Radioimmunotherapy of Relapsed B-Cell Lymphoma With Yttrium 90 Anti-Idiotype Monoclonal Antibodies

By Christine A. White, Samuel E. Halpern, Barbara A. Parker, Richard A. Miller, Homer B. Hupf, Daniel L. Shawler, Heli A. Collins, and Ivor Royston

Tumor-specific anti-idiotype (anti-Id) monoclonal antibodies (MoAbs) to B-cell lymphomas have been administered to patients, resulting in significant clinical responses. However, clinical responses have been limited by the emergence of Id-negative lymphoma. To overcome the problem of tumor heterogeneity, we conducted a pilot evaluation of the safety and effectiveness of yttrium 90 (90Y)-labeled anti-Id and shared Id (sId) MoAbs in non-Hodgkin's B-cell lymphoma. Nine patients with relapsed B-cell lymphoma in whom tumor was successfully targeted with 111In-labeled anti-Id MoAb were treated with 90Y-labeled anti-Id MoAb. A total of 19 courses (one to four per patient) were administered using 1,000 to 2,320 mg unlabeled clearing MoAb and 10 to 54 mCi 90Y MoAb per patient. Two of nine patients had a complete response, one a partial response, three stable disease, and three disease progression. Time to progression varied from 1 to 12 months. Toxicities were predominately hematologic, and only one patient developed infection and required transfusion. At progression, three of five assessable patients had Id-positive lymphoma and two had Id-negative lymphoma. Human antimouse antibodies (HAMA) did not develop in the patients after treatment. 90Y anti-Id MoAbs demonstrated excellent in vivo stability, produced significantly tumor regression in three of nine patients, exhibited acceptable toxicities, and elicited no HAMA formation. Further investigation of repetitive, low-dose 90Y anti-Id and MoAb therapy is warranted; however, the advantages of a pan B MoAb may prove the latter to be the agent of choice for the radioimmunotherapy of B-cell lymphoma. © 1996 by The American Society of Hematology.

To date, most trials with radiolabeled MoAbs in B-cell lymphoma have used the radioisotope 131I. Antibodies labeled with 131I are not ideal candidates for radioimmunotherapy, because of in vivo dehalogenation. Furthermore, γ-irradiation results in total-body irradiation to the patient and radiation exposure to the surrounding personnel, and requires hospitalization for doses greater than 30 mCi.

In the pilot trial of 90Y-labeled anti-Id MoAb therapy in relapsed B-cell lymphoma, our group chose 90Y, a pure β-emitter, because of its favorable chelation characteristics, 64-hour half-life (and therefore relatively rapid rad dose delivery rate), lack of γ-irradiation (except for Bremsstrahlung), obviating the need for extensive shielding or hospitalization, and E_{max} of 2.3 MeV and E_{moy} of 934 keV, sufficient to deliver a radiation effect over a distance of 100 to 1,000 cell diameters. Because of the field effect of radiation, radio-labeled anti-Id therapy was thought to have a potential advantage over the naked MoAb by destroying not only Id-positive target cells but also any bystander Id-negative tumor cells, theoretically overcoming the limitation of idiotype-negative relapse. For tumor targeting, 111In, a γ-emitting isotope shown by multiple animal studies to have a biodistribution nearly identical to that of 90Y-MoAb, was bifunctionally attached to the MoAb for radioimmunoscinigraphy (RIS).

Idiotype determinants have been identified that are shared by more than one lymphoma and are found in low concentrations in normal circulating Ig. MoAbs to these shared Ids (sIds) have been termed anti-sIds. In contrast, MoAbs reactive with an idiotype from an individual patient’s lymphoma and not reactive with lymphomas from other patients are called anti-private Id (anti-pId) MoAbs. Anti-sIds expand the candidate patient population for therapy by obviating the requisite lengthy waiting period for a customized anti-pId and by providing the capacity for off-the-shelf therapy for one in three patients. The anti-sIds used in this study were chosen from a panel of sIds developed for this investigation.

