High-Dose Therapy and Peripheral Blood Progenitor Cell Transplantation: Effects of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor on the Autograft

By Michael R. Bishop, James R. Anderson, John D. Jackson, Philip J. Bierman, Elizabeth C. Reed, Julie M. Vose, James O. Armitage, Phyllis I. Warkentin, and Anne Kessinger

Between June 1989 and June 1992, 144 patients participated in sequential clinical trials using peripheral blood progenitor cells (PBC) as their sole source of hematopoietic rescue following high-dose chemotherapy. All patients had received prior extensive combination chemotherapy and had marrow defects that precluded autologous bone marrow transplantation (ABMT). PBC were collected according to a single apheresis protocol. The initial 86 patients (group 1) had PBC collected without mobilization. Beginning in April 1991, PBC were mobilized solely with recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF). Thirty-four patients (group 2) received rHuGM-CSF at a dose of 125 μg/m²/d by continuous intravenous infusion, and 24 patients (group 3) received rHuGM-CSF at a dose of 250 μg/m²/d by continuous intravenous infusion. Patients underwent at least six aphereses and had a minimum of 6.5 × 10⁹ mononuclear cells (MNC)/kg collected. Cytokines were not routinely administered immediately after transplantation. A median of nine aphereses were required to collect PBC in group 1 and seven aphereses for groups 2 and 3 (P = .03). The time required to recover 0.5 × 10⁹/L granulocytes after transplant was significantly shorter (P = .0004) for the mobilized groups; the median time to recovery was 26 days for group 1, 23 days for group 2, and 18 days for group 3. Transplantation of PBC mobilized with rHuGM-CSF resulted in a shorter time to platelet (P = .04) and red blood cell (P = .01) transfusion independence. Mobilization with rHuGM-CSF alone resulted in efficient collection of PBC, that provided rapid and sustained restoration of hematopoietic function following high-dose chemotherapy. Mobilization of PBC with rHuGM-CSF alone is an effective method for patients who have received prior chemotherapy and have bone marrow abnormalities.

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Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Nonmobilized</th>
<th>Mobilized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>No. of patients</td>
<td>86</td>
<td>34</td>
</tr>
<tr>
<td>Male:female</td>
<td>43:43</td>
<td>17:17</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>38 (18-62)</td>
<td>44 (19-59)</td>
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<tr>
<td>Malignancies</td>
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<td></td>
</tr>
<tr>
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<td>7</td>
</tr>
<tr>
<td>NHL</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Each patient. Characteristics of these patients and their malignancies are presented in Table 1.

**PBC mobilization.** The initial 86 patients had PBC collected while myelopoiesis was in a steady state (group 1). Fifty-eight patients had PBC collected following mobilization with Sargramostim (Immunex, Seattle, WA), a yeast-derived form of rHuGM-CSF, administered as a continuous intravenous infusion through an ambulatory infusion pump (Model CADD+, Pharmacia-Deltex, St. Paul, MN) in an outpatient setting. From April through December 1991, 34 patients received rHuGM-CSF at a dose of 125 \mu{g}/m{\text{2}}/d (group 2). Beginning in January 1992, 24 patients received rHuGM-CSF at a dose of 250 \mu{g}/m{\text{2}}/d (group 3). For both group 2 and group 3 patients, apheresis began when the peripheral white blood cell (WBC) count reached 10 \times 10^{9}/L. The administration of rHuGM-CSF was discontinued on the day of the final apheresis procedure. The dose of rHuGM-CSF was decreased by 50% for a suspected drug toxicity or to maintain the WBC count below 25 \times 10^{9}/L. If after 5 days of rHuGM-CSF administration the WBC count had not reached 10 \times 10^{9}/L, recombinant human granulocyte colony-stimulating factor (rHuG-CSF), filgrastim (Amgen, Thousand Oaks, CA), at a dose of 5 \mu{g}/kg administered subcutaneously was substituted, and apheresis was immediately initiated.

**Stem cell collection and processing techniques.** All patients had a minimum of 6.5 \times 10^8 mononuclear cells (MNC)/kg patient weight collected with at least six apheresis procedures. The minimum number of MNC and apheresis procedures were based on results of previous trials at the University of Nebraska Medical Center using nonmobilized PBC as the sole source of hematopoietic rescue following high-dose chemotherapy.

