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original article

Effect of Priming with Granulocyte Colony-Stimulating Factor on the Outcome of Chemotherapy for Acute Myeloid Leukemia

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abstract

background

Sensitization of leukemic cells with hematopoietic growth factors may enhance the cytotoxicity of chemotherapy in acute myeloid leukemia (AML).

methods

In a multicenter randomized trial, we assigned patients (age range, 18 to 60 years) with newly diagnosed AML to receive cytarabine plus idarubicin (cycle 1) and cytarabine plus amsacrin (cycle 2) with granulocyte colony-stimulating factor (G-CSF) (321 patients) or without G-CSF (319). G-CSF was given concurrently with chemotherapy only. Idarubicin and amsacrin were given at the end of a cycle to allow the cell-cycle-dependent cytotoxicity of cytarabine in the context of G-CSF to have a greater effect. The effect of G-CSF on disease-free survival was assessed in all patients and in cytogenetically distinct prognostic subgroups.

results

After induction chemotherapy, the rates of response were not significantly different in the two groups. After a median follow-up of 55 months, patients in complete remission after induction chemotherapy plus G-CSF had a higher rate of disease-free survival than patients who did not receive G-CSF (42 percent vs. 33 percent at four years, P=0.02), owing to a reduced probability of relapse (relative risk, 0.77; 95 percent confidence interval, 0.61 to 0.99; P=0.04). G-CSF did not significantly improve overall survival (P=0.16). Although G-CSF did not improve the outcome in the subgroup with an unfavorable prognosis, the 72 percent of patients with standard-risk AML benefited from G-CSF therapy (overall survival at four years, 45 percent, as compared with 35 percent in the group that did not receive G-CSF [relative risk of death, 0.75; 95 percent confidence interval, 0.59 to 0.95; P=0.02]; disease-free survival, 45 percent vs. 33 percent [relative risk, 0.70]; 95 percent confidence interval, 0.55 to 0.90; P=0.006).

conclusions

Sensitization of leukemic cells with growth factors is a clinically applicable means of enhancing the efficacy of chemotherapy in patients with AML.

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Prevention of relapse remains a challenge in the treatment of acute myeloid leukemia (AML).[1] The high rate of recurrence is due to the reemergence of leukemia from small numbers of residual cells that have escaped the cytotoxic effect of chemotherapy.

Hematopoietic growth factors stimulate AML cells in culture, activating metabolic processes and the cell cycle. In vitro, the simultaneous exposure of leukemic cells to chemotherapy and growth factors such as granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and interleukin-3, referred to as growth-factor priming, increases the susceptibility of the cells to killing by chemotherapy, especially by the cell-cycle–specific agent cytarabine.[2-11] These observations suggest a novel therapeutic strategy for AML, but the value of such an approach has not been assessed clinically.

In previous trials of AML, G-CSF and GM-CSF have been widely used after chemotherapy to accelerate myeloid regeneration,[12-14] but there is information only from uncontrolled studies[15-17] and small, randomized studies[18-21] about their use in growth-factor priming. We conducted a randomized trial to determine whether G-CSF given only during the first two induction cycles with cytarabine plus idarubicin and cytarabine plus amsacrin improves disease-free survival in adults with newly diagnosed AML by increasing the rate of complete response, reducing the relapse rate, or both. G-CSF was not given during the aplastic phase after chemotherapy. To avoid interference of the second chemotherapeutic agent with the cell-cycle–dependent synergy between cytarabine and G-CSF, idarubicin (first cycle) and amsacrin (second cycle) were given at the end of the cycles.

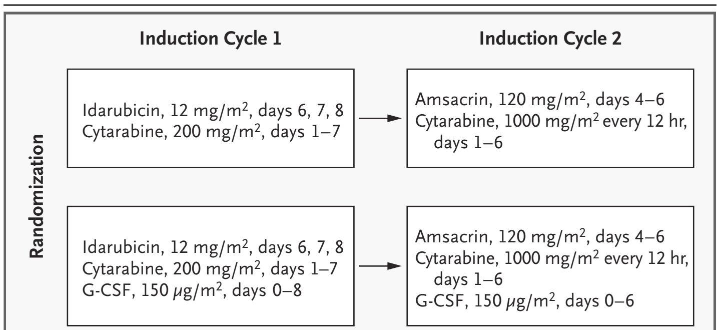

Figure 1. Treatment Regimens.

Patients were randomly assigned to receive two cycles of induction chemotherapy alone or with the addition of granulocyte colony-stimulating factor (G-CSF) beginning one day before the start of chemotherapy (day 0) through the last day of chemotherapy.

METHODS

PATIENTS

Previously untreated patients with a confirmed diagnosis of AML who were 18 to 60 years of age were eligible for the study. All subtypes of AML were included, except acute promyelocytic leukemia and blast crisis of chronic myeloid leukemia. Patients with another active cancer were not eligible, nor were patients with severe heart, lung, or neurologic disease. All patients were screened for eligibility before undergoing randomization.

The study was approved by the ethics committees of the participating institutions and was conducted in accordance with the Declaration of Helsinki. All participants gave their informed consent.

