Recombinant human granulocyte-macrophage colony-stimulating factor accelerates engraftment kinetics after allogeneic bone marrow transplantation for childhood acute lymphoblastic leukemia

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ABSTRACT

Background and Objective. The use of recombinant human granulocyte-macrophage stimulating factor (rhGM-CSF) has been shown to be well-tolerated and to reduce post-transplantation morbidity in adults undergoing HLA-identical allogeneic bone marrow transplantation (BMT). There is however, limited experience in children.

Design and Methods. We performed a prospective, comparative multicenter trial using rhGM-CSF after allogeneic BMT in children with acute lymphoblastic leukemia (ALL). The study comprised 24 patients with ALL who received rhGM-CSF and 22 patients with ALL who did not receive rhGM-CSF. There were no statistically significant differences in the demographic characteristics between the rhGM-CSF-treated and untreated groups. rhGM-CSF was given at a dose of 10 µg/kg/day infusion over 4 hours from day +1 until +28 or until the absolute neutrophil count (ANC) was ≥1×10⁹/L. All patients received HLA-identical sibling marrow and cyclosporine alone for graft-versus-host disease (GvHD) prophylaxis. The number of cells infused was similar in both groups. A software program (Statview 4.0, Abacus Concept, Inc., Berkeley, CA, USA) was used for statistical analysis.

Results. The median of days to achieve ANC ≥ 0.5×10⁹/L was shorter in the rhGM-CSF-treated patients (14 days vs 18.5 days; p<0.0001). Patients who received rhGM-CSF had a lower incidence of grade III-IV mucositis. The duration of hospital stay was significantly shorter in patients who received rhGM-CSF (31 days vs 45 days; p<0.005). No differences in GvHD severity, relapse or survival were observed. At the dose and schedule used in the present study rhGM-CSF was well-tolerated and no side effects were observed.

Interpretations and Conclusions. rhGM-CSF at a dose of 10 µg/kg/day in children with ALL undergoing allogeneic BMT is well tolerated, accelerates neutrophil and platelet engraftment, reduces the intensity and severity of mucositis and permits a more rapid discharge from hospital.

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Key words: rhGM-CSF, allogeneic bone marrow transplantation, childhood, ALL

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Patients were examined during the two weeks following completion of treatment and after every month in order to evaluate the clinical course. The study was approved by the Institutional Review Boards and Ethics Committees and parents' informed consent was obtained in all cases.

Bone marrow collection
Bone marrow was harvested from the HLA-identical donor using standard techniques on the day of transplantation (day 0). The median number of MNC administered was $3.0 \times 10^6$ (range $0.68-5.4 \times 10^6$) in the rhGM-CSF-treated group and $3.2 \times 10^6$ (range $0.21-7.3 \times 10^6$) in the untreated group.

Conditioning regimen for BMT
Fifteen patients of the rhGM-CSF-treated group and 12 patients of the untreated group were given intravenous cyclophosphamide 60 mg/kg on each of two successive days followed by 12 Gy of total body irradiation given in six fractions over three days (Cy-TBI). Two patients of the rhGM-CSF-treated group and one patient of the untreated group received busulfan orally at a dose of 16 mg/kg over 4 days and intravenous cyclophosphamide 60 mg/kg on each of two successive days (Bu-Cy). Two patients of the untreated group received busulfan orally at a dose of 16 mg/kg over 4 days, VP-16 40 mg/kg for one day and intravenous cyclophosphamide 60 mg/kg on each of two successive days. One patient of the rhGM-CSF-treated group received busulfan orally at a dose of 16 mg/kg over 4 days (days -7 to -4) and melphalan 180 mg/m² on day -2 (Bu-Me). Six patients of the rhGM-CSF-treated group and 7 patients of the untreated group received intravenous cyclophosphamide 60 mg/kg on each of two successive days, VP-16 40 mg/kg for one day and total body irradiation given in six fractions for three days.

Supportive care
Bone marrow transplantation was performed in a BMT unit. A central venous catheter was placed in each patient. Patients were cared for in a room with laminar flow or reverse isolation by positive pressure. Prophylaxis for Pneumocystis carinii (cotrimoxazole 8 mg/kg/day from day -7 to 0 and then from +50 to +150 post-transplantation) and for herpes simplex virus (acyclovir 750 mg/m² from day -7 to +24) was employed. Non-absorbable antibiotics for gut decontamination were routinely administered. Transfusions were administered for a hematocrit of <25% and platelet count of $<20\times 10^9$/L. All hemoderived transfusion products were irradiated to 1.5-2.5 Gy. Patients were started on i.v. broad spectrum antibiotics if their temperature was higher than 38°C and their neutrophil count was $<1\times 10^9$/L. If the patient continued to have fever and neutropenia on day 4-5 post-transplantation, amphotericin B (0.5-1 mg/kg/day) was added.

