Supersaturating Infusional Humanized Anti-CD33 Monoclonal Antibody HuM195 in Myelogenous Leukemia

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ABSTRACT
Humanized anti-CD33 monoclonal antibody HuM195 specifically targets myeloid leukemias in vivo and has been shown to produce molecular remissions in patients with acute promyelocytic leukemia who are in clinical remission. Previous human trials have used low intermittent dosing of HuM195 at 3 mg/m²/day, which is adequate to saturate all available CD33 sites in vivo. In the current trial, we investigated supersaturating doses of HuM195. Ten patients with relapsed or refractory myelogenous leukemia (nine acute myelogenous leukemias and one chronic myelogenous leukemia) were treated on days 1–4 and 15–18 with a 4-h daily infusion of HuM195 at three different dose levels: 12, 24, and 36 mg/m²/day. The total maximum dose of HuM195 was 576 mg. The most common toxicities were grade II fever and rigors, seen more frequently at the highest dose. Interestingly, a transient and reversible drop in hemoglobin of 1–3 g/dl was seen during the infusion in several patients. Flow cytometric analysis showed that antigen sites in the peripheral blood and bone marrow (BM) remained saturated with HuM195 during the entire 4-week trial period. At these high doses, the average plasma half-life of HuM195 was ~1 week, compared to 38 h, seen in previous studies. Human anti-HuM195 immune responses were not observed. One patient with acute myelogenous leukemia, whose disease was refractory to two rounds of chemotherapy, with <10% blasts in his BM, achieved a complete remission, lasting >32 months, at the first dose level. Another three patients showed a reduction in leukemic BM cells. These studies suggest that high doses of HuM195 achieve a long serum half-life, with tolerable toxicity and without immunogenicity. In addition, antileukemic activity was seen.

INTRODUCTION
Humanized mAbs³ have been increasingly used in clinical trials over the past decade (1–5). Complementarity-determining region-grafted HuM195 is a high-affinity, human IgG1 that is reactive with CD33, an antigen expressed on early myeloid and myelogenous leukemia cells (6). In vitro, HuM195 is capable of mediating ADCC against AML cells and is capable of fixing human complement, although complement-mediated cytotoxicity is not seen (7).

The newly acquired effector functions of HuM195 have stimulated interest in it as a therapeutic agent for myeloid leukemias when it used in its native form without radioactivity or toxins. In a Phase I trial, HuM195 showed minimal toxicity, no immunogenicity, and localization to the BM in patients with myeloid leukemias (8). After clinical trials demonstrated antileukemic activity when murine M195 was conjugated with ³¹I (9–11), ³¹I-HuM195 was substituted for M195 to avoid immunogenicity. In patients with relapsed or refractory AML or blastic CML, ³¹I-HuM195 was administered prior to busulfan and cytoxan for allogeneic BM transplant. Nine of 12 patients achieved a CR, with 2 remaining in unmaintained CR at >27 and >35 months (12).

It was previously determined from the Phase I trial that HuM195, at 3 mg/m² every 72 h, allowed reexpression of CD33 antigen sites and minimal nonspecific accumulation of antibody (8). Unconjugated HuM195 may be active in reducing or in eliminating minimal residual disease in patients with acute promyelocytic leukemia (13). Fifteen patients in first remission were treated with HuM195 at a dose of 3 mg/m² for a total of six doses over 3 weeks. Fourteen of 15 patients had minimal residual disease detectable by reverse transcriptase-PCR analysis of BM for the products of the t(15;17) translocation at the start of HuM195 therapy. Four of 13 evaluable patients converted to reverse transcriptase-PCR negative with HuM195 treatment, and 9 remained positive. None of the 15 patients had relapsed at a median follow-up of 22 months.

Passive immunotherapy using high doses of murine M195 was capable of completely eliminating HL60 cells in an athymic Swiss nude mouse leukemia model (14). Whereas the mortality of the animals treated with a control antibody was similar to untreated control groups, those animals treated with M195 were all alive at 10 weeks. Approximately 60% of the untreated animals were dead 10 weeks after the HL-60 cell inoculation. This suggests that M195 can specifically protect mice from

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1 The abbreviations used are: mAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; AML, acute myelogenous leukemia; BM, bone marrow; CML, chronic myelogenous leukemia; CR, complete remission; PR, partial remission; HAHA, human antihuman antibody; DLT, dose-limiting toxicity; PB, peripheral blood; POD, progression of disease; IL, interleukin; MDR, multidrug-resistant.

