Comparative Effects of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Granulocyte Colony-Stimulating Factor (G-CSF) on Priming Peripheral Blood Progenitor Cells for Use With Autologous Bone Marrow After High-Dose Chemotherapy

By William P. Peters, Gary Rosner, Maureen Ross, James Vredenburgh, Barry Meisenberg, Colleen Gilbert, and Joanne Kurtzberg

Two hematopoietic colony-stimulating factors, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF), have been shown to accelerate leukocyte and neutrophil recovery after high-dose chemotherapy and autologous bone marrow (BM) support. Despite their use, a prolonged period of absolute leukopenia persists during which infections and other complications of transplantation occur. We collected large numbers of peripheral blood (PB) progenitors after CSF administration using either G-CSF or GM-CSF and tested their ability to affect hematopoietic reconstitution and resource utilization in patients undergoing high-dose chemotherapy and autologous BM support. Patients with breast cancer or melanoma undergoing high-dose chemotherapy and autologous BM support were studied in sequential nonrandomized trials. After identical high-dose chemotherapy, patients received either BM alone, with no CSF; BM with either G-CSF or GM-CSF; or BM with G-CSF or GM-CSF and GM-CSF or G-CSF primed peripheral blood progenitor cells (PBPC). Hematopoietic reconstitution, as well as resource utilization, was monitored in these patients. The use of CSF-primed PBPC led to a highly significant reduction in the duration of leukopenia with a white blood cell (WBC) count under 100 and 200 cells/mL, and neutrophil count under 100 and 200 cells/mL with both GM- and G-CSF primed PB progenitor cells, compared with the use of the CSF with BM or with historical controls using BM alone. In addition, the use of CSF-primed PBPC resulted in a significant reduction in median number of antibiotics used, days in the Bone Marrow Transplant Unit, and hospital resources used. Patients receiving G-CSF primed PBPC also experienced a reduction in the median number of days in the hospital, red blood cell (RBC) transfusions, platelet transfusions, days on antibiotics, and discounted hospital charges. Phenotypic analysis of the CSF-primed PBPC indicated the presence of cells bearing antigens associated with both early and late hematopoietic progenitor cells. The use of CSF-primed PBPC can significantly improve hematopoietic recovery after high-dose chemotherapy and autologous BM support. In addition, the use of G-CSF–primed PBPC was associated with a significant reduction in hospital resource utilization, and a reduction in hospital charges.

© 1993 by The American Society of Hematology.

DURING THE PAST decade, dose intensification has been a recurrent theme in the attempt to devise new clinical approaches to various neoplasms, particularly the solid tumors. However, the intensity of therapy is often limited by myelosuppression. The concept of high-dose chemotherapy with autologous bone marrow (BM) support was initially developed to circumvent the problem of myelosuppression. Using autologous marrow allows intensification of chemotherapy doses so that the maximal tolerated dose of a chemotherapeutic regimen is determined not by extent of myelosuppression but rather by effects on the patient’s nonhematopoietic organs. Despite the use of autologous BM, high-dose chemotherapy is still associated with prolonged periods of myelosuppression, with a period of absolute leukopenia of 8 to 10 days and subsequent recovery to a leukocyte count above 1,000/mm³ in approximately 17 to 20 days depending on the chemotherapy regimen.

We and others have reported that the use of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte CSF (G-CSF) will accelerate hematopoietic recovery in patients undergoing autologous transplantation. However, the acceleration in hematopoietic recovery occurs after an obligate period of absolute leukopenia that is not materially altered by the CSFs. It is during this period of absolute leukopenia that most infections and many of the complications of high-dose therapy begin.

In an effort to try to reduce the period of absolute leukopenia, we and others proposed infusion of committed peripheral blood progenitor cells (PBPC) that bear receptors responsive to recombinant GM- or G-CSF. These cells are harvested from the PB after recovery from chemotherapy and after host priming with recombinant GM-CSF or G-CSF. Cells collected in this fashion are enriched with large numbers of circulating committed myeloid progenitors. Gianni et al have used PBPC collected during recovery after high-dose cyclophosphamide and GM-CSF to accelerate hematopoietic recovery after high-dose chemotherapy, and have found not only a reduction in the periods of absolute leukopenia, but also in the requirements for platelet transfusions. However, harvesting cells during the rebound period from chemotherapy is cumbersome in that it requires flexible scheduling and capability for 7-day-a-week apheresis to perform the collection during the optimal period. Sheridan et al have shown that the use of G-CSF–primed PB progenitors could reduce not only the period of neutropenia after high-dose busulfan and fludarabine, but also the periods of required transfusion support for platelets.

From the Duke University Bone Marrow Transplant Program, Durham, NC.

Submitted June 11, 1992; accepted December 11, 1992.

Supported in part by study grants from Sandoz Pharmaceuticals, Amgen Pharmaceuticals, and National Institutes of Health Grant No. 1 PO1 CA 47741-01A3. J.K. is a Scholar of the Leukemia Society of America.

Address reprint requests to William P. Peters, MD, PhD, Duke University Bone Marrow Transplant Program, 25101 Morris Building, Box 3961, Duke University Medical Center, Durham, NC 27710.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1993 by The American Society of Hematology.

Blood. Vol 81, No 7 (April 1), 1993: pp 1709-1719

1709
cyclophosphamide but also the time to platelet transfusion independence. In this report, we present results of sequential, nonrandomized studies using GM-CSF or G-CSF primed PBPC in patients undergoing BM transplantation who have been treated with an identical high-dose chemotherapeutic regimen. To obtain committed progenitors more easily, we chose to use CSF-priming with either GM-CSF or with G-CSF after full hematopoietic recovery had occurred after a prior course of chemotherapy. The data indicate a significant improvement in the rate of hematopoietic recovery, effective elimination of the period of absolute leukopenia, and, in some cases, a major improvement in the number of days in the hospital, days in isolation, red blood cell (RBC) and platelet transfusion requirements, requirements for antibiotics, and discounted hospital charges with the use of these CSF-primed PBPCs. These measured clinical parameters correlated with the clinical improvement and tolerance of the high-dose chemotherapy program. Such observations may make the application of the high-dose chemotherapy approach more feasible and cost effective.

