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Phase III study of all-*trans* retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia

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The purpose of our study was (i) to evaluate the impact of all-*trans* retinoic acid (ATRA) given as adjunct to chemotherapy and (ii) to compare second consolidation *vs* maintenance therapy in elderly patients with acute myeloid leukemia (AML). A total of 242 patients aged ⩾61 years (median, 66.6 years) with AML were randomly assigned to ATRA beginning on day +3 after the initiation of chemotherapy (ATRA-arm, $n=122$) or no ATRA (standard-arm, $n=120$) in combination with induction and first consolidation therapy. A total of 61 patients in complete remission (CR) were randomly assigned to second intense consolidation ($n=31$) or 1-year oral maintenance therapy ($n=30$). After induction therapy the *intention-to-treat* analysis revealed a significant difference in CR rates between the ATRA- and the standard-arm (52 *vs* 39%; $P=0.05$). Event-free (EFS) and overall survival (OS) were significantly better in the ATRA-compared to the standard-arm ($P=0.03$ and 0.01, respectively). OS after second randomization was significantly better for patients assigned to intensive consolidation therapy ($P<0.001$). The multivariate model for survival revealed lactate dehydrogenase, cytogenetic risk group, age, and first and second randomization as prognostic variables. In conclusion, the addition of ATRA to induction and consolidation therapy may improve CR rate, EFS and OS in elderly patients with AML.
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Introduction

The intensification of induction and postremission therapy has improved the outcome of younger patients with acute myeloid leukemia (AML). However, in patients over the age of 60 years treatment results have been consistently inferior with complete remission (CR) rates of 40–60% and only 10–20% of patients surviving 4 years.[1–7] The differences in response and outcome are multifactorial. There is an increase of unfavorable biological characteristics of the disease in elderly patients, such as higher proportion of unfavorable karyotypes,[8,9] higher frequency of multidrug resistance mediator P-glycoprotein expression,[10] and higher frequency of antecedent hematologic disorders or of previous treatment for another malignancy.[11]

High levels of the antiapoptotic protein bcl-2 in AML blasts were reported to be associated with an unfavorable outcome after chemotherapy.[12–15] Thus, bcl-2 represents another potential target in AML treatment. *In vitro* data suggested that the addition of retinoids, for example, all-*trans* retinoic acid (ATRA), to cultures of AML blasts in combination with cytarabine or idarubicin increased killing of clonogenic cells by down-regulation of bcl-2.[16–21] The sequence may be important when combining ATRA with cytotoxic drugs: synergistic effects were only seen when ATRA was administered after cytotoxic drug exposure, whereas preincubation with ATRA could even inhibit cytotoxic drug-induced cell killing.[16,17,21,22]

These *in vitro* data were supported by results from one clinical trial. In a small phase II study, Venditti *et al*[23] showed that the combination of low-dose cytarabine given subcutaneously in combination with ATRA resulted in a remarkably high CR rate in a poor prognosis AML patient population.

In 1998, we initiated a randomized trial to assess the effect of ATRA given in combination with age-adapted induction[24] and consolidation chemotherapy on CR rate, event-free survival (EFS) and overall survival (OS) in elderly patients with AML. Based on the *in vitro* data, ATRA was applied after exposure to the cytotoxic drugs.

Methods

Patients

Patients 61 years or older with *de novo* AML or refractory anemia with excess of blasts in transformation (RAEB-t) defined by the French–American–British (FAB) classification system,[25] secondary AML (s-AML) with a preceding history of myelodysplasia at least 3 months before the diagnosis of AML, or therapy-related AML following treatment of primary malignant disease (t-AML) were eligible for entry into the trial. Patients with acute promyelocytic leukemia (APL), patients with concomitant liver, renal or cardiac disease and patients with a performance status WHO >2 were not included. Chromosome banding analysis using standard methods was performed centrally for all patients in the Laboratory for Cytogenetic and Molecular Diagnostics of the AML Study Group Ulm (AMLSG ULM). The description of

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the karyotype followed the recommendations of the International System for Human Cytogenetic Nomenclature.[26] Written informed consent was obtained at study entry. The study was approved by the local Ethics Review Committees.

