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

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Purpose: To assess the value of granulocyte-macrophage colony-stimulating factor (GM-CSF) for induction treatment of acute myeloid leukemia (AML), both for priming of leukemic cells and for acceleration of hematopoietic recovery.

Patients and Methods: GM-CSF was administered 5 μg/kg/d by continuous intravenous (IV) infusion during induction therapy with daunorubicin (DNR) (days 1 to 3) and cytarabine (ARA-C) (days 1 to 7). A total of 102 patients were randomized onto four arms, as follows: (1) GM-CSF 24 hours before and during chemotherapy (arm +/−); (2) GM-CSF after chemotherapy until day 28 or recovery of polymorphonuclear leukocytes (PMNs) (arm −/+); (3) GM-CSF before, during, and after chemotherapy (arm +/+); or (4) no GM-CSF (arm −/−). Stopping rules were applied in case of an initial WBC count greater than 30×10^9/L or a secondary increase of circulating blast cells. Analyses were performed according to the intention-to-treat principle.

Results: The complete remission (CR) rates were 77% (arm −/−), 72% (arm +/−), 48% (arm −/+), and 46% (arm +/+). Patients randomized to receive GM-CSF after induction (arms −/+ and +/+) had a significantly lower CR rate ($P = .008$) and a trend toward accelerated recovery of neutrophils, but no fewer infections or induction deaths. The lower CR rate appeared to be related to an increased resistance rate, with persistent leukemia. The main side effects of GM-CSF were fluid retention and hypotension.

Conclusion: GM-CSF administered during induction treatment of AML with a DNR/Ara-C combination did not provide any clinical benefit. Furthermore, there was a significant decrease in the CR rate with more persistent leukemia when GM-CSF was administered during the hypoplastic phase after the chemotherapy courses.

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HEMATOPOIETIC GROWTH factors (HGFs), especially granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are currently used in cancer chemotherapy to accelerate the recovery of hematopoiesis, although their real impact in terms of cost-effectiveness is still debated. They are also increasingly used for mobilization of peripheral-blood progenitor cells in view of autologous transplantation.

In acute myeloid leukemia (AML), leukemic blast cells express receptors for HGFs, with interindividual variability in number, type, and affinity of receptors.[1] Treatment of AML with HGFs theoretically has two types of potential therapeutic effects as follows: (1) they can recruit leukemic cells into cycle and thus enhance their sensitivity to chemotherapy[2,3]; therefore, their administration before and during chemotherapy could lead to increased leukemic cell kill. (2) when administered after cytotoxic courses, they can stimulate normal hematopoietic progenitors and thus accelerate hematopoietic recovery and reduce the morbidity and eventual mortality from infection.

The presence of receptors for HGFs in both normal and leukemic cells could challenge these therapeutic effects by two possible adverse consequences: (1) when given before and during chemotherapy, recruitment of normal pluripotent stem cells in cycle may increase the fraction exposed to cycle-dependent cytotoxic drugs and result in more prolonged marrow aplasia; (2) when administered after chemotherapy courses, stimulation of residual leukemic clones may occur, with a risk of resistance to induction treatment or early relapse. Whether given during or after induction courses, HGFs might prevent chemotherapy-induced apoptosis of leukemic cells.[4,5]

Several pilot studies that combined GM-CSF during induction treatment of AML have indicated that it could result in an higher complete remission (CR) rate when compared with historical controls.[6-9] These pilot studies were followed by prospective randomized trials. In two consecutive studies, Ohno et al[10,11] have shown that G-CSF accelerates the recovery of neutrophils, with a trend

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for less documented infections, while the CR rate was not significantly increased.

Results of other cooperative trials that used GM-CSF or G-CSF have been recently published.[12-16] Unfortunately, the designs vary among studies, which makes comparisons difficult. In addition, no attempts were made to assess separately the two possible biologic effects: the priming effect by administration before and during chemotherapy courses, and the acceleration of normal hematopoietic recovery by postchemotherapy administration. The European Organization for Research and Treatment of Cancer (EORTC) Leukemia Cooperative Group has decided to study prospectively the value of these two types of administration of GM-CSF during the induction phase in previously untreated AML patients.

PATIENTS AND METHODS

The present trial (GM-CSF amendment) was designed as an extension of a large randomized study, AML 8, of the EORTC and Gruppo Italiano Malattie Ematologiche Maligne dell' Adulto (GIMEMA) Leukemia Cooperative Groups. In this study, newly diagnosed patients aged 15 to 45 years were eligible for AML 8A, which compared the following three post-CR strategies: allogeneic bone marrow transplantation (BMT), autologous BMT, and short intensive chemotherapy consolidation. The results of the AML 8A study, not including the amendment with GM-CSF, have been recently published.[17] Patients aged 46 to 60 years, in the meantime, were entered onto the AML 8B protocol, which compared the same intensive chemotherapy consolidation and standard postremission regimens of the EORTC and GIMEMA groups. The induction course used in these two trials consisted of a combination of daunorubicin (DNR) and cytarabine (ARA-C), and was repeated once if a partial remission (PR) was reached by day 28. Response to induction treatment was classified as CR if there were ≤ 5% bone marrow blast cells with a normal blood count, PR if there were near normal blood counts and 6% to 25% bone marrow blasts, early death if death occurred before completion of the first induction course, death during hypoplasia, and resistant disease if there was persistent disease in surviving patients.

