Hematopoietic Growth Factors for Graft Failure After Bone Marrow Transplantation: A Randomized Trial of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) Versus Sequential GM-CSF Plus Granulocyte-CSF

By Daniel J. Weisdorf, Catherine M. Verfaillie, Stella M. Davies, Alexandra H. Filipovich, John E. Wagner Jr, Jeffrey S. Miller, Jory Burroughs, Norma K.C. Ramsay, John H. Kersey, Philip B. McGlave, and Bruce R. Blazar

Delay in hematologic recovery after bone marrow transplantation (BMT) can extend and amplify the risks of infection and hemorrhage, compromise patients' survival, and increase the duration and cost of hospitalization. Because current studies suggest that granulocyte-macrophage (GM) colony-stimulating factor (CSF) may potenti ate the sensitivity of hematopoietic progenitor cells to G-CSF, we performed a prospective, randomized trial comparing GM-CSF (250 µg/m^2/d × 14 days) versus sequential GM-CSF × 7 days followed by G-CSF (5 µg/kg/d × 7 days) as treatment for primary or secondary graft failure after BMT. Eligibility criteria included failure to achieve a white blood cell (WBC) count ≥100/µL by day +21 or ≥300/µL by day +28, no absolute neutrophil count (ANC) ≥200/µL by day +28, or secondary sustained neutropenia after initial engraftment. Forty-seven patients were enrolled: 23 received GM-CSF (10 unrelated, 8 related allogeneic, and 5 autologous), and 24 received GM-CSF followed by G-CSF (12 unrelated, 7 related allogeneic, and 5 autologous). For patients receiving GM-CSF alone, neutrophil recovery (ANC ≥500/µL) occurred between 2 and 61 days (median, 8 days) after therapy, while those receiving GM-CSF + G-CSF recovered at a similar rate of 1 to 36 days (median, 6 days; P = .39). Recovery to red blood cell (RBC) transfusion independence was slow, occurring 6 to 250 days (median, 35 days) after enrollment with no significant difference between the two treatment groups (GM-CSF: median, 30 days; GM-CSF + G-CSF: median, 42 days; P = .24).

Similarly, platelet transfusion independence was delayed until 4 to 249 days (median, 32 days) after enrollment, with no difference between the two treatment groups (GM-CSF: median, 28 days; GM-CSF + G-CSF: median, 42 days; P = .38). Recovery times were not different between patients with unrelated donors and those with related donors or autologous transplant recipients. Survival at 100 days after enrollment was superior after treatment with GM-CSF alone. Only 1 of 23 patients treated with GM-CSF died versus 7 of 24 treated with GM-CSF + G-CSF who died between days 16 to 84 days (median, 38 days) after enrollment. Kaplan-Meier 100-day survival estimates of 96% ± 8% for GM-CSF versus 71% ± 18% for GM-CSF + G-CSF (P = .026). These data suggest that sequential growth factor therapy with GM-CSF followed by G-CSF offers no advantage over GM-CSF alone in accelerating trilineage hematopoiesis or preventing lethal complications in patients with poor graft function after BMT. GM-CSF should still be considered the standard for graft failure against which other, newer growth factors, sequential treatments, or combination therapies are tested.

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IN THE EARLY pancytopenic period after bone marrow transplantation (BMT), infection, and hemorrhage are the major morbid and life-threatening complications observed. Any delay in hematopoietic recovery can extend and exaggerate this period of risk. Poor graft function or secondary graft failure leading to secondary neutropenia may also increase these hazards and compromise patient survival. Delayed hemato logic recovery is a primary factor in extending hospitalization and markedly increasing the cost of BMT therapy. The availability of granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) as licensed products for use in stimulating hematopoietic function has allowed investigation of their effectiveness in promoting engraftment after BMT. Only limited data are available assessing their value in accelerating recovery and improving survival for patients with delayed or poorly functioning grafts after transplantation.

