Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Patients With Myelodysplastic Syndromes—A Phase I/II Trial

By A. Ganser, B. Völkers, J. Greher, O.G. Ottmann, F. Walther, R. Becher, L. Bergmann, G. Schulz, and D. Hoelzer

In a phase I/II study, 11 patients with myelodysplastic syndromes (MDS) and severe transfusion-dependent cytopenia were treated with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) to investigate the effects of rhGM-CSF on normal hematopoiesis and leukemic cells. The treatment schedule included dose escalation from 15 μg/m² to 150 μg/m² administered by continuous intravenous (IV) infusion for seven to 14 days and was repeated after a two-week treatment-free interval. The blood leukocyte counts increased dose dependently by 130% to 1,800% in ten patients; a rise of monocytes and eosinophils occurred in seven and six patients, respectively. No sustained increase in reticulocytes or platelets was observed. Lymphocyte counts increased in all patients affecting both T-helper and T-suppressor cells; however, the lymphocytes were not activated as analyzed by the expression of the interleukin-2 receptor. In four of the patients, all with >14% blast cells in the bone marrow, the percentage of bone marrow blast cells increased during treatment with rhGM-CSF. Cytogenetic data indicated induction of both proliferation and differentiation of the leukemic clones by rhGM-CSF. Toxic side effects were minor with slight fever, phlebitis at the infusion site, and bone pain in the minority of patients. In conclusion, rhGM-CSF effectively stimulates hematopoiesis in vivo in patients with myelodysplastic syndromes. However, as the leukemic cell population can be stimulated in patients with a higher initial blast cell count, the combination of rhGM-CSF with other differentiation-inducing or cytotoxic agents has to be considered.

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MATERIALS AND METHODS

Preparation of rhGM-CSF. The rhGM-CSF used in these studies has been provided by Behringwerke AG (Marburg, FRG). The recombinant protein was expressed in yeast and purified by Immunix, Inc (Seattle) as described. The rhGM-CSF is glycosylated and has a molecular weight between 14 and 22 Kd depending on the pattern of glycosylation. The rhGM-CSF used had a specific activity of approximately 5 x 10⁷ colony forming units per milligram of protein. The endotoxin concentration was <1 ng/mg protein. Sterility, general safety, and purity studies meet the Food and Drug Administration (FDA) standards.

Patient selection and study design. Only patients with clinical and hematologic confirmation of myelodysplastic syndrome (RA, RAEB, RAEB-T, CMML) as defined by the FAB-group who had not been treated with antileukemic drugs for at least 4 weeks were included. Eligibility criteria included a performance status of >50% (Karnofsky scale), life expectancy of more than 3 months, age less than 75 years, preserved hepatic, renal, and hematologic function. Patients under the age of 50 years eligible for bone marrow transplantation or conventional high-dose chemotherapy were included.

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excluded. The study was submitted to the FDA and approved by the local Ethics Committee. Informed consent was obtained from the patients before rhGM-CSF therapy.

The treatment schedule consisted of four dose levels of rhGM-CSF, ie, 15 μg/m², 30 μg/m², 60 μg/m², and 150 μg/m². The first two patients received 15 μg/m² intravenously (IV) (level 1), the next two patients received 30 μg/m² IV (level 2), and so on. Two patients were entered sequentially at dose levels 1 through 4 and received treatment from days 1 through 7 and 22 through 28. On day 1, the daily dose was administered within five minutes, while on the other days the IV infusions were administered over an eight-hour period. From dose level 4 the initial bolus injection was omitted and the first course of rhGM-CSF was extended to 14 days since, due to initial results, an effect on hematopoiesis was not expected to occur before day 7 or 8 of therapy.

