Dosimetry-based Therapy in Metastatic Breast Cancer Patients Using ^{90}Y Monoclonal Antibody 170H.82 with Autologous Stem Cell Support and Cyclosporin A[1]

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Abstract

Radioimmunoconjugates of 170H.82 (m170), a panadenocarcinoma monoclonal antibody, are effective for imaging primary and metastatic breast cancer. To evaluate m170 as a targeting agent for therapy, we developed ^{111}In- and ^{90}Y-2-iminothiolane-2-[*p*-(bromoacetamido)benzyl]-1,4,7,10 tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-m170 immunoconjugates with 99% purity by molecular sieving and immunoreactivity comparable to unmodified antibody. ^{111}In-m170 pharmacokinetic studies were performed prior to each therapy to determine the maximum dose of ^{90}Y-m170 that could be administered without exceeding a limit of 800 rad to the liver, lungs, or kidneys or 250 rad to the whole body or bone marrow for each of three cycles of treatment. Peripheral blood stem cells (PBSCs) were harvested and cyclosporin A (5 mg/kg twice daily) was administered as strategies to ameliorate myelosuppression and prevent the development of HAMA, respectively. An ^{111}In imaging/pharmacokinetic study was performed, and the ^{90}Y dose was calculated and administered. The liver was the ^{90}Y dose-limiting organ. The mean and range of calculated doses (in rad/mCi) for the five patients evaluated were as follows: whole body, 2.3 (2.1–2.4); liver, 17.8 (12.7–22.2); lung, 6.4 (4.8–7.2); kidney, 6.9 (6.3–11.5); marrow, 3.6 (1.9–4.4); and tumors (n = 25), 71.5 (14.1–141.5). Of the three patients treated, with doses of 37, 54, and 57 mCi of ^{90}Y, one had a partial response, one had measurable tumor reduction but less than a partial response, and one had stable disease for more than 1 month. PBSCs prevented prolonged myelosuppression. The therapeutic responses, coupled with an absence, thus far, of significant adverse sequelae, suggest that this dosimetry-based approach combined with PBSCs may lead to effective therapy when higher ^{90}Y doses are reached.

Introduction

RIT,[3] using systemically administered monoclonal antibodies linked to high-energy, short-range radioisotopes, is a promising new approach for treating adenocarcinoma (1–4). Because of selective biological concentration of the antibody and thus the isotope in tumor tissue, this novel modality can deliver substantial doses of radiation to tumors throughout the body, while minimizing the concomitant exposure of normal tissue (5). Pilot clinical trials in metastatic breast cancer, using the murine and human-murine chimeric antibody L6 have yielded objective responses in approximately one-half of the patients (6, 7). Myelosuppressive toxicity, the initial dose-limiting toxicity of RIT, can be ameliorated by bone marrow transplantation, as shown by Press *et al.* (8) in lymphoma, or by PBSC transplantation (9–11).

As radiopharmaceutical doses are escalated without the limitation of bone marrow suppression, toxicity to other organs such as the lungs and liver becomes dose-limiting; *e.g.*, single doses of ^{131}I-CD20 antibody coupled with stem cell transplantation have shown dose-limiting symptomatic cardiopulmonary toxicity in some lymphoma patients (12). Thus, normal organ dosimetry becomes critical in the setting of high dose RIT. In the present study, ^{111}In imaging and pharmacokinetics define the dose to normal organs anticipated from ^{90}Y-linked antibody (13–15). The aim of the study, the preliminary results of which are reported here, is to determine the maximum tolerated dose of ^{90}Y that can be administered as ^{90}Y-2IT-BAD-m170 without exceeding specified normal organ radiation dose limits and to determine the clinical effects of this treatment in patients with advanced metastatic breast cancer (16).