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2 To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, Professional Building, Phelps, 777 North Broadway, Suite 102, Sleepy Hollow, NY 10591.
HL60 leukemia as a result of specific IgG-mediated tumor suppression.

On the basis of these data, other schedules and doses for administration of HuM195 were sought. Here, we report on a Phase IB trial of HuM195 delivered at 12, 24, or 36 mg/m² over 4 h on days 1–4 and 15–18 in nine patients with relapsed and refractory AML and one patient with accelerated CML. Toxicity, pharmacology, and immunogenicity were studied. A continuous long-term saturation of CD33 sites may work via a different mechanism than ADCC, perhaps by blocking CD33 or by promoting opsonization.

PATIENTS AND METHODS

HuM195 Production and Quality Control. Clinical grade material was made under an Investigational New Drug application. Sp2/0 transfected cells secreting HuM195 were grown under Good Manufacturing Practice conditions using standard fermentation processes by Protein Design Labs (Mountain View, CA; Ref. 6). Further downstream processing provided purified drug that was tested using a battery of methods, including high-performance size exclusion chromatography, PAGE with Western blot, and isoelectric focusing. HuM195 was tested for the presence of murine viruses and endotoxin and for the absence of DNA contamination. HuM195 was placed in single-use vials at 10.5 mg/ml in sterile PBS (pH 6.0) and stored at 2–8°C.

Trial Design. All patients older than 12 years of age with myelodysplasia (French-American-British subtypes refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia), relapsed or refractory AML, or CML in chronic, accelerated, or blastic phase who had failed conventional therapy were eligible if there were <80% blasts in the BM and if >25% of BM blasts expressed CD33. Evidence of a doubling of blast counts during the previous 14 days before beginning the trial excluded patients from entry. Hydroxyurea was permitted to control PB counts up to 2 days before beginning the trial. Concurrent use of i.v. antibiotics was allowed. With the exception noted above, all chemotherapy or radiotherapy was stopped 3 weeks before HuM195 infusions began. Patients were required to have serum bilirubin and liver transaminase levels that were <3 times normal levels, a creatinine level <2 times the normal level, and no clinically significant cardiac or pulmonary disease. Patients were pretreated with acetylmethionine and diphenhydramine. A total of eight doses of HuM195 were administered as 4-h i.v. infusions on days 1–4 and 15–18. A minimum of three patients were treated at each dose level (12, 24, and 36 mg/m²/day). Total doses at each dose level were 96, 192, and 288 mg/m², respectively. Physical examinations were done once a day during treatment days and once a week during weeks 2 and 4. Complete blood counts were measured at least once daily on treatment days, whereas coagulation indices and biochemical and electrolyte values were measured once or twice a week during the protocol. Total complement, C3, and C4 levels were measured on days 4 and 18. A BM evaluation was conducted before the first dose and 1 week after the last dose. Serum for testing immunogenicity was taken before treatment and then once a month for 4 months after treatment. Toxicity was assessed according to the common criteria established by the National Cancer Institute.

Response Criteria. Patients receiving any part of one dose of HuM195 were evaluable for toxicity, and patients receiving a minimum of one course (or four doses) of HuM195 were evaluable for response. A CR was defined as a normocellular BM with <5% blasts in a patient with a hemoglobin level of >9.0 g/dl, a WBC count of >3000/μl, and a platelet count of >100,000/μl. Patients who had a 25% increase in blasts in the narrow after the administration of HuM195 or who developed new symptoms suggestive of POD were considered treatment failures. A PR was defined as ≥10% absolute decrease in BM blasts compared to pretreatment.

Pharmacology. Heparinized blood samples were drawn before and 5 min after each HuM195 infusion and once a week during weeks 2 and 4. In some patients, samples were obtained monthly for several months after the treatment ended. Plasma levels of HuM195 were measured by an ELISA using a "double-antibody sandwich" technique, using an anti-idiotype to the M195 (8). These studies were performed at Protein Design Labs.