RISK CLASSIFICATION

At diagnosis, samples of bone marrow and blood were examined for cytogenetic abnormalities with the use of standard banding techniques and classified according to the International System for Human Cytogenetic Nomenclature.[22] On the basis of the chromosomal analysis, patients were classified into three distinct prognostic categories: favorable risk, unfavorable risk, and standard risk.[23-25] Favorable risk was defined by the presence of t(8;21)(q22;22), inv16(p13q22), or t(16;16)(p13q22) and a white-cell count of less than 20×10^3 per cubic millimeter at diagnosis.[25] Unfavorable risk was defined by the presence of complex cytogenetic abnormalities (defined as at least four unrelated cytogenetic clones), monosomies, or deletions of chromosome 5 or 7 (5q–, 7q–, –5, or –7), abnormalities of the long arm of chromosome 3(q21;q26), t(6;9)(p23;q34), or abnormalities involving the long arm of chromosome 11(11q23). Leukemias that had occurred after chemotherapy or radiotherapy for a nonhematologic condition and leukemias that had occurred more than six months after a hematologic condition (secondary leukemias) were included in the unfavorable prognostic category. Patients who did not meet the criteria for favorable or unfavorable risk were classified as being at standard risk.

STUDY DESIGN AND CHEMOTHERAPY

Patients were enrolled and randomly assigned to receive G-CSF or no G-CSF during remission-induction cycles 1 and 2 (Fig. 1). Cycle 1 consisted of cytarabine (200 mg per square meter of body-surface area given by continuous infusion on days 1 through 7) and idarubicin (12 mg per square meter given intravenously over a period of 5 to 10 minutes on days 6, 7, and 8). Cycle 2 consisted of cytarabine (1000 mg per square meter given intravenously over a period of 2 hours every 12 hours on days 1 through 6) and amsacrin (120 mg per square meter given intravenously over a 60-minute period on days 4, 5, and 6). G-CSF (lenograstim, Aventis) was given subcutaneously or intravenously in a dose of 150 µg per square meter per day beginning one day before chemotherapy (day 0) and continuing until the last day of cycles 1 and 2. The administration of G-CSF was postponed or interrupted in the event of leukocytosis (more than 30×10^3 leukocytes per cubic millimeter) until the white-cell count was below 20×10^3 per cubic millimeter. Patients with standard-risk or unfavorable-risk AML who were in complete remission after cycle 2 were randomly assigned to a third cycle of chemotherapy with etoposide and mitoxantrone or high-dose chemotherapy with busulfan and cyclophosphamide followed by autologous stem-cell transplantation. Allogeneic stem-cell transplantation was performed if a suitable donor was available and the patient was younger than 55 years of age. Patients with a favorable cytogenetic profile were also to receive the third cycle of chemotherapy with etoposide and mitoxantrone.

Table 1. Characteristics of the Patients with Acute Myeloid Leukemia (AML).*

Characteristic	Chemotherapy (N=319)	Chemotherapy plus G-CSF (N=321)
Male sex (%)	50.8	50.8
Age		
Median (yr)	44.9	44.0
<35 yr (%)	25.4	30.8
35–50 yr (%)	36.4	34.9
≥50 yr (%)	38.2	34.3
White-cell count at diagnosis		
Median ($\times10^{-3}$/mm^3)	15.9	16.6
Range ($\times10^{-3}$/mm^3)	0.4–446	0.3–368
≤20×10^3/mm^3 (%)	53.3	51.7
French–American–British classification (%)		
M0	4.4	5.3
M1	16.6	16.2
M2	32.6	26.8
M4	20.4	23.1
M5	17.2	22.1
M6	4.1	5.3
M7	0.9	0.6
Mx	3.8	0.6
WHO performance score (%)†		
0	42.0	38.9
1	43.3	48.0
2	11.0	9.7
3 or 4	1.6	2.2
Unknown	2.2	1.2
Secondary leukemia (%)	5.3	7.2
Extramedullary AML (%)	12.0	15.3
Prognostic risk category (%)‡		
Favorable	7.5	5.3
Standard	73.4	71.7
Unfavorable	19.1	23.1
Postinduction therapy (%)		
Chemotherapy	31.0	28.0
Autologous stem-cell transplantation	16.3	15.6
Allogeneic stem-cell transplantation	17.9	17.1

* There were no significant differences between the groups. Adequate cytogenetic data were obtained in 87 percent of patients. Because of rounding, percentages may not total 100. G-CSF denotes granulocyte colony-stimulating factor.

† Higher scores on the World Health Organization (WHO) scale indicate poorer performance status.

‡ Favorable risk was defined by the presence of t(8;21)(q22;22), inv16(p13q22), or t(16;16)(p13q22) and a white-cell count of less than 20×10^3 per cubic millimeter at diagnosis.[25] Unfavorable risk was defined by the presence of complex cytogenetic abnormalities (defined as at least four unrelated cytogenetic clones), monosomies, or deletions of chromosome 5 or 7 (5q–, 7q–, –5, or –7), abnormalities of the long arm of chromosome 3(q21;q26), t(6;9)(p23;q34), or abnormalities involving the long arm of chromosome 11(11q23). Leukemias that had occurred after chemotherapy or radiotherapy for a nonhematologic condition and leukemias that had occurred more than six months after a hematologic condition (secondary leukemias) were included in the unfavorable prognostic category. Patients who did not meet the criteria for favorable or unfavorable risk were classified as being at standard risk.

