Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor in Combination With Standard Induction Chemotherapy in De Novo Acute Myeloid Leukemia


Based on in vitro data suggesting that recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) is capable of stimulating acute myeloid leukemia (AML) blast cells to become more sensitive to cell-cycle-specific drugs we conducted a phase I/II study in de novo AML patients (pts). rhGM-CSF (250 μg/m²/d, continuous intravenous infusion) was administered in 18 pts suffering from de novo AML in combination with standard induction chemotherapy (3 + 7 = daunorubicin 45 mg/m² days 1 through 3, cytosine-arabinoside [Ara-C] 200 mg/m² continuous infusion days 1 through 7). GM-CSF was started 48 or 24 hours before chemotherapy (prephase) in 14 pts. In four pts with high white blood cell counts (WBC) rhGM-CSF was started after chemotherapy-induced cell reduction (WBC < 30,000/mm³). During prephase GM-CSF induced an increase in neutrophil and blast cell counts in 13 of 14 and 10 of 14 pts, respectively.

In vivo recruitment of leukemic cells into drug-sensitive phases of the cell cycle could be demonstrated by multiparameter cell-cycle analyses in peripheral blood (n = 7) and bone marrow (n = 4) specimens. On day 14, complete aplasia was evident in 17 of 18 pts. GM-CSF was administered until recovery from chemotherapy-induced myelosuppression [absolute neutrophil counts (ANC) > 500/mm³]. Fifteen pts (83%) achieved complete remission, 12 did so with one cycle. A shorter duration of neutropenia was evident in these pts compared with historical controls (n = 39), (ANC > 500/mm³, day 22.5 ± 3.4 vs 25.2 ± 3.7, P < .05). Three pts achieved complete remission after a second cycle (same combination of rhGM-CSF and 3 + 7). Two pts died during bone marrow aplasia because of invasive pulmonary aspergillosis. Clinical side effects possibly related to GM-CSF, mainly fever, diarrhea, and weight gain were mild and tolerable (World Health Organization toxicity grade ≤2). Together, rhGM-CSF recruits kinetically quiescent AML cells in vivo to enter drug-sensitive phases of the cell cycle and promotes early myeloid recovery from aplasia after exposure to standard induction chemotherapy for AML.

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Submitted December 7, 1989; accepted October 16, 1990.

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0006-4971/91/7704-0019$3.00/0


G R A N U L O C Y T E - M A C R O P H A G E colony-stimulating factor (GM-CSF) is a well-defined glycoprotein involved in the control of hematopoiesis. Cloning of the human GM-CSF gene and its expression in yeast and Escherichia coli provided quantities sufficient for the evaluation of its physiologic functions and its possible therapeutical uses. GM-CSF stimulates colony formation of multipotent as well as committed myeloid stem cells and promotes survival, proliferation, and differentiation/maturaton of myeloid cells. GM-CSF also has a number of remarkable effects on the functional properties of mature myeloid cells: it primes neutrophils for enhanced phagocytosis and superoxide production and supports tumoricidal activities of monocytes and neutrophil cytotoxicity towards neoplastic cells.

Clinical trials have shown the beneficial effects of GM-CSF in promoting granulocyte recovery after autologous bone marrow (BM) transplantation, after high-dose chemotherapy, in aplastic anemia, and in bone marrow failure. Whereas GM-CSF seems useful in reducing aplasia-associated risks in these clinical settings, its use in myeloid leukemias deserves special cautions. First, in vitro studies have shown that GM-CSF stimulates growth and differentiation of malignant myeloid cells through specific receptors and, in addition, may be produced by leukemic cells under certain conditions. More recently, in patients (pts) suffering from myelodysplastic syndromes (MDS), growth stimulation of blast cells and transition to acute leukemia was observed. These experiences discouraged clinical studies in acute myeloid leukemia (AML) pts using GM-CSF as a growth factor. On the other hand, we and others were able to demonstrate that recombinant human GM-CSF (rhGM-CSF) recruits quiescent AML cells in vitro to become more sensitive to cell-cycle–specific drugs and apparently promotes drug-induced cell kill. Significantly better recruitment was found in newly diagnosed versus relapsed AML pts.

