CONCISE REPORT

Use of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Autologous Marrow Transplantation for Lymphoid Malignancies

By John Nemunaitis, Jack W. Singer, C. Dean Buckner, Roger Hill, Rainer Storb, E. Donnall Thomas, and Frederick R. Appelbaum

The use of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) following autologous marrow transplantation for lymphoid malignancies was explored in a phase I/II dose escalation study. rhGM-CSF given as a 2-hour infusion daily for 14 days was well tolerated at doses up to 240 μg/m²/day. When compared with 86 disease-matched and treatment-matched historical controls, patients receiving ≥60 μg/m²/day rhGM-CSF recovered neutrophil and platelet counts more rapidly, had fewer days with fever, and were discharged from the hospital sooner.

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G R ANULOCYTE-MACROPHAGE colony-stimulating factor (GM-CSF) is a regulatory glycoprotein necessary for the survival, proliferation, and maturation of myeloid cells. Recombinant DNA technology has allowed production of sufficient quantities of recombinant human GM-CSF (rhGM-CSF) to enable performance of clinical trials. Myeloabnormality is enhanced when rhGM-CSF is given to normal monkeys, and monkeys that receive rhGM-CSF following autologous marrow transplantation recover neutrophil counts more quickly. Initial studies in patients with AIDS-associated neutropenia also demonstrated stimulation of myeloabnormality with minimal toxicity in response to rhGM-CSF. This report describes a phase I/II dose escalation trial of rhGM-CSF in patients with lymphoid malignancies undergoing autologous marrow transplantation. rhGM-CSF (Immunex, Seattle) given as a 2-hour infusion once a day for 14 days was well tolerated at doses up to 240 μg/m²/day, and biologic activity was suggested by observation of accelerated engraftment.

MATERIALS AND METHODS

Patient selection. Patients with lymphoid malignancies, including acute lymphocytic leukemia, Hodgkin's disease, or non-Hodgkin's lymphoma, who were undergoing autologous or syngeneic marrow transplant after standard preparative regimens were eligible for study. Signed informed consent conforming to FDA and institutional review guidelines was required. No exclusions were made for disease state or stage, prior therapy, or Karnofsky performance scores.

Clinical monitoring. All patients were examined daily and had vital signs monitored at least every 6 hours. Complete blood cell counts with differentials, reticulocyte counts, chemistry, prothrombin times, platelet counts, and urine analyses were done daily just prior to and for a minimum of 3 weeks after marrow infusion. Weekly chest radiographs and electrocardiograms were also obtained during the week prior to transplant and weekly thereafter.

Study design. The study was a phase I/II dose escalation study in which patients were consecutively enrolled in groups of three starting at a dose of rhGM-CSF of 15 μg/m²/day [specific activity = 3 x 10⁵ colony-forming units/mg (CFU/mg)] for 14 days. Subsequent groups of three patients were entered, and the dose for each group was doubled, until either intolerable toxicity occurred or biologic activity was suggested. Biologic activity was defined as recovery of neutrophils to >500 cells/μL before day 14 in two of three patients at a given dose. This definition was based on our previous experience with 86 patients given autologous grafts for lymphoid malignancies, where only four patients (<5%) achieved this level of neutrophils by day 14. If no significant toxicity occurred at a dose with suggested biologic activity, we planned to investigate two more dose escalations before closing the study. rhGM-CSF was administered as a single 2-hour intravenous (IV) infusion starting within 1 hour of completion of the autologous marrow infusion (designated as day 0) and repeated daily for a total of 14 days.

Patients. Fifteen consecutive patients with lymphoid malignancy qualifying for autologous or syngeneic marrow transplant were studied. The mean age was 32 years (range 13 to 61 years). There were six females and nine males. Diagnosis, disease state, conditioning regimen, type of marrow transplant, and marrow purging are listed by patient in Table 1. All patients with a fever (T > 38°C) were treated uniformly according to Fred Hutchinson Cancer Research Center protocol with empiric antibiotic therapy and without granulocyte transfusions.