Patients and Methods

Antibody. M170.82 is a murine monoclonal IgG1 antibody reactive with the synthetic Thomsen-Friedenreich antigen family (17–19). M170 binds to a variety of human adenocarcinomas, including breast cancer, with high affinity (4×10^8 M^{-1}; Ref. 18). Eighty-five % of clinical breast cancer tissue specimens have shown *in vitro* immunohistochemical reactivity (75% of the cells positive) with m170 (20) obtained from Biomira

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[3] The abbreviations used are: RIT, radioimmunotherapy; RIC, radioimmunoconjugate; PBSC, peripheral blood stem cell; CSA, cyclosporin A; HAMA, human antimouse antibody; 2IT, 2-iminothiolane; BAD, 2-[*p*-(bromoacetamido)benzyl]-1,4,7,10 tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid.

Table 1 Clinical characteristics of patients with metastatic breast cancer imaged/treated with ^{90}Y/^{111}In-2IT-BAD-m170

Patient	Age	Site of metastases	m170-RIT		HAMA	Clinical response
			Imaging (no.), therapy (no.)	Dose mCi ^{90}Y (mCi/m^2)		
1	41	Bone, pleura, liver	I (4) T (1)[a]	54 (30)	−	Stable >1 month
2	45	Bone, liver	I (1)		+	
3	49	Nodes: supraclavicular, axilla, mediastinal	I (1) T (1)	57 (34)	+	Partial Response
4	50	Left chest wall and pleura, bone	I (2) T (1)	37 (20)	−	Objective Response <50%
5	45	Bone, liver, lung	I (1)			

[a] I, imaging; T, therapy.

(Edmonton, Alberta, Canada), where this antibody is being evaluated as a diagnostic imaging agent for breast cancer (21).

RICs (^{111}In- and ^{90}Y-2IT-BAD-m170). The RICs were prepared as described previously (22, 23). Briefly, the antibody (m170), 2IT, and BAD were combined, and the solution was incubated at 37°C for 30 min. The unlabeled immunoconjugate was then purified and incubated with a buffered solution of ^{90}Y (Pacific Northwest National Laboratory, Richland, WA). After diethylenetriaminepentaacetic acid was added to scavenge nonspecifically bound ^{90}Y, the radiopharmaceutical was purified by gel filtration. ^{111}In-2IT-BAD-m170 was prepared similarly, except that EDTA was used to scavenge nonspecifically bound ^{111}In (Amersham Pharmacia Biotech, Arlington Heights, IL). The resulting radioimmunoconjugate doses were 99% pure by molecular sieving (24, 25). Immunoreactivity was comparable to the unmodified antibody, and cellulose acetate electrophoresis showed that 98% of all RICs were in monomeric form (26, 27).

Patient Eligibility. Patients with metastatic breast cancer shown to be reactive by immunoperoxidase staining of fresh or frozen tumor tissue (20) were eligible if they had measurable disease unresponsive to or recurrent after at least one prior combination chemotherapy regimen. In addition, patients had normal complete blood counts, normal organ function, Karnofsky performance status of 70% or better, no other serious medical illnesses, no chemotherapy or radiation for at least 4 weeks, and a negative serum HAMA (28). Patients with extensive disease (>25%) of the axial skeleton, liver, or lung or evidence of central nervous system involvement were excluded. All patients who participated in the study signed written informed consent in accordance with the institutional review board guidelines as well as the Radiation Use Committee under an Investigational New Drug Authorization from the Food and Drug Administration.

The clinical characteristics of the five patients imaged with ^{111}In-2IT-BAD-m170 are shown in Table 1. The first three patients participated in an imaging/pharmacokinetic study (a regulatory requirement). Because of the extended interval from initial imaging, two of the three patients (patients 1 and 3) had a second imaging just prior to their first therapy dose. Patient 1 had transient thrombocytopenia after each of two imaging doses preceding therapy cycle 2 and did not receive further treatment. Three patients received high dose ^{90}Y-labeled m170, as indicated. Imaging and therapy doses were administered in the RIT outpatient facility. Patients were instructed on proper precautions as per Radiation Use Committee policies. Serial blood counts, chemistries, clinical examination, and tumor measurements were performed following therapy. Criteria for removal from the study included unacceptable toxicity, progressive disease, and HAMA.

