Phase II Trial of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Patients Undergoing Allogeneic Bone Marrow Transplantation From Unrelated Donors

By John Nemunaitis, Claudio Anasetti, Rainer Storb, James A. Bianco, C. Dean Buckner, Nichole Onetto, Paul Martin, Jean Sanders, Keith Sullivan, Motomi Mori, Kathleen Shannon-Dorcy, Raleigh Bowden, Frederick R. Appelbaum, John Hansen, and Jack W. Singer

The safety and possible efficacy of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) were evaluated in 40 consecutive patients who received transplants from unrelated donors. rhGM-CSF was administered by 2-hour daily intravenous infusion from day 0 to day 20 or day 27 after the marrow infusion. These patients were compared with 78 historical patients who received transplants from unrelated donors who did not receive rhGM-CSF. The rhGM-CSF-treated patients were older (P = .037) and were treated less frequently in laminar air flow rooms (P = .006) than were control patients. However, the rhGM-CSF-treated group had a higher proportion of “good risk” patients with chronic myelogenous leukemia in chronic phase (P = .006) than did the comparison group (P = .017), rendering comparisons of transplant-related complications not meaningful. rhGM-CSF was well tolerated and did not adversely increase the incidence of graft rejection or increase the incidence and severity of acute graft-versus-host disease. The median day the absolute neutrophil count reached 500/mm³ in patients who received rhGM-CSF was day 21, which was not different from that of historical patients. Nevertheless, the numbers of febrile days and septicemic episodes within the first 28 days in patients who received rhGM-CSF were less than in historical patients. The probability of nonrelapse mortality at 1 year in patients who received rhGM-CSF was 22%. In view of the retrospective nature of the control group, we cannot conclusively determine whether rhGM-CSF administration was beneficial. A prospective, randomized controlled study of rhGM-CSF is required to confirm these suggestive data.

© 1992 by The American Society of Hematology.

A LLOGENEIC bone marrow transplantation (BMT) from genotypically HLA-identical siblings is curative therapy for selected patients with hematologic neoplasia or marrow dysfunction.¹ However, an HLA-identical sibling is not available for over 60% of the patients who might benefit from BMT.² Marrow transplantation from HLA-identical, unrelated marrow donors has been successful but has been associated with a higher morbidity and mortality than HLA-identical sibling donor transplants because of an increased incidence of severe graft-versus-host disease (GVHD) and infections.³⁷

Clinical trials in patients undergoing autologous BMT suggest that infection during the first month after marrow infusion is reduced in patients who receive recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF).⁸¹¹ Also, patients who received rhGM-CSF for marrow graft failure appeared to have fewer infections and better survival than in historical patients.¹² A decrease in the incidence of early infections was also observed in recipients of genotypically HLA-identical marrow allografts when results were compared with historical patients.¹³ There was no increase in the incidence or severity of GVHD or of graft rejection compared with historical cases. The probability of relapse in patients with myeloid malignancies was not altered with rhGM-CSF.¹²¹³ Because of the important role that infections play in the outcome of marrow grafts from unrelated donors, the present phase II trial of rhGM-CSF was initiated to determine whether rhGM-CSF might decrease transplantation-associated morbidity.

MATERIALS AND METHODS

Patient selection. Patients with hematologic neoplasia or severe aplastic anemia who were undergoing allogeneic BMT from phenotypically HLA-matched or one HLA antigen-mismatched unrelated donors at the Fred Hutchinson Cancer Research Center (FHCRC) or Veterans Administration Medical Center (VAMC) from November 14, 1989 to October 25, 1990 were eligible for this study. Signed, informed consent conforming to Federal Drug Administration and Institutional Review Guidelines was required.

Characteristics of the study patients are shown in Table 1.

To aid in the interpretation of patients treated with rhGM-CSF, the results were compared with those in 78 consecutive historical patients who received transplants from unrelated donors from May 1, 1985 to February 24, 1989.⁷

The characteristics of patients who received rhGM-CSF differed from historical patients. rhGM-CSF-treated patients were older, less often treated in laminar air flow rooms, and a higher proportion had chronic myelogenous leukemia in chronic phase (CML-CP) and received lower medicine doses of total body irradiation (TBI) (Table 1).

