Favorable effect of priming with granulocyte colony-stimulating factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine

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The clinical value of chemotherapy sensitization of acute myeloid leukemia (AML) with G-CSF priming has remained controversial. Cytarabine is a key constituent of remission induction chemotherapy. The effect of G-CSF priming has not been investigated in relationship with variable dose levels of cytarabine. We randomized 917 AML patients to receive G-CSF (456 patients) or no G-CSF (461 patients) at the days of chemotherapy. In the initial part of the study, 406 patients were also randomized between 2 cytarabine regimens comparing conventional-dose (199 patients) versus escalated-dose (207 patients) cytarabine in cycles 1 and 2. We found that patients after induction chemotherapy plus G-CSF had similar overall survival (43% vs 40%, *P* = .88), event-free survival (37% vs 31%, *P* = .29), and relapse rates (34% vs 36%, *P* = .77) at 5 years as those not receiving G-CSF. However, patients treated with the escalated-dose cytarabine regimen benefited from G-CSF priming, with improved event-free survival (*P* = .01) and overall survival (*P* = .003), compared with patients without G-CSF undergoing escalated-dose cytarabine treatment. A significant survival advantage of sensitizing AML for chemotherapy with G-CSF was not apparent in the entire study group, but it was seen in patients treated with escalated-dose cytarabine during remission induction. The HOVON-42 study is registered under The Netherlands Trial Registry (www.trialregister.nl) as #NTR230. (*Blood*. 2012;119(23):5367-5373)

Introduction

Most of the younger adult patients with acute myeloid leukemia (AML) are treated with curative intent. However, prevention of relapse remains a significant challenge in the treatment of AML patients because of residual leukemic cells escaping the cytotoxic effect of chemotherapy.[1] To optimize results of standard chemotherapy, the use of G-CSF or GM-CSF concurrently with induction chemotherapy has been studied in several randomized trials.[2-15] The primary intent of these studies has been to sensitize the leukemic cells to the cytotoxic effects of the chemotherapeutic agents.[16]

In vitro studies with AML cells had demonstrated that priming with granulocytic hematopoietic growth factors, such as GM-CSF, G-CSF, and IL-3, may critically modulate cell cycle kinetics of AML blasts and render them more susceptible to the cytotoxicity of chemotherapy, especially of the cell cycle specific compound cytarabine.[17,18] A number of studies have addressed this concept in AML patients.[2-15] All these reports show that stimulation of leukemia or prolonged cytopenias resulting from sensitization of normal progenitor cells do not appear as major problems.

We have previously reported a randomized trial with 640 untreated adult AML patients in which G-CSF was given during the first 2 induction cycles together with cytarabine plus idarubicin (cycle 1) and cytarabine plus amsacrin (cycle 2).[11] We found a favorable effect of G-CSF priming on the risk of relapse after complete remission as well as an improved disease-free survival (DFS).[11] The benefit with regards to improved overall survival (OS), event-free survival (EFS) as well as DFS was limited to intermediate-risk AML patients.

In the present trial, we wished to revisit the priming question for a number of reasons: First, the previous HOVON-SAKK AML-29 study was indicative of an advantage in DFS, but not OS, in favor of the priming approach. Therefore, a confirmatory study was considered desirable for establishing G-CSF priming as an accepted treatment modality in AML. Second, in the preceding AML-29 study, the benefit of G-CSF priming had been restricted to the intermediate prognostic risk category of AML. It was considered of importance to evaluate whether this effect in relation to risk class could be reproduced in another study. Finally, in the AML-29

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study with 200 mg/m^2 cytarabine (cycle 1) and 1000 mg/m^2 cytarabine (cycle 2), there was no opportunity to assess the impact of G-CSF priming on the cytarabine dose. In the current study, we also investigated the contribution of G-CSF in addition to induction chemotherapy containing 2 differing dosing schedules of cytarabine on EFS as the primary end point.