Patients

In the present GM-CSF amendment protocol, patients were treated exactly as in the main study AML 8A or 8B, except for the randomization of GM-CSF during the induction phase. Only a limited number of pilot centers were allowed to participate in this amendment, by enrolling all of their patients consecutively hospitalized for AML, whereas the other centers continued to treat patients according to the main protocols. Selection criteria were almost the same as in the main studies. All patients aged 15 to 60 years with previously untreated AML were eligible on the basis of morphology criteria according to the French-American-British (FAB) classification.[18] Patients with blast crisis of chronic myeloid leukemia, AML that supervened after other myeloproliferative diseases, or after a myelodysplastic syndrome of more than 6 months' duration were excluded, as were patients with a severe concomitant disease or coexistent and progressive malignancy. However, contrary to the main study, patients with a poor performance status (World Health Organization [WHO] score > 2) or serum creatinine concentration greater than 1.5 times the upper limit of normal were excluded from the amendment. Informed consent was required before randomization, according to local rules. All smears were centrally reviewed for eligibility at diagnosis and for response.

Treatment Protocol

The design of the treatment protocol is shown in Fig 1. Patients received induction chemotherapy treatment that combined DNR 45 mg/m^2/d by intravenous (IV) push on days 1 to 3 and Ara-C 200 mg/m^2/d by continuous IV infusion on days 1 to 7 and were randomized to one of the four following arms: (1) control arm without GM-CSF (−/−); (2) GM-CSF starting 24 hours before induction chemotherapy and continuing until completion of the chemotherapy course on day 7 (+/−); (3) GM-CSF started immediately after completion of the chemotherapy induction course on day 8 and continuing until day 28, or for a shorter time in case of earlier neutrophil recovery with a polymorphonuclear leukocyte (PMN) count ≥ 0.5 × 10^9/L (−/+); or (4) GM-CSF started 24 hours before the chemotherapy induction course and continuing until day 28 or a PMN count ≥ 0.5 × 10^9/L (+/+). Patients who did not achieve a CR after one course, but met criteria that corresponded to a PR on day 28, received a second identical induction course, with GM-CSF administered or not according to randomization arm. GM-CSF was provided by Sandoz/Schering Plough (Basel, Switzerland) as recombinant human *Escherichia coli*–derived sterile lyophilized powder. It was reconstituted with sterile water, diluted with 0.9% sodium chloride to a maximum 50 mL total volume, and administered by continuous IV infusion through a central vein catheter at a daily dose of 5 μg/kg. According to the protocol, administration of GM-CSF was delayed in patients randomized to arms 2 and 4 in case of an initial blast count greater than 30 × 10^9/L, and was subsequently