In this report, toxicity, tumor targeting, dosimetry, and tumor response data in nine patients imaged with 111In anti-Id and treated with 90Y MoAbs are reported. MoAbs to both pIds and sIds were used for imaging and therapy.
MATERIALS AND METHODS

Patient eligibility. Patients were required to have histologically confirmed B-cell non-Hodgkin’s lymphoma and to have failed to respond to at least one standard chemotherapy. Prior radiation therapy was allowed. Each lymphoma was reactive with an available anti-sId or a customized anti-pId. Each patient had measurable disease, an Eastern Cooperative Oncology Group performance status of 0 to 2, and an age more than 18. If a pId was used, a serum pId level of less than 50 μg/mL at the time of MoAb treatment was required. This requirement was eliminated for patients treated with sl ds, since assays for circulating sl ds yielded conflicting results depending on methodology. All patients had granulocyte counts of at least 1,500/μL, platelet at least 100,000/μL, serum creatinine not greater than 2.0 mg/dL and serum bilirubin not greater than 1.5 mg/dL. Malignant cells in the bone marrow were allowed. All patients provided informed consent, and this protocol was approved by the University of California, San Diego, and San Diego Regional Cancer Center Committees on Investigations Involving Human Subjects and the Radiation Safety Committees of the University of California, San Diego, Medical Center and the San Diego Veterans Administration Medical Center. A US Food and Drug Administration Investigational New Drug Application, held by IDEC Pharmaceuticals Corp (Mountain View, CA), was obtained for this study.

Anti-Id MoAb preparation. Anti-Id MoAbs were prepared in collaboration with IDEC Pharmaceuticals Corp, as described previously. Anti-plds were generated specifically for the individual patient, and anti-sIds were generated for another patient’s lymphoma but were found to be cross-reactive with the patient’s lymphoma in this trial. Mouse hybridoma clones were screened by enzymelinked immunosorbent assay (ELISA) for anti-Id specificity. Anti-sId MoAbs were selected by a two-stage screening procedure. MoAbs were grown in vitro using hollow-fiber bioreactors. Crude MoAb-containing supernatant was purified using NH₄SO₄ precipitation or a protein A affinity chromatography step followed by ion exchange. The MoAb was more than 90% pure Ig as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and passed general safety, sterility, and endotoxin testing. The preparation was found to be free of adventitious viruses.

Preparation of ¹⁵⁵-Y-and ¹¹¹-In-labeled anti-Id MoAb. Chelation and conjugation of the anti-Id MoAbs to ¹¹¹-In and to ¹⁵⁵-Y was performed in collaboration with Hybritech Inc (San Diego, CA) as described previously. Isotiocyanatobenzyl DTPA was used as the chelating agent. Each MoAb was successively coupled with ¹¹¹-In and ¹⁵⁵-Y, and the immunoreactivity of chelated MoAbs was in excess of 65% based on the fraction of radiolabeled MoAb capable of binding to Id protein immobilized on a solid-phase matrix. Biodistribution studies in normal CD-1 mice confirmed the stability and similarity of the behavior of ¹¹¹-In and ¹⁵⁵-Y conjugates in vivo. Specific activity of the conjugates was 1.25 mCi/mg for ¹¹¹-In anti-Id MoAb and 30 mCi/mg for ¹⁵⁵-Y anti-Id MoAb on the day of calibration.

Treatment plan. Patients with detectable levels of circulating Id were treated with unlabeled MoAb in amounts sufficient to result in clearing of the pld or to result in the presence of free circulating MoAb. To identify optimal conditions for targeting, some patients received two imaging doses of ¹⁵⁵-Y MoAb and total-body scans were performed. When two doses were used, an initial clearing dose of 50 mg unlabeled, MoAb was administered followed by 0.5 mCi ¹¹¹-In-labeled MoAb, and total-body imaging was repeated. A second dose of at least 500 mg clearing MoAb was then administered, followed by approximately 5 mCi ¹¹¹-In-labeled MoAb and total-body imaging. The imaging protocol is described later. All patients were premedicated with acetaminophen and diphenhydramine.

Unlabeled anti-Id MoAb was diluted in 500 mL normal saline and infused at a maximum rate of 150 mg/h. ¹¹¹-In anti-Id MoAb was diluted in 6.5 mL aqueous buffer solution containing human serum albumin and administered intravenously (IV) as a bolus over 5 minutes at the end of infusion. ⁶⁷⁰-Y MoAb was diluted in a 2.5-mL aqueous solution containing human serum albumin and administered as an IV bolus over 5 minutes at the end of infusion. The same quantity of unlabeled anti-Id or anti-sId MoAb used preimaging was used for ⁶⁷⁰-Y MoAb infusion.

