Peripheral blood cells (PBC) can hasten hematopoietic recovery after high-dose chemotherapy. To determine if PBC apheresis after mobilization further enhance hematopoietic recovery over that achieved with autologous bone marrow (ABM) and recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF), 14 patients with metastatic solid tumors were supported by ABM and rhGM-CSF during the first course of high doses of cyclophosphamide, etoposide, and cisplatin (CVP) and 11 of these 14 patients by mobilized PBC with ABM and rhGM-CSF during the second CVP. Each patient served as his or her own control. Identical doses of CVP were administered in both courses: cyclophosphamide 5.25 g/m², etoposide 1,200 mg/m², and cisplatin 165 to 180 mg/m². PBC were collected on day 10 after mobilization with cyclophosphamide (3 g/m²) intravenously (IV) on day 1, doxorubicin (50 mg/m²) as a continuous IV infusion over 48 hours starting day 2, and rhGM-CSF as a daily 4-hour IV infusion starting day 4 at 0.8 mg/m² for 14 days. Comparing recovery in the 11 patients to receive two cycles of therapy, the median days to an absolute neutrophil count of 0.1 × 10⁹/L and 0.5 × 10⁹/L were not statistically significant between the two courses; neither was there a difference in the incidence of fever and bacteremia. The median number of days to platelet count of 0.02 × 10¹²/L unmaintained by platelet transfusion was 20 from marrow infusion for course 1 and 16 for course 2 (P = .059). The median number of days to a platelet count of 0.05 × 10¹²/L was significantly shortened: 24 and 19 days for courses 1 and 2, respectively (P = .045). Patients who received PBC required fewer number of platelet transfusions. Extramedullary toxicities were not different between the groups. Our finding of enhanced early recovery of platelets and reduced platelet transfusion requirement is in concordance with other studies.

© 1992 by The American Society of Hematology.
units (CFU-GM) and to reduce the period of neutropenia during the priming therapy.

Since 1986, we have treated patients with solid tumors using double high-dose cyclophosphamide, etoposide, and cisplatin (CVP) with meaningful response rates and a significant proportion of long-term disease-free survivors.25-27 We therefore used this double transplant approach as a model that would allow us to study the effects of addition of rhGM-CSF (first and second courses) and PBC (second course) to ABM in each patient.

MATERIALS AND METHODS

Patients. Patients with histologically confirmed metastatic solid tumors were entered into the study from March 1989 to February 1990. Patients with estrogen receptor (ER)-negative breast cancer or hormonal refractory ER-positive breast cancer were eligible. All patients had a performance status (Zubrod scale) ≤2. All patients had an ejection fraction of ≥50% by cardiac scan, forced expiratory volume in 1 second and a diffusion capacity ≥50% predicted; a serum creatinine ≤135 μmol/L (1.5 mg%) and a creatinine clearance of at least 1.00 mL/s (60 mL/min), and a total bilirubin of less than 35 μmol/L (2 mg%). All patients were negative for hepatitis-B surface antigen and human immunodeficiency viral antibody. Patients with histologic evidence of tumor cells in the BM were excluded.

Patients had not been previously exposed to nitrosourea or mitomycin. The last chemotherapy, radiotherapy, or immunotherapy must have been completed at least 3 weeks before entry into this study. All patients were able to undergo general anesthesia for bilateral BM harvesting and all had central venous access for PBC collection.

Pretreatment evaluation. All patients had history and physical examination, complete blood count, differential count, platelet count, chest radiograph, pulmonary function tests including diffusion capacity, multigated cardiac scan, electrocardiogram, bilateral BM aspirates and biopsies, 24-hour creatinine clearance, and appropriate tumor markers. All patients were restaged before entry into the study. Computerized tomography of the thorax and/or abdomen when deemed necessary were obtained to measure sites of the disease.

BM harvest and storage. Technique was as previously described.28-30 Before PBC mobilization, all patients had 800 to 1,000 mL of BM harvested from bilateral posterior iliac crests under general anesthesia. This was adequate for two courses of high-dose chemotherapy. At least 2 x 10^11 nucleated cells/kg body weight and a minimum post thaw in vitro colony growth of 2 x 10^4 CFU-GM/kg were required for entry into the study.

