A Phase 1B Trial of Humanized Monoclonal Antibody M195 (Anti-CD33) in Myeloid Leukemia: Specific Targeting Without Immunogenicity

By Philip C. Caron, Joseph J. Jurcic, Andrew M. Scott, Ronald D. Finn, Chaitanya R. Divgi, Martin C. Graham, Imad M. Jureidini, George Sgouros, Deci Tyson, Lloyd J. Old, Steven M. Larson, and David A. Scheinberg

This trial studied the biodistribution, pharmacology, toxicity, immunogenicity, and biologic characteristics of a trace-labeled, anti-CD33, humanized monoclonal antibody M195 (Hu-M195) in patients with relapsed and refractory myeloid leukemia. Hu-M195 is a computer-modeled, "complementarity-determining region-grafted," IgG1, humanized version of M195. M195 is a murine monoclonal antibody that reacts with CD33, a 67-kD glycoprotein expressed on early myeloid progenitor cells and myeloid leukemia (acute myelogenous leukemia and chronic myelogenous leukemia) cells, but not normal stem cells. 111-murine-M195 has already shown significant ability to cytoreduce patients with relapsed or refractory myeloid leukemias. Hu-M195 has higher avidity than the original mouse monoclonal antibody and, unlike murine M195, has the capability to mediate antibody-dependent cellular cytotoxicity against leukemia targets. Thirteen patients with relapsed or refractory myelogenous leukemia were treated with Hu-M195 at 4 levels of 0.5, 1.0, 3.0, and 10.0 mg/m² in a phase I trial. Patients received a total of 6 doses per patient over 18 days. Two patients were retreated for a total of 12 doses. The first dose of Hu-M195 was trace-labeled with 111I to allow detailed pharmacokinetic and biodistribution studies by serial sampling of blood, radioliminunoassays of cells, and whole-body gamma-camera imaging. Cumulative total doses of up to 216 mg of Hu-M195 were administered safely. Reversible fever and rigors were observed after infusion at the highest dose levels. The entire bone marrow was specifically and clearly imaged within hours after infusion, with optimal biodistribution occurring at the 3 mg/m² level. Adsorption of Hu-M195 onto targets in vivo was demonstrated by flow cytometry; near saturation of available sites occurred at the 3 mg/m² dose level. Plasma and whole body half lives were 38 and 51 hours, respectively, which may reflect continual replenishment of target sites on new leukemia cells. 111-Hu-M195 was rapidly internalized into the target cells in vivo within 1 hour. Human antihuman antibody responses were not observed. In conclusion, Hu-M195 can be administered safely in multiple doses, without significant toxicity or any evidence of immunogenicity, and can localize rapidly and efficiently to the bone marrow in patients with myeloid leukemias. Additional phase II trials with this agent alone or in combination with cytokines or isotopes are warranted at the optimal biologic dose.

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CD33 is a 67-kD glycoprotein antigen that is restricted to myelogenous leukemias, monocytes, and myeloid progenitor cells, but is not displayed on other normal tissues or earliest bone marrow stem cells.1-4 M195 is a nontoxic IgG2a mouse monoclonal antibody (MoAb) reactive with CD33.5,6 A pilot trial of 10 patients who were treated with escalating doses of murine M195 (trace-labeled with 111I) showed rapid and specific bone marrow targeting.2,8 111-I-M195 selectively adsorbed onto CD33+ peripheral blood leukemia cells, with saturation of antigen sites complete within 1 hour, and subsequently was documented to be internalized into the target cells.

A subsequent dose escalation trial in 24 patients of 111-I-M195 from 50 to 210 mCi/m² has shown effective cytoreduction of peripheral blood and bone marrow blasts in patients with relapsed or refractory acute myelogenous leukemia (AML) who had been heavily pretreated with chemotherapy or radiation.9 Despite evidence of effective targeting to CD33+ cells and significant cytoreduction, human-antimouse antibodies (HAMA) were elicited in patients receiving M195 in both trials.5,6 In 2 patients with HAMA who were retreated with 111-I-M195, both targeting and leukemia cell killing was blocked by HAMA. Hence, further development of murine M195 as a therapeutic agent may be limited by the immunogenicity of the mouse protein and by the need for bone marrow rescue after ablation of 111I-containing constructs.