Methods

Patients

Eligibility for the study was restricted to previously untreated patients (18-60 years of age), with a pathologically confirmed diagnosis of AML. At least 20% myeloblasts in the bone marrow were required, or the presence of refractory anemia with excess blasts and an International Prognostic Score more than or equal to 1.5.[19] All subtypes of AML were studied, including secondary AML after preceding myelodysplastic syndrome, myeloproliferative diseases, or previous chemotherapy. Patients with acute promyelocytic leukemia, primary myelofibrosis, polycythemia vera, or blast crisis of chronic myeloid leukemia were not eligible. Patients were ineligible if they had cardiologic disease defined by unstable angina, cardiac arrhythmias, reduced left ventricular function with an ejection fraction less than or equal to 50%, or myocardial infarction within the last 6 months before study entry. In addition, patients were excluded with impaired renal or hepatic function (serum creatinine, bilirubin, alanine aminotransferase, and/or aspartate aminotransferase more than or equal to 3 times upper normal value, unless probably caused by AML infiltration). The World Health Organization performance status needed to be less than or equal to 2.

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 written informed consent.

Risk classification

Bone marrow and blood samples at diagnosis from all patients were analyzed for cytogenetic abnormalities using standard banding techniques and classified according to the International System for Human Cytogenetic Nomenclature.[20] Exclusively based on the chromosomal analysis, patients were allocated to the following prognostic categories[11,21-24]: Patients in the favorable risk category had core binding factor abnormalities involving t(8;21)(q22;q22), inv(16)(p13.1;q22), or t(16;16)(p13.1;q22).[24] AML lacking any cytogenetic abnormalities or with loss of an X or Y chromosome as sole abnormality were classified as normal cytogenetics (CN-X-Y).[24] Patients in the adverse risk category (CA) lacked core binding abnormalities and showed either complex abnormalities or one of the following: t(6;9), t(6;11), t(11;19), t(9;22), 11q23, 3q, inv(3), 5q−, 7q−, −5, or −7, but did not have a monosomal karyotype.[24] Finally, patients in the very unfavorable risk category showed a monosomal karyotype, defined by the presence of 2 autosomal monosomies or 1 autosomal monosomy in combination with at least 1 structural abnormality not involving core binding factor abnormalities.[24] AML with any other cytogenetic abnormalities were classified as "CA Rest" and considered as intermediate risk.[24]

Clinical characteristics

Leukemias after chemotherapy or radiotherapy (therapy-related) or leukemias arising from myelodysplastic syndrome were classified as secondary AML. Hepatomegaly or splenomegaly assessed on physical examination, World Health Organization performance status, extramedullary disease, and white blood cell count were registered at diagnosis. Clinical characteristics of all study patients are shown in Table 1.

Study design and chemotherapy

Patients were 1:1 randomized for G-CSF priming. G-CSF (filgrastim, Amgen) at a dose of 5 μg/kg body weight subcutaneously was given on days 0 to 7 during cycles 1 and 2 as described.[11] The administration of G-CSF was postponed or interrupted in the event of leukocytosis ($> 30 \times 10^3$ leukocytes/mm^3) until the white blood cell count was less than 20×10^3 leukocytes/mm^3. Patients who were in complete remission after cycle 2 were treated with 1 consolidation course of additional chemotherapy, or autologous stem cell transplantation, or allogeneic stem cell transplantation.[11] Patients with good-risk cytogenetics were planned to receive the third cycle of chemotherapy with etoposide and mitoxantrone. Allogeneic transplantation was performed in intermediate-risk patients with an identical sibling donor or in unfavorable-risk patients with an identified donor. All other patients with no appropriate donor were treated with high-dose chemotherapy with busulfan and cyclophosphamide followed by autologous stem cell transplantation. According to the protocol treatment algorithm, those not considered eligible for allogeneic nor autologous stem cell transplantation (eg, because of no donor availability, insufficient stem cell harvest, or medical conditions) were to receive the third cycle of consolidation chemotherapy as well.

Table 1. Patients and treatment

Characteristics	−G-CSF, no. (%)	+G-CSF, no. (%)
Total	461 (100)	456 (100)
Sex male	239 (52)	237 (52)
Age, y		
≤ 35	83 (18)	82 (18)
36-50	172 (37)	167 (37)
> 50	206 (45)	207 (45)
Range	18-60	17-60
Median	49	49
Prior MDS or chemo/RT		
MDS	18 (4)	16 (4)
Chemo/RT	27 (6)	22 (5)
WHO performance status		
0	210 (46)	208 (46)
1	216 (47)	214 (47)
2	28 (6)	27 (6)
3	3 (1)	1 (0)
4	1 (0)	0 (0)
Unknown	2 (0)	6 (1)
Extramedullary disease*	56 (12)	81 (18)
WBC		
≤ 20×10^9/L	282 (61)	268 (59)
20-100 × 10^9/L	126 (27)	136 (30)
> 100×10^9/L	53 (11)	52 (11)
AML vs RAEB		
AML	445 (97)	428 (94)
RAEB	16 (3)	28 (6)
Cytogenetics		
t(8;21)	20 (4)	22 (5)
inv(16)	24 (5)	28 (6)
CN-X-Y	194 (42)	213 (47)
CA Rest	145 (31)	109 (24)
MK	58 (13)	58 (13)
Not done	20 (4)	26 (6)
Treatment		
Induction treatment		
No	0 (0)	1 (0)
1	56 (12)	69 (15)
1 + 2	405 (88)	386 (85)
Ara-C dose		
Conventional	359 (78)	350 (77)
Escalated	102 (22)	105 (23)
Postinduction treatment†		
Cycle III	106 (34)	96 (31)
Auto SCT	77 (25)	54 (18)
Allo SCT	128 (41)	155 (51)

