Radioimmunotherapy for Breast Cancer Using Indium-111/Yttrium-90 BrE-3: Results of a Phase I Clinical Trial

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BrE-3 is a murine IgG_1 monoclonal antibody that binds to 97% of human ductal breast cancer specimens. A previous study documented the ability of ^{111}In-labeled 1,4-methyl-benzyl isothiocyanate diethylenetriamine pentaacetic acid (^{111}In-MX-DTPA) BrE-3 to specifically target breast cancer tissue in patients, and the dosimetry derived from the pharmacokinetics suggested that a useful therapeutic index could be obtained with ^{90}Y-MX-DTPA BrE-3. A Phase I maximum tolerated dose study was, therefore, initiated. **Methods:** Six patients received ^{111}In/^{90}Y-MX-DTPA BrE-3, three of them receiving 6.25 and the other three receiving 9.25 mCi/m^2 of ^{90}Y. Pharmacokinetics, dosimetry, human anti-mouse antibody (HAMA), toxicity and clinical responses were evaluated. **Results:** Three of six patients demonstrated a minor and transient, but objective tumor response, and none of the patients had significant toxicity. Tumor dosimetry ranged from 39 to 167 rad/mCi of ^{90}Y (442–1887 rad/dose). HAMA response occurred in five of six patients. **Conclusion:** Minimal toxicity, dosimetric calculations and clinical assessment indicate that a useful therapeutic index can be achieved with this therapy. Indium-111/yttrium-90-MX-DTPA BrE-3 can be safely administered to patients with metastatic breast cancer, and therapy doses yielded pharmacokinetics similar to those of tracer doses. Clinical responses, albeit transient, were achieved with single-dose therapy. Rapid onset of the HAMA response will hinder multicycle therapy, unless it is prevented with immunosuppressive drugs or the use of a "humanized" antibody. Further studies are needed to determine the optimal use of BrE-3 for radioimmunotherapy.

Key Words: breast cancer; radioimmunotherapy; BrE-3; yttrium-90.

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Breast cancer strikes 182,000 people yearly in the United States, and 46,000 deaths a year are attributable to this disease (*1*). Patients with localized disease can be cured by surgery and local radiotherapy, but breast cancer is frequently disseminated and, therefore, incurable by standard therapy. Therefore, new systemic therapies capable of eliminating chemotherapy resistant metastatic disease are needed (*2,3*). Breast cancer cells are frequently sensitive to radiation, but radiation therapy has only been used for local or regional control. Systemic radiotherapy provides a new means of treating drug-resistant metastatic breast cancer with an effective therapy that may also be synergistic with other treatment modalities. Several studies have identified breast cancer-associated antigens that can serve as targets for radioimmunotherapy. Carcinoembryonic antigen and breast mucin are expressed in varying densities in many breast cancer cells and are relatively specific for carcinomas (*4*). A 20-amino acid tandem repeat sequence of breast mucin is recognized by the BrE-3 monoclonal antibody, and this antigen has been detected in abundance in more than 90% of breast cancer cell lines and in 97% of breast cancer biopsies (*5–8*). In preliminary studies, these anti-breast cancer-associated antigens have been specifically targeted using antibodies as carriers of a radionuclide (*6,9,10*).

Studies of radionuclide-conjugated BrE-3 antibody in nude mice bearing human breast cancer have shown the ability of BrE-3 to target tumor and produce an objective therapeutic response (*11–14*). Based on these results, a Phase I clinical study was performed using ^{111}In-labeled 1,4-methyl-benzyl isothiocyanate diethylenetriamine pentaacetic acid (^{111}In-MX-DTPA) BrE-3 to determine the pharmacokinetics, tumor localization and toxicity profile of the BrE-3 radioimmunoconjugates in 15 patients with metastatic breast cancer (*6*). This study showed that 86% of 70 known lesions and an additional 5 unsuspected lesions had sufficient binding of ^{111}In-MX-DTPA BrE-3 to allow detection by gamma camera imaging. The beta-phase half-life for ^{111}In-MX-DTPA BrE-3 was 56 ± 25.4 hr and 0.02%–2.56% of the injected dose localized in the tumors. Four of 15 patients experienced mild reactions, but no major adverse reactions occurred. The study supported the conclusion that targeting of breast cancer by radiolabeled BrE-3 was efficient and safe (*6*). Based on the dosimetry derived from these pharmacokinetics, the current study was designed to determine the toxicity and efficacy for therapy with ^{90}Y-MX-DTPA BrE-3 in extensively pretreated patients with advanced metastatic breast cancer. In this study, six patients with metastatic breast cancer were studied on a therapy protocol using ^{111}In/^{90}Y-MX-DTPA BrE-3.