Donor selection of historical and study patients. Histo compatibility testing for all patients and donors was performed in the FHCRC laboratory. Typing for HLA-A and -B antigens was performed according to the standard National Institutes of Health (NIH) two-stage microcytotoxicity assay.¹⁴ Antigens were assigned according to the standard World Health Organization (WHO) nomenclature. HLA-DR typing was performed using nylon-wool–purified B
cells in a modified microcytotoxicity assay.\textsuperscript{15} Antidonor lymphocytoxic antibodies were detected either by an antiglobulin microcytotoxicity crossmatch assay or by use of indirect immunofluorescence and microfluorometry. The mixed lymphocyte reaction was performed according to standard methods with reactivity expressed as a relative response.\textsuperscript{16} HLA-D region compatibility was defined by testing with HLA-D homozygous typing cells (HTC) in a standard HLA-D typing assay.\textsuperscript{17} In some cases, HLA-D specificities were assigned according to the results of informative DNA restriction fragment length polymorphism detected with DRB, DQB, and DQA probes. Donor compatibility with a recipient antigen (ie, HLA-DR4, DW4 and HLA-DR4, DW14).

Supportive care. To prevent pneumocystis carinii infection, trimethoprim-sulfamethoxazole was administered prophylactically. On day -2, restarted 2 days per week posttransplant after achieving an absolute neutrophil count (ANC) greater than 500/mm\textsuperscript{3} and administered through day 120. To prevent acquisition of primary cytomegalovirus infection, all seronegative patients who had seronegative marrow donors received red cell and platelet transfusions exclusively from seronegative blood donors. Seropositive patients did not receive screened blood. To prevent endogenous reactivation of cytomegalovirus, seropositive patients received prophylactic intravenous (IV) acyclovir (500 mg/m\textsuperscript{2} every 8 hours) from day -5 to day 30. All patients were routinely transfused to maintain hematocrits above 30% and platelet counts above 20,000/mm\textsuperscript{3} and all received prophylactic broad spectrum IV antibiotics when the ANC decreased to below 500/mm\textsuperscript{3}. Patients were monitored according to standard patient care protocols of the FHCRC and VAMC. Physical examinations, complete blood cell counts, and blood chemistries were performed at least once per day. Vital signs were monitored every 4 hours. Liver function tests and additional serum chemistries were obtained thrice weekly. Assessment of acute GVHD was performed daily and the peak grade of acute GVHD was assigned according to previously published criteria.\textsuperscript{18,19}

Definition of infection. Patients were considered to be bacteremic if at least one positive blood culture for bacteria other than coagulase-negative staphylococcus was obtained in a febrile patient. For coagulase-negative staphylococcus, two positive blood cultures were required. Fungemia was defined as at least one positive blood culture in a febrile patient. Patients who developed bacteremia, candidemia, or who had biopsy-confirmed histologic or culture evidence of bacterial or fungal organisms from closed body organs were considered to be infected.

Transplant procedure. Both historical and rhGM-CSF-treated patients received a preparative regimen consisting of cyclophosphamide (60 mg/kg/d × 2 days) followed by TBI (Table 1).\textsuperscript{1-5,7} Patients with CML-CP or aplastic anemia received 1,200 cGy of TBI in 6 daily fractions whereas patients with other malignancies received TBI as 120 cGy TID for 11 (over age 17) or 12 (under age 17) fractions. GVHD prophylaxis consistent of methotrexate was administered at a dose of 15 mg/m\textsuperscript{2} IV on day 1, then 10 mg/m\textsuperscript{2} IV on days 3, 6, and 11 after marrow infusion, and cyclosporine started on day -1 at a dose of 1.5 mg/kg every 12 hours. When patients could tolerate oral drug, cyclosporine was administered at a dose of 6.25 mg/kg every 12 hours until day 50. If the serum creatinine doubled, the dose of cyclosporine was reduced by 50%; cyclosporine was not administered if the serum creatinine exceeded 2 mg/dL. Patients who did not receive methotrexate received cyclosporine at a similar dose and schedule as described above. They also received pretransplant prophylaxis at a dose of 0.25 mg/kg IV twice a day from day 7 to 14, 0.5 mg/kg IV twice a day from day 15 to 28 and 0.25 mg/kg twice a day from day 29 to 42.

