Effect of CD34⁺ Selection and Various Schedules of Stem Cell Reinfusion and Granulocyte Colony-Stimulating Factor Priming on Hematopoietic Recovery After High-Dose Chemotherapy for Breast Cancer


We evaluated the effects of various schedules of peripheral blood stem cell (PBSC) reinfusion, granulocyte colony-stimulating factor (G-CSF) priming, and CD34⁺ enrichment on hematopoietic recovery in 88 patients with advanced breast cancer treated with high-dose chemotherapy, consisting of cisplatin 250 mg/m², etoposide 60 mg/kg, and cyclophosphamide 100 mg/kg. PBSC (7.5 × 10⁸ nucleated cells/kg) were collected following priming with G-CSF and were either immediately cryopreserved (48 patients; cohorts A and B) or were first processed for CD34⁺ enrichment (40 patients; cohorts C and D). Patients in cohorts A and C received PBSC on day 0; patients in cohorts B and D received 25% of their nucleated cells on day −2 and 75% on day 0 (split reinfusion). Patients in cohorts A, B, and C were primed with G-CSF 10 μg/kg, subcutaneously (SC), once a day; patients in cohort D were primed with 5 μg/kg G-CSF SC, twice daily (bid). Bid administration of G-CSF yielded 2.3 to 4.7 × 10⁹ nucleated cells/kg (range, 1.7 to 3.3 × 10⁹/kg) with the remaining cells reinfused on day 0 resulted in earlier granulocyte recovery to ≥500/μL when compared with reinfusion of all stem cells on day 0 (group B, median of 8 days [range, 7 to 11] vs group A, 10 days [range, 8 to 11], P = .0003); no schedule-depen-
dent difference was noted in reaching platelet independence (group B, 11.5 days [range, 5 to 21]; group A, 12 days [range, 8 to 24], P = not significant). Split schedule reinfusion of CD34⁺-selected PBSC did not accelerate granulocyte recovery. In groups D and C, the median number of days to granulocyte recovery was 12 (range, 8 to 22) and 11.5 (range, 9 to 13); patients became platelet independent by day 15 (range, 6 to 22) and 14 (range, 12 to 23), respectively. CD34⁺-selected PBSC rescue decreased the incidence of postreinfusion nausea, emesis, and oxygen desaturation in comparison to unselected PBSC reinfusion (P ≤ .005 for each). Hematopoietic recovery may be accelerated by earlier reinfusion of 2.26 × 10⁹/kg unselected nucleated cells. Earlier recovery may be triggered by components other than the progenitors included in the CD34⁺ cell population. Sustained hematopoietic recovery can also be achieved with CD34⁺-selected PBSC alone. Dosing of G-CSF on a bid schedule generates higher CD34⁺ cell yield in the leukapheresis product. Whether the administration of high-dose chemotherapy would reduce the duration of absolute granulocytopenia further while initiating sustained long-term hematopoietic recovery will require further investigation.

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We initiated a high-dose chemotherapy program for the treatment of high-risk primary and responding metastatic breast cancer in October 1988 and have published preliminary results with the first two regimens developed at the City of Hope National Medical Center.25-27 One of these regimens consists of cisplatin, 250 mg/m², etoposide 60 mg/kg, and cyclophosphamide 100 mg/kg followed by PBSC rescue. We have performed a cohort analysis on the effect of successive modifications of stem cell technology in 88 consecutively treated breast cancer patients, all of whom received the above regimen followed by PBSC. The effect of varying the schedule of administration of granulocyte colony-stimulating fac-

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tor (G-CSF) priming and PBSC and CD34⁺ selection on hematopoietic recovery is the subject of this report.

