Granulocyte Colony-Stimulating Factor to Prevent Dose-Limiting Neutropenia in Non-Hodgkin’s Lymphoma: A Randomized Controlled Trial

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The effect of granulocyte colony-stimulating factor (G-CSF) on neutropenia, infection, and cytotoxic chemotherapy administration was studied in a randomized trial in patients receiving intensive weekly chemotherapy for non-Hodgkin’s lymphoma (NHL). Eighty patients (aged 16 to 71 years) with high-grade NHL (Kiel) of any stage were randomized to receive VAPEC-B chemotherapy alone (39 patients) or with G-CSF administered as a daily subcutaneous dose of 230 μg/m² (41 patients). Prophylactic ketoconazole and cotrimoxazole were administered to all patients throughout treatment. The protocol specified identical dose modification and antibiotic treatment criteria for both groups. Neutropenia (absolute neutrophil count [ANC] < 1.0 × 10⁹/L) occurred in 15 of 41 (37%) of the G-CSF-treated patients and in 33 of 39 (85%) of the controls, giving a relative risk for control patients of 2.31 [95% confidence interval (CI), 1.61, 3.84; P = .00001]. Fever (≥37.5°C) with neutropenia (ANC < 1.0 × 10⁹/L) occurred in 9 of 41 (22%) of the G-CSF group and in 17 of 39 (44%) of the controls (relative risk for control, 2.28; 95% CI [1.01, 5.06]; P = .04). There were fewer treatment delays, with shorter duration (P = .01) in patients receiving G-CSF. Chemotherapy doses were reduced in 4 of 41 (10%) of the G-CSF patients and 13 of 39 (33%) of the controls (P = .01). The dose intensity of cytotoxic chemotherapy was significantly increased in patients receiving G-CSF (median of 95% in G-CSF group compared with 83% in control patients). Three vascular deaths occurred in the G-CSF group. Delays in the control group most commonly resulted from neutropenia (19 patients, compared with 2 patients in the G-CSF–treated group, P = .000007). Severe mucositis was the major dose-limiting toxicity in G-CSF–treated patients, but did not occur more frequently than in controls (15 patients in each group). Overall, patients randomized to receive G-CSF achieved a greater dose intensity than control patients, but this did not result in significant differences in drug toxicity (other than neutropenia), intravenous antibiotic usage, or hospitalization between the two groups.

HIGH-GRADENon-Hodgkin’s lymphoma (NHL) can be cured with combination chemotherapy. To increase the complete remission and cure rates, there has been a sustained effort to determine the most effective schedule, combination, and dosage of cytotoxic drugs. Modern regimens comprising alternating noncrossresistant drugs show a high complete remission rate, but neutropenia-related morbidity and mortality restrict the doses and limit the potential benefit of these cytotoxic regimens. Attempts to intensify treatment to improve results are prevented by very severe myelosuppression. The risk of infection is directly related to the severity and duration of neutropenia and despite the use of broad spectrum antibiotics, death from sepsis is not infrequent. In severely neutropenic patients, the morbidity associated with nonfatal infection is substantial. In a recent series of 182 patients with high-grade NHL, treated with intensive weekly VAPEC-B chemotherapy (Fig 1) in this center, 15 (8%) died of infective complications related to neutropenia, and 83 (73%) of the 113 who completed chemotherapy were subject to at least 1 week of treatment delay due to neutropenia (manuscript in preparation). The next logical step to improve treatment for high-grade NHL is to investigate the use of hematopoietic growth factors designed to ameliorate chemotherapy-induced neutropenia.

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein secreted predominantly by cells of the monocyte-macrophage lineage, but also by normal cells of mesodermal origin. G-CSF promotes the proliferation and differentiation of neutrophil precursors and enhances the effector functions of mature neutrophils in vitro and in vivo. The physiologic control of G-CSF production in vivo and its role in the maintenance of normal steady-state hematopoiesis remain unclear. The recombinant human product, r-metHuG-CSF (Amgen, Thousand Oaks, CA) is a human protein produced in an Escherichia coli expression system. The 175 amino acid protein is nonglycosylated and has a molecular weight of 18.8 Kd.

G-CSF has a molecular weight of 18.8 Kd. G-CSF as an adjunct to cytotoxic chemotherapy could improve response and cure rates in high-grade NHL in two ways. Firstly, protection from neutropenia-related infection and death would allow those patients with chemosensitive tumor to survive the induction period and achieve remission. Secondly, an increased cytotoxic dose intensity might be expected to improve overall response rates.