The aims of the present study were to demonstrate the in vivo effects of rhGM-CSF on cell-cycle kinetics of AML cells and to investigate whether rhGM-CSF is capable of shortening the time of chemotherapy-induced cytopenia in de novo AML pts.

PATIENTS AND METHODS

Eligibility. Eighteen consecutive pts with newly diagnosted (de novo) AML entered the study after written informed consent. The treatment protocol and informed consent form were approved by the Ethics Committee of the Institutional Review Board, I Medical Department, University of Vienna. Diagnoses and subclassification of AML were established according to the criteria of the French-American-British (FAB) Cooperative Group. Pts with a history of preleukemic syndrome were excluded, and none had undergone prior radiotherapy or chemotherapy. A Karnofsky score of greater than 20% was required for eligibility.

Patient characteristics. The pts’ characteristics and FAB subtypes are summarized in Table 1. Ten men and eight women ranged in age from 25 to 77 years (median 50). Severe anemia...
Prephase was shortened to 24 hours to minimize the risk of treatment protocol was modified according to initial results. The final product was formulated as lyophilized powder and expressed in E. coli. Hemorrhage (epistaxis, gum bleeding, petechiae, subcutaneous hematoma) was noted in seven pts with thrombocytopenia. In pt 3 (AML-FAB M3) requiring packed red blood cell (RBC) transfusions was found in two pts. Seven pts had fever greater than 38°C. Pt 6 had a large perirectal abscess and septicemia (Klebsiella oxytoca). Hemorrhage (epistaxis, gum bleeding, petechiae, subcutaneous hematoma) was noted in seven pts with thrombocytopenia. In pt 3 (AML-FAB M3) hypofibrinogenemia (178 mg/dL), low α-2-antiplasmin (65%), and increased D-dimer levels (12500 ng/mL) were found. Impaired kidney function was normal in all pts. The historical control group consisted of 39 de novo AML pts (excluding pts with AML following myelodysplastic syndrome), achieving complete remission (CR) after one cycle of the same chemotherapy regimen (3 + 7 = daunorubicin 45 mg/m² days 1 through 3, cytosine-arabinoside [Ara-C] continuous infusion days 1 through 7) but without rhGM-CSF. The distribution of FAB subtypes in the control group was: M1 (n = 8), M2 (n = 10), M3 (n = 3), M4 (n = 5), M5 (n = 8), M6 (n = 2). The historical controls were neither age nor sex matched.

**rhGM-CSF**. The material used was provided by Behring Company (Marburg, Germany). The human GM-CSF gene was isolated from libraries made from HUT-102 mRNA and was inserted into E. coli. The purified protein had a specific activity of 5 × 10⁶ colony forming units (CFU)/mg protein. The final product was formulated as lyophilized powder and reconstituted in aqueous buffer before use.

**Treatment protocol and study design.** Treatment included administration of E. coli-derived rhGM-CSF in combination with a standard cytotoxic remission induction chemotherapy protocol (3 + 7) as depicted in Fig 1 (see also ref 41). RhGM-CSF at a dose of 250 μg/m² daily was administered by continuous intravenous (IV) infusion and was delivered by an autosyringe pump through a central venous access line, 48 hours before chemotherapy (prephase). rhGM-CSF, continuous infusion, was administered until the neutrophil count (segmented and banded) was greater than 500/mm³ (after chemotherapy-induced aplasia) and was then changed to subcutaneous application (125 μg²/m² twice daily) for the following 3 days (pts 1 through 6). In pts 7 through 18, the treatment protocol was modified according to initial results. Prephase was shortened to 24 hours to minimize the risk of leukostasis. rhGM-CSF was stopped on day 8 and continued on day 14. Subcutaneous application of GM-CSF at the end of the regimen was omitted in pts 7 through 18. Only pts with initial white blood cell counts (WBC) less than 30,000/mm³ were eligible for prephase, otherwise rhGM-CSF was started after chemotherapy-induced WBC reduction (below 30,000/mm³) (Fig 1B). Because of an increase of serum creatinine in pt 2, rhGM-CSF dosage was decreased to 125 μg/m² beyond day 6 in this patient.