MATERIALS AND METHODS

All patients gave their written, voluntary informed consent. This study was approved by the Institutional Review Board of the City of Hope National Medical Center, Duarte, CA. Eighty-eight patients with responding stage IV or high-risk primary breast carcinoma (Stage II with 10 or more G-CSF 10

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PBSC Group A (n = 22)</th>
<th>PBSC/Split Group B (n = 26)</th>
<th>CD34⁺ Group C (n = 29)</th>
<th>CD34⁺/Split Group D (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median yr, range)</td>
<td>44 (30-56)</td>
<td>44 (27-61)</td>
<td>42 (29-55)</td>
<td>45 (35-66)</td>
</tr>
<tr>
<td>No. of prior chemotherapy regimens (median, range)</td>
<td>2 (1-3)</td>
<td>2 (1-4)</td>
<td>2 (1-5)</td>
<td>1.5 (1-2)</td>
</tr>
<tr>
<td>Prior radiation</td>
<td>12 (55)</td>
<td>6 (23)</td>
<td>11 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Stage III/IV disease</td>
<td>5 (23)</td>
<td>14 (54)</td>
<td>15 (52)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Stage IV disease</td>
<td>17 (77)</td>
<td>12 (46)</td>
<td>14 (48)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sites of distant metastases</td>
<td>       </td>
<td>       </td>
<td>       </td>
<td>       </td>
</tr>
<tr>
<td>Bone</td>
<td>1 (6)</td>
<td>1 (8)</td>
<td>4 (29)</td>
<td>NA</td>
</tr>
<tr>
<td>Liver</td>
<td>2 (12)</td>
<td>1 (8)</td>
<td>2 (14)</td>
<td>NA</td>
</tr>
<tr>
<td>Lung</td>
<td>1 (6)</td>
<td>2 (17)</td>
<td>2 (14)</td>
<td>NA</td>
</tr>
<tr>
<td>Skin, soft tissue</td>
<td>14 (82)</td>
<td>8 (67)</td>
<td>7 (50)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

Peripheral stem cell priming and processing. Peripheral stem cell collection was performed following priming with G-CSF 10 µg/kg given SC for 4 days before collection; G-CSF was continued daily, SC, through the completion of leukapheresis. Peripheral stem cell products were collected with a COBE-Spectra (Cobe Laboratories, Lake-wood, CO), or a CS-3000 Fenwall cell separator (Baxter, Deerfield, IL); approximately 10 L of blood were processed during each collection.

Patients in cohorts A (22 patients) and B (26 patients) received G-CSF 10 µg/kg, SC, once daily. Leukapheresis commenced on day 5 of G-CSF administration and was continued daily until ≥7.5 x 10⁹/kg nucleated cells were collected. Peripheral stem cell products were cryopreserved by simple immersion in a −130°C freezer, using 10% dimethyl sulfoxide and 2.5% human serum albumin as cryoprotectant. Frozen peripheral stem cell products were thawed in a 37°C water bath and infused over 10 minutes. Patients in cohort A received all of their PBSC on day 0. Patients in cohort B received 25% of their stem cells on day −2 and 75% on day 0.

Patients in cohorts C and D received PBSC rescue with products processed through a CEPRATE SC Stem Cell Concentration System (CellPro, Inc, Bothel, WA). Twenty-nine patients (cohort C) underwent priming with G-CSF 10 µg/kg, SC, once daily. Cohort D consisted of 11 patients who were primed differently, with G-CSF 5 µg/kg, SC, given twice daily. Leukapheresis commenced on day 4 and continued until collection of ≥11.5 x 10⁹ nucleated cells/kg was completed. The initial leukapheresis products (≥7.5 x 10⁹ nucleated cells/kg) were processed either immediately, or together with the collection from the following day after overnight storage at 22°C. After centrifugation at 3,000 rpm for 5 minutes in a Cobe-2991 Blood Cell Processor (Cobe Laboratories) and washing in 1,000 mL of RPMI-1640 medium (JRH Biosciences, Lenexa, KS), cells from the buffy coat were incubated for 25 minutes at 22°C with 20 µg/mL biotinylated anti-CD34 monoclonal antibody 12.8 with 150 CSF 10...
sulfoxide and 4% HSA and 10 U/mL heparin (Elkin-Sinn, Cherry Hill, NJ) in PBS to a final cell count of 100 × 10⁶ cells in a volume of 4.5 mL. The cells were stored in 5-mL vials at −196°C (Nalgene, Redmond, WA) using a controlled rate freezing method. An additional 4 × 10⁶ nucleated cells/kg were cryopreserved by simple immersion and stored as previously described for safety considerations (these cells were to be reinfused if the absolute granulocyte count remained ≤500/µL by day 16, or if a patient required platelet transfusions beyond day 28). On the day of reinfusion, the vials containing the CD34⁺ selected product were thawed by immersion in a water bath at 37°C. The CD34⁺ selected product was transferred to a 50 mL vial, and 30 mL of PBS was added slowly, over 5 minutes. The product was aspirated into a 60-mL syringe and placed in a cooler for transport to the bedside for reinfusion.

