Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor After High-Dose Chemotherapy and Autologous Bone Marrow Transplantation With Unpurged and Purged Marrow in Non-Hodgkin’s Lymphoma: A Double-Blind Placebo-Controlled Trial

By Norbert Claude Gorin, Bertrand Coiffier, Marcel Hayat, Loïc Fouillard, Mathieu Kuentz, Michel Flesch, Philippe Colombat, Pierre Boivin, Shimon Siavin, and Thierry Philip

The toxicity of autologous bone marrow transplantation (ABMT) is correlated to neutropenia. Although recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF) seems to hold promise in accelerating neutrophil recovery, few analyses from randomized studies are presently available. Ninety-one patients with non-Hodgkin’s lymphoma receiving high-dose ablative chemotherapy followed by ABMT with unpurged or purged marrow were included in a randomized, double-blind, placebo-controlled trial. Forty-four patients received 250 μg rhu GM-CSF (Escherichia coli)/m² and 47 patients received placebo. Treatment was administered daily as continuous infusion from day of ABMT until the absolute neutrophil count (ANC) reached 0.5 x 10⁹/L for 7 days or until day 30, whichever was first. With rhu GM-CSF, 50% of the patients reached an ANC count > 0.5 x 10⁹/L at day 14 as opposed to day 21 with placebo (P < .0001). Patients transplanted with marrow purged by mafosfamide also recovered earlier when treated with rhu GM-CSF (16 ± 20.5 days, P = .013). The hospitalization duration was shorter in the rhu GM-CSF group (median, 23 ± 28 days, P < .05). No difference was observed in fever, number of infections, and antibiotic administration between the two groups. The major adverse event ascribed to rhu GM-CSF was a capillary leak syndrome in three patients graded as severe in two patients, moderate in one, and reversible in all three patients. In addition, one patient in the rhu GM-CSF group died suddenly with no explanation. In long term follow-up, the relapse rate was identical in both groups and there was no significant difference in the number of deaths at 1 year (12 with rhu GM-CSF vs 9 with placebo), although deaths seemed to occur slightly earlier in the rhu GM-CSF group. We conclude that after ABMT with unpurged or purged marrow, rhu GM-CSF (E coli) significantly reduces neutropenia duration and hospitalization stay. A positive causative relation between the study drug and/or its mode of application with an increased toxicity as compared with GM-CSF from other sources and/or other modes of application cannot be deduced from the experiences in this study. Additional randomized trials would be necessary for an appropriate answer.

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MATERIALS AND METHODS

Patients and ABMT. All patients less than 55 years old with NHL included in a program of ABMT were eligible for this study if the pretransplant high-dose regimen administered was any one of the following: 

- Cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP).
- Cyclophosphamide, vincristine, procarbazine, prednisone (COPP).
- Cyclophosphamide, vincristine, procarbazine, prednisone, lomustine (CCPP).
- Prednisone, vincristine, procarbazine, cyclophosphamide (PVCP).
- Prednisone, vincristine, cyclophosphamide, cyclosporine (PVCC).

ABMT was performed using a high-dose chemotherapy regimen including cyclophosphamide (12–13 g/m²), thiotepa (250 mg/m²), 2-deoxycoformycin (80 mg/m²), and etoposide (1.5 g/m²). Patients received ABMT with unpurged or purged marrow. ABMT was performed with unpurged marrow in 41 patients and with purged marrow in 41 patients. Purged marrow was obtained with mafosfamide (10 g/m²) and filtered with a mitomycin C treatment. The patients were randomized to receive either rhu GM-CSF (E coli) or placebo (human serum albumin). The dose of rhu GM-CSF was 250 μg/m²/day as a continuous infusion from day of ABMT until the ANC reached 0.5 x 10⁹/L or for 7 days or until day 30, whichever was first.

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the following five polychemotherapy combinations: BEAM (carmustine 300 mg/m² D1, VP16 and ARAC 200 mg/m² each D2 to D5, and high-dose melphanal 140 mg/m² D6), BEAC (identical to BEAM except for cyclophosphamide 35 mg/kg D2 to D5 instead of high-dose melphanal), BETCAM (similar to BEAM with an additional administration of thiopeta 0.6 mg/kg D2 to D5 and cyclophosphamide 60 mg/kg on day 5, and high-dose melphanal at 60 mg/m² on days 6 and 7 replacing the single 140 mg/m² bolus injection on days 6 only), CBV (cyclophosphamide 1.5 g/m² D1 to D4, VP16 300 mg/m² D1 to D4, BCNU 300 mg/m² D4), BU-CY2 (busulfan 4 mg/kg D1 to D4, cyclophosphamide 60 mg/kg D5 and D6). These five regimens BEAM/BEAC/BETCAM/CBV and CBV were selected for being widely used throughout the world and universally considered as having a highly tumor cytoreductive effect in NHL with a high level of (so-called ablative) induced myelosuppression. Patients receiving total body irradiation (TBI) were not eligible for this study and were enrolled in another.28 There was no other limitation. Because several centers in this study also included their patients in the French National NHL protocol (LNH-87) in which ABMT was part of the whole therapeutic strategy, a stratification was built to achieve an overall balance of this factor between study drug and placebo. Marrow purging was optional but the random assignment was also stratified for this possible "prognostic factor."