Each patient underwent serial γ-camera RIS (Elscent [Haifa, Israel] or General Electric [Milwaukee, WI] large-field-of-view camera) at 2 hours, 24 hours, and additional time points, often through 144 hours post infusion, to evaluate changes in tracer distribution in the various organs and to determine optimal imaging time for tumor targeting. Five of nine patients underwent dosimetric estimates of radiation-absorbed doses to normal organs and to tumor sites (see below). Imaging scans were all interpreted by the same nuclear medicine physician (S.E.H.), who was aware of the patient’s site of disease, by conventional radiographic imaging. Only patients judged to have targeting of a majority of tumor sites were allowed to proceed to the therapy phase of the study.

Standard bone marrow harvesting techniques were used. The initial seven patients, after imaging of radiolabeled MoAb in tumor sites, proceeded to bone marrow harvesting (if not previously performed) as a precaution for possible severe myelosuppression. Following harvesting, patients underwent initial ⁶⁷⁰-Y-labeled anti-Id MoAb therapy beginning with a 10 to 15-mCi dose. Each patient was monitored weekly for toxicity with complete blood counts, chemistry panel, and urinalysis. Cycles of imaging with ¹¹¹-In Moab and treatment with ⁶⁷⁰-Y MoAb were repeated every 6 to 8 weeks. Doses of ⁶⁷⁰-Y MoAb were escalated by 5 mCi in patients if continued targeting could be demonstrated and if toxicities were reversible and acceptable (maximum grade 3 nonhematologic toxicity and grade 4 hematologic toxicity lasting <14 days). Cycles of imaging and therapy were administered in the outpatient setting for eight of nine patients.

Immunological monitoring and pharmacokinetic studies. Serum Id levels were determined by ELISA as previously described. The capture protein was immobilized on a 96-well plate (Dynatech Laboratories, Chantilly, VA) by incubating 100 ng/well in 0.05 mol/L carbonate buffer, pH 9.6, overnight at 4°C. Serum samples were diluted in sample buffer consisting of phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (Sigma Chemical Co, St Louis, MO), and 30 μL/well was added to the plate for 30 minutes at 37°C. The plates were washed with buffer composed of PBS supplemented with 1% Tween 20 (Sigma Chemical Co). A 1:5,000 dilution of horseradish peroxidase (HRP)-conjugated goat antihuman IgM or IgG (Sigma Chemical Co) was added to the plate at 50 μL/well for 30 minutes at 37°C. The plates were again washed with wash buffer and 150 μL 3.7-mmol/L o-phenylenediamine in 0.1 mol/L citrate buffer, pH 5.0, H₂O₂ (0.012%) was added to each well and incubated in the dark at room temperature for 15 minutes. The reaction was stopped by addition of 2.5% H₂SO₄, and absorbance at 490 nm was determined on an automatic ELISA reader (Molecular Devices, Menlo Park, CA).

Murine Ig levels were determined as described earlier, using 100 ng/well goat antimouse IgG (TAGO Inc, Burlingame, CA) as the capture antibody and a 1:5,000 dilution of HRP-conjugated goat antimouse IgG (Sigma Chemical Co) as the secondary antiserum. Human antihuman antibodies (HAMA) were determined as described earlier, using 100 ng/well anti-Id as the capture antigen and a 1:5,000 dilution of HRP-conjugated goat antihuman κ or λ light chain (Sigma Chemical Co) as the secondary antiserum. Antiserum against the non-Id light chain was used in the HAMA ELISA.

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and λ (AMAC Inc, Larabee, Westbrooke, ME) were used in the procedures. Cells (10^6 in 25 μL) were incubated with 50 μL containing 500 ng MoAb for 30 minutes at 4°C. The cells were washed twice in 1 mL PBS supplemented with 10% calf serum (Gemini Bioproducts, Calabasas, CA) and 0.1% sodium azide, and were re-suspended in 75 μL 20-μg/mL phycoerythrin-conjugated goat (Fab') anti-mouse IgG (TAGO Inc.) for 30 minutes at 4°C. The cells were again washed twice and were fixed by re-suspending in 1 mL 1% formaldehyde. The cells were then analyzed by flow cytometry on an EPICS Profile (Coulter Immunologics).

Studies of in vivo binding of anti-Id MoAbs. A biopsy of a lymphoma site was obtained from one patient (no. 5) 48 hours after infusion of 500 mg anti-pld MoAb and 4.7 mCi ^111In anti-pld MoAb, and was compared with lymphoma cells obtained before therapy. The remainder of the biopsy was then cryopreserved in dimethylsulfoxide. Indirect immunofluorescence staining of preinfusion and postinfusion lymphoma specimens was performed as described earlier. A portion of the postinfusion lymphoma specimen was weighed and then counted in a gamma counter to determine the amount of isotope present in the tissue as a percentage of injected dose per gram of tissue.