Patients in cohort C received all CD34⁺ selected PBSC on day 0. In cohort D, the CD34⁺ selected PBSC product was reinfused on a split schedule; 25% of selected stem cells were reinfused on day −2; 75% were given on day 0.

Flow cytometry. Cell fractions were incubated for 30 minutes at 4°C with phycoerythrin (PE)-conjugate anti-CD34 monoclonal antibody, and for parallel negative controls with mouse IgG-PE (both from Becton Dickinson, San Jose, CA). Propidium iodide staining served to detect dead cells. Flow cytometric analysis was performed on 100,000 cells using the Lysys II Software with a FACSscan (Becton Dickinson, San Jose, CA). CD34⁺ cells were reported as a percent of control live cells. The flow cytometry was performed to measure the percentage of CD34⁺ cells in the leukapheresis products; CD34⁺ content was analyzed further in groups C and D by measuring CD34⁺ content in buffy coat and in the postselection “absorbed” product following processing with the CEPRATE SC System.

Progenitor-cell assays. BFU-e and colony-forming unit–granulocyte-macrophage (CFU-GM) formation from buffy coat cells and from the postselection products following processing by the CEPRATE SC System was evaluated. Cells were plated in triplicate using 2.35% Iscove’s methylcellulose (Stem Cell Technology, Vancouver, BC, Canada). Buffy coat cells were plated at the following concentrations: 3 × 10⁶/mL, 1 × 10⁶/mL, and 3 × 10⁵/mL. Seeding densities for the absorbed, postselection fraction were 1 × 10⁷/mL, 3 × 10⁶/mL, and 1 × 10⁵/mL, respectively. All samples were maintained in 5% CO₂ in air and 95% humidity at 37°C for 14 days. After 14 days, plates with more than 10 and less than 100 colonies containing ≥50 cells were scored using an inverted-phase microscope.

Growth factor support. All patients received 5 µg/kg of G-CSF, intravenously (IV), bid, starting on the first day of stem cell reinfusion until an absolute granulocyte count of ≥1,000/µL for 3 consecutive days had been reached.

Treatment regimen. Patients received cisplatin 125 mg/m² and etoposide 30 mg/kg on days −12 and 5 followed by cyclophosphamide 100 mg/kg on day −3. On day −5, patients with a serum creatinine level of >1.2 mg/dL and ≤1.5 mg/dL, received 75% of their calculated day −12 cisplatin dose; a serum creatinine level >1.5 mg/dL resulted in withholding cisplatin. The number of patients requiring modification of the day −5 cisplatin dose was equally distributed among cohorts A, B, C, and D. Details of drug administration and toxicities associated with this regimen have been reported earlier.₂⁶,₂⁷

Supportive care. Patients had a central venous catheter inserted before admission and were hospitalized in private rooms equipped with positive-pressure ventilation and HEPA filter. They were placed in protective isolation when the total white blood cell count became <500/µL and remained there until the granulocyte count reached ≥500/µL. Strict low-bacteria diets were given throughout the period of isolation; parenteral nutrition and broad-spectrum antibiotics were provided as medically indicated. Blood products were filtered and irradiated at 1,500 cGy and were transfused to maintain a platelet count >20,000/µL and a hematocrit level greater than 25%. During stem cell reinfusion, a transcutaneous oxygen saturation monitor was applied to all patients; oxygen supplementation at a flow rate of 2 L/min through a nasal cannula was provided for patients whose saturation dropped to ≤90%.

