Hematopoietic growth factors allow dose escalation of chemotherapy. This approach may potentially reduce the quality and quantity of hematopoietic stem cells. The capacity of stem cells recovered after dose intensification to support myeloablative therapy is unknown. In patients with previously untreated advanced follicular lymphoma, trilineage hematopoietic engraftment was compared in two sequential trials of induction therapy (standard dose cyclophosphamide, doxorubicin, vincristine, prednisone [CHOP] without growth factors or dose intensification) CHOP supported by granulocyte colony-stimulating factor (G-CSF) followed by identical myeloablative therapy and autologous stem cell support. Neutrophil, platelet, and red blood cell (RBC) engraftment were compared on days 100, 180, and 360 after stem cell reinfusion. Despite similar patient characteristics including reinfusion of comparable numbers of marrow mononuclear cells, after stem cell transplantation, a highly significant prolongation of neutrophil and platelet engraftment was seen in patients who received high dose CHOP and G-CSF in comparison to standard dose CHOP. These findings suggest that dose intensified chemotherapy and G-CSF recruited stem cells into a proliferative phase and that G-CSF allowed treatment at a time when stem cells were susceptible to damage by cytotoxic therapy. Such inadequate hematologic engraftment after myeloablative therapy might be avoided by either shortening the time that the growth factor support is administered, lengthening the interval between cycles, or attempting to repetitively harvest additional stem cells either from the marrow or peripheral blood. Therefore, intensification of chemotherapy with growth factor support must be used with caution if stem cells are to be used to support myeloablative therapy.

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was administered in fractionated doses (200 cGy) twice daily on

RESULTS

...
had stage IV disease by virtue of BM involvement. The characteristics of the patients treated with HD-CHOP who underwent ABMT were similar to those of 77 patients with previously untreated follicular lymphoma who received SD-CHOP before ABMT (Table 1). There was no statistically significant difference between the two groups of patients.

**Treatment with HD-CHOP.** After four cycles of HD-CHOP and G-CSF, only six of the 20 patients were in a clinical CR (31%). Thirteen patients achieved minimal disease state, while one patient progressed with a high grade lymphoma within 1 month after completing cycle 4. At the time of harvest, seven patients had histologic evidence of BM involvement. The CR rate after HD-CHOP was similar to that seen in previously untreated patients with follicular NHL treated with six to eight courses of SD-CHOP before ABMT, where the CR rate was 36%. Treatment with HD-CHOP and G-CSF was associated with profound myelosuppression in these patients with follicular lymphoma (Table 2). With each successive cycle, a greater degree of leukopenia and thrombocytopenia was observed. Whereas the median nadirs for WBC and platelets were 500/µL and 70,000/µL for cycle 1, by cycle 4, these had significantly decreased to 200/µL and 18,000/µL, respectively ($P = .02$ for WBC, $P = .001$ for platelets). Despite this progressive myelosuppression, the interval between administration of each course of therapy remained quite constant, with a median of 21 days for cycle 1 to 2 and cycle 3 to 4, and 21.5 days for the interval between cycle 2 to 3. Fever associated with neutropenia requiring hospitalization was seen in 24 of 80 (30%) courses of HD-CHOP and G-CSF administered despite administration of prophylactic trimethoprim-sulfamethoxazole during each cycle. No treatment-related deaths were seen. The incidence of fever and neutropenia was not statistically different between cycles ($P = .56$).

