Adverse Effect on Bone Marrow Protection of Prechemotherapy Granulocyte Colony-Stimulating Factor Support

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Background: Increased proliferation of endogenous bone marrow progenitor cells in response to the administration of hematopoietic growth factors, followed by reduced cell cycling or entrance of the cells into a quiescent state upon withdrawal of the growth factors, may have clinically relevant effects on the tolerance of the hematopoietic system to subsequent myelotoxic treatments. Purpose: We investigated the ability of granulocyte colony-stimulating factor (G-CSF) to protect progenitor cells in the bone marrow of cancer patients from the toxic effects of subsequent treatments with chemotherapeutic agents. Methods: Thirty-six patients with histologically documented, locally advanced or metastatic breast cancer were randomly assigned to receive doxorubicin once every 3 weeks at a dose of 75 mg/m² and cyclophosphamide at a dose of 1000 mg/m², with G-CSF administered either before and after chemotherapy (18 patients) or after chemotherapy only (18 patients). For prechemotherapy administration of G-CSF, recombinant human methionyl (r-met Hu) G-CSF was administered subcutaneously to patients twice per day for 5 days at a dose of 5 μg/kg, with the last dose given 48 hours before the start of chemotherapy. For postchemotherapy administration of G-CSF, r-met Hu G-CSF was administered subcutaneously to patients once per day for 7 days at a dose of 5 μg/kg, with the first dose given 24 hours after chemotherapy. Results: The incidence or the duration of grade 4 neutropenia was not reduced in all patients by the use of prechemotherapy G-CSF; the incidence over all cycles of chemotherapy was 74% for patients treated with prechemotherapy and postchemotherapy G-CSF and 66% for patients treated with postchemotherapy G-CSF only (two-sided P, adjusted for dose = .21) and the median duration in both treatment arms was 3 days (two-sided P = .19). Unexpectedly, the incidence of grades 3 and 4 thrombocytopenia was much greater in patients who received prechemotherapy G-CSF compared with those who did not (54% versus 6%, respectively, over all chemotherapy cycles; two-sided P, adjusted for dose <.001). No difference in the decrease in hemoglobin level (adjusted for red blood cell transfusions) between patients in the two treatment arms was observed. Conclusions and Implications: No beneficial effects were associated with the administration of G-CSF to cancer patients prior to chemotherapy. The observation of more severe thrombocytopenia in patients treated with prechemotherapy G-CSF led us to conclude that the proliferation of progenitor cells was still increased 48 hours after the last dose of G-CSF and that the administration of chemotherapy at or within this time period actually worsens the toxic effects on bone marrow. This result has important ramifications for the design of clinical cancer treatment protocols, especially those that involve shortened intervals between cycles of chemotherapeutic agent administration. [J Natl Cancer Inst 1996;88:1393-8]
fore, the myeloprotective effect of prechemotherapy growth factor support should be further explored.

For this purpose, we used doxorubicin and cyclophosphamide, both given at high dose, in patients with locally advanced or metastatic breast cancer. The drug schedule and dosages were based on their ability to induce high response rates in patients with advanced breast cancer (12) and the capacity of doxorubicin and cyclophosphamide to mobilize progenitor cells on hematopoietic recovery (12). At the time of initiation and conducting the study, there were no existing data on different kinetic effects of G-CSF on the proliferation rate of progenitor cells.

Patients were randomly assigned to receive doxorubicin and cyclophosphamide chemotherapy with either prechemotherapy plus postchemotherapy administration of G-CSF or postchemotherapy only administration of G-CSF.

**Patients and Methods**

**Patients**

From October 1993 through March 1995, 36 patients who were being treated at the Rotterdam Cancer Institute were entered in the study. All patients were eligible. Eligibility criteria required that the patients have histologically documented breast cancer, either metastatic or locally advanced [stage IIIA, IIB—American Joint Committee on Cancer (AJCC) staging system (13)] disease, a performance status (World Health Organization/AJCC scale (13)) of 0 or 1, a leukocyte count $\geq 3.5 \times 10^9/L$, a neutrophil count $\geq 2.0 \times 10^9/L$, a platelet count $\geq 100 \times 10^9/L$, a creatinine level $\leq 1.4 \text{ mg/dL}$, a bilirubin level $<25 \text{ mg/dL}$, and a leukocytopenia grade $\leq 3$.

