Administration of Human Recombinant Granulocyte Colony-stimulating Factor (Filgrastim) Accelerates Granulocyte Recovery following High-dose Chemotherapy and Autologous Marrow Transplantation with 4-Hydroperoxycyclophosphamide-purged Marrow in Women with Metastatic Breast Cancer

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ABSTRACT

Stem cell contamination by tumor is common in many diseases for which autologous bone marrow transplantation is used. In in vitro models chemotherapeutic purging reduces contamination and may have an impact on clinical outcome. Purging, however, delays engraftment. Little is known about the ability of granulocyte colony-stimulating factor (G-CSF) to accelerate myelopoiesis after purged autologous bone marrow transplantation. We treated 22 women with metastatic breast cancer with high-dose cyclophosphamide and thiopeta and, following the infusion of 4-hydroperoxycyclophosphamide-purged marrow, administered G-CSF, 16 μg/kg daily, from day 0 to engraftment. Results were compared with a control population of 24 women with breast cancer who received identical chemotherapy and purged marrow but no growth factor. Neutrophil recovery was accelerated in the G-CSF-treated population. An absolute neutrophil count of 500 was reached in 19 days compared with 29 for the historic controls. The median number of days febrile was reduced (8 versus 5.5) as were the number of days of hospitalization from marrow infusion (33 versus 25). There was no difference in the number of days on antibiotics or time to last platelet transfusion. G-CSF was administered without any notable toxicity. G-CSF accelerates myelopoiesis following the infusion of 4-hydroperoxycyclophosphamide-purged autologous marrow and shortens hospitalization.

INTRODUCTION

High-dose chemotherapy requiring stem cell infusional support is curative treatment for a number of malignant disorders (1). In the treatment of metastatic breast cancer this maneuver results in high response rates and a small proportion of prolonged disease-free survivors (2-4). The exact magnitude of the therapeutic benefit produced by high-dose treatment is currently under investigation in a number of randomized trials.

Abbreviations

4-HC, 4-hydroperoxycyclophosphamide; ABMT, autologous bone marrow transplantation; ABMTR, autologous bone marrow transplantation; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IBW, ideal body weight; PBPC, peripheral blood progenitor cells.
on protocols approved by the Joint Committee for Clinical Investigation of the Johns Hopkins Medical Institution and all patients signed informed consent forms.

**Marrow Collection and Processing.** Prior to marrow collection all patients had a bone marrow biopsy to ensure both adequate reserves for harvesting and absence of histologic evidence of breast cancer in the marrow. Marrow was collected from bilateral posterior iliac crests within 6 weeks of induction chemotherapy. In all patients the targeted harvest yield was 4 × 10^8 nucleated cells/kg IBW. Bone marrow grafts from the historic controls were treated with 100 µg/ml of 4-HC, as previously described (14). Briefly, buffy coat cells were incubated with 100 µg/ml of 4-HC at a nucleated cell concentration of 2 × 10^7 cells/ml for 30 min at 37°C. The RBC content of the incubation mixtures was adjusted to obtain a mean incubation packed red cell volume of 7% (range, 6–9%). Cells were then concentrated, cryopreserved with 10% dimethyl sulfoxide in a control rate freezer, and stored in liquid nitrogen. Bone marrow grafts from the G-CSF-treated study population were collected and buffy coat concentrates were prepared in the same manner as the historic controls. How grafts from the G-CSF-treated study population were collected and buffy coat concentrates were prepared in the same manner as the historic controls. However, in this group the buffy coat cells were further purified over a density gradient solution (specific gravity, 1.077 g/ml) to remove all erythrocytes and mature granulocytes from the mixture (15). The cells were then incubated with 60 µg/ml of 4-HC at a nucleated cell concentration of 2 × 10^7 cells/ml for 30 min at 37°C and washed by using tissue culture media. Cryopreservation and storage were identical to the historic controls. For infusion, all marrow products were rapidly thawed in a 37°C water bath and infused without manipulation (50 ml over 3–5 min).