All patients had their PBC collected in an identical manner with a Haemonetics Model V50 apheresis device (Haemonetics, Braintree, MA) as previously described. PBC were collected with 4-hour apheresis procedures. For each pass, PBC collection began after half of the platelet band was discharged and ended when 40 mL of the red blood cell (RBC) band was collected. Approximately 10 to 12 passes were performed in 4 hours. At the end of the 4-hour procedure, collected cells were loaded back into the Latham bowl, and the apheresis procedure was repeated. The collection started when the platelets began to discharge and ended when 60 mL of the RBC band was collected. Apheresis procedures were repeated no more than five times weekly. If at the end of six apheresis procedures a total of 6.5 \times 10^8 MNC/kg patient weight had not been collected, the procedures were continued until the target number of MNC was obtained.

All collections were cryopreserved using a previously reported technique. Briefly, the cells were cryopreserved in a 10% concentration of dimethyl sulfoxide and stored in the vapor phase of liquid nitrogen using a controlled-rate freezer (Cryo-Med, Mt. Clemens, MI).

The colony-forming unit–granulocyte/macrophage (CFU-GM) content of the combined collections from each patient was determined both before and after cryopreservation using a modification of a previously reported culture method, where recombinant human interleukin-3 (200 U/mL), rHuG-CSF (200 U/mL), and rHuG-CSF (200 U/mL) were also added as growth factors.

**High-dose chemotherapy and PBCT.** Most patients subsequently received high-dose chemotherapy and PBCT. The high-dose therapy these patients received was determined by their underlying malignancy, but in no instance did the therapy include total-body irradiation. Fifty-one patients with Hodgkin’s disease received a single regimen of carmustine 300 mg/m{\text{2}}, etoposide 125 mg/m{\text{2}}/d administered every 12 hours for six doses, and cyclophosphamide 1.5 g/m{\text{2}}/d for 4 days. One patient with Hodgkin’s disease received cyclophosphamide 1.5 g/m{\text{2}}/d for 4 days, etoposide 125 mg/m{\text{2}}/d every 12 hours for six doses, and mitoxantrone 15 mg/m{\text{2}}/d for 3 days. Patients with non-Hodgkin’s lymphoma (NHL) were included in consecutive studies of high-dose chemotherapy regimens based on histologic diagnosis and previous tumor responsiveness. Twenty-four patients with NHL received carmustine 300 mg/m{\text{2}}, etoposide 100 mg/m{\text{2}}/d every 12 hours for eight doses, cyclophosphamide 35 mg/kg/d for 4 days, and cytarabine 100 mg/m{\text{2}}/d every 12 hours for eight doses. Twenty-six patients with NHL received carmustine 300 mg/m{\text{2}}, etoposide 150 mg/m{\text{2}}/d every 12 hours for six doses, cyclophosphamide 2.5 g/m{\text{2}}/d for 2 consecutive days, and hydroxyurea 1.5 g/m{\text{2}} every 6 hours for 12 doses. Five patients with refractory NHL received ifosfamide 3 g/m{\text{2}}/d for 4 days, carboplatin 300 mg/m{\text{2}}/d for 4 days, and etoposide 400 mg/m{\text{2}}/d for 4 days. Twenty-two patients with breast cancer received cyclophosphamide 50 mg/kg/d for 4 days, thiopeta 150 mg/m{\text{2}}/d for 4 days, and hydroxyurea 1.5 g/m{\text{2}} every 6 hours for 12 doses. Five patients with breast cancer received cyclophosphamide 60 mg/kg/d for 2 days, etoposide 150 mg/m{\text{2}} every 12 hours for six doses, and cisplatin 125 mg/m{\text{2}}. All other patients with solid tumors received high-dose chemotherapy regimens consisting of various alkylating agents.

Cytokines were not routinely administered at the time of the transplantation. Indications for the initiation of cytokines following PBCT included a documented infection during the period of absolute neutropenia or the failure to achieve an absolute neutrophil count of 0.2 \times 10^9/L by day 28 posttransplantation. Platelet transfusions were administered if the patient had clinical evidence of bleeding or if the platelet count was less than 20,000/\mu{\text{l}}. RBC transfusions were administered if the patient had a hemoglobin (Hg) level of less than 8 g/dL. The times required to recover 0.5 \times 10^9/L granulocytes and to become independent of RBC and platelet transfusions after transplantation were recorded.

**Statistical methods.** Comparisons of the number of aphereses required to reach 6.5 \times 10^8 MNC/kg, as well as a comparison of CFU-GM collected by treatment group, were made using the Wilcoxon rank-sum test. Comparisons of times required for hematopoietic recovery by treatment group were made using the log-rank test. Multivariate adjustments of these comparisons for factors prognostic for engraftment were made using the multivariate survival model of Cox.

