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The stem cell mobilizing capacity of patients with acute myeloid leukemia in complete remission correlates with relapse risk: results of the EORTC-GIMEMA AML-10 trial

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Variable numbers of CD34$^+$ cells can be harvested from the blood of AML patients in CR after G-CSF supported mobilization following consolidation chemotherapy. We hypothesized that a decreased ability to mobilize stem cells reflects a chemotherapy-induced reduction in the number of normal and leukemic stem cells. We therefore analyzed whether the mobilizing capacity of these patients was of prognostic significance. 342 AML-patients in first CR received daily G-CSF from day 20 of the consolidation course and underwent 1–6 aphereses to obtain a minimum dose of 2 x 10^6 CD34$^+$ cells/kg. Afterwards they were randomized for autologous bone marrow (BM) or blood SCT. As a surrogate marker for the mobilizing capacity, the highest yield of CD34$^+$ cells of a single apheresis was adopted. Patients could be categorized into four groups: no harvest ($n = 76$), low yield (<1 x 10^6 CD34$^+$/kg; $n = 50$), intermediate yield (1–6.9 x 10^6 CD34$^+$ cells/kg; $n = 128$) and high yield (≥7 x 10^6 CD34$^+$ cells/kg; $n = 88$). The median follow-up was 3.4 years; 163 relapses and 16 deaths in CR were reported. Autologous blood or BM SCT was performed in 36%, 64%, 81% and 88%, respectively, of the patients assigned to the no harvest, low, intermediate and high CD34$^+$ yield group. The 3-year disease-free survival rate was 46.7%, 65.0%, 50.4% and 26.9% ($P = 0.0002$) and the relapse incidence was 47.5%, 30.1%, 43.1% and 71.9% ($P < 0.0001$). Multivariate Cox's proportional hazards model showed that the CD34$^+$ yield was the most important independent prognostic variable ($P = 0.005$) after cytogenetics. Patients with the highest mobilizing capacity have a poor prognosis due to an increased relapse incidence.
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Introduction

Over the past decade, management of patients with acute myeloid leukemia has gradually improved as the result of intensification of treatment and better supportive care. Autologous bone marrow transplantation (ABMT) has been increasingly used as a treatment modality for patients with AML younger than 60 years of age. Several large randomized trials have indicated the superior antileukemic activity of ABMT.[1–5] Therefore, ABMT has become the treatment of first choice in many European centers for patients in complete remission who lack a suitable donor for allogeneic transplantation.

Prolonged pancytopenia following transplantation has emerged as a major drawback of ABMT. In an attempt to accelerate hematopoietic reconstitution following transplantation, autologous cytokine mobilized peripheral blood stem cells have been successfully used.[6–10] However, despite its widespread clinical usage, the efficacy with respect to relapse rate remains unproven. This question is now being addressed by the EORTC and GIMEMA leukemia groups in a randomized study (AML-10) comparing autologous blood stem cell transplantation (ABSCT) and ABMT.[11]

In this study, it was attempted to harvest G-CSF (lenograstim) mobilized blood stem cells in all patients recovering from consolidation chemotherapy. Patients were subsequently randomized to undergo an ABMT or ABSCT. Since we encountered a highly variable yield of mobilized CD34$^+$ cells among patients, we hypothesized that a decreased ability to mobilize stem cells may reflect a chemotherapy-induced reduction in the number of normal and leukemic stem cells. For this reason, we analyzed whether the mobilizing capacity for patients with AML in complete remission was of prognostic significance. Our results indicate that patients mobilizing high numbers of CD34$^+$ cells after consolidation chemotherapy, irrespective of whether they received a transplant or not, had an adverse prognosis and exhibited a higher relapse rate. Therefore the capacity to harvest high numbers of CD34$^+$ cells can be considered as an independent poor prognostic factor.

Patients and methods

Study design

The AML 10 trial of the EORTC and GIMEMA Leukemia Groups started in 1993 and included adult patients (≤60 years) with newly diagnosed AML. In the first randomization three different intercalating agents (daunorubicine, mitoxantrone and idarubicine) in combination with cytarabine and etoposide were compared for induction and consolidation treatment. In 1994, a second randomization was introduced comparing ABMT and autologous blood stem cell transplantation (ABSCT) in patients in complete remission who lacked a HLA-identical family donor. The protocol has been reviewed and approved by the relevant institutional ethics committees, and all patients gave informed consent. They received consolidation chemotherapy consisting of intermediate dose Ara-C (500 mg/m^2, twice daily for 6 days) and 3 days of the randomized intercalating agent.[12] Mobilization and harvest of autologous blood stem cells was planned for all these patients independent of the assignment of the second randomization and was scheduled during the recovery phase of the consolidation course. Lenograstim (150 μg/m^2) was given