**ABMT course and outcome.** Nineteen of the 20 patients underwent BM harvest and ABMT. The patient who developed a histologic transformation to high grade lymphoma died of progressive disease 3 months after completing HD-CHOP. At BM harvest, all patients had WBC above 3000/µL, while two patients had Hct less than 28, and one patient had a platelet count less than 100,000/µL. BM cellularity was assessed before harvest in both groups of patients. Patients who received SD-CHOP had a mean cellularity of 46% (range, 15% to 90%), while patients treated with HD-CHOP had mean cellularity of 69% (range, 30% to 90%). These were significantly different ($P = .0007$) using a two sample $t$-test. At the time of beginning myeloablative therapy, the median WBC, platelet count, and Hct were significantly lower than that seen in patients in the antecedent study where SD-CHOP induction was administered before ABMT (Table 3). In the 19 patients who received HD-CHOP, the mean number of anti-B-cell monoclonal antibody-purged BM mononuclear cells reinfused was $4.92 \times 10^7$/kg (range, $1.94 \times 10^7$ to $11.42 \times 10^7$), which is not significantly different ($P = .31$) from patients treated with SD-CHOP induction ($4.06 \times 10^7$/kg). CD34+ cells were not enumerated in the marrow either before or after ex vivo purging in either study.

After HD-CHOP and G-CSF induction and ABMT, there were no acute in-hospital treatment-related deaths. After SD-CHOP induction and ABMT, there were two acute in-hospital treatment-related deaths both from diffuse alveolar hemorrhage syndrome. Fever associated with neutropenia was seen in all 19 HD-CHOP patients with four documented bacterial infections. Of the 77 patients who received SH-CHOP induction, 71 patients developed fever with neutropenia and nine had documented bacterial infections. The median hospital stay was 31.5 days for patients undergoing HD-CHOP induction, which is significantly longer than that seen in patients who received SD-CHOP induction therapy before ABMT, where the median duration of hospitalization was 26 days ($P = .03$). Two patients who received HD-CHOP and were platelet and RBC transfusion-dependent have died after relapse, one of hemorrhage and disseminated candidiasis and one of gastrointestinal bleeding at 8 and 23 months post-ABMT.

**Hematologic engraftment.** The time to attain an ANC of 500/µL was not significantly greater in patients who received HD-CHOP (median, 24 days) than in patients who received SD-CHOP (median, 19 days) ($P = .13$). The time to platelet engraftment was significantly greater in patients who received HD-CHOP induction (median, 56 days; range, 17 to 570+) than in patients who received SD-CHOP (median, 27 days; range, 14 to 75) ($P = .0005$). None of the patients treated with SD-CHOP received any hematopoietic growth factors after ABMT, whereas all patients who were treated with HD-CHOP induction received hematopoietic growth factors after ABMT. The median number of days that the HD-CHOP patients received hematopoietic growth factors post-ABMT was 27 days (range, 15 to 57). Despite hematopoietic growth factor support in the HD-CHOP group, it became clear with follow-up that lineage engraftment in the patients who received HD-CHOP induction therapy was significantly prolonged as compared with patients treated with SD-CHOP induction therapy.

Hematologic engraftment was analyzed at 100, 180, and 360 days post-ABMT. The values correspond to NCI-CTC hematologic toxicities of grade 2 at 100 days, grade 1 at 180 days, and grade 0 at 360 days post-ABMT. As seen in Table 4, at 100, 180, and 360 days post-ABMT, patients who re-

