Initial Clinical Evaluation of Radiolabeled MX-DTPA Humanized BrE-3 Antibody in Patients with Advanced Breast Cancer


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ABSTRACT

To evaluate radiometal-labeled humanized BrE-3 (huBrE-3) monoclonal antibody as a radioimmunolocalization and therapeutic agent in breast cancer patients, tumor localization, pharmacokinetics, radiation dosimetry, and immunogenicity of 111In-labeled combined 1-p-isothiocyanato- benzyl 3-methyl- and 1-p-isothiocyanatobenzyl 4-methyl-diethylenetriamine pentaacetic acid (MX-DTPA) huBrE-3 were studied. Seven women with BrE-3 antigen-positive, metastatic breast carcinoma underwent 111In huBrE-3 infusion (5 mCi; 50 mg), followed by serial gamma camera imaging and plasma sampling. Region of interest analysis of images was used to make radiation absorbed dose estimates for 111In huBrE-3. Data were extrapolated to 90Y huBrE-3. Human anti-human antibody (HAHA) response was measured in serum samples obtained up to 3 months after infusion. Patients tolerated infusions well. Seventy-six percent of 105 known sites of disease were identified on planar and single-photon emission computed tomography scans. For six of seven patients, a biexponential model fit the plasma time-activity curve best with an average $T_{1/2}$ ($\alpha$) = 10.6 ± 8.5 SD h and average $T_{1/2}$ ($\beta$) = 114.2 ± 39.2 h. Radiation absorbed dose estimates for 111In huBrE-3 for whole body averaged 0.53±.08 rads/mCi. Dose estimates for 90Y huBrE-3 for marrow averaged 8.4 ± 11.9 rads/mCi, and for tumors, 70 ± 31.5 rads/mCi. Liver radioactivity uptake averaged 19.7 ± 8.8% injected dose at 24 h after infusion, translating into an average radiation absorbed dose 21.1 ± 12 rads/90Y mCi administered. Only one of seven patients demonstrated a low level of HAHA response. Although the plasma half-lives are longer and marrow dose higher for radiolabeled huBrE-3 compared with the murine construct, the excellent tumor localization, good tumor dosimetry, and low immunogenicity support the use of 90Y-huBrE-3 antibody for radioimmunotherapy of breast cancer.

INTRODUCTION

Breast cancer remains the second most common cause of cancer deaths in women in the United States today. Once a patient recurs with metastases, therapy will prolong but not improve overall survival from this disease. Response rates to second-line therapies are at best 50% and often lower. New approaches to the treatment of advanced breast cancer like radioimmunotherapy, using radiolabeled tumor-directed antibodies, hold promise for improving this prognosis.

One antibody that has shown potential in radioimmunotherapy of breast cancer is BrE-3 labeled with 90Y. The BrE-3 antibody targets the epitope of the M, 400,000 breast epithelial mucin. The epitope, which results from abnormal glycosylation of mucin in tumors, is highly abundant and prevalent in breast carcinoma (1). Radioimmunolocalization trials with 111In-MX-DTPA murine BrE-3 have shown excellent tumor localization (2). Radioimmunotherapy with 90Y-MX-DTPA murine BrE-3 has yielded objective minimal and transient responses at low doses in three of six patients (3) and partial responses in four of eight patients at high doses (4). In spite of these responses, however, retreatment has been obviated by the presence of a human anti-murine antibody response in a majority of these patients (3, 5).

Immunogenicity has proved a persistent stumbling block in radioimmunotherapy in many trials using murine immunoconjugates (6–11). Genetic engineering of antibodies to reduce the amount of mouse protein aimed first at eliminating the murine portion of constant regions of murine antibodies to form mouse/human chimerics. In clinical trials, chimeric immunoconjugates have markedly reduced anti-isotypic responses, but anti-idiotypic responses still occur (12–15). In an attempt to further reduce the immunogenicity of the antibody, humanized antibodies that incorporate human V region frameworks and C regions have been constructed. In this approach, only the CDRs remain

1 The abbreviations used are: CDR, complementarity determining region; CT, computed tomography; MX-DTPA, combined 1-p-isothiocyanatobenzyl 3-methyl- and 1-p-isothiocyanatobenzyl 4-methyl-diethylenetriamine pentaacetic acid; HPLC, high-performance liquid chromatography; SPECT, single-photon emission tomography; HAHA, human anti-humanized antibody.

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from the original murine antibody. To eliminate or reduce the immunogenicity of BrE-3, its humanization was accomplished preserving the CDRs and using consensus human frameworks but achieving over 90% homology with human IgG1 sequences (16, 17). The measured affinity of the humanized antibody is almost three times that of the murine construct. When labeled with \(^{90}\)Y, the humanized antibody shows equivalent efficacy in treatment of human breast carcinoma xenografts (16).

