High-Dose $^{90}$Y Mx-Diethylentriaminepentaacetic Acid (DTPA)-BrE-3 and Autologous Hematopoietic Stem Cell Support (AHPCS) for the Treatment of Advanced Breast Cancer: A Phase I Trial


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Abstract

This Phase I trial explores the use of high-dose $^{90}$Y conjugated to the antitumor antibody BrE-3 and autologous hematopoietic stem cell support in the treatment of women with stage four breast cancer. Nine women with heavily pretreated disease were enrolled. All of the patients had BrE-3-positive tumors by immunostaining and were treated with increasing doses of $^{90}$Y (15 mCi/m2, 3 patients), 20 mCi/m2 (six patients), and a fixed (50 mg) dose of BrE-3. In-labeled BrE-3 (5 mCi) was given simultaneously for scanning purposes. The only toxicity noted was hematologic. Grade 4 platelet toxicity requiring transfusion support occurred in four patients. Grade 4 WBC toxicity was noted in two patients that resolved in 3–9 days. All hematologic nadirs occurred approximately 25 days after treatment. Objective partial responses were noted in 4 of 8 (50%) patients with measurable tumors. Dose escalation is ongoing.

Introduction

Breast cancer continues to be the second-leading cause of cancer in American women, with more than 45,000 deaths/year. Early detection, improved surgical techniques, and more effective systemic therapy have improved quality of life and survival for selected patients, particularly those with less advanced disease. Unfortunately, patients who have metastatic tumor continue to have very poor prognosis with a median survival of <2 years (1). Although chemotherapy and hormonal therapy are effective in shrinking tumors in patients with metastases, median time to treatment failure is 8–9 months, and survival prolongation is usually <6 months (2). These disappointing results have been the impetus for numerous clinical trials. High-dose chemotherapy with autologous progenitor cell support results in durable complete remissions in selected patients with metastatic tumor (3). However, most patients still relapse after high-dose chemotherapy and autologous bone marrow transplant, suggesting a need to augment this approach.

The use of radiolabeled MoAb therapy (RAIT) is one option to improve breast cancer treatment. Radiolabeled MoAb to various tumor antigens have demonstrated efficacy for therapy of hematologic neoplasms (4). RAIT of solid tumors is more limited and has been directed primarily against gastrointestinal malignancies.

Herein we present data from an ongoing Phase I trial using the $^{90}$Y-emitting radioisotope $^{90}$Y conjugated to BrE-3, a MAb directed against an epitope of the human milk fat globule. AHPCS support is administered to all patients to avoid morbidity.

Patients and Methods

Enrollment and Eligibility

Since June 1993, nine patients with advanced breast cancer have been entered into the $^{90}$Y conjugated MoAb study. Eligibility criteria included a pathologically confirmed diagnosis of metastatic breast carcinoma with positive immunostaining with BrE-3 antibody. Serum BrE-3 antigen must be <5 µg/ml, and human antimouse antibody must be <40 ng/ml (normal range, 0–40 ng/ml). Patients with known prior exposure to mouse protein were excluded. An assay for HAMA was performed by using an ELISA assay (Dianon Systems, Inc.).

The major goals of this trial of $^{90}$Y-labeled BrE-3 with AHPCS support are to identify the nonhematologic dose-limiting toxicities and the maximally tolerated dose of this program and to obtain preliminary estimates of antitumor activity in patients with resistant metastatic disease.
Treatment Plan

Patients were admitted to the hospital and kept in isolation for at least 96 h and until their total body radioactivity was <30 mCi. All blood products (except for bone marrow or peripheral progenitor cells) were irradiated with 2739 cGy before administration. Patients were transfused to a hematocrit of >35% before administration of the isotope. Consenting patients underwent serial bone marrow biopsies or liver biopsies after treatment for dosimetric evaluation.

Each patient was premedicated with dexamethasone, cimetidine, diphenhydramine, and acetaminophen 1 h before 90Y-labeled BrE-3 infusion. The solution containing the isotope was delivered in a plexiglass shield and was administered by continuous i.v. infusion over 1 h immediately on arrival on the floor. The first three patients were treated with a dose of 1.5 mg/m² BrE-3-MX-DTPA labeled with 15 mCi/²²Na, which was added to 5 mCi of 111In-labeled BrE-3-MX-DTPA. Unlabeled BrE-3 was added to make the total protein dose 50 mg. All of these components were mixed in the same infusion bottle. The remaining six patients received 20 mCi/m² 90Y and 2 mg/m² BrE-MX-DTPA with identical amounts of 111In-labeled BrE-MX-DTPA.

