Phase I Study of $^{131}$I-Anti-CD45 Antibody Plus Cyclophosphamide and Total Body Irradiation for Advanced Acute Leukemia and Myelodysplastic Syndrome

By Dana C. Matthews, Frederick R. Appelbaum, Janet F. Eary, Darrell R. Fisher, Lawrence D. Durack, T. Edmond Hui, Paul J. Martin, David Mitchell, Oliver W. Press, Rainer Storb, and Irwin D. Bernstein

Delivery of targeted hematopoietic irradiation using radiolabeled monoclonal antibody may improve the outcome of marrow transplantation for advanced acute leukemia by decreasing relapse without increasing toxicity. We conducted a phase I study that examined the biodistribution of $^{131}$I-labeled anti-CD45 antibody and determined the toxicity of escalating doses of targeted radiation combined with 120 mg/kg cyclophosphamide (CY) and 12 Gy total body irradiation (TBI) followed by HLA-matched related allogeneic or autologous transplant. Forty-four patients with advanced acute leukemia or myelodysplasia received a biodistribution dose of 0.5 mg/kg $^{131}$I-BC8 (murine anti-CD45) antibody. The mean ± SEM estimated radiation absorbed dose (centigray per millicurie of $^{131}$I) delivered to bone marrow and spleen was 6.5 ± 0.5 and 13.5 ± 1.3, respectively, with liver, lung, kidney, and total body receiving lower amounts of 2.8 ± 0.2, 1.8 ± 0.1, 0.6 ± 0.04, and 0.4 ± 0.02, respectively. Thirty-seven patients (84%) had favorable biodistribution of antibody, with a higher estimated radiation absorbed dose to marrow and spleen than to normal organs. Thirty-four patients received a therapeutic dose of $^{131}$I-antibody labeled with 76 to 612 mCi $^{131}$I to deliver estimated radiation absorbed doses to liver (normal organ receiving the highest dose) of 3.5 Gy (level 1) to 12.25 Gy (level 6) in addition to CY and TBI. The maximum tolerated dose was level 5 (delivering 10.5 Gy to liver), with grade III/IV mucositis in 2 of 2 patients treated at level 6. Of 25 treated patients with acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), 7 survive disease-free 15 to 89 months (median, 65 months) posttransplant. Of 9 treated patients with acute lymphoblastic leukemia (ALL), 3 survive disease-free 19, 54, and 66 months posttransplant. We conclude that $^{131}$I-anti-CD45 antibody can safely deliver substantial supplemental doses of radiation to bone marrow (~24 Gy) and spleen (~50 Gy) when combined with conventional CY/TBI.

© 1999 by The American Society of Hematology.
mates demonstrated that 131I-anti-CD45 antibody could deliver relatively specific radiation to hematopoietic tissues, with 2 to 3 times more radiation delivered to marrow, up to 12 times more to spleen, and 2 to 8 times more to lymph nodes as compared with liver, lung, or kidney. We thus initiated a phase I dose escalation study combining 131I-anti-CD45 antibody with CY and 12 Gy TBI in patients with advanced AML, ALL, and MDS receiving matched related or autologous stem cell rescue. The goals of this study were to determine the biodistribution of 131I-anti-CD45 antibody in patients with leukemia in remission and relapse, to define factors influencing antibody biodistribution, and to determine the toxicity of targeted hematopoietic irradiation when combined with a conventional preparative regimen. Our initial report of the first 23 patients entered on study demonstrated that the majority had successful targeting of radiation to hematopoietic tissues and that up to 7 Gy of radiation delivered by antibody to the normal organ receiving the highest dose (liver) was well tolerated when combined with CY/TBI. We now report the completion of the study, demonstrating that 84% of 44 patients undergoing biodistribution studies had good localization of antibody and that a maximum dose of 10.5 Gy delivered by radiolabeled antibody to the liver could be tolerated in addition to CY and 12 Gy TBI.

MATERIALS AND METHODS

Patient selection. Patients referred to the Fred Hutchinson Cancer Research Center (Seattle, WA) for treatment of AML or ALL beyond first remission or for advanced MDS (refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or chronic myelomonocytic leukemia) were eligible for this study. Patients in relapse with peripheral blast counts in excess of 5,000/µL were eligible only if their blast count could be brought below that level by treatment with hydroxyurea. Patients were excluded if they had major organ dysfunction, were seropositive for human immunodeficiency virus (HIV), were allergic to mouse protein or to iodine, or had pre-existing human antimouse antibody (HAMA). Stem cell sources were either bone marrow harvested from HLA-identical family members, cryopreserved autologous marrow, or cryopreserved autologous peripheral blood stem cells. Purging of autologous marrow with 4HC7 or monoclonal antibodies and complement was allowed. Patients were informed of the potential risks and benefits of participating in this phase I study and signed a consent form approved by the Human Subjects Committee of the University of Washington and the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Antibody production, purification, and radiolabeling. The BC8 hybridoma (developed and provided by Dr Claudio Anasetti of the Fred Hutchinson Cancer Research Center) secretes a murine IgG1 antibody reactive with all CD45 isoforms. The initial lot of antibody was derived from hybridoma culture supernatant produced in hollow fiber bioreactors by Brunswick (San Diego, CA) and was purified by saturated ammonium sulfate precipitation and ion exchange chromatography. Subsequent lots were produced at the Fred Hutchinson Cancer Research Center Biologies Production Facility. Hybridoma supernatant was harvested from an Applikon bioreactor (Applikon Instruments, Schiedam, The Netherlands), filtered, concentrated, pooled, and purified using anion exchange and protein A affinity chromatography. This was followed by virus inactivation using low pH treatment, SP-Sepharose cation exchange chromatography, and diethylaminoethyl (DEAE) anion batch processing. Processed antibody was 0.2-µm sterile filtered and stored at 4°C. Twenty-nine of the first 32 patients also received nonspecific (negative control) antibodies DT or LS (produced and kindly provided by IDEC Pharmaceuticals, San Diego, CA). These antibodies are murine IgG1 antibodies reactive with idiotypes expressed by B-cell lymphomas.