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by daily subcutaneous injections from day 20 of the consolidation course until completion of the blood stem cell collections. Blood stem cell collections were performed on 1–5 consecutive days during the hematopoietic recovery phase, as soon as the leukocyte counts exceeded 2 x 10^9/l or the $CD34^+$ cells in the blood exceeded 2 x 10^7/l. According to the protocol, the total blood stem cell harvest should contain a target dose of at least 2 x 10^6/kg body weight $CD34^+$ cells. Those patients who were randomized for transplantation with bone marrow cells subsequently underwent a bone marrow harvesting procedure under general anesthesia.

As a surrogate marker for the mobilizing capacity after consolidation treatment, we adopted the highest yield of a single apheresis cycle. The total number of $CD34^+$ cells harvested could not be used for this purpose since this was influenced by the predefined target of 2 x 10^6 $CD34^+$ cells/kg.

Patients

Three hundred and ninety-six patients were candidates for this study. A total of 54 could not been included since six patients had less than 5 days of lenograstim with insufficient harvest, 35 patients started the lenograstim too late (after day 30 of consolidation course), six stopped lenograstim before the start of apheresis, one patient died at the end of the apheresis, and in six patients the number of $CD34^+$ cells was unknown. Hence 342 patients were eligible for further analysis.

Criteria of evaluation

The CALGB criteria for response to treatment and relapse were used.[13] CR was defined as morphological normal marrow with less than 5% blasts, and normal peripheral blood and differential counts. Among patients who reached CR, relapse was defined as the presence of >10% blasts in the bone marrow, or blasts in extramedullary sites. As cytogenetic classification the ISCN system has been applied.[14]. The patients with unknown, not done or unsuccessful cytogenetics were grouped together as 'unknown'.

Statistical analysis

The disease-free survival (DFS) was calculated from the start of lenograstim for mobilization of stem cells until the date of first relapse or of death in first CR; patients alive and in first CR were censored at their last follow-up. The disease-free interval (DFI) was calculated as the DFS, except that patients who died in CR were censored at that moment. The duration of survival was calculated from the date of start of lenograstim until the date of death; patients still alive were censored at their last follow-up.

Actuarial curves were calculated according to the Kaplan–Meier technique. The standard errors (s.e.) of the estimates were computed using the Greenwood formula. The estimates of the incidence of relapse and of death in CR, and their corresponding standard errors, were obtained using the cumulative incidence method, where the risks of death in CR and of relapse were considered as competing risks.[15] The differences between actuarial curves were tested for statistical significance using the two-tailed log-rank test, whereas for the cumulative incidences the Gray test has been used.[15] The Cox's proportional hazards model has been used to obtain the estimate and the 95% confidence interval of the hazard ratio (HR) of the instantaneous event rate in one group *vs* the one in another group, as specified by a given variable, and the Wald test has been used to determine the prognostic significance.[15] The Cox model has also been used to determine the independent prognostic factors among those that appeared important in univariate analyses ($P < 0.1$). All analyses were based on the intent-to-treat principle. The relationships between the four groups and patient characteristics have been tested using the generalized Wilcoxon test.[15]

The cut-off date was 8 April 2002. SAS 8.1 statistical software has been used.

Results

Mobilization characteristics

The median day to start lenograstim was 20 days (range 10–29) from the start of the consolidation course. The median duration of lenograstim administration was 8 days (range 1–37). In 76 patients the number of $CD34^+$ cells in the blood remained below the threshold for starting an apheresis in spite of a median duration of lenograstim administration of 13 days (range 5–37). In the other 266 patients the first apheresis started after a median of 2 days (range 0–9 days) of lenograstim administration, and the median number of aphereses performed was 2 (range 1–6). The median of the total number of $CD34^+$ cells in all apheresis products harvested in a particular patient (total graft) was 6 x 10^6/kg (range 0.1–246.4 x 10^6/kg). Based on the highest harvest of $CD34^+$ cells obtained during a single apheresis 50 patients were considered to have a low $CD34^+$ yield (<1 x 10^6/kg), 128 patients an intermediate $CD34^+$ yield (1–6.9 x 10^6/kg) and 88 patients a high $CD34^+$ yield (≥7 x 10^6/kg). The correlation between the highest $CD34^+$ yield, considering the cut-points at 1 and 7 x 10^6/kg, and the total yield of $CD34^+$ harvested, taking the cut-point at 2 and 10 x 10^6/kg, was extremely high (data not shown). The number of days from the day of starting of lenograstim to the first harvest was 9, 6 and 4 days, respectively for the three groups, low, intermediate and high yield.