Ninety-one patients from nine centers were included in this study between November 1988 and July 1990. Forty-four received rhu GM-CSF verum and 47 placebo. Of the 44 patients of the rhu GM-CSF group, two had a major protocol violation in that they received TBI; these patients were evaluated for rhu GM-CSF tolerability but excluded for the other parameters of the analysis. An additional patient in the same group who withdrew willingness to further participate after the first 4 hours of infusion was also excluded from analysis. Table 1 summarizes the clinical characteristics of the patients and the distribution according to pretreatment regimens, inclusion in the LNH-87 protocol, marrow purging, and dose of marrow infused. Clinical characteristics and conditioning regimens were evenly distributed except for status of disease: more patients in the rhu GM-CSF group had a still-detectable tumor (partial remission [PR]) or were beyond second complete remission (CR) at time of ABMT (45% v 21%, \( P < .05 \)). The doses of marrow infused were identical in the two strata corresponding to unpurged or purged marrow. Of the 16 patients receiving purged marrow, 14 received marrow treated in vitro with mafosfamide: in these patients the dose of marrow infused (0.018 and 0.015 10⁸ CFU/GM/kg in the rhu GM-CSF and placebo group, respectively) was as expected, more than 2 logarithms lower than in patients receiving unpurged marrow (3.1 and 2.9 10⁹ CFU/GM/kg, respectively).

**Design of the study and drug administration.** This study was a multicenter double-blind, stratified, randomized, placebo-controlled trial. The random assignment was stratified according to previous inclusion in the prospective LNH-87 regimen and according to whether or not the marrow was purged. rhu GM-CSF (Hoechst-Behring, Marburg, Germany; Escherichia coli-derived) at 250 μg/m² dose level or placebo was administered as a continuous 24-hour infusion via double-lumen central venous catheter from day of ABMT until the absolute neutrophil count (ANC) reached 0.5 x 10⁹/L for 7 days or until day 30, whichever was first. GM-CSF was to be discontinued if the ANC reached a point > 10 x 10⁹/L for 2 consecutive days.

In addition, for all patients included in the study, rhu GM-CSF could be administered if the ANC decreased to less than 0.5 x 10⁹/L for 2 consecutive days after discontinuation of the double-blind drug administration or in case it remained lower than this threshold on day 30. All reasons combined, eight patients received rhu GM-CSF verum; six in the placebo group and two in the rhu GM-CSF group. The protocol of this study was approved by the Saint Antoine Hospital institutional review committee and all patients signed informed consent before inclusion. For drug preparation, rhu GM-CSF had a specific activity of 5 x 10⁸ CFU/mg of protein. Lyophilized rhu GM-CSF or placebo was reconstituted in 1 mL sterile water and added to 50 mL normal saline with 1% human albumin. Lyophilized placebo contained glycine and NaCl in polygeline.

**Evaluation criteria.** Patients were followed up for the duration of drug administration plus an additional 14 days observation period. All patients had daily physical examinations that included recording of vital signs and a particular evaluation of fever, infections, and possible drug-related side effects. In particular, we studied the number of days with intravenous (IV) antibiotics and the number of antibiotics x days defined as the product of the number of days with IV antibiotic administration by the number of IV antibiotics administered per day. Chest x-rays were done weekly. Complete blood counts, including the white blood cell (WBC) differential count, were performed daily during treatment and twice weekly during the observation period. Blood chemistry analysis was performed twice a week during treatment and observa-

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**Table 1. Characteristics of Patients and ABMT**

<table>
<thead>
<tr>
<th>Conditioning regimen</th>
<th>rhu GM-CSF Group (n = 44)</th>
<th>Placebo Group (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>18-61</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>4-59</td>
</tr>
<tr>
<td>Grade</td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Status (%)</td>
<td>First CR</td>
<td>Second CR</td>
</tr>
<tr>
<td></td>
<td>21 (47.7)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td></td>
<td>27 (57.4)</td>
<td>10 (21.3)</td>
</tr>
<tr>
<td>Duration of disease before inclusion in months, median and range</td>
<td>8.5 (4-255)</td>
<td>10 (3-91)</td>
</tr>
<tr>
<td>Strata</td>
<td>LNH-87</td>
<td>Non LNH-87</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>Purge with mafosfamide</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Purge with MoAbs</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No purge</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>Doses of marrow CFU/GM/kg</td>
<td>10⁸ (0.06)</td>
<td>2.2 (0.30)</td>
</tr>
<tr>
<td></td>
<td>3.1 (3-56)</td>
<td>2.9 (2-30)</td>
</tr>
<tr>
<td></td>
<td>0.018 (0-0.11)</td>
<td>0.015 (0-0.37)</td>
</tr>
</tbody>
</table>

*Excluded from the efficacy analysis.