After antibody infusion, the patient's vital signs were followed every 15 min for 2 h and then hourly for 6 h. Three patients agreed to undergo serial tissue biopsies 24 and 48 h after treatment. Of these patients, two patients underwent bone marrow biopsies. One patient underwent serial liver biopsies. All patients received prophylactic antibacterial therapy with 400 mg ofloxacin p.o. twice daily as long as their ANC was <500/mI and patients were to be admitted to the floor. The first three patients were treated with a dose of 1.5 mg/m² BrE-3-MX-DTPA with 15 mCi/²²Na, which was added to 5 mCi of 111In-labeled BrE-3-MX-DTPA. Unlabeled BrE-3 was added to make the total protein dose 50 mg. All of these components were mixed in the same infusion bottle. The remaining six patients received 20 mCi/m² 90Y and 2 mg/m² BrE-MX-DTPA with identical amounts of 111In-labeled BrE-MX-DTPA. Again, sufficient unlabeled BrE-3 was added to make the total protein dose equal to 50 mg. Additional cohorts using 90Y activities of 34, 48, 62, 76, 88, 98, 106, and 114 mCi/m² are anticipated. Escalation will cease when dose-limiting hematologic toxicity is reached.

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Laboratory Procedures

Chelation. The chelating agent for this study, MX-DTPA, was prepared and bound to BrE-3 by the Coulter Corp. The product was manufactured by using FDA-approved pharmaceutical production procedures. The BrE-3-MX-DTPA conjugate was radiolabeled with 111In by admixing 5 mCi of buffered (with 0.5 ml of 0.25 m sodium acetate) 111InCl₂ (Amer sham, Arlington Heights, IL), with 2 mg of BrE-3-MX-DTPA antibody in a total volume of 2.1 ml. The mixture was incubated at room temperature for 10 min and then quenched by the addition of 0.5 ml of 5 mM EDTA to complex any unbound or loosely bound 111In. Incorporation of radiolabel into the antibody was determined by instant TLC on filter paper developed with 0.9% saline containing 5 mM EDTA. The TLC paper was eluted for 5 min. The upper and lower portions of the paper were analyzed by the gamma counter. Lower portion counts were divided by total counts to define the percentage of incorporation numbers. In this procedure, the radiolabel associated with antibody remains at the origin. All products used for clinical purposes had a labeling efficiency >95.5%. The final pharmaceutical preparations were evaluated for bacterial and pyrogen contamination before administration.

The conjugation of the 90Y to BrE-3-MX-DTPA was done by mixing 2 mg/mI of the antibody chelate with the appropriate (15 or 20 mCi/m²) amount of yttrium acetate, prepared by mixing 90Y Cl₂ in 0.4 ml of 0.5 m HCL (Amer sham) with 0.5 ml 0.25M aqueous sodium acetate. The mixture was incubated at room temperature for 10 min. After incubation, 0.5 ml of 5 mM EDTA-0.9% NaCl was added to complex any unbound or loosely bound 90Y. The labeled antibody was then transferred to a sterile, pyrogen-free BioGel P-6 column (5 x 30 cm) and eluted with physiological saline solution containing 1% human serum albumin. The final product was diluted to 250 ml in 5% human serum albumin-0.9% NaCl for patient administration. Total BrE-3 antibody (labeled and complementary "cold" antibody) concentration was 50 mg for all patient formulations in an attempt to provide comparable antibody-binding patterns among patients. The labeling efficiency, sterility, and pyrogen testing were assessed similarly to that described for the 111In-labeled product above. The radiopharmaceuticals (111In and 90Y) were obtained from Amer sham and Westinghouse Hanford Co. Both isotopes passed isotope chemical purity analysis by the respective companies according to Department of Energy isotope product acceptance guidelines.