Relationship with initial patient characteristics and initial treatment schedule

Patient characteristics of the four groups are presented in Table 1. The 50 patients in the low $CD34^+$ yield group were younger (median age 38 *vs* 46 years for all others) and had a higher initial WBC count (median of 32.9 *vs* 14 x 10^9/l for all others). The distribution regarding FAB subtype, CD34 expression on the leukemic blasts at diagnosis, the CR rate after the first induction course, and the percentage of patients not randomized were similar in the four groups. The cytogenetic prognostic subgroups were unevenly distributed between the four $CD34^+$ yield groups, ie the no harvest group contained more patients with favorable and less patients with unfavorable cytogenetics, whereas the high $CD34^+$ yield group contained less good risk patients (Table 1).

The distribution of the three initially randomized arms for either daunorubicin, mitoxantrone or idarubicine varied to a great extent per $CD34^+$ yield group (Table 2). The high $CD34^+$ yield group contained more patients randomized to the daunorubicin arm. The median duration of administration of leno-

Table 1 Patient characteristics according to CD34+ yield group

	No harvest n = 76	*Low yield n = 50*	*Intermediate yield n = 128*	*High yield n = 88*	*P-value*
Age (years) (median)	50	38	43.5	45	0.007
WBC (x109/l) (median)	12.1	32.9	13.1	15.7	0.06
Leukemic blasts	46 (60.5%)	42 (84.0%)	62 (44.9%)	45 (48.2%)	
CD34+ assessed					
<30% CD34+	25 *(54.3%)*	20 *(47.6%)*	36 *(58.1%)*	19 *(42.2%)*	0.39
≥30% CD34+	21 *(44.7%)*	22 *(52.4%)*	26 *(41.9%)*	26 *(57.8%)*	
FAB type					
Unknown	3 (3.9%)	0 (0.0%)	2 (1.6%)	0 (0.0%)	
M0	5 (6.6%)	0 (0.0%)	2 (1.6%)	2 (2.3%)	
M1	12 (15.8%)	10 (20.0%)	15 (11.7%)	17 (19.3%)	
M2	28 (36.8%)	17 (34.0%)	42 (32.8%)	26 (29.5%)	0.35[a]
M4	12 (15.8%)	10 (20.0%)	38 (29.7%)	20 (22.7%)	
M5	13 (17.1%)	10 (20.0%)	25 (19.5%)	20 (22.7%)	
M6	3 (3.9%)	3 (6.0%)	3 (2.3%)	3 (3.4%)	
M7	0 (0.0%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	
Randomization					
Not randomized	26 (34.2%)	18 (36.0%)	44 (34.4%)	28 (31.8%)	
ABMT *vs* ABSCT	50 (65.8%)	32 (64.0%)	84 (65.6%)	60 (68.2%)	0.96
Courses to CR					
1	72 (94.7%)	48 (96.0%)	119 (93.0%)	85 (96.6%)	
2	4 (5.3%)	2 (4.0%)	9 (7.0%)	3 (3.4%)	0.67
Cytogenetics					
Unknown	22 (28.9%)	10 (20.0%)	40 (31.3%)	27 (30.7%)	
Successful	54 (71.1%)	40 (80.0%)	88 (68.7%)	61 (69.3%)	
Good risk[b]	17 *(31.5%)*	12 *(30.0%)*	28 *(31.8%)*	10 *(16.4%)*	
NN or –Y only	25 *(46.3%)*	15 *(37.5%)*	33 *(37.5%)*	22 *(36.1%)*	0.025
Bad risk[c]	12 *(22.2%)*	13 *(32.5%)*	27 *(30.7%)*	29 *(47.5%)*	

Data in parentheses refer to percentages. Percentages in italic are calculated for those with a successful examination.
[a]FAB M2 or M4 vs all other FAB subtypes.
[b]Presence of t(8;21) or inv(16).
[c]Presence of –5, –7, 5q–, 7q–, 11q23, +8, t(9;22), 12p–, complex or other abnormalities.
ABMT, autologous bone marrow transplantation; ABSCT, autologous blood stem cell transplantation.