![Fig 1. Treatment protocol: rhGM-CSF in combination with standard induction chemotherapy in de novo AML pts. (A) Pts 1 through 6 received rhGM-CSF (250 μg/m²/d, continuous IV infusion) 48 hours before and during chemotherapy as well as during chemotherapy-induced aplasia. Infusion was stopped at ANC greater than 500/mm³, followed by SC application for another 3 days. (B) In pts 8, 10, 11, and 14 through 18, rhGM-CSF prephase was shortened to 24 hours, rhGM-CSF infusion was stopped on day 8, and continued on day 14 until neutrophil recovery (ANC > 500/mm³). In pts with initial high WBC counts (pts 7, 9, 12, and 13) rhGM-CSF administration was postponed until chemotherapy-induced WBC reduction (WBC < 30,000/mm³).](www.bloodjournal.org)
Table 2. Clinical Outcome in 18 AML Pts Receiving rhGM-CSF in Combination With Standard Induction Chemotherapy (3 + 7):
Comparison With Historical Controls

<table>
<thead>
<tr>
<th>Pt</th>
<th>Clinical Outcome (no. of cycles)</th>
<th>Days to &gt;1,500 Leukocytes</th>
<th>Days to &gt;500 Neutrophils</th>
<th>Days to &gt;100,000 Platelets</th>
<th>CCR</th>
<th>Survival</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CR (1)</td>
<td>24</td>
<td>23</td>
<td>494</td>
<td>568+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CR (2)</td>
<td>28/23*</td>
<td>—/23*</td>
<td>507</td>
<td>568+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CR (1)</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>045+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CR (1)</td>
<td>20</td>
<td>20</td>
<td>22</td>
<td>344</td>
<td>535</td>
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<tr>
<td>5</td>
<td>Early death (1), day 13</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>023</td>
</tr>
<tr>
<td>6</td>
<td>Early death (1), day 23</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>013</td>
</tr>
<tr>
<td>7</td>
<td>CR (2)</td>
<td>20/22*</td>
<td>23/22*</td>
<td>506</td>
<td>400+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CR (2)</td>
<td>26/23*</td>
<td>26/23*</td>
<td>304</td>
<td>375</td>
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<td>CR (1)</td>
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<td>25</td>
<td>22</td>
<td>190+</td>
<td></td>
</tr>
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<td>10</td>
<td>CR (1)</td>
<td>20</td>
<td>20</td>
<td>22</td>
<td>235</td>
<td>396+</td>
</tr>
<tr>
<td>11</td>
<td>CR (1)</td>
<td>21</td>
<td>21</td>
<td>24</td>
<td>310+</td>
<td>338+</td>
</tr>
<tr>
<td>12</td>
<td>CR (1)</td>
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<td>24</td>
<td>261+</td>
<td>288+</td>
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<td>CR (1)</td>
<td>23</td>
<td>22</td>
<td>25</td>
<td>147+</td>
<td>175+</td>
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<tr>
<td>14</td>
<td>CR (1)</td>
<td>19</td>
<td>18</td>
<td>24</td>
<td>130+</td>
<td>156+</td>
</tr>
<tr>
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<td>CR (1)</td>
<td>23</td>
<td>23</td>
<td>27</td>
<td>108+</td>
<td>136+</td>
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<td>CR (1)</td>
<td>31</td>
<td>31</td>
<td>24</td>
<td>093+</td>
<td>135+</td>
</tr>
<tr>
<td>17</td>
<td>CR (1)</td>
<td>27</td>
<td>25</td>
<td>30</td>
<td>100+</td>
<td>129+</td>
</tr>
<tr>
<td>18</td>
<td>No remission</td>
<td>22/29*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>091+</td>
</tr>
</tbody>
</table>

All pts with CR (1)
Mean 22.8 ± 3.4 22.5 ± 3.4 24.4 ± 2.5
Median 22 21.5 23

Historical controls$ (n = 39)
Mean 24.6 ± 3.6 25.2 ± 3.7 24.4 ± 4.7
Median 23 25 23

*Days from the onset of chemotherapy; the second values (pts 2, 7, and 8) represent days of the second cycle.
†Segmented and banded. At neutrophil counts >500/mm³, IV administration of rhGM-CSF was stopped in all pts.
§Thirty-nine AML patients achieving CR after one cycle of the same chemotherapy regimen but without rhGM-CSF.