Phase I/II studies with G-CSF have shown that within 4 to 5 hours of administration there is a substantial, dose-dependent increase in peripheral blood neutrophil counts. These studies also show that the drug is well tolerated and indicate the potential clinical benefit of the compound in ameliorating infectious episodes associated with neutropenia in patients receiving cytotoxic chemotherapy.

The aim of this study was to investigate the potential clinical benefits of accelerated neutrophil recovery induced by G-CSF in patients receiving weekly VAPEC-B chemotherapy for high-grade NHL. The clinical effects of G-CSF...
therapy on the infectious complications of neutropenia were examined, including the incidence and duration of antibiotic use and days of hospitalization. In addition, the cytotoxic treatment delays and dose reductions were studied. This was a single center, open-labeled, randomized study comparing the effects of G-CSF with a parallel control group. No previous randomized studies of G-CSF have been reported in NHL patients.

**PATIENTS AND METHODS**

**Patients.** Previously untreated patients aged between 16 and 71 years with histologically documented high-grade NHL (Kiell classification) of any stage and performance status were entered. Unless fever was attributed to disease, patients were required to be afebrile for at least 24 hours before entry into the study. All patients had to have normal renal and hepatic function and a normal peripheral blood count, unless the abnormal parameter was directly attributable to lymphomatous infiltration.

Patients with central nervous system involvement and those with other uncontrolled serious medical conditions were excluded. Medications likely to affect white blood cell counts were disallowed. Eighty consecutive patients were recruited between August 1989 and March 1991. The study was approved by the South Manchester District Ethics Committee, and all patients gave written informed consent for entry into the trial.

**Treatment.** Treatment was assigned to patients using unstratified permuted block randomization. Patients received treatment with either 11 weeks of VAPEC-B chemotherapy alone or VAPEC-B plus G-CSF, administered as a daily subcutaneous dose of 230 μg/m² for 13 weeks, except days preceding and during treatment with doxorubicin, cyclophosphamide, or etoposide (Fig 1). The dose of G-CSF was chosen on the basis of preliminary phase I/II data. All patients received prophylactic cotrimoxazole 960 mg twice daily (bd) and ketoconazole 200 mg bd for 13 weeks from the start of chemotherapy.

Identical cytotoxic dose modification criteria were applied to both the control and G-CSF treatment groups. These were: (1) The doses of adriamycin, cyclophosphamide, and etoposide were reduced by 50%, if the absolute neutrophil count (ANC) on the planned day of treatment was ≥0.5 x 10⁹/L and less than 1.0 x 10⁹/L. (2) If, on the planned day of treatment, the ANC was less than 0.5 x 10⁹/L or the platelet count less than 20 x 10⁹/L, chemotherapy with adriamycin, cyclophosphamide, or etoposide was delayed by 7 days. If after a further 7 days, these parameters were still below these levels, a bone marrow aspirate was performed to assess disease status. If infiltration by lymphoma was demonstrated, patients received a 100% dose. If no lymphomatous infiltrate was present in the marrow, 50% of the planned dose was administered. (3) Patients with World Health Organization (WHO) grade III neuroepathy received a 50% vincristine dose reduction. If WHO grade IV neuroepathy developed, vincristine was stopped and not restarted.

**Study procedures.** Patients kept daily diaries of their G-CSF administration and oral temperature and had a full blood count performed weekly, with additional blood counts performed if a fever occurred. Patients who developed fever with neutropenia were admitted to the hospital, had daily blood counts performed, and were treated with parenteral broad spectrum antibiotics with piperacillin and netilmicin as first-line therapy. Antibiotics were stopped when the patient had remained afebrile for at least 24 hours, the neutropenia had resolved, and there was no clinical evidence of infection.

During the 13 weeks of therapy all adverse events and concomitant medications were recorded weekly. Serum samples were collected before the study and on completion of chemotherapy, to detect any antibody production to G-CSF. Tumor response rates were documented according to WHO criteria at weeks 5, 9, and 13 and on completion of the study. On completion of chemotherapy, patients with stage I and II disease received radiotherapy to encompass their initial disease. Patients were then assessed at
2-month intervals for the first year, 3-month intervals for the second year, and every 4 months thereafter.

**Statistical methods.** The objectives of the trial were to evaluate the effect of treatment with G-CSF on neutropenia, neutropenia with fever, and cytotoxic dose intensity. Other endpoints, such as the duration and severity of neutropenia, incidence of documented infection (bacterial culture), use of intravenous (iv) antibiotics, and hospitalization, were also studied.

The sample size was chosen to provide an 80% power of detecting a difference of 30% in the incidence of fever with severe neutropenia (ANC < 0.5 x 10^9/L; temperature ≥ 37.5°C) in a one-tailed test using the .05 significance level (eg, a reduction from 40% in the control group to 10% in the G-CSF group).