**Progenitor Cell Assays.** Progenitor cell assays were performed on samples obtained before and after the 4-HC purge, using conditions previously described (16). When samples were obtained from the buffy coat concentrates (before and after treatment), light-density cells (specific gravity ≤ 1.077 g/ml) were prepared by Ficoll-Hypaque separation in 50-ml conical tubes before the cells were cultured. When the entire marrow had been processed with the use of a density gradient, the samples were plated directly (pretreatment) or washed twice (posttreatment) and then plated. For pretreatment samples, 5 × 10^6 cells were put into each plate, while 1 × 10^6 cells were used for post-treatment samples. Cultures were performed in quadruplicate and were maintained at 37°C in a humidified 5% CO2 incubator. Myeloid colonies (CFU-GM) were identified after 14 days of culture. The total number of colonies per kg was determined as follows:

\[(\text{cells/kg treated}) \times (\text{test tube Ficoll recovery, when necessary}) \times (\text{average no. colonies counted/plate)/(no. cells plated})\]

**Chemotherapy.** All patients in the study and historic control populations were treated with cyclophosphamide, 6000 mg/m^2, and thiopeta, 800 mg/m^2 IBW given as a continuous infusion from days −8 to −4 before pABMT. High-dose i.v. hydration was used as prophylaxis against hemorrhagic cystitis. The study population was also treated with the coumeromycin antibiotic, novobiocin, for 7 days bracketing the time of chemotherapy administration (Day −10 to −3). Novobiocin inhibits eukaryotic topoisomerase II and was being studied as a potential agent to reverse resistance to alkylating agents. The last dose of novobiocin was given on day −3 prior to marrow infusion. We have previously shown that hematopoietic recovery following pABMT in a breast cancer population treated with high-dose cyclophosphamide and thiopeta can be predicted from the kinetics of CFU-GM growth of a postpurge marrow sample, indicating that engraftment is the result of the infused purged marrow and not from endogenous reconstitution (16). Thus, the kinetics of engraftment is not influenced by the nature or intensity of the conditioning regimen given prior to pABMT and treatment with novobiocin should have had no influence on hematopoietic recovery.

**Supportive Care.** With the exception of treatment with G-CSF, supportive care measures were virtually identical in the study and control populations. All patients had double lumen indwelling silastic catheters inserted prior to high-dose therapy. Patients with a history of herpetiform infection or who were herpes simplex virus or cytomegalovirus antibody-positive were treated with acyclovir prophylaxis (125 mg/m^2 i.v. every 6 h). All patients received 400 mg norfloxacin p.o. every 12 h and vancomycin 500 mg i.v. every 12 h until discharge. Fungal prophylaxis was provided in the study population by fluconazole, 400 mg daily, and in the control population by miconazole, 5 mg/kg i.v., every 8 h. Patients who were neutropenic were treated with cefazidime for fever, >38.2°C, after appropriate cultures were obtained. Antibiotic and fungal coverage was broadened as dictated by the clinical and bacteriologic data available in each case.

No patient in the control population received colony-stimulating factors after marrow infusion. In the study population G-CSF (Filgrastim; Amgen, Thousand Oaks, CA) was given at a dose of 16 µg/kg/IBW as a 4-h i.v. infusion daily beginning 3 h after marrow infusion and continued until the neutrophil count reached 2000/µL, at which point the dose was reduced to 4 µg/kg daily. G-CSF was stopped 48 h later if the neutrophil count remained above 2000/µL. G-CSF was restarted at 4 µg/kg daily if the neutrophil count subsequently dropped below 1000/µL.

**Toxicity/Efficacy Evaluation.** All patients were monitored prospectively for hematopoietic recovery, transfusion requirements, infectious complications, duration of treatment with broad spectrum antibiotics, and duration of hospitalization.

**Statistical Analysis.** Comparisons of engraftment parameters and hematopoietic toxicities between study and control populations were performed by the Wilcoxon rank sum test for median differences. In all analyses, the level of significance was ≤ 0.05.

**RESULTS**

**Hematological Recovery.** Treatment with G-CSF accelerated neutrophil recovery. There was no difference between the control and study populations in time needed to attain 100 neutrophils. However, once neutrophil recovery began, it accelerated rapidly in the G-CSF-treated population, such that a neutrophil count of 500/µL was reached 10 days earlier than in the control patients (Fig. 1). In addition, patients who were treated with G-CSF were discharged on average 7.5 days earlier than the control population (Table 2). Once a neutrophil count of 2000/µL was reached, the G-CSF dose was reduced to 25% for 48 h and then stopped. This dose reduction was accompanied by a drop in absolute neutrophil count of only 4% and a further drop of 5% once G-CSF was discontinued. The overall reduction in absolute neutrophil count between the last day of full dose G-CSF and 2 days after stopping cytokine support was 12%. In no patient was therapy with G-CSF recommenced.