The purpose of this work was to study pharmacokinetics, toxicity, tumor targeting, and immunogenicity of \(^{111}\)In-MX-DTPA humanized BrE-3 (huBrE-3) in the clinical setting. Based on these results and extrapolated absorbed dose estimates, a preliminary evaluation of the potential of MX-DTPA huBrE-3 labeled with \(^{90}\)Y as a radioimmunotherapeutic agent could be made.

**PATIENTS AND METHODS**

Seven nonpregnant women, ages 33 to 61 years, with measurable or evaluable metastatic or recurrent breast cancer were studied (Table 1). All patients had localized disease at initial diagnosis of their primary. The interval between initial diagnosis and participation in the antibody study ranged from 15 months to 22 years. Each patient had tumor specimens obtained previously or current fine-needle aspirates of metastatic tumor that expressed the BrE-3 epitope in >25% of tumor cells by immunohistochemistry. All patients had normal renal and hepatic function, an adequate performance status (Karnofsky >60%), and no evidence of concurrent active collagen vascular disease or significant cardiac disease. No patient had a history of prior exposure to murine or human monoclonal antibodies. All patients were off cytotoxic therapy for at least 3 weeks before antibody infusion. Three patients were receiving hormone therapy at the time of radioimmunoconjugate administration. One patient was undergoing concurrent external beam radiotherapy to the spine. Each patient gave written informed consent as approved by the institutional review board. This study was performed under a United States Food and Drug Administration Investigational New Drug application sponsored by the Cancer Research Institute of Contra Costa.

Before administration of radioimmunoconjugate, a complete medical history was obtained, and a physical examination was performed. Complete blood counts with differentials and serum chemistries were obtained. Within 4 weeks of radioimmunoconjugate administration, chest radiographs, abdominal and pelvic CT scans, bone scans, and electrocardiograms were performed. CT scans were performed using i.v. contrast. Oral contrast was used for abdomen and pelvis studies. Slices, usually 10-mm thick on chest, abdomen, and pelvis CT and 7 mm through the liver, were contiguous. Areas of particular interest were studied with 7-mm slice thickness. Serum samples for measurement of circulating antigen reactive with humanized BrE-3 antibody and human anti-humanized BrE-3 antibody (HAHA) were also obtained at baseline.

**Antibody.** Humanized BrE-3 monoclonal antibody (huBrE-3) is an engineered immunoglobulin of human isotype IgG1 that has >90% humanized sequences (16). Only the CDR sequences remain from the original murine monoclonal antibody. The antibody reacts with an epitope on the Mr 400,000 breast epithelial mucin, which is a tandem repeat amino acid sequence (18). Its affinity for this epitope is approximately three times greater than the affinity of the murine BrE-3. The antibody was provided by the Cancer Research Institute of Contra Costa as a sterile, pyrogen-free solution and also conjugated to MX-DTPA, which is a mixture of 1-p-isothiocyanatobenzyl 3-methyl DTPA and 1-p-isothiocyanatobenzyl 4-methyl DTPA in PBS. The MX-DTPA was conjugated to huBrE-3, as reported previously by Brechbiel et al. (19). Briefly, huBrE-3 is dialyzed in the presence of 0.05 M HEPES buffer, pH 8.6, made up in 0.15 M sodium chloride, plus 1 g/l of Chelex 100 (Bio-Rad), overnight at 4°C. After this, huBrE-3 is placed in a ratio of 1:4 to MX-DTPA in the HEPES buffer plus sodium chloride and left for 19 h at room temperature. The conjugate is then dialyzed over 4 days in PBS, which is changed each day. The huBrE-3-MX-DTPA conjugate is then stored at 4°C. At this temperature, the antibody retains its original reactivity with antigen for up to 30 months thus far. The chelating activity has not decreased over this period of time. Stability in serum at 37°C for 1 week was demonstrated, with no loss of bound metal to chelate.

**Table 1** Patient clinical profiles

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>BrE-3 serum antigen (µg/ml)</th>
<th>Previous treatment</th>
<th>Known disease</th>
</tr>
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<tbody>
<tr>
<td>01HBR</td>
<td>52</td>
<td>0.89</td>
<td>CMF × 8</td>
<td>Bone, lung, liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tamoxifen Radiation-bone Megace</td>
<td></td>
</tr>
<tr>
<td>02HBR</td>
<td>61</td>
<td>0</td>
<td>FAC.*) × 6</td>
<td>Liver, lung, lymph nodes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tamoxifen Megace</td>
<td>Brain, lymph nodes</td>
</tr>
<tr>
<td>03HBR</td>
<td>59</td>
<td>0.18</td>
<td>FAC (neoadjuvant)</td>
<td>Lung, lymph nodes</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Surgery only</td>
<td>Breast, lymph nodes</td>
</tr>
<tr>
<td>04HBR</td>
<td>40</td>
<td>0</td>
<td>FAC × 4</td>
<td>Bone, liver</td>
</tr>
<tr>
<td>05HBR</td>
<td>58</td>
<td>0</td>
<td>FAC × 8</td>
<td>Chest wall, bone, lung</td>
</tr>
<tr>
<td>06HBR</td>
<td>33</td>
<td>Not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>07HBR</td>
<td>45</td>
<td>152</td>
<td></td>
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* FAC, 5-fluorouracil, Adriamycin, cytoxan.

