A Phase I Trial of Humanized Monoclonal Antibody HuM195 (anti-CD33) with Low-Dose Interleukin 2 in Acute Myelogenous Leukemia

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ABSTRACT

HuM195 is a recombinant humanized IgG1 monoclonal antibody reactive with CD33, a Mr 67,000 glycoprotein expressed on early myeloid progenitor cells and myeloid leukemia cells. HuM195 has been shown to rapidly target and saturate acute myeloid leukemia (AML) cells after i.v. infusion into patients and is capable of mediating antibody-dependent cellular cytotoxicity. This activity is enhanced in vitro when natural killer (NK) effector cells are preincubated with low concentrations of interleukin 2 (IL-2). Previous Phase I trials of HuM195 in patients with relapsed AML demonstrated safety and attainment of complete responses, but significant antileukemic activity appears limited to patients with low leukemia tumor burdens. Therefore, in the present trial, we sought to determine whether low-dose IL-2 could safely enhance the numbers of NK cells and therefore the cytotoxic capability of HuM195 via presumptive NK cell antibody-dependent cellular cytotoxicity in vivo against myeloid leukemia cells. Thirteen patients with relapsed or refractory AML and one patient with advanced myelodysplastic syndrome were treated with 0.6 × 10^6 IU/m^2 of s.c. IL-2 daily for 35 days. Starting on day 15, patients received twice weekly i.v. infusions of HuM195 (3.0 mg/m^2) for 3 weeks. Immediately after the HuM195 infusion, the patients received IL-2 i.v. infusions over 2 h at one of three escalating dose levels of 0.5 × 10^6, 1.0 × 10^6, and 2.0 × 10^6 IU/m^2. Peripheral blood mononuclear cells were quantitated and immunophenotyped by flow cytometry. Safety, tolerability, bone marrow mononuclear cell morphology, and immunophenotype, as well as responses were assessed. Of the 14 patients who entered the study, 10 were able to complete at least one cycle of therapy. Adverse effects to the s.c. IL-2 were relatively mild and included erythema and induration of the skin at the injection site and low-grade fever. Toxicity from the sequential HuM195 and i.v. IL-2 infusions included nausea, rigors, and fever. Toxicity was IL-2 dose related with dose-limiting toxicity seen at the 2.0 × 10^6 IU/m^2 dose level. Three patients had stable disease at the completion of the first cycle and went on to receive a second cycle of treatment. CD3-positive, CD56-positive, and CD33-positive cells were generally found to significantly decrease immediately after each administration of i.v. IL-2 and HuM195. CD56-expressing cells increased in 6 of 10 patients from the beginning to the end of therapy. Among the 10 evaluable patients, 2 patients had significant decreases in the percentage of blasts in the bone marrow (one of which achieved a complete bone marrow remission), 5 patients had stable levels of bone marrow blasts, and 3 had progression of disease on therapy. The combination of IL-2 and HuM195 shows modest biological activity and clinical antileukemic activity but also produced significant toxicity.

INTRODUCTION

M195 is a murine IgG2 monoclonal antibody reactive with the myeloid surface antigen CD33, a Mr 67,000 glycoprotein found on most myeloid leukemia blasts as well as committed normal myelomonocytic and erythroid progenitor cells. It is not expressed on nonhematopoietic tissues (1–3). M195 trace-labeled with I-131 has been shown to effectively target myeloid leukemia cells in vivo. CD33 surface antigen saturation occurred within 1 h of initial i.v. injection and was subsequently internalized (4). In a Phase I trial with therapeutically labeled I-131 (50–210 mCi/m^2), M195 administration resulted in significant cytoreduction of myeloid blasts from the peripheral circulation and bone marrow in patients with relapsed or refractory AML (5). The efficacy of M195 was limited in both trials by the development of human-anti-mouse antibodies that blocked M195 targeting and prevented repeat dosing. To decrease immunogenicity and add effector function, a recombinant complementary determining region-grafted HuM195 construct was developed. In contrast to M195, HuM195 has greater avidity for

