A Controlled Trial of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor After Total Body Irradiation, High-Dose Chemotherapy, and Autologous Bone Marrow Transplantation for Acute Lymphoblastic Leukemia or Malignant Lymphoma

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Infections during granulocytopenia are major complications of autologous bone marrow transplantation (ABMT). Since recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) has proved to accelerate bone marrow recovery after cytostatic chemotherapy, we studied its effects on hematopoietic regeneration and on infectious complications after total body irradiation (TBI) and high-dose chemotherapy followed by ABMT. Eighty-one patients with acute lymphoblastic leukemia (ALL) in complete remission (CR) or with non-Hodgkin's lymphoma (NHL) in CR or partial remission were randomized in a double-blind, placebo-controlled trial. They received either rhuGM-CSF 250 μg/m² (Escherichia coli-derived) daily by continuous infusion after ABMT, or placebo. Treatment was continued until the neutrophil counts reached greater than 500/μL for 1 week. The maximum treatment duration was 30 days. Thirty-nine patients in the rhuGM-CSF group and 40 patients in the placebo group were evaluable. The median time needed to reach a neutrophil count of 500/μL was 15 days with rhuGM-CSF and 28 days with placebo (P = .0001). Bacterial infections occurred in 14 (35.9%) of the patients with rhuGM-CSF and in 25 (62.5%) of the patients given the placebo (P = .024). Nine of the 14 bacterial infections in the rhuGM-CSF group and 20 of the 25 infections in the placebo group were diagnosed within the first 10 days after ABMT. Capillary leakage and a reversible fluid retention were seen in five of the rhuGM-CSF-treated patients. Patients treated with rhuGM-CSF had lower serum protein and albumin levels than patients in the placebo group. There was no statistically relevant difference in overall survival between the two groups (P = .47). Relapse occurred in 14 (34%) patients with rhuGM-CSF and in 18 (45%) patients with placebo. We conclude that continuous infusion of rhuGM-CSF after ABMT accelerates the regeneration of granulocytes and reduces the number of bacterial infections.

MYELOSUPPRESSION is often the dose-limiting toxicity following chemotherapy or radiotherapy. Therefore, the effectiveness of chemotherapy in malignant diseases is limited by two factors: drug resistance of the tumor and toxicity of the treatment. To overcome myelosuppression after myeloablative cytotoxic therapy, autologous bone marrow transplantation (ABMT) has been established as a means of providing for marrow rescue after high-dose chemotherapy and radiotherapy in patients with acute lymphoblastic leukemia (ALL) and high-risk malignant lymphoma. However, these high-dose regimens are associated with serious transplant-related morbidity and mortality due to infection, bleeding, and mucosal injury and liver toxicity. Despite ABMT, total body irradiation (TBI) and high-dose chemotherapy are usually followed by a period of severe myelosuppression for 3 to 4 weeks. During this time span, patients are at risk of acquiring bacterial or fungal infections5-8 that are more severe than after conventional chemotherapy.5-8 The number of infections increases with the duration and severity of granulocytopenia.9-11 In other studies, the incidence of bacterial infections in the early phase after ABMT was in the range of 50% to 60%.2,7-12 To diminish morbidity and mortality after bone marrow transplantation, hematopoietic growth factors have been introduced into clinical trials.13-15 It has been shown that recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) can accelerate hematopoietic regeneration and reduce infectious complications after high-dose chemotherapy and radiotherapy.5-8,15-18 In this study, the efficacy and safety of rhuGM-CSF were assessed in a randomized, double-blind, placebo-controlled trial, comparing rhuGM-CSF with placebo after ABMT.
stimulating factor (rhuGM-CSF) can stimulate the regeneration of leukocytes after bone marrow transplantation and chemotherapy.\textsuperscript{14,16,17} The application of rhuGM-CSF also shortens the duration of granulocytopenia with less than 500 neutrophils/\(\mu\)L from 20 to 30 days to 14 days in comparison to historical control patients.\textsuperscript{1,4,12,18-22} To investigate the effect of rhuGM-CSF on hematopoietic regeneration and the number of infections in patients after TBI, high-dose chemotherapy, and subsequent ABMT, a randomized, placebo-controlled, double-blind study was performed.