All analyses have been performed in an “intention to treat” manner. Incidence of neutropenia, dose delays and reductions, culture-confirmed infections, iv antibiotic administration, and hospitalization were compared using Fisher’s exact test. For each endpoint, the risk in the control group compared with the G-CSF group and its 95% confidence interval (CI) was calculated.

Kaplan-Meier survival curves were drawn for neutropenia with fever, survival, and disease-free survival and compared between treatment groups using the log-rank test. The proportion of patients having experienced an event were estimated from the survival curves. The relative risks were also estimated using survival methods.

Dose intensity was calculated separately for each cytotoxic agent. The dose intensity is the ratio of the actual dose to the planned dose, multiplied by the ratio of the planned time to the actual time. The planned dose was calculated on a cycle by cycle basis, based on the patient’s weight at the start of that cycle; the planned dose was adjusted for weight. The planned time on study was 77 days for all patients. The differences in dose intensity and duration of cycle delays between groups were compared using the Wilcoxon rank sum test.

The safety of G-CSF was also assessed. Adverse event profiles, concomitant medication usage, tumor response rates, and survival were compared in the two treatment groups.

**RESULTS**

A total of 80 patients were randomized between August 22, 1989 and March 8, 1991. Forty-one patients were randomized to the G-CSF group and 39 patients to the control group. All patients were included in the analysis of efficacy and tolerability. Pretreatment characteristics of the patients (Table 1) indicate that the groups were well matched for prognostic factors. Thirty-four patients (83%) in the G-CSF group and 29 (74%) in the control group completed 11 weeks of chemotherapy. The median follow-up was 15 months.

**Neutropenia.** The incidence of neutropenia (ANC < 1.0 x 10^9/L) in the G-CSF group was significantly reduced: 15 of the 41 (37%) G-CSF compared with 33 of the 39 (85%) control patients (P = .0001) (Fig 1). The relative risk (RR) of becoming neutropenic was 2.31 (95% CI of [1.51,3.54]).

Severe neutropenia (ANC < 0.5 x 10^9/L) was also significantly reduced in the G-CSF group. Of the 41 G-CSF-treated patients, 13 (32%) had at least one episode of severe neutropenia, compared with 28 of the 39 (72%) control patients (P = .0004). The RR of ever having severe neutropenia was 2.26 (95% CI, [1.39,3.70]). Blood counts were taken at least once weekly. More frequent counts might have shown an even greater difference.

**Infection.** The proportion of patients experiencing at least one episode of neutropenia (ANC < 1.0 x 10^9/L) with fever (≥37.5°C for 1 hour) was 23% in the group receiving G-CSF, compared to 44% in the control group, giving an RR of 2.26 (95% CI, [1.01,5.06]; P = .04). Figure 2 depicts the cumulative risk of developing fever with neutropenia.

Severe neutropenia (ANC < 0.5 x 10^9/L) with fever (≥37.5°C for 1 hour) was observed in 20% of patients receiving G-CSF, and 30% of patients in the control group, giving an RR of 1.57 (95% CI, [0.63,3.91]; P = .33). Five patients discontinued chemotherapy because of infection in the control group, compared with two in the G-CSF-treated group (Table 2).

There was little difference in the incidence of culture confirmed infections; seven patients in the G-CSF group and five in the control. Similar organisms were cultured in both groups, the majority being gastrointestinal organisms. There were no proven staphylococcal or fungal septicemias.

Patients did not routinely have central venous catheters inserted. Nine of the G-CSF patients required iv antibiotics for more than 3 days, compared with 12 of the controls.

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**Table 1. Patient Characteristics**

<table>
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<tr>
<th>Age (yr)</th>
<th>Median</th>
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<td>16-67</td>
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<tr>
<td>Control</td>
<td>53</td>
<td>22-71</td>
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<table>
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<tr>
<th>Gender</th>
<th>ECOG performance status</th>
<th>Stage of disease</th>
<th>B symptoms present</th>
<th>Histology (working formulation)</th>
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<td>9 (22)</td>
<td>15 (37)</td>
<td>High</td>
<td>15 (37) 14 (44)</td>
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<tr>
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<td>6 (16)</td>
<td>1 (7)</td>
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<td>1 (3)</td>
<td>2 (1)</td>
<td></td>
<td>Unclassified</td>
<td>5 (12) 5 (13)</td>
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</table>

**Days from Start of Study**

![Figure 2. Actuarial proportion of patients remaining free from febrile neutropenia defined by ANC less than 1.0 x 10^9/L and temperature ≥37.5°C for patients in the G-CSF group (solid line), compared with patients in the control group (stippled line). P = .04, log-rank test.](image-url)
Twenty patients from each treatment group required hospitalization for longer than 3 days. The numbers of culture-confirmed infections, days of iv antibiotics, and hospitalization were not significantly different in the two groups.