\(\ast\) FAC, 5-fluorouracil, Adriamycin, cytoxan.
Radiolabeling. Approximately 6 mCi of pharmaceutical grade $^{111}$In chloride (Mediphysics, Inc.) were buffered with 0.15 m sodium acetate and mixed with 2 mg of MX-DTPA huBrE-3 antibody for 20 min. Five mm sodium-calcium-EDTA was added to quench the reaction, and the reaction mixture was purified on a Sephadex G25 chromatography column. After purification, an average of 87 ± 8% of the total $^{111}$In incubated with the MX-DTPA huBrE-3 was recovered as antibody-bound radioactivity. Instant TLC was developed in 5 mm EDTA on the pooled fractions containing radiolabeled antibody. The $R_f$ of $^{111}$In huBrE-3-MX-DTPA is practically 0, because the radiolabeled antibody remains at the origin. Radioactivity associated with immunonjugate after purification was always >97%. The resultant product was diluted in 1% human serum albumin in normal saline, and aliquots were subjected to sterility testing and to a chromagenic assay to measure pyrogen levels, which never exceeded the upper limits of acceptability (5 EU/ml/kg). Immunoreactivity assessed by overnight incubation with antigen-coated beads was determined at antibody concentrations of 12.5 ng/ml. In all preparations, immunoreactivity was >68%.

Forty-eight mg of unconjugated huBrE-3 were diluted in 1% human serum albumin and mixed with the radiolabeled MX-DTPA huBrE-3. It was demonstrated previously that a conjugation of ~50 mg of unlabeled BrE-3 yielded optimal imaging and pharmacokinetic results (2, 3). A total volume of 250 ml was infused i.v. over 90 min. Vital signs were monitored during the infusion and for 1 h after completion of the infusion. At 72 h after infusion, repeat blood chemistries, complete blood counts, prothrombin time, and partial thromboplastin time were monitored.

Pharmacokinetics. Serial plasma samples were obtained at 5 min and 1, 2, 4, and 6 h and then at five succeeding time points through 8 days after completion of the infusion. Timed urine collections were performed through the eighth day after infusion. Plasma and urine samples were counted to measure total radioactivity. Plasma samples were further subjected to size exclusion, HPLC to identify antigen-antibody complexes, and intact antibody and breakdown products. Five hundred-$\mu$L samples were applied to a 10 × 600-mm BioSep SEC 3000 column equipped with a 10 × 50-mm guard column (Phenomenex, Torrance, CA). The mobile phase consisted of 0.05 M NaH$_2$PO$_4$, 0.1 M Na$_3$SO$_4$ buffer (pH 6.8) with an elution flow rate of 0.6 ml/min. Parallel detection was achieved with radiation detection followed by UV detection. Determination of radioactivity was performed using a BioScan Incorporated flow count detector (Bethesda, MD) equipped with an FC002 gamma flow cell. Data were processed at the 100-kilocount setting. A Perkin-Elmer LC-135 diode detector (Norwalk, CT) set at 280 nm was used for the UV detection. The 1-V output from both detector systems was interfaced to an Axxiom Chromatographic Data Controller system (Moorepark, CA). This permitted simultaneous plotting of the radioactive counts and UV absorbance. The column calibration was verified using a 20-$\mu$L injection of Bio-Rad gel filtration standard (thryoglobinulin, bovine gamma globulin, chicken ovalbumin, equine myoglobin, and vitamin B$_{12}$). Blood clearance of radiolabeled antibody was determined. The half-time for clearance of the radiolabeled antibody was obtained using a nonlinear biexponential (two-compartment) pharmacokinetic model. Initial parameter estimates were derived using the one of the three curve-stripping algorithms available within WINONLIN (Statistical Consultants, Apex, NC). These estimates were then applied to a biexponential to fit the time/concentration antibody data with respect to the duration of infusion and i.v. route of infusion. The key pharmacokinetic parameters obtained included half-lives for each compartment (e.g., α and β) and the area under the concentration versus time curve expressed as a percentage of injected dose-hours.