PBC mobilization, collection, and storage. All patients had double lumen central venous catheter (Quinton Instrument Company, Seattle, WA) inserted before priming therapy. Priming agents consisted of cyclophosphamide 3 g/m² intravenously (IV) on day 1 and doxorubicin at 50 mg/m² infusion general anesthesia over 48 hours starting day 2. On day 4, rhGM-CSF (GM-CSF; Schering, Kenilworth, NJ) was started as a 4-hour IV infusion by portable infusion pump (Travenol Laboratories, Incorporated, Deerfield, IL) at a dose of 0.6 mg/m² for 14 days. When leukocyte count reached 20 x 10^9/L, the dose of rhGM-CSF was reduced by 50% each day as long as the leukocyte count was maintained above 20 x 10^9/L. PBC collection started on day 10 to 14 of chemotherapy on 4 consecutive weekdays, each collection lasting for 4 to 5 hours.

PBC were collected from patients using continuous flow blood cell separator COBE Spectra (COBE Laboratories, Incorporated, Lakewood, CO) and Fenwal CSS3000 (Baxter Health Care Products, Deerfield, IL). Procedure parameters included the use of acid-citrate dextrose-A anticoagulant at flow rates calculated to be 1.4 times the patient's blood volume (calculated from height, weight, and sex). The ratio of acid citrate dextrose to whole blood was 1:9. Plasma flow rates were computed from patient's hematocrit. The volume of blood processed was twice the patient's calculated blood volume and ranged from 6.0 to 12.0 L. Continuous calcium chloride, diluted in normal saline was infused IV through a rate controlled pump (IMED Corporation, San Diego, CA) to prevent citrate-induced hypocalcemia. After completion of PBC collection, the Quinton catheter was changed to a double lumen subclavian catheter in preparation for high-dose CVP.

CFU-GM colony assay. BM CFU-GM were determined by culturing 1 x 10^7 nucleated cells in a double layer semisolid system described.31 The assay was performed in triplicates and sealed in boxes in a humidified atmosphere at 5% CO₂, 12% O₂, and 83% N₂ at 37°C. After 10 days, aggregates of greater than 40 cells were counted as colonies. Peripheral blood CFU-GM assay was as previously described.28

Treatment plan. As approved by the Institutional Review Board, written informed consents were obtained from all patients. Patients were nursed in private rooms. Vigorous hydration to maintain a high urine output was started 24 hours before chemotherapy and continued for 24 hours after the last day of chemotherapy. Cyclophosphamide 1.75 g/m² in 250 mL 5% dextrose in water was administered IV over 1 hour on days 1, 2, and 3; etoposide 400 mg/m² in normal saline was infused over 4 hours every 12 hours on days 1, 2, and 3; and cisplatin 60 mg/m² in 500 mL of normal saline with 40 g mannitol, 30 mEq KCl, and 8 mEq MgSO₄ was infused over 2 hours on days 1, 2, and 3. After the third patient, the dose of cisplatin was reduced to 55 mg/m² because of the frequent occurrence of peripheral neuropathy in another study using this same regimen. Antiemetics were routinely administered before and during high-dose CVP. The second course was administered as soon as the patient's ANC was greater than 1.5 x 10^9/L, the platelet count was greater than 0.1 x 10^12/L, and after all the extramedullary toxicities from course one had resolved.

A group of patients treated with similar doses of CVP and supported with ABM infusion alone served as our historical control.29

Stem cell infusion and supportive care. All patients were premedicated with hydrocortisone and diphenhydramine 30 minutes IV before autologous marrow infusion. Half of the cryopreserved ABM was rapidly thawed to 37°C at the patient's bedside and was infused on day 6 of each course of chemotherapy. On day 5 of each course of CVP, patients received rhGM-CSF at a dose of 0.6 mg/m² IV over 4 hours until the ANC of 1.5 x 10^9/L was maintained for 2 consecutive days, then the dose of rhGM-CSF is halved every day as long as ANC was maintained above 1 x 10^9/L. If the ANC decreased below 1 x 10^9/L when rhGM-CSF was tapered off, the dose of rhGM-CSF will be doubled on a daily basis until the ANC reached above 1 x 10^9/L for 2 consecutive days, then gradually reduced. In the second course on day 6 from start of chemotherapy, in addition to the ABM and rhGM-CSF, all of the PBC was rapidly thawed to 37°C, and infused to the patients.

All blood products were irradiated with 25 cGy. Patients' hemoglobin were kept above 80 g/L with packed red blood cell (RBC) transfusion and platelets above 0.02 x 10^12/L with single-donor or random-donor platelet transfusions. At the initiation of chemotherapy, all patients were placed on prophylactic trimethoprim-sulfamethoxazole and ketoconazole. During the period of neutropenia, patients with temperature was 38.5°C or higher were started immediately on vancomycin and a third-generation cephalosporin.