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3 The abbreviations used are: AML, acute myelogenous leukemia; HuM195, humanized M195; ADCC, antibody-dependent cellular cytotoxicity; IL-2, interleukin 2; NK, natural killer; LAK, lymphokine-activated killer; MoAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; HSA, human serum albumin; MDS, myelodysplastic syndrome; CR, complete remission; HLR, Hoffmann LaRoche; CMMoL, chronic myelo-monocytic leukemia.
CD33 and has been shown to mediate in vitro ADCC with human effector cells when incubated with myeloid leukemia cells (6). A subsequent Phase I trial showed HuM195 could safely and specifically target AML cells in the peripheral blood and bone marrow with internalization into target cells without eliciting a neutralizing human-anti-human antibody response even after multiple injections for up to 1 year (7). Optimal biodistribution occurred at the 3 mg/m² level with near saturation of CD33 antigen sites on leukemia cells throughout the body. In patients with relapsed and refractory AML, significant antileukemia effects were limited to patients with low tumor burdens. In addition, in patients with acute promyelocytic leukemia, elimination of residual leukemia as documented by reverse transcription-PCR has been documented (8).

IL-2 supports the proliferation of NK cells and activated T lymphocytes by interacting with its specific membrane receptor on these immune effector cells. Incubation of peripheral blood mononuclear cells in IL-2 results in proliferation of NK cells expressing the high-affinity IL-2 receptor heterotrimer and leads to the generation of LAK cells that possess enhanced cytotoxicity and the ability to lyse susceptible tumor cell lines in a non-MHC restricted manner (9–12). Patients with acute leukemia in CR treated with IL-2 produce LAK cells that can lyse autologous leukemia blasts (13). Moreover, in a murine model, treatment with LAK cells generated from a healthy donor were found to abrogate the growth of a human leukemic cell lines (14). These encouraging studies led to several clinical trials using IL-2 for patients with acute leukemia. In select cases, the administration of IL-2 resulted in responses in patients with low bone marrow blast counts (15–18). Conversely, patients with marked elevation in bone marrow blasts rarely achieved favorable responses from treatment with IL-2 (17–19).

High-dose IL-2 is associated with myriad severe toxicities that may limit its administration and, therefore, its efficacy. Hence, Caligiuri et al. (20) studied the effects of low-dose uninterrupted IL-2 infusion at doses ranging from 0.5 × 10⁹ to 6.0 × 10⁹ units/m² per day for 3 months in 21 patients with advanced malignancies. In the patients receiving 1.5 × 10⁵ and 4.5 × 10⁵ units/m² per day of IL-2, the infusion resulted in 6-fold and 9-fold expansion, respectively, of NK cells. These patients tolerated therapy well with none experiencing grade 3 or 4 toxicity. To augment immunological activity in the post bone marrow transplant setting, Soiffer et al. (21) treated 13 patients with prolonged uninterrupted IL-2 at 2.0 × 10⁹ units/m² per day. All 13 patients tolerated the IL-2 administration well, and all experienced an increase in NK cells ranging from 5- to 50-fold.

IL-2 has been shown to potentiate ADCC of human effector cells with mouse anti-melanoma MoAb (22–24) against malignant B-cell lines using the murine Lym-1 MoAb (25) and against the IL-2 receptor-positive target cells using chimeric and humanized anti-Tac MoAb (26). Moreover, in vitro studies have shown that the combination of IL-2 and HuM195 results in a 2-4-fold enhancement of PBMC ADCC of HL-60 cells compared with that seen with HuM195 or IL-2 alone (27). IL-2 concentrations as low as 20–50 units/ml were as effective as 100 units/ml. Cytotoxicity was NK cell dependent, and LAK activity represented approximately one-third of the total cell killing. In vitro assays using fresh leukemia cells from three separate patients with AML confirmed a more than additive tumoricidal effect with the combination of IL-2 and HuM195 (27).

On the basis of these results, we conducted a Phase I dose-escalation trial to determine whether low-dose IL-2 could enhance the tumoricidal effects of HuM195 by increasing the numbers and effectiveness of NK cell ADCC in patients with relapsed or refractory myeloid leukemia.

