Mobilization of Peripheral Blood Progenitor Cells by Chemotherapy and Granulocyte-Macrophage Colony-Stimulating Factor for Hematologic Support After High-Dose Intensification for Breast Cancer


High-dose therapy with autologous marrow support results in durable complete remissions in selected patients with relapsed lymphoma and leukemia who cannot be cured with conventional dose therapy. However, substantial morbidity and mortality result from the 3- to 6-week period of marrow aplasia until the reinforced marrow recovers adequate hematopoietic function. Hematopoietic growth factors, particularly used after chemotherapy, can increase the number of peripheral blood progenitor cells (PBPCs) present in systemic circulation. The reinfusion of PBPCs with marrow has recently been reported to reduce the time to recovery of adequate marrow function. This study was designed to determine whether granulocyte-macrophage colony-stimulating factor (GM-CSF)–mobilized PBPCs alone (without marrow) would result in rapid and reliable hematopoietic reconstitution. Sixteen patients with metastatic breast cancer were treated with four cycles of doxorubicin, 5-fluorouracil, and methotrexate (AFM induction). Patients responding after the first two cycles were administered GM-CSF after the third and fourth cycles to recruit PBPCs for collection by two leukapheresis per cycle. These PBPCs were reinjected as the sole source of hematopoietic support after high doses of cyclophosphamide, thiopeta, and carboplatin. No marrow or hematopoietic cytokines were used after progenitor cell reinfusion. Granulocytes ≥500/μL was observed on a median of day 14 (range, 8 to 57). Transfusion independence of platelets ≥20,000/μL occurred on a median day of 12 (range, 8 to 134). However, three patients required the use of a reserve marrow for slow platelet engraftment. In retrospect, these patients were characterized by poor baseline bone marrow cellularity and poor platelet recovery after AFM induction therapy. When compared with 29 historical control patients who had received the same high-dose intensification chemotherapy using autologous marrow support, time to engraftment, antibiotic days, transfusion requirements, and lengths of hospital stay were all significantly improved for the patients receiving PBPCs. Thus, autologous PBPCs can be efficiently collected during mobilization by chemotherapy and GM-CSF and are an attractive alternative to marrow for hematopoietic support after high-dose therapy. The enhanced speed of recovery may reduce the morbidity, mortality, and cost of high-dose treatment. Furthermore, PBPC support may enhance the effectiveness of high-dose therapy by facilitating multiple courses of therapy.

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in untreated patients and 63-fold if administered during hematopoietic recovery after the administration of chemotherapy.\textsuperscript{21,22} Gianni et al have shown that patients who received peripheral mononuclear cells (mobilized with cyclophosphamide with or without GM-CSF) and marrow rapidly recovered both myeloid and platelet function after high-dose melphalan and total body irradiation.\textsuperscript{23} This study was designed to determine whether sufficient numbers of PBPCs mobilized with GM-CSF and chemotherapy could be collected to provide reliable hematopoietic reconstitution without the use of marrow.

**MATERIALS AND METHODS**

**Eligibility**

Patients with histologically documented metastatic breast cancer, aged 18 to physiologic 55 years, and performance status 0 to 1 were eligible. The following laboratory parameters were required: WBC count \( \geq 3,000/\mu\text{L} \), platelet count \( \geq 100,000/\mu\text{L} \), creatinine clearance \( \geq 60 \text{ mL/min} \), bilirubin and aspartate aminotransferase (AST) \( \leq 1.5 \times \text{normal} \), and left ventricular ejection fraction greater than 50%. Those with tumor involving central nervous system (CNS) or marrow were excluded, as were those with prior pelvic radiotherapy or cumulative doxorubicin of over 240 mg/m\(^2\) (reduced to 180 mg/m\(^2\) midstudy). All patients gave written informed consent.