MDS indicates myelodysplastic syndrome; RT, radiation therapy; RAEB, refractory anemia with excess blasts; and SCT, stem cell transplantation.

*P = .020.

†P = .029

In the initial part of the study, patients were also randomized between conventional-dose or escalated-dose cytarabine during induction courses 1 and 2. Cycle 1 was composed of idarubicin at 12 mg/m^2 given over 3 hours on days 1, 2, and 3, and cytarabine at 200 mg/m^2 as a continuous infusion on days 1 to 7 (conventional-dose), or idarubicin with cytarabine at 1000 mg/m^2 over 3 hours every 12 hours on days 1 to 5 (escalated-dose). Cycle 2 consisted of amsacrine 120 mg/m^2 over 1 hour on days 2, 4, and 6, plus cytarabine 1000 mg/m^2 given over 3 hours twice daily on days 1 to 6 (conventional-dose), or amsacrine with cytarabine 2000 mg/m^2 given intravenously over 6 hours twice daily on days 1, 2, 4, and 6 (escalated-dose). When the trial investigating the effect of variable cytarabine dose levels had reached its target accrual, the cytarabine randomization was discontinued, and subsequently all patients (n = 511) received the conventional-dose cytarabine schedule in the second part of this study. Cycle 2 was given irrespective of the response to cycle 1. Cycle 2 was started as soon as possible in case the bone marrow still showed more than 15% blasts after cycle 1, or in case of blasts of less than 15% after hematopoietic recovery defined as platelets more than 100 g/L and neutrophils more than 1.0 g/L.

Criteria for response and definition of end points

The objective of the study was to assess the contribution of G-CSF in addition to induction chemotherapy containing 2 differing dosing schedules of cytarabine on the rate of response, EFS, risk of relapse, and OS. The primary end point was EFS. Secondary end points were complete remission rate, OS, relapse-free survival (RFS), toxicities and treatment-related mortality, time to hematologic recovery, and number and duration of platelet transfusions.

Complete response (CR) included less than 5% blasts in the bone marrow, no evidence of extramedullary leukemia, and peripheral granulocytes of at least 1.0×10^9/L and platelets of at least 100×10^9/L. EFS was defined as the interval from randomization to relapse, death, or to the date of failure to enter a complete remission within 2 cycles, whichever occurred first. OS was determined from randomization. RFS was defined for all patients who achieved CR, and it was assessed from the date of CR until relapse or death in CR whichever occurred first. Early death referred to death within 7 days of start of cycle 1, whereas induction death was death between 8 and 30 days after the start of cycle 1.

Statistical analysis

The analyses were performed according to intention to treat. Nevertheless, 11 ineligible patients were excluded: 4 patients in the −G-CSF group and 7 patients in the +G-CSF group. Reasons for ineligibility were acute promyelocytic leukemia (n = 3), lacking written informed consent (n = 2), previous autologous stem cell transplantation (n = 1), diagnosis of refractory anemia with International Prognostic Score less than 1.5 (n = 1), withdrawal of informed consent after randomization and before start of treatment (n = 1), blastic crisis of chronic myeloid leukemia (n = 1), double registration for the study (n = 1), and hypereosinophilic syndrome (n = 1). Patients were not censored when they received an allogeneic or autologous transplantation.