Hematologic end points. Days to absolute granulocyte count (AGC) >500/µL were counted from day 0 in all groups. Platelet independence was defined as the day following the last platelet transfusion. The number of platelet transfusions was totaled by counting each single unit and pooled product as one transfusion; total number of red blood cell transfusions was also recorded.

Statistical analysis. The data were summarized as medians and ranges (minimum, maximum) or frequencies and percents. To compare differences in peripheral stem cell processing and products among the four cohorts, one-way analysis of variance with Tukey’s method was performed after applying a rank transformation to the data.₂⁸ To compare differences in hematopoietic recovery end points between two cohorts, two sample t-tests were performed using the rank data. In addition, simple and multiple linear regressions were performed to identify factors associated with hematopoietic recovery end points. Factors examined included product type (CD34⁺ selected v standard PBSC), infusion schedule (split schedule v all on 1 day), prior radiation (yes/no), and total number of CD34⁺ cells infused. Regression analyses were also applied to the ranks of the data. All significance testing was done at the .05 level. The SAS/STAT software (SAS Institute Inc) was used.₂⁹

RESULTS

Stem cell processing data. Table 2 shows the total nucleated and CD34⁺ cell content of collected apheresis products. The CD34⁺ cell content of the leukapheresis product was available for 16 of 22 patients in group A. CD34⁺ cell count, as well as BFU-e and CFU-GM content of the buffy coat, and postselection products are shown for groups C and D.

The total nucleated cell counts of the products of patients who received split schedule reinfusions were significantly higher in group D than in group B (P = .0001); the number of CD34⁺ cells was similar in the products of patients in groups D and B immediately before cryopreservation (group D, 2.92 × 10⁹/kg [range, 0.83 to 6.15]; group B, 3.66 × 10⁹/kg [range, 0.36 to 16.61]). CD34⁺ cell counts in the apheresis products in group D were 3.8, 2.3, and 4.7 times higher than in groups C, B, and A, respectively (P = .001) even though the median number of leukapheresis procedures were higher in group C than in D: 5 (2 to 8) and 3.5 (range, 3 to 5), respectively (P = .05, data not shown).

Median total nucleated cell recovery following CD34⁺ selection was 0.55% of the buffy coat and 0.48% of the starting leukapheresis product in group C and 0.82% and 0.81% in group D. After CD34⁺ selection, 41.6% of CD34⁺ cells from the buffy coat (range, 24.6% to 66.6%) in group D and 51.5% (range, 21.3% to 94.1%) of CD34⁺ cells in group C were recovered; the concentration (purity) of CD34⁺ cells in the postselection products was 45% (range, 17% to 67%) in group D and 37% (range, 9% to 89%) in group C, respectively (data not shown in Table 2). The CD34⁺ cell count was higher in the group D postselection product compared with group C (medians, 2.92 × 10⁹/kg v 1.45 × 10⁹/kg; P = .01). This difference remained statistically significant (P = .03) even after accounting for the fact that 38% of patients
in group C and none of the patients in group D received prior radiation treatment. After CD34+ enrichment, 36% of BFU-e and 40% of CFU-GM from the buffy coat in group C and 35% of BFU-e and 40% of CFU-GM from the buffy coat in group D were recovered. BFU-e and CFU-GM colony formation was similar both in the buffy coat and in the postselection products in groups C and D (P = not significant [NS]).