Preclinical data suggest that early-acting growth factors such as GM-CSF, interleukin (IL)-3, or stem cell factor might increase the responsiveness of committed hematopoietic progenitors to later-acting factors such as G-CSF or erythropoietin. To evaluate the clinical value of this type of sequential growth factor therapy, we performed a formal, prospective, randomized, comparative trial of GM-CSF alone versus GM-CSF followed by G-CSF for stimulating hematologic recovery in patients with primary or secondary graft failure after BMT. This report describes the hematologic and clinical outcomes of this comparative trial.

PATIENTS AND METHODS

All patients with graft failure after BMT at the University of Minnesota (Minneapolis, MN) were eligible for this trial. Patients had received either autologous bone marrow or peripheral blood stem cells or were allogeneic transplant recipients (from either related or unrelated donors). Patients were eligible for enrollment if they met the following definitions of graft failure: (1) failure to achieve a leukocyte count of ≥100/µL by day +21 after transplantation, (2) failure to achieve a leukocyte count ≥500/µL or an absolute neutrophil count (ANC) ≥200/µL by day +28; or (3) failure to maintain a mean ANC ≥500/µL for 7 days after having previously achieved an ANC ≥500/µL at any time beyond day +28 (secondary neutropenia). Patients receiving recombinant cytokines under different study protocols at the University of Minnesota (including a trial of...
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Recombinant IL-1α23 and a trial of recombinant IL-224 were excluded until day +28, after which time they could be enrolled if still eligible because of graft failure. Patients undergoing allogeneic BMT for severe combined immunodeficiency (SCID) or familial erythrophagocytic lymphohistiocytosis (FEL), those receiving granulocyte transfusions, and those undergoing autologous BMT for acute non-lymphocytic leukemia (ANLL) and treated under Children’s Cancer Group studies that specifically excluded cytokine use were ineligible. During the study period, seven eligible patients were not enrolled due to patient refusal (n = 3), attending physician preference (n = 3), and prior adverse reaction to GM-CSF (n = 1).

Eligible, consenting patients were assigned to receive either GM-CSF (250 µg/m²/d over 2 hours intravenously × 14 days) or GM-CSF (250 µg/m²/d over 2 hours intravenously × 7 days) followed immediately by G-CSF (5 µg/kg/d intravenously over 15 minutes × 7 days). This prospective, randomized enrollment was stratified into groups of either unrelated donor marrow recipients or other recipients that included recipients of either related donor allogeneic or autologous transplantation. All patients except one were enrolled for treatment for primary graft failure.

The primary endpoint for response to cytokine therapy was the development of a sustained ANC ≥500/µL for 3 consecutive days. Secondary endpoints included recovery of red cells and platelets to transfusion-independence, adverse reactions to cytokine infusions, and 100-day survival. Patients not responding to the first 14 days of therapy were eligible for retreatment, as previously assigned, for a second full course. If graft failure was refractory to treatment, some patients subsequently received reinfusion of donor or back-up autologous marrow.25 In addition to these retransplanted patients, five others (three in the GM-CSF arm; two in the GM-CSF + G-CSF arm) continued their assigned growth factors beyond 14 days.

The trial was performed using the commercially available products (GM-CSF: Immunix Corp, Seattle, WA; and G-CSF: Amgen, Thousand Oaks, CA) with no external funding from the manufacturers. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board: Human Subjects Committee, and all patients (or their parents) gave written informed consent.

Statistical analysis. Times to hematologic recovery and survival were evaluated by Kaplan-Meier calculations, and differences between groups were compared using the Mantel-Cox test statistic. Short-term survival was evaluated censoring all patients at 100 days after enrollment. Comparisons of patient characteristics between the two study groups were performed using a x² statistic. All analyses were performed by intention-to-treat according to the randomized assignment.