No difference was seen in time to last platelet transfusion (25 versus 30 days, P = 0.5) between the control and study populations. There was a small, although statistically significant, decrease in the number of red cell transfusions required in the study population (8 versus 9.5, P = 0.01). The median duration of days on antibiotics was similar in both patient populations (20.5 in both). However, the study population had a median 2.5 fewer febrile days (defined as maximum daily temperature > 38.2°C) (5.5 versus 8, P = 0.04).

**Nonhematological Toxicity.** There were no toxic deaths in either group. Patients were evaluated for potential G-CSF-related toxicities, e.g., rash, fever, bone pain, weight gain, or edema. The only toxicity thought likely to be related to G-CSF was Grade III bone pain seen in 1 patient. Median duration of G-CSF treatment in the study population was 23 days. In no patient was therapy stopped early or dose modified because of toxicity. It was not necessary to reinstitute therapy with G-CSF in any patient.

**Cell Culture Studies.** Samples of marrow before and after in vitro purging with 4-HC were cultured to determine CFU-GM content. Results are outlined in Table 3. Similar numbers of CFU-GM (134

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**Table 1 Patient characteristics**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>G-CSF treated</th>
<th>Historic controls</th>
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<tbody>
<tr>
<td>Patient no. N = 22</td>
<td>N = 24</td>
<td></td>
</tr>
<tr>
<td>Median Age (range)</td>
<td>44 (32-53)</td>
<td>42 (30-55)</td>
</tr>
<tr>
<td>Median ECOG* performance status</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median prior chemotherapy regimens</td>
<td>2 (1-3)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
<td>7 (32%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Median no. of disease sites</td>
<td>2 (1-3)</td>
<td>2 (1-5)</td>
</tr>
</tbody>
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* ECOG, Eastern Cooperative Oncology Group.
G-CSF AND PURGED BONE MARROW

![Graph showing neutrophil recovery](image)

**Fig. 1.** Time to neutrophil recovery in G-CSF-treated study population (—) and untreated historic controls (— — —). Median absolute neutrophil count/ml is plotted against time in days following the infusion of 4-hydroperoxycyclophosphamide-purged bone marrow.

versus 120 × 10^3/kg) were collected from both patient populations. However, the numbers of CFU-GM were lower in the G-CSF-treated group following the 4-HC purge (0.2 versus 4.4 × 10^3/kg), probably reflecting the increased efficacy of purging following RBC removal.

**DISCUSSION**

Data suggesting a dose-dependent improvement in efficacy for a variety of chemosensitive malignancies treated with chemotherapy or radiation therapy have prompted the investigation of high-dose treatment with autologous stem cell rescue. This therapy is curative in some settings, although toxicity due to myelosuppression is substantial. The mortality associated with ABMT is variously reported at between 5 and 20%, depending on a number of patient and treatment variables. Around 50% of these deaths may be attributable to infectious causes. In addition, prolonged and profound myelosuppression implies extended hospitalization and intensive treatment of infectious complications. As high-dose therapy is applied to a widening circle of solid tumors (e.g., breast and ovarian cancers), where its efficacy is less certain, maneuvers to reduce morbidity become critical in the cost/benefit analysis of treatment.

The identification, cloning, and subsequent pharmaceutical production of recombinant human hematopoietic growth factors have resulted in the use of these compounds to ameliorate myelosuppression following chemotherapy. The aims of such treatment have been to reduce toxicity and increase the intensity of chemotherapy. Studies of endogenous cytokine production in patients recovering from autologous and allogeneic transplant have suggested that G-CSF has a critical role in promoting myelopoiesis in this setting (17, 18). Randomized studies of recombinant human GM-CSF following ABMT have demonstrated accelerated myeloid recovery, reduced rates of infection and hospitalization, and earlier discharge in treated patients (19). In studies utilizing historical control populations, similar effects have been seen following the use of G-CSF (20, 21). Sheridan showed accelerated neutrophil recovery, and reduced need for parenteral antibiotics and isolation, in 15 patients treated with G-CSF, 20 µg/kg/day, from the day following marrow infusion (20). Thus, there are good data to support the use of hematopoietic growth factors following the infusion of unpurged autologous bone marrow after myelotoxic therapy.