Remission induction chemotherapy (3 + 7) consisted of daunorubicin (Farmitalia Carlo-Erba, Freiburg, Germany), 45 mg/m² daily (days 1, 2, and 3) and Ara-C (Upjohn, Puurs, Belgium), 200 mg/m² by continuous IV infusion for 7 days (days 1 through 7). If CR was not achieved with one cycle of therapy, a second cycle of chemotherapy (reinduction) was administered, consisting of the same drugs as used in the first cycle (again in combination with rhGM-CSF).

Table 3. Pts Hematologic Parameters at Study Entry and Response of WBC to GM-CSF During Prephase

<table>
<thead>
<tr>
<th>Pts</th>
<th>Hemoglobin (g/dL)*</th>
<th>WBCs</th>
<th>Neutrophils</th>
<th>Blasts</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
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<tbody>
<tr>
<td></td>
<td>Platelets Before</td>
<td>After</td>
<td>Before</td>
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<tr>
<td>1</td>
<td>8.8</td>
<td>28</td>
<td>22.7</td>
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<td>&lt;0.1</td>
<td>0.3</td>
<td>21.6</td>
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<td>9.8</td>
<td>211</td>
<td>28.5</td>
<td>0.3</td>
<td>2.9</td>
<td>6.7</td>
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<tr>
<td>3</td>
<td>11.0</td>
<td>25</td>
<td>1.9</td>
<td>5.4</td>
<td>1.1</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>9.9</td>
<td>20</td>
<td>16.4</td>
<td>68.8</td>
<td>7.4</td>
<td>38.5</td>
<td>5.9</td>
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<tr>
<td>5</td>
<td>4.7</td>
<td>139</td>
<td>3.0</td>
<td>5.1</td>
<td>0.7</td>
<td>2.6</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>9.5</td>
<td>19</td>
<td>14.2</td>
<td>20.3</td>
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<td>13.0</td>
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<td>8</td>
<td>11.5</td>
<td>150</td>
<td>1.9</td>
<td>1.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.1</td>
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<td>112</td>
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<td>0.9</td>
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</tr>
<tr>
<td>14</td>
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<td>0.2</td>
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<td>145</td>
<td>26.9</td>
<td>22.7</td>
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<td>2.5</td>
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<td>45.6</td>
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<td>20.0</td>
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<td>42.0</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<tr>
<td>13</td>
<td>9.8</td>
<td>35</td>
<td>52.0</td>
<td>2.6</td>
<td>22.9</td>
<td>11.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

"Before" denotes prior rhGM-CSF administration, "after" denotes 48 hours (pts 1 through 6) or 24 hours (pts 8, 10, and 11) after rhGM-CSF prephase.
*All pts except pts 7 and 12 had required packed RBC transfusions before study entry (hemoglobin < 8 g/dL).
†These pts were not eligible for prephase; the counts were taken immediately before onset of chemotherapy, rhGM-CSF was started after chemotherapy-induced leukocyte reduction to less than 30,000/mm³ (pt 7: day 4; pt 13: day 3; pts 9 and 12: day 2).
packed RBC transfusions were administered to maintain hemo-
globin levels of more than 8 g/dL and random platelets were
administered when the platelet counts had decreased to less
than 20,000/mm³. For infection prophylaxis we used norflo-
xacin (2 × 400 mg/d orally) and to prevent fungal infections, am-
photericin B or nystatin were administered orally. An empiric
broad spectrum antibiotic regimen was administered when
systemic infection was suspected.

Clinical and laboratory monitoring. Clinical monitoring prior
during therapy included standard clinical and laboratory
investigations. Differential counts were performed daily. Toxicity
was assessed using World Health Organization (WHO) criteria.25
BM aspirates and/or biopsies were taken before and after the
GM-CSF prephase as well as on days 7, 14, 21, and 28 after the
onset of chemotherapy. CR was defined according to Cancer and
Leukemia Group B (CALGB) criteria. Short-term cultures of
peripheral blood (PB) and BM cells were performed according to
standard techniques.21,24 Karyotypes were established according to
the International System of Human Cytogenetic Nomenclature.41

Statistical analysis. The time to recovery of WBC, neutro-
phil, and platelets of the study pts achieving CR within one cycle
was compared with historical controls (see above) using the Student’s
t-test (unpaired).