**PATIENTS AND METHODS**

**Patients.** Patients over 16 years of age with relapsed or refractory AML (with or without lymphoid markers), MDS with French-American-British subtypes Refractory Anemia with Excess Blasts (RAEB), Refractory Anemia with Excess Blasts in Transformation (RAEB-T), or CMMOL, or chronic myelogenous leukemia in accelerated or myeloid blast phase were eligible. Eligibility required <75% blasts in the bone marrow (to allow for a minimum number of presumptive NK cells or progenitors) and expression of CD33 on >25% of bone marrow blasts. All chemotherapy or radiotherapy was stopped 3 weeks before initiating IL-2 with the exception of hydroxyurea, which was permitted to control peripheral blood counts up to 2 days before entering the trial. Patients were required to have a serum creatinine <2.0 mg%, bilirubin <2.5 mg%, and alkaline phosphatase and liver transaminases less than three times the upper limit of normal.

**HuM195 MoAb Production and Quality Control.** HuM195 (Protein Design Labs, Mountain View, CA) was generated using Sp2/0 hybridoma cell lines grown in vitro in serum-free medium. HuM195 was purified from concentrated supernatants by affinity chromatography followed by additional chromatographic purification steps. HuM195 was generously supplied by Dr. Daniel Levitt (Protein Design Labs) as a solution of 10.5 mg/ml and stored at 4°C. Immediately before administration, the HuM195 was diluted into 50 ml of 5% HSA in normal saline for injection.

**Recombinant Human IL-2.** IL-2 (Chiron, Emeryville, CA) was supplied by NCI in vials containing 22 × 10⁶ IU as a sterile lyophilized powder. The IL-2 was reconstituted with 2.4 ml of sterile 0.1% HSA yielding a final concentration of 9.17 × 10⁴ IU/ml. Prior to i.v. bolus therapy, IL-2 was diluted with 50–500 ml D5W with 0.1% HSA U.S.P.

**Treatment Plan.** The trial was designed as a Phase I dose-escalation with cohorts of at least three patients per dose level of i.v. IL-2. The protocol was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board, and the HuM195 was used under FDA Investigational New Drug (IND) approval. Thirteen patients with relapsed or refractory AML and one patient with advanced MDS were enrolled. All of the patients gave written informed consent. Patients received a fixed dose of daily s.c. injections of 0.6 × 10⁶ IU/m² of IL-2 for 35 days. Beginning on day 15, patients received outpatient i.v. infusions over 60 min of a fixed dose of HuM195 at 3.0 mg/m² twice weekly for 3 weeks. Immediately after each HuM195 infusion, the patients received i.v. infusions of IL-2 over 2 h at one of four escalating dose levels of 0.5 × 10⁶, 1.0 × 10⁶, 2.0 × 10⁶, and 4.0 × 10⁶ IU/m² per dose. Physical examination, complete blood counts, coagulation indices, biochemical and
Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Prior therapy</th>
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<td>1</td>
<td>60/F</td>
<td>AML</td>
<td>+8</td>
<td>Ida/Ara-C × 3</td>
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<tr>
<td>2</td>
<td>54/F</td>
<td>AML</td>
<td>+8</td>
<td>Ida/Ara-C × 2</td>
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<tr>
<td>3</td>
<td>67/M</td>
<td>AML (M4)</td>
<td>del(1),del(2),del(4),-5,-7,+8, del(12)+14,—16,—17,add(18),del(20), 5q—,+21</td>
<td>Ida/Ara-C, Ida/HiDAC, Ara-C/VP-16</td>
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<td>4</td>
<td>75/F</td>
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<td>Ida/Ara-C, Ara-C × 2</td>
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<td>5</td>
<td>62/M</td>
<td>MDS (CMMol)</td>
<td>normal</td>
<td>Dauno/Ara-C × 3</td>
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<tr>
<td>6</td>
<td>62/M</td>
<td>AML (M5b)</td>
<td>t(3;5), +4,+13</td>
<td>Dauno/Ara-C × 2, HiDAC × 3</td>
</tr>
<tr>
<td>7</td>
<td>69/F</td>
<td>AML</td>
<td>(Not known)</td>
<td>Aza</td>
</tr>
<tr>
<td>8</td>
<td>53/M</td>
<td>AML</td>
<td>+8</td>
<td>Ida/Ara-C × 2</td>
</tr>
<tr>
<td>9</td>
<td>59/M</td>
<td>MDS/AML</td>
<td>+8</td>
<td>Dauno/Ara-C × 2, CTX/VP-16</td>
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<tr>
<td>10</td>
<td>77/M</td>
<td>AML (M5b)</td>
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<td>Ida/Ara-C × 2, HiDAC × 2, CTX/VP-16</td>
</tr>
<tr>
<td>11</td>
<td>61/F</td>
<td>MDS/AML (M1)</td>
<td>t(1;1),—3,add(4),der(5),t(3;5),der(60,t(6;11), +8,del(11),—21</td>
<td>Ida/Ara-C, HiDAC/Mito/VP-16, Ara-C/VP-16 × 2</td>
</tr>
<tr>
<td>12</td>
<td>65/F</td>
<td>MDS/AML</td>
<td>add(1)(31),—2,i(2),del(4)(q21q31), del(5)(q15q31),—7,add(8)(q24), +9,add(11)(q15),add(11)(p15),—16, —17,—18</td>
<td>Ida/Ara-C</td>
</tr>
<tr>
<td>13</td>
<td>71/M</td>
<td>AML (M6)</td>
<td>—5,—13,der(17)(t;5;7)(q10p10),add(21)(q22), del(7)(q22q23)</td>
<td>Ida/Ara-C, HiDAC, Ida/Ara-C</td>
</tr>
<tr>
<td>14</td>
<td>35/M</td>
<td>AML</td>
<td>t(11;17)</td>
<td>Ida/Ara-C × 2, Hydantoin, AlloBMT, Mito</td>
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</table>

