Clinical Impact of Additional Cytogenetic Aberrations, *cKIT* and *RAS* Mutations, and Treatment Elements in Pediatric t(8;21)-AML: Results From an International Retrospective Study by the International Berlin-Frankfurt-Münster Study Group

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose

This retrospective cohort study aimed to determine the predictive relevance of clinical characteristics, additional cytogenetic aberrations, and *cKIT* and *RAS* mutations, as well as to evaluate whether specific treatment elements were associated with outcomes in pediatric t(8;21)-positive patients with acute myeloid leukemia (AML).

Patients and Methods

Karyotypes of 916 pediatric patients with t(8;21)-AML were reviewed for the presence of additional cytogenetic aberrations, and 228 samples were screened for presence of *cKIT* and *RAS* mutations. Multivariable regression models were used to assess the relevance of anthracyclines, cytarabine, and etoposide during induction and overall treatment. End points were the probability of achieving complete remission, cumulative incidence of relapse (CIR), probability of event-free survival, and probability of overall survival.

Results

Of 838 patients included in final analyses, 92% achieved complete remission. The 5-year overall survival, event-free survival, and CIR were 74%, 58%, and 26%, respectively. *cKIT* mutations and *RAS* mutations were not significantly associated with outcome. Patients with deletions of chromosome arm 9q [del(9q); n = 104] had a lower probability of complete remission ($P = .01$). Gain of chromosome 4 (+4; n = 21) was associated with inferior CIR and survival ($P < .01$). Anthracycline doses greater than 150 mg/m² and etoposide doses greater than 500 mg/m² in the first induction course and high-dose cytarabine 3 g/m² during induction were associated with better outcomes on various end points. Cumulative doses of cytarabine greater than 30 g/m² and etoposide greater than 1,500 mg/m² were associated with lower CIR rates and better probability of event-free survival.

Conclusion

Pediatric patients with t(8;21)-AML and additional del(9q) or additional +4 might not be considered at good risk. Patients with t(8;21)-AML likely benefit from protocols that have high doses of anthracyclines, etoposide, and cytarabine during induction, as well as from protocols comprising cumulative high doses of cytarabine and etoposide.

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INTRODUCTION

Core binding factor (CBF) acute myeloid leukemia (AML), which includes inv(16)(p13.1q22)/t(16;16)(p13.1;q22)/*CBFB-MYH11* and t(8;21)(q22;q22)/*RUNX1-RUNX1T1* (t(8;21)-AML), is associated with

relatively favorable outcome.¹ Although CBF-AML is a well-known cytogenetic subgroup, separate stratification of t(8;21) and inv(16) has been suggested because of their different, clinically distinct entities.^{2,3}

Although overall survival (OS) rates for pediatric patients with t(8;21)-AML specifically may reach

up to 90%,⁴ many patients continue to experience relapse. **Event-free survival** (EFS) rates seldom exceed 50% to 60%, and it has been suggested that event-free or disease-free survival might not be better than for other subgroups of AML.^{5,6} For CBF-AML, including t(8;21), high WBC count at diagnosis is often associated with an inferior outcome.^{7,8} Patients who have higher blast percentages,⁹ extramedullary or CNS involvement,^{7,10} or older age reportedly have inferior outcomes also.^{2,7,8} The prognostic impact of commonly occurring additional cytogenetic aberrations has been variably described.¹⁰⁻¹³

The different outcomes among study groups may be explained by clinical features or by the treatment schedules or doses of important agents (eg, cytarabine, anthracyclines, etoposide). Evidence is lacking to unequivocally interpret single-agent-specific effects in this cytogenetic subgroup of patients, so the collaborative International Berlin-Frankfurt-Münster Study Group aimed to study the predictive relevance of patient- and leukemia-specific characteristics and to study different treatment elements in children with t(8;21)-AML.

PATIENTS AND METHODS

Data from patients with t(8;21)-AML were retrospectively collected from 19 pediatric AML study groups between 1988 and 2010. Data on diagnostics, treatment, response, events, survival, and follow-up were gained with specifically designed forms. The study was approved by institutional review boards of the participating study groups.