Pharmacokinetics of radiolabeled MoAbs were determined by obtaining blood at specified times (maximum, five time points) following injection. Radioactivity in 1 mL serum was assayed at each time point by counting the aliquot against a standard made from injected material. The patient's blood volume was estimated from lean body mass nomograms, and the radioactivity in 1 mL serum was extrapolated to the total plasma volume. Since all radioactivity was associated with the serum, absolute values in the serum represented the total activity in the vascular compartment. Estimates of plasma volume were extrapolated from lean body mass nomograms.

The patient's serum was studied before, immediately following, and as late as 24 hours after radiolabeled MoAb infusion by standard nonreducing SDS-PAGE electrophoresis. The radioimmun conjugate was also studied alone by this method and after dispersion in nonreducing SDS-PAGE electrophoresis. The radioimmunoconjugate was then reduced to the percent injected dose of tracer incorporated per organ and per gram of tissue.

Dosimetry calculations. ^111In-labeled MoAb was initially demonstrated to have the same kinetics and distribution as ^99mTc-labeled MoAb in the nude mouse-human tumor model. This allowed the γ-emitting ^111In-labeled MoAbs to be used to predict the kinetics and distribution of ^99mTc (a pure β-emitting radiopharmaceutical). This concept has been partially validated in our human studies herein in that ^99mTc and ^111In-labeled MoAb kinetics in the vascular compartment are virtually identical.

Based on these data, we concluded that γ distribution could be predicted in a patient. A known quantity of the ^111In injectate was kept and counted daily on the gamma camera to act as a decay standard. The patient was then administered a known quantity of the radiopharmaceutical and imaged at the times previously indicated. "Spot" views were obtained over the anterior and posterior surfaces of each patient. Each day, the patient was imaged, with positioning maintained constant using a laser positioning system (accurate within 2 mm). Windows were set over the organs in question. Counts per minute per organ were then obtained over the periods indicated. These values, in turn, were converted into disintegrations per minute using the efficiency of the gamma camera for ^111In. Intravascular kinetics were determined by counting samples of the patients' blood obtained at the times indicated. Volumes of the various organs were determined based on computer tomography (CT) using 1-cm sections. Biodistributions for ^111In were estimated. The collective data were reduced using a computer program that allows quantitation of the amount of radioactivity per organ. Standard MIRD (Medical Internal Radiation Dose) calculations were then used for radiation dose calculations. Radiation absorbed-dose estimates in cGy were obtained for the dose of ^111In injected, and calculations were performed to obtain the corresponding cGy dose for 10 mCi ^99mTc, assuming identical biodistribution for the two radioisotopes.

Criteria for tumor response. The assignment of complete response was made when all clinical radiologic and scintigraphic evidence of tumor had disappeared for at least 4 weeks during which no new lesions developed. The assignment of partial response was made when the sum of the products of the perpendicular diameters (as assessed by CT) of measurable tumors had decreased by at least 50%, lasting for at least 4 weeks during which no new lesions developed. The assignment of stable disease was made when there was less than a 25% increase in the sum of the products of the perpendicular diameters of measurable tumor sites lasting for at least 4 weeks. The assignment of progressive disease was made when there was an increase of at least 25% of the sum of the products of the perpendicular diameters of measurable tumor sites, progression of any obvious lesion, or occurrence of a new lesion.

RESULTS

Patient and MoAb characteristics. A summary of the clinical observations of patients undergoing therapy is shown in Table 1.

Pharmacokinetics of administered radiolabeled MoAbs. The MoAb isotype, number of treatment cycles, MoAb mass, serum half-life, mouse IgG levels, serum IgG levels, and ^99mTc quantities delivered per cycle are shown in Table 2. When assayed, blood disappearance of mouse IgG had a time course identical to that of blood disappearance of radioactivity. Additionally, SDS-PAGE analysis of the radiolabeled MoAb injectate and the patients' serum at various times after injection was usually indicative of a circulating protein having a molecular weight consistent with IgG. In patient no. 1, there was SDS-PAGE evidence for in vivo alteration of the MoAb. Distribution of the tracer circulating in the serum also showed evidence of aggressive liver accumulation when injected in mice. These data are compatible with the formation of immune complexes in this animal model. The patients' serum Id was above 20 μg/mL at the time of MoAb injection. When small quantities of ^111In MoAb was added to a patient's serum in vitro, a high-molecular-weight species formed, even if levels of circulating Id were low. This species could be made to disappear if unlabeled MoAbs were applied to the serum before addition of ^111In-labeled MoAb. For most courses of therapy, peak mouse IgG levels were between 50 and 613 μg/mL, consistent with clearing of circulating Id.