Hu-M195 is a recombinant humanized version of the mouse MoAb M195.10 Hu-M195 is similar to the parent MoAb with respect to specificity, immunoreactivity, and internalization. Hu-M195 has a higher avidity than murine M195 and, because the murine constant regions were replaced with human Ig sequences, has acquired the capability to mediate antibody-dependent cellular cytotoxicity (ADCC) against leukemia targets in vitro.11

Prompted by the success of the M195 trials and the improved biologic function of Hu-M195 in vitro, and in an effort to eliminate immunogenicity and the need for radioisotopes, we conducted a phase IB dose-escalation trial of Hu-M195 in 13 patients with relapsed and refractory myeloid leukemia. Hu-M195 showed pharmacology and biodistribution similar to the mouse M195. Hu-M195 showed specific uptake into leukemia cells with specific bone marrow targeting and internalization of radioisotope. In contrast to the experience with murine M195, we did not observe the formation of significant human antihuman antibody (HAHA) re-

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sponses, even after 12 doses of antibody over 4 months. Therefore, Hu-M195 shows superiority over the parent murine version and will be studied in future clinical trials.

PATIENTS AND METHODS

Hu-M195 MoAb production, radiolabeling, and quality control. Hu-M195 (Protein Design Labs, Mountain View, CA) is a recombinant IgG1 MoAb that was constructed by combining the CDR regions of the M195 antibody with human framework and constant regions. Clinical grade material was made under a Food and Drug Administration-approved IND at Memorial Sloan-Kettering Cancer Center (New York, NY). Hu-M195 (1.5 mg) was labeled with approximately 8 mCi of iodine-131 (New England Nuclear, Wilmington, DE) using chloramine-T with an aseptic pyrogen-free technique. Pure MoAb was purified by exclusion chromatography and filtered through a 0.20-μm filter before clinical use.

Hu-M195 was tested for the following: for specific activity, for the presence of murine viruses and endotoxin, for the absence of DNA, and for biochemical impurities by high-performance liquid chromatography. Hu-M195 was tested for purity by polyacrylamide gel electrophoresis, and for immunoreactivity on HL60 murine cell lines. Doses of the radiolabeled MoAb for injection were greater than 98% free of unattached 131I and showed immunoreactivities of approximately 60% to 85% in one-step binding assays using an excess of antigen. 131I-Hu-M195 could be reliably detected on captured MoAb above background.

Response criteria. A complete remission (CR) was defined as the disappearance of all radiographic and clinical abnormalities consistent with the diagnosis of acute myeloblastic leukemia (AML) or acute nonlymphocytic leukemia (ANLL). This included: complete disappearance of tumor masses; no abnormal splenomegaly; a reticulocyte count of less than 1% of total peripheral leukocytes; a normal bone marrow aspirate that did not show more than 5% blasts; normal演变化 in lymphocytes, platelets, and white blood cells (WBC) counts; and negativization of positive skin tests to known AML-specific antigens (M195).

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constant regions as Hu-M195. In this assay, the positive control serum captured 25 to 50 ng of antibody versus 100 to 400 pg of capture (background) for negative control serum. The positive control was completely blocked by the addition of any of the above MoAbs sharing the CDR of M195, but not by other MoAbs.

RESULTS

Patients. Important characteristics of the 13 patients are summarized in Table 1. Seven of the 13 were able to be studied as outpatients because no major toxicities were observed. There were 7 females and 6 males. Median age was 47 years. Included in the study were 3 patients who evolved from myelodysplasia to AML, 1 patient with myeloblastic CML, and 9 patients with de novo AML. Patient no. 4 received one dose of Hu-M195 and was discontinued from the study because of the development of bacteremia and sepsis unrelated to the treatment. She was considered evaluable only for the development of an HAHA response. An additional patient was, therefore, added at this level to confirm safety and to study other parameters. One patient (no. 8) received only 2 doses of Hu-M195 before rapidly progressing; two patients (nos. 5 and 6) received 5 doses of MoAb; and the remaining 9 patients received 6 doses on the first treatment course. Patient no. 7, who had stable disease on the second dose level, was retreated with 6 doses at the next higher dose of 3 mg/m²; and patient no. 11 with erythroleukemia who showed improvement in his bone marrow blast and erythroid cell count after the first treatment was retreated at the same dose level of 10 mg/m².