Flow Cytometry. PB and BM cells were examined for quantitative expression of the CD33 antigen using an Epics Profile flow cytometer (Coulter Corp., Opa Locka, FL), as described previously (7, 8). Other myeloid cell markers, as well as the stem cell antigen CD34, lymphoid markers, and CD16 (anti-Fc γ RIIR receptor), were also measured. Samples were taken before and after the first HuM195 infusion, on day 4, and once weekly during weeks 2 and 4.

Evaluation of Human Immune Response to HuM195. A double-antigen ELISA was used to detect HAHA (8). HuM195 was adsorbed to microplate wells and blocked. Samples were diluted 1:5 in sample diluent prior to addition to the plates. After washing, enzyme-linked HuM195 was added, and bound conjugate was detected using o-phenylenediamine at pH 5. Assay controls included pretreatment specimens, untreated specimens, and serum spiked with known amounts of anti-idiotype antibody to M195 for calibration. Specificity of HAHA, if seen, for regions of HuG1 was assessed by blocking the activity of the specimen with controls, as described previously (8). This method has been demonstrated to be sensitive at 1–10 ng/ml high-affinity mouse anti-idiotypic antibody to M195.

Statistical Design. The study design was constructed as a dose escalation trial, which allowed for the termination of the study in the event of DLT. Assay ranges of variances are noted.

RESULTS

Patients. Important characteristics of the 10 patients, including PB counts and percentage of CD33-positive BM blasts pretreatment, are listed in Table 1. All 10 were able to be studied as outpatients because no major toxicities were observed. There were six females and four males. The median age was 60 years. There were three patients included in the study who evolved from myelodysplasia to AML, six patients with de novo AML, and one patient with accelerated CML. All patients had received prior chemotherapy. Patient 9 received part of one dose of HuM195 and was discontinued from the study because of the
**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Prior therapy</th>
<th>WBC (×10^9/ liter)</th>
<th>HGB (g/dl)</th>
<th>PLT (×10^9/ liter)</th>
<th>% CD33 leukemia cells</th>
<th>KPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>M</td>
<td>AML (FAB M2), refractory</td>
<td>Normal</td>
<td>HU/IDR/ARA-C × 2</td>
<td>3.0</td>
<td>11.2</td>
<td>70</td>
<td>96</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>AML (FAB M6), refractory</td>
<td>+8</td>
<td>IDR/ARA-C × 3</td>
<td>5.0</td>
<td>13.6</td>
<td>226</td>
<td>92</td>
<td>80</td>
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<td>3</td>
<td>29</td>
<td>F</td>
<td>AML (FAB M6), refractory</td>
<td>Normal</td>
<td>DNR/ARA-C × 2; HDAC/MITO/VF-16</td>
<td>1.3</td>
<td>1</td>
<td>4.2</td>
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<td>80</td>
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<tr>
<td>4</td>
<td>62</td>
<td>M</td>
<td>MDS/AML (FAB M6), refractory</td>
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<td>IDR/ARA-C; HDAC/MITO/VF-16</td>
<td>3.0</td>
<td>1.8</td>
<td>200</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>CML (accelerated phase)</td>
<td>(t9;22), −5, +16</td>
<td>IDR/ARA-C × 3; HDAC/MITO/VF-16</td>
<td>3.0</td>
<td>1.8</td>
<td>200</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>M</td>
<td>MDS/AML (FAB M0), refractory</td>
<td>+8, ins (1), (t5;19), inv (x)</td>
<td>IDR/ARA-C × 3; HDAC/MITO/VF-16</td>
<td>2.2</td>
<td>1.8</td>
<td>200</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>M</td>
<td>AML (FAB M2), refractory</td>
<td>Normal</td>
<td>DNR/ARA-C × 2; HDAC/PBSC BMT</td>
<td>1.6</td>
<td>1.8</td>
<td>200</td>
<td>2</td>
<td>80</td>
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<tr>
<td>8</td>
<td>69</td>
<td>F</td>
<td>AML (FAB M0), refractory</td>
<td>Normal</td>
<td>IDR/ARA-C × 2; ARA-C × 4; HDAC × 1</td>
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<td>1.8</td>
<td>200</td>
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<tr>
<td>9</td>
<td>63</td>
<td>F</td>
<td>AML (FAB M2), refractory</td>
<td>54–56, XX, +8, +9, +9, +13, +14, +15, +16, +21, +22 mar</td>
<td>IDR/ARA-C × 2</td>
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<td>200</td>
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<td>80</td>
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<td>10</td>
<td>69</td>
<td>M</td>
<td>MDS/AML (FAB M6), refractory</td>
<td>Normal</td>
<td>IDR/ARA-C × 2; DNR/ARA-C</td>
<td>1.8</td>
<td>1.8</td>
<td>200</td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>

