Anti-CD45 Monoclonal Antibody YAML568: A Promising Radioimmunoconjugate for Targeted Therapy of Acute Leukemia

Gerhard Glatting¹, Marguerite Müller¹, Bernd Koop¹, Kathrin Hohl², Claudia Friesen¹, Bernd Neumaier¹, Eleanor Berrie³, Pru Bird³, Geoffrey Hale³, Norbert M. Blumstein¹, Herman Waldmann³, Donald Bunjes³, and Sven N. Reske¹

¹Department of Nuclear Medicine, University Ulm, Ulm, Germany; ²Department of Biometry and Medical Documentation, University Ulm, Ulm, Germany; ³Sir William Dunn School of Pathology, Oxford, United Kingdom; and 4Department of Internal Medicine III (Hematology/Oncology), University Ulm, Ulm, Germany

The outcome of hematopoietic cell transplantation for hematologic malignancies may be improved by delivering targeted radiation to hematopoietic organs while relatively sparing nontarget organs. We evaluated the biodistribution of ¹¹¹In-labeled anti-CD45 antibody in humans using the rat IgG2a monoclonal antibody YAML568 that recognizes a common CD45 epitope present on all human leukocytes. Methods: Eight patients undergoing bone marrow transplantation received YAML568 labeled with 122 ± 16 MBq of ¹¹¹In intravenously followed by serial blood sampling, urine collection, and conjugated view planar γ-camera imaging up to 144 h after injection. Time–activity curves were obtained using region-of-interest analysis in the accumulating organs and residence times were calculated. An estimate for the radiation-absorbed doses for each organ per unit of administered activity of ⁹⁰Y was calculated using software for internal dose assessment. The first patient received no unlabeled antibody preloading. The second 2 patients received a preloading dose of 10 mg (0.15 mg/kg). The last 5 patients received a preloading dose of 30–47 mg (0.5 mg/kg). Results: No significant administration-related side effects were seen. The 3 patients receiving no antibody or low antibody preloading had an unfavorable biodistribution with a high initial accumulation of activity in the liver (37%) and the spleen (34%). For the patients receiving 0.5-mg/kg antibody preloading, the estimated radiation-absorbed doses for red bone marrow, spleen, liver, kidney, and total body were 6.4 ± 1.2, 19 ± 5, 3.9 ± 1.4, 1.1 ± 0.4, and 0.6 ± 0.1 mGy/MBq, respectively, demonstrating preferential red marrow targeting. A linear regression model showed that the amount of unlabeled antibody preloading per body weight has a strong influence on the estimated red marrow absorbed dose (P = 0.003, R² = 0.80). Conclusion: This study shows that the anti-CD45 monoclonal antibody YAML568 is suitable for delivering selectively radiation to hematopoietic tissues when labeled with ⁹⁰Y provided that a preloading dose of about 0.5 mg/kg unlabeled antibody is given.

Key Words: biodistribution; dosimetry; radiation-absorbed dose; radioimmunotherapy; anti-CD45


H igh-dose chemoradiotherapy followed by stem cell transplantation offers the only chance of cure for many patients with treatment-resistant leukemia (1,2). The concept that leukemia might be cured with high enough doses of cytotoxic therapy is based on radiosensitivity and the steep dose–response curve of leukemia to chemoradiotherapy. However, myeloablative doses are required to completely eradicate leukemic cells. In addition, nonhematologic organ toxicity limits the intensity of cytotoxic chemotherapy and total-body irradiation (2–6). Therefore, targeted radiation to leukemic progenitor and stem cells residing in hematopoietic bone marrow was developed using radioiodinated antibodies directed to epitopes expressed on leukemic cells or normal hematopoietic bone marrow cells (2,6,7).