*CFUGM/kg 10⁸ median and (range).
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Obtained 2 weeks and 4 weeks after ABMT and at the end of observation period. All toxicities were recorded on the World Health Organization (WHO) 1 to 4 graded toxicity scale. Grade 3 to 4 toxicities, possibly drug related, led to treatment discontinuation in 11 and 7 patients of the rhu GM-CSF and placebo groups, respectively.

Statistical analysis. The time to event was defined as the time from the start of blinded treatment (day 1) until the onset of an event or the last known day without an event. Kaplan-Meier product-limit estimates were used to evaluate time-to-event data such as neutrophil regeneration, dependence on erythrocyte and platelet transfusions, and patient-isolation time. Two-sided significance testing was based on the generalized Wilcoxon test statistic. Fisher's exact test (two-tailed) was used to compare distribution of events or categories in the two groups. The statistical analysis was performed with the personal computer (PC) version of Statistical Analysis Software (SAS) (release 6.04).

RESULTS

Hematopoietic recovery. Administration of rhu GM-CSF significantly accelerated the reconstitution of total leukocytes and neutrophil counts (Table 2, Figs 1 and 2) when considering the global population of 88 evaluable patients. The median day of recovery of leukocytes to 10^9/L was day 14 in the rhu GM-CSF group and day 21 in the control group (Fig 1). Following this point, the daily increase in leukocytes (slope) was considerably higher for rhu GM-CSF patients, so that by day 21 when placebo patients were reaching 10^9/L, the median leukocyte count in the rhu GM-CSF group was over threefold of this value.

Similar observations were made for neutrophils, so that by day 21 when placebo patients were reaching 10^9/L, the median neutrophil count in the rhu GM-CSF group had recovered an ANC count over two times higher than the placebo group (Fig 2). As indicated in Table 2, 50% of the patients in the rhu GM-CSF group had recovered an ANC count > 0.5 × 10^9/L by day 14 as opposed to day 21 in the placebo group, and 75% had recovered by day 16 as opposed to day 28. The median time to reach 10^9 neutrophils/L was 17 days in the rhu GM-CSF versus 30 days in the placebo group. The duration of neutropenia was also reduced by rhu GM-CSF in the population of patients transplanted with marrow purged by mafosfamide (with 50% recovery observed on day 16 v 20.5, and 75% recovery on day 16 v 25, respectively, P = .013), and there was no detectable difference whether patients were included or not in the French National LNH-87 protocol.

There was no detectable difference between the two groups in platelet reconstitution. Median time to reach platelet transfusion independency was identical (19 days) in both groups. The median time to 0.5% reticulocytes could be evaluated only in a limited number of patients. It was day 15.5 (quartiles 13 through 21) for the rhu GM-CSF in 16 patients, and day 18 (quartiles 16 through 21) for placebo in 21 patients. The median time to red blood cells (RBC) transfusion independence was day 23 in patients treated with rhu GM-CSF and day 21 in patients treated with placebo.

Fever, infection, and antibiotics. We did not find any difference in evaluation of fever, number of infections, and antibiotic administration between the two groups. Median numbers of days with fever ≥ 38.5 caused by infections were 4 and 2 in the rhu GM-CSF and placebo groups, respectively (P = not significant [NS]). Numbers of patients with documented bacterial, viral, and fungal infections were 11, 2, and 3 in the rhu GM-CSF versus 12, 6, and 4 in the placebo groups, respectively (P = NS). However, while gram-positive sepsis were evenly distributed in both groups, five of a total of six gram-negative sepsis occurred in patients receiving placebo. We detected no difference either in the number of days with IV antibiotic administration (19 days with rhu GM-CSF v 22 days with placebo, P = NS) or in the number of antibiotics × days (median 52 in both groups).

Table 2. Time to Recovery (days) of Neutrophils to 0.5 × 10^9/L in Patients Receiving GM-CSF or Placebo

<table>
<thead>
<tr>
<th>% of Patients With ANC Recovery</th>
<th>Global Population Patients Receiving Marrow Purged With Mafosfamide</th>
<th>% Patients With Neutrophils &gt; 0.5 × 10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Patients With ANC Recovery</td>
<td>GM-CSF (n = 41) Placebo (n = 47)</td>
<td>GM-CSF (n = 7) Placebo (n = 7)</td>
</tr>
<tr>
<td>25</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>50</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>75</td>
<td>16</td>
<td>28</td>
</tr>
</tbody>
</table>

Generalized Wilcoxon test: P = .0001 (generalized Wilcoxon test)
**Tolerance.** For evaluation of side effects, we cumulated grade III and IV adverse events that were either specifically related to the study drug by the investigator or classified as not assessable, and excluded those reported as related to other causes. With this definition, 33 grade III and IV adverse events were reported in 20 patients of the rhu GM-CSF group versus 14 adverse events in 11 patients in the placebo group. Details of all adverse events are given in Table 3. Adverse events more reported in the rhu GM-CSF group consisted of fever (11 v 6), bone pains (3 v 0), vomiting (3 v 0), capillary leak syndrome (3 v 0), and thrombosis (2 v 0). Liver veno-occlusive disease was reported in one patient in each group.