Cox regression analysis was used to analyze the effect of treatment group and covariates on EFS and OS. These analyses were performed with and without adjustment for covariates. The possible heterogeneity of the treatment effects in subgroups was explored posthoc by estimation of the hazard ratios for survival end points for each subgroup together with 95% CIs and tests for interaction. This was done for a limited number of subgroups: by age (3 groups of similar size), leukocytes at diagnosis (3 groups), cytogenetic risk groups (3 groups composed of core binding factor, monosomal karyotype$^+$, and Rest), and conventional versus escalated cytarabine dose. The power of these tests of interaction was limited, as the trial was not designed to test for interactions. For the tests of interaction, the nominal *P* value is reported and 1 with a Bonferroni correction for multiple testing. Competing risk analysis was applied to calculate the cumulative competing risks of failure for not achieving CR on protocol, relapse after first CR, and death in CR.

Random assignments to ± G-CSF treatment were balanced with a biased-coin minimization procedure, with the bias depending on the average imbalance between the numbers of patients allocated to both groups overall, within the participating hospital, whether the diagnosis was AML or refractory anemia, and on previous exposure to chemotherapy or radiotherapy. The expected CR rate in the conventional treatment group was 80%, with an expected 3-year (respectively 5-year) EFS of 31% (respectively 25%). The projected enrollment was 800 patients, with an additional follow-up after entry of the last patient of 1 year before final analysis. With a 2-sided log-rank test and a 5% significance level, this would give an expected number of events for EFS of 490 and a power of 86% to show an improvement of the EFS in the G-CSF group with a hazard ratio 0.76, which corresponds to an improvement of the 5-year EFS from 25% to 35%.

Hematologic recovery after cycles 1, 2, and consolidation cycle 3 was analyzed actuarially and compared between the groups with the log-rank test. In these analyses, patients were censored for hematologic recovery at death or at start of next treatment if they had not yet recovered at that time point. All *P* values were 2-sided, indicated with the letter P, and not adjusted for multiple testing, unless explicitly indicated. The study was designed by the Leukemia Working Group of the HOVON/SAKK Cooperative Groups. The HOVON Data Center was responsible for the central data management, and W.v.P. performed the analysis.

Results

Characteristics of the patients and adherence to G-CSF treatment

A total of 917 eligible and evaluable patients with AML (n = 873) or refractory anemia with excess blasts (n = 44) were randomized between remission induction therapy with no priming with G-CSF (control) versus the same therapy with G-CSF priming on days 0 to 7 in induction cycles 1 and 2. A total of 461 patients were assigned to treatment without G-CSF (treatment group A) and 456 patients to the G-CSF priming program (group B). The median follow-up of all patients still alive at the date of last contact (n = 426) is 47.0 months, with 43.9 months for group A and 50.1 months for group B. Table 1 presents the demographic characteristics of the patients. The median age was 49 years (range, 17-60 years), with 45% of patients being 50 years of age or older. The 2 groups did not differ in clinical, hematologic, and cytogenetic features, with the exception of a higher proportion of patients with extramedullary disease at diagnosis in the +G-CSF group (12% vs 18%; $P = .020$).

During the initial phase of the study, 406 eligible and evaluable patients were also randomized to receive remission induction courses 1 and 2 with conventional-dose or escalated-dose cytarabine. Thus, respectively, 199 patients on induction treatment with conventional dose cytarabine and 207 patients on the escalated dose level of cytarabine were randomized between yes or no G-CSF priming. In detail, 100 and 99 patients, respectively, in each arm (22%) received conventional-dose cytarabine and 102 and 105 patients were assigned to the escalated-dose cytarabine schedule in each arm (22% and 23%, respectively). In the final phase of the study, all randomized patients (n = 511; 259 with G-CSF vs 252 without G-CSF) received the conventional-dose cytarabine schedule.

Treatment, response, and outcome

Of 917 eligible patients, 916 (100%) received induction cycle 1. A total of 791 of 917 patients (87%) received induction cycle 2 with similar distributions (405 vs 386 patients) between treatment groups A and B (Table 1). Patients assigned to treatment without G-CSF (group A) had a slightly lower CR rate without reaching significance compared with patients with G-CSF priming (group B; 77% vs 81%; Table 2), whereas the CR rates were attained at similar frequencies within the first and second induction cycle. Of

Table 2. Outcomes

	−G-CSF, no. (%) (n = 461)	+G-CSF, no. (%) (n = 456)	*P*	OR or HR (95% CI)
Death within 30 d				
Cycle 1	50 (11)	39 (9)	.27*	
Cycle 2	27 (7)	12 (3)	.02*	
Complete remission	354 (77)	370 (81)	.11	OR = 1.30 (0.95-1.79)
Early (after cycle 1)	273 (59)	291 (64)		
Late (after cycle 2)	81 (18)	79 (17)		
Relapse†	155 (36)	156 (34)	.77	HR = 0.97 (0.77-1.21)
Death in CR1†	41 (9)	45 (10)	.80	HR = 1.06 (0.69-1.61)
EFS‡	303 (31)	286 (37)	.29	HR = 0.92 (0.78-1.08)
OS§	256 (40)	253 (43)	.88	HR = 0.99 (0.83-1.17)

OR indicates odds ratio; and HR, hazard ratio.