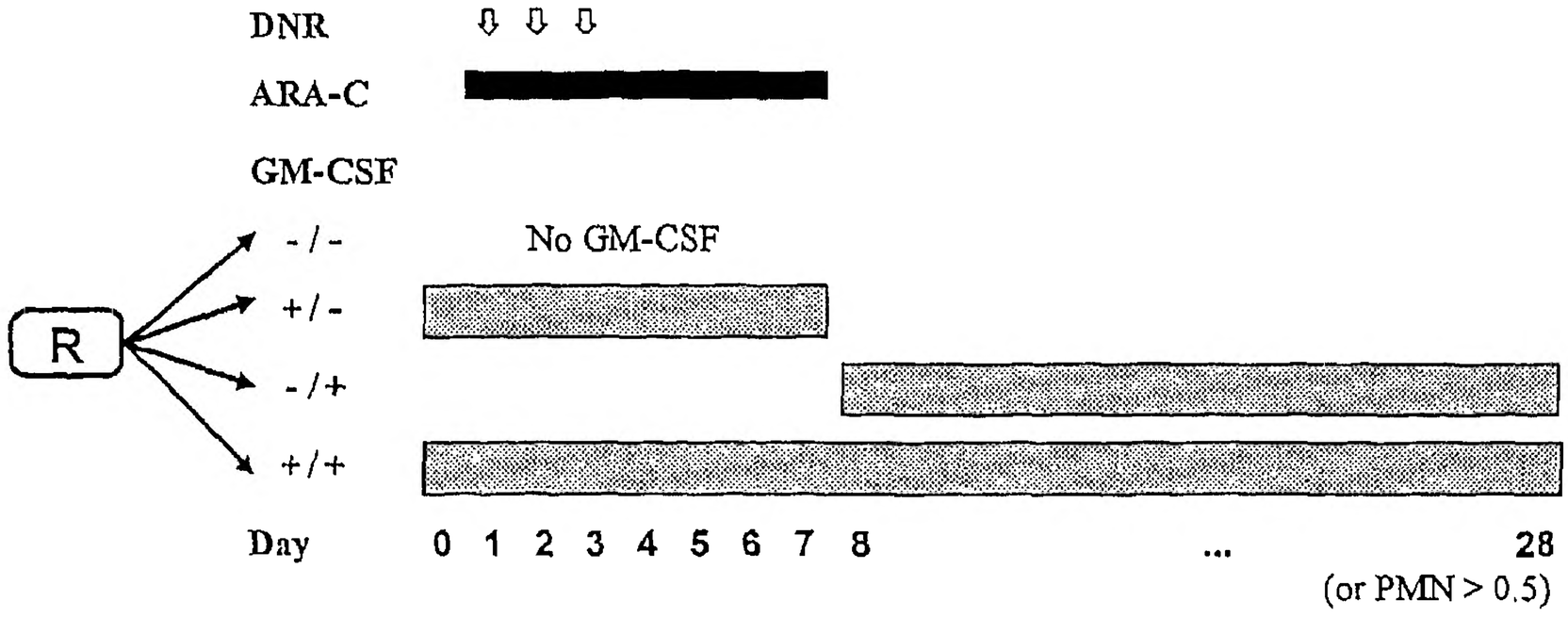

Fig 1. Protocol design. DNR 45 mg/m^2/d on days 1 to 3, Ara-C 200 mg/m^2/d by continous IV infusion on days 1 to 7. GM-CSF 5 μg/kg/d by continous IV infusion. GM-CSF delayed if circulating blast count > 30 × 10^9/L, started when < 20; Interrupted if, after chemotherapy, blasts are no longer seen and subsequently are > 1 × 10^9/L or persist and increase twofold.

started, after initiation of chemotherapy, when the circulating blast count decreased to less than 20×10^9/L. GM-CSF was also discontinued during chemotherapy if the blast count increased to 50×10^9/L, and was reintroduced when the count decreased to less than 20×10^9/L. In addition, after the end of the chemotherapy course, GM-CSF was stopped if the circulating blast cells were not or no longer detectable and subsequently reappeared to values $\geq 1 \times 10^9$/L, or if they persisted and then increased by at least twofold. Furthermore, GM-CSF was stopped at any time in case of severe side effects, or allergic or anaphylactoid reactions. To avoid fluid retention, patients received furosemide from the beginning, plus IV infusion of albumin in case of marked hypoalbuminemia. Body weight was assessed daily; GM-CSF was interrupted in case of weight increase by more than 5 kg, and reinitiated only if the body weight decreased by at least 3 kg.

No GM-CSF was administered during salvage treatment (combining mainly intermediate-dose Ara-C and idarubicin or amsacrine) in patients resistant to the induction therapy or during post-CR treatments.

Statistical Methods

Randomization was centrally performed at the EORTC Data Center in Brussels using the minimization technique, with age and treatment center being used as stratification factors.

The 2×2 factorial design allowed evaluation of two experimental groups: ·/+, GM-CSF administered after induction chemotherapy, whatever the treatment applied during induction (ie, with or without GM-CSF); and +/·, GM-CSF administered before and during induction chemotherapy, whatever the treatment applied after induction (ie, with or without GM-CSF). These two groups were compared with the two corresponding control treatment groups through an a posteriori stratification: ·/+ to ·/−, GM-CSF or not after induction chemotherapy, whatever the treatment applied during induction (ie, with or without GM-CSF); and +/· to −/·, GM-CSF or not during induction chemotherapy, whatever the treatment applied after induction (ie, with or without GM-CSF), respectively.

In the treatment evaluation, the following end points were used: response to the first induction course, response to one or two induction courses, overall response to induction or salvage treatment, event-free survival (EFS) and overall survival. EFS was defined as the time from evaluation of induction until relapse or death in CR; patients who did not reach CR after induction were considered as treatment failures at time 0. Duration of survival was calculated from the date of randomization until death, whatever the cause of death.

In all analyses, the intention-to-treat principle was used, ie, all patients were kept in the treatment arm allocated by randomization. All patients have been monitored in the same way, irrespective of treatment arm.

The relationship between treatment group and response to chemotherapy course (categorized as CR and no CR) was tested for statistical significance using the χ^2 test with continuity correction.[19] Each of the two comparisons (GM-CSF during induction, no *v* yes; and GM-CSF after induction, no *v* yes) has been adjusted mutually by each other. For a 2×2 contingency table (treatment *v* response), the odds ratio (OR) and its 95% confidence interval (CI) was calculated using the Confidence Interval Analysis program.[20] If the lower limit of the 95% CI is greater than 1, then the true OR is (with 95% chances) greater than 1, ie, the experimental group has a significant adverse impact on the response rate.

The Kaplan-Meier[19] method was used to construct EFS and survival curves. Comparison between the groups for treatment outcome was tested for statistical significance using the two-sided log-rank test. The relative risk of death in the experimental versus the control group was estimated via the OR method, along with its 95% CI.[20] For multivariate analyses with binary outcome, the linear logistic model[19] was used, and for EFS analysis, Cox's proportional hazards model[19] was used.

The initial aim of the study was to randomize 600 patients in order to detect an improvement from 65% to 75% in terms of CR rate (OR = 0.63, alpha = 0.05, beta = 0.15). Due to the risk of yielding worse results in the GM-CSF–containing groups, particularly in the ·/+ group, close monitoring of the trial was performed. After 103 patients had been recruited onto the trial, and the first 93 were evaluated for response, it appeared that the estimated OR for the comparison ·/+ versus ·/− was significantly ($P = .01$) greater than 1, and that its corresponding 99.9% CI did not contain the initial targeted value (ie, 0.63). It was therefore decided to stop the trial prematurely.