In four patients the codes were broken: one of these patients had received rhu GM-CSF and three had received placebo. This was motivated by a capillary leak syndrome in the patient receiving rhu GM-CSF and in the three receiving placebo by a sharp increase in transaminase levels (×5) in one, life-threatening gram-negative sepsis with persisting neutropenia in one, and refusal of the last patient to continue the study on day 21. During the period of drug administration, one patient died on day 17 in the rhu GM-CSF group and another one on day 19 in the placebo group. During the 14-day observation period that followed, three patients of the rhu GM-CSF group died on days 34, 35, and 38 while none in the placebo group died. The five case reports are summarized below.

In the rhu GM-CSF group the first patient, a 46-year-old man, died suddenly on day 17 of the autograft. This patient presented initially with an immunoblastic lymphoma in October 1987, and was put into first remission (CR1) after six courses of the Promace-Mopp chemotherapy regimen in July 1988. He relapsed in August 1988 and received six cycles of salvage chemotherapy and a pelvic irradiation at a total dose of 40 Gy. ABMT following the BEAM was administered for consolidation of CR2. The WBC count started to increase from day 11. The bone marrow aspirate on day 15 confirmed engraftment. Platelet support was still needed. During the morning of day 17, the patient developed a grade III reaction to amphotericin B, which he was receiving IV for a fever of unknown origin. This resolved under appropriate therapy. In the evening, he suddenly presented intense dyspnea and a bilateral mydriasis, rapidly followed by a cardiac arrest. Resuscitation was unsuccessful. The clinical course appeared consistent with two possible explanations: a cerebral hemorrhage or a massive pulmonary embolism, but the exact cause of death remained unclear because authorization for postmortem examination was not granted.

The second patient, a 38-year-old woman with a refractory follicular lymphoma with bone marrow involvement and a bulky abdominal mass, was one of the two that had a major protocol violation in that they received TBI: as indicated in Materials and Methods, she was kept in the study only for the evaluation of rhu GM-CSF tolerability and was excluded for all other parameters. Diagnosis was established in October 1987. The patient failed initial chemotherapy (12 courses over 18 months) and responded to first-line salvage therapy administered over 3 months. She was considered in PR at time of ABMT. The total dose of Adriamycin administered pretransplant was 300 mg/m² and the total dose of mitoxantrone was 30 mg/m². Cardiac evaluation was considered normal. The pretransplant regimen consisted of cyclophosphamide (60 mg/kg/d for 2 days), VP16 (300 mg/m²/d for 3 days), and TBI. The marrow infused was purged by monoclonal antibodies (MoAbs). The PMN count was 0.5 × 10⁹/L on day 18 and 8 × 10⁹/L on day 29 when rhu GM-CSF was discontinued. On day 32, the polymorphonuclear (PMN) count had dropped to 1.2 × 10⁹/L, and rhu GM-CSF was reintroduced. There was no platelet recovery. From day 13 the patient presented a nondocumented (bronchoalveolar lavages negative) bilateral diffuse interstitial pneumonitis. On day 27, she developed a cardiac failure that rapidly worsened despite the administration of dopamine-dobutamine. She died on day 34. Permission for postmortem examination was not given. Death was attributed to cardiac failure from cumulative toxicity of anthracyclines. rhu GM-CSF was not considered to be contributive.

The third patient, a 25-year-old woman, presented a diffuse large cell NHL in November 1988. She had been under oral contraceptive agents until January 1989. She received ABMT for consolidation of CR1 after CBV. By day 2 she started complaining of abdominal distension and diarrhea. By day 9 abdominal distension had increased and she had developed lower-limb edema and lethargy. On day 10 ascitis, a right pleural effusion, and a pericardial effusion were noted. It was believed that she had capillary leak syndrome; the code was broken and rhu GM-CSF verum that she was receiving was discontinued. At that time she had recovered a PMN count over 0.5 × 10⁹/L. She improved under water restriction and diuretics and after insertion of a pleural catheter and removal of 4 L of liquid over 2 days. However, from day 14 she became icteric and the liver function tests altered and worsened (bilirubin 101 μmol/L, transaminase × 6, factor V: 29%). An echographic examination indicated permeability of the sus hepatic veins. She