STATISTICAL ANALYSIS

The primary objective of the study was to determine the effect of adding G-CSF to induction chemotherapy on the rate of response, disease-free survival, relapse-free survival, and overall survival. A secondary objective was to assess the relation between the defined prognostic subgroups and the outcome. A complete response was defined by cellular marrow with less than 5 percent blasts, no Auer rods, no evidence of extramedullary leukemia, and peripheral granulocyte and platelet counts of at least 1.0×10^3 per cubic millimeter and 100×10^3 per cubic millimeter, respectively. The time to hematopoietic recovery after both cycles 1 and 2 was measured from the first day of chemotherapy. Disease-free survival was measured from the time of the first complete remission to the date of relapse or death from any cause. Relapse was defined as a recurrence of leukemia after a first complete remission. Event-free survival was measured from the date of randomization to the date of failure to enter a complete remis-

Table 2. Effect of Granulocyte Colony-Stimulating Factor (G-CSF) on the Outcome of Acute Myeloid Leukemia (AML) at Four Years.*

Outcome	No G-CSF (N=319)		G-CSF (N=321)		P Value	Relative Risk of Event (95% CI)
	No. of Events	Probability of Outcome at 4 yr	No. of Events	Probability of Outcome at 4 yr		
		%		%		
All patients						
Overall survival	207	35±3	190	40±3	0.16	0.87 (0.72–1.06)
Event-free survival	228	28±3	215	33±3	0.17	0.88 (0.73–1.06)
Complete remission	265	83±2	255	79±2	0.24	
Disease-free survival after 1st complete remission	174	33±3	149	42±3	0.02	0.77 (0.62–0.96)
Relapse after 1st complete remission	139	54±3	120	46±3	0.04	0.77 (0.61–0.99)
Death in 1st complete remission	35	13±2	29	11±2	0.29	0.77 (0.47–1.27)
	No G-CSF (N=234)		**G-CSF (N=230)**			
	No. of Events	Probability of Outcome at 4 yr	No. of Events	Probability of Outcome at 4 yr		
		%		%		
Patients with standard-risk AML						
Overall survival	155	35±3	128	45±3	0.02	0.75 (0.59–0.95)
Event-free survival	168	29±3	140	39±3	0.01	0.75 (0.60–0.93)
Complete remission	202	86±2	201	87±2		
Disease-free survival after 1st complete remission	136	33±3	111	45±3	0.006	0.70 (0.55–0.90)
Relapse after 1st complete remission	105	52±3	89	44±3	0.02	0.72 (0.54–0.96)
Death in 1st complete remission	31	14±2	22	11±2	0.11	0.64 (0.37–1.10)

* Plus–minus values are the actuarial means ±SE. Relative risks and 95 percent confidence intervals (CIs) are based on Cox regression analysis. Data on patients with a favorable risk (24 in the G-CSF group and 17 in the no–G-CSF group) and patients with an unfavorable risk (61 and 74, respectively) are not presented, but analyses of these groups showed no significant differences.

sion (set as day 1), death, or relapse, whichever came first. Overall survival was measured from the date of randomization.

Random assignments were balanced with use of a minimization procedure with the hospital as a stratification factor. We planned to enroll 600 patients over a period of five years, with an additional follow-up of two years after the enrollment of the last patient. This number of patients would give the study a power of 78 percent to show an absolute increase of 10 percent in the rate of complete remission (from 70 percent to 80 percent) with the use of G-CSF; a power of 75 percent to show an absolute increase of 10 percent in the overall survival rate (from 35 percent to 45 percent) at three years, given a relative risk of death of 0.76 and with 375 expected deaths; and a power of 81 percent to show an absolute increase of 10 percent in long-term event-free survival (from 25 percent to 35 percent) at three years, given a relative risk of 0.76 and 423 expected events, with the use of two-sided tests and a 5 percent significance level. Within 3.5 years, 655 patients had been recruited, 640 of whom could be evaluated. As of August 2002, 445 events had occurred, as defined with respect to event-free survival, and 407 patients had died.

All analyses were conducted according to the intention-to-treat principle, but 14 ineligible patients (7 in each group) were excluded, as was 1 who was lost to follow-up on day 3 and whose data could therefore not be evaluated. Reasons for ineligibility were an incorrect diagnosis (lymphoid neoplasia) in eight patients and myelodysplasia in six. Logistic regression was used to analyze the effect of G-CSF on the rate of complete remission, whereas the log-rank test and Cox regression analysis were used to analyze the differences between the two groups with respect to overall survival, event-free survival, and disease-free survival. These analyses were done before and after adjustment for age, risk category, and transplantation status during a first complete remission (as a time-dependent covariate). Competing risk analysis was used to calculate the cumulative competing risks of treatment failure among patients with a complete response (defined as relapse after a complete remission and death during a first complete remission).

The rates of hematologic recovery after cycles 1 and 2 were analyzed actuarially and compared with the use of the log-rank test. In these analyses, data on patients were censored at death or at the start of the next treatment, if hematologic recovery had not yet occurred. All P values reported are two-tailed.

Table 3. Incidence of Grade 3 or 4 Side Effects and Hematopoietic Recovery after Induction-Therapy Cycles 1 and 2.*

Variable	Cycle 1		Cycle 2	
	No G-CSF	G-CSF	No G-CSF	G-CSF
Grade 3 or 4 side effects (%)	43	47	38	41
Grade 3 or 4 infection (%)	35	38	39	41
Hematopoietic recovery				
$>1.0\times10^3$ White cells/mm³ (median no. of days)	26	26	23	24
$>0.5\times10^3$ Granulocytes/mm³ (median no. of days)	30	30	25	26
$>50\times10^3$ Platelets/mm³ (median no. of days)	27	27	28	30
$>100\times10^3$ Platelets/mm³ and $>1.0\times10^3$ granulocytes/mm³ (median no. of days)	35	34	37	37
Recovery by day 56 (% of patients)†	88	91	79	84

* The criteria of the World Health Organization were used to categorize adverse effects. The percentages of patients with any grade 3 or 4 side effect or infection are given. Side effects do not include hair loss. Infections do not include fever of unknown origin. The time to hematopoietic recovery was measured from the start of chemotherapy. G-CSF denotes granulocyte colony-stimulating factor.