The BC8 antibody was labeled with 131I (specific activity, 8.0 Ci/mg; New England Nuclear, Boston, MA), and DT or LS antibodies were labeled with 125I (specific activity, 17.2 Ci/mg; New England Nuclear) using the chloramine-T method and were purified and tested as previously described. The immunoreactivity of all 131I-labeled BC8 antibody doses was required to be at least 80% of that of an 131I-trace-labeled control aliquot of BC8 antibody.

Determination of antibody biodistribution and radiation absorbed dose. Patient serum was first tested for HAMA using an enzyme-linked immunosorbent assay (ELISA) as previously described. For patients in relapse, the expression of CD45 on leukemic blasts was determined by BC8 antibody with indirect immunofluorescence assays using a fluorescein isothiocyanate (FITC)-labeled goat-antimouse-IgG+M F(ab')2 second-stage reagent (Tago, Inc, Burlingame, CA) or by HLe-1 anti-CD45 antibody directly labeled with Peridinin chlorophyll protein (Becton Dickinson, San Jose, CA). Relapse patients were eligible if the CD45 expression on the blast cell population was clearly above that of negative control antibody. Patients in remission did not require leukemic cell phenotyping.

Organ volumes (liver, lungs, spleen, and kidney) were calculated from chest and abdominal computed tomography (CT) or magnetic resonance image (MRI) scans. Organ localization scans (technetium-99m liver-spleen, lung, and kidney) were used in the first 16 patients but were subsequently omitted because organs were easily localized (lungs), readily visualized after 131I-BC8 administration (liver and spleen), or demonstrated little uptake of radionuclide (kidney). Thyroid uptake of free 131I was blocked by oral Lugol’s solution (strong iodine solution).

Biodistribution infusions consisted of 0.5 mg/kg BC8 antibody labeled with 5 to 10 mCi 131I. For 29 patients, this was combined with 0.2 mg/kg DT or LS antibody labeled with 2 to 5 mCi 125I. Patients were premedicated with diphenhydramine at 25 to 50 mg intravenously (IV), hydrocortisone at 50 to 100 mg IV, and acetaminophen at 650 mg orally. The infusion rate for BC8 antibody varied from 5 to 10 mg/h, as tolerated, with most patients receiving a steady rate of 7.5 mg/h. Diphenhydramine doses were repeated as needed up to every 2 hours, and other medications (meperidine for chills and lorazepam for nausea) were administered as needed for symptoms. If patients developed more severe symptoms, such as throat tightness or shortness of breath, the infusion was slowed or stopped until the symptoms improved.

Blood samples, which were obtained hourly during antibody infusion and 0, 30, 60, 90, and 120 minutes afterwards and then daily for 3 to 5 days, were analyzed for BC8 and nonspecific (where administered) concentrations. Blood clearance curves were fit to determine the long-term clearance half-time and where obvious, the early phase, rapid-clearance half-time. A bone marrow biopsy was obtained the day after infusion (ie, 16 to 24 hours, where hour 0 was the end of infusion). The sample was weighed and counted against a weighed reference aliquot of the expected dose to calculate the percentage of injected dose per gram (% ID/g). For some patients, the biopsy occurred 42 to 48 hours after infusion. The marrow radioactivity clearance curves obtained by gamma camera imaging were scaled for quantitation using the bone marrow biopsy 131I uptake values. For patients receiving 125I-labeled anti-idiotypic control antibody, the marrow localization index (LI) was calculated with reference to a concomitant serum sample: LI = (Specific % ID/g [marrow]/Specific % ID/g [serum])/(Nonspecific % ID/g [marrow]/Nonspecific % ID/g [serum]).