*P value from Fisher exact test. Other *P* values from likelihood ratio test with logistic regression or Cox regression.

†Number and 5-year competing risk probability.

‡Number of events (no CR, relapse, or death in CR1) and 5-year EFS %.

§Number of deaths and 5-year OS %.

the 616 patients in complete remission undergoing consolidation treatment, 202 received a third chemotherapy cycle, 131 patients had autologous hematopoietic stem cell transplantation, and 283 patients underwent allogeneic SCT, with no differences between the 2 treatment groups (Table 1).

In group A (without G-CSF priming; n = 461), 155 (36%) patients relapsed, and 256 have died, of whom 77 died within 30 days of start of the last protocol treatment (Table 2). In the G-CSF priming group B, 156 (34%) patients relapsed and 253 patients have died, including 51 patients dying within 30 days of start of the last protocol treatment. EFS between the 2 treatment groups A and B was similar (at 5 years 31% vs 37%; $P = .29$), and the same was apparent for OS (at 5 years 40% vs 43%; $P = .88$; Table 2; Figure 1).

Prognostic factors and subgroup analysis

Table 2 shows the actuarial 5-year probabilities of OS and EFS split by treatment group. In the multivariate analysis (data not shown), karyotype abnormalities represented the strongest prognostic factor. According to cytogenetic subgroups, patients with a monosomal karyotype (n = 116) had a highly unfavorable 5-year EFS of 8% and a 5-year OS of 7% only (Table 3). The best results were apparent in the 94 patients with core binding factor abnormalities with an EFS and OS at 5 years of 52% and 67%, respectively. In addition, patients with age more than 50 years, and those with white blood cell count more than 100 g/L at diagnosis showed significantly reduced 5-year EFS and OS. Adjustment for these factors in a Cox multivariate regression analysis did not change the lack of a benefit for the G-CSF priming group B in EFS or OS compared with group A (data not shown).

To explore whether there was evidence for a possible different effect of G-CSF priming in any of the subgroups defined by the aforementioned factors, the effect of treatment was estimated separately by hazard ratios for EFS and OS with associated CIs combined with tests for interactions (Table 3). In none of these cases were the tests for interactions significant, with the exception of the cytarabine dose, with $P = .0008$ for OS (with Bonferroni correction for 4 tests $P = .003$) and $P = .023$ for EFS (with Bonferroni correction $P = .09$). Thus, these data suggest a favorable effect of G-CSF treatment limited to the group of AML patients treated with escalated cytarabine dose (Figure 2).

Adverse events

The 2 treatment groups A and B did not show differences as regards to the overall frequency of adverse effects, nor were different frequencies noted as regards to infectious complications after induction cycles 1 and 2 between both treatment groups (Table 4).

The time to neutrophil or platelet recovery between the 2 treatment groups did not differ after induction cycle 1. However, after cycle 2, neutrophils ($P = .007$) and platelets ($P = .02$) regenerated with delay in patients assigned to the G-CSF treatment regimen (Table 4). Finally, no differences in side effects and in the time to neutrophil or platelet recovery between the 2 groups were