Table 2 Induction/consolidation treatment according to CD34+ yield group

	No harvest n = 76	*Low yield n = 50*	*Intermediate yield n = 128*	*High yield n = 88*	*P value*
Randomized arm					
DNR	13 (17.1%)	8 (16.0%)	56 (43.8%)	51 (58.0%)	
MTZ	37 (48.7%)	28 (56.0%)	24 (18.8%)	22 (25.0%)	<0.0001
IDA	26 (34.2%)	14 (28.0%)	48 (37.5%)	15 (17.0%)	

DNR, daunorubicin; MTZ, mitoxantrone; IDA, idarubicin.

grastim was 5, 10 and 10 days for patients receiving daunorubicin, mitoxantrone and idarubicin, respectively. An average of 1.71, 1.68, 1.88 aphereses was performed in each treatment group, respectively. More than 7 x 10^6/kg CD34+ cells were harvested in a single apheresis in 51 (40%), 22 (20%) and 15 (15%) patients, respectively, in the three treatment groups ($P < 0.001$).

Relationship between chemotherapy toxicity and CD34+ yield (Table 3)

Hematological toxicity after the consolidation course differed considerably: the higher the CD34+ yield, the shorter the duration of neutropenia and thrombocytopenia ($P < 0.0001$). The rates of grade 3–4 infections (21%, 20%, 16%, 12%) and especially the duration of i.v. antibiotic administration ($P = 0.001$) were inversely correlated with the CD34+ yield (median of 17, 14, 11 and 13 days, respectively). The incidence and severity of other organ toxicities, such as oral and gastro-intestinal mucositis and liver toxicity were similar in the four groups.

Relationship between CD34+ yield and transplantation rate

From the 76 patients in the no-harvest group, 31 (41%) subsequently had a successful harvest of either bone marrow ($n = 23$), blood stem cells ($n = 6$) or combined ($n = 2$). Twenty-seven (35.5%) underwent a stem cell transplantation (23 ABMT, 4 ABSCT). In the remaining three CD34+ yield groups, sufficient numbers of CD34+ cells from peripheral blood or BM were harvested in the large majority of the patients to undergo an autologous stem cell transplantation (74.0%, 94.4%, and 100% in the low, intermediate and high CD34+

Table 3 Toxicity after consolidation treatment according to CD34+ yield group

	No harvest n = 76	*Low yield n = 50*	*Intermediate yield n = 128*	*High yield n = 88*	*P value*
Recovery					
Days PLT >20 (median)	33	28	23	19	<0.0001
Days PLT >100 (median)	63	52	35	25	<0.0001
Days PMN >0.5 (median)	26	25	22	21	<0.0001
Days PMN >1.5 (median)	30	27	24	23	<0.0001
Organ toxicity					
Mucositis	27 (35.5%)	22 (44.0%)	52 (40.6%)	30 (34.1%)	0.75
Diarrhea	24 (31.6%)	15 (30.0%)	40 (31.3%)	30 (34.1%)	0.85
Liver toxicity	16 (21.1%)	11 (22.0%)	29 (22.7%)	14 (15.9%)	0.62

PLT, platelet cell count (x10^9/l); PMN, polymorphonuclear cell count (x 10^9/l).

yield groups, respectively). Autologous PSC or BM transplantation rates in first CR were 64.0%, 81.3% and 87.5%, respectively. In the high CD34+ yield, 11 (12.5%) patients have not been auto-transplanted: one patient had an alloSCT (protocol violation), nine relapsed (day 26–142) and one remained in CCR (censored at day 81).

Effects of the CD34+ yield on outcome

The median follow-up was 41 months (range 1–82) and the overall DFS rate at 3 years was 45.8%. Patients with a low CD34+ yield had a higher DFS rate at 3 years, 65.0% (s.e. 6.9%), than those with no harvest, an intermediate or a high CD34+ yield: 46.7% (s.e. 6.2%), 50.4% (s.e. 4.6%) and 26.9% (s.e. 5.0%), respectively (Figure 1 and Table 4). The instantaneous event (relapse or death) rate in these last three groups was 1.56, 1.55 and 2.72, respectively, higher than in the first group ($P = 0.0002$). The cumulative incidence of relapse at 3 years was lower in the group with the low CD34+ yield (30.9%, s.e. 6.7%) compared to those with no harvest (47.5%, s.e. 6.2%), an intermediate (43.1%, s.e. 4.6%) and high CD34+ yield (71.9%, s.e. 5.2%) ($P = 0.00001$, Figure 2). The instantaneous risk of relapse was 1.58, 1.50 and 3.01 greater in these three groups, respectively, as compared with the group with the low CD34+ yield. The cumulative incidence of death in CR at 3 years was slightly lower in the group with high CD34+ yield than in the others: 1.1% *vs* 4.1% to 6.4%. The overall survival rate at 3 years from start of lenograstim was the lowest (42.9%, s.e. 5.7%) in the high CD34+ yield group and the highest (64.3%, s.e. 7.0%) in the low CD34+ yield group (overall $P = 0.07$). Using a value equal to zero for the no harvest group, the relationship between the highest CD34+ yield harvested on a single day and the DFS remained

