11 patients with intense scintigraphic tumor uptake of ^{111}In -trastuzumab demonstrated objective responses. The authors concluded that the results with ^{111}In -trastuzumab demonstrate the therapeutic efficacy and cardiotoxicity of trastuzumab therapy.

More recently, ^{111}In -MxDTPA-trastuzumab scintigraphy was performed in 17 patients with metastatic HER2-positive breast cancer by Perik et al.²² to evaluate tumor imaging and to correlate the degree of cardiac uptake with cardiotoxicity after trastuzumab therapy. ^{111}In -trastuzumab was administered and scintigraphy was performed within 24 hours of the first trastuzumab infusion and after 12 trastuzumab infusions. The tumor detection rate was 45% with new tumor lesions detected in 13 of 15 evaluable patients. Three (3) patients experienced severe cardiotoxicity, but demonstrated only modest cardiac uptake of ^{111}In -trastuzumab. The authors concluded that ^{111}In -trastuzumab was not useful in predicting trastuzumab-related cardiotoxicity but may prove useful in identifying HER2-overexpressing breast cancer lesions.

Clinical studies to date have primarily focused on assessing ^{111}In -trastuzumab as an imaging agent for tumor detection and to predict cardiotoxicity and tumor response from unlabeled trastuzumab therapy. To better define trastuzumab's potential as a radioimmunotherapeutic, organ biodistribution and tumor dose estimates are needed. The purposes of this pilot study were to evaluate the organ biodistribution, pharmacokinetics, immunogenicity, tumor targeting, and

tumor dose estimates of ^{111}In -MxDTPA-trastuzumab in patients with HER2-overexpressing breast cancer and to evaluate the feasibility and potential of ^{90}Y -MxDTPA-trastuzumab as an agent for RIT. To the best of the authors' knowledge, this is the first clinical study to do so.

^{111}In -MxDTPA-trastuzumab appeared to be well tolerated. As anticipated, this humanized antibody proved less immunogenic than murine and most chimeric-radiolabeled antibodies, with no anti-antibody responses detected in all 8 patients. This provides the potential for multiple administrations of MxDTPA-trastuzumab, which should be advantageous for therapy. However, also observed was a longer serum half life (serum $T_{1/2\beta} = 255$ hours) compared with murine and most chimeric monoclonal antibodies, which may add to circulating times, marrow dose, and RIT hematopoietic toxicity.

Organ biodistribution and dose estimates with ^{111}In -MxDTPA-trastuzumab were comparable to those seen with ^{111}In anti-CEA cT84.66 evaluated at this institution^{28,36–38} and other ^{111}In -labeled intact monoclonal antibodies reported,^{40,41} with the exception of heart wall uptake, which was higher and comparable to liver uptake. This was seen in all patients and is not surprising given the known cardiotoxicity of unlabeled trastuzumab. Higher heart wall uptake was seen in all 8 patients in this study, which appears to differ from the reports of Perik et al.²² and Behr et al.,³⁹ who noted variability in cardiac uptake between patients.

Although higher than usual cardiac uptake was observed with ^{111}In -trastuzumab, cardiac toxicity is unlikely to be dose limiting with nonmyeloablative doses of ^{90}Y -MxDTPA-trastuzumab RIT, especially if not administered concomitantly with cardiotoxic chemotherapy agents, such as doxorubicin, or in patients with previous cardiac radiotherapy. The observed cardiac uptake in this study was similar to that of the liver and comparable to liver uptake seen with other ^{90}Y -radiolabeled antibodies. As hepatic toxicity is rare after nonmyeloablative doses of RIT, and as radiosensitivity of the liver is comparable to that of the heart, cardiac toxicity should also be unlikely if nonmyeloablative activities of RIT are administered.

Tumor targeting was also observed. Of 7 patients with known tumor lesions, 3 patients demonstrated tumor imaging on nuclear scans. This appears to be comparable to that reported in the study by Behr et al.,³⁹ in which tumor imaging was observed in 11 of 20 patients. The tumor lesion detection rate was less than the 45% reported by Perik et al.,²² with 3 of 12 tumor sites imaged in this study. However, given the limited sample size, formal comparisons to previous studies evaluating ^{111}In -trastuzumab are not possible.

TABLE 4. TUMOR ABSORBED DOSE ESTIMATES

Patient no.	Tumor no.	Location	Dimensions (cm)	Volume (cc)	Volume estimated from	Y-90 Dose (cGy/mCi)
2	1	Left hemithorax	6.2 $\times$ 15.5 $\times$ 9.8	493.1	NM	24.03
5	1	Left upper chest wall	3.4 $\times$ 6.9 $\times$ 4.7	57.7	NM	21.61
5	2	Left mid chest wall	9.4 $\times$ 6.9 $\times$ 8.1	275.1	NM	28.82
8	1	Left breast mass	5.3 $\times$ 4.9 $\times$ 5.1	69.1	NM	46.80
			3.4 $\times$ 3.2 $\times$ 3.3	18.8	CT	172.03

NM, nuclear medicine scan; CT, computed tomographic scan.