Cell-cycle analysis by flow cytometry. Cell-cycle analysis was
performed using three independent methods including DNA/ RNA,
DNA/BrdU, and DNA/Ki67 flow cytometry as described.
Mononuclear cells (MNC) were isolated from heparinized PB
samples by density gradient centrifugation. Cellular DNA/RNA
content was measured using acridine orange flow cytometry, as
described.39,40 This method allows the discrimination of cells in the
G_0, G_1, S, and G_2M-phases of the cell cycle. DNA synthesis was
further investigated using bromodeoxyuridine (BrdU) incorpora-
tion into DNA-synthesizing cells. In brief, 1 × 10⁷ cells were
incubated with BrdU (final concentration 10 µg/mL) for 1 hour at
37°C with 5% CO₂. Cells were then washed and fixed in cold
ethanol. After treatment with RNase, DNA was partially denatur-
ated with heat at 85°C for 10 minutes. Anti-BrdU antibody (IU 4,
courtesy of Dr F. Dolbeare, Lawrence Livermore National Labora-
tory, Livermore, CA) and thereafter goat antimouse IgG-
fluorescein were added. Counterstaining for total DNA content
was performed using propidium iodide (for details see Vanderlaan
et al 44 and Dolbeare et al45).

To determine the growth fraction, simultaneous flow cytometric
measurement of Ki67 and cellular DNA content was performed
(Ki67 is a proliferation-associated human nuclear antigen present
in all cell-cycle phases except G_0). Cells were stained for flow
cytometric measurement of growth fraction and cell-cycle phases
as described by Larsen et al.51

All immunoassay measurements were performed using flow
cytometry (fluorescence-activated cell sorter [FACS], Becton-
Dickinson, Mountain View, CA) and analyzed using the available
Becton-Dickinson software, including Consort 30, FACSScan
Research Software, Lysis, and Paint-a-Gate.

In 11 pts, in vitro proliferation of AML blast cell populations in
response to GM-CSF was analyzed by means of thymidine-uptake
experiments.3 H-thymidine uptake assay was performed using standard
techniques. In brief, PB MNC (10⁵/well) were incubated in
5% CO₂ at 37°C in RPMI medium supplemented with 10% fetal calf serum (FCS) at
37°C in 5% CO₂. Thereafter, 1 µCi [H]-thymidine (New England
Nuclear, Boston, MA) was added. Eight hours later, cells were
harvested and the incorporated radioactivity was counted in a
liquid scintillation counter.

Table 5. In Vivo Effects of GM-CSF on Cell-Cycle Kinetics
of AML Cells

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Before % S</th>
<th>24 h After rhGM-CSF % S</th>
<th>48 h After rhGM-CSF % S</th>
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<td>PB</td>
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<td></td>
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<tr>
<td>4</td>
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<td>—</td>
<td>10.2</td>
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<td>—</td>
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<td>10</td>
<td>1.5</td>
<td>3.9</td>
<td>—</td>
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<tr>
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<td>—</td>
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<tr>
<td>18 (1)</td>
<td>4.0</td>
<td>2.8</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: % S, cells incorporating BrdU as determined by flow
cytometry, each measurement representing 10,000 cells; (1), denotes
first cycle.

RESULTS

Remission rate and follow-up. Fifteen of 18 pts (83.3%) achieved CR, 12 did so with one cycle. In three pts (2, 7, and 8), CR was obtained after a second cycle (again in combination with rhGM-CSF). Two pts (5 and 6) died in aplasia because of invasive pulmonary aspergillosis. Pt 18 achieved no remission after two cycles of therapy. After a median observation period of 9 months, nine pts are still in continuous CR (CCR) (see Table 2). Pt 3, who achieved CR after one cycle, died during consolidation therapy (Kleb-
siella pneumoniae sepsis). Six pts have relapsed within 2.0 to
16.4 months.