**Schema**

Induction doxorubicin, 5-fluorouracil, and methotrexate (AFM) was administered every 3 weeks for four cycles (Table 1). Each cycle consisted of doxorubicin 25 mg/m\(^2\)/d on days 3 through 5 by bolus infusion, 5-fluorouracil 750 mg/m\(^2\)/d on days 1 through 5 by continuous infusion, and methotrexate 250 mg/m\(^2\)/d on day 18 with leucovorin rescue.\textsuperscript{24} Due to severe mucositis and prolonged nadirs in the first six patients, the induction regimen was adjusted to deliver doxorubicin by continuous infusion on days 1 through 3 and 5-fluorouracil 600 mg/m\(^2\)/d on days 1 through 5 by bolus infusion. Induction therapy was held if the WBC was less than 3,000/\mu\text{L}, or platelets less than 75,000/\mu\text{L} on the day of treatment. Doxorubicin was decreased by 10% if the previous course was complicated by documented leukopenic infection. Methotrexate was withheld if grade 2 to 4 mucositis was present on day 18. For grade 4 oral ulceration or diarrhea, 5-fluorouracil was decreased by 25%.

GM-CSF 5 \( \mu \text{g/kg/d} \) was administered on days 6 through 15 during cycles 3 and 4 by 24-hour intravenous infusion by Pharmacia pump (Baxter Health Care Corp, Deerfield, IL). If grade 3 toxicity attributable to GM-CSF occurred during cycle 3, the dose of GM-CSF was reduced to 3 \( \mu \text{g/kg/d} \) for the fourth cycle. Acetaminophen was administered for GM-CSF–associated myalgias. Patients monitored temperature curves while on GM-CSF. Fever greater than 101°F with any change in clinical status or suspected source for infection (eg, severe mucositis) required admission for parenteral antibiotics and supportive care. GM-CSF (Schering- Sandoz, Kenilworth, NJ) and carboplatin were supplied by the National Cancer Institute (Bethesda, MD). The remaining drugs were obtained commercially.

At least a minimal response to conventional dose doxorubicin, 5-fluorouracil, and methotrexate was required to proceed to intensification.

**BM Harvest**

BM harvest was obtained after the second chemotherapy induction cycle (before exposure to GM-CSF) by standard techniques described previously.\textsuperscript{25} The reserve marrow was reinfused if less than 100/\mu\text{L} granulocytes was observed on peripheral smear on day +28 with hypocellularity on marrow biopsy, or if at any later time the patient failed to maintain hematopoietic function defined as WBC less than 3,000/\mu\text{L} and platelets less than 75,000/\mu\text{L}.

**Leukapheresis and Cryopreservation**

PBPCs were collected by two 2-hour leukaphereses between days 15 and 18 during cycles 3 and 4, provided the WBC was greater than 1,000/\mu\text{L} and increasing and platelets were greater than 30,000/\mu\text{L}. Leukaphereses were performed on a COBE Spectra Blood Component Separator (COBE, Lakewood, CO) using a single stage channel filter and disposable WBC blood tubing set. Acid citrate Dextrose-Formula A (ACD-A; Baxter, Deerfield, IL) anticoagulant, but no Ficoll or Percoll sedimenting procedures were performed. The buffy coat was washed with heparin containing medium to remove fibrin and platelets that might cause clumping during the thaw. The mononuclear cell pellet was reconstituted in 10% dimethyl sulfoxide (DMSO) and 20% autologous plasma in media 199 at a concentration of 2 to 5 \( \times 10^7 \) mononuclear cells/mL. Cryopreservation was performed using controlled rate freezing (KRYO10; Planar; TS Scientific, Perkasie, PA) at \(-1°C\) per minute to \(-20°C\), then \(-2°C\) per minute to \(-80°C\). The mononuclear cells were stored in the vapor phase of liquid nitrogen.

**Intensification Therapy**

Intensification therapy consisted of 6,000 mg/m\(^2\) of cyclophosphamide, 500 mg/m\(^2\) of thiopeta, and 800 mg/m\(^2\) of carboplatin (CTCb) by 96 hours of continuous infusion. Bladder irrigation through a 3-way Foley catheter was continued during and for 24 hours after cyclophosphamide therapy. Seventy-two hours after completion of chemotherapy, PBPCs from the four leukaphereses collected during induction were reinfused.

**Supportive Measures**

All patients received irradiated blood products (20 Gy) beginning 2 weeks before intensification. Patients were cared for in
single rooms under reverse isolation procedures until the granulocytes were ≥400/μL on two successive determinations. Prophylactic acyclovir (400 mg orally twice per day or 250 mg/m² intravenously [IV] every 12 hours) was administered during reverse isolation. Antiemetics included continuous infusion of perphenazine and diphenhydramine.