<table>
<thead>
<tr>
<th>Table 3. Number of Patients With Grade III/IV Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rhu GM-CSF</strong></td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Bone pains</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Capillary leak syndrome</td>
</tr>
<tr>
<td>Fever + hypotension</td>
</tr>
<tr>
<td>Jaundice</td>
</tr>
<tr>
<td>Thrombosis</td>
</tr>
<tr>
<td>Veno-occlusive disease</td>
</tr>
<tr>
<td>Intracranial bleeding</td>
</tr>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Polyneuritis</td>
</tr>
<tr>
<td>Rash</td>
</tr>
<tr>
<td>Weight gain</td>
</tr>
<tr>
<td>Increase in ALT</td>
</tr>
<tr>
<td>Pulmonary edema</td>
</tr>
<tr>
<td>Polypnea</td>
</tr>
</tbody>
</table>

Abbreviation: ALT, alanine aminotransferase.
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1133

received the drug verum (median 19 days, quartiles 6 through 22) than in those who received placebo (median 26 days, quartiles 21 through 30). By day 26 when 51% of patients in the placebo group were still receiving blind treatments, 93% of patients in the rhu GM-CSF group had already stopped. This also reflected directly the efficacy of rhu GM-CSF in reducing the period of neutropenia. In keeping with the design of the study, eight patients received unblinded rhu GM-CSF verum for ANC < 0.5 \times 10^9/L; six of them were in the placebo and two in the rhu GM-CSF group. The duration of stay in the isolation unit was significantly shorter in patients receiving rhu GM-CSF (median 20 days, quartiles 18 through 25) than in those receiving placebo (respective values: 24 days, 19.5 through 30, P < .05). The total hospitalization stay was similarly reduced for the rhu GM-CSF group (median and quartiles: 23 days, 20 through 29, v 28 days, 24 through 35, P < .05).

Survival and follow-up. In this trial, neither the probability of persisting remission nor survival post ABMT were preplanned study objectives. These parameters were analyzed retrospectively. The time to relapse was not different in the two groups (Wilcoxon test, P = .70). At 1 year, 12 patients in the rhu GM-CSF group and 9 in the placebo group had died (Wilcoxon test, P = .23, Fig 3), 7 in each arm primarily from tumor progression, 4 in the rhu GM-CSF, and 1 in the placebo group from early toxicity (before day 45, end of observation period), as indicated above. Causes of death are listed in Table 4. This resulted in a trend for a better early survival in the placebo group (Wilcoxon test on the first 45 days of the curve, P = .15). A retrospective careful reexamination of charts at investigator sites showed no objective difference between the two study groups, other than the status of disease at time of ABMT, with more patients with detectable tumor (PR) or beyond CR2 in the rhu GM-CSF group as already mentioned above. However, when post-stratifying the groups according to the status of disease at time of ABMT, the trend for better early survival in the placebo group still persisted (stratified Wilcoxon test on the first 45 days of the curve, P = .16). Additional prognostic factors, which may have contributed to a possible imbalance of treatment groups, could not be tested retrospectively (eg, cytogenetics, immunophenotype, etc).

Duration of treatment and hospitalization. The duration of double-blind treatment was shorter in patients who died of hepatic encephalopathy and gastrointestinal hemorrhage on day 35. The clinical diagnosis of liver veno-occlusive disease (VOD) was confirmed by a postmortem liver biopsy. A careful review of the chart with the local investigator led to the conclusion that this patient had first developed a capillary leak syndrome and then liver VOD, rather than VOD from the start. It was believed that rhu GM-CSF through the capillary leak syndrome did not contribute to death, although the question was raised.

The fourth patient, a 26-year-old woman, had a refractory diffuse large cell NHL diagnosed in August 1988. After six cycles of Promace Mopp and mediastinal irradiation (30 Gy), she entered CR1 but relapsed 2 months later in June 1989. She then received two successive salvage regimens (total six courses with a toxic cholestatic hepatitis after the first one) and was considered in PR in December 1989 when ABMT was performed following the BEAM. From day 3 the patient rapidly developed a severe mucositis, myocardial failure with pericardial effusion (which she had also developed in the past, following courses of conventional chemotherapy), bilateral pleural effusion, and abdominal distension. Both the liver and renal functions altered (bilirubin 170 \mu mol/L with normal transaminases, creatinin 160 \mu mol/L). rhu GM-CSF was discontinued on day 12 and the patient was transferred to the intensive care unit (ICU) on the same day. A drain tube was inserted in both pleural and the pericardial cavities and the patient ventilated. After a transient improvement the cardiac hemodynamics worsened. There was no sign of recovery of hematopoiesis and rhu GM-CSF was reintroduced on day 34 with no specific side effect. The patient died aplastic on day 38 from combined cardiac and pulmonary failure. It was considered that this resulted from the cumulative toxicity of previous chemotherapy and radiotherapy on top of which the BEAM was given, and that rhu GM-CSF had not contributed to death.