First and second randomization

Patients were randomized via a telephone call to the AMLSG ULM study office to either induction and consolidation chemotherapy without ATRA (standard-arm) or to the same chemotherapy plus ATRA (ATRA-arm). The induction therapy consisted of ICE (idarubicin 12 mg/m^2 i.v. days 1 and 3, cytarabine 100 mg/m^2 cont. i.v. days 1–5, etoposide 100 mg i.v. days 1 and 3) or the same chemotherapy plus ATRA (A-ICE) started after administration of idarubicin and etoposide on day 3 at a dosage of 45 mg/m^2 from day 3 to 5 and 15 mg/m^2 from day 6 to 28. Dose reduction of ATRA was carried out to avoid potential toxicities such as pancreatitis, hepatitis or cheilitis.[27] Patients achieving CR or partial remission (PR) received a second induction cycle ICE or A-ICE at the same dosage. Patients with refractory disease (RD) after first induction therapy were assigned to a second induction therapy with A-HAE (cytarabine 0.5 g/m^2/12 h i.v. days 1–3, etoposide 250 mg/m^2 i.v. days 4 and 5, ATRA 45 mg/m^2 days 3–5 and 15 mg/m^2 days 6–28). Bone marrow evaluation as well as start of the second cycle was scheduled between days 28 and 35. All patients in CR following two cycles of induction therapy were assigned to a first consolidation therapy with HAM (cytarabine 0.5 g/m^2/12 h i.v. days 1–3, mitoxantrone 10 mg/m^2 i.v. days 2 and 3) or A-HAM (along initial randomization) including ATRA at a dosage of 15 mg/m^2 from day 3 to 28. Allogeneic transplantation was allowed for patients with an HLA-identical family donor on the decision of the local investigator. For conditioning, a combination of fludarabine, cyclophosphamide, idarubicin and etoposide (FCIE) was recommended.[28] Second randomization was performed after completion of first consolidation therapy for patients in CR. Patients were randomized to either a second intensive consolidation therapy IEiv (idarubicin 12 mg/m^2 i.v. days 1 and 3, etoposide 100 mg/m^2 i.v. days 1–5) or to a 1-year oral maintenance therapy IEpo (idarubicin 5 mg p.o. days 1, 4, 7, 10, 13, etoposide 100 mg p.o. days 1 and 13; repeated on day 29 for 12 courses).

Criteria for response and definition of relapse

Response assessment during induction therapy was performed at two time points and was defined differently. The first time point was between days 28 and 35 after first induction therapy. AML patients either in CR[29] or in PR and RAEB-t with a $\geqslant$50% reduction of bone marrow blasts compared to the pretreatment status were considered as responders to the first induction cycle and were eligible for the second cycle. The second time point of response assessment was after two cycles of induction therapy. Response was defined by achievement of CR.[29] Causes of therapeutic failure were RD or death during induction therapy (early death: ED) or induced bone marrow hypoplasia (hypoplastic death: HD). Relapse was defined as a marrow with >5% blasts unrelated to recovery of blood counts from preceding course of chemotherapy or new extramedullary leukemia in patients with previously documented CR.

Statistical analysis

The primary end point for first randomization was achievement of CR after two cycles of induction therapy; secondary end points were EFS, OS, cumulative incidence of relapse (CIR) and toxicity related to ATRA. The study sample size of $n=242$ patients for the first randomization was calculated to detect a 0.2 difference in CR rate between the null hypothesis that both group proportions are 0.5 and the alternative hypothesis that the proportion in the ATRA-arm is 0.7 based on a one-sided χ^2 test with continuity correction having a power of 80% and type I error of 5%. The interim analysis in 2001 after randomization of 242 patients between the standard-arm and the ATRA-arm revealed on a *per-protocol* basis no difference in CR rate between the two arms. According to the protocol, it was decided that all further patients received the standard-arm either until the required sample size of 100 patients for second randomization was achieved or rules of stopping recommended termination of second randomization. The presented data include the final analysis of first randomization and the interim analysis of the second randomization. Based on these results, second randomization was stopped in April 2003. Focus of this report is the analysis of the 242 patients randomized for the first part of the study.