**RESULTS**

**Patients.** All patients had received considerable antitumor therapy. Fifty-four percent of patients in group 1, 58% of patients in group 2, and 17% of patients in group 3 had also received prior irradiation. All three patient groups had received a median of two different combination chemotherapy trials before being considered as candidates for high-dose therapy. Patients in the nonmobilized group and the mobilized groups had received prior chemotherapy for a
median of 8 months (range, 0 to 47 months) and 10 months (range, 3 to 48 months), respectively. The majority of patients had a diagnosis of Hodgkin’s disease, NHL, or breast cancer. Hodgkin’s disease was the predominant malignancy in the nonmobilized group, and NHL was predominant in the two mobilized groups. The most common reasons for selecting peripheral blood rather than bone marrow for autografting were either marrow involvement with malignant cells in approximately 75% of patients, or marrow hypopcellularity from prior antitumor therapy, usually secondary to pelvic irradiation.

**Mobilization.** Patients in groups 2 and 3 received rHuGM-CSF for a median of 12 days (range, 8 to 24 days) and 10 days (range, 5 to 24 days), respectively. The duration of rHuGM-CSF administration for each patient was dependent on the following three factors: (1) the time required for the WBC count to reach 10 × 10^9/L, so that PBC collections could begin; (2) the day of the week that rHuGM-CSF was started, since collections were not performed on weekends; and (3) the number of apheresis procedures required to collect 6.5 × 10^9/kg MNC. Most patients achieved a WBC count of 10 × 10^9/L after 1 day of rHuGM-CSF administration.

Seven patients in group 2 required a dose reduction of rHuGM-CSF during mobilization; four reductions were prompted by a WBC count exceeding 25 × 10^9/L. Dyspnea, which appeared during rHuGM-CSF administration in one patient, resolved following dose reduction. Vomiting prompted dose reduction in another patient. The dose of rHuGM-CSF was reduced for one patient who developed severe headaches. Three patients in group 2 required discontinuation of rHuGM-CSF and initiation of rHuG-CSF. One patient did not reach the minimum requirement of a WBC count of 10 × 10^9/L to begin PBC collection after 5 days of rHuGM-CSF administration. A second patient failed to reach the minimum number of MNC after multiple aphereses. The third patient had an adequate number of MNC collected, but the number of CFU-GM was considered unacceptable low. The collections were repeated using rHuG-CSF for mobilization.

Five patients in group 3 required unscheduled rHuGM-CSF dose reductions. Doses were reduced for WBC counts exceeding 25 × 10^9/L in four patients and for severe headaches in one. Seven patients in group 3 had rHuGM-CSF discontinued and rHuG-CSF started for mobilization. Two of these patients developed hives, and another two experienced severe “flu-like” symptoms during rHuGM-CSF administration. Two patients had rHuGM-CSF discontinued for dyspnea and recurring catheter clots, respectively. Only one patient in group 3 had rHuGM-CSF discontinued due to a low number of MNC during collection. Two patients had their collections interrupted during collections due to progressive disease. These patients resumed collections on rHuGM-CSF after completion of salvage chemotherapy and recovery from neutropenia.

**Progenitor cell product characteristics.** A median of nine apheresis procedures (range, 6 to 18) were required to collect the target number of MNC in the nonmobilized group 1 patients, in contrast with a median of seven collections for both group 2 (range, 6 to 17) and group 3 (range, 6 to 15; Table 2). Significantly fewer aphereses were required for group 2 (P = .04) and group 3 (P = .02) patients as compared with group 1 patients. The number of aphereses for group 2 and group 3 patients did not differ statistically (P = .69). This also resulted in significant financial savings as a result of performing fewer apheresis procedures and processing fewer PBC products in both group 2 and group 3 patients. The median number of MNC collected was 8.61 × 10^9/kg in group 1, 9.21 × 10^9/kg in group 2, and 7.12 × 10^9/kg in group 3 (Table 2).

There was a significant difference in the number of CFU-GM in the collections both before (P = .0001) and after (P = .0001) cryopreservation between the mobilized and nonmobilized patients, with greater numbers of CFU-GM in groups 2 and 3 (Table 2). There was no difference in the number of CFU-GM between groups 2 and 3 both before (P = .50) and after (P = .60) cryopreservation.

The number of CFU-GM were noted to increase within 1 to 2 days after initiation of rHuGM-CSF for mobilization. The peak number of CFU-GM was observed 5 to 6 days after the administration of rHuGM-CSF and subsequently decreased (Fig 1).