Effect of GM-CSF on PB and BM cells during prephase.
From the day of admission until starting rhGM-CSF, no
substantial increase of WBC was seen in any pt. Fourteen
pts were eligible for rhGM-CSF administration 48 or 24
hours before chemotherapy (see Table 3). An increase
(1.23- to 4.2-fold) in WBC occurred in 12 pts during
GM-CSF prephase. In all pts, except 14, an increase of
neutrophils (segmented and banded) was found (1.23- to
Peripheral blast cells increased in 10 pts (1.19- to 7.5-fold). In two pts (5 and 18), blast cell counts even decreased during the prephase. No effects on RBC counts, reticulocyte, or platelet counts were observed (data not shown). A comparison of the prephase GM-CSF effects on PB and BM blast cells of four pts tested is listed in Table 4.

Cell-cycle analysis. To assess the in vivo recruitment of quiescent leukemic cells cell-cycle analyses were performed before GM-CSF administration and at the end of the prephase. Cells of pts 4, 6, 8, 10, and 15 through 18 were studied with the DNA/BrdU technique and cells of pt 4, in addition, by DNA/RNA and DNA/Ki67 flow cytometry to assess the effects of rhGM-CSF on cell-cycle stages of AML cells. A substantial increase in percent BrdU-positive cells was observed after the prephase in PB (seven of seven pts) as well as in BM specimens (three of four pts) (see Table 5). The increase in percent BrdU-positive cells in BM (pts 15, 16, and 17) and PB (pts 4, 6, 10, 16, and 17) specimens was found to be associated with an increase in PB blast cell counts (Tables 3, 4, and 5). In pt 18, PB blast cell counts decreased during the prephase. At the same time the percentage of BrdU-positive BM cells decreased, whereas
GM-CSF IN AML THERAPY

The incorporation of $^3$H-thymidine into BP AML cells was studied (before administration of GM-CSF) in 11 pts (1 through 6, and 8, 10, 14, 17, and 18). An (1.2- to 17.0-fold) increase in thymidine uptake was observed in 7 of 11 pts (2, 3, 4, 6, 10, 14, and 17). In pts 1, 5, 8, and 18, no substantial increase in thymidine uptake upon stimulation with GM-CSF in vitro was found.

Response following chemotherapy. Figure 3 shows that the chemotherapy-induced decrease of peripheral WBC in the study pts was not delayed as compared with historical controls. Despite continuation of rhGM-CSF infusion, WBC decreased within 24 hours following the onset of chemotherapy in 11 pts. In three pts (11, 14, and 15) peripheral WBC increased until day 2 after onset of chemotherapy (due to elevation of neutrophils and blasts) and thereafter decreased.

In pts 7, 9, 12, and 13 (initial WBC >30,000/mm$^3$), rhGM-CSF administration was started after chemotherapy-induced WBC reduction (day 4 in pts 7 and 13, and on day 2 in pts 9 and 12). In none of them was rhGM-CSF found to elevate peripheral WBC or any leukocyte subset during chemotherapy.

Day-7 BM examination showed aplasia in 7 of 12 pts tested. In 5 of 12 pts, residual blasts were present despite BM hypoplasia. BM examination on day 14 evidenced aplasia in 17 of 18 pts (in pt 18, a small population of residual blast cells was found).

Hematopoietic recovery. Administration of rhGM-CSF was found to shorten the duration of chemotherapy-induced aplasia compared with historical controls ($n = 39$). In particular, in the 12 pts achieving CR after one cycle a neutrophil count of at least 500/mm$^3$ was reached on day 22.5 ± 3.4 (historical controls: 25.2 ± 3.7, $P < .05$).

After myeloid recovery (peripheral absolute neutrophil counts [ANC] > 500/mm$^3$), rhGM-CSF was administered SC for another 3 days in three pts (pts 1, 3, and 4), producing a maximum leukocytosis (up to 27,900/mm$^3$) at the end of rhGM-CSF application. Thereafter, WBC spon-
Fig 5.
Fig 5. BM cytology of pt 4. (A) At admission (diagnosis: FAB M2); (B) day-21 examination (hypercellularity, predominance of promyelocytes); (C) remission on day 28 (<5% blasts).