**PBPC Reinfusion**

The PBPC bags were thawed in a 37°C water bath and then reinfused rapidly. Asymptomatic gross hemoglobinuria developed after reinfusion in the first two patients. All subsequent patients received hydration at 200 mL per hour for 6 hours before and for 24 hours after PBPC reinfusion. Two bags each (100 mL volume per bag) of PBPC were infused beginning on day 0 at 4- to 6-hour intervals until all PBPC collections were reinfused.

**Laboratory Tests**

Serial samples of PB for complete blood count (CBC), differential, CFU-GM colony assay, and percent CD34* by fluorescence-activated cell sorter (FACS) analysis were obtained on days 0, 12, 15, and 17 of induction cycles 1 and 3, before each leukapheresis, and up to 6 months after CTX intensification. BM aspirates and biopsies were obtained before study, at the time of marrow harvest, at the time of completion of induction chemotherapy, at the time of recovery of neutrophils to 500/μL, and at 6 months (or before subsequent chemotherapy, if sooner). They were analyzed for cellularity, differential, and colony assays (CFU-GM, burst-forming unit-erythroid [BFU-E]). PB was simultaneously obtained for CFU quantitation.

**Definitions of Hematologic Response**

Time to reconstitution of hematologic function was defined as the number of days from reinfusion of stem cells (day 0) to recovery of neutrophils to greater than 500/μL and platelets (≥20,000/μL) and RBCs (hematocrit >25%). Hematopoietic recovery was considered durable if reconstitution persisted to the time of last follow-up or to initiation of subsequent therapy.

**Statistical Design and Analysis**

This study was designed as a pilot to investigate the feasibility of using GM-CSF and chemotherapy-mobilized PBPCs for reconstitution of BM function. The Wilcoxon signed rank test for paired data was used to test the difference between average induction nadirs, nadirs durations, and dose intensity with and without GM-CSF. These comparisons do not account for possible accumulating toxicity over the four cycles. However, because the GM-CSF cycles are the last two cycles, we feel the comparisons are biased against the research hypotheses. The difference in induction nadir depth and duration was compared between fast and slow engrafters to intensification with the Wilcoxon rank sum test.

In addition, we used the immediately prior study as a historical control group. These 29 women with stage IV breast cancer were comparable with respect to pretreatment history and received the identical intensification regimen, but with BM support only. The sample size for the pilot was based on data available from the historical control group. In this group, the time to polymorphonuclear neutrophils (PMN) greater than 500/μL appeared to follow a lognormal distribution with a geometric mean of 20 days. Assuming the pilot data from 15 patients was also lognormally distributed with the same standard deviation (0.37 logs), we would be able to detect a shift in geometric mean from 20 to 14.4 days or less with at least 80% statistical power. Due to the presence of censored data, the analysis of the time to engraftment of platelets and granulocytes after reimplantation was compared between patients receiving PBPCs and those receiving BM alone with the log rank test. Continuous data, without censoring, such as hospital stay, units transfused, antibiotic days, and febrile days, were compared between patient groups with the Wilcoxon rank sum test. Dichtomous data, such as septic events, were compared with Fisher's exact test. Statistical tests presented are two-sided.

**RESULTS**

**Patient Characteristics**

Sixteen women entered this study between May 1989 and April 1990. One patient, removed from study after two cycles of induction chemotherapy due to disease progression, is therefore unavailable for hematologic recovery and was excluded from further analysis.

The median age was 43 years (range, 29 to 57) and the median performance status was 0. Antecedent hormonal therapy had been administered to eight of nine patients with detectable hormone receptors. Six had received prior chest radiotherapy, nine patients had had adjuvant chemotherapy, and two had received prior chemotherapy for metastatic disease. Six patients had had no prior chemotherapy.

**Induction Phase**

As shown in Table 2, the depth of granulocytopenia was shortened by GM-CSF during cycles 3 and 4 of AFM compared with cycles 1 and 2 (P = .002). However, duration and depth of thrombocytopenia was cumulative through the four cycles of induction AFM with no evidence for sparing by GM-CSF. Dose reductions of doxorubicin were made principally for fever and neutropenia; reductions of 5-fluorouracil were mainly for mucositis. Dose delays, the major cause of decreased dose intensity, were principally for prolonged myelosuppression, although debility from mucositis contributed.