RESULTS

Of 472 BMT procedures performed at the University of Minnesota between January 1992 and March 1994, 47 patients met the criteria for entry, consented, and were enrolled in this randomized trial. Their clinical characteristics are shown in Table 1. The groups included 15 related-donor allogeneic transplant recipients (of 167 transplanted within the same time interval); 22 unrelated-donor recipients (of 131 transplanted), and 10 autologous recipients (of 174 transplanted). Of the 47 patients, 23 received GM-CSF for 14 days, and 24 received GM-CSF for 7 days followed by G-CSF for 7 days. Forty (85%) recovered (ANC ≥500/µL) between 1 and 61 days after initiation of cytokine therapy (median, 8 days). Seven patients were censored from analysis at the time of reinfusion of supplemental marrow (three recipients of allogeneic related donor and four unrelated donor marrow).

There were similar rates of neutrophil recovery in those treated with GM-CSF alone and those treated with GM-CSF + G-CSF (P = .39; Table 2). The neutrophil recovery times were similar in the two patient strata: those receiving unrelated donor BMT (median, 8 days; range, 1 to 61 days) and those receiving related donor or autologous BMT (median, 8 days; range, 2 to 36 days; P = .78). After 14 days, 75% ± 18% (95% confidence interval) of those treated with GM-CSF alone achieved neutrophil recovery compared with 79% ± 16% of those receiving GM-CSF + G-CSF (P > .5). Recovery to red cell transfusion-independence was delayed but occurred between 6 and 250 days after enrollment (median, 35 days). There was no difference between the two treatment arms (P = .24). Recovery to platelet transfusion-independence was also delayed and occurred between 4 and 249 days after enrollment (median, 35 days), but the two treatment arms were similarly effective (P = .38; Fig 1).

Survival. We next evaluated survival over the first 100 days after enrollment. We observed a statistically significant advantage (P = .026) favoring GM alone versus GM-CSF + G-CSF. Only 1 of 23 GM-CSF patients died (at day +34) after enrollment versus 7 of 24 GM-CSF + G-CSF patients who died between days 16 and 84 (median, 38 days) after enrollment (Fig 2). The primary and secondary causes of death in all eight patients who died were directly related to graft failure, including hemorrhage and infection in six of eight patients (Table 3). Of the patients who died, the one receiving GM-CSF alone had undergone an allogeneic related-donor transplant, while the seven GM-CSF + G-CSF deaths occurred in one of five autologous and two of seven related-donor allogeneic transplants and 4 of 12 unrelated donor transplants. Deaths attributable to infection occurred 20 to 84 days after study entry and cannot be ascribed to differences in active infections at study entry.

Adverse reactions and compliance with therapy. For all 47 patients enrolled on the trial, we assessed completeness of therapy and adverse reactions to treatment. Because treatment on both study arms was the same for the first 7 days of therapy and the therapeutic interventions differed only between days 8 and 14 of treatment, we evaluated the 2 weeks separately. Overall, 43 of 47 patients received at least

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>GM-CSF</th>
<th>GM-CSF × 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemia</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Other malignancy*</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* Includes non-Hodgkin’s lymphoma (n = 6), myelodysplastic syndromes (n = 3), and neuroblastoma (n = 1).
† Includes aplastic anemia (n = 4) and hypereosinophilic syndrome (n = 1).

Table 1. Patient Characteristics
Table 2. Hematology Recovery After Cytokine Therapy for Graft Failure

<table>
<thead>
<tr>
<th></th>
<th>Median Time to Recovery; Range (d)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>GM-CSF × 14 days + G-CSF (N = 24)</td>
<td></td>
</tr>
<tr>
<td>ANC ≥ 500/μL</td>
<td>8; 2-61</td>
<td>.39</td>
</tr>
<tr>
<td>RBC transfusion-independence</td>
<td>30; 6-124</td>
<td>.24</td>
</tr>
<tr>
<td>(first of 30+ d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet transfusion-independ</td>
<td>28; 6-127</td>
<td>.38</td>
</tr>
<tr>
<td>ence (first of 15+ d)</td>
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</tbody>
</table>