* Dauno, daunorubicin; Ara-C, cytarabine; HiDAC, high-dose cytarabine; Ida, idarubicin; Mito, mitoxantrone; Aza, azacytidine; Ctx, cyclophosphamide; Mtx, methotrexate; 5-FU, 5-fluorouracil; RT, radiation therapy; VP-16, etoposide; AlloBMT, allogeneic bone marrow transplant.

RESULTS

Patient Characteristics. Fourteen patients were enrolled in the study. The important clinical features of the patients are outlined in Tables 1 and 2. The median age was 62 years (range, 35–77 years). There were seven males and seven females. Thirteen patients had AML and one patient had CMMol. Five of the patients were noted to have AML clinically transformed from antecedent MDS. Thirteen patients were in first relapse, two of which were refractory to reinduction with anthracycline-based chemotherapy (including one relapse following six of six matched-related allogeneic bone marrow transplant that had been performed in first CR). One patient with MDS had received alkylating agent-based chemotherapy and radiotherapy for breast cancer. Twelve of 13 patients with AML had poor-risk cytogenetics. Six patients had multiple complex cytogenetic abnormalities. Patient 5 (with CMMol) was the only patient with a normal karyotype. Bone marrow blasts prior to initiating therapy on study ranged from 8 to 69% (median, 29.5%), and 7 of the 14 patients had >30% bone marrow blasts. CD33 expression was present on the surface of the leukemic cells on all 14 patients. Three patients received therapy on dose level 1, three patients on dose level 2, and eight patients on dose level 3. Of the 14 patients entered, 10 patients completed at least one cycle of therapy and were evaluable for response. Of these 10 patients, 3 received a second cycle of therapy. Four patients were not evaluable for response; one of the patients experienced toxicity to the i.v. IL-2 that resulted in his removal from study. Patient no. 8 developed toxicity to HuM195 but was able to subsequently complete two cycles of therapy with s.c. and i.v. IL-2 without HuM195. Two patients developed severe infections and were unable to complete one cycle of therapy.

Vomiting, pain, or changes in pulmonary, gastrointestinal, renal, or cardiac function nor changes in coagulation.