Study design. All patients received rhGM-CSF (specific activity, 5 × 10\textsuperscript{6} colony-forming units/mg; yeast-derived product, supplied by Immunex Corp, Seattle, WA) at a dose of 250 μg/m\textsuperscript{2} by daily 2-hour IV infusion beginning within 2 hours of completion of the marrow infusion (day 0) until either day 20 (n = 11) or 27 (n = 29) after marrow infusion.

Statistical analysis. Demographic and clinical characteristics were compared between the study patients and historical controls using the two-tailed Fisher's exact test for categorical variables (eg, sex and disease stage) and the Wilcoxon rank-sum test\textsuperscript{20} for continuous variables (eg, age and marrow cell dose). Times to achieve an ANC greater than 100/mm\textsuperscript{3}, greater than 500/mm\textsuperscript{3}, greater than 1,000/mm\textsuperscript{3}, and platelet independence were estimated by the Kaplan-Meier method\textsuperscript{21} and compared between the study and historical groups by the Wilcoxon test.\textsuperscript{22} Patients who died before achieving these levels were censored at the day of death. The number of days an ANC was less than 100/mm\textsuperscript{3} and the number of units of platelets transfused in the first 28 days were...
compared between the two groups by the Wilcoxon rank-sum test. Proportions of the patients who developed septicemia in the first 28 days were compared between the study and historical groups using Fisher's exact test, and the number of febrile days, peak serum bilirubin and creatinine between 0 and 28 days were compared using the Wilcoxon rank-sum test. The cumulative incidence of acute GVHD was estimated\(^2\) and compared between the two groups.\(^3\) Survival, nonrelapse mortality, and relapse were estimated by the Kaplan-Meier method and were compared between the two groups by the Wilcoxon test.

RESULTS

Toxicity. rhGM-CSF was well tolerated. Five patients (13%) did not receive all scheduled doses of rhGM-CSF. One patient had rhGM-CSF discontinued because of severe chest pain and a pericardial effusion which regressed who received rhGM-CSF. Three patients (8%) developed mild myalgias in association with rhGM-CSF infusions requiring additional narcotic administration. No patients developed fever and chills or other constitutional symptoms attributed to rhGM-CSF.

Hepatic and renal toxicity in patients who received rhGM-CSF was attributed to transplant-associated toxicity. The median peak bilirubin between day 0 and 28 in rhGM-CSF-treated patients was 2.7 mg/dL. Eight of the rhGM-CSF-treated patients (20%) had bilirubin elevations over 10 mg/dL. The median peak creatinine in the rhGM-CSF patients was 1.1 mg/dL.

Hematologic recovery. Parameters of hematologic recovery are presented in Table 2. Neutrophil recovery in patients who received rhGM-CSF was similar to that in historical patients. The first 11 patients received 21 doses of rhGM-CSF from day 0 to 20. Because the ANC had not reached 500/mm\(^3\) in most of the 11 patients before discontinuation of rhGM-CSF, the 29 subsequent patients received 28 doses of rhGM-CSF. The median time to reach an ANC greater than 500/mm\(^3\) was similar in patients who received 21 doses of rhGM-CSF and those who received 28 doses. However, the median time to reach an ANC greater than 1,000/mm\(^3\) was day 23 in patients who received 28 doses of rhGM-CSF compared with day 29 in those who received 21 doses of rhGM-CSF (\(P = .020\)). Two patients (5%) never attained an ANC of 1,000/mm\(^3\) before death. Two patients had a temporary decrease in ANC to less than 500/mm\(^3\) after initially achieving an ANC greater than 500/mm\(^3\). Both were administered a second course of rhGM-CSF; one again achieved an ANC greater than 500/mm\(^3\) and the other did not. Twelve of the historical patients (15%) died before reaching an ANC greater than 1,000/mm\(^3\).

The median day of independence from platelet transfusions and the number of units of platelets administered to each patient over the first 28 days after BMT are shown in Table 2. Patients who received rhGM-CSF required fewer total platelet transfusions than historical patients, although the day of platelet transfusion independence was not significantly different. Seven of the rhGM-CSF-treated patients (18%) did not become independent from platelet transfusions before day 100. Three died before day 25, one relapsed on day 43, and three require platelet transfusions more than 100 days after BMT. Forty-one percent of historical patients failed to achieve independence from platelet transfusions before death or before day 100.