Leukapheresis was performed twice between days 15 and 19 of cycles 3 and 4 when the WBC increased to ≥1,000/μL and platelets were at least 30,000/μL. The median mononuclear cell yield was 7.5 × 10⁸ cells (range, 2.2 to 38.0 × 10⁸) per leukapheresis. Typical leukapheresis differentials were comparable with differentials obtained from PB smears. The number of PBPC per kilogram collected correlated inversely with the depth (P = .02) and duration (P = .01) of the granulocyte nadir during the collection cycles (Fig 1). Thus, patients with the greatest myelosuppression despite GM-CSF had the fewest cells collected and were subsequently reinfused.

**Engraftment After Intensification and Durability of Engraftment**

All evaluable patients have been followed-up for a minimum of 12 months (range, 12 to 23) after the reinfusion of PBPCs. No late engraftment failures have been observed.

**Granulocyte engraftment.** All 15 patients were evaluable for granulocyte recovery (Table 3). The median time to granulocyte recovery to 500/μL after PBPC reinfusion was
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Table 2. Dose Intensity and Hematopoietic Toxicity of AFM Induction Chemotherapy, and the Effect of GM-CSF on Cycles 3 and 4

<table>
<thead>
<tr>
<th>Cycle of AFM</th>
<th>Without GM-CSF</th>
<th>With GM-CSF</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mean days of granulocytopenia (&lt;500/μL granulocytes)</td>
<td>6.0</td>
<td>5.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Range</td>
<td>0-18</td>
<td>0-15</td>
<td>0-11</td>
</tr>
<tr>
<td>Mean granulocyte nadir (per μL)</td>
<td>448</td>
<td>255</td>
<td>776</td>
</tr>
<tr>
<td>Range</td>
<td>0-1,650</td>
<td>0-1,080</td>
<td>100-2,438</td>
</tr>
<tr>
<td>Mean days of thrombocytopenia (&lt;100,000/μL)</td>
<td>1.8</td>
<td>2.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Range</td>
<td>0-7</td>
<td>0-8</td>
<td>0-17</td>
</tr>
<tr>
<td>Mean platelet nadir (per μL x 10^9)</td>
<td>136</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>Range</td>
<td>32-283</td>
<td>17-346</td>
<td>15-168</td>
</tr>
<tr>
<td>% Median dose intensity†</td>
<td>81</td>
<td>80</td>
<td>82</td>
</tr>
</tbody>
</table>

*P value comparing cycles 1 and 2 (without GM-CSF) with cycles 3 and 4 (with GM-CSF).
†Measured by mg/m²/week normalized against the planned dose and schedule for doxorubicin and 5-fluorouracil.

14 days (range, 10 to 57). Twelve patients recovered quickly (10 to 15 days) and three patients less quickly (26 to 57 days) (Fig 2 A). Recovery time was not related to age, prior chemotherapy, sites of disease, or performance status, nor was there apparent correlation between the dose of PBPCs per kilogram and the time to granulocyte recovery. It remains possible that a threshold phenomenon exists. The nine patients who received greater than 4.2 x 10⁸ mononuclear cells/kg engrafted quickly; in contrast, the speed of recovery was slower in three of six patients who received fewer cells. The time to engraftment appears to be inversely correlated with the depth of the granulocytopenic nadir and directly with the duration of granulocytopenia during induction, particularly for cycle 3 (P = .0001). Those patients recovering quickly had less granulocytopenia during each induction cycle than those with slower postintensiﬁcation recovery phases (Fig 3A). Response to GM-CSF also appeared to be greater in the group ultimately engrafting quickly.