To date, CD33, CD45, and CD66, addressed with monoclonal antibodies labeled with ²¹¹Bi (CD33) (8–11), ¹³¹I (CD33, CD45) (12–16), ¹⁸⁸Re (CD66) (17–22), and ⁹⁰Y (CD33, CD66) (23,24), have been explored for delivery of targeted radiation to bone marrow. Whereas α-emitting radioisotopes can eradicate individual leukemic cells (25), β-emitting radioisotopes take advantage of the cross-fire effect (26), which allows irradiation of several thousand cells adjacent to the decay position and obviates the need for addressing individual cells, provided sufficient radioiodinated antibody is deposited in the target volume.

In our previous studies the efficacy of the ¹⁸⁸Re-labeled anti-CD66 was restricted to patients in complete or very good partial remission (19,21). To target a higher leukemic tumor cell load, we decided to use an ⁹⁰Y-labeled anti-CD45 antibody in patients with more advanced disease. Here, we describe the biodistribution of YAML568, a rat IgG2a monoclonal antibody specific for the CD45 antigen, a tyrosine phosphatase present on the surface of most
nucleated hematopoietic cells, lymphoblasts, and myeloblasts and myeloid leukemic cells, but not found on non-hematopoietic cells (27,28). This antigen is expressed at high density (∼200,000 molecules per cell) and not appreciably internalized after ligand binding (13,14). In a previous study (29) we used the rat IgG2b antibody YTH24.5 but this gave a high level of uptake in the liver. This antibody binds strongly to human Fc receptors (FcR) (30) and we hypothesized that the non–FcR-binding rat IgG2a antibody might give more favorable distribution. A further advantage of the non–FcR-binding isotype is that acute reactions related to cytokine release were expected to be reduced, allowing easier administration of high doses of unlabeled antibody, if required (31). In this study we describe results using 111In-labeled YAML568 in patients before hematopoietic stem cell transplantation and show that there is still significant liver uptake that can be overcome by prior administration of the unlabeled antibody.

MATERIALS AND METHODS

Patients

In 8 consecutive patients (6 men, 2 women; median age, 54 y [range, 44–61 y]; median weight, 80 kg [range, 55–92 kg]) with refractory (5 patients) or relapsing (3 patients) acute myeloid leukemia (AML; 7 patients) or acute lymphatic leukemia (ALL; 1 patient), the biodistribution of 111In-labeled YAML568 was measured (Table 1) in preparation for conditioning before allogeneic stem cell transplantation (17–21). Patients with a favorable biodistribution then proceeded to a therapeutic infusion of 90Y-labeled YAML568 and further myeloablative conditioning with either total-body irradiation/cyclophosphamide or busulfan/cyclophosphamide as described (19,21). All patients subsequently received an unmanipulated peripheral blood progenitor cell transplant from an HLA-compatible family or unrelated donor.

Patients signed an informed consent form for this study, which was approved by the local ethics committee and the national radiation protection authorities.

Monoclonal Antibody

YAML568 recognizes a common epitope found on all isoforms of the CD45 antigen (epitope P) (27,28). The affinity constant for binding to human peripheral blood mononuclear cells was ∼4.8 × 10^9 M⁻¹ (32). The original hybridoma came from a fusion of rat spleen cells with the cell line Y3-Ag.1.2.3 (33). This expressed an irrelevant κ-light chain. To prepare the antibody for clinical use, a variant was selected that secreted only the specific heavy and light chains and a master cell bank was prepared. Cells were cultured in a hollow fiber fermenter (Acusyst-Ir). YAML568 was purified from the supernatant by affinity chromatography (protein G), cation-exchange chromatography (SP-Sepharose), and filtration through a 20-nm filter (Millipore NFP). The final product was subjected to quality control tests similar to those described for Campath antibodies (34).

In addition, it was shown by in vitro whole-blood culture that YAML568 gave substantially less release of tumor necrosis factor-α and interferon-γ compared with the rat IgG2b CD45 antibodies YTH24.5 and YTH54.12. Manufacture and quality control were performed according to good-manufacturing-practice guidelines at the Therapeutic Antibody Centre, Oxford, United Kingdom.