MATERIALS AND METHODS

Subjects

Six women with metastatic breast cancer (average age, 52.5 yr; range 43–57 yr) were treated. Previous therapy included: 2.3 (range, 1–3) courses of chemotherapy, 1.8 (range, 1–3) courses of hormonal therapy and 1.5 (range, 0–3) courses of radiation therapy directed to either the primary or metastatic disease. Tumor tissue from all six patients was shown to express the BrE-3 antigen on at least 70% of the malignant cells. Other requirements for study entry included: a Karnofsky Performance Score of ≥70, absolute neutrophil count of ≥2500, platelet count of >100,000, bilirubin levels of <2.0, serum creatinine of ≤2.0 or creatinine clearance of ≥40, normal chest radiograph or pO_2 of ≥80 mmHg, <25% of the lung parenchyma involved with tumor by chest computed tomography (CT) scan, metastatic disease of <25% of the skeleton by bone scan, negative serum human anti-mouse antibody (HAMA) and BrE-3 antigen levels of ≤10 μg/ml or less than 25 mg in the total plasma volume. Subjects must have discontinued chemother-

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TABLE 1
Dose, Efficacy and Toxicity of Indium-111/Yttrium-90-MX-DTPA BrE-3 Therapy of Metastatic Breast Cancer

Patient no.	Age	Yttrium-90 dose (mCi/m²)	Total yttrium-90 (mCi)	Total indium-111 (mCi)	Tumor response	Toxicity (grade)	Pre-treatment HAMA (μg/ml)	Post-treatment HAMA (μg/ml)	Pre-treatment BrE-3 (μg/ml)
1	50	6.25	11.2	5.2	50% reduction	0	0.30	131.0	6.2
2*	53	6.25	8.9	5.0	No response	1†	0.56	0.6	1.0
3	54	6.25	11.3	5.1	No response	0	0.04	9.0	0.9
4	55	9.25	16.5	5.0	Minor skin response	0	0.04	17.8	0.0
5*	56	9.25	17.1	5.7	No response	0	0.13	4.7	0.0
6	43	9.25	15.5	5.6	25% reduction	0	0.03	62.4	0.5

The highest HAMA titers were found 38, 7, 27, 78, 60 and 108 days after therapy for patients 1–6, respectively.
*Study was performed at New York University.
†Toxicity occurred 3 days after initiation of chemotherapy.

apy or radiation therapy at least 3 wk before the initiation of the study. Four patients (Patients 1, 3, 4 and 6) were treated at the University of California at Davis, and the other two patients were treated at New York University.

Before treatment was initiated, the serum BrE-3 antigen levels were measured and found to be 6.2, 1.0, 0.9, 0.0, 0.0 and 0.5 μg/ml for Patients 1–6, respectively. Multiplying the concentration of BrE-3 antigen by the calculated plasma volumes gave total circulating BrE-3 antigen levels of 15.5, 2.4, 2.3, 0, 0 and 1.4 mg for Patients 1–6, respectively.

BrE-3 Antibody

BrE-3 is a murine IgG_1 monoclonal antibody that recognizes an epitope on the tandem repeat of the peptide core of breast mucin. BrE-3 antibody was developed by the Cancer Research Fund of Contra Costa and reacts with over 90% of breast cancers, as well as some pancreatic and ovarian cancer cells, but it shows minimal reactivity with normal tissue (*8,15*). BrE-3 was provided by Coulter Immunology in a sterile, apyrogenic solution, both as unconjugated antibody and conjugated with MX-DTPA for labeling with ^{111}In and ^{90}Y.