Platelet engraftment. Fourteen patients were evaluable for platelet engraftment. One patient died on day +26 of congestive heart failure in a clinical setting requiring hypertransfusion of platelets. The median time to ≥20,000/μL and to ≥50,000/μL platelets after reinfusion of PBPCs was 12 days (range, 8 to 134+) and 14 days (range, 10 to 136+), respectively. Ten patients engrafted platelets greater than 50,000/μL quickly (days 10 to 22) and four slowly (days 53 to 136+). Of these latter four patients, one recovered by day +57. Three patients received marrow on days +53, +134, and +136; two for persistent platelet transfusion requirements and one to allow administration of further therapy. One of these three patients (marked by the arrow in Fig 2B) had recovered 20,000/μL platelets by day +13, but then developed antiplatelet antibodies; she subsequently became platelet transfusion-dependent 4 months later and received BM on day +136. These patients are censored at the time of marrow reinfusion for platelet engraftment endpoints. Platelet counts in all three recovered to ≥50,000/μL within 2 to 4 weeks of marrow reinfusion, but not to completely normal levels. Recovery time was not related to age, prior chemotherapy, sites of disease, or performance status. No statistical correlation can be made between time to engraftment of platelets and the number of PBPC mononuclear cells per kilogram infused; however, as was noted in granulocyte recovery, all patients who received greater than 4.2 x 10⁸ cells/kg engrafted quickly.

The 10 patients who had rapid platelet engraftment (>50,000/μL) after high-dose therapy also had significantly less thrombocytopenia during AFM induction (mean, 2.7 days/cycle <100,000/μL platelets; range, 0 to 7.7). The four patients with poor platelet engraftment tended to have a longer average duration of thrombocytopenia during AFM (mean, 7.1 days/cycle <100,000/μL platelets; range, 1.5 to 13.0) (P = .12) and greater cumulative thrombocytopenia (P = .02) (Fig 3B). Slow engraftment also correlated with delay in treatment cycles; mean days from initiation of induction to intensification was 128 days for the slow group and 113 days for those with rapid engraftment (P = .011;
Wilcoxon rank sum test). The patients who had poor platelet recovery received 75% and 61% and those who had rapid platelet recovery received 88% and 82% of the planned dose intensities of doxorubicin ($P = .01$) and 5-fluorouracil ($P = .12$), respectively. Thus, patients who required marrow reinfusion could be identified, in retrospect before high-dose therapy.

**Marrow Cellularity and CFU-GM Assays**

The median marrow cellularity before induction was 50% (20% to 65%). All three patients who later required marrow infusion for poor platelet engraftment had low marrow cellularity (20% to 30%) before induction treatment. These three all had prior chemotherapy 6 to 24 months earlier, with unusually prolonged myelosuppression noted in a single patient. All other patients had normocellular marrow. Cellularity of the marrow obtained at the time of recovery to greater than 500/μL PMN ranged between 10% and 80% (median, 35%).

Day 14 assays for CFU-GM and FACS analysis for percent CD34+ cells were performed on PB serially during cycles 1 and 3 and on leukapheresis samples from cycles 3 and 4. While a significant increase over baseline was occasionally observed between days 12 and 17, no correlation of the number of colonies or percent CD34+ cells with subsequent reconstitution was evident.

**Hospitalization.** The median hospital stay was 24 days (range, 19 to 34). The median number of RBC and platelet units transfused were 8 (4 to 15) and 22 (10 to 237), respectively. The median number (range) of febrile days, days on intravenous antibiotics, and days on Amphotericin were 3 (0 to 10), 10 (0 to 24), and 3 (0 to 21), respectively. Of the three patients who developed central line infections, two had documented *Staphylococcus epidermidis* bacteremia. No other patients became bacteremic.

**DISCUSSION**

This trial shows that PBPCs recruited with chemotherapy and GM-CSF as the sole source of hematopoietic support provide rapid and sustained reconstitution of all cell lineages in the majority of patients. While granulocyte recovery was universal, recovery of platelet function was less consistent. Thrombocytopenia (<50,000/μL), persisting to day 50 after PBPC reinfusion, was observed in 4 of 14 patients and in three cases required reinfusion of the stored marrow. Long-term reconstitution appeared durable in the 11 patients who recovered adequate marrow function with PBPC alone as well as in the three who received their back-up marrow. In retrospect, the patients requiring use of the reserve marrow could have been identified by marrow cellularity greater than 30% before induction therapy and poor platelet recovery after conventional dose therapy.