† Recovery was defined by the presence of both a granulocyte count of more than 1.0×10^3 per cubic millimeter and a platelet count of more than 100×10^3 per cubic millimeter.

RESULTS

CHARACTERISTICS OF THE PATIENTS AND ADHERENCE TO G-CSF TREATMENT

Between March 1995 and January 1999, 319 patients were assigned to induction chemotherapy without G-CSF, and 321 patients were assigned to chemotherapy combined with G-CSF. As of the time of the data analysis, the median follow-up was 55 months, and 90 percent of the patients had been followed for more than 40 months. Thirteen patients were lost to follow-up or were last seen more than one year before the analysis. Of these 13 patients, 7 had been followed for more than three years.

The two treatment groups were evenly matched with respect to various factors, including assignment to postinduction therapy (Table 1). As for the prognostic risk groups, most patients were in the standard-risk category, and approximately 20 percent were in the unfavorable-risk category (Table 1). Only about 7 percent of all the patients presented with prognostically favorable AML.

In cycle 1, G-CSF was not given to 16 of the 321 patients who were assigned to receive G-CSF; treatment with G-CSF was delayed (median period, four days) in 120 of the patients and interrupted (median period, two days) in 30 patients. The primary reason for these deviations was leukocytosis (in 75 percent of cases), as prespecified in the protocol. Other reasons, based on decisions by local physicians, were usually related to medical problems (e.g., infections, hemorrhage, liver-function abnormalities, and urticaria). Of the 279 patients in the G-CSF group who proceeded to cycle 2, G-CSF was not given to 23 patients because of leukocytosis, persistent leukemia, or deviations from the protocol, including 1 patient because of chemotherapy-associated toxicity during cycle 1. In 19 patients treatment with G-CSF was postponed (median period, one day) because of leukocytosis or deviations from the protocol.

RESPONSE AND ADVERSE EFFECTS

The rates of complete remission were 83 percent in the group that did not receive G-CSF and 79 percent in the group that received G-CSF (P=0.24) (Table 2). In both groups, 73 percent of the complete remissions occurred after cycle 1. The rates of complete remission in the two groups did not differ significantly according to age or risk group. The frequencies of various grade 3 (severe) or grade 4 (very severe) adverse effects (according to World Health Organization criteria) after cycles 1 and 2 and the times to hematopoietic recovery after cycles 1 and 2 were similar in the two groups (Table 3). There were more deaths within 50 days after cycles 1 and 2 among patients who received G-CSF than among patients who did not receive G-CSF (Table 4).

RELAPSE AND SURVIVAL

Among patients who had a complete remission, the disease-free survival rate at four years was higher in the G-CSF group than in the group that did not receive G-CSF (42 percent vs. 33 percent; relative risk of relapse or death, 0.77; 95 percent confidence interval, 0.62 to 0.96; P=0.02) (Table 2 and Fig. 2). This difference was related to a lower relapse rate in the G-CSF group (46 percent vs. 54 percent; relative risk, 0.77; 95 percent confidence interval, 0.61 to 0.99; P=0.04) (Table 2). At four years there were no statistically significant differences between the two groups in the rates of overall and event-free survival (Table 2 and Fig. 2). The unadjusted Cox regression analysis and an analysis adjusted for age, risk category, and presence or absence of subsequent stem-cell transplantation during a first complete remission (time-dependent covariates) yielded similar estimates for the hazard rates associated with treatment results and P values (data not shown).

Table 4. Mortality Rates Associated with Induction Chemotherapy with Granulocyte Colony-Stimulating Factor (G-CSF) and without G-CSF.*

Outcome	No. of Deaths	Death from Treatment Resistance†	Death from Infection	Death from Other Causes‡
		no. of patients		
After cycle 1				
Early death (day 0–8)§				
No G-CSF	7¶	NA	NA	NA
G-CSF	11¶	NA	NA	NA
Day 9–50				
No G-CSF	11	4	3	4
G-CSF	19	2	10	7
After cycle 2				
Day 0–50				
No G-CSF	16	9	7	0
G-CSF	25	13	8	4

* Numbers and causes of deaths were calculated during induction chemotherapy cycles 1 and 2 and within 50 days afterward. Overall, there were 34 deaths during induction in the group that did not receive G-CSF and 55 in the group that received G-CSF (P=0.02).

† Death was classified as due to treatment resistance only if there was pathological documentation of persistent leukemia.

‡ Death from other causes includes cardiac causes (two in the G-CSF group), hepatic causes (one in the G-CSF group), hemorrhage (two in the no–G-CSF group and six in the G-CSF group), pulmonary causes (one in the no–G-CSF group), and other causes (one in the no–G-CSF group and two in the G-CSF group).

§ Leukemia-related deaths were not distinguished from treatment-related causes of death during the first eight days of treatment. NA denotes not assessed.

¶ Three patients in each group had excessively high white-cell counts at diagnosis ($>170\times10^3$ per cubic millimeter; range, 182×10^3 to 344×10^3 per cubic millimeter).