Patients were monitored daily and all constitutional symptoms were recorded. Vital signs were checked before injections and at 1, 2, 4, and 6 hours after the start of infusion on days 1, 2, 5, 8, 22, and 29. A history and physical examination were performed before the initial dose and weekly thereafter. Patients were weighed weekly. An electrocardiogram and chest x-ray were performed before the study and after the final dose. A serum chemistry profile, coagulation profile, and urinalysis were obtained before treatment and on days 1, 2, 5, 8, 15, 22, 26, 29, and 32. A complete blood count, including differential and reticulocyte counts, was performed before treatment and on days 1 through 8, 15, 22, 24, 26, 29, and 32.

Lymphocyte subpopulations (CD4 and CD8) and the presence of the interleukin-2 receptor on the lymphocytes were estimated in the peripheral blood on days 1, 8, 15, 22, and 29 using a commercial kit (provided by Behringwerke AG, Marburg, FRG) and 1 unit of recombinant human erythropoietin (specific activity 173,000 U/mg protein; provided by Boehringer Mannheim GmbH, Mannheim, FRG).37 The culture plates, set up in quadruplicate, were incubated for 14 days at 37°C and 5% CO2 in humidified atmosphere and scored in situ under an inverted microscope. Colonies derived from multipotent progenitors CFU-GEMM (colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte) contained at least granulocytic/monocytic and erythroid elements; colonies derived from erythroid progenitors BFU-E (burst-forming unit-erythroid) contained >300 cells, whereas those derived from myeloid progenitors CFU-GM (CFU-granulocyte, macrophage) contained >50 cells.

Toxicity was graded according to the World Health Organization (WHO) criteria.36 Dose-limiting toxicity was generally defined as toxicity of grade 3 or higher by WHO criteria, excluding hematopoietic toxicity. The maximally tolerated dose was defined as the dosage in which approximately 75% of patients achieved a reversible grade 3 toxicity. Red cell concentrates were administered if the hemoglobin level dropped below 8 g/dL or if there were symptoms related to anemia. Platelet transfusions were only administered in cases of bleeding. Analgesics or antipyretics were not administered unless the body temperature rose to >39°C. Progression of disease was defined as fulfillment of the criteria of acute nonlymphoblastic leukemia in 4 weeks after the end of GM-CSF therapy.

RESULTS

Eleven patients with MDS were entered in the study: seven men and four women, ranging in age from 46 to 75 years (median age, 64 years). Three patients had RA, four had RAEB, two had RAEB-T, and two had CMML. The median time from diagnosis to treatment with rhGM-CSF was 9 months (range, 4 to 144). The performance status based on the Karnofsky scale was ≥70% in all patients. All patients were anemic, seven were neutropenic, and eight were thrombocytopenic (Table 1). All but two patients required regular blood transfusions, ie, at least 2 U/mo. One patient had been pretreated with low-dose cytosine arabinoside 3 months before treatment with rhGM-CSF.

Hematologic effects. The effect of rhGM-CSF on circulating leukocytes is shown in Table 2. An increase in the leukocyte counts was observed in ten of the 11 patients. In

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics Before Treatment With rhGM-CSF*</th>
</tr>
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<tbody>
<tr>
<td><strong>Patient No.</strong></td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

*The data on segmented neutrophils and blast cells are provided in Table 2.
four patients this increase was substantial with a rise of more than 10,000/μL. The rise in leukocyte counts was usually seen after three to five days. After discontinuation of rhGM-CSF infusions, the leukocyte counts gradually declined within two to ten days to reach pretreatment levels in most, but not all, patients. The increase in leukocyte counts was related to the dosage of rhGM-CSF administered (Fig 1, Table 2). With regard to the differential counts, the increase in leukocytes was mainly due to an increase in the neutrophilic granulocyte series. However, in patients no. 9 and 10, the increase in leukocytes was, in addition, due to a substantial rise of blast cells, promyelocytes, myelocytes, and metamyelocytes (Fig 2).