**Hematopoietic recovery following transplantation.** One hundred thirty-four of the 144 patients received high-dose chemotherapy followed by PBCT. Eighty patients received nonmobilized PBC (group 1), 31 received PBC mobilized with rHuGM-CSF at a dose of 125 pg/m^2/d (group 2), and 23 received PBC mobilized with rHuGM-CSF at a dose of 250 pg/m^2/d (group 3). Five patients in group 1 experienced early deaths and were censored for time to hematopoietic recovery at their time of death. One patient in group 2 failed to achieve 0.5 × 10^9/L granulocytes, but did become RBC and platelet transfusion-independent. The patient died of progressive disease 161 days after transplantation. There were no graft failures in group 3.

With the exception of four patients in group 1, cytokines were not administered at the time of transplantation. Nine patients in group 1 (9%), nine patients in group 2 (25%), and
three patients in group 3 (13%) received cytokines (rHuGM-CSF or rHu-G-CSF) 1 week or more following PBCT (Table 3). The most common reason for cytokine administration post-PBCT was failure to achieve $0.2 \times 10^9$/L granulocytes by day 28 after transplantation.

The median number of days until the appearance of $0.5 \times 10^9$/L granulocytes in the circulation after PBCT was 26 for group 1, 23 for group 2, and 18 for group 3 (Fig 2A). There was a statistical difference in granulocyte recovery ($P = .002$) between the nonmobilized and mobilized groups (Table 4).

The number of CFU-GM both before and after cryopreservation correlated with the rate of hematopoietic recovery. Patients with greater than $2.5 \times 10^9$ CFU-GM/kg patient weight had a more rapid recovery of $0.5 \times 10^9$/L circulating granulocytes ($P < .001$). There was no difference in the recovery of $0.5 \times 10^9$/L granulocytes in either group 2 or group 3 patients who were switched from rHuGM-CSF to rHuG-CSF during PBC collection. There were no differences in hematopoietic recovery between patients who received cytokines following transplantation and those who did not.

The median time to platelet transfusion independence for both groups 1 and 2 was 24 days (Fig 2B). Patients in group 3 became independent of platelet transfusions at a median time of 15 days, which was statistically significant ($P = .01$) as compared with groups 1 and 2.

Results for time to RBC transfusion independence were similar to those for platelet transfusion independence (Fig 2C). Patients in group 3 became independent of RBC transfusions at a median of 15 days, as compared with 22 days for group 1 and 27 days for group 2. In addition, the range of recovery times was decreased in the mobilized PBC patients, so overall, a smaller proportion of these patients experienced prolonged pancytopenia (Table 4).

**DISCUSSION**

For the last 8 years, PBCT following high-dose therapy has been performed with increasing frequency for patients with a variety of malignancies. The use of mobilized PBC to augment ABMT has resulted in earlier hematopoietic recovery than with ABMT alone.\textsuperscript{13-15} PBC, mobilized by a combination of chemotherapy and cytokines, has permitted the scheduled administration of relatively higher doses of conventional chemotherapy.\textsuperscript{8} However, patients in these clinical situations had received minimal or no prior therapy and had bone marrows without significant defects such as metastases. Chemotherapy has failed to mobilize PBC in patients who have received extensive prior therapy, despite the addition of hematopoietic cytokines.\textsuperscript{3,5} Because bone marrow metastases and extensive prior chemotherapy have predicted for refractoriness to mobilization attempts, patients have been excluded from clinical trials who may have potentially benefitted from high-dose therapy.\textsuperscript{17,18}

This report describes our initial experience with the recombinant human hematopoietic cytokine, rHuGM-CSF, for the mobilization of PBC in patients who were ineligible for ABMT due to the presence of bone marrow metastases and/or bone marrow hypocellularity. The patients shared the common characteristic of having received extensive prior therapy, had PBC collected in an identical manner, and received essentially the same number of MNC after administration of high-dose chemotherapy. As cytokines were not routinely administered following PBCT, the mobilization effects of rHuGM-CSF on the autograft could be more readily recognized.

The expression of CD34 surface antigen has been used by other investigators to determine the optimal time to initiate PBC collection following various mobilization methods.\textsuperscript{19} Although this technology was not available in a practical manner when these trials were conducted, it does not appear necessary, since the administration of rHuGM-CSF for mobilization permitted the relatively immediate initiation of PBC collection. Mobilization solely with rHuGM-CSF also avoided the toxicity and the delay in initiation of PBC collection while waiting for recovery from neutropenia associated with mobilization attempts using cytotoxic agents.

Overall, rHuGM-CSF was relatively well tolerated by the majority of patients. Dose reductions were required by protocol in eight of 12 patients for a WBC count in excess of $25 \times 10^9$/L. Dose reduction resulted in a prompt resolution of signs and symptoms attributed to rHuGM-CSF.