Quantitative gamma images were collected with a dedicated GE 400 AT large-field-of-view camera (General Electric Medical Systems, Milwaukee, WI) with a high energy collimator at hour 0 (end of infusion) and then daily for 2 to 3 days (Fig 1). Regions of interest (spleen, liver, lungs, kidneys if visible, and at least 2 marrow sites) were imaged using a 180° opposing view quantitative planar technique.
Results were compared with an 131I imaging standard for quantitation and were corrected for whole-body thickness attenuation and radioactive decay. The time-activity curves for each source organ were integrated to obtain residence times. Because organ dose is roughly inversely proportional to mass, corrections were made for patient weight and organ mass when actual weights were known from CT or MRI. This correction was made by multiplying the observed source-organ residence time by the ratio of the defined reference man or woman organ mass to the known organ mass. Radiation absorbed doses were then estimated using methods consistent with those recommended by the Society of Nuclear Medicine’s special committee on Medical Internal Radiation Dose,41,42 as previously described.43 The marrow clearance curve was scaled by correcting the biopsy-determined % ID/g of 131I-BC8 by a multiplication factor of 2, because antibody cannot bind to the trabecular bone and fat that make up approximately half of the total biopsy weight.44,45 For dosimetry purposes, patient marrow volumes were normalized to the MIRD model values of 1,120 grams for an adult male and 1,050 grams for an adult female. For consistency, the same S values42 were used for all marrow dose calculations throughout the study. Statistical comparisons between disease type or stage and between anti-CD45 and control anti-iodiotype antibody used the Student’s t-test (SPSS for Windows 8.0; SPSS Inc, Chicago, IL).

**Therapy.** Patients in whom the biodistribution study of 131I-BC8 showed that the target organs of marrow and spleen would receive a greater estimated radiation absorbed dose than liver, lung, or kidney were said to have favorable biodistribution and were eligible for a therapy dose of antibody. Patients were retested for HAMA the day before administration of the therapy dose and were treated only if the test was negative. The therapy dose was labeled with the amount of 131I calculated to deliver a specified dose to the normal organ receiving the highest radiation absorbed dose. The starting dose level delivered to this normal organ by 131I-BC8 antibody was 3.5 Gy, and the dose was escalated by 1.75 Gy if 0 of 3 or not more than 1 of 6 evaluable patients developed...
grade III (life-threatening) or IV (fatal) regimen-related toxicity at the previous dose level.

The therapy dose, administered at the same rate as the biodistribution dose, was administered on day 1−14 of the transplant regimen. This dose, generally 9 days after the trace-labeled biodistribution dose, was administered in lead-lined radiation isolation rooms, where patients remained until the total body 131I activity was less than 30 mCi (usually 3 to 6 days). Patients were then admitted to a marrow transplant ward, where they received CY 60 mg/kg IV on days −8 and −7, followed by TBI administered in daily 2 Gy fractions from days −6 to −1. TBI was delivered at a dose rate of 7.36 Gy/min from 2 opposing 60Co sources. Stem cells were infused on day 0. Cyclosporine and methotrexate were used for graft-versus-host disease (GVHD) prophylaxis in allogeneic recipients. All patients received routine posttransplant supportive care. Results are current as of September 15, 1998.

RESULTS

Biodistribution studies. Forty-four patients were entered on study and received a biodistribution dose of trace 131I-labeled BC8 antibody. The median age was 38 years (range, 16 to 55 years). Thirty-one had AML (9 remission and 22 relapse), 10 had ALL (5 remission and 5 relapse), and 3 had MDS (Table 1).

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Disease</th>
<th>Stage</th>
<th>No. of Patients Studied</th>
<th>No. of Patients Treated</th>
<th>Stem Cell Source (allogeneic/autologous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>All stages</td>
<td>31</td>
<td>22</td>
<td>14/8</td>
</tr>
<tr>
<td></td>
<td>1° refractory</td>
<td>4</td>
<td>2</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>Untreated first relapse</td>
<td>13</td>
<td>9</td>
<td>5/4</td>
</tr>
<tr>
<td></td>
<td>Refractory first relapse</td>
<td>2</td>
<td>2</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>Second remission</td>
<td>8</td>
<td>6</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td>Second relapse</td>
<td>3</td>
<td>2</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>Third remission</td>
<td>1</td>
<td>1</td>
<td>1/0</td>
</tr>
<tr>
<td>MDS</td>
<td>RAEB</td>
<td>2</td>
<td>2</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>RAEBT</td>
<td>1</td>
<td>1</td>
<td>1/0</td>
</tr>
<tr>
<td>ALL</td>
<td>All stages</td>
<td>10</td>
<td>9</td>
<td>6/3</td>
</tr>
<tr>
<td></td>
<td>1° refractory</td>
<td>3</td>
<td>3</td>
<td>3/0</td>
</tr>
<tr>
<td></td>
<td>Second remission</td>
<td>4</td>
<td>4</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>Refractory relapse</td>
<td>2</td>
<td>2</td>
<td>2/0</td>
</tr>
<tr>
<td></td>
<td>Third remission</td>
<td>1</td>
<td>0</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Grade I−III side effects were experienced by 75% of patients receiving the biodistribution dose of 131I-BC8 antibody. Most common were shaking chills and nausea or vomiting. Fever greater than 38.5°C occurred in 4 patients. Grade I−II hypotension developed in 11 patients and generally responded to IV crystalloid administration. Side effects usually developed after the first hour of infusion and responded to slowing of the infusion and treatment with meperidine, diphenhydramine, and lorazepam. Twenty-five percent of patients experienced respiratory symptoms including wheezing or sensations of tightness in the chest or throat. These symptoms usually improved after the antibody infusion was temporarily slowed or halted and diphenhydramine was administered, but in 3 patients, the antibody infusion was stopped after a total dose of 0.2 mg/kg because of these symptoms. In 2 of these patients, antibody infusion rates were greater than 7.5 mg/h when they developed symptoms. After the initial 21 patients, antibody infusion was stopped after a total dose of 0.2 mg/kg.