Imaging and Image Analysis. Quantitative planar gamma camera imaging was performed to determine normal organ and tumor biodistribution, to identify tumor localization, and to make radiation absorbed dose estimates (20). All images were obtained with a digital large field of view gamma camera fitted with a medium energy collimator. Prior to radioimmunoconjugate administration, an $^{111}$In transmission scan was acquired. Conjugate regional spot views or whole-body images were acquired at 4, 24, and 48–72 h and 7–8 days after infusion. Dual energy 20% windows centered on 173 and 247 keV, the energy windows of $^{111}$In, were used. For whole-body imaging, scan speed was 5 cm/min. For regional spot imaging, 5-min acquisitions were performed for time points up to 72 h after injection and 7.5 min for more delayed images.

Whole-body counting was performed for those patients who underwent regional spot view imaging. For patients who underwent whole-body imaging, the whole-body images were used to obtain whole-body counts of radioactivity.

SPECT scans were obtained at the third (48–72 h) and fourth (7–8 day) imaging sessions. Either a triple-headed large field of view camera (Triad, Trionix Corp., Twinsburg, OH) acquired a total of 120 25-s projections, or a dual-headed camera (GCA7200; Toshiba Corp.) acquired a total of 90 40-s views over a 360° circumferential interval around the patient. Raw projection data were reconstructed into transaxial slices using filtered backprojection, and Chang's method of attenuation correction was applied. Resulting slices were 2 pixels thick (7.1 mm, Triad; 8.6 mm, GCA7200). Data were then reformatted into 2-pixel-thick coronal and sagittal slices.

Images were interpreted by a nuclear medicine physician with full knowledge of the extent of disease as assessed by physical examination and conventional imaging modalities. Only those lesions that showed increased uptake, even in the liver, were considered positive. Because this was an early Phase I study whose objective was to assess $^{111}$In-MX-DTPA huBrE-3 localization in known tumors, blinded readings were not performed.

Using the planar images, region of interest and background templates were generated over normal organs and tumors for all imaging time points (21). Background subtraction, attenuation correction, and camera sensitivity derived from an $^{111}$In standard of known activity were applied to all normal organ regions of interest to calculate the fraction injected activity (20, 22). Background subtraction and correction for camera sensitivity were applied to all tumor regions of interest. Attenuation correction was applied to the tumor regions when appropriate. The geometric mean of anterior and posterior activity was determined for large organs (i.e., liver and lung). For smaller organs, e.g., kidney, and for tumors the counts from a single planar projection (anterior and posterior) were used (20). Using the
fraction injected activity calculated for each time point, the biological and the effective half-lives of the radioimmunoconjugate were calculated for each normal organ and for measurable tumors that were visible on planar scans. A mono-exponential curve was assumed in all cases. Whole body remainder was calculated using both whole-body scans and urine clearance values, and the results were compared. The residence time was then calculated as follows:

Residence time = Maximum fraction injected activity

× 1.44 × $T_{1/2\text{eff}}$

Following the MIRD formulation and based on the $^{111}$In-MX-DTPA huBrE-3 biodistribution, the radiation absorbed dose (per mCi or MBq administered) for the $^{111}$In-labeled immunoconjugate was estimated using an implementation of the MIRDSE program (23). By extrapolation from the $^{111}$In biodistribution data to $^{90}$Y-MX-DTPA huBrE-3, absorbed doses for the $^{90}$Y-labeled immunoconjugate were estimated. Blood dose to marrow was calculated using the concentration of radioactivity in blood, assuming 25% of the marrow volume was blood and using the “S” factor for marrow dose from marrow (non-penetrating; Ref. 24). For $^{111}$In, this calculated dose was added to the absorbed dose to marrow from other organs and the remainder of the whole body to determine red marrow absorbed dose (25).

For $^{90}$Y, the radiation absorbed dose from other organs and whole body were not included because the contribution from $^{90}$Y in these sites to marrow constitutes <1% of the total marrow dose.

Radiation absorbed dose was calculated for at least one tumor that was both measurable on CT and identifiable on planar scintigraphy for each patient. The tumor size was determined by measurement of the tumor of interest in three planes on CT scans. Regions of interest were generated over these tumors on the sequential scans, and fraction injected activity and biological and effective half lives for radioactivity were calculated as described above. For tumors, a single planar projection was used to determine the activity within that region of interest. Tumor radiation absorbed doses from radioactivity accumulation in tumor was calculated by finding the volume based on the closest regular geometric shape, then calculating the radius of a sphere with the same volume and obtaining the absorbed fraction in water for a sphere of that radius (26).

**HAHA.** Serum samples for determination of HAHA were obtained at baseline, 8 days, 5 weeks, and 3 months after antibody infusion. HAHA response was assessed by incubating patients’ serum with $^{125}$I-labeled huBrE-3. After incubation, the mixture was run on an HPLC equipped with a guard column and two separation columns BioSep-SEC 3000 (Phenomenex) placed in series. Presence of HAHA was determined by detection of a decrease in transit time of a portion of the radiolabeled huBrE-3 representing the antibody-antibody immune. The level of this antibody-antibody complex was quantitated using a standard curve constructed by incubating 0.6–16 ng/ml of mouse anti-BrE-3 idiotype with the $^{125}$I-labeled huBrE-3.