Conjugation, Radiolabeling, and Characterization of Antibody

HPLC (High-Performance Liquid Chromatography). HPLC was performed on a Dionex HPLC system. Size-exclusion chromatography (SEC) was performed at room temperature at 1 mL/min with a BioSep-SEC-S 3000 column (300 × 7.8 mm; Phenomenex) using phosphate-buffered saline as solvent (0.1 mol/L, pH = 6.8). Ultraviolet (UV) absorbance was measured at 280 nm; radioactivity was measured with a Na(I) scintillation detector (Raytest) connected to the effluent of the UV system.

**TABLE 1****

**Patient Data**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Disease</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Unlabeled Ab mg</th>
<th>mg/kg</th>
<th>Red marrow leukemic blast infiltration (%)</th>
<th>Peripheral blood leukemic blast counts/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AML, refractory</td>
<td>61</td>
<td>F</td>
<td>0.00</td>
<td></td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>AML, relapsing</td>
<td>56</td>
<td>M</td>
<td>0.16</td>
<td></td>
<td>80</td>
<td>8,800</td>
</tr>
<tr>
<td>3</td>
<td>ALL, refractory</td>
<td>48</td>
<td>M</td>
<td>0.14</td>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>AML, relapsing</td>
<td>54</td>
<td>F</td>
<td>0.48</td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>AML, refractory</td>
<td>44</td>
<td>M</td>
<td>0.49</td>
<td></td>
<td>50</td>
<td>800</td>
</tr>
<tr>
<td>6</td>
<td>AML, refractory</td>
<td>58</td>
<td>M</td>
<td>0.51</td>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>ALL, refractory</td>
<td>53</td>
<td>M</td>
<td>0.48</td>
<td></td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>AML, relapsing</td>
<td>49</td>
<td>M</td>
<td>0.51</td>
<td></td>
<td>80</td>
<td>800</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>—</td>
<td>53 ± 6</td>
<td>—</td>
<td>—</td>
<td></td>
<td>59 ± 28</td>
<td>1,325 ± 3,040</td>
</tr>
</tbody>
</table>

**HPLC (High-Performance Liquid Chromatography).** HPLC was performed on a Dionex HPLC system. Size-exclusion chromatography (SEC) was performed at room temperature at 1 mL/min with a BioSep-SEC-S 3000 column (300 × 7.8 mm; Phenomenex) using phosphate-buffered saline as solvent (0.1 mol/L, pH = 6.8). Ultraviolet (UV) absorbance was measured at 280 nm; radioactivity was measured with a Na(I) scintillation detector (Raytest) connected to the effluent of the UV system.

**Conjugation of Isothiocyanato-Benzyl-Methyl-Diethylenetriaminepentaacetic Acid (Isothiocyanato-Benzyl-MX-DTPA) to Anti-CD45.** All buffers were prepared with high-purity water (Fluka) and filtered steriley through a 0.22-μm-pore size Millex-GV filter (Millipore). All preparations were performed aseptically in a laminar flow hood. Aminobenzyl-MX-DTPA (2-(4-aminobenzyl)-6-methyl-diethylenetriaminepentaacetic acid) was obtained from Macroyclics. Aminobenzyl-MX-DTPA was converted into isothiocyanato-benzyl-MX-DTPA (35).

Isothiocyanato-benzyl-MX-DTPA (5 mmol/L in H2O) was added to anti-CD45 in N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) buffer (50 mmol/L, pH = 9.5) in a 5-fold molar excess. The reaction mixture was incubated for 20 h at 23°C. The conjugated anti-CD45 was separated from unconjugated isothiocyanatobenzyl-MX-DTPA by PD 10 column chromatography (Pharmacia) using isotonic saline as eluent. Protein concentration and identity were determined via HPLC. The final product was...
filtered steriley through a 0.22-μm-pore size Millex-GV filter (Millipore) and stored at −20°C.