For the assessment of first randomization, EFS and OS were measured from study entry. For EFS, events were defined by ED/HD during induction therapy (failure), no CR after two cycles of induction therapy (failure), relapse (failure), death in CR (failure), allogeneic transplantation in CR (censored) and alive in CR at last follow-up (censored). OS times of patients being alive at last follow-up or at the date of allogeneic transplantation were censored. The analyses of CIR and the cumulative incidence of death in CR (CID) included only patients achieving CR with time calculated from date of CR until event (relapse or death in CR). CIR, CID, their standard errors (s.e.) and differences between groups were estimated using the method of Gray.[30] The median duration of follow-up was calculated according to the method of Korn.[31] Survival distributions were compared using the log-rank test. For multivariate analysis, an extended Cox model was used including the second randomization as time-dependent covariate.[32] The statistical analyses were performed with the statistical software packages SAS (SAS Institute Inc., Cary, NC, USA) and R, version 1.6.2[33] together with the Design software library.[34]

Results

Accrual of patients

Between February 1998 and September 2001, 253 patients were registered for the study. Of these patients, 10 had concomitant disease (heart failure, $n=5$; other cancer, $n=3$; hepatic insufficiency, $n=1$; renal failure, $n=1$) and one patient was not randomized due to thrombocytopenia unresponsive to transfusions. Thus, 242 patients were randomized, 120 to the standard-arm and 122 to the ATRA-arm. Table 1 shows the distribution of clinical parameters by up-front treatment assignment. The trial is summarized in the flow diagram in Figure 1.

Induction and first consolidation therapy

Response: Response to the first cycle of induction is summarized in Table 2. There was a significantly higher

Table 1 Distribution of factors by up-front treatment assignment

	ICE (n = 120)	*ATRA-ICE (n = 122)*	*P-value*
Sex (no. of patients)			
Male	62	66	0.79
Female	58	56	
Age (years)			
Median	65.8	66.7	0.61
Range	61–84.5	61–78	
Type of AML (no. of patients)			
De novo	83	82	0.81
t-AML	15	12	
s-AML	17	22	
RAEB-t	5	6	
WBC count (10^9/l) (n = 240)			
Median	6.5	5.7	0.73
Range	0.4–210	0.4–303	
LDH (U/l) (n = 232)[a]			
Median	343	369	0.87
Range	83–3953	113–2347	
Platelet count (10^9/l) (n = 240)			
Median	65	57	0.63
Range	6–435	4–445	
Cytogenetic risk group[b] *(no. of patients)*			
Low-risk	7	7	0.70
Intermediate-risk	79	84	
High-risk	14	12	
Not evaluable	20	19	

[a]Upper limit of normal 240 U/l.
[b]According to MRC criteria.[35]

response rate including PR and CR in favor for the ATRA-arm with 60% compared to the standard-arm with 43.5% (Fisher's exact test, $P = 0.01$, estimated OR 1.94, 95% CI 1.13–3.36). Of 52 patients assigned to a second ICE in the standard-arm, 46 received a second induction therapy (ICE, $n = 44$; other intensive therapy, $n = 2$) resulting in 42 CRs, one death and three RDs. Of 73 patients assigned to a second A-ICE in the ATRA-arm, 68 received a second induction therapy (A-ICE, $n = 66$; other intensive therapy, $n = 2$) resulting in 55 CRs, one death and 12 RDs. Of the 86 patients with resistant disease after the first induction cycle, 33 patients in the standard-arm (A-HAE, $n = 22$; other intensive therapy, $n = 11$) and 22 patients in the ATRA-arm (A-HAE, $n = 16$; other intensive therapy, $n = 6$) had intensive salvage therapy: after salvage therapy, there were four CRs, three deaths and 26 RDs in the standard-arm, and eight CRs and 14 RDs in the ATRA-arm. Following two cycles of induction therapy excluding patients receiving an allogeneic transplantation after first induction therapy ($n = 4$), CR rates analyzed on an *intention-to-treat* basis were 39% (46/117) in the standard-arm and 52% (63/121) in the ATRA-arm (Fisher's exact test, $P = 0.05$; estimated OR 1.67, 95% CI 0.97–2.90).

For first consolidation therapy, 36 and 50 patients received the assigned treatment in the standard-arm and the ATRA-arm, respectively.

Toxicity: In the ATRA-arm, five and four patients intermittently discontinued ATRA during first and second induction therapy, respectively; there was no case of ATRA discontinuation during first consolidation therapy. The causes of ATRA discontinuation were fever, weight gain, exanthema and edema, appearing in all cases at the same time with the onset of infection. None of the patients receiving ATRA developed pancreatitis, hepatitis, cheilitis or an ATRA syndrome. There was no difference in toxicity and supportive care for induction and first consolidation therapy between the two treatment arms. The results for the first induction cycle are summarized in Table 3.