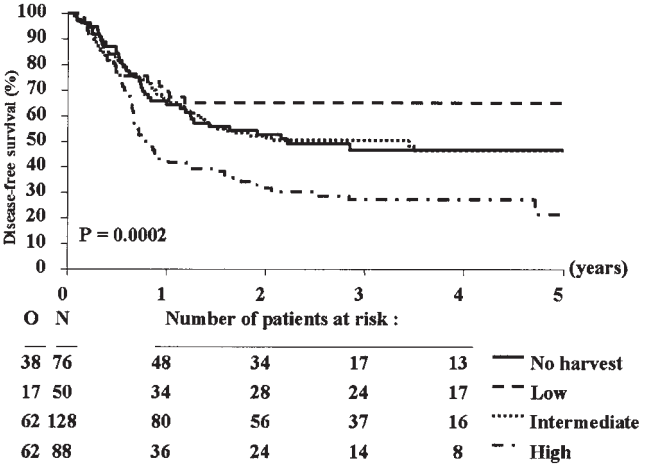

Figure 1 Disease-free survival from start of lenograstim according to CD34+ yield group. N, number of patients; O, observed number of events (relapse or death in first CR). *P* value given by the logrank test.

important by using the median (2.1 x 10^6/kg) as a cut-point ($P = 0.03$), or considering it as a continuous variable ($P = 0.002$) or the log of the continuous variable ($P = 0.001$) in a Cox model. The total of CD34+ yield also showed a strong relationship with the DFS, when one considers it is as a categorized variable, with cut-points at 2 and 10 x 10^6/kg ($P = 0.0007$), or as a continuous variable ($P = 0.004$).

Restricting the analysis to 266 patients who have been harvested, the highest CD34+ yield appeared to be highly predictive for the DFS, time to relapse and even for duration of survival (Table 4). Similar results have been obtained by

Table 4 Estimated rates or incidences at 3 years according to CD34+ yield group

Endpoint	*No harvest*	*Low yield*	*Intermediate yield*	*High yield*	*P value*[a]
Disease-free survival rate[b]	46.7 (±6.2)	65.0 (±6.9)	50.4 (±4.6)	26.9 (±5.0)	0.0002 (<0.0001)
Relapse incidence[c]	47.5 (±6.2)	30.9 (±6.7)	43.1 (±4.6)	71.9 (±5.2)	<0.0001 (<0.0001)
Death in CR incidence[c]	5.9 (±2.9)	4.1 (±2.9)	6.5 (±2.2)	1.1 (±1.1)	0.23 (0.11)
Survival rate[b]	55.3 (±6.0)	64.3 (±7.0	59.9 (±4.7)	42.9 (±5.6)	0.07 (0.014)

Data are expressed as percentage (±s.e.).
[a]*P* value given by the logrank test or Gray test; between brackets, *P* value given by the logrank test for linear trend or Gray test comparing the outcome of the three ordered CD34+ groups: low, intermediate and high yield.
[b]Estimate using the Kaplan–Meier method.
[c]Estimate of the cumulative incidence using the competing risk method.

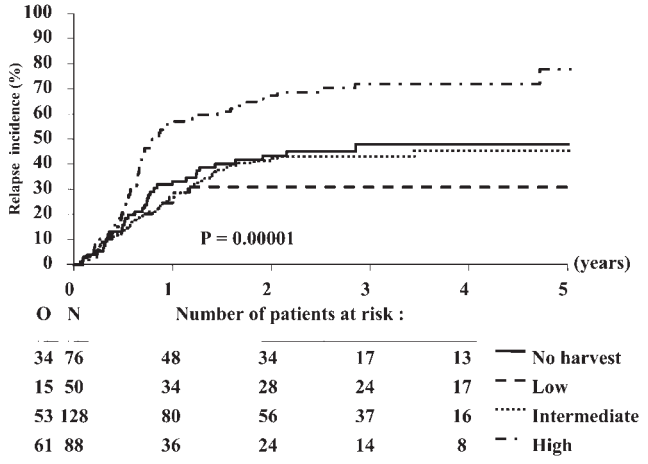

Figure 2 Cumulative incidence of relapse from start of lenograstim according to CD34+ yield group. N, number of patients; O, observed number of relapses. *P* value given by the Gray test.

considering the CD34+ yield as a continuous variable ($P = 0.003$) or the log of the continuous variable ($P = 0.001$).