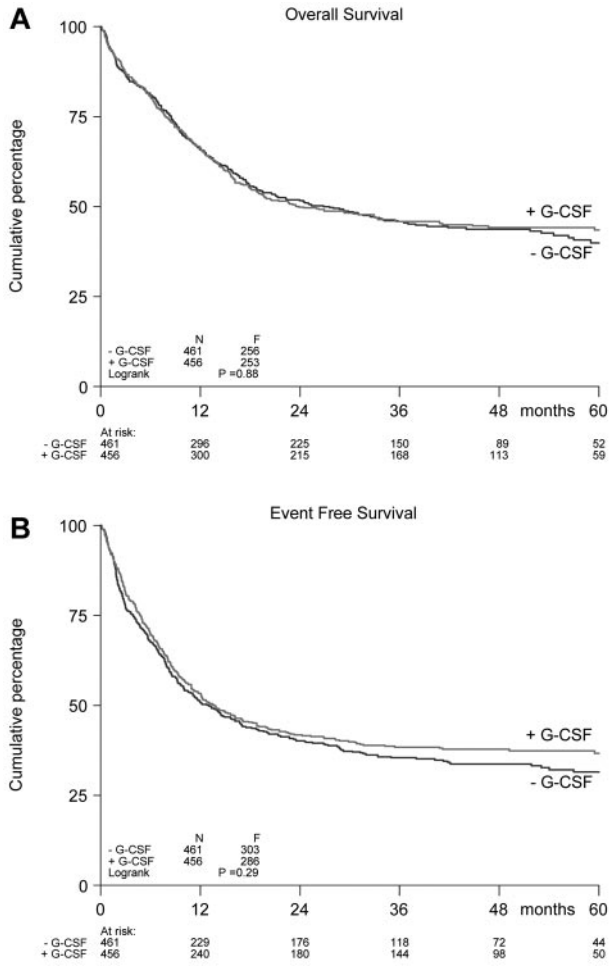

Figure 1. Survival according to the assigned G-CSF treatment. OS (A) and DFS (B) are shown according to the assigned treatment (with or without G-CSF treatment). *P* values were calculated using the log-rank test.

Table 3. Subgroup analysis and tests for interaction

			OS					EFS					
	N	Dead	−G-CSF (5-y), %	+G-CSF (5-y), %	HR	95% CI	*P*	Event	−G-CSF (5-y), %	+G-CSF (5-y), %	HR	95% CI	*P*
Total	917	509	40	43	0.99	0.83-1.17	.88	589	31	37	0.92	0.78-1.08	29
Age, y							*P*-int = .24						*P*-int = .35
≤ 35	165	81	43	55	0.77	0.50-1.20	.24	94	32	50	0.70	0.46-1.05	.088
36-50	339	174	47	43	1.19	0.88-1.60	.26	208	37	38	0.99	0.75-1.30	.94
> 50	413	254	33	39	0.94	0.74-1.21	.64	287	26	30	0.95	0.75-1.20	.67
WBC							*P*-int = .71						*P*-int = .39
≤ 20 × 10^9/L													
20-100 × 10^9/L	550	293	43	48	0.97	0.77-1.22	.80	350	32	39	0.91	0.74-1.13	.41
> 100 × 10^9/L	262	143	39	43	0.94	0.68-1.31	.73	161	35	39	0.84	0.61-1.14	.26
≤ 20 × 10^9/L	105	73	27	25	1.18	0.75-1.88	.47	78	23	22	1.22	0.78-1.90	.39
Cytogenetics							*P*-int = .82						*P*-int = .52
CBF	94	27	72	63	1.05	0.49-2.25	.90	42	51	52	0.83	0.45-1.52	.55
Rest	707	376	42	46	1.01	0.82-1.24	.93	440	33	39	0.95	0.78-1.14	.56
MK	116	106	6	8	0.88	0.60-1.29	.52	107	6	10	0.74	0.51-1.09	.13
Ara-C dose							*P*-int < .001						*P*-int = .023
Conventional	709	382	43	41	1.16	0.95-1.42	.14	451	33	35	1.02	0.85-1.22	.86
Escalated	207	126	30	50	0.59	0.41-0.84	.003	137	28	43	0.65	0.46-0.91	.012

N indicates number of patients; Dead, number of deaths; Event, number of events (no CR, relapse, or death in CR1); HR, hazard rate; CI, confidence interval; *P*-int, *P* value for test of interaction between factor and G-CSF treatment group; and *P*, other *P* values for test of no difference between G-CSF groups within row subgroup.

observed after consolidation chemotherapy cycle 3 with mitoxantrone and etoposide (data not shown).