RESULTS

Hematologic responses. The days on which the patients achieved an absolute neutrophil count (ANC) of >500 cells/μL and platelet independence are shown in Table 1. No patient receiving rhGM-CSF at 15 or 30 μg/m²/day achieved an ANC >500 cells/μL before day 14, whereas five of eight evaluable patients receiving ≥60 μg/m²/day did so. Of the five patients who reached 500 neutrophils/μL by day 14, four temporarily decreased their ANC to <500/μL within 24 to 72 hours after stopping rhGM-CSF. The mean maximum reduction of ANC after stopping rhGM-CSF (dose ≥60 μg/m²/day) was 35% (4% to 63%). Table 2 compares the retrospective control group with study patients receiving ≤30 μg/m²/day and ≥60 μg/m²/day rhGM-CSF. Patients receiving ≥60 μg/m²/day showed earlier granulocyte and platelet recovery than the retrospective control group. The average number of days with a temperature ≥38.0°C and the number of platelet transfusions in the first
30 days after transplantation appeared to be least in patients receiving ≥60 µg/m²/day of rhGM-CSF. Two of 14 evaluable patients (UPN 3990 not evaluable) had incomplete engraftment. Both received ≤30 µg/m²/day of rhGM-CSF. One patient, (UPN 3810) now 100 days post-BMT. The other patient (UPN 3647) received a 4-µg/m² dose of rhGM-CSF were observed: Three patients had abdominal cramps, two of whom had symptoms associated only with the first dose. One patient had a constant low-grade temperature occasion before reaching an ANC of 500/tL. UPNs (3990 and 3945). No hepatic, pulmonary, neurologic, or renal toxicities occurred. However, one patient (UPN 3647) developed acute tubular necrosis with a transient rise in creatinine to 2.5 mg/dL believed to be caused by a rapid infusion of acyclovir. He was also receiving rhGM-CSF at the time. The rhGM-CSF was held for 2 days and was restarted without recurrence of renal function abnormalities.

Two of 15 (13%) patients had positive blood cultures before reaching an ANC of 500/µL. Both positive blood cultures occurred in the first week after transplant. Patient 3990 died with an ANC of 20 cells/µL on day 8 of Pseudomonas septicemia presumably secondary to an intestinal wall abscess found at autopsy. Thirty percent of the retrospective control group of patients had a positive bacterial/fungal blood culture with clinical evidence of septicaemia or the same organism isolated on more than one occasion before reaching an ANC of 500/µL.

**DISCUSSION**

rhGM-CSF was well tolerated at doses up to 240 µg/m² given by slow IV infusion in patients undergoing autologous marrow transplantation for lymphoid malignancy. Furthermore, five of eight patients surviving 14 days treated with rhGM-CSF at doses ≥60 µg/m²/day achieved an ANC of 500 cells/µL before day 14. Because only 5% of 86 patients

### Table 1. Day of Myeloid and Megakaryocytic Engraftment in rhGM-CSF-Treated Patients

<table>
<thead>
<tr>
<th>Unique Patient No.</th>
<th>Diagnosis/Disease State</th>
<th>Conditioning Regimen* TBI Dose (cGy)/days</th>
<th>Type of Marrow</th>
<th>Dose of rhGM-CSF (µg/m²)</th>
<th>Day After Marrow Infusion Patient Independent of Platelet Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>3769</td>
<td>NHL/remission</td>
<td>200 × 6</td>
<td>A None</td>
<td>15</td>
<td>18</td>
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<tr>
<td>3647</td>
<td>ALL/remission</td>
<td>200 × 6</td>
<td>A 4HC†</td>
<td>15</td>
<td>26</td>
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<tr>
<td>3810</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A Pan B‡</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>3806</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>3854</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>S None</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>3895</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>30</td>
<td>41</td>
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<tr>
<td>3919</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A Pan B</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>3918</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>3935</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A Pan B</td>
<td>60</td>
<td>12</td>
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<tr>
<td>3990‡</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>120</td>
<td>—</td>
</tr>
<tr>
<td>3922</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>120</td>
<td>18</td>
</tr>
<tr>
<td>3945</td>
<td>ALL/relapse</td>
<td>360 × 4</td>
<td>A None</td>
<td>120</td>
<td>13</td>
</tr>
<tr>
<td>3984</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>240</td>
<td>12</td>
</tr>
<tr>
<td>3992</td>
<td>NHL/relapse</td>
<td>200 × 6</td>
<td>A None</td>
<td>240</td>
<td>13</td>
</tr>
<tr>
<td>3908</td>
<td>ALL/relapse</td>
<td>0*</td>
<td>S None</td>
<td>240</td>
<td>18</td>
</tr>
</tbody>
</table>

NHL, non-Hodgkins lymphoma; ALL, acute lymphocytic leukemia; A, autologous; S, syngeneic.

*All conditioning regimens included cyclophosphamide (60 mg/kg/day × 2 days). TBI, total body irradiation delivered by dual opposing cobalt 60 source. Patient 3945 received TBI by linear accelerator. Patient 3908 received Busulfan (4 mg/kg/day × 4 days) and no TBI.

†Autologous marrow was treated with 100 µg/mL 4-hydroperoxycyclophosphamide.

‡Marrow treated in vitro with a pan-B cell antibody plus complement.

§Death on day 8.