Multivariate analyses for disease-free survival (DFS) and disease-free interval (DFI)

Prognostic factor analyses for the DFS revealed that patients in the cytogenetic good risk group had a better outcome than those with normal karyotype or –Y only (HR, 0.38; $P = 0.0005$). The following factors were associated with a poor outcome: cytogenetic bad risk group (HR, 1.74; $P = 0.004$), FAB subtype different from M2 and M4 (HR, 1.64; $P = 0.001$), CR reached after the second cycle of induction (HR, 1.76; $P = 0.06$), patients not randomized (HR, 1.33; $P = 0.07$), presence of diarrhea during consolidation (HR, 1.45; $P = 0.02$). A trend towards a worse prognosis was observed for those patients with a shorter duration of pancytopenia after the consolidation course, ie neutrophils $>1.5 \times 10^9$/l (≤ 22 *vs* 23–27 vs ≥ 28 days: $P = 0.04$) or platelet count $>100 \times 10^9$/l (≤ 28 *vs* >28 days: $P = 0.11$). Age ($P = 0.42$), WBC ($P = 0.22$), the type of intercalating agent used in the induction and consolidation treatment ($P = 0.35$) had no prognostic value. Patients with unknown cytogenetic analysis ($n = 99$) had a prognosis very similar (HR, 0.94; $P = 0.76$) to those with normal NN karyotype or –Y. The 3-year DFS rates for cytogenetic good, intermediate (NN or –Y) and bad risk groups were 74.1%, 41.6% and 24.9%, respectively, whereas for the group with an unknown cytogenetics it was 49.2%.

Multivariate Cox's proportional hazards model indicated that cytogenetic risk group (low *vs* intermediate *vs* bad *vs* unknown) was the most important prognostic factor ($P < 0.0001$). The Cox model stratified by cytogenetic risk group showed that the CD34+ yield group was the most important prognostic variable ($P = 0.005$) after the cytogenetic risk group. The only additional variable which might have been included was FAB type (HR, 1.30), but it was only marginally significant ($P = 0.095$). The estimated hazard ratios (95% confidence intervals) of the no harvest, intermediate and high CD34+ yield groups were 1.67 (0.94, 2.97), 1.59 (0.93, 2.73) and 2.46 (1.43, 4.23), respectively taking the low CD34+ yield group as reference, and stratifying by cytogenetics. Table 5 shows the joint prognostic importance of cytogenetics and CD34+ yield group using a proportional hazards Cox model. For the DFI analysis stratified by cytogenetic group, the estimated hazard ratios for the three CD34+ risk groups were 1.71 (0.93, 3.15), 1.55 (0.87, 2.76) and 2.77 (1.57, 4.90), respectively ($P = 0.0006$), and the cumulative incidences stratified for cytogenetics were different as well ($P = 0.0003$).

Table 5 Results of the Cox model regarding the disease-free survival where the two significant factors have been retained (cytogenetics and CD34+ yield group)

Variable	*Hazard ratio*[a]	*(95% CI)*	*P value*[b]
Cytogenetics			
Good risk	0.38	(0.22, 0.69)	0.0006
Intermediate	1		
Bad risk	1.62	(1.11, 2.36)	0.01
Unknown	0.89	(0.61, 1.32)	0.57
CD34 group			
No harvest	1.70	(0.96, 3.02)	0.07
Low yield	1		
Intermediate yield	1.60	(0.93, 2.74)	0.09
High yield	2.49	(1.45, 4.27)	0.001

CI, confidence interval.
[a]A value >1 indicates that the outcome is worse in that category as compared with the baseline.
[b]*P* value given by the Wald test.

Another parameterization of the CD34+ yield (continuous variable or its logarithmic transformation, categorical variable by using other cut-points) did not affect the conclusions of these analyses. Interestingly, a significant interaction ($P = 0.03$) between the highest CD34+ yield considered as a continuous variable and the cytogenetic risk group has been detected: the prognostic importance of the highest CD34+ yield regarding the DFS was quite low in bad-risk cytogenetic group (see Figure 3). The same lack of prognostic importance has been noted for the FAB subtype. On the contrary, in the remaining group of patients ($n = 261$) without bad cytogenetic features, univariate analyses showed that the CD34+ yield was of highly prognostic importance ($P = 0.0005$), cytogenetic group ($P = 0.0009$) and FAB ($P = 0.004$). The Cox model stratified by cytogenetic group (low, intermediate or unknown)