MATERIALS AND METHODS

Patient Population

PBPCs. Forty-eight patients were entered between April 1989 and June 1990. All are evaluable, analyzed, and presented. The characteristics of patients entered in the study and the dates of study are presented in Table 1. The schedules for PBPC harvest and reinfusion are presented in Table 2. Sixteen patients were treated with recombinant human G-CSF (rHuG-CSF) to accomplish CSF-priming. Thirty-two patients were primed with rHuGM-CSF to augment PBPC harvesting. Forty-seven patients had histologically proven breast cancer (31 stage II with 10 or more involved axillary lymph nodes; 16 with measurable, hormone receptor negative or hormone non-responsive, metastatic breast cancer.) Stage II patients had previously received four cycles of induction chemotherapy using CAF (cyclophosphamide, 600 mg/m², day 1; doxorubicin, 60 mg/m², day 1; 5-fluorouracil, 600 mg/m², days 1 and 8). BM harvesting was performed after the third cycle of CAF. PBPC harvesting was performed after hematologic recovery (leukocyte count >3,000/mm³) after the fourth cycle of CAF. Patients with stage IV breast cancer received 2 to 4 cycles of induction chemotherapy before the autograft using the Duke AFM regimen and had BM harvested after the last chemotherapy cycle. CSF-primed PBPCs were collected just before high-dose chemotherapy and autologous BM support. One patient with stage II melanoma, who received no prior chemotherapy, was entered.

Comparison Populations

Three populations of patients that did not receive PBPCs as part of supportive care after high-dose chemotherapy are used for comparison (Table 2). The first was a historical control group of 23 patients with breast cancer (15 patients) or melanoma (8 patients) treated between January 1985 and November 1986 with the identical high-dose chemotherapy regimen of cyclophosphamide, cisplatin, and carmustine and autologous BM support that has been previously described. These patients received no CSF as supportive care. The second and third comparison populations consisted of 13 patients (breast cancer—stage IV, 8 patients; stage II, 4 patients; and melanoma, 1 patient) treated between February 1988 and November 1988 with the same high-dose chemotherapy and autologous marrow support who additionally received identical doses and schedules of either GM-CSF after BM infusion (7 patients; GM3) or G-CSF (6 patients; G3), as described in Tables 1 and 2. These patients did not receive PBPCs.

Bone Marrow Harvest

Marrow was collected as described previously from the posterior iliac crests under general or regional anesthesia. Auffy-coat concentrate was prepared using a Cobe 2991 cell washer (Cobe Industries, Lakewood, CO), mixed with 20% autologous plasma and 10% dimethylsulfoxide and frozen using a controlled rate program technique. No BM purging technique was used. Marrow was stored in the liquid-phase of liquid nitrogen until administration. BM was thawed rapidly at 37°C in a water bath and infused over 10 minutes without further

Table 1. Demographic Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Size</th>
<th>Dates Treated</th>
<th>Number Stage II (%</th>
<th>Age (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>23</td>
<td>1/85-11/86</td>
<td>0 (0%)</td>
<td>8 melanoma</td>
<td>37 (29-56)</td>
</tr>
<tr>
<td>G3</td>
<td>6</td>
<td>2/88-11/89</td>
<td>3 (50%)</td>
<td>1 melanoma</td>
<td>44 (32-51)</td>
</tr>
<tr>
<td>G4</td>
<td>8</td>
<td>8/89-1/90</td>
<td>5 (62%)</td>
<td>37 (32-43)</td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>6</td>
<td>10/89-12/89</td>
<td>6 (75%)</td>
<td>38 (31-48)</td>
<td></td>
</tr>
<tr>
<td>GM-CSF schedule</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM3</td>
<td>7</td>
<td>2/88-11/89</td>
<td>2 (29%)</td>
<td>39 (34-57)</td>
<td></td>
</tr>
<tr>
<td>GM4</td>
<td>8</td>
<td>4/89-7/89</td>
<td>4 (50%)</td>
<td>38 (29-44)</td>
<td></td>
</tr>
<tr>
<td>GM5</td>
<td>9</td>
<td>5/89-8/89</td>
<td>5 (50%)</td>
<td>44 (31-59)</td>
<td></td>
</tr>
<tr>
<td>GM6</td>
<td>8</td>
<td>12/89-3/90</td>
<td>5 (62%)</td>
<td>41 (32-52)</td>
<td></td>
</tr>
<tr>
<td>GM7</td>
<td>7</td>
<td>4/90-6/90</td>
<td>6 (86%)</td>
<td>37 (32-48)</td>
<td></td>
</tr>
</tbody>
</table>

* Refers to number of stage II breast cancer patients; the numbers of remaining patients are either stage IV breast cancer or melanoma (as indicated).