OUTCOME AMONG DISTINCT PROGNOSTIC SUBGROUPS

Among patients with standard-risk AML (72 percent of all patients), treatment with G-CSF reduced the probability of relapse and improved overall, event-free, and disease-free survival (Fig. 3A and 3B and Table 2). G-CSF did not, however, significantly affect overall, event-free, or disease-free survival among the 135 patients with unfavorable-risk AML (Fig. 3C and 3D and Table 2). In the 41 patients with favorable-risk AML, G-CSF priming had no effect.

DISCUSSION

Incubation of AML cells with G-CSF, GM-CSF, or interleukin-3 and the cell-cycle–dependent agent cytarabine increases intracellular levels of the active metabolite cytosine arabinoside triphosphate, incorporation of cytarabine into cellular DNA,[5,7] and the killing of leukemic blasts and leukemic progenitor cells by the drug.[2-11] We evaluated the clinical efficacy of growth-factor priming in patients with previously untreated AML. G-CSF was given beginning one day before the start of chemotherapy of cycles 1 and 2 and continued through the last day of induction cycles 1 and 2. Among patients who received G-CSF and who had a complete remission, the relapse rate was lower than that among patients in complete remission who did not receive G-CSF. Moreover, in the group of patients who had a complete remission, the disease-free survival rate at four years was 42 percent in the G-CSF group and 33 percent in the group that did not receive G-CSF (P=0.02). The difference in overall and event-free survival did not reach statistical significance. The significant difference in disease-free survival probably resulted from G-CSF–mediated activation of subpopulations of leukemic cells that were initially insensitive to cytosine arabinoside. Elimination of the primed cells may have reduced the frequency of relapse.

The ability of colony-stimulating factors to activate AML cells has been directly demonstrated in vivo: injection of G-CSF or GM-CSF 18 to 72 hours before the beginning of chemotherapy drives AML cells into the cell cycle.[26,27] This effect is consistent with the notion that G-CSF receptors, because of their high binding affinity, require minimal levels of ligands for activation.[28]

From our data, we cannot determine whether the sensitization effect was mediated by increasing the efficacy of cytarabine, idarubicin and amsacrin, or the combined chemotherapeutic agents we used. In any case, the efficacy of a chemotherapy regimen that included cytarabine at doses of 200 mg per square meter as well as 1000 mg per square meter was enhanced by the addition of G-CSF priming. Studies of the dose effect of cytarabine have shown that doses of 3 g per square meter[29,30] were more effective than doses of 200 or 400 mg per square meter in preventing relapse but did not result in an increased rate of remission. Similarly, in this study, only the duration of remission, not the number of remissions, changed as the result of G-CSF sensitization.

We used a dose of 1 g of cytarabine per square meter in cycle 2. The comparative effect of a dose of 1 g per square meter and a dose of 3 g per square meter has not been established in AML therapy. It would be of interest to know whether G-CSF priming would have a similarly positive effect on the probability of relapse in regimens containing a dose of 3 g of cytarabine per square meter.

The fact that our results do not suggest a benefit of G-CSF priming in patients with favorable-risk AML might relate to the small numbers of cases, or it might indicate that the dose of 1 g of cytarabine per square meter was optimal in terms of its ability to kill neoplastic cells in this subgroup. After the two induction cycles with or without G-CSF, approximately one third of patients received a third cycle of chemotherapy and another third went on to high-dose chemotherapy followed by stem-cell transplantation. It is unlikely that the postinduction treatment influenced the outcome of G-CSF treatment. The two groups were evenly matched in terms of assignment to postinduction therapy. Besides, Cox regression analysis with autologous and allogeneic transplantation during a first remission as time-dependent covariates yielded results similar to those of the unadjusted analysis.

The fact that more deaths occurred during induction among patients who received G-CSF may explain the slightly reduced rate of complete remission in this group. These deaths had several causes and were thus not due to a common problem. However, because of a reduction in the incidence of later deaths, the overall death rate among patients in the G-CSF group was lower than that among those treated with chemotherapy alone.

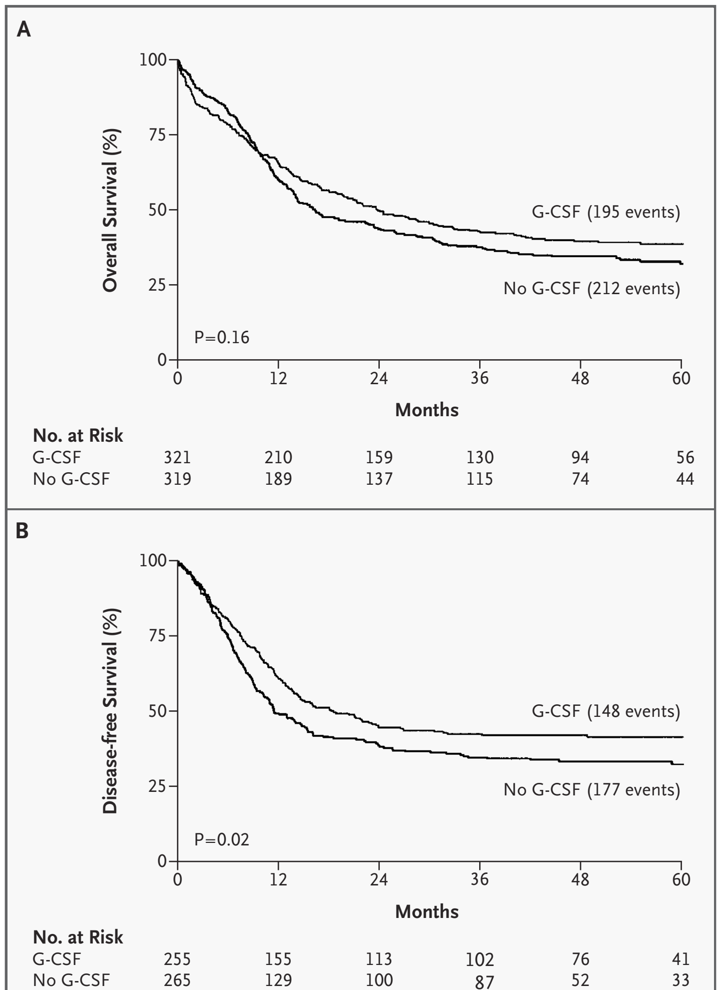

Figure 2. Cumulative Rate of Overall Survival (Panel A) and Disease-free Survival (Panel B), According to the Assigned Treatment.