Estimated radiation absorbed doses. The mean estimated radiation absorbed doses calculated from the biodistribution dose of trace-labeled antibody are shown in Fig 2. The mean radiation absorbed doses (centigray per millicurie of 131I administered ± SEM) were 6.5 ± 0.5 for marrow, 13.5 ± 1.3 for spleen, 2.8 ± 0.2 for liver, 1.8 ± 0.1 for lung, 0.6 ± 0.04 for kidney, and 0.4 ± 0.02 for the total body. Thus, the mean ratio...
of estimated radiation dose was 2.3 for the marrow as compared with the liver, 3.6 for the marrow as compared with the lung, and 11 for the marrow as compared with the kidney. The liver was the normal organ receiving the highest estimated radiation absorbed dose, except in 1 patient with an unusually long retention of radiiodine in the lungs. Overall, favorable biodistribution was seen in 37 of 44 patients studied (84%).

Patients with AML in relapse (including MDS) had higher estimated radiation absorbed doses to marrow (7.8 v 4.9 cGy/mCi, P = .04) and to spleen (16.5 v 8.3 cGy/mCi, P = .001) as compared with patients with AML in remission. There was no significant difference in the estimated radiation absorbed dose to the liver between patients with active disease and those in remission, and therefore the ratio of radiation delivered to target as compared with normal organs was higher for both marrow (2.7 v 2.0, P = .053) and spleen (5.4 v 3.3, P = .012) for patients with AML in relapse. However, even for patients in relapse, there was appreciable variability in estimated radiation dose to marrow and spleen resulting from variation in initial uptake, half-time, or both. For patients with ALL, there were no differences between estimated radiation dose to marrow, spleen, or nontarget organs between patients in remission or relapse (data not shown).

Although the liver was the normal organ receiving the highest radiation dose per micromille of 131I in all but 1 patient, there was great variability between patients in the estimated radiation absorbed dose to liver, ranging from 1.7 to 7.1 cGy/mCi 131I. No correlations between clinical features such as disease state, type of leukemia, or number of circulating blasts and the estimated liver dose were apparent in this study.

Seven patients did not have favorable biodistribution of 131I-BC8 antibody, with a lower estimated radiation dose to marrow or spleen than to liver or lung. Two were in remission and 5 were in relapse at the time of study. Whereas 3 had relatively low marrow cellularity (50% of normal), the other 4 had cellularity ranging from 100% to 200% of normal. The average weight of the 7 patients with unfavorable biodistribution was greater than that of the patients with favorable biodistribution (105.4 v 76.0 kg, P < .001), and the patients with unfavorable biodistribution were overweight (actual body weight divided by ideal body weight) by a higher percentage than those with favorable biodistribution (141% v 107%, P < .02). Of 15 patients weighing 89 kg or more, 7 had unfavorable biodistribution. In contrast, all 29 patients weighing less than 89 kg had favorable biodistribution. Because the antibody dose administered was calculated on actual as opposed to ideal body weight, obese patients received a higher average dose of antibody in relationship to circulating blood volume and to total CD45 antigen. We found a negative correlation (Pearson correlation = −.372, P = .018) between weight and the percentage of injected dose per gram of marrow at biopsy.

An eighth patient had favorable biodistribution of antibody to marrow and spleen but had a low concentration of 131I-BC8 antibody in a skin biopsy from a site of leukemia cutis and thus was not treated on study. The remaining 2 patients that were not treated with radiolabeled antibody included 1 that was positive for HAMA on the day before the planned therapy (8 days after receiving the biodistribution dose) and 1 that received only 0.2 mg/kg BC8 antibody and had an estimated radiation absorbed dose of 1.3 cGy/mCi for liver. This patient, whose antibody dose had been limited by side effects, was not treated because the amount of 131I required to deliver dose level 5 (830 mCi) would have resulted in a specific activity of more than 50 mCi/mg, a level previously shown to damage the immunoreactivity of BC8 antibody. Patients not receiving a therapy dose of 131I-BC8 antibody were treated with alternative marrow transplant preparative regimens.