Toxicity. No significant toxicity was seen at the first and second dose levels. Patient no. 3 had a grade I headache, possibly related to one infusion. At the third dose level, one elderly female (patient no. 8) experienced grade II dyspnea, bronchospasm, fever, and rigors 2 hours after the end of the infusion of Hu-M195. Although patient no. 7 did not have any toxicity when treated at the second dose level, he did complain of myalgias and had a grade II fever when retreated at the third dose level. Five of six patients at the two highest dose levels showed a grade II fever. Two of three patients at the highest dose level experienced grade II rigors within 2 hours after the infusion ended. On retreatment at the same dose level, patient no. 11 had grade I vomiting. Patient no. 13 sustained asymptomatic grade II hypotension with a decrease in systolic pressure of 20 points; her blood pressure returned to normal after 1 L of normal saline was administered.

Dose-limiting toxicity was not seen. Rigors were reversible on administration of acetaminophen and diphenhydramine. On subsequent MoAb doses, further rigors were prevented by premedication with acetaminophen and diphenhydramine, except for patient no. 11, who on retreatment required an additional dose of diphenhydramine on 3 out of 6 occasions before the rigors subsided. No hematologic toxicity was noted in evaluable patients at any dose level.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Prior Therapy</th>
<th>Initial Complete Blood Cell Count</th>
<th>Initial Bone Marrow Marrow</th>
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<tr>
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<td></td>
<td></td>
<td>WBC Blast % ANC HgB PLAT</td>
<td>Blast % KPS</td>
</tr>
<tr>
<td>1</td>
<td>20/F</td>
<td>AML-M2 t(8;21)</td>
<td>IDA-ARA-C × 3; CBDCA × 3; Aza; MITO – VP15</td>
<td>2.3 27 0.9 9.6 29</td>
<td>26 80</td>
</tr>
<tr>
<td>2</td>
<td>38/F</td>
<td>AML-M2</td>
<td>HDAC; MITO-VP-16 ARA-C; CBDCA-aza</td>
<td>4.8 3 2.5 11.2 142</td>
<td>9 80</td>
</tr>
<tr>
<td>3</td>
<td>47/M</td>
<td>AML-M2 (5q–)</td>
<td>IDA-ARA-C × 2</td>
<td>2.8 3 1.2 12.9 68</td>
<td>32 90</td>
</tr>
<tr>
<td>4</td>
<td>29/F</td>
<td>AML-M5 t(11; 12; 16)</td>
<td>IDA-ARA-C × 2; HDAC – CTX; WBRT; TBI – AZO-CTX-BMT</td>
<td>0.8 16 0.4 7.6 10</td>
<td>14 70</td>
</tr>
<tr>
<td>5</td>
<td>69/F</td>
<td>RAEB-AML-M2 (refractory) (trisomy 8)</td>
<td>Danacrine; IDA-ARA-C; MITO-VP-16-ARA-C; CBDCA-aza</td>
<td>1.5 20 0.1 8.0 28</td>
<td>60 70</td>
</tr>
<tr>
<td>6</td>
<td>35/M</td>
<td>AML-M1</td>
<td>IDA-ARA-C × 2; HDAC × 4; MITO-VP-16</td>
<td>5.3 46 0.1 11.6 31</td>
<td>72 80</td>
</tr>
<tr>
<td>7</td>
<td>41/M</td>
<td>AML (refractory) (inv 3q)</td>
<td>IDA-ARA-C; MITO-VP-16-ARA-C</td>
<td>4.2 2 1.8 7.9 219</td>
<td>15 80</td>
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<tr>
<td>8</td>
<td>80/F</td>
<td>AML-M4</td>
<td>IDA-ARA-C; IDA-MITOX</td>
<td>5.9 60 3.8 8.2 12</td>
<td>36 70</td>
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<td>9</td>
<td>50/M</td>
<td>AML-M5b</td>
<td>HU; IDA-ARA-C × 2; MITO/VP16/Ara-C DNR-TAMOX</td>
<td>4.6 28 1.1 7.4 10</td>
<td>7 90</td>
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<tr>
<td>10</td>
<td>56/M</td>
<td>RAEB → AML-M2 (refractory)</td>
<td>IDA-ARA-C; MITO-VP-16-ARA-C</td>
<td>9.3 94 2.4 8.4 35</td>
<td>45 90</td>
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<tr>
<td>11</td>
<td>66/M</td>
<td>RAEB → AML-M2 (refractory) (20q–)</td>
<td>IDA-ARA-C; MITO-VP-16-ARA-C</td>
<td>1.5 1 0.4 9.8 8</td>
<td>18 (63%) erythroid</td>
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<tr>
<td>12</td>
<td>58/F</td>
<td>CML-blast (&gt;1Ph Chromo) (Trisomy 8)</td>
<td>Hu; etF; Fludarabine-ARA-C G-CSF, Topotecan</td>
<td>1.6 50 0.4 9.0 24</td>
<td>45 70</td>
</tr>
<tr>
<td>13</td>
<td>34/F</td>
<td>AML-M2 (refractory) t(3;3)</td>
<td>DNR-ARA-C; HDAC-DNR × 2</td>
<td>13.1 13 8.6 7.6 833</td>
<td>46 80</td>
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</table>