PBSC Mobilization and Collection. Prior to imaging/therapy, PBSCs were harvested by apheresis following G-CSF mobilization (10 μg/kg/day) as described previously (10). The goal was to collect a total of 6×10^5 granulocyte-macrophage colony-forming units, and 6×10^6 CD34+ cells per kg of patient weight to support the planned three therapy cycles. Cells were cryopreserved (10), and an aliquot (approximately 1/3 of the cells, containing at least 2×10^6 CD 34+ cells/kg) was transfused 7 days after a therapy dose in patients 1 and 3 and 14 days after therapy in patient 4. Blood levels of radioactivity were less than 0.25 μCi/ml.

Pharmacokinetics and Radiation Dosimetry. Because ^{90}Y has β emissions not suitable for imaging, ^{111}In was used for pharmacokinetic studies, for imaging, and to calculate the radiation dosimetry for equivalent ^{90}Y-immunoconjugates (29-31). After an imaging dose of ^{111}In-2IT-BAD-m170 (~5 mCi of ^{111}In on 5–10 mg of m170), serial blood and urine specimens and whole body scans were obtained: immediately after injection and at approximately 4, 24, 48, 72, and 144 h. Methods for collecting the pharmacokinetic data and calculating radiation doses have been described previously (13, 32). The study was designed to deliver the maximum mCi dose of ^{90}Y-m170 that would not exceed specified organ limits for each of three planned doses: 800 rad to the liver, lungs, or kidneys (total, 2400 rad) and 250 rad to the whole body or bone marrow (750 rad for 3 cycles; see Figs. 2 and 3). Two weeks after the imaging dose, the ^{90}Y dose was administered along with a second tracer dose of ^{111}In to further define pharmacokinetics with data collection as for the imaging dose. Five patients had pharmacokinetic and imaging studies, and three patients received one therapy dose (Table 1).

CSA and HAMA. CSA was administered p.o. at a dose of 5 mg/kg every 12 h beginning 3 days before the imaging dose and continuing, following each dose of antibody, for 11 days (patients 1–3) initially. After HAMA occurred in two of the initial three patients, the duration of CSA was extended for an additional week. CSA trough levels were measured twice weekly by HPLC and maintained between 150 and 250 ng/ml. Pretherapy and posttherapy HAMA values were quantitated using a standard enzyme-linked immunoabsorbent assay as described previously (28).

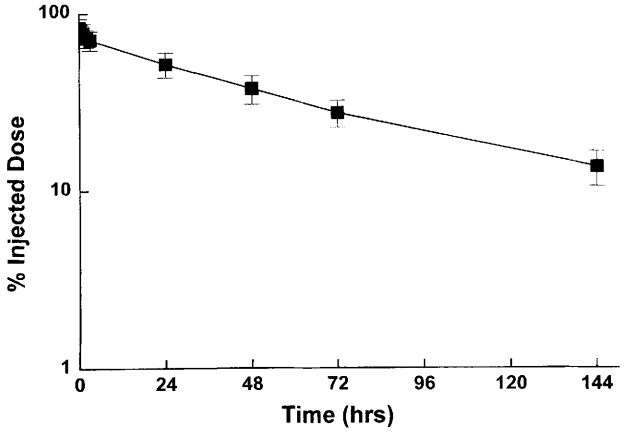

Fig. 1 Blood clearance (mean ± SD) of ^{111}In-2IT-BAD-m170 in studies from five patients. Radioactivity is expressed as a percentage of the injected dose (*%ID*).

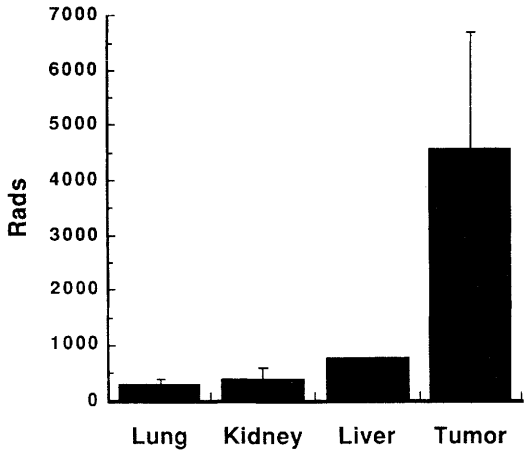

Fig. 2 Dosimetry for ^{90}Y-2IT-BAD-m170 in three patients indicating the calculated dosage to each organ (mean ± SD) and to tumors based on pharmacokinetics and imaging following a tracer dose of ^{111}In-2IT-BAD-m170. The starting organ dose limit in this Phase I study was 800 rad (per cycle of treatment) to the liver, lungs, or kidney or 250 rad to the whole body or bone marrow. The dose-limiting organ was the liver.