RIS. In all patients, tumor targeting was observed in most sites detected by CT. Sites were also seen with RIS that were not observed with CT. Six patients were imaged
two, once with a small clearing dose (50 mg) of MoAb before 0.5 mCi $^{111}$In, and a second time with a larger clearing dose (500 mg) of MoAb before imaging with approximately 5.0 mCi $^{111}$In. In all cases studied, the 500-mg clearing dose provided better imaging resolution, and optimal imaging results were obtained on films at or later than 72 hours following $^{111}$In MoAb. Examples of tumor targeting seen in patient no. 2 (treated with an anti-pId MoAb) and patient no. 3 (treated with an anti-sId MoAb) are shown in Figs 1 and 2, respectively. In general, MoAb scans appeared more sensitive than CT in the detection of tumor. Lesions that were at least 1 cm in diameter were imaged.

Dosimetry. Dosimetry estimates from the first five patients were made by injecting $^{111}$In MoAb, assuming near-identical distribution for $^{90}$Y MoAb and manipulating the equations as though 10 mCi $^{90}$Y MoAb was administered. These data are shown in Table 3. Eighty to 430 cGy/10 mCi $^{90}$Y MoAb injected was delivered to tumor sites in patients with tumor sites that were technically assessable by dosimetry. Patient no. 5 underwent a lymphoma biopsy 48 hours after receiving treatment.

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### Table 1. Clinical Characteristics

<table>
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<th>Patient No.</th>
<th>Age/Sex</th>
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<th>Prior Therapy</th>
<th>Maximum Tumor Cells No.</th>
<th>Bone Marrow</th>
<th>Circulating Tumor Cells</th>
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<td>CVP, ChlPred</td>
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<td>Cytoxan + IFN, Chl VP</td>
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Abbreviations: Pos, positive; Neg, negative; FSC, follicular small cleaved; DM, diffuse mixed; FM, follicular mixed; FLC, follicular large cell; XRT, radiation therapy; CVP, cyclophosphamide, vincristine, and prednisone; CED, cyclophosphamide, etoposide, and dexamethasone; IFN, interferon alfa; cytoxan, cyclophosphamide; COPP, cyclophosphamide, vincristine, procarbazine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CHOP, CHOP with etoposide; ChlPred, chlorambucil and prednisone; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin; m-BACOD, methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone.

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### Table 2. Therapeutic Courses

<table>
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<tr>
<th>Patient No.</th>
<th>Cycle</th>
<th>Clearing MoAb (mg)</th>
<th>MoAb Isotype</th>
<th>Blood Clearance $t_2$ (h)</th>
<th>Peak Mouse IgG $^{111}$In (pg/mL)</th>
<th>Serum IgG $^{111}$In (pg/mL)</th>
<th>Total $^{90}$Y (mcCi)</th>
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<td>$9^{90}$Y 38 15</td>
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<td>22</td>
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<td>24</td>
<td>$1^{111}$In 24 24 0.3 19</td>
<td>$9^{90}$Y 38 15</td>
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Abbreviation: NA, not assessable.

* $^{111}$In and $^{90}$Y injected simultaneously.
Fig 1. (A) and (B) RIS scans of patient no. 2 after injection of 5.0 mCi $^{111}$In anti-pld MoAb before course 1 of 10 mCi $^{90}$Y anti-pld MoAb. Arrows indicate tumor in axillae and iliac node regions. A mediastinal mass and superclavicular nodes are also evident, as are numerous other inguinal and para-aortic nodes. (C) and (D) RIS scans 6 weeks later of patient no. 2 after injection of 4.8 mCi $^{111}$In MoAb just before course 2 of $^{90}$Y anti-pld MoAb. No targeting is seen in the axillae, and diminished targeting is observed in the para-aortal and iliac regions. After injection of 4.7 mCi $^{111}$In MoAb during the imaging phase, and calculations showed 0.0028% of the injected dose of $^{111}$In incorporated per gram of lymphoma tissue.

Tumor response and toxicity of therapy. The therapeutic results and observed toxicity are shown in Tables 4 and 5, respectively. CT scans of the chest and pelvis of patient no. 5 before, during, and after therapy are shown in Figs 3A and B. Patient no. 6 achieved a complete response in three cycles of treatment (Fig 4) and relapsed at 10 months from protocol entry. The time from protocol entry to progression of disease varied from 1 to 12 months for the nine patients studied.