**MATERIALS AND METHODS**

**Patient Eligibility**

Patients with ALL in complete remission (CR) or with non-Hodgkin's lymphoma (NHL) in CR or partial remission were eligible for this trial. The preparative regimen was performed in accordance to the protocol of each center. Only the application of TBI with a dosage of 8 Gy or higher was a prerequisite for enrollment into this trial. Bone marrow purging was optional. Participation in another experimental trial was excluded.

**Study Design**

This was a controlled, randomized, stratified, double-blind, multicenter trial with 40 patients in each treatment arm. Twenty centers in Europe and one in Israel participated in the trial. The study was approved by the ethics committee at each institution. Informed consent was obtained from all patients.

**Randomization**

Separately for each center, eligible patients were randomized by telephone by the study monitor. Stratification was performed according to ALL or NHL, and within these groups according to patients with and without bone marrow purging. The randomization procedure was a modification of the "minimization method" described by Pocock.\textsuperscript{23}

**Preparation of rhuGM-CSF and Placebo**

Complementary DNA encoding human GM-CSF was cloned and expressed in *Escherichia coli* by Behringwerke, Marburg, Germany.\textsuperscript{24} RhuGM-CSF had a specific activity of 5 \(\times\) 10^7 colony-forming units (CFU) per milligram of protein. The agent was more than 95% pure and had no measurable endotoxin by limulus amebocyte assay. Lyophilized rhuGM-CSF or placebo was provided in vials labeled identically for rhuGM-CSF or placebo and contained glycine and NaCl in polygeline. A set of vials was prepared and numbered for each patient. The drugs were reconstituted with 0.9% normal saline and diluted with 1% human albumin in 25 to 50 mL 0.9% saline.

**Treatment Plan With rhuGM-CSF**

Two hundred fifty micrograms rhuGM-CSF per square meter or placebo were administered per day by continuous intravenous infusion over 24 hours. The continuous infusion of rhuGM-CSF was chosen because phase I/II trials with this drug have shown higher levels of granulocytes with 24-hour infusions than after 2-hour infusions.\textsuperscript{25} The first dose was administered within 24 hours after the completion of the bone marrow infusion. Therapy with rhuGM-CSF continued until the absolute neutrophil count reached 500/\(\mu\)L or for a period of at least 7 consecutive days. The maximal treatment duration was defined as 30 days.

**Evaluation and Criteria for Response**

The objectives of this trial were defined as follows: (1) major study objective: time until the neutrophil count reached a level \(\geq 500/\mu\)L for 7 consecutive days; (2) minor study objectives: (a) time to first reach a neutrophil count of \(\geq 1,000/\mu\)L, (b) incidence and severity of infections after ABMT, including number of days with fever of 38.5°C or greater, (c) effects on platelet or erythrocyte recovery, and (d) toxicity according to World Health Organization (WHO) criteria.

**Statistical Analysis**

The data were analyzed using standard statistical methods with the assistance of the PC version of the Statistical Analysis System (SAS) release 6.03 (SAS Institute, Cary, NC) and BMDP statistical software 1990 (Cork Technology Park, Ireland, UK). Kaplan-Meier product-limit estimates were used to evaluate time-to-event data such as neutrophil regeneration, occurrence of infections, dependence on erythrocyte and platelet transfusions, survival, and patient isolation time.\textsuperscript{26} 95% confidence intervals of median time-to-event were derived using the smoothed modified reflected method.\textsuperscript{27} Two-sided significance testing was based on the log-rank test.\textsuperscript{28} The analysis of treatment effects adjusted by known or suspected covariates was performed using Cox's proportional hazards model. Regression coefficients were tested by means of likelihood ratio tests.\textsuperscript{29}

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. Fisher's exact test (two-tailed) was used to determine whether the frequency of events in the two groups differed. \(P\) values for the difference in blood counts at a particular time were computed using the two-tailed Wilcoxon-Mann-Whitney test.\textsuperscript{30}

**Bone Marrow Aspiration and Storage**

Bone marrow was aspirated under general or regional anesthesia from the posterior iliac crests with suitable needles and was anticoagulated with preservative-free heparin. The marrow buffy coat was cryopreserved in 10% dimethylsulfoxide and 20% autologous or pooled AB plasma and stored at \(-196°C\).