Abbreviations: DNR, daunorubicin; ARA-C, cytarabine; MITO, mitoxantrone; CBDCA, carboplatinum; VP16, etoposide; IDA, idarubicin; HDAC, high-dose cytarabine; Aza, azacytidine; AZQ, aziridinylbenzoquinone; CTX, cyclophosphamide; TBI, total-body irradiation; HU, hydroxyurea; 2-CDA, 2-chlorodeoxyadenosine; TAMOX, tamoxifen; RT, radiation therapy; etF, etoposide; HgB, hemoglobin B; ANC, absolute neutrophil count; PLAT, platelet; KPS, Karnofsky Performance Status.
Fig 1. Whole-body gamma-camera imaging of patients 4 hours after infusion with $^{131}$I-Hu-M195 at levels of (A) 0.5 mg/m$^2$, (B) 1.0 mg/m$^2$, (C) 3.0 mg/m$^2$, and (D) 10.0 mg/m$^2$. Anterior and posterior images are shown. Bone marrow uptake was seen at all dose levels, with marked uptake in long bones in (B) and (C). Liver and spleen showed activity, except for patient B, who had undergone a splenectomy. Cardiac blood pooling was seen at the highest dose level (D).

Radiolocalization and biodistribution. Anterior and posterior whole-body imaging showed marked uptake of $^{131}$I-Hu-M195 in all patients, at every dose level, into areas of bone marrow, including the skull, sternum, ribs, vertebrae, pelvis, and the long bones of the upper and lower extremities. Representative patients imaged 4 hours after MoAb administration are shown at each dose level (Fig 1A through D). Images were clearly seen within several hours after injection and lasted for 4 days in patients, as was seen in the murine M195 phase I trial. The most specific images were seen at the intermediate dose level of 3 mg/m$^2$. At the highest dose level, excess Hu-M195 resulted in blood pooling. Serial images of patient no. 10, with a hypercellular bone marrow and elevated peripheral blood blast count, illustrate the rapid and distinct bone marrow imaging that persisted with a time-dependent decline throughout the first 4 days, without blood pooling (Fig 2). Earlier dose levels showed little blood pooling, whereas excess antibody was visualized in the blood (iliac veins, vena cava, and heart) at the higher doses. However, the bone marrow still imaged clearly. Although thyroid uptake of iodine was blocked, radioactivity was seen in the thyroid by 2 to 4 days, indicating some free radioactive iodine in the blood. Excretion of free iodine could also be seen in the bladder by day 3 at all doses.