**Immunohistochemistry.** Immunohistochemical analysis of the expression of BrE-3 epitope in patient tumor samples was determined on formalin-fixed, paraffin-embedded tumor samples from breast and axillary lymph nodes and on cell smears derived from fine-needle aspiration of bone or visceral metastases. Immunohistochemical assays were performed using a modified avidin-biotin immunoperoxidase technique (Vectastain; Vector Laboratories, Burlingame, CA) with 3,3'-diaminobenzidine as a chromogen. Briefly, 5-μm tissue sections from surgical specimens were applied to poly-L-lysine-coated slides, and cell smears from fine-needle aspirates were prepared on coated slides, fixed in formalin for 5 min, and then held at −4°C in saline. Biotinylated humanized BrE-3, diluted to a final concentration of 25 μg/ml, was applied for 1 h to slides in an automated immunostainer (Cadenza; Shandon-Lipshaw, Pittsburgh, PA). A positive control (derived from formalin-fixed, paraffin-embedded sections of a breast carcinoma characterized previously) and a negative control (where primary antibody was substituted for serum) were run for each case.

Immunostained sections or cell smears were evaluated for positive immunoreactivity and scored from 1 to 4, depending upon intensity of staining. In addition, the percentage of immunoreactive tumor cells was determined and expressed in 10% increments. All seven patients demonstrated positive staining on immunohistochemistry of tumor tissue obtained previously or fine-needle aspirates.

**RESULTS**

**Toxicity.** No evidence of allergic reactions was observed among the seven patients evaluated in this study. One patient developed a grade 3 thrombocytopenia 9 days after administration of $^{111}$In-MX-DTPA huBrE-3. Although this was in proximity to the administration of radioimmunoconjugate, it was felt that this thrombocytopenia could be attributed to the combination of concurrent external beam radiation to the spine and extensive bone marrow metastases as identified by bone scan and bone marrow biopsy. Analysis of blood failed to identify a serological basis for platelet destruction. No other significant alterations in blood chemistries or blood counts were identified.

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**Table 2** Average percentage of injected dose (%ID) ± SD at 4, 24, and 72 h after injection and $T_{1/2\text{eff}}$ (h) for $^{111}$In MX-DTPA huBrE-3 antibody

<table>
<thead>
<tr>
<th>Organ</th>
<th>4 h</th>
<th>24 h</th>
<th>72 h</th>
<th>8 days</th>
<th>$T_{1/2\text{eff}}$ (h)</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>16.7 ± 7.1%</td>
<td>19.7 ± 8.8%</td>
<td>19.3 ± 7.7%</td>
<td>18.7 ± 7.7%</td>
<td>63.6 ± 3.4</td>
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<tr>
<td>Spleen</td>
<td>1.8 ± 0.4%</td>
<td>1.9 ± 0.4%</td>
<td>1.7 ± 0.4%</td>
<td>1.7 ± 0.5%</td>
<td>58.5 ± 8.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.7 ± 1.7%</td>
<td>4.4 ± 1.5%</td>
<td>4.4 ± 1.7%</td>
<td>4.1 ± 1.4%</td>
<td>50.4 ± 10.8</td>
</tr>
<tr>
<td>Lung</td>
<td>5.9 ± 2.4%</td>
<td>5.6 ± 1.9%</td>
<td>4.3 ± 1.7%</td>
<td>3.9 ± 1.7%</td>
<td>58.8 ± 6.1</td>
</tr>
</tbody>
</table>

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Biodistribution and Pharmacokinetics. The uptake of radioactivity at 4, 24, and 72 h and 8 days in normal organs is shown in Table 2. The average liver uptake over the course of imaging was ~20% of the injected dose of radioactivity. In fact, for all normal organs analyzed, the percentage of injected dose varied little over the course of the imaging. The mean effective half-life for the $^{111}$In-MX-DTPA huBrE-3 for the whole body was 61.2 ± 2.1 h (Table 3).

The pharmacokinetics of radiolabeled antibody as determined by HPLC of plasma fit a biexponential model in six of seven patients. In these six patients, the $T_{1/2a}$ averaged 10.6 ± 8.5 h, and the $T_{1/2b}$ averaged 114.2 ± 39.2 h. One patient (01HBR) demonstrated first-order kinetics with a $T_{1/2bio}$ of 24.1 h. This patient also demonstrated a relatively high percentage of radioactivity present as antigen-antibody complex (15.6%) in her plasma, although her baseline circulating antigen level was fairly low (Table 1). The average area under the curve for plasma radiolabeled antibody over time was 198.5 ± 139.6 %ID h for all seven patients.