* HGB, hemoglobin; PLT, platelets; KPS, Karnofsky performance status; FAB, French-American-British; MDS, myelodysplasia; HU, hydroxyurea; DNR, daunorubicin; HDAC, high-dose cytarabine; ARA-C, cytarabine; VP-16, etoposide; MITO, mitoxantrone; IDR, idarubicin; Auto, autologous; BMT, bone marrow transplant; PBSC, peripheral blood stem cell.

**Table 2** Toxicity

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Level I</th>
<th>Level II</th>
<th>Level III</th>
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</thead>
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<tr>
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<td>1a</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pain</td>
<td>1a</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Rigors</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LFT elevation</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cough</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* Numbers represent grade levels.

**Pharmacology.** Heparinized blood samples were drawn before and 5 min after each HuM195 infusion and once weekly after treatment. The peak plasma HuM195 level attained during the first 4 days always occurred after the fourth dose. Peak levels of HuM195 correlated with the dose level, i.e., the highest doses showed the highest peak serum concentrations of HuM195. The highest plasma concentration (175 μg/ml) occurred on day 15 in patient 8 at the highest dose level. In cases in which HuM195 levels were measured for several months after treatment ended, it took 4 months before nondetectable levels of HuM195 were seen.

Levels of HuM195 (μg/ml) measured during treatment were shown for a representative patient at the lowest dose level (Fig. 1). Serum antibody levels rose steadily during the 4 days of development of severe back pain and sustained hypotension. She was considered evaluable only for the toxicity and development of a HAHA response. An additional patient was, therefore, added at this level to confirm safety and to study other parameters. One patient (patient 5) with accelerated CML received the first course (4 days) of treatment with HuM195 but was discontinued from the study due to POD requiring hydration. All other patients (eight total) were able to receive both courses of HuM195.

**Toxicity.** Toxicity was tolerable and seen at all dose levels (Table 2). No grade IV toxicity was noted in this study. Toxicity was mild and most commonly seen on the first dose, despite premedication with acetaminophen and diphenhydramine. No hematological toxicity was noted at any dose level. DLT was not seen. The most common toxicities seen were pain, fever, and rigors, which were seen more often at the highest dose level. Rigors were reversible on administration of meperidine. Hypertension was seen in two patients at the highest dose level. Substernal pain without electrocardiographic changes occurred in one patient at the highest dose level on the first day and resolved spontaneously. Patient 9 was discontinued from the study due to severe lower back pain (grade III) and grade II hypotension that lasted for several hours, occurring 1 h into the infusion. Asymptomatic hypotension occurred in one patient at the intermediate dose level. Other minor episodes of pain of the leg, ear, and hip were also noted. Hyperbilirubinemia and elevation of liver function tests were seen in two and three patients, respectively, but in both cases, toxicity was either grade I or II. Hyperglycemia occurred in three patients, of which one case was grade III toxicity due to an increase in glucose from 140 to 275 mg/dl. However, it was not clinically symptomatic. Headache and cough were also seen.

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treatment and peaked after the last dose on day 4. During the week between treatments, HuM195 levels decreased significantly. The average estimated combined initial and terminal half-life of HuM195 was ~7 days for each of the patients shown. The range of half-lives for HuM195 of all patients, excluding patient 9, who only received part of one dose, was 4–10 days. No significant difference in half-life could be seen among the dose levels, but there was a trend toward a longer half-life during the second week of treatment.