**Hematopoietic recovery and transfusion requirements.**

Table 3 shows the hematopoietic recovery and blood product requirements for patients undergoing high-dose chemotherapy. The median (range) number of days required to reach each end point is reported. One patient in group C died of sepsis-associated cardiac failure on day 22 without becoming platelet independent. Two patients in group C received their “backup” peripheral stem cells on days 19 and 7 (this later patient received “backup” stem cells to hasten recovery in the setting of bacteremic sepsis); these two patients reached an AGC of ≥500/μL within 3 and 5 days from the day of “backup” reinfusion, making it likely that hematopoietic recovery was predominantly due to the CD34+ selected stem cell product. All patients (except the one patient who died of sepsis) recovered and sustained trilineage hematopoietic function.

Median number of days to granulocyte recovery >500/μL occurred 2 days earlier following split reinfusion of standard PBSC product in group B compared with group A patients who received all PBSC products on day 0 (P = .0003).

Table 3 Hematopoietic Recovery and Transfusion Support

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBSC Group A (n = 22)</th>
<th>PBSC/Split Group B (n = 26)</th>
<th>Group A v Group B</th>
<th>P Value¹</th>
<th>Group C v Group D</th>
<th>P Value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to AGC ≥500/μL</td>
<td>10 (8-11)*</td>
<td>8 (7-11)</td>
<td>.0003</td>
<td></td>
<td>12 (8-22)</td>
<td>11 (9-13)</td>
</tr>
<tr>
<td>Days to platelet independence</td>
<td>12 (8-24)</td>
<td>11.5 (5-21)</td>
<td>.61</td>
<td></td>
<td>15 (6-221)</td>
<td>14 (12-23)</td>
</tr>
<tr>
<td>No. of RBC transfusions</td>
<td>4 (3-7)</td>
<td>4 (2-11)</td>
<td>.84</td>
<td>.03</td>
<td>6 (3-11)</td>
<td>4 (3-14)</td>
</tr>
<tr>
<td>No. of platelet transfusions</td>
<td>7.5 (3-33)</td>
<td>6 (2-52)</td>
<td>.50</td>
<td>.50</td>
<td>9 (2-26)</td>
<td>9 (6-22)</td>
</tr>
</tbody>
</table>

Abbreviation: RBC, red blood cell.

* Median (range).

† One patient died before reaching platelet independence.

‡ P value derived from linear regression analysis applied to the ranks of data.
patients of groups A and B; a slightly prolonged median of 10 days (range, 8 to 11) was needed to reach an AGC of >500/µL after reinfusion of <1 × 10^6 CD34+ cells/kg in comparison to the rest of patients in these two groups. The time to reach platelet independence and transfusion requirements was not influenced by the CD34+ cell content of the PBSC product.

**Toxicities associated with peripheral stem cell reinfusion.** Table 5 shows the toxicities associated with PBSC reinfusion. There was a significant difference in favor of CD34+ enriched PBSC products. Nausea and emesis occurred in 13% and oxygen supplementation was provided for 2.5% of patients receiving CD34+ selected products; in contrast, 48% of patients receiving standard peripheral stem cell reinfusion complained of nausea (P = .0002), 31% experienced emesis (P = .0005), and 23% (P = .005) received oxygen supplementation following reinfusion, respectively.

**Other toxicities.** One patient in group C developed *S viridans* bacteremia and subsequently died of sepsis with associated cardiac failure.

Toxicities specific for the high-dose chemotherapy regimen consisted primarily of high-frequency hearing loss, reversible renal electrolyte wasting, and neutropenic and thrombocytopenic side effects.26,27

**DISCUSSION**

We set out to improve on the beneficial effects of peripheral blood stem cell rescue following high-dose chemotherapy. First, we tested whether reinfusion of relatively small numbers of nucleated cells given shortly after completion of high-dose chemotherapy could accelerate hematopoietic recovery. We found that reinfusion of <2.5 × 10^8 nucleated cells/kg given 2 days before the remaining stem cell product shortened absolute granulocyte recovery.