The effects of PBPCs on hematopoietic reconstitution were compared with 29 historical control patients who received only autologous BM (Table 3). These 29 women with metastatic breast cancer had responded to standard-dose induction therapies (half had received AFM and the other half CAF [cyclophosphamide, doxorubicin, and 5-fluorouracil]) and then received the identical intensification regimen (CTCb) using autologous marrow support alone.
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These patients had similar pretreatment chemotherapy and radiotherapy exposures (Table 3). The substitution of PBPCs for marrow significantly shortened the median number of days from reinfusion to neutrophil and platelet engraftment by 7 days ($P = .038$) and 12 days ($P = .057$), respectively. Median hospital stay was reduced by 8 days ($P < .001$). Antibiotic requirements ($P = .003$) and RBC transfusions were both significantly reduced ($P = .032$). Platelet transfusion requirements were markedly reduced ($P = .001$). Failure of platelet engraftment has been observed in 9% to 23% of patients when using autologous marrow as support and was observed in one of 29 breast cancer patients using CTCb with marrow.

The adequacy of specific cell lineage progenitor collection and subsequent engraftment could be predicted by specific cell-lineage toxicity caused by conventional-dose induction chemotherapy. For example, patients with slower granulocyte recovery after high-dose cyclophosphamide, thiotepa, and carboplatin had protracted, profound granulocytopenia during all four conventional-dose AFM induction cycles. Parallel findings of prolonged thrombocytopenia during conventional induction chemotherapy were observed for patients with delayed platelet recovery after high-dose therapy. These two groups only partially overlapped. Thus, the toxicity generated by induction chemotherapy within a particular cell lineage may imply inadequate collection of progenitor cells within that particular line. If this predictive model is confirmed, patients likely to recover marrow function quickly and those who may be better served by additional collection of PBPCs or by reinfusion of marrow can be easily identified prospectively. Indeed, even within the subset of six patients with less than $4.2 \times 10^9$/kg PBPCs reinused, the three who engrafted more slowly were those with more profound myelosuppression during induction chemotherapy than the three who recovered quickly.

PBPCs collected without mobilization by chemotherapy or growth factors produce complete and permanent reconstitution of marrow function after high-dose therapy even after potentially myeloablative regimens such as cyclophosphamide and total body irradiation. However, the 7 to 10 phereses required tax patient, blood bank, and cryopreservation resources. We had previously shown that administration of GM-CSF increased the number of circulating CFU-GM a median of 13-fold (2-fold to 200-fold) in untreated patients and 63-fold during hematopoietic

Fig 2. The dose of PBPCs per kilogram does not correlate with time to granulocyte (A) or platelet (B) recovery, but a threshold phenomenon may exist. The arrow in (B) indicates a patient who recovered greater than 20,000/μL platelets by day +13, developed antiplatelet antibodies, and subsequently received BM on day +136.

Fig 3. GM-CSF shortens granulocytopenia (A), but does not prevent cumulative thrombocytopenia (B). The time to engraftment of the particular cell lineage correlates directly with the durations of granulocytopenia (A) and thrombocytopenia (B) during induction. (−), Cycles 1 and 2 without GM-CSF; (+), cycles 3 and 4 with GM-CSF. The patients who are “fast” and “slow” in (A) and (B) are defined by the patient distribution shown in Fig 2A and B, respectively.
recovery after the administration of chemotherapy.\textsuperscript{21,22} In combination with autologous marrow, cytokine- or chemotherapy-mobilized PBPCs also enhanced hematopoietic recovery.\textsuperscript{23,35,36} Gianni et al determined that marrow plus PBPCs from 2 to 3 leukaphereses during recovery after 7 g/m\textsuperscript{2} of cyclophosphamide (with and without GM-CSF) produced early reconstitution of both myeloid and platelet function after high-dose melphalan and total body irradiation.\textsuperscript{23} The number of CFU-GM per kilogram infused correlated well with the time to absolute neutrophil count (ANC) recovery. Peters et al have combined GM-CSF-mobilized PBPCs and marrow after high-dose cyclophosphamide, carmustine, and cisplatin.\textsuperscript{35} GM-CSF alone increased circulating CFU-GM 10-fold and CD34\textsuperscript{+} (a cell surface antigen carried by the progenitor cell population) cells up to 11\% of the leukapheresed mononuclear cell fraction. These patients experienced fewer days of absolute granulocytopenia (ANC < 100/\mu L) compared with controls who received marrow alone followed by GM-CSF.\textsuperscript{35} G-CSF also appears to mobilize CFU-GM into the peripheral circulation. Investigators in Melbourne have observed more rapid ANC and platelet recovery in patients receiving both PBPCs collected after G-CSF and autologous marrow.\textsuperscript{37}