Discussion

For decades, the cell cycle-dependent agent cytarabine has been a cornerstone in the treatment of adults with AML. Exposure of AML cells to cytokines, such as G-CSF, GM-CSF, or IL-3, together with cytarabine increases the intracellular levels of the active metabolite cytosine arabinoside triphosphate, elevates the incorporation of cytarabine into cellular DNA, and enhances the killing of leukemic blasts and leukemic progenitor cells by the antimetabolite.[16-20,25,26] We previously reported a randomized trial with 640 untreated adult AML patients in which G-CSF was given during the first 2 induction cycles together with cytarabine plus idarubicin in cycle 1 and cytarabine plus amsacrin in cycle 2.[11] In the latter study, we found a favorable effect of G-CSF priming on the risk of relapse after

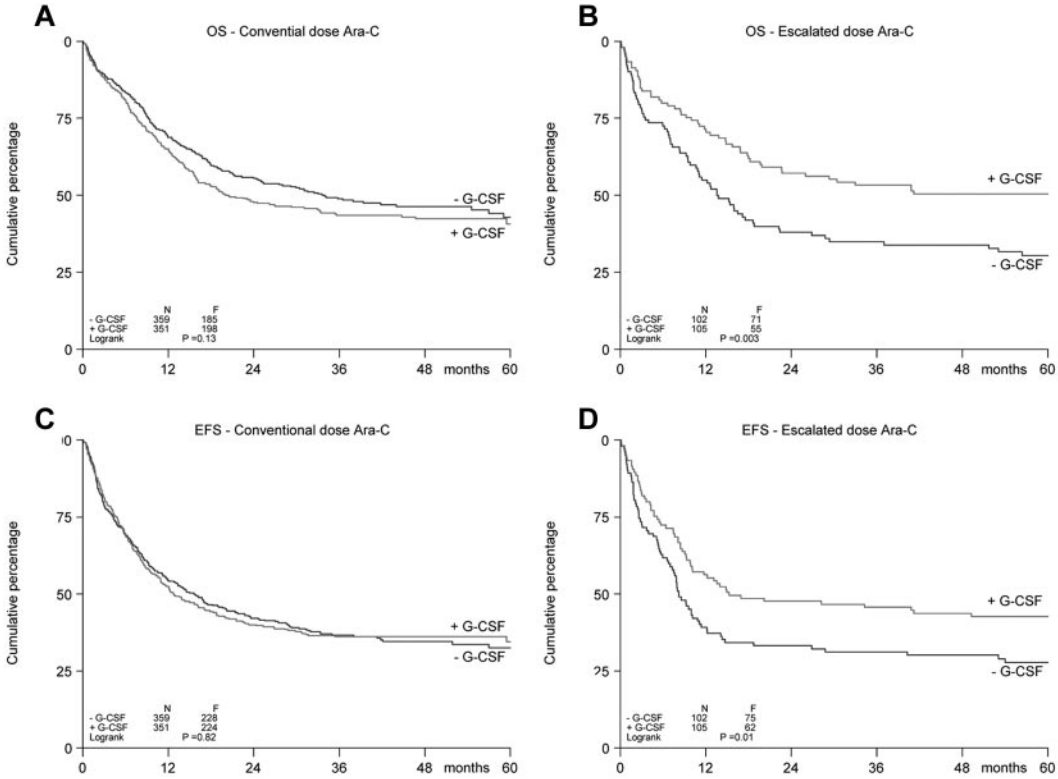

Figure 2. Survival according to the assigned cytorabin treatment. OS (A-B) and EFS (C-D) are presented according to the cytarabine dose given. In the initial part of the protocol, 406 AML patients were randomized between yes and no G-CSF, whereas in the final part of the protocol, all 511 AML patients received conventional-dose cytarabine.

Table 4. Adverse events and hematopoietic recovery

	−G-CSF	+G-CSF	*P*
Adverse events grade 3 or 4, %			
Cycle 1			
Side effects, %	52	55	.18
Infections, %	88	92	.14
Cycle 2			
Side effects, %	63	64	.91
Infections, %	90	92	.70
Hematopoietic recovery, d (median)			
Cycle 1			
ANC $> 0.5 \times 10^9$/L	29	29	.70
Platelets $> 50 \times 10^9$/L	27	27	.56
Cycle 2			
ANC $> 0.5 \times 10^9$/L	28	31	.007
Platelets $> 50 \times 10^9$/L	35	40	.02

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.

complete remission as well as an improved DFS.[11] However, a benefit with regard to improved OS in addition to EFS and DFS was only evident in patients with cytogenetically defined intermediate-risk AML.[11] In the current randomized trial, we addressed the priming question again in an independent cohort of 917 untreated AML patients. In this study, we found no significant differences in OS, EFS, or RFS between patients with or without G-CSF priming in cycles 1 and 2.