Secondly, we tested a CD34+ selection technology using the CEPRATE SC System. Recovery of CD34+ cells (median, 51.5% [group C] and 41.6% [group D]) and purity of products (median, 37% [group C] and 45% [group D]) after the selection process were similar to those reported by the group from the University of Colorado (mean recovery, 52% and purity, 42% [range, 21% to 72%]).22 Slightly higher mean recovery (73.2% ± 24.6%) and purity (61.4% ± 19.7%) were reported by investigators from the University of Freiburg in a less heavily pretreated patient population; in addition, their patients were primed with a combination of chemotherapy and G-CSF, which may explain the difference.30

Despite the advantage of early reinfusion of unselected stem cells, we observed no benefit with split schedule reinfusion of CD34+ selected cells (group D) when compared with reinfusing all CD34+ products the same day (group C). Since the number of CD34+ cells before cryopreservation was similar in the product reinfused in group D patients compared with that in the unselected product in group B and the total nucleated cell count was reduced to <1 × 10^6/kg after selection in Group D compared with a total nucleated cell count of approximately 9 × 10^6 nucleated cells/kg in group B, it is possible that the presence of cellular elements other than those contained within the CD34+ population are required to enhance the early phase of hematopoietic recovery.

Sustained hematopoietic recovery was achieved in patients with either standard or CD34+ selected peripheral blood stem cell support. Our findings suggest that long-term hematopoietic recovery following high-dose chemotherapy can be achieved following reinfusion of as few as <1 × 10^5 nucleated cells/kg with a CD34+ cell content as low as 0.45 × 10^6/kg. Contrary to previous reports suggesting that a minimum of 2.5 × 10^6/kg CD34+ cells are required for rapid granulocyte and platelet recovery, we could not define a clinically significant threshold number of CD34+ cells.31

Without gene marking, one cannot rule out the possibility of self-regeneration of bone marrow function after nonmyeloablative therapy. However, the rapidity of hematopoietic recovery is enhanced by peripheral blood stem cell rescue, and this effect can be further improved by administering G-CSF following stem cell reinfusion.32 The varying speed of hematopoietic recovery in patients supported with standard PBSC versus CD34+ selected products (groups A and B v C and D) cannot be explained by either spontaneous bone

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**Table 4. Relationship of Hematopoietic Recovery to CD34+ Cell Content in Patients Supported With Unselected (PBSC) or CD34+ Selected (CD34+) Products**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of CD34+ Cells x 10^6/kg</th>
<th>No.</th>
<th>PBSC (n = 42) Median (range)</th>
<th>P Value*</th>
<th>No.</th>
<th>CD34+ (n = 40) Median (range)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to AGC &gt; 500/µL</td>
<td>&lt;1 6</td>
<td>10.0 (9.0-11.0)</td>
<td>.006</td>
<td>9</td>
<td>12.0 (11.0-20.0)</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>Days to platelet independence</td>
<td>&gt;1 25</td>
<td>13.0 (11-24)</td>
<td>.09</td>
<td>18</td>
<td>15.0 (12.0-20.0)</td>
<td>.37</td>
<td></td>
</tr>
</tbody>
</table>

* P value from one-way analysis of variance with Tukey’s multiple comparison tests after applying a rank transformation to the data.
† One patient died before reaching platelet independence.

**Table 5. Toxicities Associated With PBSC Reinfusion**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBSC Group A (n = 22)</th>
<th>PBSC/Split Group B (n = 26)</th>
<th>CD34+ Group C (n = 29)</th>
<th>CD34+ Split Group D (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen required</td>
<td>5 (23)*</td>
<td>6 (23)</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>8 (36)</td>
<td>15 (58)</td>
<td>4 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (32)</td>
<td>8 (31)</td>
<td>1 (3)</td>
<td>0</td>
</tr>
</tbody>
</table>

* No. of patients (percent).
marrow recovery or G-CSF effect, as the only difference between groups A versus C and B versus D was the type of stem cell support.