<table>
<thead>
<tr>
<th>Table 3. Reason for Cytokine Administration Following Transplantation</th>
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<tbody>
<tr>
<td>Nonmobilized patients</td>
</tr>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>Fever                                            2</td>
</tr>
<tr>
<td>Neutropenia                                       7</td>
</tr>
<tr>
<td>Mobilized patients</td>
</tr>
<tr>
<td>Group 2</td>
</tr>
<tr>
<td>Fever                                            3</td>
</tr>
<tr>
<td>Sepsis                                           1</td>
</tr>
<tr>
<td>Neutropenia                                       4</td>
</tr>
<tr>
<td>Group 3</td>
</tr>
<tr>
<td>Fever                                            2</td>
</tr>
<tr>
<td>Sepsis                                           1</td>
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</table>
Evaluation of rHuGM-CSF was required in a notable number of patients in group 3 for symptoms that have previously been described with rHuGM-CSF administration. Only one patient had rHuGM-CSF discontinued for failure to achieve a minimum number of MNC for transplantation.

The use of rHuGM-CSF for mobilization decreased the number of apheresis procedures required to collect a target number of MNC compared with historical controls. This was not only significant in terms of convenience to patients, but it was also useful financially by decreasing the costs of additional collections, processing, and cryopreservation. Recent reports have described the collection of a sufficient number of PBC with one to three apheresis procedures.\(^7\,^{26}\)

In general, these have been performed in patients with minimal prior therapy using apheresis procedures in excess of 4 hours.

The transplantation of PBC mobilized with rHuGM-CSF resulted in a shorter period required to recover greater than \(0.5 \times 10^9/L\) granulocytes, as compared with nonmobilized PBC. In addition, the mobilization of PBC with rHuGM-CSF resulted in earlier platelet and RBC transfusion independence.

Cytokines were not routinely administered immediately following PBCT. Cytokines were used in less than 15% of patients later in their hospital course, primarily for delayed hematopoietic recovery or suspected sepsis. However, the use of cytokines following PBCT did not affect the rate of hematopoietic recovery between the nonmobilized and mo-
HIGH-DOSE THERAPY AND PBC TRANSPLANTATION

Fig 2. (Cont’d) (C) Time to RBC transfusion independence.

- - - Group 1; - - - group 2;
- - - - group 3.

bilized groups. Only one patient failed to achieve a granulocyte count greater than $0.5 \times 10^9/L$ within 50 days of PBCT. This patient experienced progression of disease while receiving high-dose therapy.

The number of CFU-GM was higher in PBC collections mobilized with rHuGM-CSF, both before and after cryopreservation. The number of CFU-GM correlated directly with the rate of engraftment. This suggests that patients receiving PBC mobilized with rHuGM-CSF engrafted earlier as a result of the larger number of available progenitors.

The mobilization effect of rHuGM-CSF, as measured by the number of required PBC collections, CFU-GM, and hematopoietic recovery after PBCT were statistically significant as compared with nonmobilized PBC. These results were obtained in a heterogenous patient population relative to the variety of malignancies reported. However, these patients shared important clinical characteristics of having received extensive prior combination chemotherapy and having marrow defects that precluded ABMT. These characteristics have been associated with difficulty in mobilization using cytotoxic chemotherapy with or without cytokines. These data suggest that rHuGM-CSF can effectively mobilize PBC in patients who are at risk for failure or who have failed mobilization attempts with cytotoxic chemotherapy.

Table 4. Hematopoietic Recovery

<table>
<thead>
<tr>
<th>Group</th>
<th>Median No. of Days Until Recovery</th>
<th>ANC &gt; 0.5 $\times 10^9/L$</th>
<th>Ptt &gt; 20,000 $/\mu L$</th>
<th>Hb &gt; 8 g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 80)</td>
<td>26 (11-93)</td>
<td>24 (9-266)</td>
<td>22 (4-135)</td>
<td></td>
</tr>
<tr>
<td>2 (n = 31)</td>
<td>23 (12-49)</td>
<td>24 (7-102)</td>
<td>27 (2-74)</td>
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<tr>
<td>3 (n = 23)</td>
<td>18 (10-46)</td>
<td>15 (8-41)</td>
<td>15 (6-41)</td>
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</tbody>
</table>

Abbreviations: ANC, absolute neutrophil count; Ptt, platelet count.

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High-dose therapy and peripheral blood progenitor cell transplantation: effects of recombinant human granulocyte-macrophage colony-stimulating factor on the autograft

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