Table 2. Dose and Schedule of Hematopoietic CSF Administered

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CSF</th>
<th>Dose (µg/kg/d)</th>
<th>Pheresis (day of CSF)</th>
<th>BM</th>
<th>PBPC</th>
<th>CSF</th>
<th>Dose (µg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>6</td>
<td>G-CSF</td>
<td>6 s.c.</td>
<td>6, 8, 9</td>
<td>+1</td>
<td></td>
<td>G-CSF</td>
<td>20 IV × 21 d</td>
</tr>
<tr>
<td>G4</td>
<td>8</td>
<td>G-CSF</td>
<td>6 s.c.</td>
<td>6, 8, 9</td>
<td>+1</td>
<td>+1, +2, +3</td>
<td>G-CSF</td>
<td>20 IV × 21 d</td>
</tr>
<tr>
<td>G5</td>
<td>8</td>
<td>G-CSF</td>
<td>6 s.c.</td>
<td>6, 8, 9</td>
<td>+1</td>
<td>-1, 0, +1</td>
<td>G-CSF</td>
<td>20 IV × 21 d</td>
</tr>
<tr>
<td>GM3</td>
<td>7</td>
<td>GM-CSF</td>
<td>8 IV</td>
<td>6, 8, 9</td>
<td>+1</td>
<td></td>
<td>GM-CSF</td>
<td>16 × 7 d; 8 × 14 d</td>
</tr>
<tr>
<td>GM4</td>
<td>8</td>
<td>GM-CSF</td>
<td>8 IV</td>
<td>6, 8, 9</td>
<td>+1</td>
<td>+1, +2, +3</td>
<td>GM-CSF</td>
<td>16 × 7 d; 8 × 14 d</td>
</tr>
<tr>
<td>GM5</td>
<td>9</td>
<td>GM-CSF</td>
<td>8 IV</td>
<td>6, 8, 9</td>
<td>+1</td>
<td>-1, 0, +1</td>
<td>GM-CSF</td>
<td>16 × 7 d; 8 × 14 d</td>
</tr>
<tr>
<td>GM6</td>
<td>8</td>
<td>GM-CSF</td>
<td>16 IV</td>
<td>6, 8, 9</td>
<td>+1</td>
<td>-1, 0, +1</td>
<td>GM-CSF</td>
<td>16 × 7 d; 8 × 14 d</td>
</tr>
<tr>
<td>GM7</td>
<td>7</td>
<td>GM-CSF</td>
<td>16 IV</td>
<td>12, 14, 15</td>
<td>+1</td>
<td>-1, 0, +1</td>
<td>GM-CSF</td>
<td>16 × 7 d; 8 × 14 d</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, colony stimulating factor used; s.c., subcutaneous injection; IV, intravenous infusion over 4 hours; BM, day on which bone marrow is infused; PBPC, days during which PBPCs are administered.
G-CSF and GM-CSF

G-CSF (filgrastim) was expressed in Escherichia coli and provided to us formulated in diluent (Amgen, Thousand Oaks, CA). During priming, it was administered as a subcutaneous injection in the afternoon. After infusion of the PBPCs or BM, G-CSF was administered as a daily 4-hour infusion diluted in D5W supplemented with 0.25% albumin. Note that the dosage administered as reported in this paper differs slightly from that reported in our abstracts, since a refinement of the analytic technology at Amgen, Inc showed a different extinction coefficient and as such, the administered doses were actually 20% higher than previously reported.

GM-CSF (regramostim; Sandoz Pharmaceuticals, East Hanover, NJ) was expressed in Chinese hamster ovary cells (CHO) and was glycosylated. The dosage presented in this report is based on the actual weight of GM-CSF used, including glycosylation. Table 2 indicates the comparative dosing when expressed in terms of glycosylated protein, and doses in protein by amino acid analysis can be obtained by multiplying by 0.61. GM-CSF was administered as a daily 4-hour infusion during both the CSF-priming period and during the posttransplant period. To prevent absorption onto the surface of the administration sets, 0.25% human serum albumin was added to the infusion solution.

CSF-Primig and PBPC Harvest and Infusion

After leukocyte recovery to greater than 3,000/mm³ from the previous cycle of chemotherapy, CSF administration was initiated. The treatment schema are outlined in Table 2. Sixteen patients were treated with G-CSF at 6 µg/kg/d as a daily subcutaneous injection for 8 days. Thirty-two patients were treated with GM-CSF as three doses and schedules. Nine patients received GM-CSF at 8 µg/kg/d intravenously (IV), and seven patients received 16 µg/kg/d IV for 8 days administered in the afternoon. IV GM-CSF was administered as a 4-hour infusion. These patients underwent three leukaphereses on the mornings after the fifth, seventh, and eighth doses of cytokine (days 6, 8, and 9). Seven patients received GM-CSF, 16 µg/kg/d IV for 14 days. These patients were leukapheresed after the 11th, 13th, and 14th doses (thus, on days 12, 14, and 15). Leukaphereses were performed via a central catheter processing approximately 9 L of blood over 3 hours using a Cobe Spectra (Cobe Industries). Approximately 150 mL of cellular concentrate was obtained and then cryopreserved using 10% dimethylsulfoxide (DMSO) and frozen in a controlled-rate freezer at −1°C/min and stored in the liquid phase of nitrogen until used. BM was always administered on day +1 after rapid thaw at 37°C. During this study, the PBPCs were administered on two different schedules. In eight patients, each primed with either GM-CSF or G-CSF, PB progenitors were administered on the day of BM infusion (day +1) and for 2 days thereafter (days +2 and +3) (schedules GM4 and G4). The remaining patients received their PBPCs on the day of marrow infusion and for 2 days preceding this (days −1, 0, +1) (schedules GM5, GM6, GM7, and G5). PB progenitors were thawed rapidly at 37°C and administered over 5 to 10 minutes IV. At least 3 hours were allowed to elapse before the initiation of the cytokine on the day of administration of the PBPCs. On days on which both BM and progenitors were administered, they were infused 3 hours apart, and again 3 hours were allowed to elapse before cytokine began. Cytokine was begun on the first day of progenitor cell or BM administration and continued up to day +21, or until the neutrophil count was greater than 2,500/mm³ on 3 successive days.