RESULTS

Characteristics of Treatment Arms and Groups

A total of 103 patients from eight centers were included from December 1990 to November 1992. All patients were eligible for entry onto the study. One patient randomized to the +/+ arm, who had severe complications from the central venous access device that allowed only palliative chemotherapy without GM-CSF, was not assessable for response. The main characteristics of the remaining 102 eligible and assessable patients are listed in Table 1. The control group is characterized by slightly fewer unfavorable prognostic features, such as WBC count greater than 100×10^9/L and fever at diagnosis, and more patients with Auer rods present in blast cells. Cytogenetics were performed in 58 patients and showed an even distribution in the four treatment arms when grouped into good, intermediate, and poor prognostic categories according to Keating's classification.[21]

Four patients randomized to the +/−, −/+, and +/+ arms did not receive GM-CSF because of leukostasis, vasculitis and lung infiltration, skin vasculitis, and leukemic pleuritis (Table 2). All four patients received the chemotherapy according to the AML 8 protocol and were kept for analysis in their treatment arm allocated by randomization. Table 2 also lists the number of patients in whom the start of GM-CSF was, according to the protocol, delayed or prematurely stopped for increased blast cells or toxicity. Due to an initial high WBC count, the start of GM-CSF was postponed in 20 patients. In five patients who received GM-CSF before induction chemotherapy (arms +/− and +/+), an increased WBC count by twofold to 15-fold was observed on day 1 or 2. This increase during GM-CSF did not seem to correlate with response (three in CR and two with resistance). In addition, GM-CSF was temporarily interrupted in eight patients and prematurely stopped in 43. The reasons for premature stopping of GM-CSF are also listed in Table

Table 1. Patient Characteristics at Diagnosis by Treatment Arm

Characteristic	Treatment Arm			
	−/− (n = 26)	+/− (n = 25)	−/+ (n = 27)	+/+ (n = 24)
Age, years				
Median	45	45	42	42
Range	26-59	22-58	17-59	21-55
Sex ratio (male/female)	0.86	1.08	1.08	1.18
Performance status grade 2 v 0 or 1	3	4	3	4
Fever > 38°C at entry	4	8	6	8
WBC count ($\times 10^9$/L)				
30-99	8	7	7	9
≥ 100	0	2	2	3
Auer rods positive	12	10	10	9
FAB subtype				
M1/M2/M3	2/7/1	4/9/2	4/6/0	2/7/1
M4/M5/M6/M7	7/8/1	2/6/2	7/8/2	9/4/0/1
Cytogenetic risk group (good/intermediate/poor)	3/9/4	2/6/6	3/7/8	3/4/3

2. The more frequent reasons were early PMN recovery (16 patients) and increased circulating blast cells (11 patients), which led to stopping GM-CSF on days 16 to 26 (median, 22.6) and 11 to 24 (median, 19.3), respectively. The other reasons were side effects assumed to be due to GM-CSF (mainly fever, flu-like syndrome, fluid retention, and cardiac failure), and, in six cases, severe infection associated with serious clinical problems.

Response to Induction and Salvage Treatments

Response to induction treatment, according to randomization, is listed in Table 3. The highest CR rate after the first induction course was observed in the control arm. The three treatment arms showed a trend for a lower CR rate and an increased resistance rate, especially in the two arms −/+ and +/+.

The effect of GM-CSF was analyzed by treatment group (Table 4) after the first induction course or after the whole induction treatment (one or two courses). The differences between CR rates of the groups randomized to receive or not receive GM-CSF during induction chemotherapy were not significant, whether one considers the results after the first cycle only (51.0% *v* 60.4%) or the overall results (59.2% *v* 62.3%). In contrast, the CR rate was significantly lower for the group randomized to receive GM-CSF (group ·/+) during the postchemotherapy period, compared to the group ·/−: 43.1% versus 68.6% ($P = .015$) after the first course and 47.1% versus 74.5% ($P = .008$) after one or two courses. These differences were mainly attributable to an increased resistance rate in the group ·/+ compared with ·/−. On the basis of the treatment protocol, administration of GM-CSF was delayed in case of an initial WBC count greater than 30 $\times 10^9$/L. However the CR rate in the group ·/+ was still significantly lower than in the group ·/− after adjustment for WBC count.

If one takes into consideration several factors that may influence the CR rate after induction, such as age, sex, FAB cytology subtype, fever, WBC count, platelet count, performance status, Auer rods, and treatment group, the linear logistic model showed that treatment group (·/− *v* ·/+) was the most predictive variable ($P = .005$), fol-

Table 2. Patients in Whom GM-CSF Was Not Given, Delayed, or Prematurely Stopped by Treatment Arm

Variable	Treatment Arm						Total (N = 76)
	+/− (n = 25)		−/+ (n = 27)		+/+ (n = 24)		
	No.	%	No.	%	No.	%	
GM-CSF not given	1	4	2	7	1	4	4
GM-CSF delayed	9	36	—		11	46	20
GM-CSF prematurely stopped	2	8	21	78	20	83	43
Main reason for interruption							
Early PMN recovery			11	41	5	21	16
Increase of blast cells			4	15	7	29	11
Supposed toxicity	2	8	3	11	2	8	7
Cardiac failure			1	4	2	8	3
Severe infection/other			2	7	4	17	6

Table 3. Response to Induction Treatment by Treatment Arm

Variable	Treatment Arm							
	−/−		+/−		−/+		+/+	
	No.	%	No.	%	No.	%	No.	%
First course								
CR	20	76.9	15	60.0	12	44.4	10	41.7
PR	0	0	2	8.0	1	3.7	2	8.3
Resistance	4	15.4	7	28.0	13	48.1	10	41.7
Death	2	7.7	1	4.0	1	3.7	2	8.3
1 or 2 courses								
CR	20	76.9	18	72.0	13	48.1	11	45.8
Resistance	4	15.4	6	24.0	13	48.1	11	45.8
Death	2	7.7	1	4.0	1	3.7	2	8.3
Total	26		25		27		24	

lowed by the presence of Auer rods ($P = .02$) and fever, which was marginally important ($P = .09$).