Maturing neutrophils were the dominant leukocyte population found in PB smears in all pts achieving CR, including myeloid precursors (promyelocytes + myelocytes + metamyelocytes up to 32%). After the end of rhGM-CSF administration, myeloid precursor cells disappeared spontaneously (a representative course of WBC and differential counts is depicted in Fig 4). This transient increase of immature cells in PB corresponded with the BM morphology findings. On day-21 BM examination, in four of seven pts achieving CR after one cycle marked hypercellularity with increased myeloid: erythroid ratio and predominance of immature cells, in particular promyelocytes (up to 35%), was found (a representative follow-up of BM morphology is shown in Fig 5). Day 28 BM smears showed maturation indicating complete remission (blast cells <5%; promyelocytes + myelocytes: 11.2% to 22% v segmented + banded: 20.4% to 37%), CR was confirmed cytogenetically in all pts. BM eosinophilia was present in four pts (8% to 39.2% eosinophils).

Platelet counts of more than 100,000/mm³ were reached on day 24.4 ± 2.5 in the rhGM-CSF–treated pts versus day 24.4 ± 4.7 in the historical controls (not significant). Effects on RBC counts and reticulocytes with respect to early recovery were not found in any pts (data not shown).

In pts 2, 7, and 8, who did not enter CR after one cycle (15.2% to 27% blasts on day-28 BM examination), only a slight increase of WBC was observed during the recovery period (Table 2). CR was achieved in these three pts after a second cycle of treatment (same combination with rhGM-CSF, beginning between day 33 and 44 from the onset of the first cycle). Myeloid recovery was characterized by a steep WBC increase with ANC greater than 500/mm³ on days 22 (pts 7 and 8) and 23 (pt 2) of the second cycle with differential count features analogous to the seven pts described above. Pt 18 presented with greater than 25% BM blasts on day 28 of the second cycle and was therefore classified as treatment failure despite normalization of PB counts.

Adverse reactions and clinical course. Side effects related to rhGM-CSF were mild (WHO toxicity grade ≤2) and no patient failed to complete a course of treatment because of suspected toxicity. The predominant side effects related to rhGM-CSF were fever greater than 38°C (the increase coincident with the onset of rhGM-CSF) in 13 of 18 pts and mild diarrhea in 10 of 18 pts. Serum albumin decreased in
all pts within 1 week (53.8% to 83% of pretreatment values), accompanied by peripheral edema and weight gain in 10 of 18 pts. Hypoalbuminemia persisted as long as rhGM-CSF was administered and then resolved spontaneously. Decreased levels of albumin were associated with low serum pseudocholinesterase levels and a prolonged prothrombin time. The follow-up of these laboratory parameters suggests an rhGM-CSF-mediated effect (Fig 6). These phenomena were not observed in the historical controls.

Transient serum creatinine elevation up to 256.4 μmol/L (2.9 mg/100 mL) was observed in pt 2, and disappeared after dose reduction of rhGM-CSF (half dosage beyond day 6). In five other pts (4, 7, 13, 14, and 18) a transient increase of serum creatinine (WHO toxicity 1) was observed. Severe infections were documented in seven pts. Pts 5 and 6 died because of invasive pulmonary aspergillosis during BM aplasia. Hepatic and splenic abscesses were accountable for a prolonged period of fever after myeloid recovery in four pts. Septicemia because of K acyctoca was diagnosed in one pt. Four pts experienced transient exanthemas. Myalgias and bone pain were not observed in any pt.

**DISCUSSION**

Recent in vitro studies have shown that rhGM-CSF triggers AML blasts to enter the G1- and S-phases of the cell cycle and thereby renders them more susceptible for subsequent kill by cell-cycle–specific drugs. In a phase-I/IT study we now used rhGM-CSF in de novo AML pts in combination with standard induction chemotherapy. Our study demonstrates that rhGM-CSF can safely be administered in combination with standard induction chemotherapy in AML pts to recruit leukemic blast cells and to shorten the time of chemotherapy-induced aplasia. Hepatic and splenic abscesses were accountable for a prolonged period of fever after myeloid recovery in four pts. Septicemia because of K acyctoca was diagnosed in one pt. Four pts experienced transient exanthemas. Myalgias and bone pain were not observed in any pt.