Chemotherapy

All patients were treated with a single chemotherapy program of cyclophosphamide, cisplatin, and carbustine. Cyclophosphamide was administered at a dose of 1,875 mg/m²/d for 3 days (days −6, −5, −4) as a daily 1-hour IV infusion (total cyclophosphamide dose 5,625 mg/m²). Cisplatin was given at 55 mg/m²/d × 3 days (days −6 through −4) as a continuous IV infusion (total dose 165 mg/m²). Carbustine, 600 mg/m², was given IV at 5 mg/m²/min on day −3 beginning at the end of the cisplatin infusion. Details of the chemotherapy administration have been described elsewhere.11

Supportive Care

All patients were treated in private rooms in which air was cycled through high-efficiency particle aeration (HEPA) filters. Patients re-
mained in isolation from the start of therapy until the peripheral neutrophil count was greater than 500/mm³ on two separate determinations 12 hours apart. The unit protocol prescribed two broad-spectrum antibiotics at initial febrile event during neutropenia; if, after 48 hours, fever did not resolve or if culture results dictated, vancomycin was added. If fever persisted after 5 full days of antibiotics, amphotericin B was added. Patients received empiric antibiotics by Unit protocol from the time of first fever after the onset of leukopenia until neutropenia resolved and isolation was discontinued. All antibiotics were, in general, discontinued at this time unless the patient had positive cultures for bacteria or fungus; in these cases, antibiotic therapy was continued after neutrophil recovery for a brief but variable period, depending on clinical judgment. To minimize heterogeneity in transfusion procedure, all patients were transfused with packed red blood cells (RBCs) to keep the hematocrit greater than 42% before initiation of chemotherapy and maintained at this level until day 25 or until the neutrophil count exceeded 2,500/mm³ and the patient was platelet transfusion independent. Irradiated, single-donor pheresed platelets were administered when the platelet count fell below 25,000/mm³. Lymphocytotoxic antibodies were monitored weekly during therapy. Patients were discharged from the hospital after full hematopoietic recovery manifested by a peripheral neutrophil count greater than 2,500/mm³, platelet transfusion independence, and adequate performance status. No patient was treated with pentoxifyllin.

Characterization of PBPCs

During the CSF priming, peripheral leukocyte counts were evaluated daily and mononuclear cell preparations made by centrifugation of blood through Ficoll-Hypaque (American Red Cross, Washington, DC) and collection of the interface. Light-density mononuclear cells were assayed for the presence of clonal hematopoietic progenitor cells by quantitation of colony forming units-GM (CFU-GM) in semisolid medium (methylocellulose) by previously described methods. Assay cultures were plated in duplicate or triplicate per the laboratory routine, and representative colonies were plucked, washed, cytocentrifuged, and Wright-stained for morphologic confirmation of colony composition. Colonies were scored on day 14.

Antibodies

Monoclonal antibodies (MoAbs) IgG1-fluorescein isothiocyanate (FITC), IgG2-phycoerythrin (RD1) negative control, 3A1-RD1 (CD7), MY9-RD1 and MY9-FITC (CD33) were purchased from Coulter Immunology (Hialeah, FL) while MY10-FITC (CD34) was purchased from Amac, Inc (Westbrook, ME).

Phenotypic Analysis

Cell surface expression of CD7, CD33, and CD34 antigens were quantitated in the G-CSF and GM-CSF PBPC collections by incubation of aliquots of the cells with MoAbs directly labeled with the fluorescent markers FITC, or RD1, as previously described. Cells obtained via leukapheresis were fixed in 0.1% paraformaldehyde for 15 minutes before staining with the indicated antibodies to eliminate nonspecific Fc receptor binding. Dual-labeled immunofluorescent studies were performed by co-incubating the cells with two directly conjugated antibodies (one labeled with FITC and the other with RDI) simultaneously gated. Cells were analyzed using a Coulter Profile II flow cytometer (Coulter Electronics, Hialeah, FL). Forward and side-scatter properties were used to gate on all nucleated cells (lymphocytes + blasts + myeloid/monocytic cells). Nonspecific background staining that was quantitated with irrelevant class-matched MoAbs was subtracted. Absolute values (percent positive sample – percent positive control) are presented.

Hospital Charges

Actual hospital charges for patients were retrieved and discounted to 1985 dollars using actual average annual hospital charge increase rates. No charge for CSF is included in those analyses because during the conduct of these studies, the CSFs were provided without charge because they were not approved by the Food and Drug Administration (FDA). Estimated current charges at our institution associated with the G-CSF dose and schedule used for the 8-day priming would be $2,128, and for the 21 days of G-CSF administered after reinfusion of BM/PBPC would be $12,033, although most patients required courses of therapy less than 21 days; a 15-day course would result in charges of $8,595. The charges associated with the GM-CSF dose and schedule can only be approximated from those of Yeast-derived GM-CSF which is marketed (this study used Chinese hamster ovary derived GM-CSF), and if this were similar would be approximately for the 8-day priming schedules $2,243 and $4,480, and for the 14-day schedule $7,840. The estimated charges associated with GM-CSF for 21 days after transplant would be approximately $6,701; a patient requiring 15-day course as given in this study would be charged $4,797. The costs associated with leukapheresis and cryopreservation of the PBPCs (approximtely $5,000) was included in the charge. The charges associated with BM harvesting and cryopreservation (approximately $6,200) were from a separate hospitalization and performed in all patients, did not meaningfully vary by patient subgroup, and were not included in these charge determinations because this admission is often associated with other cancer treatment charges and events not directly associated with transplant. An average overall increase in hospital charges was used for discounting purposes as a review of a sample of hospital bills and did not indicate any significant change in the charge distributions over the time period examined or major variations in the rate increases between various categories of charges. Hospital charge data for the control patients are performed on a subset of 18 patients, because five other patients were treated on a charge structure that differed from that of the remaining patients.

Statistical Considerations

All pairwise comparisons were tested for significance with the Wilcoxon Rank Sum Test (with continuity corrections). All P values quoted are nominal two-sided and have not been adjusted for multiple comparisons. The number of endpoints tested in Table 4 is 11. There were 16 comparisons tested for significance (some are not shown). Thus, using the Bonferroni correction to protect against falsely declaring a difference between groups in this study, one might want to compare the quoted P values to 0.00284 and declare a difference statistically significant at the .01 level if the P value is below this number. Average values are presented ± standard error.