The mechanisms by which hematopoietic cytokines increase circulating PBPCs are not well understood. They may provide a proliferative stimulus to expand the population of progenitor cells within the marrow and PB compartments, a differentiation stimulus to increase circulating progenitor cells (at the expense of stem cells responsible for self-renewal), or an alteration in adhesion molecules on the cell membrane that ordinarily regulate the release of cells from the marrow into the circulating compartment.\textsuperscript{38,39} The fact that G-CSF-stimulated PBPCs together with marrow appear to enhance platelet engraftment\textsuperscript{37} despite its lack of stimulation of thrombopoiesis suggests that the release of progenitor cells by alterations in adhesion molecules may be at least partly the explanation. Mobilization by chemotherapeutic agents may be due to high marrow cytokine concentrations in response to relative aplasia and may not be specific for a given chemotherapy agent itself.

The timing of GM-CSF within the cycle of induction chemotherapy may be important to optimize collection of mobilized PBPC. Gianni et al showed that GM-CSF is more effective in ameliorating myelosuppression due to cyclophosphamide when begun on day +1 than on day +5.\textsuperscript{40} In a companion study of the same patients,\textsuperscript{41} Siena et al reported that the number of CD34\textsuperscript{+} cells per kilogram (in particular, the CD34\textsuperscript{+}/CD33\textsuperscript{+} subset; a phenotype describing more mature progenitor cells) correlated with the time to early hematopoietic recovery.\textsuperscript{42} The presence of these committed progenitor cells correlated strongly with the growth of day 14 CFU-GM. The rebound of CD34\textsuperscript{+} cells and CFU-GM was observed when GM-CSF was begun on day +1, but not if delayed to day +5 of the cycle.\textsuperscript{43} These studies suggest that mobilization of PBPCs and enhanced maturation by GM-CSF optimally requires exposure over a period of days. In our study, we were unable to document a consistent rebound effect on CD34\textsuperscript{+} cells in the circulation after cycles 3 and 4, perhaps because GM-CSF was begun on day +6 of the cycle. The intrapatient variation of CFU-GM colonies rendered this assay problematic for analysis. Despite the lack of a rebound effect on CD34 cells, early reconstitution was observed clinically.

Rapid engraftment possible with PBPC support should enhance the evaluation of dose-intensive therapy. Early reconstitution should reduce serious infectious and bleeding complications, antibiotic courses, hospital stays, and fiscal costs. Despite the four phereses to collect PBPCs per patient, the use of pheresis resources in the blood bank actually decreased because of these patients' dramatically reduced requirements for platelet support. Decreased risks and cost should permit the study of dose-intensive therapy in earlier stage poor prognosis diseases.

On a more fundamental level, PBPCs may allow for the safe administration of sequential high-dose regimens. Single courses of dose-intensive therapy currently cure only a minority of patients. Curative conventional-dose chemotherapy has been successful in situations in which active drugs are used in combination for multiple courses. Multiple cycles of high-dose chemotherapy have been toxic, expensive, and impractical. A preliminary report by Shea et al indicates that PBPCs and GM-CSF adequately supported repeated cycles of 1,200 mg/m\textsuperscript{2} of carboplatin, doses that could not be repeated for three cycles due to profound thrombocytopenia when using GM-CSF alone.\textsuperscript{44} Thus, testing the concept of dose-rate becomes feasible. On a cautionary note, these trials must consider the possibility that PBPCs may contain enough mature progenitors to support patients through acute myelosuppression of multiple high-dose courses, but might not be sufficient to prevent cumulative myelosuppressive effects.\textsuperscript{45}

Unanswered questions regarding the optimal technique for collection of PBPCs remain. Optimal scheduling of the growth factor with respect to the chemotherapy may enhance the number of circulating PBPCs and the efficiency of collection. Other cytokines and/or combinations may optimize mobilization. In the laboratory, interleukin-3 increases circulating CFU-GM and is synergistic in this effect with GM-CSF.\textsuperscript{46,48} Preclinical modelling may also guide determination of optimal chemotherapy agents for inducing a rebound in circulating CFU-GM or CD34\textsuperscript{+} cells and provide an understanding of the regulatory mechanisms of progenitor cell traffic between the BM and PB compartments.

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