The immunoreactivity was determined by incubating polystyrene beads coated with saturating amounts of antigen or BSA after diluting the product to 25 mg/ml of protein. Two-hundred μl of product were put into triplicate sets of tubes containing antigen-coated beads or BSA-coated beads (nonspecific binding) or no beads. The samples were then incubated overnight at 3-5°C. One-hundred μl of supernatant from each tube were diluted with 10 μl of deionized water and counted using a β counter. The bead:supernatant ratio to the total count supernatant (no beads) minus nonspecific binding (BSA beads) counts was expressed as a percentage to determine the immunoreactivity. A level of 60% was required for use of the radiolabeled product.

PKs. Blood was obtained in 5-ml heparinized tubes for PK analysis 1, 2, 4, 8, 12, 24, 48, 72, and 96 h after treatment for determination of radioactivity and BrE-3 plasma levels. Plasma was obtained, and radioactivity was determined by using liquid scintillation counting to quantitate 90Y and γ counting for 111In. The counts were corrected for decay and emission overlap from the second isotope. The resultant data were modeled by using PCNONLIN version 4.0.
The initial three patients received 15 mCi/m² 20Y (cohort 1). The type of cell support was determined by the availability of previous autologous hematopoietic cell rescue. The total body activity was determined by fitting the total body counts to a monoexponential function. The total body activity was corrected for attenuation or underlying activity. The total body activity was determined and recorded before the marrow reinfusion.

Dosimetry was performed by using the major source organs that localize radioactivity. These include ROIs over the whole body, liver, spleen, and kidneys. If the lungs were visible, they were considered as an additional source organ. Cumulated activities were combined with the MIRD formalism (8) to estimate organ doses. No attempts were made to correct for the differences in organ mass between the reference man and the individual patient. Correspondence between the predicted dose and patient outcome were maintained to validate the dose estimates. Total body activity was determined and recorded before the marrow reinfusion.

Tumor dosimetry was performed by using nuclear medicine ROI data reported for each patient and the estimated tumor volumes obtained from the patient chart. Volumes were calculated by using rectangular (box) models. A tumor density of 1 was assumed. The dimensions reported in the patient chart were assumed to represent the length of tumor from one side to the other. When a dimension was not reported in the chart, the smallest of the dimensions reported was used as the estimate of the missing dimension. The activity curves for each tumor ROI were fit to a monoeponential function. If the tumor was resolved on only the anterior or posterior view, the sequence of points corresponding to that view was used in the fit. Otherwise, the geometric mean of the two views was used in the fit. Individual activity points were estimated by taking the ratio of counts in the tumor ROI to the counts in the total body image and multiplying by the activity in the total body. No attempt was made to correct for attenuation or underlying activity. The total body activity was determined from evaluating the monoexponential equation governing the elimination of activity from the whole body: $A(t) = A_0 e^{-\lambda t}$, where $\lambda$ was determined by fitting the total body counts to a monoexponential function.

**Immunostaining.** The BrE-3 immunostaining was performed on tissue biopsies obtained from each patient by using a method described previously (9), modified to include only BrE-3 antibody.

### Results

**Toxicities.** Nine patients have been treated in this Phase I study. The initial three patients received 15 mCi/m² 20Y (cohort 1). The remaining six patients received 20 mCi/m² (cohort 2). Two patients received bone marrow, and seven patients received PBPC support. The type of cell support was determined by the availability of previously frozen cells of one or the other type. Demographics of the patients are described in Table 1. There were no acute toxic side effects or allergic reactions from administration of the radioisotope antibody. The only delayed toxicities equal to or more than grade 1 were hematologic. Four patients experienced transient grade 4 platelet toxicity (Table 2) requiring transfusion support (cohort 1: 1 patient; cohort 2: 3 patients). Two patients from cohort 2 had grade 4 WBC toxicity, which resolved in 3 and 9 days, respectively (Table 2). All hematological nadirs occurred approximately 3.5 weeks after treatment. No fevers or infectious episodes were noted, and no hospitalizations were required as a result of the toxic effects of this regimen.

**BrE-3 Blood PKs.** The half-life of the 90Y and 111In were determined by the one-compartment or two-compartment models and assumed a constant infusion rate and first-order output. The elimination half-lives for both isotopes are shown in Table 3.