Immunohistochemistry

Tumor tissue was analyzed for BrE-3 antigen expression by immunohistochemistry using a modified avidin-biotin-peroxidase technique (*6,8*). Dilutions of BrE-3 antibody were applied to formalin-fixed, paraffin-embedded tissue on poly-L-lysine-coated slides that were subsequently scored for reactivity with the antibody. In addition, routine histopathologic staining and examination were performed on the specimens.

Radiolabeling

Indium-111/yttrium-90-MX-DTPA BrE-3 antibodies were prepared as previously described for ^{111}In-MX-DTPA BrE-3 (*6*). One vial containing 5 mCi of ^{111}In or 20 mCi of ^{90}Y was used for each radiolabeling. The radiometal solutions were buffered in sodium acetate (pH 7) and incubated with 2 mg of MX-DTPA BrE-3 conjugate at room temperature for 20 min. The mixture was challenged with 5 m*M* EDTA for 5 min and then purified by column chromatography using a sterile slurry of P6 stationary phase and 1% human serum albumin in saline as the mobile phase. The purified ^{111}In/^{90}Y-MX-DTPA BrE-3 product was confirmed to be greater than 95% radioimmunoconjugate monomer using thin-layer chromatography, high-performance liquid chromatography and cellulose acetate electrophoresis. Each radiopharmaceutical preparation was filtered through a 0.22-μm filter and diluted to 50 ml with sterile saline containing 5% human serum albumin. The immunoreactivity of each preparation was measured in vitro using BrE-3-coated beads. Binding averaged 74 ± 4 and 84 ± 2 for ^{90}Y and ^{111}In radiopharmaceuticals, respectively. Pyrogen levels in the preparations were below the acceptable limit, 1.0 endotoxin units/ml.

Antibody Administration

Therapy was administered in the outpatient setting after signed informed consent was obtained. Unlabeled BrE-3 antibody preloads, 50 mg to Patients 1, 3, 4 and 6 and 40 mg to Patients 2 and 5, were diluted in 100 ml of 0.9% saline and infused at 1 mg/min (Patients 2 and 5 received an additional 6 mg of unlabeled BrE-3 along with the radiopharmaceutical). After 30 min, approximately 5 mCi of ^{111}In-MX-DTPA BrE-3 and either 6.25 or 9.25 mCi/m² of ^{90}Y-MX-DTPA BrE-3 were coinfused over 30 min (Table 1). Patients were continuously monitored during the infusion.

Pharmacokinetics and Dosimetry

Pharmacokinetics were performed as previously described (*6*). Blood and 24-hr urine samples were obtained during the week after infusion. Yttrium-90 was measured by counting in a sodium iodide gamma well counter. Decay-corrected radioactivity in the blood or urine sample was expressed as μCi/ml. By using standards with a comparable volume, ^{90}Y counts were corrected for attenuation as well as decay (^{90}Y window = 320-1500 keV; ^{111}In window = 150–320 keV). Biexponential modeling was used to determine the blood clearance of the radioimmunoconjugate. Indium-111 was measured by sequential quantitative imaging of the whole body to provide an estimate of whole-body retention of the radioactivity and compared to urinary ^{111}In clearance.

Quantitative imaging was performed sequentially during the week after infusion. Planar conjugate views were acquired with a dual-head camera equipped with medium-energy collimators (University of California at Davis) or a single-headed camera (New York University). Each energy window was centered on the primary emission photon energy (171 and 245 keV, respectively) and was 15% in width. Images were acquired an average of 3.5 (range, 2–4) times during the first 24 hr and an average of 3.8 (range, 3–6) additional times up to 8 days after treatment at 1- to 3-day intervals. The amount of activity in organs and tumors was determined using the geometric mean (*16,17*) or effective-point-source methods (*18*), depending on whether the source object could be identified on both conjugate views or not (*19*). Data were corrected for attenuation of photons by using transmission images obtained just before radioimmunoconjugate administration using a source filled with ^{111}In. Biodistribution data for ^{90}Y-MX-DTPA BrE-3 were estimated from that of ^{111}In-MX-DTPA BrE-3 by assuming equivalent biodistribution. Radiation doses to organs and tumors were calculated using the Medical Internal Radiation Dose (MIRD) formalism (*20,21*). A uniform distribution of radionuclide