A dose-related increase in the absolute eosinophil and monocyte counts was observed in the majority of patients (Table 2, Fig 1) with a mean increase of eosinophils from 122,000/μL in patient no. 9, this patient's platelet counts gradually fell from 317,000/μL to 31,000/μL on day 13, i.e., two days after GM-CSF had been discontinued. The platelet counts reached 60,000/μL on day 20 when the WBC counts had fallen to 73,600/μL (with 5% blast cells) and recovered to >150,000/μL within the following 4 weeks.

The increase in the number of circulating lymphocytes was directly correlated to the dosage of rhGM-CSF administered (Fig 1) and affected both CD4- and CD8-positive T-lymphocyte populations. The mean CD4/CD8 ratio was not changed and was 1.53 (range, 0.67 to 3.94) before and 1.40 (range, 0.30 to 3.22) after treatment with rhGM-CSF. No activation of the lymphocytes, as determined by the expression of the interleukin-2 receptor, was observed. There was no change in the serum levels of the immunoglobulins IgG, IgA, and IgM.

In order to examine the effect of rhGM-CSF on the blast cell compartment in the bone marrow, cytologic examination of the bone marrow was performed before and after each course of therapy. The differentiation ratio of mature myeloid cells (myelocytes plus metamyelocytes plus bands plus segmented neutrophils) to immature myeloid cells (blasts plus promyelocytes) did not change and was 3.5 (range, 0.9 to 16.2) before and 4.1 (range, 0.2 to 15.4) after treatment with rhGM-CSF. An increase in the percentage of bone marrow blasts was found in four of the 11 patients.
Table 3. Bone Marrow Changes During Treatment With rhGM-CSF*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>% Blast Cells</th>
<th>% M₄+</th>
<th>Maturation Index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>4.5</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>36</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>15</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>54.5</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>16.5</td>
<td>8.8</td>
<td>1.5</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>21</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Numbers under each straddle rule indicate the day of treatment course.

*: rhGM-CSF from day 1-14 and 22-28, patients no. 1-7; rhGM-CSF from day 1-14 and 22-28, patient no. 8; rhGM-CSF from day 1-14, patients no. 10 and 11; rhGM-CSF from day 1-11, patient no. 9.

†: M₄, metamyelocyte; M₅, juvenile; M₆, band neutrophil; M₇, segmented neutrophil.

‡: (Myelocytes + metamyelocytes + juveniles + bands + segmented neutrophils)/blasts + promyelocytes.

From 150 μg/m² to 75 μg/m² after day 1 and was unable to continue beyond 48 hours of the second course at dose level 3 because of repeated severe chest pain.

In general, treatment with rhGM-CSF was well tolerated. The side effects associated with rhGM-CSF are shown in Table 6. They included fever reaching 38.6°C in one patient, because of repeated severe chest pain.

Table 4. Effect of In Vivo Treatment With rhGM-CSF on Cytogenetically Defined Clones in Patients With MDS

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Dosage (μg/m²)</th>
<th>Before</th>
<th>After</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 RAEB</td>
<td>15</td>
<td>11</td>
<td>2</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>3 CMML</td>
<td>30</td>
<td>3</td>
<td>0</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>7 RAEB-T 150/60</td>
<td>4</td>
<td>1</td>
<td>46,XY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 RAEB</td>
<td>150</td>
<td>0</td>
<td>1</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>10 RA</td>
<td>150</td>
<td>11</td>
<td>12</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>11 RA</td>
<td>150</td>
<td>3</td>
<td>6</td>
<td>46,XY</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Hematopoietic Progenitor Cell in the Bone Marrow per 10⁶ (Mean ± SEM)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Day</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.5 ± 0.5</td>
<td>2</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>0</td>
<td>2 ± 2</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>0</td>
<td>11.5 ± 0.5</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>0</td>
<td>12.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0.5 ± 0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>0.5 ± 0.5</td>
<td>11.5 ± 0.5</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>12.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>0</td>
<td>2 ± 2</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>11</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>10.5 ± 0.5</td>
</tr>
</tbody>
</table>