Statistical methods. Median days to recovery of a given ANC and platelet count and the difference in transfusion frequency for
patients in the two arms of the study were compared using the Wilcoxon signed rank. Differences in the characteristics of patients between the two arms and difference in the number of febrile days and episodes of bacteremia were analyzed by χ² analysis. A P value < .05 was considered significant.

RESULTS

Fourteen patients were registered in the study. Three patients did not receive the second course of high-dose CVP because of severe toxicity in one, progressive disease in the second, and refusal by the third patient to continue in the study, leaving 11 patients evaluable for a comparison of hematopoietic recovery for the two courses. Tumor types of the evaluable patients were as follows: breast cancer 5, lung cancer 3, sarcoma 1, melanoma 1, and adenocarcinoma of unknown primary 1. The median age of the group was 46 (range, 35 to 65). The median number of prior cycles of chemotherapy was three (range, 0 to 14); one patient had prior radiation. One patient underwent two priming courses because of inadequate mononuclear cells collected. All patients underwent apheresis and tolerated PBC collection without complications. The median number of apheresis was five (range, 4 to 6); each collection lasted for an average of 4 hours. The median number of weeks between the two courses for the whole group was 5 weeks.

With the priming course, the median number of days to a leukocyte count (white blood cell [WBC]) of $1.0 \times 10^{9}$/L was 14, to an ANC of $0.1 \times 10^{9}$/L and to $0.5 \times 10^{9}$/L were 14 and 15 days, respectively. Three patients had platelet count decreased to $\leq 0.02 \times 10^{12}$/L.

Hematopoietic recovery of the two courses are shown in Table 1. The median numbers of days to an ANC of $0.1 \times 10^{9}$/L, $0.5 \times 10^{9}$/L, and $1.0 \times 10^{9}$/L from first day of chemotherapy were 16, 18, and 21 (10, 12, and 15 from the day of ABM infusion, day 0) with course 1 of high-dose CVP and 16, 17, and 18 (10, 11, and 12 from ABM + PBC) for course 2 of CVP.

Twenty-three historical controls with comparable characteristics were treated in course 1 with identical doses of CVP with ABM support alone.29 When comparing recoveries during the first course using ABM + rhGM-CSF with our historical group of patients, the neutrophil recovery to $0.1 \times 10^{9}$/L, $0.5 \times 10^{9}$/L, and $1.0 \times 10^{9}$/L from the first day of chemotherapy were 3 days earlier (19, 21, and 24, respectively) than with ABM alone. With ABM + rhGM-CSF + PBC, the median numbers of days to an ANC of $0.1 \times 10^{9}$/L, $0.5 \times 10^{9}$/L, and $1.0 \times 10^{9}$/L was 1, 3, and 4 days earlier than with ABM alone.29

The median number of days to platelet count of $0.02 \times 10^{12}$/L unmaintained by platelet transfusion was 20 from first day of chemotherapy (14 from ABM) for course 1 and 16 (10 from ABM + PBC) for course 2, which did not reach statistical significance ($P = .59$). The median number of days to a platelet count of $0.05 \times 10^{12}$/L was statistically significant; for course 1, 24 days (18 from ABM) and for course 2, 19 days (13 from ABM) ($P = .045$). Platelet recovery to $0.10 \times 10^{12}$/L was 26 and 29 days for course 1 and course 2, respectively ($P = .575$). At our institution, 4 U of random-donor platelets constituted one transfusion. In this study, all patients uniformly received 4 U of random-donor platelets for each transfusion. The median number of platelet transfusions (random-donor and single-donor platelets) as shown in Table 2 were five for course 1 (in all patients and also in those to receive two courses) and three for course 2 ($P < .05$). Except for three patients in the second course, all patients had shorter duration of platelet count less than $0.02 \times 10^{12}$/L and less than $0.05 \times 10^{12}$/L during the second course than on the first course.