Cytogenetic and Molecular Analyses

Cytogenetic reports of each patient were submitted to the coordinating Dutch Childhood Oncology Group. All reports were centrally reviewed and **karyotypes** were described according to the International System for Human Cytogenetic Nomenclature (2009).¹⁴ Data from patients without proof of t(8;21) presence and patients with absent or incomplete karyotypes were excluded from analyses.

Aberrations arbitrarily occurring in 10 or more patients were regarded sufficiently recurrent to define an additional cytogenetic subgroup. Karyotype complexity was determined by counting the number of unrelated chromosomal abnormalities within the karyotypes (≥ 3 or ≥ 5 abnormalities).

Fig 1. Study enrollment. AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; AML, acute myeloid leukemia; AML1-ETO, RUNX1-RUNX1T1; BFM-A, Berlin-Frankfurt-Münster Study Group Austria; BFM-G, Berlin-Frankfurt-Münster Study Group Germany; CCG, Children's Cancer Group; CCR, continued complete remission; CPH, Czech Paediatric Hematology Working Group; CR, complete remission; CR1, first complete remission; EORTC-CLG, European Organization for Research and Treatment of Cancer-Children's Leukemia Group; FISH, fluorescent in situ hybridization; GATLA, Grupo Argentino de Tratamiento de la Leucemia Aguda; Hong Kong, Hong Kong Paediatric Haematology and Oncology Study Group; JPLSG, Japanese Paediatric Leukemia/Lymphoma Study Group; LAME, Leucémies Aiguës Myéloblastiques de l'Enfant Cooperative Group; MRC, Medical Research Council; NOPHO, Nordic Society of Paediatric Haematology and Oncology; PINDA, National Program for Antineoplastic Drugs for Children; POG, Pediatric Oncology Group; PPLLSG, Polish Pediatric Leukemia Lymphoma Study Group; Russia, Russian AML Study Group; St Jude, St Jude Children's Hospital; Turkey, separate Turkish data provided by Professor Adalet Meral Günes, Uludag University, Bursa, Turkey.

Table 1. Demographic and Clinical Characteristics of Pediatric Patients With t(8;21) and Full Karyotype

Characteristic*	No./Total No. (%) of Patients†
Year of diagnosis	
1988-1997	352/838 (42.0)
1998-2010	486/838 (58.0)
Sex	
Male	495/838 (59.1)
Female	343/838 (40.9)
Mean $\pm$ SD age, years	9.2 $\pm$ 4.2
< 12	552/838 (65.9)
$\geq$ 12	286/838 (34.1)
Mean (IQR) WBC at diagnosis, $\times 10^9/L$	13.6 (6.9-28.5)
≤ 20	525/832 (63.1)
> 20	307/832 (36.9)
Mean $\pm$ SD hemoglobin, g/dL	7.7 $\pm$ 2.6
Median (IQR) platelets, $\times 10^9/L$	36 (18-69)
Mean $\pm$ SD BM blasts, %	57 $\pm$ 22
< 60	394/756 (52.1)
≥ 60	362/756 (47.9)
FAB classification	
M2	660/814 (81.1)
M other	154/814 (18.9)
CNS involvement	52/669 (7.8)
Cytogenetic additional aberrations	
≥ 1 additional aberration	573/838 (68.4)
Loss of sex chromosome	381/573 (66.5)
Deletion chromosome 9q	104/573 (18.2)
Gain of chromosome 8	49/573 (8.6)
Abnormalities chromosome 7q	41/573 (7.2)
Gain of chromosome 4	21/573 (3.7)
Complex karyotype (≥ 3 aberrations)	207/838 (24.7)
Complex karyotype (≥ 5 aberrations)	22/838 (2.6)
Molecular aberrations	
Available for mutational analysis	228/838 (27.2)
cKIT mutation present	53/222 (23.9)
Exon 8	8/53 (15.1)
Exon 10	3/53 (5.7)
Exon 17	42/53 (79.2)
RAS mutation present	29/188 (15.4)
N-RAS	17/29 (58.6)
K-RAS	5/29 (17.2)
Unknown	7/29 (24.1)
Induction regimen	
Course 1	
Anthracycline > 150 mg/m ²	244/838 (29.1)
Cytarabine $> 1,400$ mg/m ²	243/838 (29.0)
Etoposide > 500 mg/m ²	270/838 (32.2)
Courses 1 and 2	
Anthracycline > 300 mg/m ²	99/838 (11.8)
Cytarabine $> 5,000$ mg/m ²	267/838 (31.9)
HD cytarabine 3 g/m ² †	236/838 (28.3)
Etoposide $> 1,000$ mg/m ²	261/838 (31.1)
Overall treatment	
Anthracycline > 400 mg/m ²	371/838 (44.3)
Cytarabine > 30 g/m ²	323/838 (38.5)
Etoposide $> 1,500$ mg/m ²	389/838 (46.4)
Allo-SCT in first complete remission	104/760 (13.7)
Maintenance	198/838 (23.6)