**Complement Levels.** No change in complement levels were seen after treatment as compared to baseline levels.

**Flow Cytometry.** Immunophenotyping of PB and BM was done following HuM195 administration. To account for the total number of CD33 sites in the cell population, the data are expressed as the CD33 labeling index, i.e., the mean peak channel × the percentage of CD33-positive cells (Table 4). In 7 of 7 evaluable patients, the CD33 labeling index in the blast population of the PB consistently showed a >97% decrease after the first 4 days and remained so throughout the 4-week treatment period. In those patients for whom data were available, CD33 sites became saturated after the first dose of HuM195 and remained so throughout this period. No other myeloid markers, i.e., CD16 (FcγRIII), CD14, CD13, or CD11b, showed significant changes during treatment.

The BM blasts in patient 1 were characterized by both an exceptionally high mean peak fluorescence for CD33 (data not shown) and a high percentage of CD33-positive cells (Table 1). The percentage of PB blasts was negligible except for patient 6, who had >50% PB blasts. In this patient, the absolute blast count in the PB dropped from 1.2 to 0.3, and PB blasts that dually stained for CD33/CD34 decreased from 21 to 3% immediately after the first dose of HuM195. CD33 sites were still saturated up to 1 month after HuM195 was completed. Patient 5, with CML characterized by an expanded early myeloid population in the PB, demonstrated a >97% decrease in the CD33 index after the first treatment. Restoration of CD33 sites on PB cells did not occur until 50 days after HuM195 ended, while the patient was on hydroxyurea.

Flow cytometric results of the BM cells were also consistent. Dramatic decreases in the CD33 labeling index were seen in six of seven evaluable patients when compared before and after the 4-week treatment schedule (Table 4). In patient 1, who achieved a CR, CD34 decreased from 10 to 2% but showed little or no change in all other patients.

**Transient Drops in RBC Number.** Following each dose of HuM195, transient drops in RBC number (or Hgb) were seen. This effect was most pronounced during the first week of infusion and was consistent during the first dose. Although this drop in hemoglobin was occasionally seen during the second week of infusions, it was much less frequent. In patient 2, RBC number steadily decreased over the 4 days (Fig. 2). Levels returned to close to normal before each infusion. The most dramatic decrease in hemoglobin was seen in patient 3, in whom a drop of 2.4 g/dl from 8.2 to 5.8 g/dl (or RBC number drop from 2.8 to 1.9 x10^12/μl) was seen. Levels of RBC number returned to 90% of pretreatment levels by 2 h after the dose of HuM195 ended. Changes in WBC were seen to some degree in all patients and usually only during the first infusion. Drops in platelets were seen, but they were not consistent and were more variable among patients.

A hemolysis work-up (i.e., Coomb’s test, bilirubin, haptoglobin, LDH, and reticulocyte count) was done in several patients, and no evidence of hemolysis was seen. A 51Cr-labeled RBC scan was performed in patient 3 to determine whether RBCs were sequestered in the spleen or liver following administration of HuM195. Nuclear medicine scans done prior to infusion, midway during the infusion, and postinfusion did not show any changes in the pattern of distribution of 51Cr-tagged RBC, suggesting that sequestration did not cause the changes.

**Biological Effects.** Patients that received at least four doses of HuM195 were evaluable for response. Of 10 patients, there were 1 CR, 3 PRs, 1 nonevaluable patient, and 5 patients with POD. Of the patients with PR, although all eventually

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**Table 3 Pharmacology**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>HuM195 dosage (mg/m²/day)</th>
<th>Maximum plasma HuM195 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>9</td>
<td>36</td>
<td>NE</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>99</td>
</tr>
</tbody>
</table>

* Received 1 week of HuM195 and was then taken off study.
* Discontinued after first dose of HuM195.
* NE, not evaluable.
progressed with disease, one did remain clinically stable for 8 months. The patient who achieved a CR was a 75-year-old male who presented with AML (French-American-British M2) with biopsy-proven skin and lymph node involvement. After two rounds of induction therapy with idarubicin and cytarabine, the BM showed refractory disease with 8% blasts when he entered onto our study. After receiving HuM195 at the first dose level, the percentage of BM blasts decreased to <5%, but the patient remained thrombocytopenic and, therefore, could not be called a CR. The platelets gradually rose, and 10 months after beginning the study, he had a documented CR by BM and PB examination. He remained in unmaintained CR >32 months after starting therapy with HuM195.