P values represent Mantel-Cox tests of significance between the two groups.

five of the seven planned doses of GM-CSF during the first treatment week, 19 of 23 received at least five of the seven randomly assigned GM-CSF doses, and 20 of the 24 received at least five of the planned seven G-CSF doses. Of those 10 patients who received less than the scheduled therapy, eight were stopped according to protocol because neutrophil recovery made cytokine therapy no longer necessary. No significant adverse reactions (e.g., fevers, rash, serositis, bone pain) led to discontinuation of either GM-CSF or G-CSF on either treatment arm. Graft-versus-host disease (GVHD) was similarly frequent in both treatment arms. Fourteen of the 18 allogeneic transplant recipients receiving GM-CSF and 16 of 19 receiving GM-CSF + G-CSF developed acute GVHD.

DISCUSSION

Bone marrow graft failure or poor hematologic function after BMT is frequent, can contribute to greater post-BMT mortality, and often complicates and prolongs hospitalization, thus increasing treatment costs. In a retrospective evaluation of delayed engraftment at our institution that preceded initiation of this trial, of 591 patients transplanted, 196 (33%) had not achieved an ANC ≥500/μL by 28 days after BMT. This one-third rate of delayed hematologic recovery (defined by a less stringent endpoint than that of the current study) was similar in all three cohorts (autologous, related-donor, and unrelated-donor BMT) before hematopoietic growth factors were available as therapeutic tools. However, we also observed that 6-month survival was equivalent for those with or without neutrophil recovery to ≥500/μL by day 28 (70% vs. 62%, respectively; P = .18), although hospital stay was often prolonged secondary to persisting neutropenia. With that institutional background data available, we initiated the current trial in which we evaluated sequential therapy of GM-CSF followed by G-CSF compared with GM-CSF alone to reduce the early morbidity and improve survival for patients with graft failure.

Numerous reports suggest that several hematopoietic growth factors, including GM-CSF, G-CSF, IL-1α, IL-3, and some growth factor combinations can accelerate engraftment when used immediately after BMT, although data demonstrating improvements in survival after growth factor administration are limited. In contrast, only a few reports describe the use of growth factors for treatment of delayed hematologic recovery or secondary graft failure. For example, Nemunaitis et al first reported that 21 of 37 patients responded to GM-CSF administered for graft failure and suggested superior 100-day and 1-year survival rates in the GM-CSF–treated patients compared with institutional historical controls. Similar results with growth factor therapy for delayed engraftment have been reported by others using either GM-CSF or G-CSF, but no conclusive data are available comparing GM-CSF to G-CSF for acceleration of

![Fig 1. Shown are the hematologic recovery rates (time to ANC ≥500/μL; to red blood cell (RBC) independence, and to platelet independence) for graft failure patients treated with GM-CSF × 14 days (n = 23) or GM-CSF × 7 days followed by G-CSF × 7 days (n = 24).](image-url)
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hematologic recovery after transplantation. Therefore, we initiated the comparative trial reported herein.

Current understanding of the physiologic maturation of hematopoietic cells suggests that GM-CSF–primed progenitors may be more susceptible to secondary stimulation with G-CSF. In addition to the early reports and Food and Drug Administration-licensed indications for the use of GM-CSF for graft failure, we reasoned that the known endogenous high circulating levels of G-CSF (but not GM-CSF) in the plasma accompanying post-BMT neutropenia and the sequential and potentially synergistic role these two factors have in stimulating hematopoietic differentiation and proliferation suggested that GM-CSF as primary therapy followed by G-CSF might effect superior recovery and reduce the morbidity and mortality associated with delayed engraftment.