Toxicities to the i.v. IL-2 and HuM195 are listed in Table 3. (Only toxicities for which a patient reached grade 2 or greater are shown.) Additional patients were treated with bolus infusions of IL-2 at 2.0 × 10^6 IU/m^2/dose to better define the toxicities at this dose level. Of the 14 patients treated, 4 experienced grade I or II nausea and vomiting.
Eleven patients had grade I or II fever, including 3 patients on dose level 1 (100%), 3 patients at dose level 2 (100%), and 5 patients at dose level 3 (63%). Seven patients had mild to moderate rigors associated with the i.v. therapy. Fever and rigors were reversible with administration of acetaminophen and diphenhydramine except for patient no. 14, who on retreatment required indomethacin on four occasions to alleviate fever. Patient no. 5 developed grade II hypotension with a 70 mmHg decrease in systolic blood pressure that returned to normal after a 1-liter i.v. fluid bolus. Patient no. 1 had fleeting substernal chest pain at the onset of the IL-2 infusion. Patient no. 8 developed grade II substernal chest pain radiating to the left shoulder, dyspnea, and wheezing 5 min after the infusion of HuM195. These symptoms subsided after discontinuation of the antibody infusion and the administration of diphenhydramine and hydrocortisone. He subsequently tolerated two cycles of s.c. and i.v. IL-2 therapy without significant toxicity. Patient no. 10 sustained grade III hypotension with a decrease in systolic pressure of 35 mmHg at the end of the IL-2 infusion. This event was further complicated by transient hypoxemia and subsequently by grade II increase in prothrombin time, grade I increase in partial thromboplastin time, and grade I nephrotoxicity characterized by an increase in serum creatinine. The hypotension and hypoxemia responded favorably to i.v. fluids and oxygen delivered via mask, respectively. Patient no. 12 developed polymicrobial sepsis after a single administration of i.v. IL-2 and HuM195. Patient no. 13 completed two weeks of

Table 2  Patient initial hematological data

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Initial complete blood cell counta</th>
<th>Initial bone marrow blast</th>
<th>Pretreatment bone marrow immunophenotype</th>
<th>IL-2 dose (IU/m²)</th>
<th>Duration of therapy (wk)</th>
</tr>
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<tr>
<td>1</td>
<td>2.3 0 1.4 12.3 92 23</td>
<td>CD13, CD33, CD34, CD45</td>
<td>0.5 x 10^6</td>
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<tr>
<td>2</td>
<td>2.8 12 0.73 11.7 49 20</td>
<td>CD25, CD33, CD34</td>
<td>0.5 x 10^6</td>
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<tr>
<td>3</td>
<td>2.7 27 0.59 10.4 31 62</td>
<td>CD3, CD33, CD34, CD56</td>
<td>0.5 x 10^6</td>
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<tr>
<td>4</td>
<td>23.3 10 6.99 8.5 47 8</td>
<td>CD13, CD33, CD34</td>
<td>1 x 10^6</td>
<td>10</td>
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<tr>
<td>5</td>
<td>6.7 24 4.29 11.1 49 21</td>
<td>CD33, CD34</td>
<td>1 x 10^6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.5 0 0.1 9.7 88 33</td>
<td>CD13, CD14, CD33, CD34</td>
<td>1 x 10^6</td>
<td>5</td>
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</tr>
<tr>
<td>7</td>
<td>1.1 0 0.2 10.4 25 42</td>
<td>CD25, CD33, CD34</td>
<td>2 x 10^6</td>
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<tr>
<td>8</td>
<td>2.8 15 0.06 9.4 8 16</td>
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<tr>
<td>9</td>
<td>3 0 0.8 9.7 24 32</td>
<td>CD3, CD25, CD33, CD34</td>
<td>2 x 10^6</td>
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<tr>
<td>10</td>
<td>21.6 1 2.2 11.1 102 57</td>
<td>Ia, CD4, CD11b, CD13, CD14, CD33, CD34, CD45</td>
<td>2 x 10^6</td>
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<tr>
<td>11</td>
<td>1.5 8 0.1 10.8 20 27</td>
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<td>CD33, CD34</td>
<td>2 x 10^6</td>
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a ANC, absolute neutrophil count; Hgb, hemoglobin.
b Successfully completed two cycles of IL-2 alone.

Table 3  Toxicities of grade 2 or higher after i.v. IL-2 and HuM195 in combination

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Nausea/Vomiting</th>
<th>Fever</th>
<th>Rigors</th>
<th>Hypotension</th>
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<th>Pain</th>
<th>PTb</th>
<th>Infection</th>
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</table>

The toxicity was graded according to National Cancer Institute Common Toxicity Criteria: 0, grade 0; 1, grade I; 2, grade II; 3, grade III; 4, grade IV.
b PT, prothrombin time.
c Chest pain.
d Shortness of breath, wheezing 5 min after infusion of HuM195.
e Chest pain radiating to the left shoulder and bilateral leg pain.
f Transient apneic episode.
g Sepsis.
Phase I Trial of HuM195 with IL-2 in AML

obtained at the completion of therapy demonstrated decreases in CD33-positive cells in 10 patients and an increase in one patient. Patient no. 8, who did not receive HuM195, also had increases in CD33-positive cells in his bone marrow sample.