**DISCUSSION**

In this trial, we report the results of a high-dose, supersaturating daily infusion of HuM195. As compared to the highest dose level of 10 mg/m² delivered over 1 h for six doses over an 18-day period that was used previously (8), here, we administer ~5 doses that dose over the same time period without any significant increase in toxicity when patients were treated at our highest dose level (i.e., 36 mg/m²). At the highest dose level, the total amount of HuM195 used in this trial was more than double that used in the initial Phase I trial in a patient who had been retreated (576 versus 216 mg). Such high levels of plasma HuM195 would make it possible to use a high-dose weekly infusion of HuM195 and still maintain a steady-state saturation of antigen sites for many weeks with detectable levels of HuM195 in the plasma for several months.

One of the most significant findings to come from this trial is the extension of half-life of HuM195 when it is delivered as a high-dose infusion. In this trial, the plasma half-life of HuM195 was ~7 days, as compared to 38 h for the intermittent dosing in the Phase IB trial (8). Levels of HuM195 rose during each of the four daily doses and then slowly fell until the start of the next infusion. The half-life of native human IgG in plasma is ~21 days. The half-life of HuM195 in monkeys, which do not express CD33, is 14 days. Therefore, it is likely that the half-life seen in this trial was a consequence of temporary saturation of available CD33 sites, followed by absorption and clearance of HuM195 as new CD33-positive cells (normal and leukemic) are formed in the BM.

Although several trials using HuM195 have now been reported, all have used an intermittent dosing schedule of 3 mg/m² every 72 h to allow for the reexpression of CD33 antigen sites (8, 10–13). The fate of the bound antibody has been previously studied for intermittent doses of HuM195. In vitro (7) and in vivo measurements of ¹³¹I-HuM195 (8) and ¹³¹I-M195 (9) showed that almost half of the bound mAb was internalized by 1 h after infusion and that the internalized radioactivity persisted for 24 h. A saturating dose of HuM195 was not reached in the Phase I trial until the highest dose of 10 mg/m² was given (8). At that dose, CD33 levels decreased abruptly after the first infusion and remained at low levels throughout the 3-week period.

Although internalization does occur, it is expected that the doses of HuM195 used in this trial would saturate all antigen sites on leukemia cells. Here, a continuous infusion of HuM195 for several hours daily allowed for constant saturation of CD33 sites, as demonstrated by the consistent decrease in CD33 labeling index (mean peak channel × percentage CD33-positive cells) in both the PB and BM populations. In one patient (patient 8), the CD33 index only decreased by 59%, rather than 90%. Reasons for a low saturation rate may be inavailability of antigen sites due to modulation or expansion of CD33-positive cells because of POD. One patient (patient 6) who had peripheral blasts and another (patient 5) with CML demonstrated reexpression of CD33 sites several weeks after the infusion had ended and their disease had progressed. It is difficult to determine how long CD33 sites would have remained saturated if the number of CD33 sites had not expanded, especially as is characteristic of the chronic phase of CML.

Table 4  Reduction in available CD33 antigen sites after treatment

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>% decrease in CD33 index a% CD33-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>NE b</td>
</tr>
<tr>
<td>5</td>
<td>NE</td>
</tr>
<tr>
<td>6</td>
<td>&gt;99</td>
</tr>
<tr>
<td>7</td>
<td>&gt;99</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
</tr>
<tr>
<td>9</td>
<td>NE</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
</tr>
</tbody>
</table>

 a CD33 index, mean peak channel × % CD33-positive cells.
 b NE, not evaluable.