Prechemotherapy G-CSF was started 24 hours after chemotherapy at a dose of $5 \mu g$ subcutaneously twice daily, for a total of 3 days, the last dose to be given 48 hours before the administration of chemotherapy. The prechemotherapy dose of G-CSF was based on its greater ability to stimulate endogenous marrow progenitor cells (14).

Postchemotherapy G-CSF was started 24 hours after chemotherapy at a dose of $5 \mu g$ subcutaneously once daily for 7 days; this period was extended if neutrophil counts were less than $0.5 \times 10^9/L$ at that time, until recovery above that value. If postchemotherapy G-CSF had to be continued for more than 3 days, the next cycle of prechemotherapy G-CSF and doxorubicin and cyclophosphamide chemotherapy was postponed to keep a minimum of 4 days between the last postchemotherapy G-CSF administration and the first dose of prechemotherapy G-CSF. No escalations or reductions in the dose of G-CSF were necessary or applied.

**Duration of Therapy**

At least two cycles were to be given to all patients unless there was evidence of rapidly progressive disease after one course. Patients who demonstrated a response or no change and no more than a single 25% dose reduction of doxorubicin and cyclophosphamide were kept on protocol treatment for six cycles.

**Statistical Analyses**

A randomized phase III study design was used. The primary aim was to detect whether the incidence and duration of (febrile) grade 4 neutropenia (Common Toxicity Criteria [CTC] issued by the National Cancer Institute [NCI], Bethesda, MD; CTC-NCI grading) could be reduced by using the sequence of prechemotherapy plus postchemotherapy G-CSF during doxorubicin and cyclophosphamide chemotherapy. A reduction in the incidence of grade 4 neutropenia by 50% was deemed to be of clinical importance in view of the costs of pre-emptive G-CSF usage and the potential alternative measure of bone marrow support by harvesting and reinforcement of progenitor cells. By applying a triweekly schedule of $75 \text{ mg/m}^2$ doxorubicin and $1000 \text{ mg/m}^2$ cyclophosphamide plus postchemotherapy G-CSF support, grade 4 neutropenia was expected in 90% of the patients after the 1st cycle. Therefore, the incidence of grade 4 neutropenia was to be reduced from 90% to 45%. Hopes to demonstrate such reduction with 80% probability, while ensuring that the probability of wrongly concluding that there was a difference of more than 45% did not exceed 5% (one-sided test with $\alpha = 0.05$, $\beta = 0.20$), approximately 16 patients were required in each arm, i.e., a total of 32 assessable patients. Nadir blood cell counts were measured three times per week in all patients. Patients were considered assessable after the completion of one cycle of chemotherapy plus postchemotherapy G-CSF (with or without prechemotherapy G-CSF). Secondary end points were the incidence and duration of grade 3 plus 4 or grade 4 thrombocytopenia, anemia, and the number of red blood cell transfusions. The incidence of grade 4 neutropenia, grade 4 leucocytopenia, grades 3 plus 4 or grade 4 thrombocytopenia, both in cycle 1 and over all cycles, were compared by use of the Fisher’s exact test for a two-by-two table. The duration of these events was compared with the Mann–Whitney $U$ rank sum test. For comparisons that involved adjusting for 25% chemotherapy dose reductions, the Mantel–Haenszel test for combining 2 $\times$ 2 tables was used (15).

The influence of the type of treatment on the decline of hemoglobin levels during the course of treatment, adjusted for numbers of red blood cell transfusions administered, was studied with the use of a multivariate regression analysis. All $P$ values resulted from the use of two-sided statistical tests.