**Treatment Protocol**

All patients received TBI with at least 8 Gy, combined with any high-dose chemotherapy. After 1 or 2 days of rest after the ablative treatment, the patients received the thawed marrow in the form of a rapid intravenous infusion.

Patients were isolated in single rooms. Blood cell support was supplied according the protocols of the different centers.

**Definition and Classification of Infections**

By summarizing the reported data, infections were defined as follows: (1) microbiologically defined infection classified according the causative organism: (a) bacteremia, fungemia or septicemia microbiologically verified by one or more positive blood cultures with signs of infection, or by more than one positive blood culture with the same organism, even without signs of infection; (b) local infection verified microbiologically in samples from sites with signs of infection. (2) Clinically defined infection: a clinical local infection was diagnosed if no organism could be recovered from an infected site.
Fever was an oral temperature of 38.5°C or greater and categorized as either due to a microbiologically or clinically defined infection, or as having an undetermined infectious origin.

Evaluating Infections

Infections were classified into four categories: (1) bacterial, (2) fungal, (3) viral, and (4) clinically documented. Each patient was evaluated in every category just once. Therefore, in case patients suffered from three bacterial infections, they were noted as a “patient with infection” only once in the category bacterial infection. When a patient suffered from a bacterial and, for example, a fungal infection, he was counted as “patient with infection” in each category. For this reason, the number of infections was higher than the total number of patients in this study.

Infection per patient-day was calculated as the total number of infections observed divided by the total number of treatment days of all patients from day 1 to 30 after ABMT.

Patient Characteristics

Between April 1988 and October 1989, 81 patients were included in the trial and randomized according to the protocol. Forty-one patients were assigned to the rhuGM-CSF group. Following the intention-to-treat method, none of the patients were excluded from the safety analysis. For analysis of the efficacy, two of the patients had to be excluded from the analysis, one who had not received TBI and one ALL patient who was not in CR. Forty patients were assigned to the placebo group. Demographic data, remission status, diagnosis of the patients, and the antineoplastic therapy before ABMT are summarized in Table 1.

In 14 patients with ALL and six with NHL treated with rhuGM-CSF, and in 14 patients with ALL and five with NHL who received placebo, bone marrow in vitro treatment was performed to remove residual malignant cells by chemotherapy, monoclonal antibodies, or physical methods. The total mean (±SD) number of transfused mononuclear bone marrow cells per kilogram body weight was 0.94 (±0.78) × 10^6 in patients receiving rhuGM-CSF treatment and 1.04 (±0.94) × 10^6 in patients receiving placebo.

RESULTS

Hematopoietic Recovery

Regeneration of neutrophils. The mean numbers (±2 SE) of neutrophils at weekly intervals after ABMT are shown in Fig 1. They reached a nadir on day 8. After that cut-off point, the neutrophils were higher in the rhuGM-CSF–treated patients (Table 2).

During the 30-day study period, the means (±SD) of the highest absolute neutrophil counts were 3,350 ± 3,180 neutrophils/μL on day 21 in patients treated with rhuGM-CSF, and 820 ± 650 cells/μL on day 23 in patients receiving placebo (P = .0001).

Patients treated with rhuGM-CSF needed significantly less time to reach greater than 500 neutrophils/μL for 7 consecutive days (P = .0001). For all patients in this group, this level of neutrophils was reached after a median of 15 days, with a 95% confidence interval of 11.8 to 17.5 days. The placebo patients reached this level of myeloid recovery after a median of 28 days (23.5 to >42 days). Patients suffering from ALL achieved this hematological end-point after a median of 18 days, with a 95% confidence interval of 12.3 to 19.5 days for rhuGM-CSF patients and 27 days (21.8 to >42 days) for the placebo patients. In patients with NHL, these times were 13 days (11.1 to 17.1 days) in the rhuGM-CSF group and 28 days (22 to >42 days) in the placebo group.