![Fig. 2](image-url)
One would expect that patients with a higher tumor burden
(as reflected by percentage of BM blasts and level of CD33
expression) adsorbed more HuM195 and, therefore, had lower
levels of circulating HuM195. However, this was not observed
at the doses used here. Although most patients with advanced
AML have high PB counts, this was not true of our population
of patients. Many of our patients were heavily pretreated, and
this may be one of the contributing factors to the low WBC
counts. However, earlier studies showed that 131I-M 195 destroyed
up to 1 kg of leukemia (9) at smaller doses than those used in
this study. Maximum serum HuM195 levels did not correlate
with the extent of disease. At each dose level, a high serum level
of HuM195 did not correlate with a lower morphological per-
centage of either BM blasts or BM blasts positive for CD33 or
CD33/CD34. The percentage of PB blasts was negligible in all
patients except for patient 6, who had >50% PB and 70% BM
blasts, and maximal serum levels of HuM195 were higher than
in the other two patients. Patients at dose level III had the
highest serum concentrations of HuM195, yet they also had the
highest leukemic burden in the BM, as reflected by the highest
percentage of CD33-/CD34-positive stem cells.

Although the only toxicity seen with murine M 195 was
bone pain and low-grade fever, the toxicity recorded here
closely resembles that seen in earlier trials of HuM195 (8, 10,
12). No DLT was seen in this trial. In the previous trial, all
patients treated at the highest dose level (10 mg/m²) experienced
grade 2 fever, and two of three patients had grade 2 rigors.
Toxicity in this study was also dose dependent. Grade 2 fever
and rigors were mostly seen at dose levels II and III; no rigors
were noted at dose level I. Some toxicities that were seen
previously were more commonly seen in this trial, such as back
and leg pain, nausea/vomiting, and hypotension. These side
effects were seen mostly at level III. Patient 9 was taken off the
study due to severe back pain (grade III) and asymptomatic
hypotension. The only other grade III toxicity that was seen
was hyperglycemia (glucose = 275 mg/dl) and was not clinically
significant.

Although our study used a longer daily intermittent infu-
sion, we cannot extrapolate these results to a continuous infu-
sion of HuM195. A comparison of bolus versus continuous
infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in
patients with B-cell lymphoma showed that the toxicity was
related to the serum level of the immunotoxin (15). The maxi-
imal tolerated dose was similar, and both regimens achieved
comparable peak serum concentrations of the immunotoxin.
When the anti-CD25 ricin A-chain immunotoxin RFT5-SMPT-
dgA was administered in patients with refractory Hodgkin’s
lymphoma as a 4-h bolus infusion every 48 h over 8 days (16),
the response, toxicity, and pharmacokinetics were similar to
trials with the anti-CD22 immunotoxin, RFB4-SMPT-dgA.
When RFB4-SMPT-dgA was administered as a 4-h bolus over
8 days (17) or as a continuous infusion over 8 days (18), no
significant differences were seen.

It is possible that the side effects of fever and hypotension
seen in our study may be attributed indirectly to the stimulation
of immunomodulatory cytokines. In a Phase Ia/Ib trial using
MDX-210, a bispecific mAb against type I Fc receptors and the
HER-2/neu oncogene product, a rise in plasma concentrations of
cytokines such as IL-6, neopterin, granulocyte colony-stimulat-
ing factor and tumor necrosis factor-α that occurred from 1 to
6 h after treatment was thought to be responsible for the main
side effects of fever, malaise, and hypotension (19). A recent
trial using CAMPATH-1H, a humanized anti-CD52 mAb,
which was administered as a 30-mg 2-h infusion thrice weekly
for a maximal period of 12 weeks, showed fever and rigors
during the early infusions (20), similar to our experience with
HuM195. Nausea was also a common side effect, and hypoten-
sion that required temporary withdrawal or a slower infusion
rate was also seen. In our study, nausea and asymptomatic
hypotension were seen mostly at the highest dose level.

A consistent observation among the patients was a drop in
hemoglobin or RBC number that was usually most pronounced
in the first week, especially after the first infusion. This phe-
nomenon could not be explained by hemolysis (as measured by
Coomb’s test, haptoglobin, LDH, reticulocyte count, and bilir-
ubin) and was short lived. In fact, the hemoglobin concen-
tration returned to normal within hours after the infusion ended.