Table 6. They included fever reaching 38.6°C in one patient, because of repeated severe chest pain.
infusion occurred in two other patients in whom a small vein had been chosen for venous access. One patient (no. 4) developed a pulmonary infiltrate on the third day of the second course of therapy accompanied by fever to 38.2°C. The pulmonary infiltrate completely resolved within five days after starting antibiotic treatment with piperacillin and tobramycin. In one patient with a prior history of stable angina, chest pain as well as pain of the lower back developed after bolus injection at day 1, and necessitated dose deescalation. Repeated severe chest pain at the lower dose level and the development of a reversible complete left bundle branch block resulted in discontinuation of rhGM-CSF therapy in this patient. One patient who had been unilaterally nephrectomized for a hypernephroma 10 years earlier experienced a rise in the creatinine level when his leukocyte counts increased to >100,000/μL. The creatinine level returned to normal after institution of allopurinol and increased fluid intake.

DISCUSSION

Eleven patients with MDS who had long-lasting cytopenias and required frequent transfusions of RBCs or platelets, were treated with rhGM-CSF in an attempt to restore hematopoiesis in order to decrease the risk of infection and bleeding. Treatment with rhGM-CSF clearly induced a dose-related leukocytosis in all but one patient mainly consisting of increases in neutrophilic granulocytes and bands. However, a rise of more immature cells, including blast cells, was observed in seven patients. A dose-related increase in monocytes and eosinophils was also seen similar to previous reports in patients with AIDS or MDS. Only one patient had an infection during the study; however, since repeated infections were a problem before GM-CSF therapy in only one of our patients, it is too early to conclude whether the rate of infection can be reduced in this patient population by rhGM-CSF.

Several factors may contribute to the increase in leukocyte counts. An initial component could be demargination and mobilization of neutrophils from the vessel wall or bone marrow leading to the regularly occurring early rise of mature leukocytes. A second component could be increased proliferation of morphologically recognizable precursors of granulopoiesis, mainly promyelocytes and myelocytes as has been shown in vitro. In two of our patients, this mechanism might at least, in part, have been responsible for the rapid expansion of myelocytes, metamyelocytes, and bands in the peripheral blood. The sustained rise in leukocyte counts and the subsequent increase of bone marrow counts points to an additional effect of rhGM-CSF on the hematopoietic progenitor cell compartment. Although during treatment with rhGM-CSF the incidence of the granulocyte/macrophage progenitors CFU-GM remained unchanged in the bone marrow, there could well be an absolute increase in the number of progenitors due to the increase of bone marrow cellularity. On the other hand, in agreement with previous observations in MDS multilinage progenitors CFU-GEMM and erythroid progenitors BFU-E were found at highly reduced numbers before as well as after rhGM-CSF therapy. Therefore, we could not draw any conclusion regarding whether normal progenitors could at all be activated in our patients.

A rise in the reticulocyte counts only occurred at the highest concentrations of rhGM-CSF tested in our trial. However, in contrast to previous preclinical and clinical studies, a persistent rise in reticulocytes and, subsequently, hematocrit was not observed. All patients continued to require RBC transfusions at the same frequency as before therapy. Similarly, except for a transient rise in one patient, the platelet counts did not improve. Thus, we could not confirm a previous report of an increase in platelet counts in patients with MDS. Our findings are, however, in accordance with the experience of others after treatment of patients with AIDS with rhGM-CSF.

The reasons for these discrepancies concerning stimulation of erythropoiesis and thrombopoiesis are unclear but could be related to differences in the dosages, in the method of administration of rhGM-CSF, ie, an intermittent daily eight-hour infusion or continuous infusion, or in the number of treatment courses. However, since three of the eight patients treated by Vadhan-Raj et al had a secondary MDS and five cases had received chemotherapy up to 4 weeks before entering the study, physiologic recovery of the marrow may have biased their results. All but one of our patients had been treated with only transfusion before entering the trial. However, since our patients only received one to two courses of therapy, further studies with larger groups of patients and repeated cycles of GM-CSF therapy will have to clarify whether rhGM-CSF may act as a multilineage stimulator in vivo. As a result of future trials, a combination of rhGM-CSF with other hematopoietic growth factors, eg, erythropoietin or interleukin-3, might become a feasible approach to obtain a multilineage stimulation.