Results

Pharmacokinetic and imaging studies were performed in five patients with metastatic breast cancer; three patients were subsequently treated with the ^{90}Y-immunoconjugate. The blood clearance of ^{111}In-2IT-BAD-m170 is shown in Fig. 1, demonstrating a biexponential curve: 50% of the injected dose was cleared within 24 h and 90% by 6 days after treatment. Approximately 10% of the injected dose was excreted in the urine by 6 days after therapy (data not shown).

Based on the pharmacokinetic studies above and serial whole body imaging, the highest dose that would not exceed the specified organ dose limits was determined. Fig. 2 shows that the dose-limiting organ was the liver (800 rad) in the three patients treated. The doses (in rad/mCi) predicted by ^{111}In imaging for ^{90}Y therapy for all five patients are shown in Fig. 3.

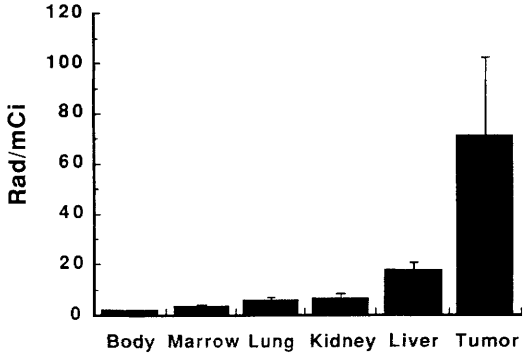

Fig. 3 ^{90}Y dose (mean ± SD rad/mCi) to normal organs and tumors in five patients studied based on ^{111}In-2IT-BAD-m170 pharmacokinetics and imaging. The therapeutic index (dose to tumor/dose to normal tissue) was at least 4.

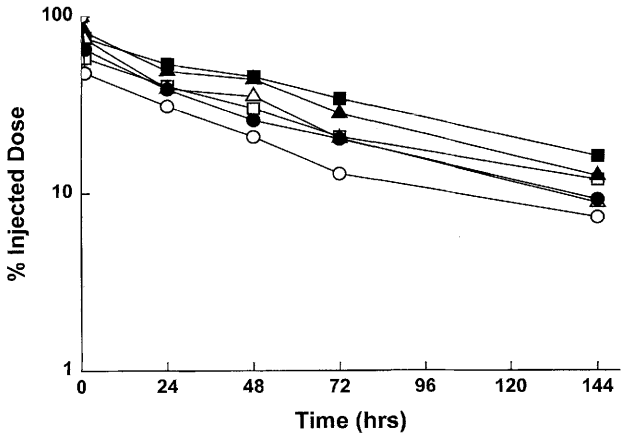

Fig. 4 Blood clearance of ^{111}In (●, ▲, and ■) and ^{90}Y (○, △, and □) after co-administration of ^{90}Y/^{111}In-2IT-BAD-m170 in the three patients (patient 1, ■ and □; patient 3, ▲ and △; patient 4, ● and ○) given therapy doses of 37–57 mCi of the ^{90}Y radiopharmaceutical along with a tracer dose of ^{111}In-m170.

As expected, the liver received the highest radiation dose of the normal solid organs, approximately 18 rad/mCi, compared to less than 7 rad/mCi for the kidneys and lungs. However, the tumor dose was over 70 rad/mCi, resulting in a good therapeutic index.