![Fig 1. Mean values ±2 SE of neutrophil granulocytes/μL.](image-url)
The median time interval to reach more than 1,000 neutrophils/µL for at least 1 day was 18 days (13.5 to 19.3 days) with rhuGM-CSF treatment and 33 days (28 to 42 days) with placebo.

Multivariate analysis of factors with possible influence on neutrophil reconstitution. Various factors such as diagnosis (ALL or NHL), remission status, age, number of transfused mononuclear cells, bone marrow purging, single or fractionated dose of TBI, kind of chemotherapy for conditioning, Karnofsky index, and treatment with the study drug were screened for their impact on the neutrophil reconstitution. A multivariate Cox regression analysis was stratified by participating centers showed that the study drug had the strongest impact on neutrophil regeneration (P = .0001).

Regeneration of eosinophils, monocytes, lymphocytes, and basophils. The number of eosinophils was higher in rhuGM-CSF-treated patients at days 15 and 22. However, the number of monocytes was only slightly higher. There was no major difference as far as lymphocyte and basophil counts were concerned.

Regeneration of erythropoiesis and thrombopoiesis. The platelet counts were slightly lower on days 22 and 29 in patients treated with rhuGM-CSF. Reticulocyte counts were similar in both groups.

Blood cell support. Patients treated with rhuGM-CSF reached platelet transfusion independence after a median of 39 days (22.9 to 73.9 days). Patients in the placebo group reached platelet transfusion independence after a median of 31 days (21.7 to 43.2 days) (P = .39). Independence from erythrocyte transfusion was reached on day 52 (29.3 to 81.1 days) in the rhuGM-CSF group and on day 28 (20.5 to 46.5 days) in the placebo group (P = .36).

Infections, Fever, Antibiotic Therapy, and Patient Isolation

Fever of 38.5°C or greater occurred in 31 of 39 patients (79.5%) receiving the rhuGM-CSF treatment and 31 of 40 patients (77.5%) receiving the placebo. The total number of patients with any kind of microbiologically documented infection was smaller in the rhuGM-CSF (E coli) group (Table 3). Unexplained fever was observed in five (12.8%) patients treated with rhuGM-CSF and in none of the patients on placebo.

During the first 30 days, the number of patients with bacterial infections was lower with rhuGM-CSF treatment (P = .024).

The major difference was seen in infections due to gram-positive organisms. Eleven (28.2%) patients treated with rhuGM-CSF developed infections, compared with 21 (52.5%) patients (P = .024) in the placebo group.

The total number of all infectious episodes from day 1 until day 30 was 0.723 per patient-day with rhuGM-CSF and 0.942 per patient-day with placebo; the corresponding figures for exclusively bacterial infections were 0.568 and 0.713 per patient-day.

Intravenous antibiotic treatment was necessary in 38 (97%) of the 39 evaluable patients treated with rhuGM-CSF and in all 40 (100%) of the placebo group patients, with a mean (±SD) duration of 19.4 (±7.57) and 19.2 (±6.41) days, respectively.

Patient isolation and discharge from hospital. The median duration of patient isolation (with 95% confidence intervals) was 26 days (21.7 to 28.8 days) for the rhuGM-CSF–treated patients and 30 days (24.6 to 34.7 days) for the placebo patients (P = .22). The median time to discharge from hospital was 30 days (24.9 to 38.3 days) for patients treated with rhuGM-CSF and 31 days (28.9 to 35.6 days) for patients in the placebo group.