Allogeneic transplantation and second randomization

Of the 242 randomized patients, 11 received dose-reduced conditioning followed by allogeneic transplantation from an HLA-identical family donor. Seven patients were transplanted in first CR and four patients were transplanted with refractory AML. Two of the latter patients achieved a CR after transplantation. Two patients died from treatment-related toxicity (cardiac failure, aspergillus pneumonia) and two patients relapsed 10 and 11 months after transplantation. Five patients are in continuous CR ranging from 18 to 101 months from the time of transplantation.

A total of 61 patients in CR after first consolidation were randomized between a second intensive consolidation therapy with IEiv ($n = 31$) and a 1-year maintenance therapy with IEpo ($n = 30$). Baseline characteristics of the 61 randomized patients were well balanced between the two arms.

Analysis of survival and cumulative incidence of relapse

The estimated median follow-up time for OS was 34 months. Out of the 242 patients, 204 have died. The median OS time for the entire group was 10 months (95% CI 7.4–11.9 months) and OS after 34 months was 14% (95% CI 10–20%). For the 115 patients who entered CR, CIR after 34 months was 80% (s.e. 4.1%). The total number of administered intensive chemotherapy cycles within or outside the protocol was a median of 3 cycles (1–8) with no difference between the two treatment arms (ATRA-arm, standard-arm). The comparison of EFS distributions according to first randomization on the *intention-to-treat* principle showed a statistically significant benefit ($P = 0.03$) for the ATRA-arm compared to the standard-arm. This led to a statistically significant better OS ($P = 0.01$) with estimated median survival times of 11.3 and 7.0 months in the ATRA- and the standard-arm, respectively (Figure 2). There were no statistically significant differences in CIR ($P = 0.35$) or CID ($P = 0.27$) between the two treatment arms. End points for second randomization were survival and CIR beginning from date of randomization. On the *intention-to-treat* principle, there was a statistically significant reduced CIR for patients randomized to a second intensive consolidation therapy compared to maintenance therapy ($P = 0.002$) with a CIR at 12 months after randomization of 39% (s.e. 9%) for the IEiv-arm and 80% (s.e. 8%) for the IEpo-arm translating into a significantly ($P < 0.001$) better survival for patients randomized to a second intensive consolidation therapy.

Multivariate analysis for survival included all patients ($n = 242$) examined, Log(WBC), Log(LDH), age, bone marrow blasts, platelet count, cytogenetic group according to the MRC classification,[35] type of AML (*de novo vs* s-AML/t-AML or RAEB-t), first randomization and second randomization as time-dependent covariates. This analysis revealed LDH ($P = 0.005$), cytogenetic group (high-risk *vs* others) ($P = 0.005$), age ($P = 0.007$), first randomization ($P = 0.01$) and second randomization ($P < 0.001$) as statistically significant prognostic variables (Table 4).