**Table 2. Biodistribution and Pharmacokinetics for 131I-BC8 Antibody**

<table>
<thead>
<tr>
<th>Patient Group and Marrow Status</th>
<th>Marrow</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hr 0% ID/g</td>
<td>t1/2 (h)</td>
<td>Hr 0% ID/g</td>
</tr>
<tr>
<td>0.028 ± 0.012</td>
<td>44.2 ± 14.7</td>
<td>35.3 ± 10.3</td>
<td>0.013 ± 0.004</td>
</tr>
<tr>
<td>0.028 ± 0.014</td>
<td>46.7 ± 15.8</td>
<td>30.2 ± 4.7</td>
<td>0.013 ± 0.004</td>
</tr>
<tr>
<td>0.025 ± 0.008</td>
<td>35.5 ± 6.5</td>
<td>30.1 ± 4.7</td>
<td>0.013 ± 0.004</td>
</tr>
<tr>
<td>0.024 ± 0.005</td>
<td>37.4 ± 6.3</td>
<td>30.2 ± 4.7</td>
<td>0.013 ± 0.004</td>
</tr>
<tr>
<td>0.020 ± 0.005</td>
<td>32.1 ± 6.1</td>
<td>30.2 ± 4.7</td>
<td>0.013 ± 0.004</td>
</tr>
<tr>
<td>0.020 ± 0.003</td>
<td>35.2 ± 2.1</td>
<td>29.3 ± 1.1</td>
<td>0.013 ± 0.003</td>
</tr>
<tr>
<td>0.020 ± 0.015</td>
<td>36.3 ± 4.6</td>
<td>31.1 ± 4.7</td>
<td>0.013 ± 0.003</td>
</tr>
</tbody>
</table>

Values are the means ± SD. % ID/g was estimated by extrapolation to time 0 for marrow.

*P = .001 for difference between patients with AML/MDs and ALL.

**P = .009 for difference between patients with AML/MDs and ALL.

†P < .001 for difference between remission and relapse in patients with AML/MDs.

§P = .01 for difference between remission and relapse in patients with AML/MDs.

||P = .013 for difference between remission and relapse in patients with AML/MDs.

* Therapy, toxicities, and engraftment. Thirty-four patients proceeded to the transplant phase of the study and were treated with the same dose of BC8 antibody received during the biodistribution study labeled with the amount of 131I activity calculated to deliver an estimated radiation dose to the normal organ receiving the highest dose (i.e., the liver) of 3.5 (dose level 1) to 12.25 Gy (dose level 6). The 131I activity administered and estimated radiation absorbed doses delivered to marrow and spleen at each dose level are summarized in Table 3 together with regimen-related toxicities at each dose level. Side effects during the therapeutic infusion of antibody were similar to those experienced with the biodistribution dose. Some patients, particularly those treated at higher dose levels, experienced mild to moderate nausea and vomiting during the first few days after administration of the therapeutic dose of antibody. Patients remained in radiation isolation for 3 to 7 days after treatment.

Most regimen-related toxicities were typical for conventional
marrow transplant regimens. All patients developed at least grade II mucositis (ie, requiring narcotic therapy). Grade III mucositis occurred in both patients receiving dose level 6 (12.25 Gy to liver) and thus was the dose-limiting toxicity in this study (see below).

A patient treated at dose level 1 (3.5 Gy to liver) developed ileus and hemorrhagic enterocolitis 16 to 18 days posttransplant and thus was considered to have grade III gastrointestinal toxicity. This patient went on to develop gut GVHD that may have been the etiology of his symptoms.

A patient treated at dose level 3 (7 Gy to liver) did not engraft by the time of her death from candida pneumonia 29 days posttransplant, despite the administration of granulocyte colony-stimulating factor (G-CSF). Minimal myeloid and erythroid engraftment was present in a postmortem marrow biopsy, and the presence of macrophages and eosinophilic debris suggested marrow stromal damage. This patient received an estimated radiation absorbed dose to the bone marrow of 31 Gy from $^{131}$I-BC8 antibody, in addition to 12 Gy external beam TBI. It is possible that this high total radiation dose delivered to marrow damaged the marrow microenvironment, and this patient was considered to have grade IV marrow toxicity. In subsequent patients, the estimated marrow dose from $^{131}$I-BC8 antibody was limited to 28 Gy. Three subsequent patients with high ratios of radiation delivered to marrow as compared with liver thus received estimated liver radiation doses that were less than those specified by the dose escalation schema to limit the marrow dose to 28 Gy.

One of 6 patients treated at dose level 5 (10.5 Gy to liver) developed grade III hepatic toxicity, with a maximum serum bilirubin concentration of 11.7 mg/dL and severe ascites requiring paracentesis. Two of 2 patients treated at dose level 6 (12.25 Gy to liver) developed grade III mucositis as defined by the need for endotracheal intubation to protect the airway or by the development of aspiration pneumonia. The first of these patients developed significant mucositis by the fifth dose of TBI; thus, the sixth dose was omitted. This patient was intubated because of severe mucositis and oropharyngeal bleeding on day 0 and remained intubated for 3 days. He also developed portal vein thrombosis with ascites and a peak serum bilirubin concentration of 8.0 mg/dL, which was complicated by renal failure secondary to hepatorenal syndrome, but recovered. The second patient developed fever and cough 9 days before transplant and a naso-pharyngeal culture grew respiratory syncytial virus (RSV). He developed both severe mucositis and progressive pulmonary impairment and was intubated on day 6 after transplant. Squamous cells were demonstrated in pulmonary fluid obtained by broncho-alveolar lavage, documenting aspiration. The patient died 8 days posttransplant from progressive RSV pneumonia despite treatment with Ribavirin.