The liver and spleen were imaged in all the patients except in patient no. 5, who had undergone a splenectomy (and had no splenic uptake) (Fig 1B), and in patient no. 11, who had reduced liver uptake and significant stomach excretion for unknown reasons. The average liver uptake (10% of the injected dose) was similar or lower than that found for the murine M195. No imaging was seen for lymph nodes.

Pharmacology. Pharmacokinetics were based on the first trace-labeled dose of Hu-M195. Single Hu-M195 doses ranged from 0.8 to 18 mg (0.5 to 10 mg/m$^2$), and total doses ranged from 5 to 216 mg (on retreatment) (Table 2). Two-phase kinetics were seen. Mean ± standard deviation plasma half-lives $T_{1/2}\alpha$ and $T_{1/2}\beta$ for Hu-M195 were 0.3 ± 0.4 hours (patient no. 5 not included because of a significant variation from the mean) and 38 ± 9 hours, as compared with 1.3 ± 0.7 and 56 ± 21 hours, respectively, for murine M195. An average of 27% of the total dose per liter was found in the serum at time 0, representing the amount of radioactivity at the end of a 20-minute infusion. However, the amount of radioactivity found in the serum was dose-dependent; the mean injected dose per liter detected in the serum for the first 3 dose levels was 22%, as compared with a range of 34% to 52% for the highest dose level. This is consistent with the increased blood pooling. Total body half-life for Hu-M195, as measured by gamma-camera imaging, ranged from 34 to 68 hours, with a mean of 51 ± 13.1 hours. The percentage of the initial injected dose that was found in the liver ranged from 7.2% to 13.8%, with a mean ± standard
deviation of 9.8% ± 2.5%, whereas the initial spleen uptake represented 3.7% ± 1.3% of the total injected dose.

Saturation of CD33 antigen sites. To determine the effect of Hu-M195 on the availability of cell surface CD33 levels, flow cytometric analysis of CD33 expression on peripheral blood mononuclear cell targets of 9 patients were obtained. Remaining CD33 on the surface of target cells posttreatment showed dose-dependent decreases. At 0.5 and 1.0 mg/m², there were small changes in available CD33 levels 1 hour after treatment; 20% to 70% decreases in CD33 sites were seen at 3 mg/m². At the highest dose of 10 mg/m², 100% of available antigen sites were saturated (ie, no available CD33 was found) as soon as 1 hour after treatment as measured by flow cytometry. Plasma levels of Hu-M195 at 1 hour at the 3 mg/m² dose level were in the range of 200 to 500 ng/mL. Hence, a single dose between 3 and 10 mg/m² would be expected to deliver saturating amounts of MoAb as shown by flow cytometry.

There were also dose-dependent differences in the timing of reexpression of antigen sites depending on the dose of Hu-M195. Flow cytometric data showing CD33 sites on the peripheral blood blast population are shown for patient no. 10 (dose level 3) before and after each of 6 treatments (Fig 3). At 3 mg/m², as measured by the cell mean peak fluorescence, re-expression of available CD33 sites occurred every 72 hours to nearly pretreatment levels before the next dose. One hour after the first dose, CD33 levels decreased by approximately 50%, and then by about 80% for all subsequent doses. However, at the 10 mg/m² level, CD33 sites decreased immediately to zero after the first treatment and never returned to baseline pretreatment levels until the administration of Hu-M195 was discontinued. Hence, the 3 mg/m² level appeared to provide brief saturating plasma levels of Hu-M195, whereas the 10 mg/m² level resulted in prolonged CD33 saturating plasma levels of MoAb.

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Table 2. Pharmacology of 131I-Hu-M195

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose (mg/m²)</th>
<th>Plasma T1/2 (h)</th>
<th>Time 0% Injected Dose per Liter</th>
<th>Whole Body T1/2 (h)</th>
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<td>0.5</td>
<td>43</td>
<td>14</td>
<td>22</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; NF, no fit.
Phase I Trial of Humanized M195 (Anti-CD33)

Fig 3. Flow cytometric analysis of CD33 expression on peripheral blood leukemic blasts for patient no. 10 treated with $^{111}$I-Hu-M195 at a dose level of 3 mg/m$^2$ during 6 treatments. The number of available CD33 sites decreased by almost half 1 hour after the first dose, then increased slightly before dose no. 2, and decreased by approximately 80% after each subsequent dose. Arrows indicate the time of dosing.