Cytotoxic chemotherapy administration. Different numbers of patients required dose delays in the two groups (Fig 3). Ten patients in the G-CSF group experienced total delays of 8 days or longer, compared with 20 patients in the control group (RR, 2.10; 95% CI, [1.31, 3.91]; \( P = .02 \)). The median duration of cycle delays was reduced from 8 days in the control to zero in the G-CSF–treated patients (\( P = .01 \)). The main reason for treatment delay in the control group was neutropenia, for which 19 patients (31 cycles) had treatment delayed, compared with two patients (2 cycles) in the G-CSF–treated group (\( P = .000007 \)) (Table 3). Similar numbers of patients from each group required treatment delays because of an infectious event: seven patients (7 cycles) in the G-CSF group and nine patients (11 cycles) in the control group. Patients in the G-CSF group were protected from neutropenia and as a result of this were more likely to have treatment delayed for mucositis, the next dose-limiting toxicity (Table 3). However, it is important to note that the overall incidence of WHO grade III and IV mucositis was equivalent in both groups (15 patients in each group).

Four of the 41 G-CSF patients and 13 of the 39 control patients experienced at least one dose reduction of their myelosuppressive drugs (\( P = .01 \)). One of the 41 G-CSF patients had a neutropenia-related dose reduction of myelosuppressive chemotherapy, compared with 12 of the control group (\( P = .0006 \)). Dose reductions for vincristine neuropathy were equivalent in the two groups.

As a result of these differences in dose delays and dose reductions, the patients on G-CSF received a greater dose intensity (Fig 4). The median dose intensities of adriamycin, cyclophosphamide, and etoposide were 96%, 96%, and 94%, respectively, in the G-CSF group, and 85%, 83%, and 82% in the control group (with respective \( P \) values of .0004, .0001, and .02).

Tumor response and survival. Ninety percent of patients in the G-CSF–treated group achieved either a complete or partial tumor response, compared with 92% of control patients. There were no significant differences in disease-free survival (\( P = .78 \)) (Fig 5) or in overall survival (\( P = .68 \)) (Fig 6). Eighty percent of patients survived 1 year from start of treatment.

Safety of G-CSF administration. Chemotherapy-associated side effects included nausea and vomiting, alopecia, dyspepsia, peripheral neuropathy, constipation, and ane-
There were no apparent differences in the incidence of these events between the two treatment groups. Musculoskeletal pain was the only event reported as possibly or probably related to G-CSF (7 patients). This was controlled by simple analgesics and did not interrupt treatment. No patient refused G-CSF injections. There was no evidence of production of G-CSF antibodies.

Despite the increase in dose intensity in the G-CSF group, there was no increased incidence of thrombocytopenic or hemorrhagic complications. Although median platelet counts and hemoglobin values on treatment days were slightly lower in the G-CSF group in later cycles, the incidence of WHO grades III and IV thrombocytopenia and anemia were similar in the two groups, and transfusion requirements were similar.

In the G-CSF group, there were six deaths (including one patient who completed chemotherapy but died of myocardial infarction 1 month later) and two patients were withdrawn. In the control group, four patients died and six patients were withdrawn. Reasons for withdrawal and the causes of death for both groups are listed in Table 2.

DISCUSSION

This is the first randomized study of G-CSF during induction chemotherapy for high-grade NHL. This study has shown that G-CSF is well tolerated in an outpatient setting, and is effective in reducing the morbidity associated with chemotherapy-induced neutropenia. In patients with high-grade NHL, G-CSF significantly reduced the incidence of neutropenia and infection as manifest by neutropenia with fever even though prophylactic antimicrobial and antifungal drugs were administered. Our results also show that by using G-CSF it is possible to improve the dose delivery of cytotoxic chemotherapy in a group of patients with highly chemosensitive tumors.

Bodey et al.1 showed a direct correlation between the duration of neutropenia (ANC < 1.0 × 10⁹/L) and the risk of infection in leukemia patients. Neutropenia with fever has been shown to be the most reliable indication of infection in patients receiving chemotherapy.24,25 The 2.3-fold reduction in the incidence of neutropenia and neutropenia with fever in patients receiving G-CSF in the present study was not accompanied by a significant difference in iv antibiotic use or hospitalization. Several factors may account for this.