Toxicity

Without analyzing their causes or relationship to the study drug, the major side effects are listed in Table 4. Capillary leakage syndrome occurred in five rhuGM-CSF–treated patients, whereas it was not noted in the placebo group. Edema was noted for six rhuGM-CSF–treated patients and for three patients in the placebo group. Hepatomegaly occurred in seven rhuGM-CSF–treated patients and in one patient in the placebo group, and splenomegaly in five rhuGM-CSF–treated patients and in

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**Table 2. Mean Values and Standard Errors of Neutrophil Counts per Microliter**

<table>
<thead>
<tr>
<th>Day</th>
<th>rhuGM-CSF (neutrophils/µL)</th>
<th>Placebo (neutrophils/µL)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>1,377</td>
<td>401</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>38</td>
<td>1,443</td>
<td>269</td>
</tr>
<tr>
<td>22</td>
<td>33</td>
<td>1,428</td>
<td>210</td>
</tr>
<tr>
<td>29</td>
<td>30</td>
<td>1,889</td>
<td>331</td>
</tr>
</tbody>
</table>

*Wilcoxon test, two-sided.

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**Table 3. Patients With Infections**

<table>
<thead>
<tr>
<th></th>
<th>rhuGM-CSF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial infection and organism (no. of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis (7)</td>
<td>Staphylococcus aureus (1)</td>
<td></td>
</tr>
<tr>
<td>S aureus (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S epidermidis (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S epidermidis (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S faecalis (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S pneumonia (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S aureus (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S epidermidis (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S aureus (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S faecalis (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P aeruginosa (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious organism: no. of patients (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>14 (36.9%)*</td>
<td>25 (62.5%)*</td>
</tr>
<tr>
<td>Fungi</td>
<td>3 (7.7%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Viruses</td>
<td>3 (7.7%)</td>
<td>6 (15.5%)</td>
</tr>
<tr>
<td>Total patients with microbiologically documented infections</td>
<td>18 (46.2%)</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Patients with clinically documented infections</td>
<td>11 (28.2%)</td>
<td>8 (20%)</td>
</tr>
</tbody>
</table>

Those with different kinds of infectious organisms or clinically defined infections are counted in each of the respective categories. *P = .024.
none of the placebo group patients. The numbers for other events were similar in the two groups.

There was no relevant difference between the two groups with respect to the following laboratory serum parameters: alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatinine, alkaline phosphatase, or uric acid. However, the mean values of total protein and of albumin were lower during the treatment in the rhuGM-CSF group than in the control group.

**Survival**

Survival was not a preplanned study objective, but was analyzed retrospectively. The median overall survival was 309 days (95% confidence interval, 164 to 917 days) in the rhuGM-CSF group, and 640 (308 to 910) days in the placebo group. The log-rank test showed no major difference in overall survival between rhuGM-CSF–treated and placebo-treated patients ($P = .47$). Comparing the Kaplan-Meier survival curves of patients in first remission, nearly identical curves were demonstrated with rhuGM-CSF or placebo ($P = .63$). However, in patients not in first remission, the median survival was shorter in rhuGM-CSF–treated patients ($P = .24$) (Fig 2).

**Causes of Death**

The median observation period (range) was 637 days (464 to 917 days) for rhuGM-CSF–treated patients and 585 days (448 to 910 days) for the placebo group. On March 1, 1991, 22 (56%) rhuGM-CSF–treated patients and 21 (52.5%) patients in the placebo group had died. Fourteen patients (34%) died from underlying malignancy in the rhuGM-CSF group and 18 (45%) patients in the placebo group. The median progression-free survival after ABMT was 215 days with rhuGM-CSF and 245 days with placebo. Death due to causes other than malignancy occurred in eight (20%) patients in the rhuGM-CSF group and in three (7.5%) patients in the placebo group (Table 5). During the study and subsequent observation period up to day 42, one patient in the rhuGM-CSF group died of interstitial pneumonia due to cytomegalovirus on day 22 and one patient in the placebo group died of pseudomonal sepsis on day 8.