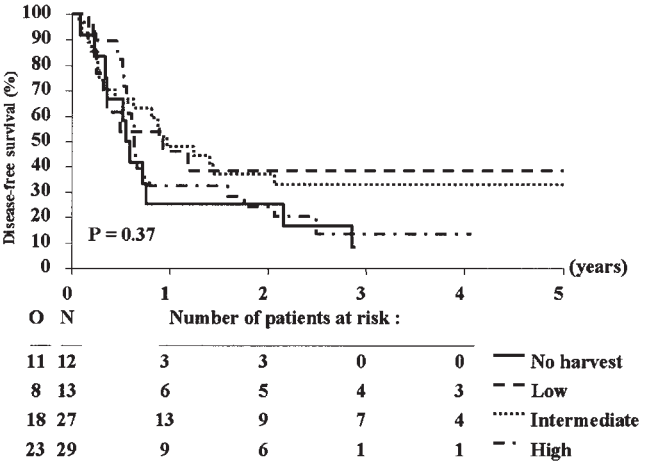

Figure 3 Bad risk cytogenetic subgroup: disease-free survival from start of lenograstim according to CD34+ yield group. N, number of patients; O, observed number of events (relapse or death in first CR). *P* value given by the logrank test.

Table 6 Patients without bad cytogenetic features

Variable	*Hazard ratio*[a]	*(95% CI)*	*P value*[b]
Cytogenetics			
Good risk	0.44	(0.25, 0.78)	0.0045
Intermediate	1		
Unknown	0.87	(0.58, 1.30)	0.49
CD34 group			
No harvest	1.69	(0.78, 3.63)	0.18
Low yield	1		
Intermediate yield	2.21	(1.07, 4.56)	0.03
High yield	3.52	(1.69, 7.31)	0.0007
FAB			
M2 or M4	1		
Other subtypes	1.41	(0.96, 2.08)	0.08

Results of the Cox model regarding the disease-free survival where the three factors (cytogenetics, $CD34^+$ yield group and FAB) have been considered.
CI, confidence interval.
[a]A value >1 indicates that the outcome is worse in that category as compared with the baseline.
[b]*P* value given by the Wald test.

showed that the hazard ratios of the no harvest, intermediate and high $CD34^+$ yield groups were 1.83 (0.86, 3.91), 2.09 (1.02, 4.28) and 3.53 (1.70, 7.33), respectively, taking the low $CD34^+$ yield group as reference ($P = 0.001$). The joint prognostic importance of cytogenetics, CD34 yield and FAB subtype (marginally significant) are shown in Table 6. Figure 4 shows the prognostic importance of the $CD34^+$ yield group in patients with low, intermediate or unknown cytogenetics.

Discussion

The present study was undertaken to test the prognostic significance of the capacity to mobilize hematopoietic stem cells after consolidation chemotherapy and lenograstim in patients with AML. This was based on the assumption that the mobilizing capacity after consolidation chemotherapy is a reflection of the size of the stem cell pool and that the latter may be inversely related with the sensitivity to chemotherapy. We found a relationship between the mobilizing capacity after intensive chemotherapy and lenograstim administration and the subsequent risk of relapse. Patients with the highest yield of $CD34^+$ cells ($\geq 7 \times 10^6$/kg) in a single apheresis had the highest relapse incidence and the shortest disease-free survival, whereas patients with the lowest number of $CD34^+$ cells ($<1 \times 10^6$/kg) in the harvest showed the longest disease-free survival. The strong prognostic importance of the $CD34^+$ yield remained valid in a multivariate analysis, after adjusting for possible confounding factors, such as cytogenetics[16,17] and FAB subtype. Thus, although a significant correlation existed between the $CD34^+$ yield and cytogenetics, the $CD34^+$ yield represents an important independent risk factor for time to relapse and DFS. If one distinguishes a very bad risk cytogenetic group containing those with –5/5q–, –7/7q–, 11q23, +8, t(9;22), 12p– or complex abnormalities, and/or considering other FAB grouping (M2 or M4E *vs* other subgroups) the results were extremely similar (data not shown). Different statistical analyses which have been performed regarding the highest $CD34^+$ or the total $CD34^+$ yield, considering them as a categorical or a continuous variables, all pointed in the same direction: the higher the $CD34^+$ cell count, the worse the prognosis. This appeared to be particularly true in patients without bad risk cytogenetic features.