Three patients received therapeutic doses of 37–57 mCi of ^{90}Y-2IT-BAD-m170 (20 to 34 mCi/m^2). To verify the ability of ^{111}In to track the dose of ^{90}Y, a tracer dose of ^{111}In-linked m170 was administered simultaneously with the ^{90}Y-linked m170. Pharmacokinetics are shown in Fig. 4. The blood clearance of the two isotopes was comparable in all three patients, with the ^{111}In clearing somewhat more slowly than the ^{90}Y in all cases. (The area under the curve for ^{90}Y was 22,000 ± 3,400 μCi-hr/mCi (mean ± SD), and for ^{111}In, it was 29,500 ± 6,400.) The average liver dose in these three patients was 800 rad, and the tumor dose was 4600 rad. One patient had a partial response (70% reduction in the sum of products of perpendicular diameters), one had objective tumor shrinkage (48%), and one had stable disease for more than 1 month. Although three treatments

Table 2 Hematologic toxicity following ^{90}Y-2IT-BAD-m170 therapy

Patient	Duration (days)		
	Neutrophils (<1000/<500)	Platelets (<50/<25)	Platelet transfusions
1	0/0	35/4	2
3	1/0	8/0	0
4	0/0	30/0	0

were planned, the three patients treated received a single course of therapy due to progressive disease (1), HAMA (1), and patient decision due to lack of objective response (disease was stable) and treatment delay due to transient thrombocytopenia following repeat imaging doses (1).

Acute toxicity from the radiopharmaceutical infusion was minimal (grade 1 chills and grade 1 rise in blood pressure in one patient). Despite PBSC infusion following therapy, transient myelosuppression occurred, as expected (Table 2). Neutropenia was mild; only 1 patient had grade 3 neutropenia, and that lasted only 1 day. All patients had grade 3 thrombocytopenia, but only one had grade 4 toxicity for 4 days; that patient received two platelet transfusions. Platelets exceeded 100,000/μl by days 63, 38, and 48 after stem cell infusion (for patients 1, 3, and 4 respectively).

Nonhematological toxicities were mild. Transient bilirubin elevations, to 1.4–1.9 mg/dl, were observed in 3 of 5 patients, between 6 and 21 days after an imaging dose (5 events) and 3-6 days after a treatment dose (3 events). The bilirubin returned to normal within 1 week. One patient, with a bilirubin of 1.4 mg/dl just prior to imaging, was subsequently found to have adenocarcinoma-induced hemolysis and went off study following the imaging study due to rapidly progressive disease. The fifth patient had normal liver chemistries throughout. Renal function remained normal in all patients, except for a transient creatinine of 1.4 mg/dl in one patient following an imaging dose.

Despite CSA administration, HAMA was detected in two of the five patients given m170, one after her first imaging dose (patient 2), and a second after her initial therapy dose (patient 3).

Discussion

RIT is a novel approach that can produce objective clinical responses in approximately one-half of patients with metastatic breast carcinoma, most of whom have been heavily pretreated and are chemotherapy-refractory (1, 6, 7, 10, 11). In this Phase I trial, two of three patients treated at the starting dose level had objective reduction in tumor size. Like chemotherapy, RIT is systemic tumoricidal therapy. However, unlike chemotherapy, the antibody on the radiopharmaceutical accumulates gradually in tumors, with relatively rapid clearance from normal tissues, resulting in a high ratio of radiation to tumor when compared to normal tissue.

In prior studies with ChL6, ^{90}Y-linked antibody had a better therapeutic index than the ^{131}I radioimmunoconjugate (32). ^{90}Y delivers high-energy pure β particles (2.3 MeV maximum) with a maximum range of 1.2 cm in soft tissue (5). Tumor cells without tumor-antigen expression can thus be killed by ^{90}Y bound to adjacent antigen-expressing cells. The high energy of ^{90}Y β particles, the relatively short half-life (64 h) of ^{90}Y, and the absence of γ-rays and hence reduced risk of radiation exposure to distant organs and to health care personnel favor its use over ^{131}I, which has a longer physical half-life (8 days) and lower particulate energy (~600 keV) and decays predominately as long-range γ-rays (81%) that require inpatient radiation safety monitoring. Because ^{90}Y has no γ-emissions, the surrogate γ-emitting ^{111}In is used for imaging and dosimetry for ^{90}Y radiopharmaceuticals. Similar pharmacokinetic behavior for both ^{111}In and ^{90}Y isotopes in blood, urine, and bone marrow samples have been reported in studies with ^{90}Y/^{111}In-DOTA-peptide-ChL6 (32). In the present study of ^{90}Y/^{111}In-2IT BAD-m170, ^{111}In and ^{90}Y pharmacokinetic curves followed each other closely (see Fig. 4). ^{111}In consistently clears somewhat more slowly than ^{90}Y, as has been reported previously (32). The use of ^{111}In as a surrogate for ^{90}Y in determining doses thus results in selection of a conservative dose of ^{90}Y. The ^{111}In-2IT-BAD-m170 pharmacokinetics in initial imaging studies paralleled the behavior of ^{111}In-2IT-BAD-m170 co-administered with the ^{90}Y-2IT-BAD therapy dose.