Thus, the maximum tolerated dose estimated by this study was dose level 5 (10.5 Gy delivered to the normal organ estimated to receive the highest dose from radiolabeled antibody in addition to CY and 12 Gy TBI).

Engraftment was analyzed for the patients receiving allogeneic marrow who survived more than 30 days after transplant and did not relapse in the first month. Of 19 such patients, an absolute neutrophil count of 500/µL (first day of 3 sustained days >500/µL) was achieved a median of 21 days (range, 9 to

![Fig 2. Estimated radiation absorbed doses per milliCurie of $^{131}$I administered for (A) all patients, (B) patients with AML or MDS, and (C) patients with ALL. Values are the means ± SD. Estimated radiation absorbed doses to marrow were calculated using published methods. Recently proposed marrow S values incorporated in MIRDOS3 software would result in lower marrow doses than noted here. AML-Remission group includes a patient in marrow remission with leukemia cutis. *P = .04 for difference between marrow remission and relapse in patients with AML/MDS; **P = .001 for difference between marrow remission and relapse in patients with AML/MDS.](attachment://Fig2.png)
Table 3. 131I Activity Administered, Total Radiation Absorbed Doses, and Grade III/IV Regimen-Related Toxicities

<table>
<thead>
<tr>
<th>Dose to Liver (Gy)</th>
<th>Dose to Marrow (Gy)</th>
<th>Dose to Spleen (Gy)</th>
<th>Grade III/IV Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>(dose level)</td>
<td>mCi 131I*</td>
<td>(dose level)</td>
<td>(no.)</td>
</tr>
<tr>
<td>3.5 (Level 1)</td>
<td>148 ± 42</td>
<td>7.3 ± 1.7</td>
<td>14.4 ± 5.0</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(76-206)</td>
<td>(4-9)</td>
<td>(7.5-22.1)</td>
</tr>
<tr>
<td>5.25 (Level 2)</td>
<td>215 ± 55</td>
<td>12.6 ± 2.5</td>
<td>16.6 ± 4.0</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(142-309)</td>
<td>(10.5-17.7)</td>
<td>(9.3-21.7)</td>
</tr>
<tr>
<td>7.0 (Level 3)</td>
<td>250 ± 83</td>
<td>24.8 ± 6.1</td>
<td>36.5 ± 13.8</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(149-389)</td>
<td>(13.2-30.6)</td>
<td>(22.9-59.2)</td>
</tr>
<tr>
<td>8.75 (Level 4†)</td>
<td>304 ± 92</td>
<td>20.4 ± 7.3</td>
<td>52.4 ± 10.0</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>(226-405)</td>
<td>(14.7-28.6)</td>
<td>(41.7-61.5)</td>
</tr>
<tr>
<td>10.5 (Level 5†)</td>
<td>255 ± 73</td>
<td>18.3 ± 5.5</td>
<td>61.7 ± 21.1</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(148-350)</td>
<td>(11.6-24.0)</td>
<td>(34.0-92.8)</td>
</tr>
<tr>
<td>12.25 (Level 6)</td>
<td>549</td>
<td>26.0</td>
<td>55.2</td>
</tr>
<tr>
<td>(n = 2)</td>
<td>(486-613)</td>
<td>(24.1-28.0)</td>
<td>(46.7-63.7)</td>
</tr>
</tbody>
</table>

Values are the mean ± SD, with ranges in parentheses. Abbreviation: NE, not evaluable.

*Amount of 131I calculated to give the designated dose to liver. Actual dose administered, as determined by gamma counting of the intravenous bag and tubing before and after administration of the dose, differed from the calculated dose in some cases. The average of the percentage of calculated dose actually administered was 101.8%, with a SD of 8.8%.

†Estimated radiation absorbed doses to marrow were calculated using published methods. 41,42 Recently proposed marrow S values incorporated in MIRDose3 software56 would result in lower marrow doses than those noted here.

‡Three patients scheduled to be treated at these dose levels (2 at level 4 and 1 at Level 5) had their radiation absorbed dose to liver limited so that dose to marrow would not exceed 28 Gy. These patients received 28, 27, and 459 mCi 131I delivering estimated radiation doses to liver of 4.8, 6.1, and 9.2 Gy, respectively. These patients had no grade III/IV toxicities and were not counted as evaluable patients for purposes of dose escalation.