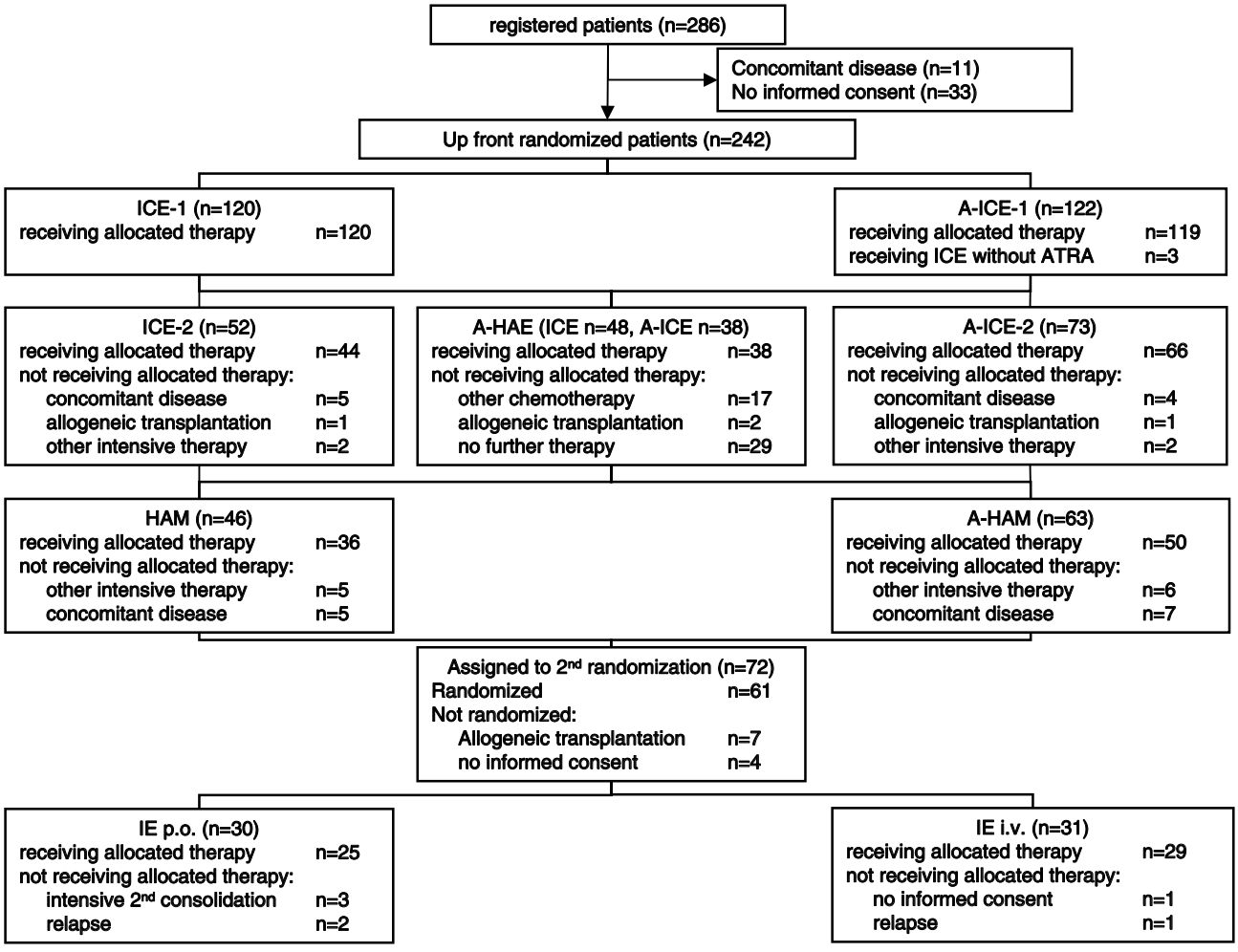

Figure 1 Summary of the AML HD98B treatment trial.

Table 2 Response to first induction therapy

	ICE (n = 120)	*ATRA-ICE (n = 122)*	
CR	33 (27.5%)	46 (38%)	
PR	19 (16%)	27 (22%)	
CR and PR	52 (43.5%)	73 (60%)	$P = 0.01$
RD	48 (40%)	38 (31%)	
ED/HD	20 (16.5%)	11 (9%)	

Discussion

The principle conclusion of this study is that the addition of ATRA to standard induction and consolidation therapy may improve response rate, EFS and OS in elderly patients with AML. When considering CR and PR after the first induction therapy, there was a significantly higher response rate in the ATRA-arm, indicating a higher initial bone marrow blast clearance (Table 2). This higher initial response may have led to the significantly higher CR rate in the ATRA-arm following two cycles of induction therapies. This difference could be demonstrated in an intention-to-treat analysis – also taking into account further intense treatment outside the protocol – but not in a per-protocol analysis. Therefore, the addition of ATRA to first induction therapy seems to be crucial for this beneficial effect since the results of the ATRA containing salvage regimen (A-HAE) were disappointing. The rationale for the addition of ATRA was to increase the sensitivity of leukemic cells for the effect of the

Table 3 Toxicity and supportive care requirements during induction course 1 by up-front treatment assignment

	ICE (n = 120)	*ATRA-ICE (n = 122)*
Hematological toxicity		
Median duration of neutropenia $<0.5 \times 10^9$/l (days)	19	19
Median duration of thrombopenia $<20 \times 10^9$/l (days)	16	15
Nonhematological toxicity, WHO grade III/IV (no.)		
Hemorrhage	2	2
Infection	36	34
Diarrhea	8	15
Cardiac failure	4	3
Pulmonary failure	9	7
Nausea/vomiting	8	11
Supportive care		
Units of packed red cells, median	8	9
Units of random platelets, median[a]	32	28

[a]HLA-matched platelet units were counted as 16 random platelet units.