Tumor ^{90}Y dose estimates ranged from 21.6 to 172 cGy/mCi, which are comparable to those seen with other radio-metal-labeled intact radioimmunotherapeutics.^{40,41} As with other radiolabeled antibodies, doses of this magnitude alone are unlikely to result in clinically significant results in macroscopic disease, but have the potential to further improve results if combined with other systemic therapies in a combined modality setting, particularly in the setting of minimal disease because tumor uptake of radiolabeled antibody increases exponentially with decreasing tumor size.

Conclusions

In summary, biodistribution and dosimetry results from this pilot study indicate that ^{90}Y -MxDTA-trastuzumab is an appropriate agent to further evaluate in therapy trials. Pharmacokinetics and organ biodistribution are comparable to other radiometal-labeled antibodies, which have been evaluated in RIT clinical trials. No evidence of an immune response to ^{111}In -MxDTA-trastuzumab was detected in all patients in this trial, predicting for the ability to administer multiple therapy cycles. Cardiac uptake is higher than most radiolabeled monoclonal antibodies to date, but does not appear to be prohibitively high as to result in dose-limiting cardiac toxicities if delivered as a radioimmunotherapeutic.

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Disclosure Statement

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References

1. American Cancer Society. Cancer Facts and Figures, Atlanta, GA, 2009.
2. Slamon DJ, Clark J, Wong YC, et al. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177.
3. Slamon DJ, Goeckeler W, Jones M, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;244:707.
4. Hynes NE. Amplification and overexpression of the erb-2 gene in human tumors: Its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy. *Semin Cancer Biol* 1993;4:19.
5. Hynes NE, Stern DF. The biology of erb-2/neu/HER-2 and its role in cancer. *Biochim Biophys Acta* 1994;1198:165.
6. Drebin JA, Link VC, Stern DF, et al. Down modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell* 1985;41:695.
7. Drebin JA, Link VC, Greene MI. Monoclonal antibodies reactive with distinct domains of the neu oncogene-encoded p185 molecule exert synergistic anti-tumor effects *in vivo*. *Oncogene* 1988;2:273.
8. Drebin JA, Link VC, Greene MI. Monoclonal antibodies specific for the neu oncogene product directly mediate anti-tumor effects *in vivo*. *Oncogene* 1988;2:387.
9. Fendly BM, Winget M, Hudziak RM, et al. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Res* 1990;50:1550.
10. Aboud-Pirak E, Hurwitz E, Pirak ME, et al. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. *J Natl Cancer Inst* 1988;80:1605.
11. Pietras RJ, Fendly BM, Chazin VR, et al. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* 1994;9:1829.
12. Hancock MC, Langton BC, Chan T, et al. A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 1991;51:4575.
13. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783.
14. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-over-expressing metastatic breast cancer that progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999;17:2639.
15. Crow DM, Williams L, Colcher D, et al. Combined radio-immunotherapy and chemotherapy of breast tumors with Y-90-labeled anti-Her2 and anti-CEA antibodies with taxol. *Bioconjug Chem* 2005;16:1117.
16. Kotts CE, Hutchins BM, Leddy CA, et al. Internalization of humanized monoclonal antibody against p185-HER2. *Proc Annu Meet Am Assoc Cancer Res* 1993;34:A2839.
17. Chen KT, Lee TW, Lo JM. *In vivo* examination of ^{188}Re (I-tricarbonyl)-labeled trastuzumab to target HER2-over-expressing breast cancer. *Nucl Med Biol* 2009;36:355.
18. Akabani G, Carlin S, Welsh P, et al. *In vitro* cytotoxicity of ^{211}At -labeled trastuzumab in human breast cancer cell lines: Effect of specific activity and HER2 receptor heterogeneity on survival fraction. *Nucl Med Biol* 2006;33:333.
19. Luo T-Y, Tang I-C, Wu Y-L, et al. Evaluating the potential of ^{188}Re -SOCTA-trastuzumab as a new radioimmunoagent for breast cancer treatment. *Nucl Med Biol* 2009;36:81.
20. Rasaneh S, Rajabi H, Babaei MH, et al. Radiolabeling of trastuzumab with ^{177}Lu via DOTA, a new radiopharmaceutical for radioimmunotherapy of breast cancer. *Nucl Med Biol* 2009;36:363.
21. Milenic DE, Garmestani K, Brady ED, et al. Potentiation of high-LET radiation by gemcitabine: Targeting HER2 with trastuzumab to treat disseminated peritoneal disease. *Clin Cancer Res* 2007;13:1926.
22. Perik PJ, Lub-De-Hooge MN, Gietema JA, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *J Clin Oncol* 2006;24:2276.
23. Borchardt PE, Yuan RR, Miederer M, et al. Targeted Actinium-225 *in vivo* generators for therapy of ovarian cancer. *Cancer Res* 2003;63:5084.
24. Costantini DL, Chan A, Cai Z, et al. ^{111}In -labeled trastuzumab (Herceptin) modified with nuclear localization sequences (NLS): An auger electron-emitting radiotherapeutic