Methotrexate doses and GVHD. Among 23 recipients of autologous marrow, 18 received all 4 scheduled doses of methotrexate for GVHD prophylaxis. Two patients received 3 doses, and 1 patient each received 2, 1, or no scheduled doses. Twenty-one autologous marrow recipients lived at least 30 days and were evaluable for the development of acute GVHD. Twelve patients had no (n = 8) or grade I GVHD (n = 4). Nine patients developed grade II-IV GVHD (grade II, 3; grade III, 5; and grade IV, 1). Sixteen patients were evaluable for the development of chronic GVHD. Eight patients developed clinical extensive chronic GVHD, 6 developed subclinical chronic GVHD, and 2 did not develop chronic GVHD.

Outcome. Of 25 patients with advanced AML and MDS, 3 died of infection in the early posttransplant period and a fourth died of infection 4 months posttransplant. One patient who had had poor engraftment after receiving a 4-HC–purged autologous marrow required chronic transfusions and G-CSF support and died with infection 54 months posttransplant. This patient was treated at dose level 2 with an estimated marrow dose of 12 Gy delivered by antibody (24 Gy total). Her course was felt to be consistent with the poor engraftment seen in a minority of AML patients whose autologous marrows have been incubated with 4-HC. Thirteen patients relapsed 2 to 77 months posttransplant, and 7 patients survive disease-free 15 to 89 months (median, 65 months) posttransplant (Fig 3A). One of the 5 patients transplanted for AML in second or third remission and surviving the first 100 days relapsed after transplant, as compared with 11 of 15 patients transplanted in relapse and 1 of 3 patients with MDS. Of the 9 patients with ALL, 2 died of infection and 4 relapsed 0.5 to 11 months posttransplant. Three patients died of infection 23, 58, and 70 months posttransplant (Fig 3B). Two of the survivors were transplanted in second remission and a third had primary refractory disease.

Fifteen of 18 evaluable patients became hypothyroid, as indicated by an elevated thyroid stimulating hormone, at a median of 13 months (range, 1 to 40 months) posttransplant and were treated with thyroid hormone.

DISCUSSION

We have demonstrated that 131I-labeled anti-CD45 antibody BC8 can deliver more radiation to marrow and spleen than to any normal organ in most patients with acute leukemia whether in remission or relapse. On average, 131I-BC8 antibody delivered estimated radiation doses to marrow that were 2.3-fold greater than liver and doses to spleen that were 4.8-fold greater than liver. The ratios of radiation delivered to marrow and spleen as compared with lung, kidney, and total body were even greater. The maximum tolerated dose suggested by this phase I study was 10.5 Gy delivered by 131I-BC8 antibody to the normal organ receiving the highest dose. Although the liver was the normal organ receiving the highest dose in all treated patients, the dose-limiting toxicity was mucositis. Based on the average estimates of radiation absorbed dose, this dose level would deliver an average of 24 Gy to marrow and 50 Gy to spleen, combined with CY and 12 Gy TBI.

The ability to deliver such supplemental doses of radiation to sites of leukemic involvement in marrow and spleen may improve the cure rate by decreasing the risk of relapse. As noted, a 3.75 Gy increase of TBI dose (from 12 to 15.75 Gy) decreased the relapse rate after matched related marrow trans-
plant for both AML in first remission and CML in chronic phase. The estimates of radiation dose to marrow were based on marrow biopsy and the measured retention half-times of radioiodine in marrow from serial gamma scans. Because radiolabeled antibody distribution may be heterogeneous in marrow, these may represent overestimates or underestimates of radiation doses delivered at various skeletal marrow sites. Also, the relative biological effectiveness (RBE) of radiation delivered by antibody at low dose rate may be lower than that of external beam TBI, such that we would not anticipate the leukemic cell killing of 24 Gy delivered by antibody to be comparable to the killing provided by the same dose of TBI.

Although it is an area of controversy, several studies suggest that hematopoietic progenitor or stem cells possess some capacity for repair of sublethal DNA damage and that there is apparent heterogeneity in both radiation sensitivity and self-repair capacity in their malignant counterparts. Although other factors, including accumulation of cells in the radiosensitive G2 stage of the cell cycle, can impact radiation sensitivity and dose-rate effects, leukemic cells with such repair ability will generally sustain less cell kill when a given radiation dose is delivered at a low dose rate, as compared with TBI. However, we would predict that in some patients these substantial supplemental doses of hematopoietic irradiation delivered by antibody added to CY/TBI would decrease the risk of relapse as compared with CY/TBI alone.

This phase I study was not designed to determine the efficacy of the preparative regimen of 131I-anti-CD45 antibody combined with CY/TBI. Given the small number of patients treated at each dose level and the variability in disease stage and hematopoietic stem cell source (allogeneic vs autologous), it was not possible to correlate the risk of relapse with the amount of radiation delivered by antibody to the normal organ receiving the highest dose or with the estimated radiation dose delivered to marrow.

There was significant variation in the estimated radiation absorbed dose to liver per millicurie of 131I delivered by BC8 antibody. This variation could not be explained by disease stage, disease type, or number of circulating blasts. This observed variability supports the need to determine antibody biodistribution in each patient to avoid delivery of excessive or inadequate radiation dose. Biodistribution studies also identified the minority of patients with unexpectedly low estimated radiation doses to the bone marrow, which generally resulted from unexplained initial low uptake of 131I-BC8 antibody in marrow.