The potential problem of tumor cell contamination of the infused stem cell product has implications for the toxicity of ABMT and the role of growth factors in ameliorating this toxicity. That bone marrow is contaminated by malignant cells, in a variety of diseases where ABMT is utilized, is not debated. However, the implications of such contamination and the role of marrow purging are not established. Nevertheless, some indirect evidence does point to a useful role for marrow purging. Rowley et al. (7) have shown that the relapse rate is lower following ABMT for AML if the recovery of CFU-GM from the postpurge specimen is less than 1%. In addition, historically con-

**Table 2** Comparison of engraftment parameters and hematopoietic toxicities in study (G-CSF treated) and control populations

<table>
<thead>
<tr>
<th>G-CSF treated</th>
<th>Historic controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to ANC ≥ 100</td>
<td>17.5 (13-27)</td>
<td>19.5 (12-30)</td>
</tr>
<tr>
<td>Days to ANC ≥ 200</td>
<td>18 (13-30)</td>
<td>24 (12-36)</td>
</tr>
<tr>
<td>Days to ANC ≥ 500</td>
<td>19 (16-31)</td>
<td>29 (14-40)</td>
</tr>
<tr>
<td>Days with fever</td>
<td>5.5 (1-14)</td>
<td>8 (1-22)</td>
</tr>
<tr>
<td>Days of hospitalization</td>
<td>25 (20-46)</td>
<td>33 (18-43)</td>
</tr>
<tr>
<td>Days on antibiotics</td>
<td>20.5 (7-36)</td>
<td>20.5 (12-39)</td>
</tr>
<tr>
<td>No. of RBC transfusions</td>
<td>8 (7-13)</td>
<td>9.5 (6-18)</td>
</tr>
<tr>
<td>No. of platelet transfusions</td>
<td>95 (47-205)</td>
<td>127 (37-369)</td>
</tr>
<tr>
<td>Days to last platelet transfusion</td>
<td>25 (14-46)</td>
<td>30 (16-47)</td>
</tr>
</tbody>
</table>

*ANC, absolute neutrophil count; NS, not statistically significant, P > 0.05.

**Table 3** Nucleated cell and CFU-GM recoveries before and after 4-HC treatment of study and control marrows

<p>| Predicted and actual neutrophil recoveries in both populations. All numbers are medians. |
|-----------------------------------------------|-----------------|-----------------|---|</p>
<table>
<thead>
<tr>
<th>G-CSF treated</th>
<th>Historic controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM (×10^3/kg)</td>
<td>134</td>
<td>120</td>
</tr>
<tr>
<td>Pre-4-HC treatment</td>
<td>0.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Post-4-HC treatment</td>
<td>0.2%</td>
<td>3.3%</td>
</tr>
<tr>
<td>Predicted day to 500 granulocytes</td>
<td>41</td>
<td>29</td>
</tr>
<tr>
<td>Actual day of 500 granulocytes</td>
<td>19</td>
<td>29</td>
</tr>
</tbody>
</table>

*NS, not statistically significant, P > 0.05.
trolled data from the European Bone Marrow Transplant Registry have suggested that patients with AML treated with mafosfamide-purged marrow will have lower relapse rates than patients who receive unpurged grafts (8). No randomized data exist, however, to support the role of marrow purging in AML or any other disease. While the advantages of purging are uncertain, the drawbacks are clear. Chemotherapeutic purging with 4-HC may destroy up to 3 logs of tumor cells in experimental models but it will also reduce the number of committed progenitors in the graft (9, 22). This results in delayed engraftment, with a concomitant increase in cost and morbidity of therapy. For patients with metastatic breast cancer, this delay is probably in the order of about 7 days based on comparisons between disparate studies (2, 23).

Little data exist concerning the utility of recombinant growth factors in the acceleration of myelopoiesis following the infusion of purified marrow. In 1989 Blazar et al. (10) reported on the use of GM-CSF in 25 patients undergoing ABMT for acute lymphocytic leukemia who received marrows purged with 4-HC and a variety of monoclonal antibodies. Accelerated neutrophil recovery (as compared with historic controls) was only seen in patients who received $\geq 7.2 \times 10^3$ CFU-GM/kg, suggesting that GM-CSF was efficacious only if the committed progenitor (CFU-GM) content of the graft was high. By extrapolation, the kind of vigorous marrow purging which may be associated with improved antineoplastic effects would render the graft insensitive to the beneficial effects of GM-CSF.