Seven of the 11 patients required fewer platelet transfusions in the second course than in the first, three required more, and one patient the same. The median number of packed RBC transfusions was four for both courses in the 11 patients who received both cycles (Table 2). Platelet recovery supported with ABM + rhGM-CSF in course 1 was not significantly different when compared to the historical group supported with ABM alone in course 1 (median day to a platelet count of $0.02 \times 10^{12}$/L, $0.05 \times 10^{12}$/L, and $0.10 \times 10^{12}$/L were 19, 21, and 24, respectively). We have previously reported the consistent delayed platelet recovery after CVP of the same dose intensity as the current study with ABM support alone in course 229 with median day to platelet count of $0.02 \times 10^{12}$/L, $0.05 \times 10^{12}$/L, and $0.10 \times 10^{12}$/L being 22, 26, and 35, respectively, which are 6, 7, and

### Table 1. Hematopoietic Recovery

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Course 1</th>
<th>Course 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP + ABM</td>
<td>0.1 x 10^9/L</td>
<td>16 (13-20)</td>
</tr>
<tr>
<td>CVP + ABM</td>
<td>0.5 x 10^9/L</td>
<td>18 (17-20)</td>
</tr>
<tr>
<td>CVP + ABM</td>
<td>1.0 x 10^9/L</td>
<td>21 (17-29)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Course 1</th>
<th>Course 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP + ABM</td>
<td>0.02 x 10^12/L</td>
<td>20 (16-28)</td>
</tr>
<tr>
<td>CVP + ABM</td>
<td>0.05 x 10^12/L</td>
<td>24 (18-31)</td>
</tr>
<tr>
<td>CVP + ABM</td>
<td>0.10 x 10^12/L</td>
<td>26 (20-35)</td>
</tr>
</tbody>
</table>

Abbreviation: GM, rhGM-CSF.

*From the first day of chemotherapy.
6 days later than the platelet recovery in this study during the second course using ABM + rhGM-CSF + PBC (Table 1). There were no major episodes of bleeding.

During the first course, the median number of cells infused in the BM was $1.5 \times 10^8$/kg and the median number of CFU-GM infused was $3.5 \times 10^4$/kg. During the second course, the median number of cells infused in the BM was $1.6 \times 10^8$/kg and the median number of CFU-GM infused was $3.2 \times 10^4$/kg and $0.75 \times 10^4$/kg. Nonhematologic toxicities of high-dose CVP with rhGM-CSF and stem cell support were not different. In decreasing order, nausea and vomiting, diarrhea, and mucositis were among the most common toxicities. There were no treatment-related deaths. The median number of febrile days were 7 (range, 1 to 19) and 7 (range, 0 to 15) for courses 1 and 2, respectively. There was one episode of gram-negative bacteremia and two episodes of gram-positive bacteremia during the priming therapy. There were three gram-negative bacteremia in each of the courses; 5 and 2 gram-positive infections during courses 1 and 2, respectively. No fungal infection occurred during the first course, whereas there were two episodes of fungemia during the second course. Both patients had no major organ involvement by fungus and infection cleared with the administration of Amphotericin-B.

**DISCUSSION**

Fever and infection are directly related to the length of time patients have an ANC of less than $0.1 \times 10^9$/L.\textsuperscript{20} Although most studies report ANC recovery to $0.5 \times 10^9$/L, the most crucial period in neutrophil recovery for resolution of fever after high-dose chemotherapy is probably that to greater than $0.1 \times 10^9$/L. The onset of fever generally occurs during the first 2 to 5 days of absolute neutropenia. The usual pattern of neutropenia is a decrease of the ANC to below $0.1 \times 10^9$/L on day 8 to 10 after the initiation of high-dose chemotherapy, which is 2 days after ABM infusion followed by an increase to $0.1 \times 10^9$/L on day 19 to 21 after chemotherapy. Hence, the absolute neutropenic period ($0.1 \times 10^9$/L) is approximately 9 to 13 days. Fever and serious infections occur before the reappearance of the first neutrophil. Therefore, the goal of ameliorating fever and infection would be best served if the absolute neutropenia ($0.1 \times 10^9$/L) could be abrogated. There is no doubt that growth factors hasten neutrophil recovery over that of ABM infusion alone. They accelerate predominantly the terminal part of neutrophil recovery (ANC to $1 \times 10^9$/L). Our trial,\textsuperscript{22} as well as those of others,\textsuperscript{19,20,31} using growth factors with ABM after high-dose chemotherapy, showed faster recovery of ANC to $0.5 \times 10^9$/L and $1 \times 10^9$/L when compared with controls; however, incidence of febrile episodes and infection was largely unchanged. One study reported a decrease in episodes of bacteremia, although the recovery of ANC to $0.1 \times 10^9$/L was similar to that of control patients.\textsuperscript{23} The majority of the studies, except that of Nemunaitis et al,\textsuperscript{32} demonstrated no benefit on platelet recovery. With growth factor, early recovery of ANC by only 4 days still leaves a persistent period of approximately 7 to 8 days of absolute neutropenia.