Recent data suggest that CD34+ selected allogeneic bone marrow and peripheral stem cell products are capable of inducing sustained hematopoietic recovery following ablative therapy.33 We found that CD34+ selected peripheral stem cell rescue resulted in slight, but significantly prolonged, hematopoietic recovery when compared with unselected stem cell reinfusion. This is not surprising because the number of total nucleated cells and CD34+ cells is reduced in the postselection product. Additionally, CD34+ selected products might be more susceptible to the damage inflicted by cryopreservation and thawing since, as opposed to unselected PBSC, controlled rate freezing is mandatory to maintain sufficient viability (unpublished data). Another consequence of ≥2 logs of “purging” of the non-CD34+ cellular components such as the CD3+ cell population,33 could be a decrease in the number of facilitator or accessory cells, which might be needed for early recovery. Accessory cells within the lymphocyte and monocyte population could potentially be contributing to hematologic recovery following chemotherapy and to the sustenance of hematopoiesis in the setting of human immunodeficiency virus (HIV) infection.34,35 We observed diminished toxicities with CD34+ selected PBSC rescue. The possible explanations for our findings include both the reduced volume of CD34+ selected products and decreased DMSO content. Since the absolute cell content is decreased in the CD34+ selected products, one can theorize a reduction in cytokine release from cells entering different phases of activity following thawing and reinfusion. An additional benefit of CD34+ selection is the ability to use these cells for gene markings and transfer studies.36 Gene marking of tumor cells from patients suffering from a variety of malignancies have demonstrated that these cells survive cryopreservation and may be responsible for late relapse.37,38 At the University of Colorado following CD34+ selection, which resulted in a 2-log reduction of the starting nucleated cell count, investigators could not detect breast cancer cells in three products, which were previously “contaminated” with immunohistochemically detectable cancer cells.22 We observed that CD34+ selection resulted in a ≥2-log decrease in total nucleated cell count, however the presence of breast cancer cells was not evaluated. The clinical significance of either negative “purging” or positive selection, including ex vivo expansion of CD34+ selected cells needs to be tested in prospective, randomized trials.39-41

We have also compared split schedule versus once a day administration of G-CSF 10 µg/kg; SC priming with G-CSF given as 5 µg/kg bid for patients in cohort D resulted in higher yields of CD34+ cells, while permitting a smaller number of leukapheresis procedures. Our observations are consistent with previous reports favoring prolonged exposure to G-CSF ≥10 µg/kg/d for priming, although we need to point out that patients in cohort D were less heavily pre-treated (none received prior radiation therapy) and their disease status was less advanced than the other three cohorts.14,42

In summary, administration of relatively high dose G-CSF on a bid schedule yields a peripheral stem cell product with higher percentage of CD34+ cells. Peripheral blood stem cell rescue following CD34+ selection provides for sustained hematopoietic function, but may result in slightly delayed recovery. Toxicities associated with reinfusion of unselected peripheral stem cell products are reduced following CD34+ selected stem cell rescue. Finally, we have demonstrated that a relatively small dose (≤2.5 × 10^6/kg) of peripheral blood-derived nucleated cells can initiate early hematopoietic recovery, and when followed 2 days later by reinfusion of a larger number of stem cells, may also contribute to the sustenance of hematopoietic function. One can theorize that such a small “sacrificial” dose of PBSC could be reinfused concomitantly with high-dose chemotherapy. At least a fraction of pluripotent stem cells and committed progenitors could survive the effects of nonablative chemotherapy, either because they are dormant or through various mechanisms of drug resistance (aldehyde dehydrogenase, MDR1)43,44 and enter a state of division/differentiation immediately after completion of chemotherapy. If this hypothesis is correct, further acceleration of early hematopoietic recovery would be the outcome. This same theory could also explain why patients receiving growth factors during induction therapy experience shorter periods of absolute granulocytopenia.45 Presently, we are testing our hypothesis in breast cancer patients treated with the same high-dose chemotherapy regimens described in this report.

ACKNOWLEDGMENT

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