Other attempts to deliver targeted radiation to marrow have included 131I-labeled anti-CD33 antibody and, recently, anti-CD33 antibody labeled with 213Bismuth, which emits a high-energy α particle of short path length. In our experience with conventionally iodinated p67 antibody, only modest ratios of radiation were delivered to marrow compared with the highest normal organ (average ratio, 1.2:1). The use of 131I-p67 antibody was limited by the short retention of 131I in the marrow (t1/2, 21.4 hours), presumably because of the rapid internalization of the 131I-p67-CD33 complex into the cell with subsequent dehalogenation and excretion of 131I from the cell. Furthermore, because of restricted CD33 antigen expression, CD33 antigen was saturated at relatively low antibody doses (0.05 mg/kg), not allowing attachment of sufficient 131I for treatment of patients at the higher doses used. However, using a different anti-CD33 antibody, 131I-M195, Scheinberg et al. found longer retention of 131I in the marrow (t1/2, 21.4 hours), presumably because of the rapid internalization of the 131I-p67-CD33 complex into the cell with subsequent dehalogenation and excretion of 131I from the cell. The studies by Scheinberg et al. also demonstrated that relatively low doses of antibody (3 mg/m2) resulted in saturation of CD33 antigen.

Whether the delivery of supplemental hematopoietic irradiation using 131I-anti-CD45 antibody will benefit patients with advanced leukemia remains unknown, but the experience reported here demonstrated that the approach is feasible; accordingly, phase II studies are underway. Based on the safety of this approach as detailed in this report, we are also conducting a clinical trial in which 131I-BC8 antibody is combined with busulfan and CY for patients with AML in first remission receiving HLA-matched transplants. Ninety percent of patients have had favorable biodistribution of 131I-BC8.
antibody, and 24 patients in first remission have received 131I-BCS antibody labeled with the amount of 131I estimated to deliver 3.5 Gy (first 4 patients) to 5.25 Gy to the liver and 6 to 16 Gy to marrow. Eighteen of these 24 patients are surviving disease-free 10 to 63 months (median, 42 months) after transplant, with 4 nonrelapse deaths and 2 relapses (manuscript in preparation). Accrual continues on this study to better define the efficacy and toxicity of this preparative regimen.

In addition to its use for delivery of supplemental antileukemic doses of radiation, radiolabeled anti-CD45 antibody may also provide immunosuppressive effects, because CD45 is expressed by virtually all lymphoid cells. Preclinical studies are underway to determine the marrow ablative and immunosuppressive effects of 131I anti-CD45 antibody. In murine transplant models, we have determined that 131I anti-CD45 antibody can replace TBI when the donor and recipient differ only with respect to CD45 allotype and can partially replace TBI when transplanting T-cell-depleted H2-mismatched marrow. These preclinical studies suggest that it may be possible to increase the proportion of radiation delivered by antibody and decrease the dose of TBI and/or high-dose chemotherapy when transplanting allogeneic marrow without increasing the probability of rejection. Such an approach should allow the delivery of a higher total radiation dose to sites of leukemic involvement with less toxicity.

In summary, substantial supplemental doses of radiation can be delivered to bone marrow and spleen by 131I-anti-CD45 antibody when combined with CY and 12 Gy TBI, with acceptable toxicity. Phase II studies of this approach are underway for patients with advanced AML/MDS and with ALL. These studies will include both recipients of matched related as well as matched unrelated marrow and should better define the toxicity and efficacy of this approach. The ability to increase the radiation doses delivered to leukemic cells may decrease the rate of relapse and thus improve the outcome of marrow transplantation for acute leukemia.

ACKNOWLEDGMENT

The authors are indebted to Eileen Sickle and Sharon Bush for their expert nursing assistance and to Minna Zheng and Jennifer Smith for their expert technical assistance. We also acknowledge the excellent care provided to these patients by the physicians and nurses of the marrow transplant teams, as well as the work of the staff in the Long Term Followup office.

REFERENCES


20. Scheinberg DA, Lovett D, Divgi CR, Graham MC, Berman E,


41. Society of Nuclear Medicine: MIRD Primer for Absorbed Dose Calculations. New York, NY, Society of Nuclear Medicine, 1988


50. Ploemacher RE, van Os R, van Beurden CA, Down JD: Murine hematopoietic stem cells with long-term engraftment and marrow
repopulating ability are more resistant to gamma-radiation than are spleen colony forming cells. Int J Radiat Biol 61:489, 1992


Phase I Study of $^{131}$I-Anti-CD45 Antibody Plus Cyclophosphamide and Total Body Irradiation for Advanced Acute Leukemia and Myelodysplastic Syndrome

Dana C. Matthews, Frederick R. Appelbaum, Janet F. Eary, Darrell R. Fisher, Lawrence D. Durack, T. Edmond Hui, Paul J. Martin, David Mitchell, Oliver W. Press, Rainer Storb and Irwin D. Bernstein