Clinical Response to Therapy. Table 5 lists the changes in the percentages of bone marrow blasts for each patient. Of the 10 patients evaluable, 1 patient achieved a complete bone marrow response to therapy. In Patient no. 1, bone marrow blasts decreased from 23 to 9% after her first cycle of therapy and to 5% after her second cycle of therapy. Her peripheral blood counts showed evidence of relapse 4 months after beginning therapy. In two patients, the percentage of bone marrow blasts decreased; five patients had stable bone marrow blast counts after therapy; and three patients progressed on therapy. Patient no. 9 remained stable and transfusion independent for 4 months after the completion of her second cycle of therapy until her disease progressed.

**DISCUSSION**

The CD33 cell surface glycoprotein possesses many unique features that make it well suited as a target for immunological antineoplastic therapy. Foremost, the antigen is selectively expressed on malignant myeloid blasts and on a subset of normal myelomonocytic and erythroid progenitors. The humanized monoclonal antibody HuM195 has demonstrable cytotoxic activity against myeloid leukemia cells bearing CD33 via ADCC.

Clinical studies with HuM195 have suggested that its ability to mediate an effective response against leukemic cells may be most applicable for patients with low tumor burdens. The rationale of this trial incorporates both the immunomodulatory effects of low-dose IL-2 to potentiate the effectiveness of HuM195-targeted NK cell ADCC and intrinsic antileukemic properties of each agent alone. Because these agents had never before been combined in humans in vivo, this Phase I trial was designed to assess their safety, tolerability, and possible biolog-
immunotherapies (31, 32).

present in these heavily pretreated patients, are also resistant to

We have reported the 13 patients with AML had poor-risk cytogenetics, of which

after allogeneic bone marrow transplantation. In addition, 12 of

chemotherapy. Seven patients had relapsed disease after consol-

involved with high-dose cytarabine, and one patient had relapsed

itoriness of the very poor prognosis patients included in the

been further limited in this study because of the relative refrac-

were found to have stable disease upon completion of the combined

therapy. Because the majority of patients with AML generally
tends to progress rapidly, the duration of treatment with low-

ICCs and low numbers of nonneoplastic progenitor cells

study had advanced AML. High burdens of bone marrow leu-


tors. In addition, because these patients had active AML, which

possible reasons for this difference. In sharp contrast to the

previous trials has not been a result of CD33 negative cells

expression, precluding further therapy.

Of the 10 patients who completed at least one cycle of

therapy with IL-2 and HuM195 and who were evaluable for

responses, two had significant decreases in bone marrow blasts. Both of these patients received two complete cycles of therapy,

resultant declines in their bone marrow blasts from 23 to

5% and 32 to 17%, respectively. Five additional patients were

found to have stable disease upon completion of the combined

therapy. Because the majority of patients with AML generally

have progressive increases in their number of bone marrow

blasts over a similar time interval, this may reflect a modest

clinical effect as well. Moreover, previous trials of both IL-2

and HuM195 showed that effects were greatest in patients with

relatively low bone marrow leukemia burdens (7, 29, 30).

The ability of the HuM195 and IL-2 to eliminate leukemia may have

been further limited in this study because of the relative refrac-
toriness of the very poor prognosis patients included in the

study. This is reflected first by the fact that the majority of the

patients had received multiple cycles of anthracycline-based

chemotherapy. Seven patients had relapsed disease after consol-
idation with high-dose cytarabine, and one patient had relapsed

after allogeneic bone marrow transplantation. In addition, 12 of

the 13 patients with AML had poor-risk cytogenetics, of which

had multiple chromosomal abnormalities. We have reported

previously that multidrug-resistant cells, which are likely to be

present in these heavily pretreated patients, are also resistant to

immunotherapies (31, 32).