Toxicities were mild and well tolerated, as shown in Table 5. All patients except no. 1 were treated in the outpatient setting. The most severe infusion-related toxicity was transient hypotension in patient no. 1, related to a rapid infusion rate (150 mg/h) of unlabeled clearing MoAb before injection of a $^{111}$In MoAb imaging dose. This patient was known to have high levels of circulating Id. The patient was treated with fluids, benadryl, and epinephrine, and monitored overnight in the hospital. There were no further sequelae. The patient had only mild mucosal congestion on repeat MoAb infusion at a slower rate. Further study revealed ongoing complement fixation, presumably secondary to Id–anti-Id immune complex formation. Patient no. 7 had late development of a progressive mixed motor and sensory neuropathy after receiving two cycles of $^{90}$Y anti-Id approximately 8 weeks apart. Muscle and nerve biopsies failed to demonstrate either MoAb or immune complexes, and the patient’s neuropathy did not respond to steroids. This same patient developed progressive pancytopenia 6 months following a second course of $^{90}$Y MoAb therapy, after having recovered to grade 0 neutropenia and grade 1 thrombocytopenia at week 11, cycle 2. Repeat bone marrow analysis demonstrated hypo-

<table>
<thead>
<tr>
<th>Site</th>
<th>4.8 mCi $^{111}$In cGy</th>
<th>4.3 mCi $^{111}$In cGy</th>
<th>4.5 mCi $^{111}$In cGy</th>
<th>4.7 mCi $^{111}$In cGy</th>
<th>4.4 mCi $^{111}$In cGy</th>
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</thead>
<tbody>
<tr>
<td>Lung</td>
<td>70</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Kidney</td>
<td>130</td>
<td>130</td>
<td>180</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td>Liver</td>
<td>250</td>
<td>200</td>
<td>450</td>
<td>380</td>
<td>170</td>
</tr>
<tr>
<td>Spleen</td>
<td>70</td>
<td>120</td>
<td>160</td>
<td>110</td>
<td>100</td>
</tr>
<tr>
<td>Marrow</td>
<td>90</td>
<td>130</td>
<td>150</td>
<td>140</td>
<td>60</td>
</tr>
<tr>
<td>Tumor</td>
<td>310</td>
<td>NA</td>
<td>430</td>
<td>180/230</td>
<td>80/200*</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not assessable.

* More than 1 tumor site evaluated.
plasia. Autologous bone marrow was transfused, but re-
graftment ultimately failed—the patient died 7 months after
initiation of therapy with stable disease. No opportunistic
infections, hospitalizations, or need for blood transfusions
occurred except in patient no. 7. Abnormal liver function
tests were observed only in patient no. 7. In addition to the
hypotension reported, nonhematologic toxicities included
mucosal congestion (present in each course administered to
patient no. 7). Abnormal liver function tests were observed only in patient no. 7. In addition to the
hypotension reported, nonhematologic toxicities included
mucosal congestion (present in each course administered to
patient no. 7), which was easily controlled with oral diphen-
hydramine, and arthralgias experienced by patient no. 5,
which were easily managed with nonsteroidal antiinflamma-
tory agents. Patient no. 6 had nausea and vomiting requiring
treatment with antiemetics during the first and second but
not the third course of treatment. One further patient (data
not shown) developed mild hypotension in response to an
initial clearing dose (small in quantity) of MoAb, and did
not receive further treatment. The etiology of the hypoten-
sion was not solved, and the decision was made to exclude
the subject from the study.

All but one patient continued with their normal daily rou-
tines with a performance status of 100%. HAMA were moni-
tored each cycle and were not observed during therapy or
follow-up evaluation for 19 courses administered to nine
patients.