TABLE 2
Tumor Pharmacokinetics and Dosimetry for Indium-111-MX-DTPA BrE-3 and Yttrium-90-MX-DTPA BrE-3

Patient no.	Tumor site	Tumor mass (g)	Peak %ID/g	Biologic $T_{1/2}$ (hr)	Indium-111 (rad/mCi)	Yttrium-90 (rad/mCi)	Yttrium-90 (rad/dose)
1	Liver	50	0.08	Cubic	4.9	122	1362
1	Chest wall	10	0.06	6.7	2.7	64	720
3	Pelvis (bone)	15	0.03	Cubic	1.5	49	555
3	Liver	35	0.12	Cubic	6.5	167	1887
3	T1 vertebra	4	0.03	Cubic	2.4	57	643
3	T12 vertebra	6	0.03	Cubic	2.8	68	772
3	L1 vertebra	4	0.02	Cubic	1.8	39	444
3	L2 vertebra	8	0.02	Cubic	1.8	39	442
3	L5 vertebra	5	0.03	Cubic	1.8	44	503
4	Shoulder (soft tissue)	7	0.06	23.4	3.5	84	1378
4	Chest wall	10	0.07	28.2	4.2	101	1666
4	Chest wall	30	0.08	29.0	4.3	104	1716
4	Pelvis (soft tissue)	5	0.07	29.2	4.2	105	1732
6	Axilla (soft tissue)	8	0.07	11.1	4.2	104	1612
6	Xiphoid (soft tissue)	30	0.08	2.1	2.8	56	860
6	Shoulder (soft tissue)	15	0.04	39.8	2.8	66	1028

in the organ and tumor was assumed, and the standard man assumption was used for the organ S factor using MIRD data (*22*). For tumor dosimetry, the S value for self and nonpenetrating radiation was the total mean nonpenetrating energy emitted per transition divided by tumor mass. The size of palpable tumors was determined with calipers, and the size of nonpalpable tumors, including bone metastasis, was determined by CT or magnetic resonance imaging. In the one instance where CT detected a clearly necrotic area in a large tumor, the necrotic area was not included when calculating tumor volume or area of radioactive uptake. Only tumors ≥2 cm in diameter were reported in this study to maintain quantitative accuracy; 16 tumors with diameters of ≥2 cm and clearly defined locations and borders were analyzed. Eight tumors had daily measurements so that a cubic spline fit could be used; otherwise, the monoexponential fit method was used. Eleven tumors were not reported: 3 of these were <2 cm in diameter or lacked clear definition, and the other 8 were not seen on CT scan, magnetic resonance imaging or physical exam but did correlate with uptake on a standard bone scan.

The total radiation absorbed dose received by the bone marrow was calculated as previously described (*23–25*). Two components comprise the total bone marrow dose delivered by ^{90}Y-MX-DTPA BrE-3: the dose to marrow from radionuclide in the blood is adjusted to reflect a specific activity for marrow blood that is 25% that of peripheral blood (*23,24*); and direct uptake of radioactivity in the entire bone marrow was extrapolated from measurement of radioactivity in lumbar vertebrae L2, L3 and L4 by imaging (*25*). The fraction of total marrow represented by the three lumbar vertebrae was assumed to be 4.6%. The ^{90}Y dose to marrow from the whole body was negligible and not included in the total dose to marrow calculation because of the nonpenetrating nature of the ^{90}Y emission.

Measurement of Serum Levels of BrE-3 Antigen and HAMA

Serum BrE-3 antigen was quantitated by a competitive serum assay with BrE-3 antigen as the solid-phase reagent. Stoichiometric quantities of BrE-3 antibody and serum dilutions were added to microtiter plates coated with BrE-3 antigen. After overnight incubation, radioiodinated anti-mouse immunoglobulin was added to the wells, and the amount of radioiodine bound was counted, compared to a standard curve and expressed as μg/ml of protein equivalent of breast epithelial mucin (*6*).