In the placebo group the only patient who died from early toxicity was a 30-year-old man who presented a diffuse large cell NHL in February 1989. He was autografted in August 1989 in CR1 obtained after five courses of ACBV. He received a BEAM regimen before ABMT with marrow purged with mafosfamide. On day 9 local erythema appeared at the catheter insertion site. The patient complained of myalgia and fever on day 10. Despite the withdrawal of the catheter and antibiotic administration, his condition worsened with drowsiness, nuchal rigidity, hypotension, and purpura; the drug was stopped on day 11. Five blood cultures were positive with both gram-positive and gram-negative bacteria. He was transferred to ICU because of polypnea and hypoxemia on day 15, and he died on day 19 from lesional pulmonary oedema caused by sepsis. There was no sign of hematologic recovery.

Overall, in the four patients in the rhu GM-CSF group who died within the observation period, a relationship to rhu GM-CSF administration was believed to be unlikely in two (second and fourth) and questionable in two (first and third).
hematologic malignancies and solid tumors. In a recent
mented bacterial sepsis varies from 30% to 60%. Of a total
improvement in patient care, ABMT is still associated with
in 1989 has been evaluated as greater than 4,000, of which
attributed directly or indirectly to infection occurring in the
develops in almost all patients and the incidence of docu-
first 30 days.1-8,19,31
creases with increasing counts of granulocytes with two
behave like normal individuals.20 Further, in neutropenic
patients with gram-negative sepsis, massive leukocyte trans-
fusions have, in the past, considerably decreased the fatality
rate.32

ABMT is now routinely used for the treatment of several
hematologic malignancies and solid tumors.30 In a recent
international survey the total number of ABMT performed
in 1989 has been evaluated as greater than 4,000, of which
1,000 concerned patients with NHL.12,13 Despite constant
improvement in patient care, ABMT is still associated with
a significant morbidity and mortality. In particular, fever
develops in almost all patients and the incidence of docu-
mented bacterial sepsis varies from 30% to 60%. Of a total
death rate reported from 5% to 15%, more than half is
attributed directly or indirectly to infection occurring in the
first 30 days.1,8,19,31

The incidence and severity of infectious episodes de-
creases with increasing counts of granulocytes with two
critical levels: patients with counts below 0.5 x 10^9/L have
the highest risk of developing potentially lethal infections
while those with counts higher than 10^9/L (or 1.5 x 10^9/L)
behave like normal individuals.20,21 Further, in neutropenic
patients with gram-negative sepsis, massive leukocyte trans-
fusions have, in the past, considerably decreased the fatality
rate.32

Patients with NHL who undergo ABMT remain neutro-
openic for 3 to 4 weeks irrespectively of whether or not
marrow infused has been unpurged, or purged with MoAbs
or cyclophosphamide.1,8 An important area of clinical
research in the past 10 years has been the identification of
means to accelerate hematopoietic engraftment in an effort
to reduce the period of neutropenia and the risk of
infection. Recently, several CSFs have been cloned and
expressed as recombinant glycoproteins. rhu GM-CSF in
particular33 has been extensively tested in vitro, in numer-
ous preclinical animal models and in humans. rhu GM-CSF
is a 21-Kd protein that is either unglycosylated or glycosy-
lated according to whether the recombinant product is
produced by E coli or yeast. It promotes the proliferation
and differentiation of myeloid and monocyte progenitors
and stimulates the function of mature granulocytes and
monocytes.33 It also induces early neutrophil recovery
without substantial toxicity in nonhuman primates after
ABMT.34,35 In humans, rhu GM-CSF has been shown to
increase neutrophil counts in acquired immunodeficiency
syndrome (AIDS) patients treated with zidovudine (AZT)36
and to increase neutrophil recovery after chemotherapy37
or ABMT for solid tumors24 and lymphomas.26,38 Recently
rhu GM-CSF has been shown to be effective in patients
with failure to engraft32 and even has been able to replace
ABMT in highly selected cases of NHL with progressive
disease and bone marrow involvement, after a high-dose
polychemotherapy regimen, the BEAM.39 However, all of
these were nonrandomized phase I/II studies in which
comparisons were made with historical controls: although
they provided evidence that rhu GM-CSF accelerates
neutrophil recovery, they suffered from the usual limita-
tions of such studies, mainly the risks of a selection bias
in patients; in addition, they also provided some discordant
results with, for instance, acceleration of platelet recovery
observed in one study36 but not in most others.

Our study was a multicenter double-blind randomized
placebo-controlled trial that we applied to the most com-
mon ABMT setting, namely patients with NHL trans-
planted after a non-TBI high-dose regimen. The study
was designed in accordance with information available in Jan-
uary 1988 including preliminary results of a phase II trial
performed with the E coli-derived rhu GM-CSF from the
same company40: (1) The dose of rhu GM-CSF (250 μg/m^2)
was selected from phase I studies both for optimum efficacy
and tolerance. (2) The route and schedule of administra-
tion, ie, continuous IV infusion over 24 hours, was selected
over others (SC, 2 hours and 6 hours IV infusion) despite a
possible increase in toxicity and a previous report suggest-
ing that when so given rhu GM-CSF might decrease
neutrophil migration to areas of inflammation41 with the
hope that ensuring a constant rhu GM-CSF serum level
would rather augment efficacy. (3) The duration of adminis-
tration (from day 0 to an ANC >0.5 x 10^9/L for 7
consecutive days or until day 30, whichever was first) was
longer than in previous studies to avoid an ANC decrease
below 0.5 x 10^9/L at disconnection of the drug, as had
been previously observed by others with a 14-day infusion
schedule.26 (4) Finally, to provide maximum security, all
patients enrolled were scheduled to receive the drug verum
in case the ANC remained lower than 0.5 x 10^9/L on day
30, which was felt to be more likely to occur in those
receiving placebo. This comforted those patients who had a
personal prejudice in favor of rhu GM-CSF and were
reluctant to take the chance of receiving placebo.