GVHD prophylaxis
Cyclosporin A alone was administered i.v. at a dose of 3 mg/kg from day -1 until oral intake was resumed. CyA was then given orally at a dose of 12.5 mg/kg until day 50, after which it was gradually tapered off and was discontinued by 6-8 months post-transplantation.

Definitions
Neutrophil recovery was defined as the days to achieve ANC of $\geq 0.5 \times 10^9$ for three consecutive days. Platelet recovery was defined as the time to achieve $\geq 20 \times 10^9$ without requiring transfusion. Hospital stay was defined as days from day 0 to hospital discharge. Clinically documented infectious episodes were defined as the presence of symptoms and signs of infection. Fever was categorized as a clinically or microbiologically defined infection. Bloodstream infection was defined as one or more blood cultures positive for any organism. Interstitial pneumonitis was diagnosed if bilateral infiltrates on chest X-ray were associated with significant hypoxemia. Acute GVHD was diagnosed and graded according to the Seattle criteria. The grading of regimen-related toxicity was classified according to criteria reported by Bearman et al.

Statistical analysis
A software program (StatView 4.0, Abacus Concept Inc., Berkeley, CA, USA) was used for the statistical analysis. The data are expressed as median and range. The statistical significance was determined by Student’s t-test. Results were considered significant if the p value was $\leq 0.05$. Probabilities of achieving a neutrophil count of $\geq 0.5 \times 10^9$ and a platelet count of $\geq 20 \times 10^9$ and $\geq 50 \times 10^9$ were calculated using the method of Kaplan and Meier and the comparison between them by the log-rank test. Disease free survival and the probability of relapse were calculated using the Kaplan Meier method.
**Results**

**Hematopoietic recovery**

All patients achieved an ANC of $\geq 0.5 \times 10^9$ cells/L within 28 days after marrow infusion. The patients who received rhGM-CSF reached myeloid engraftment before the patients who did not receive rhGM-CSF. The median of days to ANC $\geq 0.5 \times 10^9$ cells/L was 14 days for the rhGM-CSF-treated group versus 18.5 days for the untreated group, the difference being statistically significant ($p < 0.0001$).

rhGM-CSF significantly influenced platelet recovery. The medians of days to platelet count $\geq 20 \times 10^9$ platelets/L and $\geq 50 \times 10^9$ platelets/L without platelet infusion were 16 days and 23 days in the patients who received rhGM-CSF versus 26 days and 40 days in the patients who did not receive rhGM-CSF ($p < 0.04$ and $p < 0.02$, respectively) (Table 2). Figures 1 and 2 show the myeloid and platelet engraftment probabilities for both groups.

**Fever and infection**

The median duration of fever was 6 days in the patients who received rhGM-CSF versus 8 days in the patients who did not receive rhGM-CSF ($p < 0.2$). Six patients of the untreated group and 7 patients of the rhGM-CSF-treated group developed clinically documented infection ($p < 0.2$) (Table 2).

**Toxicity**

The rhGM-CSF-treated patients had a lower incidence of severe mucositis (6 patients vs 17 patients of the untreated group, $p < 0.003$) (Table 2). There were no differences in non-hematologic toxicity (renal, hepatic, neurological, digestive and cardiac) in both groups.

**Hospital stay**

The median duration of hospital stay was shorter in patients who received rhGM-CSF than in patients who did not receive rhGM-CSF (31 days vs 45 days; $p < 0.003$).

**Relapse**

Three patients who received rhGM-CSF relapsed within the first 12 months post-transplantation. The probability of relapse in this group was $15.55 \pm 8.2\%$.

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**Table 2. Hematologic and clinical values.**