Radiolabeling. All buffers were prepared with high-purity water (Fluka) and filtered steriley through a 0.22-μm-pore size Millex-GV filter (Millipore); sterile equipment was used for the preparations and all preparations were performed aseptically in a laminar flow hood. 111In-InCl₃ in 0.05 mol/L HCl (Tyco Healthcare) was diluted with the 2-fold amount of a citrate/acetate-buffer (pH 5.5, 0.05 mol/L sodium citrate/0.05 mol/L sodium acetate). The buffered 111In solution was added to 1 mg of the antibody conjugate. After 20 min of incubation at room temperature the antibody was diluted with isotonic saline. A small aliquot was analyzed by SEC to determine the radiochemical purity and identity of the radioimmunonconjugate preparation (100 MBq/mg protein, >95% 111In bound to the antibody). Sterility and apyrogenicity were assessed retrospectively according to the European Pharmacopeia and the Limulus amoebocyte lysate test after allowing 30 d for radionuclide decay. Immunoreactivity of the antibody was evaluated by fluorescence-activated cell sorter analysis and was determined to be >97%.

Antibody Biokinetics
Patients received an intravenous injection of 0–47 mg unlabeled antibody between 34 and 2 min before the intravenous injection of 122 ± 16 MBq 111In-labeled anti-CD45 antibodies as a slow intravenous push.

Blood samples were obtained at the end of the antibody infusion and at 5, 10, and 30 min, 1 and 2 h, 1, 2, 3, or 5 d, and 6 d after injection. The 111In activity was measured using a γ-counter (Auto-γ-5003; Canberra Packard) and the total serum activity was expressed as percentage of injected dose using the formula of Dubois (29).

Planar whole-body scintigraphies (anterior and posterior) with a double-head γ-camera (ECAM; Siemens) were performed at 2 and 4 h, 1, 2, 3, or 5 d, and 6 d after injection. The 111In activity was measured using a γ-counter (Auto-γ-5003; Canberra Packard) and the total serum activity was expressed as percentage of injected dose using the formula of Dubois (29).

Blood clearance was different in the 2 groups (Fig. 1; Table 2). For the patients with zero/low preloading, about 83% ± 12% of the activity was eliminated within 10 min from blood. Thus, the main difference between the dose groups with zero/low preloading and 0.5-mg/kg preloading is reflected by the reduced magnitudes (α, β) of the exponential elimination pattern (Table 2).

Correspondingly, the tissue distribution of 111In-labeled YAML568 was also different between doses of zero/low

Results
No acute side effects were seen after administration of unlabeled (10–47 mg) or 111In- or 90Y-labeled YAML568 (1 mg) despite relatively rapid intravenous injection within 1 min.

Biodistribution
The biodistribution was different between the dose groups with zero/low preloading (patients 1–3) and with 0.5-mg/kg preloading (patients 4–8).

Blood clearance was different in the 2 groups (Fig. 1; Table 2). For the patients with zero/low preloading, about 83% ± 12% of the activity was eliminated within 10 min from blood. Thus, the main difference between the dose groups with zero/low preloading and 0.5-mg/kg preloading is reflected by the reduced magnitudes (α, β) of the exponential elimination pattern (Table 2).

Correspondingly, the tissue distribution of 111In-labeled YAML568 was also different between doses of zero/low

Estimation of Radiation-Absorbed Doses
The residence times calculated for 90Y were applied to the appropriate phantom dosimetry model contained in the OLINDA/EXM software (37). Internal radiation-absorbed doses were then calculated using the method recommended by the MIRD Committee of the Society of Nuclear Medicine (37).

Statistical Analysis
Descriptive statistics of the data are presented as mean ± SD. To examine the influence of each investigated parameter on organ absorbed dose per administered activity (mGy/MBq) a simple linear regression was performed. The following parameters were investigated: preloading of unlabeled antibody per body weight (mg/kg), peripheral leukemic blast count/μL, and leukemic infiltration of the marrow (%). P < 0.05 was considered statistically significant. The analyses were performed using Origin software (version 7.0273, OriginLab Corp.).