**Dosimetry.** Table 4 represents the total body, normal organ, and tumor doses from three measurable tumors in two separate patients. The data illustrate the greater radiation dose to tumor compared to normal organs experienced during these treatments. Serial bone marrow biopsies demonstrated a 1.7-fold 90Y accumulation in bone marrow at 48 h compared to that at 24 h. In contrast, 111In content decreased by 50% during the same time interval. 90Y accumulation in bone marrow likely reflects progressive inclusion in the bone matrix, whereas 111In may be bound to marrow tissues. The serial liver biopsy showed a decline in 90Y-specific activity of 0.85 over the 24-h period, roughly consistent with physical decay. Total 90Y urinary excretion varied from 7 to 11% in the nine patients over 72 h after dosing.

**Scanning.** In eight of nine patients, known tumors were visualized on scans. This included brain metastases in two patients.

**Tumor Responses and Survival.** Of the nine patients enrolled in the study, eight were available for antitumor response. The ninth patient had tumor limited to bone and experienced transient decrease in bone pain. Four of the eight patients with measurable disease achieved partial responses (as defined by a >50% shrinkage of measurable tumor for at least 1 month). Responding tumor sites were lymph node (4), skin (1), and marrow (1). One patient achieved a complete clinical response of cutaneous tumor, but surgical biopsy revealed microscopic residua.

**Immunostaining.** Forty-one patients desiring entry to this trial submitted histological sections for BrE-3 immunostaining. All tissues

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### Table 1 Pretreatment patient characteristics

<table>
<thead>
<tr>
<th>Age</th>
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<th>Range</th>
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<tr>
<td># of prior treatments</td>
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<td>3-8</td>
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<tr>
<td>Sites of disease</td>
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<td>2-6</td>
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<tr>
<td>Relapse-free interval</td>
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<td>20-67 months</td>
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### Table 2 Hematologic toxicities of therapy

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<th>Cell type</th>
<th># of grade 4 toxicity</th>
<th>Duration of toxicities (days)</th>
<th>Median days to nadir</th>
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<td>Platelets</td>
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<td>20 (median), (range 5 to &gt;30)</td>
<td>27</td>
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<tr>
<td>WBC</td>
<td>2</td>
<td>3, 9 days</td>
<td>25</td>
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### Table 3 Isotope half-lives of the nine patients at different 90Y doses

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Infused 90Y activity (mCi/m²)</th>
<th>Infused 111In activity (mCi)</th>
<th>90Y half-time (h)</th>
<th>111In half-time (h)</th>
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<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>5</td>
<td>70</td>
<td>25</td>
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<td>60</td>
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<tr>
<td>9</td>
<td>20</td>
<td>5</td>
<td>75</td>
<td>47</td>
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</tbody>
</table>

### Table 4 Mean doses (cGy/mCi) to normal tissues in nine patients, and the doses from three known tumors in two patients.

<table>
<thead>
<tr>
<th>Organ (n = 9)</th>
<th>Mean</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Total body</td>
<td>3</td>
<td>1-6</td>
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<tr>
<td>Liver</td>
<td>16</td>
<td>6-27</td>
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<tr>
<td>Spleen</td>
<td>25</td>
<td>9-59</td>
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<tr>
<td>Lungs</td>
<td>23</td>
<td>10-40</td>
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<tr>
<td>Kidney</td>
<td>14</td>
<td>5-26</td>
</tr>
<tr>
<td>Heart</td>
<td>11</td>
<td>5-21</td>
</tr>
<tr>
<td>Tumor (n = 3)</td>
<td>49</td>
<td>35-56</td>
</tr>
</tbody>
</table>

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* Aeros, centiGray; mCi, millicurie.
showed detectable staining with this technique with a median intensity of +3 (range, +1→+4), with +4 being the most positive.