Recent in vitro studies have shown that rhGM-CSF triggers AML blasts to enter the G1- and S-phases of the cell cycle and thereby renders them more susceptible for subsequent kill by cell-cycle–specific drugs. In a phase-I/IT study we now used rhGM-CSF in de novo AML pts in combination with standard induction chemotherapy. Our study demonstrates that rhGM-CSF can safely be administered in combination with standard induction chemotherapy in AML pts to recruit leukemic blast cells and to shorten the time of chemotherapy-induced aplasia. In vitro studies have shown that rhGM-CSF promotes growth and survival of normal as well as malignant hematopoietic cells. The potential hazards of in vivo stimulation of leukemia cell growth by rhGM-CSF have prohibited its clinical use in AML pts so far. In this study, rhGM-CSF administered before cytotoxic chemotherapy (in 14 pts) induced a substantial increase in WBC counts caused by elevation of both AML blasts and neutrophils, whereas no apparent growth-promoting effect of GM-CSF on AML blast cells was observed during or after chemotherapy. Elevation of PB blast cell counts during prephase most likely was the result of a direct effect of GM-CSF on malignant AML blasts. This effect of GM-CSF was associated with proliferation of leukemic cells and probably also with redistribution of cells from the BM into the blood stream.

In vivo recruitment of AML blast cells from G0-phase into the chemotherapy-sensitive G1- and S-phases upon stimulation with rhGM-CSF could be demonstrated in all pts tested. In one pt (PB cells) a decrease in G0-cells and an increase in G1- and S-phase cells was assessed by three independent double-labeling techniques (DNA/RNA-, DNA/BrdU-, and DNA/Ki67 analyses). The in vivo increase in S-phase positive blast cells was observed in BM as well as PB specimens. Together, these data provide evidence that rhGM-CSF is capable of recruiting quiescent leukemic AML cells in vivo to enter the chemotherapy-sensitive phases of the cell cycle. In vitro studies have shown that the increase in S-phase induced by GM-CSF is associated with an increased cell kill. In our study, complete aplasia was observed in all but one pt on day 14 and no delay in chemotherapy-induced blast cell kill (ie, decrease in PB leukocytes during chemotherapy) was seen. We also observed a high rate of CR (83%) with no signs of GM-CSF–induced leukemia cell regrowth. Whether a longer survival time will be obtained in pts using our new protocol remains to be evaluated in ongoing randomized trials.

Recent studies have shown that rhGM-CSF shortens the chemotherapy-induced phase of aplasia in tumor pts. In this study, the duration of chemotherapy-induced neutropenia was significantly shortened in GM-CSF-treated pts (achieving CR after one cycle) compared with historical controls (identical chemotherapy, CR after one cycle) (P < .05). Similar data on the effect of GM-CSF on myeloid recovery have previously been reported in relapsed
AML pts.\textsuperscript{5,5} Despite early recovery of neutrophils, severe infections occurred. Therefore, a possible influence of GM-CSF-induced acceleration of neutrophil recovery on the incidence and/or severity of infections also remains to be determined in forthcoming trials. Recovery of platelets and restitution of erythropoiesis was not accelerated by administration of GM-CSF compared with historical controls.

Clinical side effects related to rhGM-CSF were mild and tolerable. Fever, mild diarrhea, and peripheral edema were the most dominant complaints. Impaired kidney function occurred in three pts receiving rhGM-CSF. However, the increase in creatinine observed in these pts could not definitely be linked to administration of GM-CSF because other nephrotoxic drugs were given simultaneously. The rapid onset of hypalbuminemia (observed in all pts) accompanied by peripheral edema and weight gain was probably the result of endothelial cell damage and/or because of impaired liver cell function. The underlying mechanisms for such cellular dysfunctions are at present not known. We believe that in addition to a direct effect of GM-CSF on such cells, a preexisting cellular dysfunction caused by the presence of leukemic cells and/or their products could have been a cofactor for GM-CSF-induced side effects. The above mentioned leukemia cell products may for example represent cytokines such as interleukin-6 (IL-6)\textsuperscript{5,6} because in preliminary investigations we were able to detect increased serum IL-6 levels in pts treated with GM-CSF compared with pretreatment values (data not shown).

We conclude that rhGM-CSF is a potentially useful agent in the treatment of AML in combination with standard induction chemotherapy. Further studies are necessary to evaluate the long-term effects of this new therapeutic concept in AML.

ACKNOWLEDGMENT

We wish to thank Roswitha Kaltenbrunner for excellent technical assistance.

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Recombinant human granulocyte-macrophage colony-stimulating factor in combination with standard induction chemotherapy in de novo acute myeloid leukemia

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