**Assessment of tumor at relapse.** Patient no. 1 had a tran-
sient therapeutic response followed by progression. A lymph
node aspirate demonstrated persistent Id-positive lymphoma.
Patient no. 2 underwent a complete remission following three
courses of therapy; however, he relapsed in a new site 12
months after protocol enrollment. He had no response to a
fourth course of 90Y anti-Id MoAb. Subsequent biopsy of
his relapsed lymphoma revealed transformation to diffuse
large-cell lymphoma (histology at entry was follicular mixed
lymphoma), and flow-cytometric evaluation with a panel of
MoAbs revealed no reactivity with his anti-Id MoAb, consis-
tent with Id-negative lymphoma. Subsequent molecular stud-
ies of this lymphoma documented the same JH rearrangement
data not shown) consistent with relapse of the original
lymphoma. Patient no. 4 was evaluated when she had disease
progression after further chemotherapy and a relapse in the
bone marrow. Immunophenotyping of the patient’s bone
marrow revealed Id-positive disease; however, the patient
had severe symptomatic cytopenias and refused further 90Y
MoAb therapy. Patient no. 5 had a partial response following
two courses of treatment, but because of persistent grade 2
thrombocytopenia, he could not be re-treated with 90Y
MoAb. At that time, his peripheral disease progressed in
prior sites of involvement, and rebiopsy of the lymphoma
confirmed persistent Id-positive lymphoma. Patient no. 6,
who had a complete response, relapsed at 10 months in a
new site with Id-negative cells derived from the original
malignant clone.

*In vivo binding of anti-Id.* Lymph node biopsies were

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cycle</th>
<th>Granulocytes</th>
<th>Platelets</th>
<th>Other Toxicity</th>
<th>HAMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3,270</td>
<td>83,000</td>
<td>Hypotension</td>
<td>Neg</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2,176</td>
<td>86,000</td>
<td>Mucosal cong.</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1,110</td>
<td>88,000</td>
<td>Mucosal cong.</td>
<td>Neg</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1,180</td>
<td>60,000</td>
<td>Arthralgias</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1,360</td>
<td>84,000</td>
<td>Arthralgias</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2,210</td>
<td>68,000</td>
<td>Nausea/vomiting</td>
<td>Neg</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1,768</td>
<td>76,000</td>
<td>Neuropathy, aplasia,* liver dysfunction</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1,771</td>
<td>58,000</td>
<td>Fever, splenic pain</td>
<td>Neg (3 weeks later)</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1,600</td>
<td>74,000</td>
<td>Back pain</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Abbreviation: Neg, negative.

* Occurred 4 months after cycle 2.
performed on patient no. 5 before and 48 hours after infusion of 500 mg anti-pld MoAb, and were analyzed by indirect immunofluorescence. The fluorescence indicated prior binding of anti-pld MoAb to tumor cells in vivo (data not shown).

In vitro incubation of postinfusion cells with additional anti-pld MoAb resulted in increased fluorescence. This suggests that the cells had not been saturated by anti-pld MoAb in vivo. Preinfusion and postinfusion biopsies showed similar expression of CD20, an antigen found on this patient’s lymphoma cells. However, in the postinfusion biopsy, fewer cells expressed λ and pld, suggesting that in vivo anti-pld MoAb induced SIg modulation.

**DISCUSSION**

Both anti-slgs and anti-plds were used in this study. No apparent differences were noted between the radiopharmaceuticals with regard to tumor targeting, pharmacokinetics, or toxicity. Characteristics of the tumor immunophenotype and treatment isotype (IgG1 vs IgG2a) did not result in obvious targeting or response differences.

The variable that appeared to correlate with tumor targeting was the attainment of free circulating mouse IgG.22,26 The presence of high levels of circulating antigen has been associated with poor antitumor responses in previous studies,27 and probably contributed to the limited clinical benefit in patient no. 1. In the remaining pld MoAb-treated patients, clearing of circulating Id was easily attained and tumor targeting was accomplished, with high peak levels of free mouse IgG documented in all patients assayed.

The administered radiolabeled MoAbs appeared stable based on similarity to the pharmacokinetics of the unlabeled mouse IgG. This is confirmed by the results of the nonreducing serum SDS-PAGE. The serum half-life of radioactivity in the vascular compartment was 10 to 24 hours. Only one
Fig 4. CT scans of abdomen and pelvis of patient no. 6 before (A) and after (B) 3 courses of \(^{99m}\)Tc anti-Id MoAb therapy. The patient’s remission lasted 10 months.

Patient displayed a significant difference in the serum half-life of \(^{111}\)In MoAb versus \(^{90}\)Y MoAb (patient no. 5, course 2). Based on the above data and the degree of toxicity in vivo, dissociation of \(^{90}\)Y or \(^{111}\)In from the MoAbs was considered unlikely. Reports of serum half-life values of other radiolabeled intact murine MoAbs have varied from 24 to 48 hours and occasionally longer. In the majority of the cases, levels of circulating antigen were much lower and far fewer antigen binding sites were available for complex formation than in this patient group. It should also be remembered that complex formation of serum antigen by unlabeled MoAb does not necessarily mean that antigen existing in the interstitial fluid spaces of normal tissues has also been bound. The possibility exists that antigen in the interstitial fluid spaces could have acted as a sump, thus shortening the serum half-life of the radiopharmaceutical.