(continued in next column)

Table 1. Demographic and Clinical Characteristics of Pediatric Patients With t(8;21) and Full Karyotype (continued)

Characteristic*	No./Total No. (%) of Patients†
Outcome	
Median (IQR) follow-up time, years	5.7 (2.6-9.4)
Complete remission after two courses	767/838 (91.5)
Relapse	227/838 (27.1)
Death	228/838 (27.2)
Patients with event(s)	355/838 (42.4)

Abbreviations: allo-SCT, allogeneic stem-cell transplantation; AML, acute myeloid leukemia; BM, bone marrow; FAB, French-American-British classification of AML; HD, high dose; IQR, interquartile range; SD, standard deviation.

*Mean values $\pm$ SD are presented for normally distributed data; otherwise, median values (IQR) are presented.

†Percentages may not add up to 100% because of rounding.

‡HD was defined as 3 g/m².

Genomic DNA was isolated from cytospin slides, frozen cell pellets, or cells cryopreserved in liquid nitrogen for mutational analysis. Available samples ($n = 228$) were screened for *cKIT*, *N-RAS*, and *K-RAS* mutations by high-resolution-melting analysis. Samples with aberrant profiles were validated by Sanger sequencing as previously described.^{15,16} This method implies that mutations are designated positive only when they were confirmed by sequencing, which has a sensitivity of approximately 20%.

Therapy

Induction was defined as the first two courses of chemotherapy. Except for one protocol, the treatment protocols consisted of an anthracycline- and cytarabine-based induction with or without other agents, such as etoposide. The prognostic impact of both induction and cumulative doses of anthracyclines, etoposide, and cytarabine were evaluated. Combinations of these agents were always assimilated in the multivariable models. To overcome the widely ranged doses within the different protocols, commonly used or the most discriminating cutoff values for these agents were determined (Appendix Table A1, online only). Patients who were not treated according to their applicable protocols were excluded from the final analyses. Allogeneic stem-cell transplantation (SCT) was evaluated for protocols that contained first-line SCT in first complete remission (CR1) only.

End Points

Outcomes were evaluated by using the probabilities of complete remission (pCR), cumulative incidence of relapse (CIR), probabilities of EFS (pEFS), and probabilities of OS (pOS; Appendix Table A2, online only).

Statistics

Mutual univariable analyses of characteristics were conducted by using the Wilcoxon test for quantitative variables and Fisher's exact test or the χ^2 test for qualitative variables. To identify factors associated with pCR, variables significant at $P < .10$ in univariable analyses and elements of induction treatment entered a logistic regression model with limited backward elimination ($\alpha < .05$). Results were presented as odds ratios and 95% CIs.

The Kaplan-Meier method was used to estimate survival probabilities, and associations with covariates were tested by the log-rank test. Cox proportional hazard regression analyses were used to assess effects of covariates on pOS and pEFS. Variables significant at $P < .10$ in univariable analyses entered an explorative multivariable Cox regression model. After a backward elimination procedure, the remaining important covariates were used in a multivariable model to assess the effects of induction elements (induction model) and overall therapy elements (overall model). To overcome multicollinearity, the most relevant course per agent entered the induction model.

For CIR, subdistributional hazards were calculated to correct for death or secondary malignancies as competing events for relapse. The Fine and Gray multivariable regression model, with similar conditions as the survival models,

Fig 2. Outcomes for patients with t(8;21) and significantly important additional cytogenetic aberrations. (A) Complete remission (CR) rates for patients with del(9q) [n = 104] or without del(9q) [n = 734]. (B) Cumulative incidence of relapse for patients with gain of chromosome 4 (n = 21) or without gain of chromosome 4 (n = 817). (C) Event-free survival for patients with gain of chromosome 4 (n = 21) or without gain of chromosome 4 (n = 817). (D) Overall survival for patients with gain of chromosome 4 (n = 21) or without gain of chromosome 4 (n = 817).

was used to assess the effects of both induction therapy and overall therapy on CIR. Estimates for (subdistributional) hazard ratios and corresponding 95% CIs were obtained for each significant factor.