Radiometals, such as ^{111}In and ^{90}Y, are retained longer in normal tissue, particularly the liver, than iodinated radioconjugates (33–35). When used in high doses, ^{90}Y-RIT could result in radiation damage to normal organs. In the present study, we have used the method of patient-specific dosimetry as opposed to the usual mCi/m^2 dosing strategy. This is done to optimize the dosage used in the MTD trial, while assuring the safety of administration by limiting the dose per cycle to lung, liver, kidney, bone marrow, and whole body. In this way, there is a means of preventing excessive radiation doses to normal tissues in patients who happen to have diffuse tumor involvement, for example, in the liver or lung. As expected, the liver was the dose-limiting organ in all three patients treated, *i.e.,* the mCi dose administered delivered the protocol-specified first level limit of radiation (800 rad) to the liver and did not reach set limits for other organs. The limits set are based on a dose per cycle with three planned cycles of treatment, so the proposed total dose to liver lung and kidney would be 2400 rad. In prior studies with ^{131}I linked ChL6 given in three fractions, we were able to administer a total lung dose of 3100 rad with no sign of cardiopulmonary toxicity, whereas Press *et al.* (12), using a single high dose, found that the maximum tolerated dose was 2700 rad, suggesting that the fractionation strategy permits administration of higher radiation doses, as has been seen in external beam radiation therapy (5) and in preclinical RIT trials (36, 37).

Development of antibodies directed at the targeting antibody is an obstacle for the fractionated approach to RIT (38). In our prior studies of ^{131}I- or ^{90}Y-DOTA-peptide-linked ChL6 (10), the administration of CSA from 3 days before the initial antibody exposure (*i.e.,* the imaging dose) until 11 days after the treatment dose prevented HAMA and corroborated results of other investigators (39, 40). Using the same timing with ^{90}Y-2IT-BAD-m170 in our first three patients resulted in two patients developing HAMA (one after imaging alone, one after the initial therapy dose). The blood clearance of ^{111}In-2IT-BAD-m170 was significantly slower than observed in our prior studies with (ChL6), in which over 80% was cleared by 24 h and over 90% by 48 h (32), compared to 50% clearance in 24 h and 90%

clearance in 6 days with ^{111}In-2IT-BAD-m170. Because of this, the duration of CSA administration has been extended an additional week. Although it is too early to draw conclusions, HAMA has not developed in subsequent patients. It is likely that the transient bilirubin elevations noted in some patients were related to CSA and not to the RIT itself because abnormalities occurred with both imaging only and imaging plus treatment doses.

Outpatient-based fractionated intensive dose RIT with PBSC support and CSA for HAMA prevention is being evaluated in this Phase I trial in metastatic breast cancer. Preliminary results indicate that the therapy is well tolerated with only transient grade 3/4 myelosuppression and minimal transient nonhematological toxicity. Multidose therapy with dose escalation is in progress. Additional strategies to improve the efficacy of therapy include development of new chemical linkers to attach the antibody to the radioisotope in such a way that radiopharmaceutical in normal tissues can be readily metabolized and excreted to reduce normal organ exposure (41). Further enhancement of RIT may be achieved by combination of RIT with radiation-sensitizing chemotherapy. Recent studies have shown marked schedule dependent synergy between RIT and low doses of Taxol (42). A clinical trial of a carefully sequenced combination of multidose Taxol and RIT with stem cell support is being developed and may lead to improved therapy for metastatic breast cancer (43).

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