Radiation Dose Estimates. For $^{111}$In-MX-DTPA huBrE-3, the absorbed dose to the whole body averaged 0.53 ± 0.08 rads/mCi administered (Table 4). The average absorbed dose to the liver was 2.92 ± 1.40 rads/mCi. These estimates are comparable with that seen for the murine antibody. The biodistribution of $^{111}$In-MX-DTPA huBrE-3 was used to extrapolate $^{90}$Y-MX-DTPA huBrE-3 absorbed dose estimates. Whole-body absorbed doses averaged 2.46 ± 0.31 rads/mCi of $^{90}$Y administered. This was similar to those calculated for the murine construct labeled with $^{90}$Y (2, 3). However, again assuming that marrow irradiation came only from circulating radioimmunoconjugate, red marrow absorbed doses averaged higher (8.43 ± 11.88 rads/mCi) compared with the murine radioimmunoconjugate. This is at least in part due to the longer biological half-life of the humanized MX-DTPA BrE-3.

On the other hand, with one exception (patient 7 with 13 rads/mCi), we found consistently high tumor absorbed doses (43–101 rads/mCi). Tumor absorbed doses averaged 70.0 ± 31.5 rads/mCi of administered $^{90}$Y. This gives an average tumor:marrow absorbed dose ratio of 8.3:1.

Imaging Results. Altogether, 105 sites of disease were identified by conventional diagnostic modalities including physical examination, bone scintigraphy, and CT. Of these, 76.2% were identified on $^{111}$In-MX-DTPA huBrE-3 whole-body and SPECT scans (Table 5). Sensitivities for lymph node metastases (88.9%) and lung metastases (84.6%) were the highest. Sensitivity for bone metastases was lower (71.9%). Five of 16 bony lesions in patient 6 were false negatives, possibly because many of these sites fell within the radiation therapy port. At the time of the antibody administration, this patient had already received a week of external beam radiation treatments. In other patients, bony lesions were obscured by underlying or nearby nonspecific uptake such as liver or blood pool. Among the 105 lesions, two sites, one lymph node and one bony, had not been identified previously. These were confirmed by repeat CTs (Fig. 1). Almost all liver lesions seen on antibody scan were identified by increased uptake (Fig. 2). The one exception was a cold lesion with a rim of mildly increased uptake, presumably representing necrosis surrounded by tumor. For most liver lesions, SPECT imaging was needed to identify the sites of abnormal uptake.

HAAAA. Only one of the patients’ serum showed detectable levels of HAAHA. These very low levels were detected at 1 week (26.4 ng/ml) and 3 months (28 ng/ml) after antibody infusion in one patient. No other patients demonstrated levels of HAAHA above the baseline average at any of the time points after huBrE-3 administration (8 days, 5 weeks, and 3 months).

DISCUSSION

In the seven patients whom we studied, $^{111}$In-MX-DTPA huBrE-3 administration was tolerated extremely well. No allergic or antibody-related toxic reactions were identified. In the one patient who experienced a significant thrombocytopenia in temporal proximity to radioimmunoconjugate administration, no anti-platelet antibodies could be identified and, in fact, bone marrow examination revealed extensive tumor infiltration as the probable cause of her thrombocytopenia. As with the murine form of this antibody (2), we administered 50 mg to each patient without difficulty. Up to 500 mg of antibodies targeting similar antigens have been administered in earlier trials without complication (27). In the clinical trials of murine BrE-3, a few mild allergic reactions occurred (2, 3). Possibly, the humanization of the antibody may have reduced the likelihood of these reactions. This report provides still further evidence that toxicity from the antibody itself will not be problematic, possibly even with

<table>
<thead>
<tr>
<th>Patient</th>
<th>Whole-body $T_{1/2bio}^a$ (h)</th>
<th>Blood $T_{1/2bio}^b$</th>
<th>Ag-Ab complex$^c$ (%)</th>
<th>AUC$^d$ (%ID × h)</th>
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<tr>
<td>01HBR</td>
<td>55.3</td>
<td>24.1</td>
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<td>16.8</td>
<td>192.6</td>
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<tr>
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<tr>
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<td>1.6</td>
<td>119.3</td>
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<tr>
<td>07HBR</td>
<td>62.4</td>
<td>16.1</td>
<td>106.5</td>
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<td>Mean ± SD</td>
<td>61.2 ± 2.1</td>
<td>10.6 ± 8.5</td>
<td>114.2 ± 39.2</td>
<td>2.6 ± 5.8</td>
</tr>
</tbody>
</table>

$^a$ $T_{1/2bio}$, effective half-life.
$^b$ $T_{1/2bio}$, biologic half-life.
$^c$ Ag-Ab, antigen-antibody complex.
$^d$ AUC, area under the curve.
repeated administrations. Immunogenicity may still be an issue in that setting, however.