**Results**

All 36 patients entered in the study were eligible and assessable. Patient characteristics are shown in Table 1. The pretreatment characteristics were well balanced. Of a total of 151 cycles of chemotherapy given, 61 were preceded by prechemotherapy G-CSF, of which 36 cycles were given at a 100% dose and 25 were given at a 75% dose. Ninety cycles were given with postchemotherapy G-CSF only, of which 55 were given at a 100% dose and 35 were given at a 75% dose. The imbalance in the number of cycles in the two treatment arms was caused by the more frequent second dose reductions in patients receiving pre-emptive G-CSF. This need for a second dose reduction excluded the patients from the study and is detailed in the subsections below. With two exceptions (in both arms), chemotherapy was delivered at the scheduled intervals. In two patients in the experimental arm, chemotherapy was postponed for 2-3 days for one and two cycles, respectively, because of continuation of postchemotherapy G-CSF until the neutrophils recovered to a count of $2.0 \times 10^9/L$.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prechemotherapy and postchemotherapy (n = 18)</th>
<th>Postchemotherapy G-CSF only (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>44 (30-64)</td>
<td>41 (29-66)</td>
</tr>
<tr>
<td>Performance status 0/1</td>
<td>5/13</td>
<td>6/12</td>
</tr>
<tr>
<td>Locally advanced disease</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predominant site</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Visceral</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Bone</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Blood values at study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level, mmol/L (range)</td>
<td>8.1 (6.7-9.4)</td>
<td>8.1 (6.7-9.2)</td>
</tr>
<tr>
<td>Leukocyte count × 10^9/L (range)</td>
<td>6.4 (4.6-9.1)</td>
<td>5.8 (3.0-9.6)</td>
</tr>
<tr>
<td>Platelet count × 10^9/L (range)</td>
<td>269 (173-520)</td>
<td>236 (152-356)</td>
</tr>
<tr>
<td>Response† to chemotherapy</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

*G-CSF = granulocyte colony-stimulating factor. Unless otherwise specified, values = number of patients.†Response denotes measurable disease regression.

greater than 0.5 × 10^9/L. Since objective response rate was not an end point and lack of measurable or evaluable disease was not an exclusion criterion, one third of the patients could not be evaluated according to standard World Health Organization response criteria. Overall, there was measurable disease regression in 14 of 18 patients who received prechemotherapy plus postchemotherapy G-CSF and in 16 of 18 patients who received postchemotherapy G-CSF only.

Incidence and Duration of Grade 4 Neutropenia and Leukocytopenia

As expected, the incidence of grade 4 neutropenia by doxorubicin and cyclophosphamide chemotherapy at this dose and schedule with postchemotherapy G-CSF support was high, 95% in the first cycle and 66% in 90 cycles, despite a

25% chemotherapy dose reduction in 25 cycles. However, the incidence of grade 4 neutropenia was similar in the patients receiving both prechemotherapy G-CSF and postchemotherapy G-CSF, 100% in cycle 1 and 74% in 61 cycles, of which 35 were given at a 25% reduced dose. The incidence of grade 4 neutropenia was thus not reduced by the use of prechemotherapy G-CSF (P = .37) for the comparison over all cycles. When adjusting for chemotherapy dose delivered, these results were again not different (Mantel–Haenszel test for combining 2 × 2 tables [P = .21]) (Table 2). In addition, the duration of grade 4 neutropenia was not significantly different; with the use of prechemotherapy plus postchemotherapy G-CSF, the median duration was 3 days (range, 1-7 days), with postchemotherapy G-CSF alone, the median duration was 3 days (range, 1-6 days) in cycle 1, and in both arms the median duration was 3 days (P = .19; over all cycles).

Similarly, the incidence of grade 4 leukocytopenia over all cycles was not different with 83% grade 4 leukocytopenia in the postchemotherapy G-CSF arm and 94% in the prechemotherapy plus postchemotherapy G-CSF arm having been observed (P = .60). There was an equivalent incidence of neutropenic fever in both treatment arms, with 16 (19%) of 90 and 12 (20%) of 61 case subjects, respectively, experiencing this toxic effect.

Incidence and Duration of Grades 3 Plus 4 and Grade 4 Thrombocytopenia

The incidence of grades 3 plus 4 thrombocytopenia with postchemotherapy G-CSF support over all cycles was 6%, and grade 4 thrombocytopenia was observed in only 2% of all cycles (Table 3).

Unexpectedly, the incidence of both grades 3 plus 4 and grade 4 thrombocytopenia was considerably higher in the patients who also received G-CSF prechemotherapy; grades 3 plus 4 were observed in 54% of all cycles, and grade 4 was observed in 25% of all cycles (P < .001) for both comparisons. When adjusting for chemotherapy dose delivered, this highly significant difference was maintained (P < .001) for both comparisons.