After salvage treatment—without GM-CSF—was administered to patients with resistant disease, the overall CR rate of the group ·/+ was still lower, but not significantly ($P = .11$), than that of the group ·/−: 66.7% versus 80.4%. A similar nonsignificant trend ($P = .34$) was observed for the comparison +/· versus −/·: 67.3% versus 77.4%.

EFS and Survival

Table 5 lists treatments given after the completion of induction treatment. Of 62 patients who achieved a CR, 12 received standard maintenance and 16 received one or two cycles of intensive chemotherapy consolidation, whereas 20 have been transplanted. There were a few more patients allografted in the +/− arm, but in general there was a good distribution of postinduction treatments among the four arms. Table 5 also indicates that fewer relapses have been reported in the control arm (−/−), and more in the GM-CSF–containing arms. The highest rate of death in first CR has been reported in the +/− arm, a finding which is probably due to the highest incidence of allografts. The limited number of patients in the treatment arms and groups precludes any valid comparison of the duration of CR and of disease-free survival.

The EFS time from evaluation of the last induction course was significantly shorter (log-rank $P = .02$) for the ·/+ group than for the ·/− group, whereas the difference between groups −/· and +/· was not significant ($P = .16$) (Fig 2). Using Cox's model, after adjustment for the presence of Auer rods, which was the only significant

Table 4. Response to Induction Treatment by Treatment Group

Variable	Treatment Group							
	·/−		·/+		−/·		+/·	
	No.	%	No.	%	No.	%	No.	%
First course								
CR	35	68.6	22	43.1	32	60.4	25	51.0
PR	2	3.9	3	5.9	1	1.9	4	8.2
Resistance	11	21.6	23	45.1	17	32.1	17	34.7
Death	3	5.9	3	5.9	3	5.7	3	6.1
P^*		.015				.41		
OR		2.93				1.53		
95% CI		1.29-6.61				0.68-3.46		
1 or 2 courses								
CR	38	74.5	24	47.1	33	62.3	29	59.2
Resistance	10	19.6	24	47.1	17	32.1	17	34.7
Death	3	5.9	3	5.9	3	5.7	3	6.1
P^*		.008				.86		
OR		3.31				1.18		
95% CI		1.43-7.64				0.51-2.70		
Total	51	100	51	100	53	100	49	100

*χ^2 (2 × 2).

Table 5. Post-CR Treatment and Outcome After Induction Treatment by Treatment Arm

Variable	Treatment Arm −/− No.	%	+/− No.	%	−/+ No.	%	+/+ No.	%	Total No.	%
Treatment										
Toxicity → no treatment	1		0		1		2		4	
Standard maintenance	4		4		1		3		12	
Intensive consolidation	11		7		5		3		16	
Autologous BMT	1		2		4		2		9	
Allogeneic BMT	3		5		2		1		11	
Outcome										
Continued CR	14	70.0	5	27.8	5	38.5	5	45.4	29	46.8
Bone marrow relapse	5	25.0	8	44.4	7	53.8	5	45.4	25	40.3
CNS relapse	0	0.0	1	5.6	0	0.0	0	0.0	1	1.6
Death in first CR	1	5.0	4	22.2	1	7.7	1	9.1	7	11.3
Total	20	100	18	100	13	100	11	100	62	100

prognostic factor for EFS ($P = .015$), similar results were obtained regarding the treatment groups.

The overall survival rate from randomization was 32% at 3 years, with a median estimation of 15 months and a median follow-up duration of 34 months. The total numbers of deaths in the four arms were 10, 17, 19, and 17, respectively. A trend for a higher death rate (log-rank $P = .07$) was observed in the ·/+ group versus ·/− group (OR = 1.51; 95% CI, 0.92 to 2.49). For the comparison +/· group versus −/· group, the difference was smaller (log-rank $P = .37$; OR = 1.26; 95% CI, 0.76 to 2.07).

Time to Recovery of Neutrophils

The time from start of induction to recovery of a neutrophil count greater than 0.5×10^9/L was studied by treatment arm and group, for patients who achieved a CR after the first induction course. Comparison among the four treatment arms showed no significant difference ($P = .28$). However, a trend was observed with a shorter duration of neutropenia in the +/+ arm and a longer duration in the control arm (Fig 3). In addition, there was a nearly significant inverse correlation between treatment arm (arm 1 to 4) and duration of neutropenia (log-rank test for linear trend, $P = .09$).

Toxicities

The main toxicities observed by treatment arm are listed in Table 6, which also provides the number of clinically or microbiologically documented infections in the four randomized arms. Fluid retention, weight gain, and hypotension were mainly observed in the two arms with postchemotherapy administration of GM-CSF. On the other hand, the number of infections was not reduced, and the number of days with fever, antibiotics, and time spent in the hospital in the three treatment arms were not lower than in the control arm. Platelet and RBC transfusions were similar in the four arms (data not shown).