FIGURE 1. Clearance of 111In-labeled YAML568 from blood is shown for 2 patients, with (patient 5) and without (patient 1) prior administration of unlabeled antibody (Table 1).
and high preloading. Patients who received a dose of zero/low preloading showed an initial high uptake in liver (37% ± 9%) and spleen (34% ± 9%) and low red marrow activity (16% ± 2%) (Figs. 2 and 3). The ratio of the activity in red marrow to liver was between 0.3 and 1.0, and the ratio of red marrow to spleen was between 0.04 and 0.11. The residence times for red bone marrow, spleen, liver, kidneys, and remainder of body were 13 ± 3, 22 ± 3, 21 ± 6, 0.3 ± 0.1, and 11 ± 4 (MBq × h/MBq), respectively. Patients who received a preloading dose of 0.5-mg/kg showed a relatively low uptake in liver (14% ± 11) and spleen (7% ± 5%) and a high red marrow uptake (37% ± 8%) (Figs. 2 and 3). The ratio of the activity in red marrow to liver was between 3 and 5, and the ratio of red marrow to spleen was between 0.3 and 2. The residence times for red bone marrow, spleen, liver, kidneys, and remainder of body were 31 ± 6, 10 ± 5, 13 ± 3, 0.6 ± 0.2, and 19 ± 10 (MBq × h/MBq), respectively.

The cumulated urinary excretion after 4 h, 2 d, and 6 d was 2% ± 1%, 9% ± 2%, and 31% ± 2%, respectively. No difference was found between the groups receiving zero/low and high preloading doses.

Estimated Radiation-Absorbed Dose
The radiation-absorbed doses for 90Y-labeled YAML568 calculated assuming the biokinetics were identical to the 111In-labeled species are given in Table 3. For the patients with zero/low preloading doses, the radiation-absorbed dose in the liver would have been larger than in the target organ red marrow.

For the patients receiving a preloading dose of 0.5 mg/kg, the radiation-absorbed dose for red marrow and spleen was higher than that for nontarget organs (Table 3). The liver was the nontarget organ with the highest estimated radiation dose in all 5 patients. However, the estimated radiation-absorbed dose was between 2.6 and 9.6 times greater for the spleen (median, 6.1) and between 1.1 and 2.4 times greater for the red marrow (median, 1.8) compared with that of the liver. The dose to spleen was between 10 and 53 times greater (median, 14) and the dose to marrow was between 4 and 10 times greater (median, 5) than that to the kidneys, the nontarget organ receiving the second highest radiation dose.

Linear regression showed that the estimated radiation-absorbed doses for red marrow, spleen, and liver correlated with the preloading antibody dose (Table 4). According to the slope of the regression equation, a higher preloading dose increases the radiation-absorbed doses of the red marrow but reduces that of the spleen and the liver. The coefficients of determination ($R^2$) of the explorative linear regression models show that the preloading antibody dose seems to be able to explain a greater part of the variety of the red marrow ($R^2 = 0.80$) than of the spleen ($R^2 = 0.64$) or of the liver ($R^2 = 0.49$).

Peripheral blood leukemic blast counts and leukemic marrow infiltration did not show a significant effect on estimated organ radiation-absorbed dose. The lack of significance may be due to the small sample size or other confounding variables—for example, due to binding sites outside the bone marrow or blood.

Clinical Application
Four patients with a favorable biodistribution proceeded to a therapeutic antibody infusion. All 4 patients engrafted and achieved a complete remission. Follow-up was too short for assessment of the clinical outcome.

DISCUSSION
In this study the suitability of radiolabeled anti-CD45 monoclonal antibody YAML568 for radioimmunotherapy

### TABLE 2
Blood Clearance vs. Preloading Dose for 111In-Labeled YAML568 Antibody

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zero/low preloading</th>
<th>0.5-mg/kg preloading</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ (%)</td>
<td>14 ± 11</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>$\beta$ (%)</td>
<td>3 ± 1</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>38 ± 20</td>
<td>11 ± 10</td>
</tr>
</tbody>
</table>

SDs reflect differences between patients.