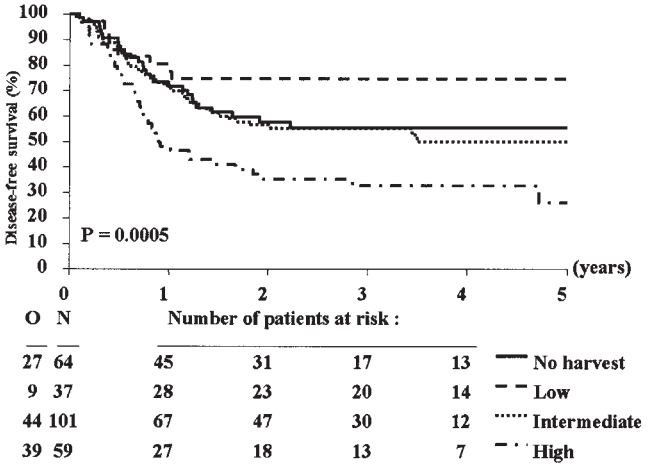

Figure 4 Low, intermediate or unknown cytogenetic risk subgroup: disease-free survival from start of lenograstim according to $CD34^+$ yield group. N, number of patients; O, observed number of events (relapse or death in first CR). *P* value given by the logrank test.

To explain this phenomenon we considered several possibilities. One hypothesis is that increasing numbers of leukemic cells present in the graft result in a higher relapse risk after transplantation.[18] Although we cannot exclude this possibility it is unlikely for several reasons. Firstly, the proportion of $CD34^+$ blast cells at diagnosis was not different between patients in the high $CD34^+$ yield and the low $CD34^+$ yield group. Secondly, dynamics of relapse in the $CD34^+$ high yield group was similar to the other groups within the first 5 months after the start of lenograstim, and only thereafter, especially within 6–12 months, there was a divergence of the DFS and relapse curves. These data suggest that the high relapse risk for patients in the high $CD34^+$ yield group was unlikely to be due, exclusively, to contamination with leukemic cells. 'Late' divergences of the DFS curves, between 1 and 2.5 years, have been observed in the previous AML-8A study, where patients randomized to received a second cycle of consolidation had a continuously higher risk of relapse than those randomized to an ASCT group.[4] Therefore an insufficient *in vivo* purging is a more likely explanation for the worse prognosis observed in the high yield group, although one may not exclude completely the first explanation.

The group with the highest $CD34^+$ yield had the shortest duration of neutropenia and thrombocytopenia after the consolidation course, whereas patients with the lowest $CD34^+$ yield exhibited the longest duration of pancytopenia. Thus, the low yield may reflect a more effective *in vivo* purging, resulting in less normal and leukemic stem cells. Conversely, the highest $CD34^+$ yield, which coincides with the shortest pancytopenia and the highest relapse risk, could be due to a relatively low sensitivity for chemotherapy of the leukemic, as well as the normal progenitor cells. Thus, the high $CD34^+$ counts in the harvest is associated with a high occult leukemia burden in the patient. A relationship with other organ toxicities could not be detected. These findings may be in line with a yet undetermined polymorphism of the hematopoietic progenitor cells for intracellular drug metabolism, resulting in reduced sensitivity for chemotherapy. The conditioning before transplantation did not result in a high eradication of leukemic cells, since the group of patients with the highest CD34 yield

had the highest and fastest relapse rate despite the highest percentage of performed autologous transplantation (88%).

The average relapse rate and disease-free survival and the longest lasting pancytopenia of the patients in whom the mobilization failed completely may be explained by the possibility that this group consists of a mixture of different kinds of patients. Some may be highly chemotherapy sensitive; the preceding consolidation course results in an extended bone marrow aplasia with a prolonged neutropenia and thrombocytopenia and a very low relapse risk. Some other patients may have persisting leukemia in the bone marrow which, for unknown reasons, prevents the normal bone marrow repopulation, blood cell recovery and $CD34^+$ mobilization. Attempts to discriminate the prognosis of the no harvest patients according to the duration of neutropenia or thrombocytopenia failed so far (data not shown). The trend towards a poorer prognosis of the patients with no harvest as compared with those with a low $CD34^+$ yield may also be explained by a difference in their subsequent transplantation rate (36% *vs* 64%). These patients tended to be older (median, 50 years); however age *per se* did not appear to be of prognostic importance in this study.

We conclude that mobilization of high numbers of $CD34^+$ cells after intensive consolidation treatment is an independent poor prognostic factor. We hypothesize that this may be explained by a genetic polymorphism that determines the sensitivity for chemotherapy. This observation may have implications for additional treatment before and/or after transplantation for this subgroup with a high risk of relapse, especially in those with an intermediate cytogenetic risk group. Other treatment modalities, such as immunotherapy or allogeneic stem cell transplantation, should also be considered.

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