Internalization of $^{131}$I-Hu-M195. The fate of $^{131}$I-Hu-M195 after binding to CD33$^+$ cells was examined ex vivo as previously described. Seven of the 13 patients that had peripheral leukemic involvement were studied. At 1 and 24 hours after administration of the first trace-labeled dose of $^{131}$I-Hu-M195, a range of 37% to 56% of the label was found inside the cells. None of the patients at the first dose level were included in this data.

HAHA response. One of the primary concerns in developing humanized MoAbs is to avoid HAHA, and thus to allow for multiple doses of MoAb to be administered. Sera from a total of 96 time points were measured up to 19 weeks after therapy administration (Fig 4). Multiple samples from each patient were examined for an HAHA response. There were no obvious positive results. However, four serum samples of the total (from 3 different patients) showed cpm slightly above the control background levels (outside of 2 standard deviations of the control population mean). Positive serum binding was 25- to 250-fold higher than these results. These responses did not appear significant, but, because any evidence of HAHA would be important in our application of this drug, additional competition assays were conducted to determine if the activity was indeed specific. The 4 sera were tested against a panel of MoAbs sharing various regions of homology, as described in Materials and Methods. Although the positive control serum could be competed by 98%, specific competition could not be demonstrated for the 4 sera from the current trial. These competition assays indicated that these low levels of activity above background were most likely nonspecific. Moreover, analysis of peak and trough Hu-M195 levels before and after each dose (up to 12 doses in patient no. 7) showed no evidence for rapid serum clearance of Hu-M195 that would be indicative of the presence of HAHA (unpublished data).

Biologic effects. Patient no. 11 with erythroleukemia (French-American-British [FAB]-M6) showed a decrease in bone marrow blasts from 18% (49% of nonerythroid cells) to 6%, as well as a decrease in the total bone marrow erythroid cell number from 63% to 34%, after one complete cycle of therapy. After completion of the second cycle of therapy, the bone marrow blast count increased slightly to 11%, and the erythroid component also increased. This patient had an antecedent history of a myelodysplastic syndrome (MDS) that evolved into FAB-M6 erythroleukemia. The Hu-M195 treatment appeared to have reverted his status from acute FAB-M6 AML back to MDS, which persisted after treatment for several months. Another patient (no. 7)

Fig 4. Map of time points of serum samples assayed for HAHA. HAHA was measured using a radioimmunoassay “double-antibody sandwich” technique. No positive responses were seen in sera from 96 samples up to 19 weeks after initiation of therapy with Hu-M195. Patient numbers are shown on the X axis. Each circle represents a sample assayed. The narrow arrowhead indicates dose no. 1 in all patients. Wider arrows indicate retreatment in patients no. 7 and 11.
lympholysis, the side effects seen here at the highest dose patients shortly after the start of the first infusion, which CAMPATH-lH, fever, rigors, and nausea developed in all of MoAb may be immune mediated and may be caused by toxicities seen with murine M195 was bony pain and low-grade fever and low-grade pain and low-grade fever in several cases. In contrast, dose-dependent minor reversible adverse effects of Hu-M195 were noted. All patients at 10 mg/m² experienced grade 2 fever and 2 of 3 patients at this dose had grade 2 rigors. Rigors were prevented by premedication with diphenhydramine and acetaminophen, except in patient no. 11, who on repeated treatment required further doses of diphenhydramine to control rigors.

In this trial, no hematologic toxicity was seen and maximum tolerated dose was not reached. Thus, toxicity was not a limiting factor for repeated treatments (up to 12 doses at this time) of Hu-M195. In a trial of humanized MoAbs in clinical trials stems first from an effort to avoid neutralizing HAMA, thus allowing continuing treatment; and, second, from attempts to improve leukemia cell killing by more efficient immune effector functions via the humanized Fc region. Hu-M195 is a high-affinity, recombinant MoAb capable of binding human complement and, importantly, killing leukemia cells in vitro by ADCC using human effector cells.