In particular, this study could not confirm the previous results of the HOVON-SAKK AML-29 trial,[11] which had indicated that G-CSF priming confers a benefit on the outcome of intermediate-risk AML patients. Moreover, in the current study, no prognostic subgroup of AML could be identified with improved outcome with G-CSF priming, even not if the possible confounding effect of cytarabine dose level was taken into account. Indeed, the therapeutic outcome was similar between escalated and conventional cytarabine dose schedules in the unselected study population as well as in the favorable-risk, intermediate-risk and adverse-risk subsets of patients if the G-CSF priming effect was disregarded. The reasons for the discrepant results obtained in our 2 successive G-CSF priming studies remain unclear. We have used filgrastim instead of lenograstim in the current study; but because both G-CSF preparations have very similar biologic effects, we consider the choice of filgrastim as an unlikely explanation for the lack of an apparent therapeutic benefit in the current study. Because inclusion and exclusion criteria were kept unchanged, differences in patient selection and patient characteristics between the 2 studies also offer unlikely explanations for the inability to reproduce our prior G-CSF priming effects, although the median age of the study population in the current study was somewhat higher (median age 49 vs 44 years). Finally, our strategy of allocating patients to the various postremission treatments (chemotherapy vs autologous vs allogeneic transplantation) has been maintained.

The lack of a favorable effect of G-CSF priming in this trial is consistent with recent reports from several collaborative groups.[2-15,27-31] Similarly, we also observed that G-CSF treatment was not associated with an increase in grade 3 or 4 side effects or infectious complications. Noteworthy, we found a delay in the time to neutrophil (> 0.5 g/L) recovery of 3 days and to platelet (> 50 g/L) recovery of 5 days in the G-CSF treatment group. This effect was limited to cycle 2 only, and no differences in hematopoietic recovery were observed in those patients who underwent subsequent chemotherapy cycle 3 with mitoxantrone and etoposide.

In previously reported G-CSF priming studies, the cytarabine dose as a possibly important codeterminant of outcome has remained a neglected issue. In the same sense, in the HOVON-SAKK AML-29 study,[11,32] it has remained unresolved whether the favorable sensitization effect of G-CSF treatment had been mediated by increasing the efficacy of cytarabine, idarubicin, or amsacrin, or the combined chemotherapeutic agents that were used. In the latter study, 200 mg/m^2 cytarabine (cycle 1) and 1000 mg/m^2 cytarabine (cycle 2) had been administered (conventional dose). During the initial phase of the present study, 406 patients were also randomized to receive remission induction courses I and II with conventional dose or intensified dose levels of cytarabine.[33] The results of the study revealed the notable finding of a highly significant interaction between G-CSF and cytarabine dose, especially with respect to OS and even after Bonferroni correction for multiple testing. AML patients treated with an escalated dose schedule of cytarabine had improved EFS ($P = .01$), and OS ($P = .003$) when they were also primed with G-CSF. The reason for this remains unresolved, apart from the possibility of a chance finding in a subgroup. Future studies will be warranted for confirmation of our findings. However, it could also, for instance, be related to limitations set by the nucleoside transporter that has appeared important in transmitting the cellular cytotoxic effects of cytarabine. In addition to a direct sensitizing effect of G-CSF on the AML blast cell population, recent data have suggested a second possible mechanism for enhanced cytotoxicity related to the dissociation of leukemic blasts from their protective microenvironment because of the mobilizing abilities of G-CSF, and such effects have been described both for CXCR4 inhibitors (eg, AMD3100) and for G-CSF.[34-36] This has prompted clinical studies with G-CSF and CXCR4 inhibition in AML that are currently in progress. To this end, the data presented herein are indicative of a dose-dependent favorable effect of cytarabine in association with G-CSF priming. They raise the provocative possibility of a therapeutic strategy of relapse risk reduction via growth factor chemosensitization of leukemia that is strongly cytarabine dose-dependent. Such a concept would obviously warrant critical testing in specific studies and would also require validation in a prospective manner.

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Authorship

Contribution: T.P. and B.L. designed research, analyzed data, and wrote the paper; E.V., H.C.S., C.G., M.-C.V., B.B., P.S., J.P., L.V., M.-C.L., M.T., E.J., M.B., and J.M. analyzed data and contributed vital material; W.v.P. analyzed data and wrote the paper; and G.J.O. designed research, analyzed data, and contributed vital material.

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For a list of HOVON/SAKK participants, see the supplemental Appendix (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

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