![FIGURE 2. Biodistribution of 111In-labeled YAML568 (left, mirrored anterior view; right, posterior view) is shown for 2 patients, with (A, patient 5) and without (B, patient 1) prior administration of unlabeled antibody (Table 1).](image-url)
of high-risk leukemia was examined in 8 patients with refractory or relapsing AML or ALL. Intravenous injection of the $^{111}$In-labeled antibody without or with a very low preloading dose of 0.15 mg/kg resulted in a very fast blood clearance, a pronounced antibody uptake in the liver and spleen, and a low uptake in red marrow, unsuitable for radioimmunotherapy. In contrast, preloading with 0.5 mg/kg produced preferential and high uptake in red marrow, significantly reduced liver and spleen uptake, and increased red marrow to liver uptake (Table 3). Kidney and lung doses always remained low. Focal marrow lesions or extramedullary lesions are rare in AML and were not detected on dosimetry. Preferential antibody localization in the red marrow is a prerequisite for treating high-risk leukemia, which can be achieved using radiolabeled YAML568 combined with an unlabeled predose.

The $^{111}$In-labeled YAML568 was very well tolerated. None of our patients developed a cytokine release reaction because of the inability of the rat IgG2a antibody to interact with FcRs on patient cells. This is an important advantage of YAML568, as we were able to inject up to 47 mg of the antibody within a minute without observing adverse effects. In contrast, the murine IgG1 anti-CD45 antibody BC8 has to be infused at 5–10 mg/h because of adverse effects (13).

The estimated radiation-absorbed dose in red bone marrow for $^{90}$Y-labeled YAML568 of 6.4 mGy/MBq is similar to the values of 6.8 mGy/MBq obtained for the $^{90}$Y-anti-CD66 (BW250/183) (24) or for the $^{90}$Y-anti-CD33 (HuM195) (10). The red marrow-to-liver ratio of radiation-absorbed doses is the relevant measure to compare antibodies carrying different radionuclides. Here we obtained a value of 1.8 ± 0.5 (Table 3), similar to the results with $^{90}$Y- and $^{213}$Bi-labeled anti-CD33 antibody (1.7) (10) but slightly lower compared with $^{188}$Re-labeled anti-CD66 (2.3–3.0) (19,22), $^{131}$I-labeled anti-CD45 (2.2–2.6) (13,14,16), and $^{131}$I-labeled anti-CD33 (2.2) (15). The spleen-to-red marrow ratio of $^{90}$Y-labeled YAML568 is 3.0, similar to $^{131}$I-labeled anti-CD45 antibody (2.7). Corresponding values were 1.1–1.4 for $^{188}$Re-labeled anti-CD66 (19,22), 1.1 for $^{213}$Bi-labeled anti-CD33 (10), and 1.8 for $^{131}$I-labeled anti-CD33 (15).

As shown by the high absorbed doses in the liver and spleen after zero/low preloading, YAML568 had a specific distribution to liver (and spleen). This non–FcR-binding isotype appeared to offer no advantage in this regard compared with previous results using the rat IgG2b antibody (29). This may be attributed to direct binding of antibody to CD45 antigen expressed by tissue macrophages and lymphocytes in the liver (13,38). Consistent with these findings, blood clearance was very fast in these patients (Fig. 1). In contrast, preloading at 0.5 mg/kg considerably delayed blood clearance (Fig. 1) and shifted antibody distribution to red marrow (Table 4). This is a well-known effect, because the preceding administration of unlabeled antibody can saturate binding sites (specific or nonspecific) and thereby reduce binding of radiolabeled antibody to the liver (39). Preloading was facilitated using an antibody that did not cause cytokine release or infusion-related toxicity. However, the preloading dose of 0.5 mg/kg used in this study may not be optimal. Either a higher preloading dose would further increase the ratio of bone marrow to liver due to better saturation of the CD45 antigens expressed in the liver or very high doses of unlabeled antibody could supersaturate the binding sites in the bone marrow and thus decrease tumor targeting. Also, the optimal preloading dose is probably dependent on the individual patient. Nemecek et al. (40) demonstrated in a macaque model using anti-CD45 and preloading doses of 0.5 and 4.5 mg/kg that both doses improve biodistribution compared with no preloading. Thus, the subject of future work will be to collect more data using different preloading doses to develop adequate models for the prediction of an optimal preloading dose of unlabeled antibody in this system.
TABLE 3
Estimated Radiation-Absorbed Dose per Injected Activity (mGy/MBq) and Therapeutic Ratio of Red Marrow to Liver for $^{90}$Y-Labeled YAML568 Antibody