In this trial, 13 patients with myeloid leukemia were treated with increasing doses of trace-labeled Hu-M195. Because mouse M195 is effective at marrow targeting and ablation when coupled to ⁴¹Ir⁴¹ I, this trial was designed to assess biodistribution and leukemia cell targeting as well as to collect conventional phase I data and immunogenicity. The only toxicity seen with murine M195 was bony pain and low-grade fever in several cases. In contrast, dose-dependent minor reversible adverse effects of Hu-M195 were noted. All patients at 10 mg/m² experienced grade 2 fever and 2 of 3 patients at this dose had grade 2 rigors. Rigors were prevented by premedication with diphenhydramine and acetaminophen, except in patient no. 11, who on repeated treatment required further doses of diphenhydramine to control rigors.

In this trial, no hematologic toxicity was seen and maximum tolerated dose was not reached. Thus, toxicity was not a limiting factor for repeated treatments (up to 12 doses at this time) of Hu-M195. In a trial of humanized MoAb CAMPATH-lH, fever, rigors, and nausea developed in all patients shortly after the start of the first infusion, which were thought to be related to lympholysis and mediated by the release of cytokines. Although Hu-M195 does not cause lympholysis, the side effects seen here at the highest dose of MoAb may be immune mediated and may be caused by the release of cytokines or other mediators on binding or sequestration of target cells.

The pharmacology of Hu-M195 was similar to that of M195 as seen in its phase I trial. However, the serum T₁/₂ of Hu-M195 ranged from 23 to 54 hours, similar to that of the mouse version. This is comparable to the half-life of CAMPATH-lH when used in patients with lymphoma.

In the absence of target antigen or with a large MoAb excess, one would expect the half-life of humanized MoAb to be much longer than that of the murine MoAb. The findings in this trial may be explained by the following: (1) The first dose of MoAb in this trial was not saturating until the highest dose level was reached, as shown by the flow cytometric data and image analysis. (2) At time zero, there is enough antigen on leukemia cells to absorb 2 to 4 mg of MoAb. (3) There is a constant production and turnover of leukemia cells in vivo where new antigen sites are continuously being produced and capable of binding MoAb. In monkeys (n = 6) that do not express CD33 antigen, the β half-life of Hu-M195 is 14 days, supporting our conclusion that CD33 expression has a significant role in shortening the half-life (unpublished data). Measurement of a true plasma half-life in the absence of antigen may require infusions of larger doses into patients in remission.

Based on imaging and flow cytometry, approximately 3 mg/m² appears to be the optimal biologic dose for future trials. Hu-M195 rapidly and specifically targeted to the bone marrow as seen in whole-body gamma imaging. Blood pooling was seen most obviously at the highest dose level, but not to any degree at the 2 lowest dose levels, indicating that targeting had occurred in the bone marrow and that excess radiolabeled MoAb was circulating in the blood at the higher doses. At higher doses, isotope or toxin conjugated to M195 would be in excess and could lead to increased toxicity.

These studies showed that about 80% of CD33 sites on the surface of the target cells were saturated by 1 hour after infusion at the 3 mg/m² level. At this level, surface CD33 levels returned to pretreatment levels within 72 hours, allowing full expression of the antigen before the next dose of Hu-M195. Therefore, approximately 3 mg/m² every 72 hours appears to be the most appropriate dosage schedule. The optimal biologic dose of 3 mg/m² is similar to that found for the murine M195.

In contrast, at the highest dose of MoAb (10 mg/m²), CD33 levels decreased abruptly after the first infusion and remained at low levels throughout the full 6 doses. Because the degree of internalization was similar at the high dose, it is likely that the high repeated doses failed to allow reexpression of antigen because saturation of antigen sites had occurred and excess MoAb was in the plasma. Because immune-mediated mechanisms of killing require cell surface CD33 antigen and antibody, higher doses that resulted in more permanent downregulation of available CD33 sites appear less useful.