Fever and infection. Patients who received rhGM-CSF had a median of 4 febrile days (temperature \(\geq 38.5^\circ\text{C}\)) within the first 28 days. Three rhGM-CSF-treated patients (8%) developed bacteremia or fungemia during the first 28 days after BMT. Two patients (5%) who had candidemia died before day 30, while one patient with staphylococcus aureus bacteremia from an infected Hickman catheter responded to antibiotics and catheter removal. One GM-CSF-treated patient had a progressive aspergillus nodule before BMT that continued to expand in size during and after BMT.

GVHD. Eighty-two percent of rhGM-CSF-treated patients received all four projected doses of methotrexate. Ninety-three percent of the rhGM-CSF-treated patients received at least three doses of methotrexate. The median amount of IV cyclosporine administered to the rhGM-CSF patients was 3.0 mg/kg/d through day 28. The overall incidence of grade II to IV acute GVHD was 88% (Table 3). The incidence of grade III or IV acute GVHD in the rhGM-CSF–treated patients was 25% (Fig 1).

Relapse. Two patients transplanted for acute lymphocytic leukemia (ALL) in relapse and one transplanted for CML in blast crisis relapsed within 100 days after BMT. rhGM-CSF was discontinued in one patient with persistent leukemia proven by cytogenetic analysis 22 days after BMT. Eleven percent (95%, confidence interval [CI]: 1% to 21%) of patients who received rhGM-CSF relapsed after day 100 (\(P = .580\)).

Table 2. Hematologic Recovery (median values)

<table>
<thead>
<tr>
<th>GM-CSF Schedule</th>
<th>No. of Patients</th>
<th>No. of Days ANC &lt; 100/mm(^3)</th>
<th>Day ANC &gt; 100/mm(^3)</th>
<th>Day ANC &gt; 500/mm(^3)</th>
<th>Day ANC &gt; 1,000/mm(^3)</th>
<th>Day Platelet Independent</th>
<th>Units of Platelets (day 0-28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 doses</td>
<td>11</td>
<td>12</td>
<td>19</td>
<td>24</td>
<td>29*</td>
<td>29</td>
<td>80</td>
</tr>
<tr>
<td>28 doses</td>
<td>29</td>
<td>12</td>
<td>17</td>
<td>21</td>
<td>22*</td>
<td>26</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>12</td>
<td>18</td>
<td>21</td>
<td>25</td>
<td>26</td>
<td>90‡</td>
</tr>
<tr>
<td>Historical group</td>
<td>78</td>
<td>11</td>
<td>16</td>
<td>21</td>
<td>24</td>
<td>31</td>
<td>118‡</td>
</tr>
</tbody>
</table>

*The only category achieving statistical significance between patients receiving 21 or 28 doses of rhGM-CSF was in day ANC > 1,000/mm\(^3\) (\(P = .02\), Wilcoxon test).

†Comparison between historical patients and all patients who received rhGM-CSF, \(P = .0066\) (Wilcoxon rank sum test).
Table 3. Number of Patients Who Developed Acute GVHD

<table>
<thead>
<tr>
<th>Severity of GVHD</th>
<th>No. of GM-CSF Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0-1</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>25 (62)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>9 (23)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Survival.** Five patients (13%) who received rhGM-CSF died before day 100. Two patients died of venoocclusive disease of the liver and one each died of relapse, infection, and multiorgan failure. The probability of nonrelapse mortality at day 100 in patients receiving rhGM-CSF was 13% (95% CI: 2% to 24%) (Fig 2). At 1 year, the overall mortality was 22% (95% CI: 8% to 36%) for the rhGM-CSF-treated patients. All 19 patients with CML-CP survived to day 100. In a historical series of 17 patients transplanted with CML-CP from unrelated donors, four died before day 100 ($P = .04$, Fisher's exact test) (Table 4).

**DISCUSSION**

The purpose of this study was to test the safety and possible efficacy of rhGM-CSF when administered to patients after BMT from unrelated donors. One concern with the use of rhGM-CSF in unrelated donor grafts was that it might increase the risk of marrow graft rejection. Although survival was improved, mice administered recombinant murine GM-CSF after allogeneic T-cell-depleted BMT had an increase in the frequency of autologous recovery, suggesting that GM-CSF might increase immunologic graft rejection. However, none of the 40 patients given rhGM-CSF in this series developed graft rejection.