The major aim of this study was to reduce the duration
of granulocytopenia after ABMT and this goal was reached:
patients receiving rhu GM-CSF recovered both a leukocyte
count >10^9/L and a neutrophil count greater than 0.5 x
10^9/L 7 days earlier than those receiving placebo. Fur-
more, the time to reach a neutrophil count over 10^9/L was
13 days shorter in the rhu GM-CSF group. This was also
indirectly reflected by the duration of the double-blind drug
verum/placebo administration which, again, was 7 days
shorter in the rhu GM-CSF group, and also indirectly

<table>
<thead>
<tr>
<th>Table 4. Cause and Time of Death (day)</th>
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| Unknown (possible subarachnoidal hemor-
  rhage and/or massive pulmonary embolism?) | (17) Sepsis (gram + and gram –)       | (19) |
| Irreversible cardiac failure            | (34) Tumor progression                | (127) |
| Veno-occlusive disease                  | (35) CMV IP/liver veno-occlusive       | (135) |
| disease                                 |                                       |       |
| Polys visceral failure                  | (38) Tumor progression                | (157) |
| Tumor progression                       | (54) Tumor progression                | (165) |
| Tumor progression                       | (65) Tumor progression                | (208) |
| Infection                               | (108) Tumor progression               | (252) |
| Tumor progression                       | (106) Tumor progression               | (294) |
| Tumor progression                       | (109) Tumor progression               | (352) |
| Tumor progression                       | (207)                                 |       |
| Tumor progression                       | (209)                                 |       |
| Tumor progression                       | (340)                                 |       |

At 1 year posttransplant.
Abbreviations: CMV, cytomegalovirus; IP, interstitial pneumonitis.

DISCUSSION

The major aim of this study was to reduce the duration
of granulocytopenia after ABMT and this goal was reached:
patients receiving rhu GM-CSF recovered both a leukocyte
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reflected by the fact that after study drug, six patients in the placebo group but only two in the rhu GM-CSF group had to receive rhu GM-CSF verum, according to the protocol, for a persisting ANC count below $0.5 \times 10^9$/L. Both hospitalization in the isolation unit and total hospitalization durations were 5 days shorter in the group receiving rhu GM-CSF. Because the initial design of the study was to administer rhu GM-CSF for 7 days after ANC recovery followed by a subsequent observation period of 14 days, it is likely that some patients, especially in the rhu GM-CSF arm, were kept hospitalized for unnecessarily longer periods than would have been required otherwise. Therefore, it is our hope that the median total hospitalization duration of 23 days as observed in the rhu GM-CSF group of the present study will be further reduced when rhu GM-CSF is routinely administered outside the frame of a randomized study.

The efficacy of rhu GM-CSF on recovery of neutrophils is dependent on the dose of stem cells infused: in a total of 16 ALL patients receiving autologous marrow purged with 4 hydroperoxycyclophosphamide (4 HC) and rhu GM-CSF, Blazar et al.\(^4\) observed only five responses and 11 failures. Failures were associated with a dose of CFU-GM progenitors infused below $7.2 \times 10^3$/kg. Similarly, in a total of 37 patients receiving an allograft or an autograft, Nemunaitis et al.\(^7\) observed that none of seven patients who received chemically purged autologous marrow grafts responded to rhu GM-CSF. In our own study, 14 patients received marrow purged with mafosfamide consisting of seven in each group. Of particular interest in this respect was our observation that, despite purging, the duration of neutropenia was nonetheless reduced by rhu GM-CSF, although the benefit was somewhat smaller than in the global study (4.5 days v 7 days). Purging with mafosfamide was done in two of the nine participating institutions at levels adjusted to the individual CFU-GM sensitivity to spare 5% CFU-GM (CFU-GM LD 95): this may in turn have accounted for the efficacy of rhu GM-CSF thanks to the persistence of a responsive residual, albeit reduced, stem cell pool that may not have been present in patients reported above by others.