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### Table 2. Mean Values of Study Parameters

<table>
<thead>
<tr>
<th>rhGM-CSF Dose (µg/m²/day)</th>
<th>No. of Patients</th>
<th>Day of AGN &gt; 100*</th>
<th>Day of AGN &gt; 500*</th>
<th>Day of AGN &gt; 1,000*</th>
<th>Day Platelet Transfusion Independent*</th>
<th>Day of Discharge* (T &gt; 38°C)</th>
<th>No. of Fabreg Days (T &gt; 38°C)</th>
<th>No. of Platelets Units Transfused From day 0 to day 301*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>86</td>
<td>16 × (6-8.41)</td>
<td>26 × (10.10-60)</td>
<td>29 × (17-64)</td>
<td>38 × (13-118)</td>
<td>41 × (18-129)</td>
<td>12 × (7-30)</td>
<td>84 × (122-170)</td>
</tr>
<tr>
<td>≤30</td>
<td>6</td>
<td>13 × (5-8.22)</td>
<td>22 × (11-41)</td>
<td>30 × (12-53)</td>
<td>30 × (22-41)</td>
<td>30 × (6-22)</td>
<td>11 × (6-20)</td>
<td>78 × (42-154)</td>
</tr>
<tr>
<td>≥60†</td>
<td>9</td>
<td>10 × (2-13)</td>
<td>14 × (2-18)</td>
<td>22 × (4-22)</td>
<td>29 × (4-16)</td>
<td>29 × (18-51)</td>
<td>6 × (2-11)</td>
<td>67 × (16-90)</td>
</tr>
</tbody>
</table>

*Mean ± SD (range).

†Retrospective control group of comparably matched patients with lymphoid malignancy who underwent autologous BMT at FHCRC prior to the start of this study.

‡Patient 3990 who died on day 8 was unevaluable and is not included.
treated in a similar fashion but without rhGM-CSF reached this level before day 14, biologic activity at these doses was strongly suggested. However, a prospective placebo-controlled trial will be necessary to prove this.

rhGM-CSF may also have had an influence on platelet recovery. However, we cannot determine whether this was a direct or indirect effect. In vitro studies have suggested mild stimulation of megakaryocyte proliferation with GM-CSF which could be significantly augmented when combined with other growth factors.8,9 Other researchers have reported shortened platelet recovery times after autologous BMT in monkeys treated with rhGM-CSF.4 In addition, two of eight patients with myelodysplasia became platelet transfusion independent after treatment with rhGM-CSF.10 However, in other patients with normal baseline platelet counts, GM-CSF had no effect on platelet counts.3 Erythroid stimulation has also been observed in vitro and in animals with increased erythropoietin levels.7,11 No clear effect of rhGM-CSF on erythropoiesis was observed in our study.

Toxicity with rhGM-CSF was mild and predominantly limited to muscle cramps and bone pain. The mechanisms of toxicity are unknown. GM-CSF may induce prostaglandin E production by macrophages.12

Our study raised three concerns. First, a temporary reduction in ANC to <500 cells/μL occurred in four patients within 1 to 3 days after the last dose of rhGM-CSF. This observation is consistent with previous studies showing the short stimulatory effect of rhGM-CSF. Second, not all patients had sustained hematopoietic recovery. This raises the further concern that rhGM-CSF may cause differentiation of early stem cells and interfere with the self-renewal required for long-term reconstitution. Metcalf and colleagues reported data in mice showing reductions in CFU-GM and bone marrow cellularity after mice were given daily intraperitoneal GM-CSF.13 Third, the effect of rhGM-CSF on tumor cell regrowth is unknown. Patients with myeloid leukemia were excluded from this study because myeloid tumors have rhGM-CSF receptors on their cell surface and the effects of exposure to rhGM-CSF on possible surviving tumor cells after transplant have not been determined. Because most lymphoid malignancies are not believed to express rhGM-CSF receptors, we considered it reasonable to explore the molecule in this setting. Nonetheless, some lymphoid tumor cells may express rhGM-CSF receptors or rhGM-CSF may influence tumor growth indirectly.

This study evaluated the short-term effects of rhGM-CSF after autologous BMT for lymphoid malignancy. rhGM-CSF was well tolerated and appeared to have biologic activity when compared with a retrospective control group at doses ≥60 mg/m2/day given as a 2-hour daily infusion. We cannot conclude by this trial whether a particular cytoreduction regimen, marrow-purging technique, or underlying disease affects the responsiveness to rhGM-CSF. Larger prospective randomized phase III trials are needed to define more precisely the possible therapeutic and economic advantages offered by this molecule and to uncover any serious untoward effects.

REFERENCES

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