Several predicted biological responses were observed dur-

and after the treatment with IL-2 and HuM195. Mononuclear

cells expressing CD33 in the peripheral blood were found to

decline substantially in 9 of the 12 evaluable patients. As we

have observed in previous trials (7, 8), this may reflect a direct

effect of the HuM195 binding to the antigen and thus competi-

tively blocking the fluorescence-labeled antibody (M195) used

for immunophenotyping. In addition, the decreased CD33 may

be due to eradication of CD33 expressing leukemic blasts via

ADCC or because of a global decrease in CD33 expression

because of modulation of CD33 off the cell membrane after

binding to HuM195 (4, 6). Failure of therapy in this trial or in

previous trials has not been a result of CD33 negative cells

growth. CD33-expressing cells decreased immediately after
each administration of IL-2. This may reflect an IL-2-induced

margination of T lymphocytes (33, 34). Although the number of

CD56-expressing cells was found to generally decrease imme-
diately after each infusion of the IL-2, the levels of CD56-

expressing cells increased modestly over the course of the

4-week treatment program in the majority of patients, possibly

reflecting IL-2 induced activation and expansion of NK cells. In

this study, we were not able to reproduce the large increases in

NK cell expansion by low-dose IL-2 originally reported by

Caligiuri et al. (20) or Soiffer et al. (21). There are several

possible reasons for this difference. In sharp contrast to the

patients treated by Caligiuri, which included a significant num-

ber of patients with solid tumors, the patients treated in this

study had advanced AML. High burdens of bone marrow leu-

kemic blasts and low numbers of nonneoplastic progenitor cells

probably resulted in lower absolute numbers of NK cells and

progenitors. Moreover, many of these patients were heavily

pretreated with chemotherapy toxic to hematopoietic progeni-
tors. In addition, because these patients had active AML, which

tends to progress rapidly, the duration of treatment with low-
dose IL-2 was substantially shorter in this study compared with

that used by Soiffer in the post bone marrow transplant setting.

Another possible reason for our observed decreased bio-

logical effects may relate to our using the Chiron preparation of

IL-2 compared with others who used HLR IL-2. Hank et al. (35)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Bone marrow blasts (%) before therapy</th>
<th>Bone marrow blasts (%) after cycle 1</th>
<th>Bone marrow blasts (%) after cycle 2</th>
<th>Change in bone marrow blasts</th>
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<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>9</td>
<td>5</td>
<td>78% reduction</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>70</td>
<td>7D</td>
<td>POD</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>61</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>7</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>26</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>82</td>
<td>POD</td>
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<td>7</td>
<td>42</td>
<td>37</td>
<td>SD</td>
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<td>9</td>
<td>32</td>
<td>30</td>
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<td>47% reduction</td>
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<td>10</td>
<td>57</td>
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<tr>
<td>11</td>
<td>27</td>
<td>41</td>
<td>POD</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>69</td>
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<tr>
<td>14</td>
<td>57</td>
<td>66</td>
<td>SD</td>
<td></td>
</tr>
</tbody>
</table>

POD, progression of disease; SD, stable disease; NA, not available.

a Patient no. 8 received IL-2 without HuM195.
described recently the results of several studies comparing the clinical activity and toxicity as well as biological effects of these two agents. In patients treated with 96-h continuous infusions using equivalent doses of Chiron IL-2 and HLR IL-2, absolute lymphocytosis and CD56 expression were both found to be significantly greater after the administration of HLR IL-2 compared with the Chiron IL-2. Moreover, patients who received HLR IL-2 1.5 × 10⁶ units/m²/day experienced far more toxicities than those who received an identical 1-week course of Chiron IL-2, despite a higher dose of 4.5 × 10⁶ units/m²/day. In vitro proliferation assays using the IL-2-dependent Tf1-β cell line and PBMCs obtained from patients treated with IL-2 showed greater responses after incubation with HLR IL-2 compared with Chiron IL-2. Approximately 3–6 IU of Chiron IL-2 was required to induce the same biological effects as HLR IL-2.

In conclusion, this study showed that the combination of low-dose IL-2 and HuM195 shows modest biological activity and clinical antileukemic activity but also produces significant toxicity, largely IL-2-related. These effects may be better elucidated in a Phase II study, possibly with a different preparation of IL-2, in patients with AML or advanced MDS with low bone marrow tumor burdens. The evidence of activity also encourages the use of this or similar regimens in the setting of consolidation or maintenance therapy, when longer courses of low-dose IL-2 might be used to further increase NK cell numbers before antibody infusion commences.

REFERENCES


A Phase I Trial of Humanized Monoclonal Antibody HuM195 (anti-CD33) with Low-Dose Interleukin 2 in Acute Myelogenous Leukemia


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