<table>
<thead>
<tr>
<th></th>
<th>GM-CSF (n=24)</th>
<th>Controls (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to neutrophil</td>
<td>14</td>
<td>18.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>count $&gt;0.5 \times 10^9$</td>
<td>9-24</td>
<td>11-29</td>
<td></td>
</tr>
<tr>
<td>Days to neutrophil</td>
<td>16</td>
<td>24.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>count $&gt;1 \times 10^9$</td>
<td>18-28</td>
<td>13-40</td>
<td></td>
</tr>
<tr>
<td>Days to platelet</td>
<td>16</td>
<td>26</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>count $&gt;20 \times 10^9$</td>
<td>11-72</td>
<td>(14-120)</td>
<td></td>
</tr>
<tr>
<td>Days to platelet</td>
<td>23</td>
<td>40</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>count $&gt;50 \times 10^9$</td>
<td>(17-120)</td>
<td>(18-150)</td>
<td></td>
</tr>
<tr>
<td>Febrile days</td>
<td>6</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0-21</td>
<td>(0-30)</td>
<td></td>
</tr>
<tr>
<td>Parenteral antibiotics</td>
<td>12</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>(days)</td>
<td>(6-41)</td>
<td>(0-49)</td>
<td></td>
</tr>
<tr>
<td>Time in hospital</td>
<td>31</td>
<td>45</td>
<td>0.005</td>
</tr>
<tr>
<td>(days)</td>
<td>(18-63)</td>
<td>(21-135)</td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>7</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>of clinically documented infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis 0-I</td>
<td>18</td>
<td>5</td>
<td>0.003</td>
</tr>
<tr>
<td>II-IV</td>
<td>6</td>
<td>17</td>
<td>0.003</td>
</tr>
</tbody>
</table>

The median number of days of i.v. antibiotics in the rhGM-CSF-treated patients was 12 (range 6-41) compared with 15 (range 0-49) days in the untreated group ($p < 0.2$) (Table 2).

**GvHD**

The incidence and severity of acute GvHD was not different between the two groups (Table 3).

**Hospital stay**

The median duration of hospital stay was shorter in patients who received rhGM-CSF than in patients who did not receive rhGM-CSF (31 days vs 45 days; $p < 0.003$).

**Relapse**

Three patients who received rhGM-CSF relapsed within the first 12 months post-transplantation. The probability of relapse in this group was $15.55 \pm 8.2\%$.
The median number of days to achieve ANC ≥ 0.5 × 10^9 cells/L was lower in the rhGM-CSF-treated patients (14 vs 18.5 days, \( p < 0.0001 \)). These observations are similar to the findings reported elsewhere.\(^{3,24,25} \) The faster neutrophil recovery in our patients treated with rhGM-CSF resulted in fewer febrile days and a shorter duration of antibiotic use, although the differences were not significant (\( p < 0.2 \)). Unlike the findings of the study by Nemunaitis et al.,\(^{13} \) we did not find a decrease in the number of infectious episodes. We did, however, find that platelet recovery was influenced by the administration of rhGM-CSF. In our study the medians of days to platelet ≥ 20 × 10^9/L and ≥ 50 × 10^9/L were significantly shorter in patients who received rhGM-CSF. The foregoing finding has not been observed by other authors in patients undergoing allogeneic BMT.\(^{13,14} \) but has been observed in patients with different malignancies who received high dose chemotherapy and autologous BMT.\(^{26} \) This effect on platelet recovery may be due to the fact that rhGM-CSF is capable of stimulating megakaryocytic progenitor cell growth.\(^{27} \)

Like other studies,\(^{10,13} \) we found that the incidence and severity of GVHD did not increase. Growth factor administration has been reported to produce moderate side effects.\(^{28} \) However, at the dose and schedule used in the present study, rhGM-CSF was well-tolerated and no side effects were observed, as we have also reported in a previous study on rhG-CSF.\(^{29} \)

Although some authors have reported that the relapse rate is lower in patients undergoing allogeneic BMT who receive rhGM-CSF,\(^{11} \) we did not observe a decrease in the relapse rate in our homogeneous group of patients. On the other hand, we have found a lower incidence of mucositis. This finding may be explained by an increase in the residual function of host macrophages.\(^{13} \)

The duration of hospitalization was shorter for the rhG-CSF-treated patients, which might be ascribable to the earlier neutrophil and platelet recovery. A shorter hospital stay implies an economic benefit, although cost reduction also depends on reducing the costs of supportive patient care. Although growth factor administration implies higher costs, to our knowledge no cost analysis concerning pediatric patients is available in the literature.

From the present data, we can conclude that rhG-CSF at a dose of 10 \( \mu \)g/kg/day in children with ALL undergoing allogeneic BMT is well-tolerated, accelerates neutrophil and platelet engraftment, reduces the intensity and severity of mucositis and permits earlier discharge from hospital.

**Contributions and Acknowledgments**

The study was designed and coordinated by LM, AM, JJO and AM collected the clinical and analytical data. IB, TO and PG were responsible for the data handling. MAD was responsible for the statistical analyses and references. LM wrote the manuscript which was submitted to the rest of the authors for their approval. The order of appearance of the names is based on the importance of each individual contribution, as previously established and accepted by all of the authors.

**Disclosures**

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.
References