RESULTS

BM and PB Progenitor Harvesting

A bulk BM harvest was obtained before CSF priming. The numbers of nucleated cells obtained from the BM harvest and the three leukapheresis collections are described in Table 3. During the administration of CSF for priming, the peripheral leukocyte counts increased. The mean peripheral leukocyte counts for patients receiving 8 or 16 µg/kg/d of GM-CSF did not meaningfully differ, increasing from a combined mean 4,680/mm³ ± 270/mm³ to 11,190/mm³ ± 820/mm³ after 8 days of IV cytokine and to 20,566 ± 4,680/mm³ after 14 days of cytokine. G-CSF increased peripheral counts to a mean 39,300/mm³ ± 2,310/mm³ after 8 days of treatment. The average peripheral leukocyte count on each day
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Controls</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>P Value*, v Controls</th>
<th>G6</th>
<th>G7</th>
<th>P Value*, v Controls</th>
<th>G8</th>
<th>G9</th>
<th>P Value*, v Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median days WBC &lt; 100/mm³</td>
<td>6</td>
<td>3.5</td>
<td>.1033</td>
<td>1</td>
<td>1 &lt;0.001</td>
<td>4</td>
<td>.028</td>
<td>2.5</td>
<td>1 &lt;0.001</td>
<td>0.5</td>
<td>2 &lt;0.001</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(4-7)</td>
<td>(3-4)</td>
<td>(0-3)</td>
<td>(0-1.5)</td>
<td>.0041</td>
<td>(4-6)</td>
<td>(1.5-3.5)</td>
<td>(0-1)</td>
<td>.0008</td>
<td>(0-1)</td>
<td>(0-2) .0002</td>
</tr>
<tr>
<td>Median days ANC &lt; 100/mm³</td>
<td>10</td>
<td>7.5</td>
<td>.0072</td>
<td>2.5</td>
<td>1 &lt;0.001</td>
<td>8</td>
<td>.0115</td>
<td>5</td>
<td>3 &lt;0.001</td>
<td>8.6</td>
<td>9 &lt;0.001</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(8-9)</td>
<td>(7-8)</td>
<td>(1.5-5)</td>
<td>(0.5-3)</td>
<td>.0008</td>
<td>(7-8)</td>
<td>(4-5.5)</td>
<td>(1-4)</td>
<td>.0005</td>
<td>(3-3)</td>
<td>(2-9) .0007</td>
</tr>
<tr>
<td>Median days ANC &lt; 200/mm³</td>
<td>11</td>
<td>7.5</td>
<td>.0020</td>
<td>8</td>
<td>6 &lt;0.001</td>
<td>11</td>
<td>.6555</td>
<td>8.6</td>
<td>8 &lt;0.001</td>
<td>9.6</td>
<td>9 &lt;0.001</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(10-12)</td>
<td>(7-9)</td>
<td>(6-8.5)</td>
<td>(6-6.5)</td>
<td>.0632</td>
<td>(10-14)</td>
<td>(6-10.5)</td>
<td>(7-8)</td>
<td>.0023</td>
<td>(9-8)</td>
<td>(8-9) .0007</td>
</tr>
<tr>
<td>Median days in hospital</td>
<td>37</td>
<td>49</td>
<td>.0620</td>
<td>24.5</td>
<td>28 &lt;0.001</td>
<td>39</td>
<td>.8634</td>
<td>41.5</td>
<td>41 .7527</td>
<td>31.5</td>
<td>32 .1690</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(35-54)</td>
<td>(40-82)</td>
<td>(23.5-26)</td>
<td>(25-36)</td>
<td>.0008</td>
<td>(34-41)</td>
<td>(26.5-46)</td>
<td>(31-46)</td>
<td>.5299</td>
<td>(26-36.5)</td>
<td>(29-35) .3593</td>
</tr>
<tr>
<td>Median number of RBC transfusions</td>
<td>22</td>
<td>26.5</td>
<td>.2684</td>
<td>17.5</td>
<td>18 &lt;0.001</td>
<td>19</td>
<td>.0493</td>
<td>30</td>
<td>23 .4677</td>
<td>18.5</td>
<td>18 .7132</td>
</tr>
<tr>
<td>Median number of platelet transfusions</td>
<td>29</td>
<td>38</td>
<td>.2580</td>
<td>9.5</td>
<td>12.5 &lt;0.001</td>
<td>27</td>
<td>.7126</td>
<td>28.5</td>
<td>30 .8598</td>
<td>21.5</td>
<td>19 .4692</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(29-50)</td>
<td>(29-69)</td>
<td>(8.5-16)</td>
<td>(10-16.5)</td>
<td>.0020</td>
<td>(26-28)</td>
<td>(24-35.5)</td>
<td>(29-39)</td>
<td>.5889</td>
<td>(14-28)</td>
<td>(8-29) .7006</td>
</tr>
<tr>
<td>Median number of infections</td>
<td>18.5</td>
<td>16.5</td>
<td>.8333</td>
<td>10</td>
<td>6 &lt;0.001</td>
<td>15</td>
<td>.0138</td>
<td>19</td>
<td>12 .6672</td>
<td>12</td>
<td>11 .9009</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(16-20)</td>
<td>(14-21)</td>
<td>(8-10.5)</td>
<td>(5-7)</td>
<td>.0016</td>
<td>(12-15)</td>
<td>(17-23.5)</td>
<td>(10-21)</td>
<td>.2019</td>
<td>(9-16.5)</td>
<td>(10-19) .8257</td>
</tr>
<tr>
<td>Median number of antibiotics used</td>
<td>5</td>
<td>5</td>
<td>.5836</td>
<td>2</td>
<td>3 &lt;0.001</td>
<td>4</td>
<td>.0235</td>
<td>4</td>
<td>4 &lt;0.001</td>
<td>4</td>
<td>4 &lt;0.001</td>
</tr>
<tr>
<td>(interquartile)</td>
<td>(4-5)</td>
<td>(4-6)</td>
<td>(2-3)</td>
<td>(3-3)</td>
<td>.0027</td>
<td>(4-4)</td>
<td>(4-5)</td>
<td>(3-4)</td>
<td>.0100</td>
<td>(3-3)</td>
<td>(3-5) .5000</td>
</tr>
<tr>
<td>Median hospital charges</td>
<td>$85,845</td>
<td>$123,677</td>
<td>$65,311</td>
<td>$56,734 &lt;0.001</td>
<td>$83,629</td>
<td>$73,92</td>
<td>$106,210</td>
<td>$84,478</td>
<td>$7791</td>
<td>$78,857</td>
<td>$54,610</td>
</tr>
</tbody>
</table>

All treatment group values are expressed as medians with interquartile ranges below.