A second concern was that rhGM-CSF might increase the severity of acute GVHD. rhGM-CSF induces production of interleukin-1 (IL-1) and tumor necrosis factor, which may amplify the T-cell response to alloantigens and increase the severity of GVHD. However, rhGM-CSF also downregulates IL-2 receptor expression on monocytes and increases serum levels of soluble IL-2 receptor and soluble CD8 in patients. These factors may moderate the graft-versus-host reaction. In the present study, the overall incidence of acute GVHD was similar to that of historical patients. Importantly, the incidence of grade III to IV GVHD in rhGM-CSF-treated patients was lower than in historical patients. GVHD of this severity is highly correlated with poor survival.

A hypothesis that may explain these observations is that suppression of endotoxemia may suppress GVHD. Patients undergoing BMT in protected environments with gut decontamination have a lower incidence of infection, and reduced severity and delayed onset of acute GVHD. rhGM-CSF may decrease endotoxemia in neutropenic patients through activation of residual phagocytic cells. The decrease in the number of febrile days provides suggestive evidence for this. Because endotoxin is a major stimulus for the modulation of GVHD through induction of inflammatory cytokines, suppression of endotoxemia may decrease its severity. However, because comparison of patients who received rhGM-CSF was made to a historical control group which differed in age, dose of TBI, and disease state, the effect of rhGM-CSF on the severity of acute GVHD cannot conclusively be determined from these data. Randomized, prospective studies are required.

Patients who receive rhGM-CSF after autologous BMT have fewer infections than placebo-treated patients. The reduction in infection could not be attributed to earlier neutrophil recovery, because in this trial, most infections

---

**Fig 1.** Cumulative incidence of grade III to IV acute GVHD in patients who received rhGM-CSF.

**Fig 2.** A Kaplan-Meyer estimate of nonrelapse mortality of 40 patients who received rhGM-CSF.
occurred when the ANC was less than 100/mm³ and the number of days that the ANCs were at this level were fewer in the present trial, as in the autologous BMT study in allogeneic BMT patients who received HLA-identical marrow from family donors. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) had little effect on neutrophil recovery in patients who received methotrexate for GVHD prophylaxis. When rhGM-CSF is administered to neutropenic mice before induction of bacterial or fungal sepsis, residual myeloid cell functions are enhanced and survival is improved. It is likely that rhGM-CSF contributed to the low incidence of infection during the neutropenic period in the present trial, as in the autologous BMT trial, by enhancing function of residual phagocytic cells. Other cytokines such as rhM-CSF may also protect against infection in neutropenic hosts, possibly by functionally activating radioresistant monocytes and tissue macrophages.

From the present data, we conclude that rhGM-CSF at a dose of 250 μg/m²/d in patients undergoing BMT from unrelated donors is well tolerated. rhGM-CSF did not accelerate engraftment, but the incidence of severe infections and nonrelapse mortality may have been lower than in historical cases. Although the overall incidence of GVHD was not affected, there was a trend toward a decrease in the occurrence of life-threatening GVHD. Because these conclusions may be erroneous because historical patients were used for comparison, these data will require a randomized, placebo-controlled trial for confirmation.

ACKNOWLEDGMENT

We give special acknowledgment to Pam Jones for data collection assistance, to Wally Meyer and Carol Crittendon for assistance in computer analysis, and to the attending staff, nurses, physician assistants, and fellows of the Veterans Affairs Medical Center Marrow Transplant Program and of the Fred Hutchinson Cancer Research Center for assisting with the care of patients on this study.

REFERENCES


34. van Bekkum DW, Knaan S: Brief communication: Role of bacterial microflora in development of intestinal lesions from graft-versus-host reaction. J Natl Cancer Inst 58:787, 1977


Phase II trial of recombinant human granulocyte-macrophage colony-stimulating factor in patients undergoing allogeneic bone marrow transplantation from unrelated donors

J Nemunaitis, C Anasetti, R Storb, JA Bianco, CD Buckner, N Onetto, P Martin, J Sanders, K Sullivan and M Mori