HAMA was quantitated using a standard enzyme-linked immunoabsorbent assay as previously described (*6*). A standard curve was constructed using affinity-purified cynomolgus monkey anti-mouse IgG. Serum samples were obtained before and 8, 14, 35 and 90 days after BrE-3 administration. A positive HAMA was defined as one being >5 μg/ml.

RESULTS

Tumor Pharmacokinetics/Dosimetry

Total ^{90}Y radiation absorbed dose delivered to tumors ≥2 cm in diameter ranged from 39 to 167 rad/mCi (442–1887 rad/dose) (Table 2). Excellent tumor uptake of radiolabeled BrE-3 is demonstrated in Figure 1 [images of a liver (A) and chest wall (B) breast cancer metastases in Patients 3 and 4, respectively]. Bone metastasis had a radiation dose range of 39–68 rad/mCi from ^{90}Y, whereas all other tumors ranged from 56 to 167 rad/mCi.

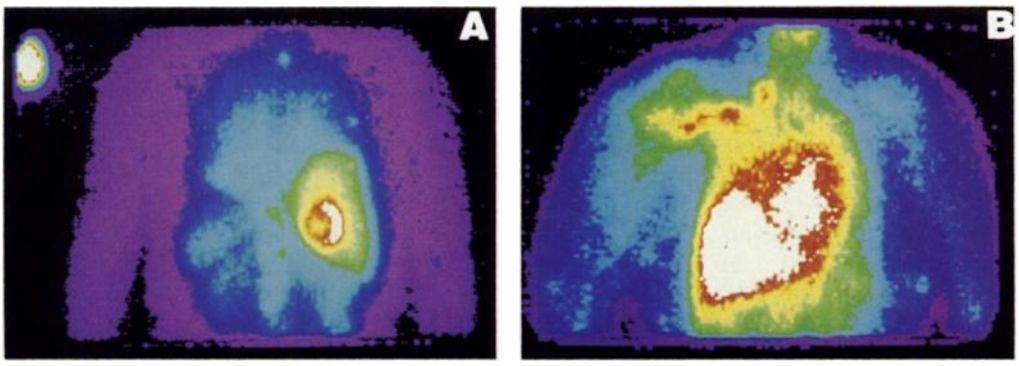

FIGURE 1. Imaging using ^{111}In-MX-DTPA BrE-3 in patients with metastatic breast cancer. (A) Day 2 posterior abdominal view of ^{111}In-MX-DTPA BrE-3 localization in a liver metastasis of Patient 3. (B) Day 4 anterior chest view of ^{111}In-MX-DTPA BrE-3 localization in metastases to the right upper thorax and axilla of Patient 4.

TABLE 3
Indium-111/Yttrium-90-MX-DTPA BrE-3 Blood Pharmacokinetics

Patient no.	Indium-111-MX-DTPA BrE-3 (μCi · hr)	Indium-111-MX-DTPA BrE-3 $T_{1/2}\beta$ (hr)	Yttrium-90-MX-DTPA BrE-3 (μCi · hr)	Yttrium-90-MX-DTPA BrE-3 $T_{1/2}\beta$ (hr)
1	1.1×10^5	95	1.4×10^5	70
2	8.5×10^4	68	9.7×10^4	66
3	1.4×10^5	29	2.3×10^5	48
4	3.2×10^5	153	4.9×10^5	128
5	2.1×10^5	75	2.3×10^5	71
6	2.2×10^5	133	2.8×10^5	141

Organ Pharmacokinetics/Dosimetry

The $T_{1/2}\beta$ values for ^{111}In-MX-DTPA BrE-3 and ^{90}Y-MX-DTPA BrE-3 are shown in Table 3. Based on imaging data, the dose received by the total body or specific organ in rad/mCi of ^{111}In could be measured and used to predict the rad/mCi of ^{90}Y, assuming equivalent biodistribution of the two forms of radionuclide-labeled MX-DTPA BrE-3 (Table 4).