**Influence of Prognostic Factors on Survival**

The influence of potential prognostic factors was evaluated using Cox’s proportional hazards model with the following covariates: Karnofsky index at ABMT, previous central nervous system irradiation, remission status at time of ABMT (first CR $v$ second CR $v$ higher than second CR or partial remission), duration of underlying malignancy before ABMT ($> 12$ $v$ $12$ months), conditioning chemotherapy (cyclophosphamide $v$ etoposide $v$ other combinations). In addition, treatment (placebo $v$ rhuGM-CSF) was ana-

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**Table 4. Major Toxicity Observed in Patients Receiving rhuGM-CSF or Placebo**

<table>
<thead>
<tr>
<th>Toxic Effect</th>
<th>No. of Subjects With Toxic Effects (%)</th>
<th>rhuGM-CSF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin rash</td>
<td></td>
<td>3 (7.7)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Capillary leakage</td>
<td></td>
<td>5 (12.8)</td>
<td>0</td>
</tr>
<tr>
<td>Lung edema</td>
<td></td>
<td>3 (7.7)</td>
<td>0</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td></td>
<td>3 (7.7)</td>
<td>0</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td></td>
<td>6 (15.4)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td>1 (2.6)</td>
<td>5 (12.6)</td>
</tr>
<tr>
<td>Chills</td>
<td></td>
<td>7 (18)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td></td>
<td>5 (12.8)</td>
<td>0</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
<td>0</td>
<td>5 (12.6)</td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td>4 (10.3)</td>
<td>5 (12.6)</td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td>3 (7.7)</td>
<td>5 (12.6)</td>
</tr>
</tbody>
</table>

**Table 5. Causes of Death After ABMT**

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse</td>
<td>14</td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>Veno-occlusive lung and liver disease</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
</tr>
</tbody>
</table>

**Fig 2. Probability of survival in patients with ALL and NHL after ABMT with rhuGM-CSF or placebo in first remission or more advanced stage of the disease.**
lyzed. Karnofsky index less than 100%, as well as not being in first remission at the time of ABMT, had a prominent adverse effect on survival \( (P < .05) \).

**DISCUSSION**

The aim of this study was to reduce the duration of granulocytopenia and the number of infections after TBI and high-dose chemotherapy followed by ABMT in patients with ALL and NHL. In this regard, rhuGM-CSF was highly superior to placebo. Several studies have shown that bone marrow treatment to eliminate residual leukemic cells has no obvious effect on the kinetics of bone marrow regeneration.\(^1,3,12,20,22\) This could be confirmed in our trial, where in addition the bone marrow purging had no impact on marrow recovery triggered by rhuGM-CSF.

According to published data, granulocytopenia lasts 22 to 26 days after the corresponding myeloablative treatment in NHL.\(^3,4,21,31\) regardless of bone marrow treatment. In our study, no difference in regeneration kinetics was found between patients with ALL and those with NHL, so these two patient groups were evaluated jointly. Based on our own phase II study\(^19\) and in contrast to several other studies,\(^14,18\) we continued to give rhuGM-CSF for 7 days after the target level of 500 neutrophils/\( \mu L \) was reached. Other studies with rhuGM-CSF after ABMT have reported an initial increase in the number of neutrophil granulocytes followed by a decrease\(^14\) if rhuGM-CSF was discontinued on day 15.\(^15\) In contrast, the neutrophil count between day 14 and day 28 was notably higher in the rhuGM-CSF group than in the control group in our trial. We also observed a decrease in the number of neutrophils after the discontinuation of rhuGM-CSF, but the mean values remained above the critical level of 500 neutrophils/\( \mu L \). A Cox multivariate analysis\(^29\) showed no correlation between granulocyte recovery and age, diagnosis, remission status, number of transfused mononuclear cells, bone marrow purging, single- or fractionated-dose TBI, kind of chemotherapy for conditioning, or Karnofsky index. RhuGM-CSF had no relevant effect on the time period for which erythrocyte or thrombocyte transfusions were necessary. This finding differs from those of phase II trials in patients after ABMT or chronic bone marrow failure, where rhuGM-CSF proved to accelerate the regeneration of thrombopoiesis\(^18\) or of erythropoiesis,\(^30\) and animal studies with primates after ABMT where the recovery of platelets was accelerated as well.\(^33\)

The number of patients with bacterial infections was essentially reduced to 35.9% by the administration of rhuGM-CSF, whereas in the control group 62.5% of patients had a bacterial infection. This effect was caused by the significantly shorter phase of severe granulocytopenia and by the markedly greater number of granulocytes. RhuGM-CSF–treated patients performed better in this respect before neutrophil reconstitution could be ascertained in peripheral blood. However, one might speculate that the early reduction of infections reflects the positive effects of rhuGM-CSF on the number or activity of monocytes or macrophages.