Discussion

Despite extensive research, the present treatment of metastatic breast cancer remains unsatisfactory. Although RAIT has historically been disappointing for the treatment of solid tumors, treatment of advanced-stage breast cancers provides an attractive area for research. Because the BrE-3 antibody reacted with cell surface antigen in 41 of 41 patient breast cancer biopsies, it seems likely that BrE-3 may be generally applicable in breast cancer patients. 90Y-labeled BrE-3 antibody can deliver radioactivity to widely dispersed tumor metastases when administered i.v. The virtual absence of BrE-3 reactivity to normal tissues (data not shown) increased the probability of favorable distribution to tumor. The effective tumor-killing capacity of 90Y for tumors within 0.5 cm (10) should compensate for labeling heterogeneity within a given tumor mass. A major potential problem with using 90Y conjugated with MX-DTPA is the disassociation of radiolabel from the antibody chelating molecule in vivo and deposition of the 90Y in bone. Important hematological toxicities have resulted from irradiation of adjacent marrow (11) as shown in Table 3. To minimize the potential prolonged myelosuppression and toxicity and to maximize the radiation dose administered, we used AHPSC in conjunction with G-CSF 15 days after the administration of the radiolabeled Br-E-3. There have been no treatment-associated fatalities, bleeding, or important infectious complications in this heavily pretreated group of patients.

Dosimetry studies suggest that hepatic or pulmonary toxicity are likely to be the dose-limiting toxicities at higher doses of 90Y. The risk of hepatic and pulmonary toxicity with higher levels of radiation is well known. The fact that hepatic or pulmonary toxicity are not dose-limiting features of many high-dose chemotherapy regimens used for breast cancer is encouraging for future trials of combined chemotherapy and RAIT.

The 90Y blood half-life appears shorter (not statistically significant) than that of 111In. Faster decomposition of the chelate with liberation of free 90Y may be the explanation. Additional tissue biopsies will be necessary to obtain accurate dosimetry, as using 111In scanning to predict 90Y tissue distribution is inaccurate. These inaccuracies are reflected in the plasma half-life differences. Accumulation of 90Y in bone while it is simultaneously being lost from liver further emphasizes these difficulties.

Applebaum et al. (12) treated dogs with very high doses of 199Ho-EDTMP, a β-emitting radioisotope with rapid localization to the bone marrow, followed by marrow support. Estimated marrow doses were as high as 15,000 cGy during that study. All the animals had adequate recovery of their marrow, suggesting that marrow stromal toxicity should not be a dose-limiting factor for this study. Reinfusion of the marrow or the peripheral blood progenitor cells typically results in reingraftment of granulocytes and platelets at approximately 10 and 14 days after reinfusion (6). Thus, it is reasonable to hypothesize that the granulocyte suppression, in particular, was lessened by the AHPSC support. The purpose of giving AHPSC support is to shorten the period of myelosuppression, not to protect from irreversible myelotoxicity.

The 15-day interval from treatment to marrow reinfusion was estimated from theoretical calculations assuming 100% of the remaining total body activity was deposited uniformly throughout the skeleton. Only physical decay of the radioactivity was considered, providing an extremely conservative upper limit to the potential dose experienced by the reinfused marrow. Source activity contributions from both the cancellous bone and red marrow indicated that the dose contribution to red marrow of 100 mCi of 90Y declines to 380 rads after day 7 (when the only the cumulative dose from that time forward is considered). Using a tolerance dose of 250 rads to bone marrow as the threshold for suppression, bone marrow reinfusion at day 15 was judged to provide a safe interval so that the reinused marrow was not compromised by the activity remaining in the body. On the basis of 90Y half-life in these patients and the results of serial marrow biopsies, it may be possible to safely administer marrow sooner without risk of injury to the marrow graft at the doses of 90Y-labeled radioimmuno-conjugate used in this study.

The dosimetry evaluation suggests that a favorable distribution of dose to tumor relative to normal tissues is achieved with this treatment. Additional studies of tissue biopsies are planned to better define the predictability of 111In scanning for dosimetric data derived from tissue biopsies. These data are critical because the mean blood half-lives for 111In and 90Y may differ (although not significantly in this study), suggesting differences in distribution or elimination.

The 50% response rate with minimal toxicities in this group of heavily pretreated patients was encouraging, even though the number of patients treated to date is small. If confirmed in future patient cohorts, combining RAIT with other treatment modalities may improve antitumor effects. However, additional dose escalation will be necessary to determine the dose-limiting organ toxicity before efficacy can be assessed. Patient accrual will continue until dose-limiting toxicity is defined.

Acknowledgments

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