### Table 2. Toxicity After HD-CHOP Induction

<table>
<thead>
<tr>
<th>Cycle</th>
<th>WBC nadir $\times 10^3$/µL (median, range)</th>
<th>Platelet nadir $\times 10^3$/µL (median, range)</th>
<th>Fever/neutropenia (no. of episodes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500 (100-4,500)</td>
<td>70 (5-127)</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>400 (100-3,400)</td>
<td>52 (11-130)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>300 (100-1400)</td>
<td>22 (6-82)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>200 (&lt;100-700)</td>
<td>18 (7-128)</td>
<td>5</td>
</tr>
</tbody>
</table>
ceived HD-CHOP had marked delay in achieving the expected neutrophil count at those time intervals, of 1,200/µL, 1,600/µL, and 2,000/µL, respectively. This is highly significantly different from the patients who received SD-CHOP induction before ABMT, where the vast majority attained the expected neutrophil count for those intervals. In marked contrast, the patients who received HD-CHOP, failed to develop adequate numbers of neutrophils. At 1 year, 63% of the HD-CHOP patients had an ANC of under 2,000/µL, whereas 89% of the SD-CHOP patients had an ANC of 2,000/µL or above. Similarly, platelet engraftment was markedly delayed in the patients who received HD-CHOP, whereas by 360 days, 75% had a platelet count of less than 100,000/µL (Table 5). In contrast, 89% of patients who received SD-CHOP induction therapy had a platelet count of 100,000/µL or greater at 1 year. Red blood cell (RBC) engraftment was also markedly impaired in the 19 patients who received HD-CHOP before ABMT (Table 6). Although not statistically significant, RBC engraftment was delayed as compared with patients who received SD-CHOP. At 1 year post-ABMT, 31% of the HD-CHOP patients did not have an Hct of greater than 30 as compared with 19% of the SD-CHOP patients.

At 1 year post-ABMT, four of 18 HD-CHOP patients in remission were still platelet transfusion-dependent and three of these four patients were also RBC-dependent. The marked impairment in platelet engraftment is reflected by the observation that two of 15 patients who are alive and in remission are still platelet and RBC transfusion-dependent at 26 months post-ABMT. In contrast, none of the 77 patients treated with SD-CHOP induction were platelet or RBC transfusion-dependent at or beyond 1 year post-ABMT. We examined the relationship between BM cellularity before harvest and engraftment in both groups of patients. We found that low cellularity (less than 50%) was not significantly associated with delayed neutrophil engraftment in either patients treated with SD-CHOP or HD-CHOP induction. Among the patients receiving HD-CHOP, low BM cellularity before harvest was significantly associated with delayed platelet engraftment ($P = .02$), whereas no such association was seen among the patients treated with SD-CHOP. Moreover, there was no significant association between the marrow cellularity before harvest and number of BM mononuclear cells per kg reinfused among the HD-CHOP treated patients (not shown). This suggests that the engraftment problems of the HD-CHOP patients with low marrow cellularity may represent poor marrow stem cell reserve, rather than simply a problem of transfused cell dose numbers.

**DISCUSSION**

We report a complication of the use of high dose induction therapy with hematopoietic growth factor support not previously observed in clinical stem cell transplantation. Despite the significant myelosuppression observed after each cycle of HD-CHOP and G-CSF support, trilineage hematologic recovery occurred with acceptable median WBC, RBC, and platelet counts at admission for ABMT. Therefore, before ABMT, there was no indication that hematologic engraftment would be different from that seen in patients with follicular NHL undergoing ABMT at our institution after SD-CHOP induction therapy or, in fact, all other induction regimens used during the past decade. The limited WBC and platelet engraftment clearly present by 100 days post-ABMT is evidence for injury to both committed and primitive stem cells. Furthermore, the lack of adequate hematologic reconstitution at 1 year and beyond after ABMT suggests significant damage to primitive stem cells in a sizeable subset of these patients, as it is presumably the primitive stem cells that are responsible for maintaining long-term hematopoiesis after transplantation.

Experimentally, hematopoietic stem cells vary greatly in their self-renewal and proliferative capacity. Most primitive stem cells under steady state conditions are proliferatively inactive. Under normal physiologic conditions, there is a large functional reserve in the primitive stem cell population to prevent marrow exhaustion. Based on animal models, there are a number of situations where apparent stem cell exhaustion or depletion can be elicited. These include the serial transplantation of marrow into lethally irradiated mice; exposure of marrow to radiation or cytotoxic drugs that damage stem cells; exposure of the marrow to great

| Table 4. Percent of Patients Not Achieving Expected Absolute Neutrophil Count Post-ABMT |
|-----------------------------------|------------------|------------------|------------------|
|                                   | Day 100          | Day 180          | Day 360          |
| ANC expected value               | 1,200/µL         | 1,600/µL         | 2,000/µL         |
| SD-CHOP                          | 3                | 0                | 11               |
| HD-CHOP                          | 37               | 47               | 63               |
| $P$ value                         | .0016            | .00007           | .0001            |