P values were calculated with use of the log-rank test. G-CSF denotes granulocyte colony-stimulating factor.

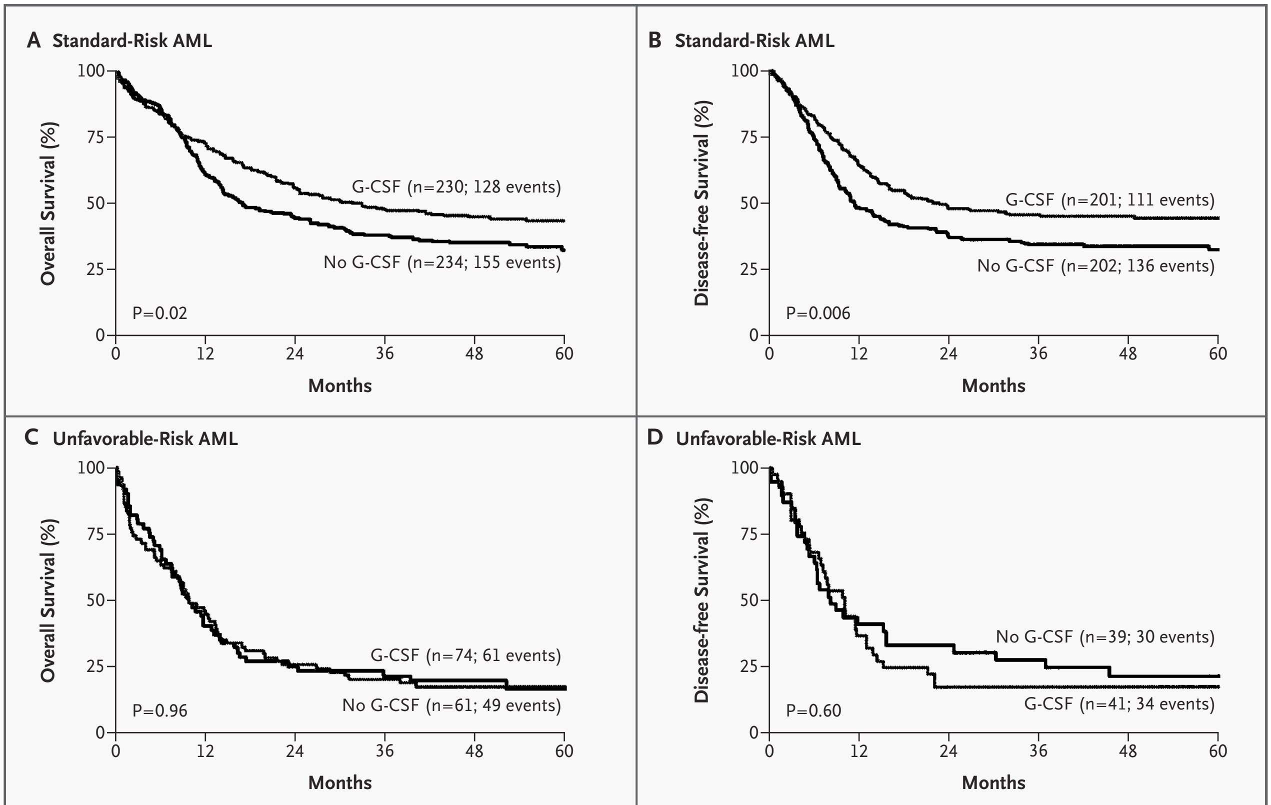

Figure 3. Cumulative Rates of Overall Survival (Panels A and C) and Disease-free Survival (Panels B and D) among Patients with Standard-Risk Acute Myeloid Leukemia (AML) and Unfavorable-Risk AML.

P values were calculated with use of the log-rank test. G-CSF denote granulocyte colony-stimulating factor.

Previous studies of G-CSF and GM-CSF in AML have been almost entirely confined to the ability of these growth factors to accelerate hematopoietic recovery and reduce morbidity and mortality due to infection after chemotherapy. The efficacy of these agents in modulating chemotherapy has, however, been evaluated in controlled[18-21] and uncontrolled[15-17] studies involving limited numbers of patients. In two relatively large, randomized studies, GM-CSF was administered concomitantly with and after chemotherapy.[31,32] These studies involved older patients, most of whom had AML with an unfavorable prognosis. One of these studies reported a higher rate of disease-free survival among the patients who received GM-CSF than among those who did not receive GM-CSF,[32] but it was not possible to distinguish the effect of priming from the effect of enhanced hematopoietic recovery. By contrast, our study selectively focused on the effect of growth-factor priming in AML and was conducted in young and middle-aged adults with previously untreated leukemia.