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Red marrow</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lungs</th>
<th>Total body</th>
<th>Red marrow/liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>31</td>
<td>5.5</td>
<td>1.0</td>
<td>0.1</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>29</td>
<td>6.8</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>35</td>
<td>6.7</td>
<td>0.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>6.4</td>
<td>19</td>
<td>6.0</td>
<td>1.4</td>
<td>0.1</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>8.2</td>
<td>12</td>
<td>4.6</td>
<td>1.2</td>
<td>0.1</td>
<td>0.6</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
<td>26</td>
<td>2.7</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>21</td>
<td>3.4</td>
<td>1.3</td>
<td>0.2</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>8</td>
<td>6.8</td>
<td>18</td>
<td>2.8</td>
<td>1.3</td>
<td>0.2</td>
<td>0.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Mean ± SD* 5.0 ± 2.2 24 ± 8 4.8 ± 1.7 0.9 ± 0.4 0.1 ± 0.1 0.6 ± 0.1 1.3 ± 0.8  
Mean ± SD† 6.4 ± 1.2 19 ± 5 3.9 ± 1.4 1.1 ± 0.4 0.1 ± 0.1 0.6 ± 0.1 1.8 ± 0.5

*All patients.  
†Only patients with 0.5-mg/kg–preloading dose (patients 4–8).

For red marrow we obtained a positive correlation of the radiation-absorbed dose with the preloading dose (Table 4). This is most probably a consequence of the competing antibody binding between liver and red marrow mediated by blood flow: If no preloading antibody is administered, a large part of the labeled antibody will localize in the liver because of its large blood flow of approximately 24% of the cardiac output. However, if the liver binding sites are saturated by a preloading of unlabeled antibody, the net accumulation of labeled antibody in red marrow will increase: First, because of the low blood flow through red marrow (a few percent), only a small part of the unlabeled antibodies will reach the red marrow and occupy binding sites; second, because of the large antigen number in red marrow, the number of binding sites occupied by unlabeled antibodies is negligible.

The observed negative correlation of the spleen radiation-absorbed doses with the preloading dose is very similar to that of the liver. This is not a surprise, as both organs are well perfused and contain a large amount of specific binding sites.

CONCLUSION

YAML568 can be labeled successfully with $^{90}$Y and is extremely well tolerated with no infusion-associated cytotoxicity release reaction. Our biodistribution data of $^{111}$In-labeled YAML568 show that preloading with unlabeled antibody is required to achieve a favorable dosimetry. The regression analyses suggest that the use of higher doses of unlabeled antibody may further increase the therapeutic ratio of this radioimmunoconjugate.

ACKNOWLEDGMENTS

The authors gratefully acknowledge grants by the Deutsche Forschungsgemeinschaft (German Research Foundation, grant KFO 120/1-1, GL 236/6-1), the Deutsche José Carreras Leukämie-Stiftung (German José Carreras Leukemia Foundation, grant DJCLS H04/05), Deutsche Krebshilfe (German Cancer Aid, grant 70-3249-Bu 2), the U.K. Medical Research Council, Millennium Pharmaceuticals Inc, TolerRx Inc., and the EPAbrahams Trust. The authors are indebted to Karen Tucker, Emma Bolam, Steve Yates, Tony Gallagher, and Gavin McKinley for assistance with the manufacture of YAML568 and to Regine Feurer for her expert technical assistance.

REFERENCES