In vitro measurements of the fate of ¹³¹I-Hu-M195 substantiated that, as soon as 1 hour after infusion, almost half of the bound MoAb was internalized; internalization persisted for at least 24 hours. Comparisons of the data from the Hu-M195 trial here with the data from the phase I M195 trial, in which effective cytoreduction was seen, suggest that the Hu-M195, when appropriately labeled with ¹³¹I, will
also be an effective cytoreductive agent in leukemia. In another study using radiolabeled anti-CD33 MoAb p67 before bone marrow transplantation, the half-life of the radiolabel in the bone marrow was 9 to 41 hours, because of the rapid modulation and release of $^{131}$I from the marrow space.18

Despite a humanized IgG1 Fc region, no increased liver uptake was seen. In fact, the percentage of injected dose of radioactivity in the liver was similar or slightly less than that seen with the murine MoAb.2 This result was not surprising because these Fc receptors are already bathed in 10,000- to 100,000-fold excess of circulating IgG. Previous studies had shown that nonspecific Fc receptor binding did not occur in vitro.21

One of the major reasons to develop humanized MoAbs is to avoid HAMA formation, which prevented effective retreatment with murine M195 in the previous trial.9 In the current trial no patient developed HAHA. Each patient was tested several times, up to 19 weeks after the beginning of the initial 6 doses of therapy, and in two instances after a total of 12 doses. In the CAMPATH-1H trial, 3 of 4 patients developed antiglobulins, but only after retreatment.16

Although no CRs were seen, patient no. 11 with refractory erythroleukemia (AML-M6) showed a decrease in bone marrow blasts and in total erythroid marrow cells; another refractory patient (no. 7) showed stable disease. Both of these patients were retreated with another course of Hu-M195, one at a higher dose level. Neither patient required any further therapy for more than 6 weeks after the end of their second antibody treatment (15 to 19 weeks). In future studies, it may be necessary in stable or responding patients to continue antibody dosing until the disease progresses. The absence of HAHA in the Hu-M195 trial should allow for retreatment without adverse effects on Hu-M195 efficacy.

Multiple trials using murine MoAbs in leukemia and lymphoma have been reported,19 but only recently are clinical trials with humanized MoAbs for the treatment of human malignancies and prevention of organ graft rejection beginning to emerge. It has been previously shown that CAMPATH-1H produced remissions in 2 lymphoma patients20 and has shown clinical benefits for patients with rheumatoid arthritis.10 Preliminary results of humanized MoAb trials with anti-TAC specific for the interleukin-2 receptor indicate it is active in the treatment of acute graft-versus-host disease.21 Preliminary results from a phase I/II trial of CAMPATH-1H showed activity in non-Hodgkin’s lymphoma and chronic lymphocytic leukemia.17 Other preclinical studies using humanized MoAb have been reported.22-25

Anti-CD33 MoAbs covalently linked to ricin toxin (anti-MY9-blocked-ricin)20 or in combination with rabbit complement and chemotherapy22,28 have been used to purge bone marrow ex vivo. Patients who received autologous marrow treated ex vivo with anti-MY9 and complement after myeloablative therapy had durable engraftment.20 If unlabeled Hu-M195 does not prove to be effective in vivo, Hu-M195 conjugated with radiolabels or toxins may be used. Myeloablative doses of M195 labeled with a long-range beta emitter $^{131}$I with a wide field of kill effect are currently being used as part of a conditioning regimen for allogeneic bone marrow transplant in patients with refractory AML. Based on the trial presented here, Hu-M195 will be substituted for M195.

In cases of minimal disease, Hu-M195 linked to short-lived radionuclides with short-range auger electron emissions, or short-lived alpha-particle emitting radionuclides, may be clinically useful. If tumor burden is low, a higher dose of Hu-M195 where saturation and internalization are maximal may avoid the need for repeated doses. If repeated doses of Hu-M195 are found to be necessary, the absence of HAHA will prove Hu-M195 to be superior to murine M195. At the current time, phase II trials in the setting of minimal disease are also planned.

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A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity

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