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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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 × 10⁸ 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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MATERIALS AND METHODS

Patient characteristics. The trials reported here are sequential. Patient eligibility requirements for high-dose therapy included malignancies that were refractory to conventional curative strategies, age less than 60 years, a Karnofsky performance status of at least 70, and no major organ dysfunction. Eligibility requirements for peripheral blood progenitor cell transplantation (PBCT) included the presence or history of morphologic tumor contamination of the bone marrow or bone marrow hypocellularity. Patients with evidence of circulating malignant cells on peripheral smear were ineligible for these trials. Between June 1989 and June 1992, 144 patients who were candidates for high-dose, marrow-ablative chemotherapy and PBCT at the University of Nebraska Medical Center were eligible to participate in these trials. Written informed consent for PBCT collection with or without cytokine mobilization, as appropriate, and for autologous transplantation was obtained from...
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), rHuGM-CSF (200 U/mL), and rHuG-CSF (200 U/mL) were also added as growth factors.\textsuperscript{11}

### 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\textsuperscript{2}, etoposide 125 mg/m\textsuperscript{2} administered every 12 hours for six doses, and cyclophosphamide 1.5 g/m\textsuperscript{2}/d for 4 days. One patient with Hodgkin's disease received cyclophosphamide 1.5 g/m\textsuperscript{2}/d for 4 days, etoposide 125 mg/m\textsuperscript{2}/d every 12 hours for six doses, and mitoxantrone 15 mg/m\textsuperscript{2}/d for 3 days. Patients with non-Hodgkin's lymphoma (NHL) were included in consecutive studies of high-dose chemotherapies based on histologic diagnosis and previous tumor responsiveness. Twenty-four patients with NHL received carmustine 300 mg/m\textsuperscript{2}, etoposide 100 mg/m\textsuperscript{2}/d every 12 hours for eight doses, cyclophosphamide 35 mg/kg/d for 4 days, and cytarabine 100 mg/m\textsuperscript{2}/d every 12 hours for eight doses. Twenty-six patients with NHL received carmustine 300 mg/m\textsuperscript{2}, etoposide 150 mg/m\textsuperscript{2}/d every 12 hours for six doses, cyclophosphamide 2.5 g/m\textsuperscript{2}/d for 2 consecutive days, and hydroxyurea 1.5 g/m\textsuperscript{2}/d every 6 hours for 12 doses. Five patients with refractory NHL received ifosfamide 3 g/m\textsuperscript{2}/d for 4 days, carboplatin 300 mg/m\textsuperscript{2}/d for 4 days, and etoposide 400 mg/m\textsuperscript{2}/d for 4 days. Twenty-two patients with breast cancer received chemotherapy, and hydroxyurea 1.5 g/m\textsuperscript{2}/d every 6 hours for 12 doses. Five patients with breast cancer received cyclophosphamide 60 mg/kg/d for 2 days, etoposide 150 mg/m\textsuperscript{2}/d every 12 hours for six doses, and cisplatin 125 mg/m\textsuperscript{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 0.5 × 10\textsuperscript{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 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 × 10\textsuperscript{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 × 10\textsuperscript{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 hypocellularity 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 $10^9/L$, 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 $\times 10^8$/kg MNC. Most patients achieved a WBC count of 10 $\times 10^9/L$ to begin PBC collection after 5 to 2 days after initiation of rHuGM-CSF for mobilization. Eight patients in group 2 and group 3 (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 $\times 10^9$/kg in group 1, 9.21 $\times 10^9$/kg in group 2, and 7.12 $\times 10^9$/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 µg/m²/d (group 2), and 23 received PBC mobilized with rHuGM-CSF at a dose of 250 µg/m²/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 $\times 10^9$/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

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### Table 2. PBC Product Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Median No. of Aphereses</th>
<th>Median No. of MNC $\times 10^8$/kg</th>
<th>Pre-Cryopreservation</th>
<th>Post-Cryopreservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 86)</td>
<td>9 (6-18)</td>
<td>8.01 (4.03-32.1)</td>
<td>0.7 (0.04-29.7)</td>
<td>0.24 (0.01-13.4)</td>
</tr>
<tr>
<td>Group 2 (n = 34)</td>
<td>7 (6-17)</td>
<td>9.21 (6.5-28.8)</td>
<td>3.6 (0.68-57.7)</td>
<td>1.9 (0.2-102.8)</td>
</tr>
<tr>
<td>Group 3 (n = 24)</td>
<td>7 (6-15)</td>
<td>7.12 (6.1-10.8)</td>
<td>8.8 (0.62-104.3)</td>
<td>10.2 (0.30-104.3)</td>
</tr>
</tbody>
</table>

Ranges are in parentheses.
HIGH-DOSE THERAPY AND PBC TRANSPLANTATION

three patients in group 3 (13%) received cytokines (rHuGM-CSF or rHuG-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.13-15 PBC, mobilized by a combination of chemotherapy and cytokines, has permitted the scheduled administration of relatively higher doses of conventional chemotherapy.6 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.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.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.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. Discontin-

![Graph](image_url)

Table 3. Reason for Cytokine Administration

<table>
<thead>
<tr>
<th>Following Transplantation</th>
<th>Nonmobilized patients</th>
<th>Mobilized patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Fever</td>
<td>Group 2</td>
</tr>
<tr>
<td></td>
<td>Neutropenia</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>Neutropenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
<td></td>
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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.15 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 m

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Fig 2. Hematopoietic recovery after PBCT. (A) Time required to produce 500 granulocytes. (B) Time to platelet transfusion independence.
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 heterogeneous 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANC &gt;0.5 x 10^9/L</td>
</tr>
<tr>
<td>1 (n=80)</td>
<td>26 (11-93)</td>
</tr>
<tr>
<td>2 (n=31)</td>
<td>23 (12-49)</td>
</tr>
<tr>
<td>3 (n=23)</td>
<td>18 (10-46)</td>
</tr>
</tbody>
</table>

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

**ACKNOWLEDGMENT**

The authors would like to thank Kim Schmit-Pokorny for the coordination and patient care in these trials. We would also like to thank Kathleen Petersen for data collection and management.

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