Somewhat disappointingly, within this trial both treatments produced similar rates of trilineage hematologic recovery after initial graft failure. Compared with GM-CSF alone, sequential therapy with GM-CSF followed by G-CSF was of no advantage in stimulating more rapid or more frequent neutrophil recovery, while red cell and platelet transfusion-dependence persisted for a median of 5 weeks after enrollment. The observation that this delayed erythrocyte and platelet transfusion-dependence persisted for many weeks in many patients indicates that additional measures are needed beyond either single or sequential growth factor therapy to satisfactorily promote trilineage hematologic recovery in those with initially poor graft function.

Importantly, a significant difference in 100-day survival was observed favoring GM-CSF alone as compared with sequential therapy with GM-CSF + G-CSF. The primary and secondary causes of death were related to graft failure in all patients and can in no way be attributed to treatment with G-CSF during the second therapeutic week, and we can offer no sound explanation for this observed difference. However, there were no findings observed in this trial that favored changing growth factor treatment to G-CSF after the first week of GM-CSF. Because neither hematologic recovery of red cells, neutrophils, or platelets nor survival favored GM-CSF + G-CSF, this sequential cytokine therapy cannot be accepted as advantageous, at least in this dose and schedule. GM-CSF treatment for delayed engraftment should remain the standard against which other, newer growth factors, sequential treatments, or combination therapies are tested.

Finally, it must be remembered that in addition to hematopoietic growth factors that should be initiated promptly if engraftment delay is suspected or observed, strong consideration must be given to second marrow infusion if engraftment delay persists. In the current report, 80%+ of all patients who eventually responded with satisfactory neutrophil recovery did so within 21 days of growth factor therapy (usually by day +42 post-BMT). We have previously reported that second infusions of bone marrow after graft failure have led to successful engraftment in over half of patients, and 24% of reinfused patients survive at 1 year. Although still disappointing, these data show the demonstrable value of second marrow infusion as a legitimate therapeutic intervention when recombinant cytokine therapy is unsuccessful.

Table 3. Causes of Death Within 100 Days After Cytokine Therapy for Graft Failure

<table>
<thead>
<tr>
<th>Day of Death Post-Enrollment (days post-BMT)</th>
<th>Primary Causes of Death</th>
<th>Secondary Causes of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF × 14 days</td>
<td>Multisystem organ failure</td>
<td></td>
</tr>
<tr>
<td>24 (54)</td>
<td>Primary: Multisystem organ failure</td>
<td></td>
</tr>
<tr>
<td>GM-CSF + G-CSF</td>
<td>Pulmonary hemorrhage; hepato-renal failure, GVHD</td>
<td></td>
</tr>
<tr>
<td>16 (37)</td>
<td>Secondary: Liver failure; DIC, GI bleeding, GVHD</td>
<td></td>
</tr>
<tr>
<td>20 (46)</td>
<td>Primary: Pulmonary hemorrhage; hepato-renal failure, GVHD</td>
<td></td>
</tr>
<tr>
<td>26 (47)</td>
<td>Secondary: Liver failure; DIC, GI bleeding, GVHD</td>
<td></td>
</tr>
<tr>
<td>38 (59)</td>
<td>Primary: Pulmonary hemorrhage; hepato-renal failure, GVHD</td>
<td></td>
</tr>
<tr>
<td>39 (61)</td>
<td>Secondary: Liver failure; DIC, GI bleeding, GVHD</td>
<td></td>
</tr>
<tr>
<td>70 (90)</td>
<td>Multisystem organ failure</td>
<td></td>
</tr>
<tr>
<td>84 (140)</td>
<td>Aspergillosis; GVHD</td>
<td></td>
</tr>
</tbody>
</table>

All patients also had delayed engraftment as a contributing cause of death.

Abbreviations: DIC, disseminated intravascular coagulation; GI, gastrointestinal.

Fig 2. Shown are 100-day survival rates after cytokine therapy for graft failure with GM-CSF alone or GM-CSF followed by G-CSF (P = 0.026).

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