Data were analyzed by using the intention-to-treat principle. To check for lead-time bias, the overall models were tested for the whole study population and for patients in CR1 only.

The proportional hazards assumptions were tested by assessing the Schoenfeld residuals. Two-sided *P* values less than .05 were considered statistically significant. Statistical analyses were performed by using IBM SPSS Statistics for Windows, version 20.0, and Stata 11.0 (StataCorp LP).

RESULTS

The prevalence of t(8;21) among patients with AML from the 19 participating study groups was 6% to 16%. In total, data on 916 patients were collected. Data from 78 patients without proven t(8;21) presence or with incomplete karyotypes were excluded. Data from 12 patients were excluded in final multivariable models; nine patients were excluded because they had not received cytarabine, etoposide, or anthracyclines, and three patients were excluded because it was not known whether they received one of the agents. Figure 1 gives details on study enrollment, and Table 1 provides baseline characteristics for included patients.

Approximately 68% (n = 573) of patients had one or more additional chromosomal aberration. Appendix Table A3 (online only) gives clinical characteristics by the number of additional cytogenetic aberrations. Loss of a sex chromosome (LOS), deletion of the long arm of chromosome 9 (del(9q)), gain of chromosome 8 (+8), gain of chromosome 4 (+4), and abnormalities of the long arm of chromosome 7 (abn(7q)) occurred in more than 10 patients (Appendix Fig A1, online only, shows distribution of these aberrations). Differences in clinical characteristics and treatment aspects between patients with these aberrations are shown in Appendix Table A4 (online only). Patient characteristics among samples that were or were not evaluated for *cKIT* and *RAS* mutations did not significantly differ; however, several treatment aspects were significantly different (Appendix Table A5, online only) because not all study groups provided samples.

cKIT mutations were identified in 24% (n = 53) of the 222 successfully evaluated samples. No significant differences between patients with or without *cKIT* mutations were found with respect to age, sex, WBC count, or blast percentage. Seventy percent (n = 10) of the patients with +4 had *cKIT* mutations, compared with 21% (n = 46) of the patients without +4 (*P* = .002). Patients with *cKIT* mutations received significantly more cytarabine and more

Table 2. Univariable and Multivariable Analyses of Prognostic Factors for Achieving Complete Remission in t(8;21)-Positive Patients With AML and Full Karyotype