We found that G-CSF improved overall and disease-free survival in the group with standard-risk AML. There were too few patients in the group with a favorable prognosis to allow a meaningful analysis. There was no indication that G-CSF priming improved the outcome among patients with chemotherapy-refractory, unfavorable-risk AML. This lack of benefit explains why overall survival was not significantly better in the G-CSF group as a whole.

Additional studies of G-CSF priming in specific subgroups of patients and regimens of combination therapy seem warranted. The results of our study provide proof of the principle that chemotherapy and sensitization of leukemia cells by hematopoietic growth factors is a plausible strategy for reducing the risk of relapse in patients with AML.

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APPENDIX

The following centers and persons participated in the study: *Study coordinators* — B. Löwenberg, M.A. Boogaerts, and J. Gmür; *Statistician* — W. van Putten (Dutch–Belgian Hemato-Oncology [HOVON] Cooperative Group Data Center, Rotterdam, the Netherlands); *Cytogenetic review* — A. Hagemeijer (Leuven, Belgium), S.L. Bhola (Leiden, the Netherlands), and M. Jotterand-Bellomo (Lausanne, Switzerland); *Cytology review committee* — M.B. van 't Veer (Rotterdam, the Netherlands), J. Fehr (Zurich, Switzerland); *Central data management* — A.J.M. Meurisse, M. van Os (HOVON Cooperative Group Data Center) and B. Rufener (Swiss Group for Clinical Cancer Research Trial Office Center, Bern, Switzerland); *Participating centers (and investigators)* — **the Netherlands:** Free University Medical Center, Amsterdam (P.C. Huijgens, G.J. Ossenkoppele); University Hospital, Groningen (S.M.J.G. Daenen, E. Vellenga); University Hospital, Utrecht (A.W. Dekker, L.F. Verdonck); Erasmus Medical Center and Daniel den Hoed Cancer Center, Rotterdam (B. Löwenberg, P. Sonneveld, G.E. de Greef); University Hospital, Maastricht (H.C. Schouten); University Hospital, Amsterdam (J. van der Lelie); Leijenburg Hospital, The Hague (P.W. Wijermans); Hospital Eemland, Amersfoort (S. Wittebol); Medical Center Twente, Enschede (M.R. Schaafsma); Sophia Hospital, Zwolle (M. van Marwijk Kooy); Antonius Hospital, Nieuwegein (D.H. Biesma); Antoni van Leeuwenhoekhuis, Amsterdam (J.W. Baars); Diaconessen Hospital, Meppel (H. de Korte); Hospital Reinier de Graaf, Delft (E. Maartense); Medical Center, Leeuwarden (P. Joosten); Catharina Hospital, Eindhoven (W.G. Peters); and Hospital Gooi Noord, Blaricum (H.P. Muller); **Belgium:** Hospital Gasthuisberg, Leuven (M.A. Boogaerts, G. Verhoef); Cliniques Universitaires Saint-Luc, Brussels (A. Ferrant); Cliniques Universitaires de Mont-Godinne, Yvoir (A. Bosly); and Hôpital de Jolimont, Haine-St. Paul (A. Delannoy); **Germany:** Johannes Gutenberg-University Hospital, Mainz (M. Theobald, J. Beck); and Nordwest Hospital, Frankfurt am Main (A. Knuth); **Switzerland:** University Hospital, Zurich (J. Gmür, E. Jacky); University Hospital, Bern (M.F. Fey, A. Tobler); University Hospital, Lausanne (T. Kovacsovics); University Hospital, Basel (A. Gratwohl, A. Tichelli); Kantonsspital, St. Gallen (U. Hess); Hôpital Cantonal Universitaire, Geneva (B. Chapuis); University Hospital, Neuchâtel (D. Piguet); Hospital St. Giovanni, Bellinzona (L. Leoncini); Kantonsspital, Winterthur (T. Kroner); and Kantonsspital, Arau (M. Wernli).

REFERENCES

1. Löwenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med 1999; 341:1051-62. [Erratum, N Engl J Med 1999; 341:1484.]

2. Bhalla K, Birkhofer M, Arlin Z, Grant S, Lutzky J, Graham G. Effect of recombinant GM-CSF on the metabolism of cytosine arabinoside in normal and leukemic human bone marrow cells. Leukemia 1988;2:810-3.

3. Lista P, Porcu P, Avanzi GC, Pegoraro L. Interleukin 3 enhances the cytotoxic activity of 1-β-D-arabinofuranosylcytosine (ara-C) on acute myeloblastic leukaemia (AML) cells. Br J Haematol 1988;70:121-3.

4. Miyauchi J, Kelleher CA, Wang C, Minkin S, McCulloch EA. Growth factors influence the sensitivity of leukemic stem cells to cytosine arabinoside in culture. Blood 1989;73:1272-8.

5. Tanaka M. Recombinant GM-CSF modulates the metabolism of cytosine arabinoside in leukemic cells in bone marrow. Leuk Res 1993;17:585-92.

6. Cannistra SA, Groshek P, Griffin JD. Granulocyte-macrophage colony-stimulating factor enhances the cytotoxic effects of cytosine arabinoside in acute myeloblastic leukemia and in the myeloid blast crisis phase of chronic myeloid leukemia. Leukemia 1989;3:328-34.

7. Bhalla K, Holladay C, Arlin Z, Grant S, Ibrado AM, Jasiok M. Treatment with interleukin-3 plus granulocyte-macrophage colony-stimulating factors improves the selectivity of Ara-C in vitro against acute myeloid leukemia blasts. Blood 1991;78:2674-9.