*Comparison is between schedule G3 and control patients using Wilcoxon Rank Sum Test; bars are placed around comparisons with a nominal P value < .01.
†Comparison is between schedules (G4 and G5) and controls in the first line and G3 in the second line using Wilcoxon Rank Order Statistic.
‡Comparison is between schedule G3 and control patients.
§Comparison is between schedules (G4M and G5M) and control patients in the first line and schedule G3M patients in the second line.
||Comparison is between schedules (G4M and G5M, G6M, and G7M) and control patients in the first line and schedule G6M patients in the second line.
¶Control charges are based on 18 patients (rather than 23) since a change in charge structure was introduced after 5 patients entered that was adopted for all subsequent patients.
of CSF-priming is presented in Fig 1. In this patient population, G-CSF resulted in a more rapid increase in leukocyte counts.

Three leukaphereses of PBPCs resulted in the collection of an average $10.2 \times 10^8$ nucleated cells/kg for patients receiving G-CSF and $4.1 \times 10^8$ nucleated cells/kg for patients receiving GM-CSF during the priming period (Table 3). The range of CFU-GM collected was 0.0 to $72.6 \times 10^4$/kg. There was considerable heterogeneity between individual patients and between individual days of collections for any given patient. There was no one day of collection that clearly and reliably produced better cell or progenitor collection among patients leukapheresed on days 6, 8, or 9.

The total numbers of nucleated cells per kilogram and progenitor and phenotype characterization in the BM collections and the leukapheresis collections are shown in Table 3. The majority of these cells were mononuclear cells with contaminating mature neutrophils $19.3\% \pm 8.6\%$ for GM-CSF primed preparations and $7.9\% \pm 6.0\%$ for leukapheresis collections from patients primed with G-CSF. There is no difference in the number of nucleated BM cells $\times 10^9$/kg that were harvested in any of the subgroups of patients studied. The average number of nucleated PB cells per kilogram harvested in patients receiving G-CSF was approximately 2.5 times greater than the number of nucleated cells $\times 10^9$/kg harvested in patients receiving GM-CSF. However, the number of CFU-GM $\times 10^4$/kg in these PB progenitor collections was at a ratio of 1.4:1, reflecting a higher number of CFU-GM per $10^5$ nucleated cells collected in the GM-CSF primed patients.

Total numbers of CD34, CD33, and CD7 positive cells collected per kilogram are reported in Table 3, and were higher for patients primed with G-CSF than GM-CSF. Examination of dual antigen expression showed that patients receiving G-CSF had a similar phenotype on $6.0\% \pm 3.5\%$ of leukapheresed cells.

**Hematopoietic Reconstitution**

The addition of CSF-primed PBPC resulted in a significant reduction of the period of time of absolute leukopenia, compared with patients receiving BM alone or to patients receiving BM with either G- or GM-CSF. Figure 2 displays the mean leukocyte counts for patients treated with both schedules of PBPCs, as well as for control patients and those receiving BM alone and CSF as support. The period of time in which the leukocyte count is below 100/mm$^3$ is reduced in the patients receiving PBPCs and hematopoietic CSF, either G- or GM-CSF. In addition, there is an enhancement in the rate of hematopoietic reconstitution, indicated by a shift to the left of the reconstitution curve. Of note, G-CSF or GM-CSF (Fig 2D), coupled with BM alone, did not significantly alter the period of time in which the mean leukocyte count was below 100/mm$^3$. With G-CSF, administering the PBPCs on days $-1, 0$, and $+1$ was associated with fewer days with an absolute neutrophil count (ANC) under 100/mm$^3$ or 200/mm$^3$ ($P < .05$) with a trend toward fewer days with white blood cell (WBC) counts under 100/mm$^3$ or 200/mm$^3$. For GM-CSF, administering the PBPCs on days $-1, 0$, and $+1$ resulted in a significant reduction in the number of days the WBC count was below 100/mm$^3$ or 200/mm$^3$ ($P < .02$), with a trend toward fewer days with an ANC count under 100/mm$^3$ or 200/mm$^3$. Thus, we consider administration on days $-1, 0$, and $+1$ to be the optimum schedule.