| Table 5. Percent Patients Not Achieving Expected Platelet Count Post-ABMT |
|---------------------------------|------------------|------------------|------------------|
|                                 | Day 100          | Day 180          | Day 360          |
| Platelet count expected value   | 50,000/µL        | 75,000/µL        | 100,000/µL       |
| SD-CHOP                         | 6                | 4                | 11               |
| HD-CHOP                         | 58               | 65               | 75               |
| $P$ value                       | .0023            | .0039            | .000003          |
proliferative stress; and most recently, exposure of marrow to combinations of cytotoxic drugs and cytokines. Therefore, our working hypothesis is that high dose chemotherapy and G-CSF recruited both committed and primitive stem cells into a proliferative phase, and that G-CSF allowed us to retreat patients at a time interval when these stem cells were still susceptible to damage by cytotoxic therapy.

Using the treatment approach described above, it appears that dose escalation and G-CSF support for induction therapy before ABMT resulted in a severe and potentially irreparable toxicity. This marked delay in engraftment to date has not been associated with excess bleeding or infection. To date, five patients who have received SD-CHOP have developed myelodysplasia/acute myeloblastic leukemia. The follow-up in patients who received HD-CHOP is too short to assess this toxicity. Although no clinically relevant toxicity has been observed in patients who received HD-CHOP and G-CSF, our results suggest that one must be prudent in the use of high dose induction therapy and hematopoietic growth factor support before myeloablative therapy. Inadequate hematologic engraftment might be avoided by either shortening the time that growth factor support is administered, administering cytokines that might protect stem cells, lengthening the interval between cycles, or attempting to repetitively harvest additional stem cells either from the marrow or peripheral blood. Sufficient numbers of stem cells could potentially be harvested after the first cycle of dose intensified treatment, an approach that has been successfully done for patients with intermediate and high grade lymphomas. However, it would be important that a tumor-free stem cell product be obtained through either ex vivo purging and/or stem cell enrichment procedures. Although it is presently not known whether the peripheral blood stem cell compartment would be similarly depleted by multiple cycles of high dose CHOP and G-CSF, there is no reason to believe that it would not. A recent study suggested that stem cell toxins had a greater effect on peripheral blood stem cell progenitors than on BM progenitors. Therefore, attention to animal models and markers of hematopoietic stem cell function should be evaluated in all attempts to dose intensify induction therapy with growth factor support.

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REFERENCES


Table 6. Percent of Patients Not Achieving Expected Hct Post-ABMT

<table>
<thead>
<tr>
<th></th>
<th>Day 100</th>
<th>Day 180</th>
<th>Day 360</th>
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<tr>
<td>Hct expected value</td>
<td>28</td>
<td>30</td>
<td>&gt;30</td>
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<tr>
<td>SD-CHOP</td>
<td>14</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>HD-CHOP</td>
<td>37</td>
<td>24</td>
<td>31</td>
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<tr>
<td>P value</td>
<td>0.021</td>
<td>0.198</td>
<td>0.3136</td>
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</tbody>
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patients with a history of low grade B cell non-Hodgkin’s lymphoma.

Blood 77:2524, 1991


Cyclophosphamide, Doxorubicin, Vincristine, Prednisone Dose Intensification With Granulocyte Colony-Stimulating Factor Markedly Depletes Stem Cell Reserve for Autologous Bone Marrow Transplantation

Arnold Freedman, Donna Neuberg, Peter Mauch, John Gribben, Robert Soiffer, Kenneth Anderson, Michael Robertson, David C. Fisher, Robert Schlossman, Mary Kroon, Catherine Rhuda, Caroline Kuhlman, Jerome Ritz and Lee Nadler

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