A major point for the clinical application of rhGM-CSF in patients with MDS is related to the question of the growth stimulation of leukemic subclones. The infusion of rhGM-CSF resulted in an increase in leukemic blast cells in the bone marrow of four of our patients. In three additional patients blasts increased in the peripheral blood. In contrast to a previous study, we achieved neither a decrease in the percentage of bone marrow blast cells nor an improvement in the ratio of differentiation. In only two patients with an excess of blasts in the marrow was the differentiation ratio increased. Again, these differences could be due to different timings of bone marrow analysis, although our data do not
support this hypothesis. Cytogenetic analysis before and after treatment suggests that rhGM-CSF did not eradicate but preferentially stimulated the abnormal clone even in patients with no measurable increase in bone marrow blasts. The presence of only abnormal metaphases and the absence of normally growing hematopoietic progenitor cells, however, suggests that in the individual patient the increase of mature cells after treatment with rhGM-CSF might have been due to differentiation from the abnormal clone. Direct proof for it would require cytogenetic analysis on mature neutrophils, isoenzyme marker studies, or analysis of restriction fragment length polymorphism. The in vivo stimulation of leukemic clones corresponds to in vitro observations that have shown that GM-CSF, besides inducing stimulation of leukemic clones corresponds to in vitro observations that have shown that GM-CSF, besides inducing differentiation in some of the established myeloid leukemic cell lines, promotes the proliferation of leukemic progenitor cells. The discrepancy between our results and those reported in a previous trial regarding the stimulation of the leukemic cells can be partially explained by different patient characteristics, ie, our patients in general had a higher percentage of blast cells in the bone marrow before treatment with rhGM-CSF and were not pretreated with cytostatic chemotherapy. An increase in the percentage of bone marrow blasts only occurred in patients with an initial blast cells percentage >14%, ie, in patients with a higher initial leukemic cell burden. These patients, especially those with CMML who had progression to frank acute leukemia in our trial should, therefore, not be included in further trials with rhGM-CSF alone at the present time.

Although lymphoid cells lack receptors for rhGM-CSF, GM-CSF has been demonstrated to act directly on T-cell lines. Therefore, the dose-dependent rise in lymphocyte counts that was seen in all patients receiving ≥60 μg rhGM-CSF/m² and that has also been observed in previous trials, could be a direct effect of rhGM-CSF. In addition, it may be indirect through mobilization from yet unknown sites or through stimulation of accessory cells, eg, macrophages or antigen-presenting cells, to produce further cytokines. Both CD4-positive helper T lymphocytes and CD8-positive suppressor T lymphocytes increased at the same rate excluding a selective effect of rhGM-CSF. In addition, the lymphocytes were not activated as demonstrated by the absence of the interleukin-2 receptor.

The toxicity of the treatment schedule presented was only mild. As in previous trials, bone pain of unknown mechanism also occurred in some of our patients. Phlebitis at the site of IV infusion was a further problem if small veins were used for infusion, but can be prevented by using larger cubital veins or a central venous catheter for access. Subcutaneous application of rhGM-CSF is presently under investigation and should facilitate the application and clinical use of rhGM-CSF.

In summary, our results indicate that rhGM-CSF effectively stimulates the production of neutrophilic granulocytes in vivo without major toxic effects at the tested dose schedule. In patients with MDS and low initial leukemic cell load, prospective studies are needed to establish the optimal treatment schedule and to examine whether severe infections can be prevented by rhGM-CSF. In patients with a higher initial leukemic cell burden and the risk of an acceleration of the leukemia, a combination of rhGM-CSF with other differentiation-inducing drugs or cytotoxic agents should be further evaluated.

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