DISCUSSION

The aim of the present trial was to improve the outcome of induction treatment and the EFS in AML, by administration of GM-CSF, using various schedules. However, our results appeared disappointing: none of the combined modalities of GM-CSF and chemotherapy was superior to the standard DNR/Ara-C regimen. With the present dose schedules of GM-CSF, it is unlikely that the CR rate in AML could be improved by addition of GM-CSF. On the contrary, there are indications that GM-CSF, when administered during the postinduction chemotherapy period, could increase the risk of resistance to induction chemotherapy. Unfortunately, this conclusion is based on a limited number of patients, since the trial had to be stopped prematurely. Confirmatory studies would be useful, but administration of GM-CSF after chemotherapy courses to patients with residual leukemic cells should be discouraged. However, this precaution might reduce the chance of confirmation of our negative results.

The control arm contained, to a certain extent, more favorable prognostic factors in comparison with the other randomized treatment arms. Random bias may occur in limited series, but with such central randomization, systematic biases are avoided and the validity of statistical tests guaranteed. A posteriori stratification by important prognostic factors has been performed to adjust for possible imbalances of known factors between the treatment groups, without changing the initial conclusion.

Statistical analysis was performed on the basis of the intention-to-treat principle. In several patients random-

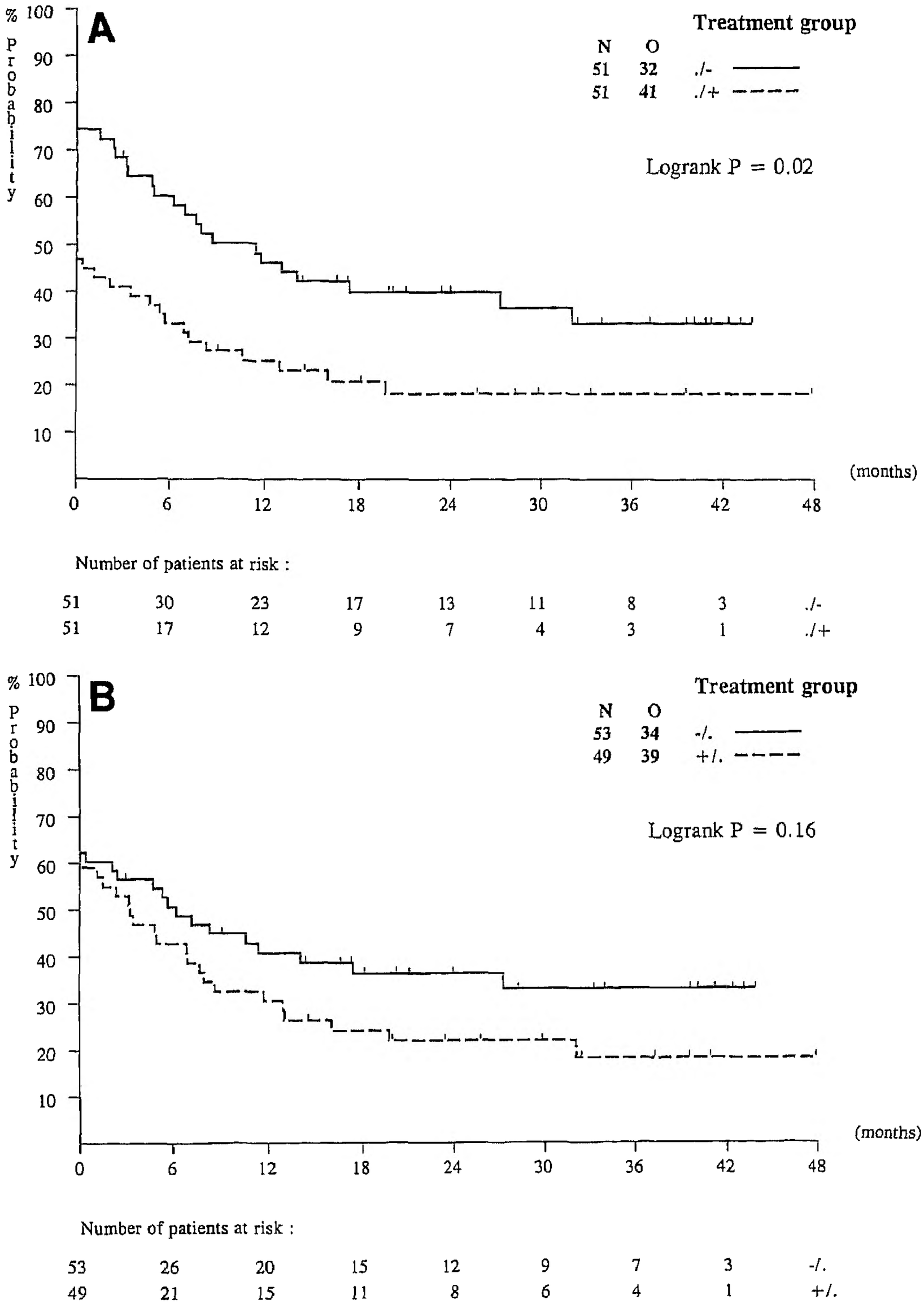

Fig 2. EFS by treatment group. (A) Group · /− (arms −/− and +/−) v · /+ (arms −/+ and +/+); (B) group −/ · (arms −/− and −/+) v +/ · (arms +/− and +/+). N, number of patients; O, observed number of events (no CR after induction courses, relapse or death in first CR).