Bone marrow radiation calculated for ^{90}Y from extrapolation of imaging of ^{111}In and blood pharmacokinetics for Patients 1, 3, 4 and 6 were 2.8, 6.6, 3.2 and 2.8 rad/mCi (17.5, 41.2, 29.6 and 25.9 rad), respectively (Table 4). Patient 3 had tumor in the lumbar vertebrae that was used to quantify marrow uptake, thus the marrow-to-marrow component of total marrow rad/mCi was relatively high in this patient. Patients 2 and 5 had metastatic disease in vertebrae, which precluded a meaningful assessment of the marrow-to-marrow dose.

Efficacy and Toxicity

Three of six patients had objective evidence of response to therapy that lasted 3–8 wk (Table 1). Of three patients in the 6.25 mCi/m^2 group, one (Patient 1) had a reduction in measurable disease (liver metastasis) by approximately 50% (Fig. 2). Patients 2 and 3 had progressive disease; however, Patient 2 was lost to follow-up within 1 mo of therapy. In the 9.25 mCi/m^2 group, one patient had a temporary reduction in skin lesions and arm edema, one had an approximately 25% reduction in a liver tumor and the third patient had progressive disease. Because of progressive disease or development of HAMA, none of the six patients received more than one cycle of therapy.

Only one patient experienced drug-related hematologic toxicity, which was grade 1 toxicity (a platelet count of 87,000 beginning 3 days after the patient received chemotherapy for progressive disease). No patient experienced a nonhematologic toxicity greater than grade 2; two patients experienced transient urticaria with pruritis. All six patients had a negative HAMA before therapy (range, <0.04–0.56 μg/ml) (Table 1). Five of six patients had developed positive HAMA titers (range, 4.7–131.0 μg/ml) at 4–6 wk after therapy, although the highest HAMA titers were not measured until days 78, 60 and 108 in Patients 4–6 (Table 1). Patient 2 could only be tested 1 wk after infusion of BrE-3 because she was soon lost to follow-up; however, when she was last tested, the HAMA was negative (0.56 μg/ml). One patient was treated at New York University with ^{90}Y-MX-DTPA BrE-3 at a dose level of 12.25 mCi/m^2, and this patient experienced grade 4 thrombocytopenia and received platelet transfusion and reinfusion of her peripheral blood stem cells.

DISCUSSION

Cure of metastatic breast cancer remains an elusive goal. Although standard therapy frequently provides temporary relief, even palliative options become limited with time. Therefore, new therapeutic modalities are needed. Radioimmunotherapy has shown considerable promise for therapy of disseminated cancers that require a systemic approach (*26*). Radiolabeled monoclonal antibodies have the potential to specifically target sites of disease and deliver radiation while relatively sparing normal tissue, thus minimizing toxicity. Radioimmunotherapy may prove to be of use in treating chemotherapy-resistant metastatic breast cancer because radiation and chemotherapy have different mechanisms of action. Furthermore, studies are underway to assess the ability of targeted delivery of radiation by radioimmunoconjugates to synergize with chemotherapeutic agents or cytokines.

The therapeutic radionuclide used in this study, ^{90}Y, has several useful attributes for the treatment of metastatic breast cancer. The high energy beta particle (2.3 MeV maximum) has a range of 5.3 mm in soft tissue. Thus, radiation can penetrate small solid tumor deposits without irradiating distant normal organs. The 64-hr half-life of ^{90}Y permits time for adequate

TABLE 4
Yttrium-90-MX-DTPA BrE-3 Dosimetry Extrapolated from Indium-111-MX-DTPA BrE-3 Imaging (rad/mCi)

Patient no.	Total body	Liver	Kidneys (left/right)	Lungs (left/right)	Spleen	Marrow (m→m/b→m)*
1	1.8	16.3	6.1/5.5	6.5/6.8	11.7	0.9/1.9
2	3.6	5.8	8.8/8.8	—†	6.6	—‡/5.0
3	1.9	12.4	7.9/7.9	7.6/7.1	14.4	4.5§/2.1
4	2.1	11.3	9.5/9.4	12.9/13.5	15.0	1.1/2.1
5	2.1	9.5	2.0/2.0	5.0/5.0	3.0	—‡/2.0
6	2.0	15.2	6.8/6.8	13.3/10.3	20.0	1.2/1.6

*The total bone marrow dose was the sum of marrow-to-marrow (m→m) and blood-to-marrow (b→m) doses. The m→m dose for ^{90}Y was extrapolated from ^{111}In uptake in lumbar vertebrae 2, 3 and 4.