Table 4 lists the median number of days with a leukocyte count under 100, 200, and the number of days with a median ANC less than 100 or 200 for patients treated with various schedules. In each case, the use of CSF-primed progenitor cells on either schedule significantly reduced the frequency of leukopenic or neutropenic days for patients receiving CSF-primed progenitor cells compared with marrow only controls.
Fig 2. Mean leukocyte counts versus day from BM infusion for all treated patients. In each panel the solid line without markers is the historical controls (n = 23); the standard errors of the observations at days −5, 0, 5, 10, and 15, respectively, are 0.49, 0.20, 0.02, 0.02, 0.14. (A) Schedules G4 (\(\triangledown\) --- \(\triangledown\)) and G5 (\(\bigcirc\) --- \(\bigcirc\)); standard errors for G4 at days −5, 0, 5, 10, and 15, respectively, are 0.97, 0.24, 0.03, 1.0, 5.51; standard errors for G5 at days −5, 0, 5, 10, and 15, respectively, are 0.05, 0.53, 0.03, 1.84, 1.34; (B) schedules GM4 (\(\square\) --- \(\square\)) and GM5 (\(\bigtriangleup\) --- \(\bigtriangleup\)); standard errors for GM4 at days −5, 0, 5, 10, and 15, respectively, are 0.91, 0.23, 0.02, 0.18, 0.72; standard errors for GM5 at days −5, 0, 5, 10, and 15, respectively, are 1.01, 0.46, 0.03, 0.12, 0.42. (C) Schedules GM6 (\(\bigtriangledown\) --- \(\bigtriangledown\)) and GM7 (* --- *); standard errors for GM6 at days −5, 0, 5, 10, and 15, respectively, are 0.82, 0.20, 0.03, 0.02, 0.88; standard errors for GM7 at days −5, 0, 5, 10, and 15, respectively, are 0.52, 0.62, 0.03, 0.17, 0.87. (D) Schedules GM3 (\(\bigtriangleup\) --- \(\bigtriangleup\)) and G3 (\(\bigtriangledown\) --- \(\bigtriangledown\)); standard errors for GM3 at days −5, 0, 5, 10, and 15, respectively, are 0.55, 0.18, 0.01, 0.02, 0.17; standard errors for G3 at days −5, 0, 5, 10, and 15, respectively, are 0.77, 0.25, 0.02, 0.03, 0.25, 0.95.
and compared with patients receiving BM and CSF after high-dose chemotherapy. Again of note, the use of CSF with BM (schedules G3 and GM3) did not significantly reduce the periods of absolute leukopenia and neutropenia with the exception of the median number of days with a WBC count under 200 and with an ANC under 100 for patients with G-CSF, or the median number of days with a WBC count under 200 for GM-CSF.

Figure 2 displays the hematopoietic recovery in terms of the average leukocyte count as a function of the day from BM reinfusion. Figures 2A and 2B show the hematopoietic reconstitutions for Schedules G4 or G5 (Fig 2A) or GM4 and GM5 (Fig 2B), each compared with patients receiving BM alone without CSF (group 1). There was a significant reduction in the number of days with a WBC count under 100 or 200 compared with patients receiving either BM alone, or BM plus CSF but no PBPCs (GM3, G3) (Table 4). The pattern of hematopoietic reconstitution using hematopoietic progenitors collected with schedules GM6 or GM7 did not appear to differ from the results obtained with the other schedules of GM-CSF primed PBPC.

Other Measures of Clinical Benefit

Table 4 presents the median values, interquartile ranges, and the results of statistical comparisons for additional clinical parameters of patient care, including days in the hospital, days in the BM transplant unit, number of RBC and platelet transfusions, number of days on antibiotic therapy, the number of antibiotics used, and the median hospital charges discounted to 1985 dollars. The data indicate that for these patients of similar diagnosis and treatment, the use of G- or GM-CSF by itself, coupled with BM, did not in general significantly influence the parameters measured. The data do show that there is a highly significant decrease in each of the measured parameters in patients who received G-CSF primed PBPC compared with BM only control populations and to BM plus G-CSF patients control patients. Significant reductions in number of days in the Bone Marrow Unit were seen with all schedules of GM-CSF primed progenitor cells and, as well, in the median number of antibiotics used compared with control populations for GM-CSF.

DISCUSSION

After high-dose chemotherapy and autologous BM support, there is a profound and lengthy period of leukopenia and neutropenia during which most of the infections and other complications of transplant occur. The recent molecular cloning, in vitro expression, and formulation of the hematopoietic CSFs have offered the potential of accelerating hematopoietic recovery in this patient population. We and others have previously reported that both G- and GM-CSF accelerate hematopoietic recovery following high-dose chemotherapy and autologous BM support. Nevertheless, we and others have noted that despite the use of G- or GM-CSF after transplantation, there remains a period of absolute leukopenia that persists for approximately 8 days before evidence of hematopoietic recovery. Thus, the ability of G-CSF or GM-CSF to accelerate hematopoietic recovery occurs only after this obligate period of absolute leukopenia. The presumed mechanism for this delay in recovery relates to the limited number of late-committed progenitors present after high-dose chemotherapy and BM infusion that are able to proliferate and differentiate in response to exogenously administered G- or GM-CSF. Hence, after reinfusion of autologous marrow, replication and expansion of both the early stem cell and early progenitor compartments must occur before committed progenitors would become responsive to exogenously administered G-CSF or GM-CSF. In the studies reported here, we tried to overcome this period of absolute leukopenia by using large numbers of committed progenitors derived by leukapheresis that were collected from the patient after administration (“priming”) with CSF and who were leukapheresed during a period in which large numbers of committed progenitors are circulating in the PB.

The data presented here indicate that the addition of CSF-primed PBPCs with BM can accelerate hematopoietic recovery and effectively eliminate the period of absolute leukopenia after high-dose chemotherapy and autologous BM support. The use of G-CSF or GM-CSF in patients after hematopoietic recovery from standard-dose chemotherapy allows collection of large numbers of committed hematopoietic progenitors that are capable of accelerating hematopoietic recovery compared with BM alone or BM with either GM-CSF or G-CSF. In addition to the reduction of leukopenia there was also a significant reduction in the duration of neutropenia, below 100 or 200/mm³, although substantial periods of neutropenia were still seen in all patients treated. In this study, the PBPCs were administered over a 3-day period in an effort to minimize toxicity from infusion of large numbers of cells and to distribute over time the “mature” progenitors and thereby minimize the chance for exhaustion of the mature progenitor pool before full marrow recovery. Other investigators have infused BM and the PBPCs on a single day and achieved similar results.

This study was designed to attempt to collect committed PB progenitors that would be capable of responding to GM-CSF or G-CSF when infused. The finding that the leukapheresis collections contained large numbers of CD34+ cells, and particularly CD34+/CD33- cells, a phenotype marking early hematopoietic progenitor cells, was unexpected and is of potential clinical utility. Whether these cells would be capable of providing “stem cell” function is not addressed by these studies and should not be assumed. All patients treated also received BM presumed to provide stem cell recovery; whether the PB cells collected in the manner used alone would result in some patients having late graft failure is unknown and possible. Further, the design of this study does not address other important issues such as whether postinfusion growth factors are necessary given the large number of PBPCs that were administered. Whether sufficient levels of endogenous cytokine are present posttransplant to permit the survival and proliferation of these progenitors is unstudied.