Development of human anti-murine antibody has been a major obstacle to the clinical deployment of radioimmunotherapy with BrE-3 (3), preventing retreatment with the murine antibody in patients who developed an immune response even when clinical tumor responses were observed. Engineering of antibody proteins like humanized BrE-3 to reduce the foreign immunogenicity of these antibodies in some cases.

Chimerization of other antibodies has proved effective in reducing the immunogenicity of these antibodies in some cases. For instance, chimeric 17-1A elicited very little immune response (12, 14). On the other hand, chimeric B72.3 elicited significant anti-chimeric immune responses in 7 of 12 patients (13) as did anti-CEA chimeric T84.66 in 2 of 3 patients (28). Chimeric NR-LU-13 elicited anti-chimeric immune responses in six of eight patients (15), albeit at a very low level.

Thus far, humanization of antibodies has reduced immunogenicity more consistently. Repeated dosing with humanized M195 elicited no measurable HAHA response in 13 patients, although 4 patients may have had very low-level responses (29). In a comparison of repeated dosing with murine anti-TAC antibody to a similar regimen with humanized anti-TAC in primates, an anti-humanized response was elicited later and at a lower level than the response to the murine anti-TAC regimen (30). With humanized BrE-3 antibody, only one patient showed any HAHA, and this very low level of HAHA may not have any clinical significance. This suggests that repeated dosing might be possible over a short interval without an alteration in pharmacokinetics. The incidence and magnitude of the HAHA response to BrE-3 may increase with repeated administrations. Both the influence of these low HAHA responses on pharmacokinetics and the effect of repeated administration will be examined in the upcoming dose-fractionation radioimmunotherapy trial.

Humanization of an antibody may be accompanied by reduced affinity and, in vivo, by changes in pharmacokinetics and biodistribution compared with the murine constructs. In the case of BrE-3 antibody, humanization was accomplished without loss of in vitro affinity for its antigen (16). By scintigraphy in our patients, we observed a slightly lower tumor localization rate (76%) than we did with the patients receiving the murine BrE-3 (87%; Ref. 2). At least in part, this may have related to higher background ratios because humanized BrE-3 showed overall a longer half-time for clearance from blood than murine BrE-3. Persistent blood pool may have obscured uptake in some of the known lesions. It is less likely that this is related to antibody binding to tumor because fairly consistently high radiation doses were estimated for tumors in the group receiving humanized antibody (average, 70.0 ± 31.5 rads/mCi administered; range for six tumors, 43–101 rads/mCi; one tumor, 13 rads/mCi). Estimated radiation absorbed dose in those tumors studied with the murine antibody ranged widely from ~2 rads/mCi administered to 590 rads/mCi in our earlier trial (2). The relatively high tumor doses found here are based only on imaging data. Biopsy after antibody administration was not available for validation of fraction injected dose estimates made from the planar images. Inaccuracies may be introduced when using image analysis for radiation absorbed dose because of unavailability of correct background measurements and inaccurate edge detection when generating regions of interest. Smaller tumor volumes may worsen these problems. The accuracy of this method is >10% for 131I (20) but may be less for 111In. For a 10-mI tumor, the reproducibility has been found to be 7.7% (20).

The pharmacokinetics of 111In-MX-DTPA huBrE-3 appear to differ from the murine as well. The biodistribution in normal organs showed somewhat higher uptake in the liver than the murine construct. The average value for liver uptake was skewewed somewhat by patient 1, who showed ~37% ID in the liver at 24 h. One explanation in this patient may be related to circulating antigen, although a higher level was observed in patient 7. The absolute amount of antigen measured was 0.89 μg/ml (Table 1), and HPLC showed 15.6% radioactivity present in plasma as antigen-antibody complex 5 min after infusion (Table 3). Clearance of antigen-antibody complex into the liver may have contributed to the high accumulation in liver. More likely, however, the high accumulation in the liver may have been due to occult but diffuse metastatic disease. Although this patient’s liver CT was without evidence of tumor at the time of imaging result, 2.5 months later, diffuse liver metastases were found. Possibly, some of the antibody uptake might be attributable to localization in diffuse microscopic metastases in this patient. The other three patients studied with liver metastases, patients 2, 3, and 6, also had relatively higher liver uptake (21.4, 24.3, and 20.8%, respectively) than the other patients because these metastases were included in the regions of interest used for analysis. The average percentage of uptake of the humanized radioimmunoconjugate in the spleen was slightly
higher in the seven patients studied compared with patients receiving murine BrE-3. Compared with the normal organ biodistribution of the murine radioimmunoconjugate, the values for the humanized radioimmunoconjugate showed little decrease over the first week. This may be related to the longer blood clearance observed for the humanized construct.