PBC not only can reconstitute hematopoiesis in lieu of ABM after high-dose chemotherapy, but when collected in a timely fashion after expansion of the progenitor cells can also accelerate hematopoietic recovery. This finding has generated interest in the use of PBC in conjunction with ABM and growth factors in the transplant setting. Gianni et al reported the near attenuation of the absolute neutropenic period after high-dose melphalan and total body irradiation with a combination of ABM, rhGM-CSF, and mobilized PBC collected during the overshoot of hematopoiesis from high-dose cyclophosphamide and rhGM-CSF.\textsuperscript{10} With this background, we designed this protocol to determine in a comparative fashion the contribution of PBC over that of ABM and growth factors within the same patients in the hematopoietic recovery after high-dose chemotherapy. Taking advantage of our extensive experience with double high-dose CVP and with the knowledge of the pattern of hematopoietic recovery with this regimen,\textsuperscript{29} we used ABM and rhGM-CSF with the first CVP while applying primed PBC in addition to ABM and rhGM-CSF with the second CVP. We have previously shown in a randomized study of ABM versus endogenous recovery (no-ABM) using double CVP that ABM enhanced neutrophil recovery, particularly the early component (ANC to $0.1 \times 10^9$/L), with no significant clinical impact on febrile neutropenic episodes. There was a trend, although not significant, towards more rapid neutrophil recovery to $0.5 \times 10^9$/L and $1 \times 10^9$/L on the ABM arm during the second CVP, suggesting that indeed a transfusion product can overcome the depletion of endogenous myeloid progenitor cells. In contrast, there was a consistent delay in platelet recovery with the second CVP with or without ABM.\textsuperscript{29} Because of the favorable reports of PBC on platelet recovery,\textsuperscript{2,10} it was hoped that by adding mobilized PBC to the other stem cells (ABM and rhGM-CSF), we could shorten not only the period of absolute neutropenia but also the period of thrombocytopenia and thereby decrease platelet transfusion and avoid bleeding complications and platelet alloimmunization, which develops with multiple platelet transfusions.

In this study, we confirmed our initial conjecture and Gianni et al’s impression that mobilized PBC enhanced the early part of platelet recovery with resultant reduction in platelet transfusion requirement. The fact that rhGM-CSF with ABM did not hasten platelet recovery over that of patients treated with same regimen with ABM support alone and the finding of significantly rapid platelet recovery in the second course, which in our historical controls using ABM alone was consistently delayed, suggest that the positive effect may be that produced by PBC rather than that by rhGM-CSF. We, however, failed to demonstrate any statistically significant difference in neutrophil recovery and reduction in the incidence of infection.

This transplant approach is not without problems. The performance of repeated aphereses can be costly. The therapy for mobilization may not always be appropriate for the treatment of the disease. The timing of the collection of PBC may be off resulting in unnecessary aphereses. Siena et
al described a direct immunofluorescence assay for circulating hematopoietic progenitor cells (CD34/CD33). This could aid investigators in the appropriate time and duration of apheresis. The timing of the infusion of PBC in relation to ABM infusion is also crucial and has not yet been definitely established. Another concern is tumor contamination of the PBC. Although tumor cells could not be detected in the PBC harvest by one group, another study suggested tumor contamination of PBC collections in a quarter of their patients. However, there was no correlation between bone marrow involvement and contamination of PBC in the latter study. Long-term follow-up of patients who undergo PBC transplantation is warranted. The next important question is will the enhancement of platelet recovery continue to be an issue and will the gain of lesser platelet transfusions be worth the risk of possible tumor cell reinfusion and other potential negative factors that have yet to surface with the increased use of PBC. The use of newer growth factors may circumvent this problem. Refinement of stem cell support requires true and properly designed comparative trials that will also have to account for all the variables in supportive care such as methods of nursing reinfusion and other potential negative factors that have yet to surface with the increased use of PBC.

REFERENCES

15. Cline MJ, Golde DW: Mobilization of hematopoietic stem cells (CFU-U) into the peripheral blood of man by endotoxin. Exp Hematol 5:186, 1977


From www.bloodjournal.org by guest on October 23, 2017. For personal use only.
Influence of mobilized peripheral blood cells on the hematopoietic recovery by autologous marrow and recombinant human granulocyte-macrophage colony-stimulating factor after high-dose cyclophosphamide, etoposide, and cisplatin

SD Huan, J Hester, G Spitzer, JC Yau, FR Dunphy, RO Wallerstein, K Dicke, V Spencer, CF LeMaistre and BS Andersson