†Not available because of interference from overlying chest wall tumor.

‡Metastatic disease precluded calculation of m→m dose in normal lumbar vertebral marrow in these patients.

§L2, L3 and L4 vertebrae contained deposits of metastatic breast cancer.

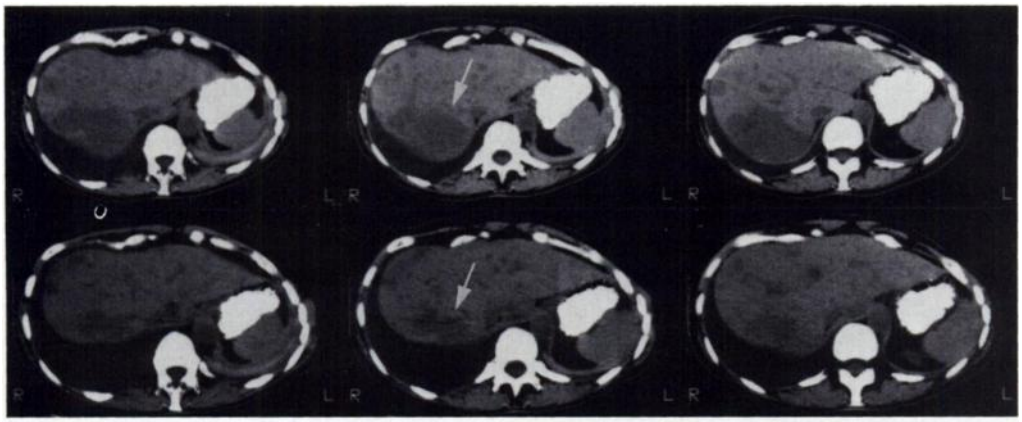

FIGURE 2. CT scan of Patient 1 demonstrating regression of liver metastasis 5 wk after therapy with ^{90}Y-MX-DTPA BrE-3. Upper, before therapy; lower, after therapy. Note the decreased size of the large liver metastasis after therapy (arrow).

localization of the radioimmunoconjugate and effective irradiation of targeted tumor. Although the absence of gamma emissions decreases the radiation exposure to distant normal organs and health care personnel, it also precludes the use of standard imaging techniques to assess the dosimetry of ^{90}Y radioimmunoconjugates. In our study, we used pharmacokinetic data from imaging of ^{111}In-MX-DTPA BrE-3 to estimate normal organ and tumor radiation absorbed dose from ^{90}Y. The current clinical trial demonstrated that blood pharmacokinetics for ^{111}In-MX-DTPA BrE-3 were comparable to those measured for ^{90}Y-MX-DTPA BrE-3, although more definitive assertions cannot be made from this small, six-patient study. There was no unexpected or unusual localization of the radioimmunoconjugate (Table 4). Estimates for radiation dose to kidney and lung were similar to those calculated for patients with metastatic breast cancer in our previous Phase I imaging trial of ^{111}In-MX-DTPA BrE-3, suggesting that there is a consistent and predictable biodistribution of the radioimmunoconjugate. Some variability among patients in organs such as the liver and spleen was expected because of the varying degrees of metastatic tumor in these organs. The calculated radiation absorbed dose to the bone marrow from ^{90}Y-MX-DTPA BrE-3, based on blood pharmacokinetics and ^{90}Y dosimetry extrapolated from ^{111}In bone marrow imaging data, falls within a range (17.5–41.2 rad) that correlated with the minimal hematologic toxicity observed clinically. The use of ^{111}In radioimmunoconjugates as surrogates for the ^{90}Y radioimmunoconjugates appears to be the best system available for the estimation of ^{90}Y dosimetry at present. However, the ability of ^{111}In-MX-DTPA BrE-3 imaging data to allow estimation of biodistribution and calculate ^{90}Y dosimetry does not negate the desirability of developing techniques that provide direct measurement of ^{90}Y. In the future, imaging techniques that would allow the direct measurement of ^{90}Y-labeled radioimmunoconjugates may be used for providing dosimetry from ^{90}Y therapy (*19,27*).