Firstly, the increase in cytotoxic dose intensity achieved in the G-CSF group may have reduced the observable benefit in terms of infection. That is, if the dose intensity administered had been equivalent in the two groups, the observed difference in infection-related endpoints might have been greater.

Secondly, the risk of infection was reduced in both patient groups by the use of prophylactic antimicrobial and antifungal therapy.

Thirdly, the incidence of infectious complications was lower than in our previous series of 182 patients with high-grade NHL treated with VAPEC-B chemotherapy. This may be due to a number of factors, including better performance scores, more rigorous exclusion of patients with concurrent medical problems, and fewer patients with stage III and IV disease. Such factors may have resulted in the selection of patients less prone to infection. Because the incidence of infections was lower than expected in both groups, the power of this study to detect a difference was lower than planned.

Finally, our threshold for commencing broad spectrum iv antibiotics in patients with fever ≥37.5°C and ANC less than 1.0 × 10⁹/L, and continuing until the neutropenia and any associated infection have resolved, is lower than in many other studies, and although it may have enhanced patient safety, it may also have diminished the observed benefit to be derived from the G-CSF administration. Patients were not admitted for nonneutropenic infections. In patients with refractory or relapsed leukemia treated with G-CSF, Ohno et al.26 also found reduced neutropenia, but little difference in the incidence of febrile episodes, although documented infections were significantly less frequent.

The only two large randomized studies of G-CSF reported to date are one European and one North American study, both in small cell lung cancer.27,28 The lung cancer studies were designed to be placebo-controlled, but patients who experienced febrile neutropenia were un-
blinded. In the European study, placebo was then discontinued, and in the North American study G-CSF was substituted for placebo. This limits interpretation of the data, as the treatment policy was dependent on the clinical course of the patient. The reduction in risk of fever with neutropenia for patients receiving G-CSF in the present study is similar to those seen in the small cell lung cancer studies, although we used prophylactic antimicrobial and antifungal therapy and our policy allowed no cumulative dose reductions.

The use of G-CSF enabled more patients to complete therapy and allowed delivery of the planned dose on time in a greater proportion of patients. Our aim was to deliver the full doses of cytotoxic drug on time, and not to increase absolute dose or reduce the treatment period. G-CSF-treated patients received on average 12% greater dose intensity (Fig 4). Despite the increase in dose intensity, toxicities in the G-CSF group were not significantly increased.

As the response rates to chemotherapy in this patient group are so high, differences in survival must be studied for possible benefits from the increase in dose intensity allowed by G-CSF. However, the median follow-up is only 15 months, with 80% of the patients alive at 1 year, so it is too early to expect the difference in achieved dose intensity to be reflected in a survival difference. Nevertheless, these results indicate that G-CSF may facilitate the delivery of chemotherapy at reduced intervals or increased dose, and permit quantification of the relationship between dose intensity and cure. Uncontrolled studies suggest that highly intensive treatments using ablative chemotherapy followed by hematopoietic rescue in high-grade NHL patients with poor prognostic features are associated with improved long-term relapse-free survival. The use of G-CSF in this setting should now be fully evaluated in appropriately designed clinical trials.

Toxicities other than neutropenia may become dose-limiting with dose intensification. Although G-CSF has been reported to reduce the incidence of mucositis after chemotherapy, this complication was the next factor limiting dose intensity with the VAPEC-B regimen. In this study, the incidence of grade III and IV mucositis was equivalent in both groups, but patients in the G-CSF group were protected from neutropenia and as a result of this were more likely to have treatment delayed for mucositis. New approaches are required to deal with this problem.

The occurrence of three vascular deaths in the G-CSF group is of concern. One patient died of intracerebral hemorrhage while thrombocytopenic. Despite normal electrocardiograms at entry to the study, established atherosclerosis was found at autopsy in the two myocardial deaths. These findings suggest that cardiovascular risk factors need to be monitored in patients receiving intensified treatments.

Clearly, not all oncology patients require hematopoietic growth factor support. Large randomized studies are needed to identify those patients who will benefit most from the administration of G-CSF and whose treatment will be cost effective. The beneficial effects of G-CSF in this trial included a significant reduction in the incidence of neutropenia with fever, with fewer cytotoxic drug dose reductions and treatment delays. This permitted a greater administered dose intensity in the G-CSF group and it seems likely that further dose intensification is possible. The potential role of G-CSF in dose intensification warrants further study.

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REFERENCES


Granulocyte colony-stimulating factor to prevent dose-limiting neutropenia in non-Hodgkin's lymphoma: a randomized controlled trial

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