In view of the imbalance in the total numbers of cycles administered, we also calculated the median platelet count nadirs for each cycle separately (Table 4). The median nadir counts between the two treatment groups were compared within each cycle, adjusted for chemotherapy dose delivered, with bivariate linear regression analysis. For cycles 1-4, there was a significant difference between the

Table 2. Incidence of grade 4 neutropenia*

<table>
<thead>
<tr>
<th>G-CSF arm</th>
<th>Chemotherapy cycles at 100% dose</th>
<th>Chemotherapy cycles at 75% dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prechemotherapy and postchemotherapy G-CSF</td>
<td>Postchemotherapy G-CSF only</td>
</tr>
<tr>
<td>No. of cycles of chemotherapy</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>No. of patients with grade 4† (%)</td>
<td>30 (83.3)</td>
<td>15 (60.0)</td>
</tr>
</tbody>
</table>

*G-CSF = granulocyte colony-stimulating factor.
†Comparison of the incidence of grade 4 neutropenia, adjusting for chemotherapy delivered, Mantel–Haenszel test for combining 2 × 2 tables; P = .21.
treatment groups ($P<.05$). For cycles 5 and 6, the numbers were too small to calculate $P$ values. In addition, the difference in grades 3 plus 4 thrombocytopenia between the treatment groups, adjusted for dose delivered, was tested within each cycle with logistic regression analysis and was again significant ($P<.05$) for cycles 1-3.

The duration of grades 3 plus 4 thrombocytopenia was not significantly different; with the use of prechemotherapy plus postchemotherapy G-CSF, the median duration was 7 days (range, 2-12 days), with postchemotherapy G-CSF, the median was 5 days (range, 1-7 days) in cycle 1, and in both arms the median was 7 days ($P = .76$) over all cycles.

It is noteworthy that the imbalance between the two treatment arms with regard to the total numbers of cycles given at a full dose or at a 25% reduced dose was caused by the difference in grades 3 plus 4 thrombocytopenia. This necessitated a second dose reduction, which excluded the patients from the study.

### Decline in Hemoglobin Levels During the Course of Treatment

The decline in hemoglobin levels, adjusted for numbers of red blood cell transfusions administered over all cycles (mean decline, 1.17 mmol/L; mean number of red blood cell units given, 2.6), did not depend on the type of treatment; $P = .91$.

### Table 3. Incidence of grades 3 plus 4 and grade 4 thrombocytopenia*

<table>
<thead>
<tr>
<th>G-CSF arm</th>
<th>Chemotherapy cycles at 100% dose</th>
<th>Chemotherapy cycles at 75% dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prechemotherapy and postchemotherapy G-CSF</td>
<td>Postchemotherapy G-CSF only</td>
</tr>
<tr>
<td>No. of cycles of chemotherapy</td>
<td>36</td>
<td>55</td>
</tr>
<tr>
<td>No. of patients with grades 3 plus 4 (%)</td>
<td>16 (44.4)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>No. of patients with grade 4 (%)</td>
<td>6 (16.6)</td>
<td>1 (1.8)</td>
</tr>
</tbody>
</table>

*G-CSF = granulocyte colony-stimulating factor.
†Comparison of the incidence of grades 3 plus 4 thrombocytopenia and comparison of the incidence of grade 4 thrombocytopenia, adjusting for chemotherapy dose delivered, Mantel-Haenszel test for combining $2 \times 2$ tables; $P \leq .001$.

### Discussion

In the present study, we investigated whether, with the addition of prechemotherapy G-CSF to a schedule of chemotherapy plus conventional postchemotherapy G-CSF support, the incidence and duration of bone marrow suppression could be reduced. The design of the study was based on previous findings of an abrupt progenitor proliferation arrest within 24 hours after withdrawal of the hematopoietic growth factor GM-CSF (5). This would allow for a chemoprotective effect on progenitor cells as was supported by initial clinical results of reduced neutropenia obtained with the use of prechemotherapy GM-CSF for 5 days in a 3-week schedule of topotecan followed by postchemotherapy GM-CSF support (9). In the present study, we explored the myeloprotective effect of such prechemotherapy use of G-CSF in a triweekly regimen of chemotherapy with doxorubicin and cyclophosphamide (both given at high dose) plus postchemotherapy G-CSF in patients with locally advanced or metastatic breast cancer. In this study, prechemotherapy G-CSF was also given for 5 days, with the final dose administered 48 hours before scheduled chemotherapy. We found no beneficial effect of the prechemotherapy administration of G-CSF in this schedule on the incidence and duration of the main end point, neutropenia. In contrast to what was expected, significantly more severe thrombocytopenia was observed in patients receiving prechemotherapy G-CSF as compared with the patients who received postchemotherapy G-CSF support only. No difference was observed in the decrease in hemoglobin levels, adjusted for red blood cell transfusions, during the course of chemotherapy cycles.