More recent data on the use of GM-CSF following pABMT in small numbers of patients suggest that this compound does have an effect in accelerating myelopoiesis in this setting. Carlo-Stella et al. (11) have reported outcome for 10 patients receiving mafosfamide-purged marrows who were treated with GM-CSF, $10 \mu g$/kg/day, and compared them with 10 historic controls. The median time to 500 neutrophils was reduced by 11 days, and there was a reduction in hospitalization of 8 days. Gorin et al. (12) treated 91 patients with non-Hodgkin's lymphoma with high-dose therapy and ABMT and randomized the patients to GM-CSF, $250 \mu g$/m$^2$/day, or placebo from the day of infusion. A subgroup of 14 patients received marrow purged with mafosfamide. Median time to neutrophil recovery (500/µl) was reduced by 4.5 days, which was statistically significant despite the small sample size.

This report describes the results of treatment with G-CSF following the infusion of 4-HC-purged marrow in a group of 22 patients with metastatic breast cancer receiving high-dose cyclophosphamide and thiotepa. Engraftment is compared with historic controls treated with identical chemotherapy and 4-HC pABMT but not G-CSF. In the G-CSF-treated population the number of CFU-GM collected was similar to that of the control population. However, following purging with 4-HC, the CFU-GM count in the study population was significantly lower. Our group has previously shown that the number of CFU-GM in the postpurge product predicts for the kinetics of engraftment (16). However, similar predictive models have not been possible with other types of bone marrow-purging techniques (24). The 4-HC engraftment projection model was created from the CFU-GM and engraftment data obtained from a group of 40 patients with assorted diseases who did not receive growth factors after transplant. This model was then used to predict engraftment based on the number of CFU-GM that survived the 4-HC purge. The time to myeloid engraftment was projected to be 41 days in the study group compared with 29 days for the control population. This longer predicted day of recovery is logical considering the lower number of progenitors surviving the 4-HC purge procedure $(0.2 \times 10^3 \text{ versus } 4.4 \times 10^3 \text{ CFU-GM/µl, respectively.)}$

Despite the reduced CFU-GM content in the bone marrow grafts of the treated population and the projected delay in engraftment, neutrophils engrafted significantly more rapidly in the patients treated with G-CSF than in the untreated controls. The median time to 500 neutrophils/µl was 19 rather than 29 days. This translated into a reduction of 7.5 days in hospitalization following marrow infusion. There was a small decrease in the red cell requirements and no difference in the day to last platelet transfusion (30 versus 25 days) in the study population. No difference in number of days on antibiotics was seen between the two patient groups, although the G-CSF-treated patients had fewer days of fever (5.5 versus 8).

It is not likely that major discrepancies in patient characteristics account for the significant difference in time to neutrophil recovery. Patients in the 2 groups were very similar in age, disease status, and extent of prior therapy, all factors which might have an impact on engraftment kinetics. In addition, it is highly unlikely that the incorporation of novobiocin in the induction regimen of the study group was responsible for the shortened length of myelosuppression. In preclinical models, novobiocin enhances the cytotoxicity of alkylating agents (25). Thus, if it were influencing myelosuppression, an increase in the duration of aplasia would be expected. Similarly, differences in the purging techniques would be expected to have resulted in delayed recovery in the G-CSF-treated population by virtue of the reduced CFU-GM recovery in that group. Based on the CFU-GM recovery seen, the time to myeloid engraftment was projected to be 41 days, based on historical experience in patients with breast cancer who received 4-HC-treated bone marrow grafts without growth factor support. The actual engraftment time of 19 days observed when G-CSF was used suggests that G-CSF is effective in accelerating myelopoiesis following the infusion of 4-HC-purged grafts. A second projection model created from the G-CSF-treated patient's engraftment and progenitor cell data could be used to predict engraftment kinetics for future patients who receive growth factors after transplant.

Data from a number of investigators have suggested that the use of chemotherapy and/or growth factor-stimulated PBPC either alone or in combination with bone marrow will result in accelerated hematopoiesis following high-dose therapy (26, 27). In particular, this approach may facilitate thrombopoiesis which is unaffected by G-CSF or GM-CSF. However, data on the long-term viability of such stimulated PBPC grafts are not yet available. In addition, many of the concerns which pertain to possible tumor infusion in unpurged bone marrow may also apply to the use of PBPC (6). The feasibility or necessity of purging PBPC and the effects of such purging on the speed of engraftment are unknown. Careful large-scale clinical studies will be required before the optimal stem cell sources, defined in terms of rapidity of engraftment and freedom from tumor cell contamination, are identified.

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