There was no substantial difference in fungal and other infections between the two patient groups. The severity or duration of fever did not differ between the two patient groups. This can be explained by the mucositis, which is almost always present, viral infections, or rhuGM-CSF itself, which might have produced fever.\(^17,34\)

As a rule, neutropenic patients with fever are treated with antibiotics, even when no pathogenic organism is identified.\(^8,35\) This explains why the duration of antibiotic therapy was the same despite a major reduction in bacterial infections. Our results clearly show that with rhuGM-CSF the infectious morbidity after TBI and high-dose chemotherapy in ABMT could be reduced by significantly shortening the regeneration phase of granulopoiesis.

There was no particular increase in serum levels of bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and creatinine, and no relevant difference between the two patient groups. However, total serum protein and albumin were lower in patients receiving rhuGM-CSF. This clearly confirms earlier observations by other investigators.\(^36\) This may in part reflect the higher capillary permeability and loss of albumin into the extravascular space, mainly reported when rhuGM-CSF was applied after aggressive chemotherapy and radiotherapy.\(^37\)

The most extreme form may be the syndrome of capillary leakage observed in some of our patients treated with rhuGM-CSF. These symptoms were described earlier after ABMT and could be caused by functional activation of granulocytes,\(^38\) the release of tumor necrosis factor,\(^14,17\) interferon gamma, or interleukin-1,\(^36\) and consecutive endothelial lesions. However, in another study after ABMT conducted with rhuGM-CSF (yeast), no increased toxicity and no capillary leakage were reported.\(^15\)

The mode of application by continuous infusion of rhuGM-CSF may cause more severe side effects than 2-hour or 6-hour infusions used in other trials.\(^15,18,30\) The higher and dose-dependent toxicity of rhuGM-CSF with continuous infusion has already been reported from phase I/II trials.\(^14,17,25,40\) This effect may be more relevant after ABMT by intensifying the markedly procedure-related multimorgan toxicity. One might speculate that even the way of producing recombinant GM-CSF with _E coli_ used in our study, which leads to an unglycosylated product in contrast to yeast-derived rhuGM-CSF, might play a role in these effects, but there is no trial comparing _E coli_- with yeast-derived rhuGM-CSF.

The similar relapse rates in both groups support the assumption that GM-CSF does not promote growth of lymphoid neoplasias. We analyzed the causes of deaths in all patients in order to elucidate the high number of early death not due to the malignancy in the group of rhuGM-CSF–treated patients. None of these patients died for reasons that could be considered drug-related. Furthermore, survival was evaluated retrospectively. Indeed, status of remission and Karnofsky index were the only parameters that correlated with a higher risk of death for all study patients. Similar observations have been reported by oth-
ers. Diverse studies with rhu-GM-CSF confirmed that it is possible to shorten the time period of engraftment after ABMT and to reduce the frequency of infections. For patients with bone marrow failure after allogeneic BMT, long-term survival could be greatly improved by treatment with rhuGM-CSF. Future developments of hematopoietic stimulating factors will allow their combination and the use of new factors with activity on other cell lines.

ACKNOWLEDGMENT

The study drug was provided by Behringwerke AG, Marburg, Germany.

REFERENCES


A controlled trial of recombinant human granulocyte-macrophage colony-stimulating factor after total body irradiation, high-dose chemotherapy, and autologous bone marrow transplantation for acute lymphoblastic leukemia or malignant lymphoma

H Link, MA Boogaerts, AM Carella, A Ferrant, H Gadner, NC Gorin, I Harabacz, JL Harousseau, P Herve and J Holldack