- agent for HER2/neu-amplified breast cancer. *J Nucl Med* 2007;48:1357.
25. De Santes K, Slamon D, Anderson SK, et al. Radiolabeled antibody targeting of the HER-2/neu oncoprotein. *Cancer Res* 1992;52:1916.
 26. Tsai SW, Sun LE, Williams LE, et al. Biodistribution and radioimmunotherapy of human breast cancer xenografts with radiometal-labeled DOTA conjugated anti-HER2/neu antibody 4D5. *Bioconjug Chem* 2000;11:327.
 27. Milenic DE, Garmestani K, Brady ED, et al. Targeting of HER2 antigen for the treatment of disseminated peritoneal disease. *Clin Cancer Res* 2004;10:7834.
 28. Wong JYC, Thomas GE, Yamauchi DM, et al. Clinical evaluation of Indium-111-labeled chimeric anti-CEA monoclonal antibody. *J Nucl Med* 1997;38:1951.
 29. Odom-Maryon TL, Williams LE, Chai A, et al. Pharmacokinetic modeling and absorbed dose estimation for a chimeric anti-CEA antibody (cT84.66) in humans. *J Nucl Med* 1997;38:1959.
 30. Loevinger R, Berman M. MIRD Pamphlet No.1. A schema for absorbed-dose calculations for biologically-distributed radionuclides. *J Nucl Med* 1968;9 (supplement 1):7.
 31. Stabin MG. MIRDose: Personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med* 1996;37:538.
 32. Siegel JA, Wessels BW, Watson EE, et al. Bone marrow dosimetry and toxicity for radioimmunotherapy. *Antibody Immunoconj Radiopharm* 1990;3:213.
 33. Thomas SR, Maxon HR, Kereiakes JG. *In vivo* quantitation of lesion radioactivity using external counting methods. *Med Phys* 1976;3:253.
 34. Loevinger R, Japha EM, Brownell GL. Discrete radioisotope sources. In: Hine GJ, Brownell GL (eds.). *Radiation Dosimetry*. New York: Academic Press, Inc., 1956:732.
 35. Loevinger R, Holt JG, Hine GJ. Internally administered radioisotopes. In: Hine GJ, Brownell GL (eds.). *Radiation Dosimetry*. New York: Academic Press, Inc., 1956:824.
 36. Wong JY, Chu DZ, Yamauchi D, et al. Dose escalation imaging trial evaluating indium-111-labeled anti-CEA chimeric monoclonal antibody (cT84.66) in presurgical colorectal cancer patients. *J Nucl Med* 1998;39:2097.
 37. Wong JYC, Chu DZ, Yamauchi DM, et al. Phase I radioimmunotherapy trials evaluating Y-90 labeled anti-CEA chimeric T84.66 in patients with metastatic CEA-producing malignancies. *Clin Cancer Res* 2000;6:3855.
 38. Wong JYC, Somlo G, Odom-Maryon T, et al. Initial results of a phase I trial evaluating yttrium-90 (⁹⁰Y) Chimeric T84.66 (cT.84.66) anti-CEA antibody and autologous stem cell support in CEA-producing metastatic breast cancer. *Clin Cancer Res* 1999;5:3224s.
 39. Behr TM, Behe M, Angerstein C, et al. Does pretherapeutic immunoscintigraphy allow for diagnostic predictions with respect to the toxicity and therapeutic efficacy of cold immunotherapy with trastuzumab (Herceptin)? *J Nucl Med* 2000;41:73P.
 40. Wong JYC. Systemic targeted radionuclide therapy: Potential new areas. *Int J Radiat Oncol Biol Phys* 2006; 66:S74.
 41. Meredith R, Wong JYC, Knox SJ. Targeted radionuclide therapy. In: Gunderson LG, Tepper JE (eds.). *Clinical Radiation Oncology*, 2nd ed. Pennsylvania: Churchill Livingstone, 2006:407.