Investigators have shown previously that the use of GM-CSF could cause a release into the PB of large numbers of committed progenitors responsive to further expansion with G- or GM-CSF. Further, Siena et al and Gianni et al have shown that PBPCs obtained during recovery from an intensive chemotherapy program, coupled with simultaneous
administration of GM-CSF, could accelerate hematopoietic recovery in patients treated with autologous BM transplantation. These studies have shown marked reduction in the period of absolute leukopenia, as well as a reduction in the requirements for platelet transfusions in patients treated with these treatment modalities. Nonetheless, the collection of hematopoietic progenitors in this manner is cumbersome and complex in that leukapheresis must be performed during a period when increased numbers of CD34-positive hematopoietic progenitors circulate. This produces a technical requirement for the ability to perform leukapheresis at not completely predictable intervals following the initiation of chemotherapy with the ability to monitor CD34 and/or hematopoietic progenitors to provide a guide to the optimum time for apheresis.

Because of the technical difficulties of performing leukapheresis during periods of chemotherapy rebound, we chose to study the ability of CSFs to prime for the collection of hematopoietic PBPCs after full hematopoietic recovery from a prior course of standard-dose chemotherapy.

The optimal time for administration of the CSF-primed progenitor cells seems to be on days −1, 0, and +1, which appears to offer more rapid hematopoietic reconstitution than administering the cells on days +1, +2, and +3. This is consistent with the premise that it is the CSF-primed progenitor cells that affect early hematopoietic reconstitution, because providing precursor cells responsive to the cytokine would be predicted to shift the hematopoietic reconstitution curve in this manner and, as well, reduce the period of absolute leukopenia more efficiently. The provision of the CSF-primed progenitor cells on several sequential days was done to minimize toxicity related to product infusion and to divide the mature progenitors over several days to increase the probability of circulating effector cells maturing from these progenitors over a several-day period. Other studies using a single-day infusion schedule of BM and PBPCs did not report toxicity.

In this study, the patients treated were of similar diagnosis and received similar prior chemotherapy before entry into these studies. All but two of the patients who received CSFs, either alone or in combination with PBPCs, had received prior chemotherapy for metastatic disease using one of two programs (doxorubicin, 5-fluorouracil and methotrexate [AFM], or cyclophosphamide, doxorubicin, and 5-fluorouracil [CAF]). All the patients had progenitors collected at a steady state, after full hematopoietic reconstitution. In our observations, despite the relatively small numbers of patients studied, the results were dramatic. Hematopoietic reconstitution was accelerated, and the use of G-CSF primed PBPCs resulted in improvement in major parameters of clinical follow-up, including antibiotics, transfusion usage, hospital days, and, as a final common denominator, discounted hospital charges.

The identification of this simple technique for collection of PBPCs may in part account for the more rapid and sustained engraftment seen.

In a large, randomized study in lymphoma where the preparative chemotherapy was different, the use of GM-CSF posttransplant by itself has been shown to reduce leukopenia, antibiotic usage, and hospitalization requirements. Our results, which are non-contemporaneous and non-randomized, do not display these effects at the level of statistical significance (P < .01), although similar trends to the previous studies are present (Table 4). Further, our results differ in one way from those of Siena et al, in which GM-CSF primed hematopoietic progenitors were collected during recovery from high-dose cyclophosphamide; in that study, hematopoietic reconstitution was improved and platelet transfusion requirements were reduced, whereas in ours, platelet transfusions were not significantly affected. Results from Sheridan et al report similar findings to ours for G-CSF–primed progenitors. Our data differs from that of Gulati and Bennett, who showed in a small series selected from a randomized trial reduction in neutropenia duration, platelet transfusion dependence, and adjusted hospitalization costs with E.coli GM-CSF alone administered after high-dose chemotherapy in patients with relapsed Hodgkin’s disease. The cause of these differences is unclear, but may result from differences in the patient’s disease, the method of collecting progenitor populations, the preparative chemotherapy, prior chemotherapy experience, type of GM-CSF used, methods of cost accounting, or other factors.

The data contained in this report provide clear evidence that the use of these CSF-primed progenitors can effectively eliminate the period of absolute leukopenia after high-dose...
chemotherapy and autologous BM support. Further, the data provide evidence that meaningful reduction in the periods of time of hospitalization, intensive care, and isolation, as well as the number of RBC and platelet transfusions, occurred with the use of G-CSF primed PBPCs. Antibiotic usage according to our protocols, as well as the number of antibiotics used, was substantially reduced in patients receiving G-CSF primed progenitor cells. An estimate of the cumulative financial impact of these findings derives from the observation that median hospital charges were reduced approximately $30,000 (in 1985 dollars) for patients receiving G-CSF primed PBPCs, coincident with the reduction in the number of days in hospital and the reduced number of platelet and RBC transfusions. The charges associated with therapy will vary from institution to institution but are presented here from an institution with a stable charge structure to provide an estimate of the magnitude of toxicity reduction associated with the use of the CSF and PBPCs. This approach has been used in other settings at our center to provide a useful estimate of acute morbidity. These parameters provide objective evidence supporting the clinical observations of reduced toxicity in patients receiving CSF primed PBPCs.

ACKNOWLEDGMENT

We particularly thank Drs Dagmar Oette (Sandoz) and Larry Souza (Amgen) for their help in the conduct of these studies. We thank the many members of the Bone Marrow Transplant Team for their help in the conduct of these studies.

REFERENCES


Comparative effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on priming peripheral blood progenitor cells for use with autologous bone marrow after high-dose chemotherapy

WP Peters, G Rosner, M Ross, J Vredenburgh, B Meisenberg, C Gilbert and J Kurtzberg