ized to the +/− and +/+ arms, GM-CSF was delayed, according to the protocol, because of a high initial WBC count. Also, once started, GM-CSF was prematurely stopped in 41 of 51 patients randomized to the −/+ or +/+ arms, mainly because of increased blast cells or early PMN recovery. In addition, four patients did not receive the growth factor at all, because of persisting significant levels of circulating blast cells or symptoms related to leukostasis. Despite these precautionary measures, the CR rate was significantly lower when GM-CSF was administered during the postinduction period, and this difference remained highly significant after adjustment for initial WBC count.

All smears were centrally reviewed, especially for response. This review, and the subsequent hematologic evaluations allowed us to rule out a misinterpretation of

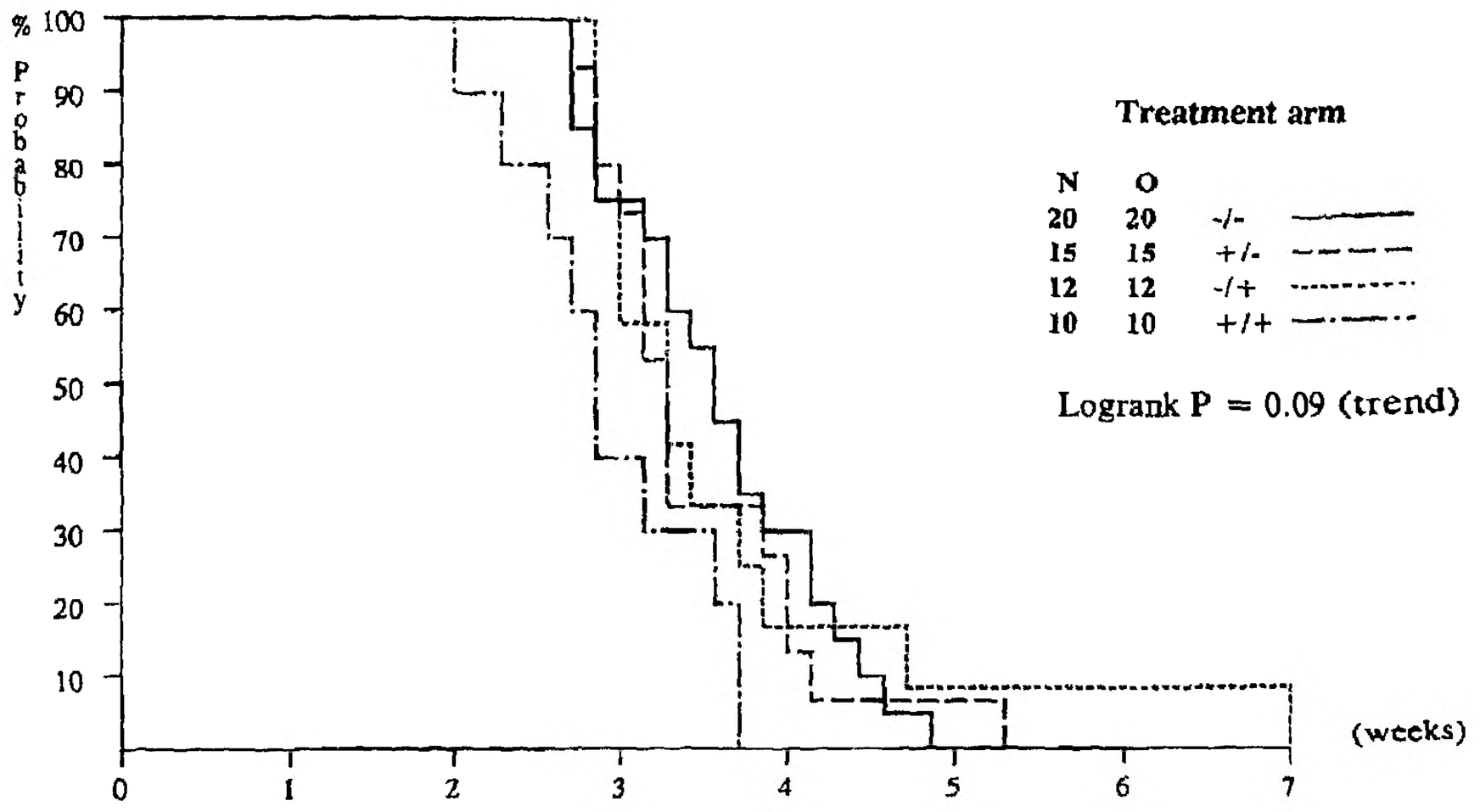

Fig 3. Time to recovery of PMN count ≥ 0.5 × 10⁹/L in the 4 randomized arms, during the first induction course, for patients who achieved a CR after this course. Median time in days: 24.5 (arm −/−), 22.2 (arm +/−), 22.0 (arm −/+), and 19.5 (arm +/+). N, number of patients; O, observed number of patients who reached a PMN count ≥ 0.5 × 10⁹/L.

responses. Such risk of erroneous evaluation had been pointed out, with respect to possible cytologic consequences of the administration of HGFs at the bone marrow level: an underestimation of the CR rate may result from stimulation of early normal progenitor cells that could simulate leukemic blast cells, or by a transient stimulation of leukemic cells, which could disappear after stopping the growth factor, as reported by Büchner et al.[7] On the other hand, stimulation of mature granulopoiesis could dilute the residual leukemic cells and lead to an overestimation of the CR rate. In fact, these cytologic modifications could mainly lead to confusion between CR and PR, but our observation of a lower response rate is still found when PR and CR are considered together, with a lower total response rate in patients allocated to receive GM-CSF during the post induction courses.