8. Te Boekhorst PA, Löwenberg B, Sonneveld P. Hematopoietic growth factor stimulation and cytarabine cytotoxicity in vitro: effects in untreated and relapsed or primary refractory acute myeloid leukemia cells. Leukemia 1994;8:1480-6.

9. Te Boekhorst PA, Löwenberg B, Vlastuin M, Sonneveld P. Enhanced chemosensitivity of clonogenic blasts from patients with acute myeloid leukemia by G-CSF, IL-3 or GM-CSF stimulation. Leukemia 1993;7: 1191-8.

10. Inatomi Y, Toyama K, Clark SC, Shimizu K, Miyauchi J. Combinations of stem cell factor with other hematopoietic growth factors enhance growth and sensitivity to cytosine arabinoside of blast progenitors in acute myelogenous leukemia. Cancer Res 1994;54:455-62.

11. Reuter C, Auf der Landwehr U, Schleyer U, et al. Modulation of intracellular metabolism in cytosine arabinoside in acute myeloid leukemia by granulocyte-macrophage colony-stimulating factor. Leukemia 1994; 8:217-25.

12. Terpstra WE, Löwenberg B. Application of myeloid growth factors in the treatment of acute myeloid leukemia. Leukemia 1997; 11:315-27.

13. Schiffer CA. Hematopoietic growth factors as adjuncts to the treatment of acute myeloid leukemia. Blood 1996;88:3675-85.

14. Ohno R. Granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor in the treatment of acute myeloid leukemia and acute lymphoblastic leukemia. Leuk Res 1998;22:1143-54.

15. Rossi HA, O'Donnell J, Sarcinelli F, Stewart FM, Quesenberry PJ, Becker PS. Granulocyte-macrophage colony-stimulating factor (GM-CSF) priming with successive concomitant low-dose Ara-C for elderly patients with secondary/refractory acute myeloid leukemia or advanced myelodysplastic syndrome. Leukemia 2002;16:310-5.

16. Frenette PS, Desforges JF, Schenkein DP, Rabson A, Slapack CA, Miller KB. Granulocyte-macrophage colony stimulating factor (GM-CSF) priming in the treatment of elderly patients with acute myelogenous leukemia. Am J Hematol 1995;49:48-55.

17. Estey E, Thall PF, Kantarjian H, et al. Treatment of newly diagnosed acute myelogenous leukemia with granulocyte-macrophage colony-stimulating factor (GM-CSF) before and during continuous-infusion high-dose ara-C + daunorubicin: comparison to patients treated without GM-CSF. Blood 1992;79:2246-55.

18. Ohno R, Naoe T, Kanamaru A, et al. A double-blind controlled study of granulocyte colony-stimulating factor started two days before induction chemotherapy in refractory acute myeloid leukemia: Kohseisho Leukemia Study Group. Blood 1994;83: 2086-92.

19. Zittoun R, Suciu S, Mandelli F, et al. Granulocyte-macrophage colony-stimulating factor associated with induction treatment of acute myelogenous leukemia: a randomized trial by the European Organization for Research and Treatment of Cancer Leukemia Cooperative Group. J Clin Oncol 1996;14:2150-9.

20. Löwenberg B, Boogaerts MA, Daenen SM, et al. Value of different modalities of granulocyte-macrophage colony-stimulating factor applied during or after induction therapy of acute myeloid leukemia. J Clin Oncol 1997;15:3496-506.

21. Estey EH, Thall PF, Pierce S, et al. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin +/– all-trans retinoic acid +/– granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. Blood 1999;93:2478-84.

22. Mitelman F, ed. ICSN 1995: an international system for human cytogenetic nomenclature (1995). Basel, Switzerland: Karger, 1995.

23. Mrozek K, Heinonen K, de la Chapelle A, Bloomfield CD. Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol 1997;24:17-31.

24. Wheatley K, Burnett AK, Goldstone AH, et al. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial: United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Br J Haematol 1999;107:69-79.

25. Löwenberg B. Prognostic factors in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001;14:65-75.

26. Cannistra SA, DiCarlo J, Groshek P, et al. Simultaneous administration of granulocyte-macrophage colony-stimulating factor and cytosine arabinoside for the treatment of relapsed acute myeloid leukemia. Leukemia 1991;5:230-8.

27. Baer MR, Bernstein SH, Brunetto VL, et al. Biological effects of recombinant human granulocyte colony-stimulating factor in patients with untreated acute myeloid leukemia. Blood 1996;87:1484-94.

28. Löwenberg B, Touw IP. Hematopoietic growth factors and their receptors in acute leukemia. Blood 1993;81:281-92.

29. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia: Cancer and Leukemia Group B. N Engl J Med 1994;331:896-903.

30. Bishop JF, Matthews JP, Young GA, et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. Blood 1996;87:1710-7.

31. Löwenberg B, Suciu S, Archimbaud E, et al. Use of recombinant GM-CSF during and after remission induction chemotherapy in patients aged 61 years and older with acute myeloid leukemia: final report of AML-11, a phase III randomized study of the Leukemia Cooperative Group of European Organisation for the Research and Treatment of Cancer and the Dutch Belgian Hemato-Oncology Cooperative Group. Blood 1997;90:2952-61.

32. Witz F, Sadoun A, Perrin MC, et al. A placebo-controlled study of recombinant human granulocyte-macrophage colony-stimulating factor administered during and after induction treatment for de novo acute myelogenous leukemia in elderly patients. Blood 1998;91:2722-30.