This infusion-related decrease in hemoglobin may be at-
tributed to a cytokine effect. Primates that received IL-6 devel-
oped anemia more rapidly than controls, and once IL-6 was
discontinued, the anemia resolved. In addition, the combination
of IL-3 and IL-6 delayed and diminished the decrease in hemo-
globin levels compared to IL-6 alone (21). In a Phase I trial of s.
IL-6 administered to patients with advanced malignancies,
there was a dose-dependent decrease in hemoglobin and RBCs
that occurred 24–48 h after the initiation of IL-6 that eventually
required blood transfusions (22). Further studies using radio-
isotope dilution assays to elucidate the mechanism of IL-6-
associated anemia were done (23). Mean hemoglobin concen-
trations recovered 48–72 h after the completion of IL-6 therapy.
It was shown that the anemia was mostly due to hemodilution
secondary to a significant increase in plasma volume.

However, the rapidity of hemoglobin recovery in our study
argues against a cytokine effect. A more likely explanation
for these drops in RBC number would be a sequestration phenom-
emon in which RBCs may be opsonized and sequestered by
complement fixed to HuM19S that was bound to targets in the
BM, spleen, or liver. Although HuM19S binds to normoblasts
and early myeloid precursors (but not stem cells), it is not
known to bind to RBCs, platelets, neutrophils, or lymphocytes.
However, it does bind monocyes (7). Although a chromium-
tagged RBC scan in patient 3 did not show any changes, on this
particular first dose, a significant concomitant drop in hemoglo-
bin was not seen. The same mechanisms may be responsible for
the less reproducible decreases in the WBC and platelets.

HuM195 in its native form is biologically active. Evidence
that HuM195 is capable of producing molecular remissions in
patients with acute promyelocytic leukemia who are in clinical
remission from retinoic acid has been reported (13). In this
study, one patient (patient 1) with refractory AML that had
minimal disease (<10% blasts) after two rounds of chemother-
apy achieved a clinical remission after being treated at the first
dose level of HuM195. The patient has not required any addi-
tional therapy for almost 3 years and remains in CR. Three other
patients with AML also showed improvements in their BM
blasts for 1–8 months after administration of high dose
HuM195. Perhaps continuation of therapy would have resulted in further benefit.

When HuM195 was given as an intermittent bolus, the mechanism of biological action was thought to be ADCC, which theoretically would require surface antigen availability. Complement-mediated killing has been shown to occur in vitro but was dependent on antigen density (7). However, in this study, other mechanisms must be sought to explain its clinical activity. Whether blocking the function of CD33 may inhibit a crucial cellular function is still unknown. The only function described thus far for CD33 has been that of a sialic acid-dependent cell adhesion molecule (24).

All of our patients in this study had refractory or relapsed disease. All but one of our patients received an anthracycline and, therefore, may have developed drug resistance. A MDR HL60 myeloid leukemia cell line that overexpresses the p-glycoprotein was susceptible to killing by ADCC or by lymphokine-activated killer cells with HuM195 but not to complement-mediated cytotoxicity (25, 26). The achievement of a CR in patient 1, who had minimal disease before HuM195 therapy, may have been due to the ability of HuM195 to overcome MDR cells. The high antigen density in patient 1, as reflected by a high mean peak fluorescence of CD33, may also have contributed to the achievement of a CR. However, although it was shown that MDR HL60 cells had a 2-fold higher density of CD33 antigen expression than the parental HL60 cells (25, 26), this could not explain the difference in sensitivity of MDR cells to killing by HuM195.

Additional ways to improve the biological activity of unconjugated HuM195 are also being investigated. HuM195 conjugated with gelonin immunotoxins showed cytotoxic activity against HL60 cells in vitro and may be used for in vivo or ex vivo BM purging of myeloid leukemias (27). It was previously shown that BM depleted of CD33 with anti-M9 and complement sustains durable but delayed engraftment after myeloblastic therapy (28). IL-2 has been shown to potentiate the antileukemia activity of HuM195 in vitro (25). The use of IL-2 with HuM195 is currently being studied in clinical trials at Memorial Sloan-Kettering Cancer Center in patients with myelodysplasia and myeloid leukemias.

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Supersaturating infusional humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia.

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