The half time for blood clearance for humanized BrE-3 \((T_{1/2\text{bioP}} = 114.2 \pm 39.2)\) averaged slightly more than twice as long as the blood clearance half-time for the murine antibody \((T_{1/2\text{bioP}} = 56 \pm 25.4; \text{Ref. } 2)\). Prolonged half-time for blood clearance has been reported for some other humanized antibodies (30) and for chimeric B72.3 (13, 31), NR-LU-13 (15), and 17-1A (12). Longer half-times may translate into prolonged access to tumor for the radioimmunoconjugate. On the other
The variability in tumor and marrow absorbed dose estimates in our patients raises a further point about the use of radioimmunolocalization in radioimmunotherapy. Because $^{90}Y$ is difficult to image, we have chosen to codeadminister $^{111}$In-labeled antibody to trace normal organ and tumor uptake. In the tumors, as with these other antibodies, the prolonged half-time in blood for huBrE-3 translates into a higher radiation absorbed dose to marrow. Marrow absorbed dose estimates for $^{90}Y$-labeled huBrE-3 in six patients ranged from 1.9 to 9.9 rads/mCi $^{90}Y$ administered. In one patient, the absorbed dose estimated was 34.5 rads/mCi $^{90}Y$. In our patients, an average tumor: marrow absorbed dose ratio for $^{90}Y$-MX-DTPA huBrE-3 of 8.3:1 was found. However, the individual tumor:marrow ratios ranged from 2.0 to 50.2. These ratios, which are an indication of therapeutic index, may be sufficient to achieve tumor responses in some patients.

The variability in tumor and marrow absorbed dose estimates in our patients raises a further point about the use of radioimmunolocalization in radioimmunotherapy. Because $^{90}Y$ is difficult to image, we have chosen to codeadminister $^{111}$In-labeled antibody to trace normal organ and tumor uptake. In the

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**Fig. 2** A 61-year-old woman with progression of disease while on Tamoxifen. In A, a posterior planar image at 72 h after infusion of $^{111}$In-MX-DTPA huBrE-3 shows a distinct focus of uptake at the top of the liver, which corresponded to a lung metastasis at the right base (open arrow) and a less well-defined area of increased uptake in the mid portion of the right lobe (arrow). In B, a transaxial SPECT slice through the liver metastasis shows this increased uptake in the posterior aspect of the right lobe more distinctly (open arrow). In C, CT slice at the same level obtained at the time of the antibody scan shows multiple hypodense liver metastases, the largest one of which corresponds to the abnormal uptake within the liver.
setting of our Phase I trial, where pharmacokinetics and biodistribution are important end points, this has been the only means of estimating the behavior of the therapeutically labeled (90Y) immunoconjugate. However, in the setting of more routine clinical therapy, pretherapy imaging may be a sounder strategy to predict which patients’ tumors will be targeted by radioimmunoconjugate and in which patients excessive normal organ radiation absorbed dose would make radioimmunotherapy unacceptable toxic.

The utility of the latter approach will depend on our ability to use 111In radioimmunoconjugate to predict the behavior of 90Y radioimmunoconjugate. It is recognized that free 111In and 90Y will behave differently in vivo, with 90Y seeking bone mineral and 111In binding to marrow-associated proteins. It is also expected that the stability of the 111In conjugate bonds may differ from that of 90Y conjugates. The validity of using 111In to predict 90Y radiation absorbed dose will also depend on the reproducibility of the behavior of radioimmunoconjugate between two closely spaced administrations. Analysis of blood and urine pharmacokinetics for 90Y-MX-DTPA huBrE-3 and 111In-MX-DTPA huBrE-3 when they are coadministered in the context of the ongoing dose fractionation therapy trial will answer some of these questions.

In conclusion, this initial radioimmunolocalization trial has shown that the engineered humanized construct of BrE-3 antibody is much less immunogenic than the murine form. Tumor localization is comparable, although background clearance is slower. The prolonged blood clearance appears to have implications for increasing red marrow absorbed dose. Although this is not a serious consideration for antibody labeled with a diagnostic amount of 111In, this may decrease the therapeutic index of 90Y-labeled huBrE-3. Initial estimates of tumor absorbed dose for 90Y-labeled huBrE-3 give an average tumor:marrow ratio of 8.3 with a fair degree of interpatient variability. We anticipate that the reduced immunogenicity of humanized BrE-3 will permit repeated administration and improve the possibility of prolonged therapy with 90Y-labeled huBrE-3. Strategies in radioimmunotherapy that may offer some marrow protection of prolonged therapy with 90Y-labeled huBrE-3 may be feasible with this reduced immunogenicity. The efficacy of such an approach is now being tested in a radioimmunotherapy trial. However, it may be necessary to incorporate other types of marrow support to achieve tumoricidal effects. Alternatively, strategies to enhance the radiation effect at the tumor site may prove effective.

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