Although the major action of rhu GM-CSF concerns myeloid and monocytic proliferation and differentiation, there is considerable evidence at least in animal models that rhu GM-CSF is also required to induce proliferation of multipotent, erythroid (BFU-E), and megakaryocytic (CFU-Meg) progenitors.\(^{43-48}\) In humans, acceleration of platelet recovery has been found in one nonrandomized study\(^{26}\) but not in other subsequent studies. In the present study, rhu GM-CSF had no detectable influence on recovery of RBCs and platelets and no influence on the amount of RBCs or platelet transfusions needed. Nonetheless, a few patients were felt to be platelet responsive by one investigator, consistent with the proposed explanation that such patients may benefit, for some reason, from higher circulating levels of other cytokines with which exogeneously administered rhu GM-CSF acts in synergy.

While this study clearly demonstrated the reduction of the neutropenic period post ABMT induced by rhu GM-CSF, it failed to show any detectable benefit in term of a reduction of infectious episodes. We found no difference by evaluation of number of days with fever, numbers of documented bacterial, viral, and fungal infections, number of days with administration of IV antibiotics, or number of IV antibiotics x days. The only possible, albeit important, difference was the nature of the documented sepsis, because five of six gram-negative sepsis occurred in patients receiving placebo while gram-positive sepsis, which are at least in part cathether related, were evenly distributed in both groups. Patients included in this study were aggressively treated with antibiotics introduced very early after transplant. IV antibiotics were administered as a general rule as soon as fever appeared and rapidly modified within 48 hours to reach stable appexia. No single antibiotic was then discontinued until recovery of myelopoiesis.

This attitude explains the figures for both groups in numbers of days with IV antibiotics (19 and 22 days for rhu GM-CSF and placebo, respectively), and numbers of IV antibiotics x days (52 in each group) and, conversely, the low numbers of days with fever above 38.5 (4 and 2 only). In the randomized study recently published by Nemunaitis et al.\(^7\) on a similar population of patients with lymphoid cancer, the duration of antibiotics administration was slightly longer (24 days with GM-CSF and 27 days in the control group). This may possibly reflect the longer duration of aplasia in their study (days 19 and 26 and days 14 and 21 in our own study), which may in part be caused by their use of TBI in over 70% of the patients. We believe that the very design of our study may have been improper for an evaluation of infectious bacterial episodes in relation to rhu GM-CSF, despite a suggestion that rhu GM-CSF may reduce the incidence of gram-negative sepsis.

Forty-five percent of the patients in the rhu GM-CSF group experienced at least one grade III or IV adverse event versus only 23% in the placebo group ($P = .03$). Adverse events reported more frequently (fever) or exclusively (capillary leak syndrome in 3, bone pains in 3, thrombosis in 2) in our GM-CSF group have been previously mentioned by others in nonrandomized studies in patients receiving glycosylated rhu GM-CSF by continuous IV infusion at higher dosages of 32 μg/kg or more.\(^{24,37}\) On the other hand, in the recent randomized study of Nemunaitis et al.\(^{29}\) in a similar population of patients autografted for lymphoid cancers who received glycosylated GM-CSF at the same dosage as in our study but over a shorter period of 2 hours only, there were no differences between groups in the frequency of side effects associated with the study drug and the toxicity of rhu GM-CSF could not be distinguished from that related to marrow transplantation.

Although the evolution of lymphoma and the overall survival were not questions addressed prospectively in this study, we also were concerned retrospectively by the existence of a trend (Wilcoxon test on the first 45 days of the curve, $P = .15$) that might indicate a better early survival in patients receiving placebo. However, the relapse rate and progression of lymphoma were identical in both groups, with no indication whatsoever that rhu GM-CSF promoted tumor growth. Indeed, the difference in early survival resulted from four early deaths before day 45 in the rhu
GM-CSF group compared with only one in the placebo group; for two of the four patients dying in the rhu GM-CSF group a relationship to rhu GM-CSF administration, although unlikely, could not be ruled out. A retrospective analysis pooling the data from this study with data from two other studies performed with the same E coli rhu GM-CSF administered with the same schedule (continuous IV infusion) by ABMT, both using unpurged marrow and marrow purged with mafosfamide at levels individually adjusted in patients with NHL. rhu GM-CSF administration reduces both the duration of hospitalization in the isolation unit and total hospitalization. Despite a possible reduction in gram-negative sepsis, this study did not show a significant reduction in the number of infectious episodes. Because of the occurrence of some adverse events, mainly three capillary leak syndromes in the GM-CSF group and a concern about the existence of a trend (albeit with no statistical significance) in favor of a better early survival in the placebo group, some caution should be raised on the potential toxicity of unglycosylated material administered by continuous IV infusion. However, the group of investigators concluded that, overall, rhu GM-CSF was reasonably well tolerated in this study.

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Recombinant human granulocyte-macrophage colony-stimulating factor after high-dose chemotherapy and autologous bone marrow transplantation with unpurged and purged marrow in non-Hodgkin's lymphoma: a double-blind placebo-controlled trial

NC Gorin, B Coiffier, M Hayat, L Fouillard, M Kuentz, M Flesch, P Colombat, P Boivin, S Slavin and T Philip