Previous studies of metastatic breast cancer biopsies have demonstrated abundant BrE-3 antigen (*8*). This may explain, in part, the good in vivo targeting of BrE-3 antibody for breast cancer observed in the organ and tumor dosimetry data. The high density of BrE-3 target antigen on breast cancer cell facilitates the binding of large numbers of radiolabeled BrE-3 antibodies, thus increasing the radiation dose delivered to malignant cells. No organ received a radiation dose as high as could be observed for any of the tumor metastasis. In the current study, each of the four patients that could be closely evaluated had at least one site of metastatic disease with greater than 100 rad/mCi delivered by ^{90}Y (Table 2), whereas the highest organ dose was 20 rad/mCi (the spleen of Patient 6) (Table 4). The estimated %ID/g localized in tumors, as assessed by region of interest image analysis, varied from 0.02%ID/g to 0.12%ID/g (Table 2); the previous study demonstrated %ID/g from 0.004% to 0.28% (*6*). The excellent tumor uptake and tumor-to-normal tissue ratio achieved by radiolabeled BrE-3 serve to emphasize the therapeutic potential of this monoclonal antibody for treatment of metastatic breast cancer. There was good radioimmunoconjugate delivery to bone metastasis, a frequent site of disease in patients with breast cancer (Table 2). Because normal marrow may receive substantial bystander irradiation when diffuse bone metastases exist, doses of ^{90}Y higher than those used in this study may require autologous bone marrow or peripheral stem cell support. However, escalation of the dose of ^{90}Y with or without autologous bone marrow or peripheral blood stem cell support may increase the number and durability of the clinical response to treatment with ^{90}Y-MX-DTPA BrE-3. The encouraging therapeutic effect of ^{90}Y-MX-DTPA BrE-3 could not be enhanced by additional cycles of radioimmunotherapy because a HAMA response developed in patients tested 4–6 wk after the infusion. Humanization of the antibody or immunomodulation with cyclosporin A to prevent development of HAMA may make multiple administrations of this radioimmunoconjugate an achievable goal. BrE-3 has been humanized such that only the complimentarity-defining regions remain from the murine antibody (*28*), and preliminary clinical trials to evaluate the immunogenicity and pharmacokinetics of the humanized construct have recently begun.

Another means of enhancing the therapeutic index of radioimmunoconjugates may be to combine radioimmunotherapy with other modalities, such as chemotherapy or biological response modifiers. The use of autologous bone marrow or stem cell support may allow an increase in doses, and combination of synergistic agents with targeted radiation may increase the response rate without untoward hematologic toxicity (*29*). With maximization of the dose, stem cell support and a humanized form of the radioimmunoconjugate, we anticipate that clinical efficacy in the treatment of currently unresponsive metastatic breast cancer may be possible. Further improvement in tumor targeting and faster clearance of nontumor targeted radionuclide will result in an even greater therapeutic index in the future. This form of targeted, relatively low-dose-rate radiation may ultimately become a key part of multimodality therapy for patients with metastatic breast cancer.

CONCLUSION

Although patients in this study received only a single, modest dose of ^{90}Y-MX-DTPA BrE-3, a decrease in measurable disease was observed in three of six patients, albeit of brief duration. In addition, radiolabeled BrE-3 monoclonal antibody shows an excellent tumor-to-normal organ ratio. The therapy

was well-tolerated, and the therapeutic index for ^{90}Y-MX-DTPA BrE-3 compares favorably to most standard chemotherapeutic regimens given to heavily pretreated patients with metastatic breast cancer. HAMA developed in five of six patients. Multiple doses of ^{90}Y-MX-DTPA BrE-3 at higher doses, in combined modality therapy, could result in more frequent and durable responses of this lethal disease.

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