It is frequently assumed that the administration of GM-CSF before induction chemotherapy courses might stimulate proliferation of leukemic cells and/or induce recruitment into the cycle. This biologic effect has been observed by cell kinetics methods in some studies[2,22,23] and could enhance leukemic cell kill. Cell kinetic studies were not performed in our study. An increase of the WBC count cannot be simply attributed to stimulation of leukemic proliferation. A noticeable increase was observed only in five patients, and did not correlate with an unexpectedly high CR rate, or, conversely, resistance rate. In their study, Ohno et al[11] did not observe a greater increase of bone marrow blasts in patients who received G-CSF 2 days before the start of induction chemotherapy than in those who received placebo. In fact, the optimum timing of administration HGFs for eventual priming of the leukemic cells remains largely unknown. The administration of GM-CSF during several days before the start of

Table 6. Main Side Effects, Number of Documented Infections, and Supportive Care by Treatment Arm

	Treatment Arm							
	−/− (n = 26)		+/− (n = 25)		−/+ (n = 27)		+/+ (n = 24)	
Variable	No.	%	No.	%	No.	%	No.	%
Bone pain grade 3/4	0	0	0	0	2	7	2	8
Fluid retention	6	23	8	32	11	41	12	50
Weight gain (≥ 5 kg)	2	8	0	0	4	15	4	17
Hypotension	1	4	2	8	6	22	5	21
Cardiac	0	0	0	0	2	7	5	21
Infection	19	73	19	76	22	81	22	92
No. of days with fever								
Median	5.5		7		7		10.5	
Range	0-63		0-19		0-26		3-30	
No. of days of antibiotics								
Median	12		18		14		18	
Range	0-29		0-37		0-32		0-56	

chemotherapy could induce a hyperleukocytosis, with eventual pulmonary infiltrates.[23] An early start of GM-CSF, 4 to 7 days before induction chemotherapy, might explain the relatively low CR rate in patients reported by Estey et al,[24] when compared with a group of matched historical controls. However, these patients also received GM-CSF during the postchemotherapy period, which, according to the present study, may increase the risk of persistent leukemia. An interesting combination of GM-CSF with timed-sequential chemotherapy has been proposed by Archimbaud et al.[25] In this combination, GM-CSF was administered during a short period of 5 days between two short courses of chemotherapy to increase the recruitment and cell kill of residual leukemic cells.

Our study showed a trend for earlier recovery of PMNs in patients who received GM-CSF. A significantly shorter duration of neutropenia was observed in most other studies with GM-CSF[13,14,26] or G-CSF.[10,15,27] However, in our series, as in most others, this slightly accelerated recovery did not result in a significant reduction of the rate of documented infections or mortality during hypoplasia, or of the number of days with fever, duration of administration of antibiotics, and duration of stay in the hospital. Only in the Eastern Cooperative Oncology Group (ECOG) study was there a significant decrease of grade 4 to 5 infections, with a trend for lower therapy-related mortality and a higher CR rate. The reasons for this discrepancy with the other studies of GM-CSF[12,14,16] remain to be explored, taking into account the slightly higher daily dose (250 $\mu g/m^2$ *v* 5 $\mu g/kg$) and the use of a yeast-derived growth factor. Also in this study, Rowe et al[13] did not observe an increased rate of early relapse in patients who received GM-CSF during induction and consolidation; however, the risk of promoting the regrowth of leukemic cells was reduced in this protocol, with GM-CSF being started only on day 11 and in patients with no residual blood or bone marrow blast cells.

Another study with positive results in favor of a combination with growth factor in AML was reported by Dombret et al,[15] who used G-CSF in elderly AML patients from day 9 until day 28 or earlier hematopoietic recovery. These investigators observed a significantly higher CR rate in patients who received the growth factor, without reduction of the mortality rate from infection. The higher CR rate was related to less resistance of leukemia, especially in patients with adverse prognostic factors. This result led to the hypothesis of an antileukemic effect of G-CSF by stimulation of terminal differentiation of residual leukemic cells. If confirmed, our results might indicate a different, adverse effect of GM-CSF. The combination of GM-CSF with antileukemic cytotoxic drugs might have other disadvantages over G-CSF with regard to intracellular metabolism of Ara-C: the in vivo formation of cytarabine triphosphate (Ara-CTP) is decreased by GM-CSF,[28] and there is a decreased sensitivity to Ara-C of leukemic clonogenic cells after exposure to this growth factor,[4] despite the observed increases in labeling index and of numbers of nucleoside transporters.[22]

Further randomized studies to compare the various HGFs at different dose schedules are warranted. Our results indicate that outside such controlled prospective studies, the use of HGF should be avoided in AML, at least during the induction period, thus supporting the recent recommendations of an expert panel.[29]

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