The lack of benefit of prechemotherapy G-CSF and especially the more severe thrombocytopenia in patients treated with prechemotherapy G-CSF suggests that the accelerated progenitor cell proliferation is still augmented 48 hours after the last dose of G-CSF and that, by administering chemotherapy at or within this time period, the toxic effects on the bone mar-

### Table 4. Median platelet count nadirs for each cycle

<table>
<thead>
<tr>
<th>Dose, %</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
<th>Cycle 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prechemotherapy and postchemotherapy G-CSF</td>
<td>100</td>
<td>18/50</td>
<td>10/56</td>
<td>5/48</td>
<td>2/119</td>
<td>1/119</td>
</tr>
<tr>
<td>75</td>
<td>0/—</td>
<td>6/83</td>
<td>7/22</td>
<td>2/37</td>
<td>3/26</td>
<td>2/44</td>
</tr>
<tr>
<td>Postchemotherapy G-CSF only</td>
<td>100</td>
<td>18/84</td>
<td>13/119</td>
<td>10/100</td>
<td>7/89</td>
<td>4/74</td>
</tr>
<tr>
<td>75</td>
<td>0/—</td>
<td>5/155</td>
<td>7/102</td>
<td>8/102</td>
<td>8/96</td>
<td>7/86</td>
</tr>
</tbody>
</table>

*G-CSF = granulocyte colony-stimulating factor.
†Numbers are too small to calculate $P$ value.
row are actually increased. Possible detrimental effects of prechemotherapy G-CSF on neutrophil recovery in this study may have been masked by the high incidence of severe neutropenia in both study arms as well as a beneficial effect on neutrophil recovery by post-chemotherapy G-CSF support. Although not statistically significant, there was a trend of more severe neutropenia in the prechemotherapy G-CSF study arm. The apparent lack of a difference in the development of anemia and red blood cell transfusion requirements may have been caused by the more frequent chemotherapy dose reductions applied in the prechemotherapy G-CSF arm because of the more severe thrombocytopenia.

Both the initial clinical data of an abrupt progenitor proliferation arrest (5) and the finding of reduced neutropenia with the prechemotherapy use of a hematopoietic growth factor (9) were obtained with GM-CSF. At the time of initiating and conducting this study, there were no existing data on possible differences between GM-CSF and G-CSF with regard to progenitor proliferation kinetics. Of note, it was recently reported (16) that the period of cycling of progenitor cells is prolonged to at least several days after withdrawal of r-met Hu G-CSF.

Our observation of increased bone marrow toxicity by the prechemotherapy use of G-CSF until 48 hours before chemotherapy has important consequences for the design and conduct of clinical studies aimed at shortening treatment intervals with the use of hematopoietic growth factors, since these growth factors are then administered until the day before chemotherapy (17-25). It is generally assumed that hematopoietic growth factors may be safely administered until the day before the resumption of chemotherapy (26), but our results clearly demonstrate that this may not be the case for at least several days. The cumulative thrombocytopenia observed in several of these studies (18,24,25) and ascribed to the chemotherapy dose intensification may actually be related to increased damage to progenitor cells.

In conclusion, we found no beneficial effects of the prechemotherapy use of G-CSF. On the contrary, administering G-CSF until 48 hours before chemotherapy significantly enhanced the toxic effects on the bone marrow, as evidenced by significantly more severe thrombocytopenia. This finding has an important impact on the design of clinical studies of closely spaced chemotherapy with the start of the next cycle early after the final administration of a hematopoietic growth factor.

References

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