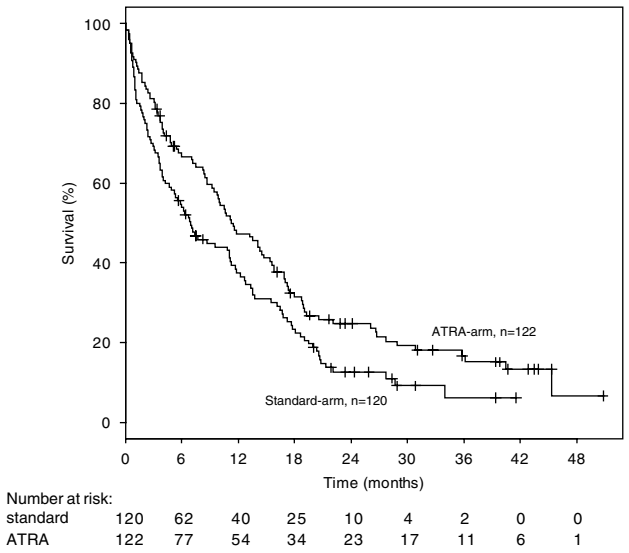

Figure 2 OS for the up-front randomized treatment arms; the difference between the curves was statistically significant ($P = 0.01$).

cytotoxic drugs. *In vitro*, such a sensitizing effect had been shown for the antileukemic agents cytarabine and idarubicin,[16,19,21,22] which were both administered in induction therapy of the current study. The initial *in vitro* data suggested that ATRA may exert its effect by downregulation or by post-translational modification of the antiapoptotic protein bcl-2.[21,36] More recently, further proteins, for example, survivin as a member of the inhibitors of apoptosis family, have been described as potential targets of ATRA.[37]

There have been three other trials evaluating ATRA in non-APL AML (Burnett *et al*, *Blood* 2002; **100**: 582; abstract).[38,39] In the two randomized trials, there was no significant effect on survival; however, there are relevant differences among the trials with respect to patient selection and scheduling of ATRA along with the cytotoxic drugs. Estey *et al* studied a high-risk population, including patients over the age of 70 years (32%), with s-AML (66%) and t-AML (26%). Interestingly, in the study of Estey *et al*, univariate analysis revealed a statistically significant better OS in the two arms containing ATRA, whereas multivariate analysis suggested no effect of ATRA. This phase II trial with four treatment arms with 53 patients in each arm had a significantly lower statistical power to detect differences in survival compared to our phase III study. Multivariate analysis in our phase III study indicated a statistically significant benefit for ATRA on survival, which was independent of the effect of second randomization. In the MRC-12 trial, ATRA was evaluated in younger AML patients (up to the age of 55 years); the analysis may be complicated by the multifactorial design of the entire trial evaluating in parallel the impact of autologous transplantation and the impact of a fifth cycle of treatment. A second and potentially more important issue relates to the scheduling of ATRA: in the study reported by Estey *et al,* ATRA was commenced at day −2, that is, prior to the start of induction chemotherapy, and in the MRC-12 trial as well as the study reported by Bolanos-Meade *et al*[39] ATRA was started simultaneously with chemotherapy. In contrast, in our study, ATRA was administered at day +3, that is, at a time point where a significant proportion of the cytotoxic drugs had already been administered to the patient. This schedule was based on *in vitro* data showing that the sensitizing effect of ATRA was only seen when ATRA was given after the cytotoxic drugs.[16,19,21,22] Thus, the initial higher blast clearance as seen in our study using such a time-sequential schedule may parallel the *in vitro* observations.

Table 4 Multivariate model for survival ($n = 242$)

	Hazard ratio	*95% CI*
LDH (difference of 100 U/l)	1.16	1.05–1.28
Age (difference of 5 years)	1.19	1.05–1.36
High-risk cytogenetics	1.84	1.20–2.83
Assigned treatment with ATRA	0.71	0.55–0.93
Assigned treatment with IE i.v.	0.32	0.21–0.48

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