Tumor targeting by RIS was accomplished in all patients, with no apparent differences between five patients receiving anti-Id MoAbs and four patients receiving anti-sId MoAb. The 500-mg clearing dose of unlabeled MoAb provided optimal tumor targeting in comparison to the 50-mg clearing dose. The necessity for a large clearing dose of MoAb in a patient with a low circulating Id level is most likely due to the need to clear or mask nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAbs in other tumor systems. The liver is normally the most apparent organ on the scan, even in the absence of circulating antigen, partly due to its size and partly due to nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAbs in other tumor systems. The liver is normally the most apparent organ on the scan, even in the absence of circulating antigen, partly due to its size and partly due to nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAbs in other tumor systems. The liver is normally the most apparent organ on the scan, even in the absence of circulating antigen, partly due to its size and partly due to nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAbs in other tumor systems. The liver is normally the most apparent organ on the scan, even in the absence of circulating antigen, partly due to its size and partly due to nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAbs in other tumor systems. The liver is normally the most apparent organ on the scan, even in the absence of circulating antigen, partly due to its size and partly due to nonspecific intravascular binding sites or antigen sequestered in normal tissues. Indeed, little is known of antigen secretory rates and degradation rates in B-cell lymphoma, but such rates in other antigen-production systems can vary from static to extraordinary. The general distribution of \(^{111}\)In MoAb in the patients was consistent with that observed with other \(^{111}\)In MoAb targets.

Lymphoma biopsy 48 hours after \(^{111}\)In MoAb administration (patient no. 5) yielded 0.0028% of injected \(^{111}\)In per gram of tissue, similar to observations in other studies involving lymphoma, as well as other tumor systems. The calculation was made from a single biopsy site, and tracer uptake may have varied from site to site. Animal model studies strongly indicate that uptake will vary not only with any specific tumor but also with the size of the tumor site. There are several theoretical causes of this phenomenon, but blood flow variances are probably the dominant feature.

Three of nine patients had significant clinical responses. Significant clinical responses in B-cell lymphoma have been reported with non marrow-ablative doses of \(^{131}\)I Lym-1 MoAb, marrow ablative doses of \(^{131}\)I MB-1 MoAb, and comparatively low doses of a \(^{131}\)I-labeled anti-CD20 MoAb. It has been demonstrated that the imaging dose of an anti-CD20 MoAb indicated a reduction in tumor size in some patients with low-grade B-cell lymphoma (M.S. Kaminski, personal communication, September 1995). Thus,
it is possible that some of our tumoricidal responses are secondary to the antibody working in concert with the radiation. The finding of Id-negative disease at relapse in a new site in two patients suggests the possibility of a sanctuary site of Id-negative cells. Patients no. 1, 4, and 5 underwent biopsy at progression and all had Id-positive disease, suggesting that an inadequate amount of the radiolabeled anti-Id MoAb had been administered to eradicate Id-positive disease.

Our report documents the first experience with non-marrow-ablative, repetitive, anti-Id 99mTc MoAb therapy in B-cell lymphoma. In general, the therapy was well tolerated. Eight of nine patients were treated as outpatients, with no significant radiation risk posed to health personnel or families and no requirements for patient shielding. These data, together with other studies using radiolabeled pan B MoAbs, suggest that radiolabeled MoAbs may play a role in the treatment of B-cell lymphoma.

ACKNOWLEDGMENT

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REFERENCES


3. Hale G, Dyer MJ, Clark MR, Phillips JM, Marcus R, Reichmann L, Winter G, Waldmann H: Remission induction in non-Hodgkin lymphoma. In general, the therapy was well tolerated. Eight patients no. 1, 2, 3, 4, and 5 underwent biopsy at progression and all had Id-positive disease, suggesting that an inadequate amount of the radiolabeled antibody working in concert with the radiation risk posed to health personnel or families and no requirements for patient shielding. These data, together with other studies using radiolabeled pan B MoAbs, suggest that radiolabeled MoAbs may play a role in the treatment of B-cell lymphoma.


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Radioimmunotherapy of relapsed B-cell lymphoma with yttrium 90 anti-idiotype monoclonal antibodies

CA White, SE Halpern, BA Parker, RA Miller, HB Hupf, DL Shawler, HA Collins and I Royston