Factor	No. of Patients	Univariable Analysis		Multivariable Analysis	
		OR (95% CI)	P	OR (95% CI)	P
Year of diagnosis					
1988-1997	352	Ref		Ref	
1998-2010	486	1.88 (1.15 to 3.07)	.012	1.71 (1.01 to 2.87)	.044
Sex				NA	
Male	495	Ref			
Female	343	1.30 (0.78 to 2.17)	.307		
Age, years					
< 12	552	Ref			
≥ 12*	286	0.60 (0.37 to 0.99)	.044		
WBC count at diagnosis, × 10 ⁹ /L				NA	
≤ 20	525	Ref			
> 20	307	1.01 (0.61 to 1.68)	.959		
≤ 50	737	Ref			
> 50	95	0.77 (0.38 to 1.55)	.461		
≤ 100	804	Ref			
> 100	28	1.43 (0.51 to 3.96)	.497		
Blasts in BM, %					
< 60	394	Ref			
≥ 60*	362	1.57 (0.93 to 2.63)	.091		
FAB type†				NA	
M2	663	Ref			
M other	83	0.38 (0.12 to 1.25)	.113		
CNS involvement				NA	
No	617	Ref			
Yes	52	0.71 (0.29 to 1.73)	.446		
Cytogenetic aberration				NA	
t(8;21) only	265	Ref			
+2 additional aberrations	154	0.50 (0.07 to 3.82)	.501		
+3 additional aberrations	31	0.44 (0.06 to 3.52)	.441		
+4 or more aberrations	22	0.32 (0.03 to 3.09)	.326		
Specific aberration					
Loss of sex chromosome	381	1.15 (0.71 to 1.89)	.570	NA	
Deletion chromosome 9q	104	0.49 (0.27 to 0.90)	.022	0.43 (0.23 to 0.82)	.010
Gain of chromosome 8	49	1.04 (0.36 to 2.99)	.936	NA	
Abnormalities chromosome 7q	41	0.85 (0.29 to 2.45)	.762	NA	
Gain of chromosome 4	21	0.88 (0.20 to 3.84)	.861	NA	
Complex karyotype (≥ 3)	207	0.82 (0.48 to 1.42)	.479	NA	
Complex karyotype (≥ 5)	22	1.97 (0.26 to 14.87)	.511	NA	
Mutational analysis					
<i>CKIT</i> mutation present*	53	7.38 (0.97 to 56.23)	.054		
<i>RAS</i> mutation present	29	1.51 (0.33 to 6.95)	.596	NA	
Induction regimen					
Course 1 anthracycline, mg/m ²					
≤ 150	594	Ref		Ref	
> 150	244	2.13 (1.13 to 4.04)	.020	1.99 (1.04 to 3.82)	.039
Course 1 cytarabine, mg/m ²					
≤ 1,400	595	Ref			
> 1,400*	243	1.05 (0.61 to 1.79)	.872		
Course 1 etoposide, mg/m ²					
≤ 500	568	Ref			
> 500*	270	1.44 (0.83 to 2.51)	.198		
Courses 1-2 anthracycline, mg/m ²					
≤ 300	739	Ref			
> 300*	99	0.92 (0.44 to 1.91)	.814		
Courses 1-2 cytarabine, mg/m ²					
≤ 5,000	562	Ref			
> 5,000*	276	1.76 (0.99 to 3.14)	.054		

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Table 2. Univariable and Multivariable Analyses of Prognostic Factors for Achieving Complete Remission in t(8;21)-Positive Patients With AML and Full Karyotype (continued)

Factor	No. of Patients	Univariable Analysis		Multivariable Analysis	
		OR (95% CI)	P	OR (95% CI)	P
Courses 1-2 HD cytarabine†					
No	599	Ref		Ref	
Yes	236	2.28 (1.8 to 4.41)	.015	2.37 (1.18 to 4.78)	.015
Courses 1-2 etoposide, mg/m ²					
≤ 1,000	577	Ref			
> 1,000	261	1.48 (0.84 to 2.61)	.173	§	

Abbreviations: BM, bone marrow; FAB, French-American-British classification system of acute myeloid leukemia; HD, high dose; NA, not applied, because they were not significant in univariable analyses; OR, odds ratio; Ref, reference category.

*Excluded from the multivariable model because $P > .05$.

†FAB type M2 included three occurrences that were classified as FAB type M1/M2.

‡HD cytarabine was defined as ≥ 3 g/m².

§Etoposide > 1,000 mg/m² correlated highly with etoposide > 500 mg/m² and therefore was omitted from the model.

anthracyclines during the first induction course (Appendix Table A6, online only).

RAS mutations were identified in 15% ($n = 29$) of 188 successfully evaluated samples. These patients had higher WBCs at diagnosis ($P = .034$), but lower blast percentages ($P = .001$), than those without RAS mutations. There were no clinical differences between patients with *N-RAS* ($n = 17$) or *K-RAS* ($n = 5$) mutations (data not shown).

Outcome

Complete remission (CR) was achieved by 92% of the patients ($n = 767$), and 59% ($n = 452$) remained in CR1 until the last date of follow-up. The median overall time from diagnosis to achievement of CR was 38 days (interquartile range, 32 to 56 days). The time until CR did not significantly differ between patients with or without events.

Of the 838 patients, 228 patients (27%) died: 11 patients died during induction, 30 died as a result of refractory disease, 121 died after they experienced relapse, and 66 patients died in CR during or after treatment. The most-reported causes of death were progressive disease ($n = 89$), infection ($n = 70$), transplantation-related death ($n = 21$), and other toxicity ($n = 9$).

The 5-year pEFS was 58% (SE, 1.7%); the 5-year pOS, 74% (SE, 1.5%); and the 5-year CIR, 26% (SE, 1.5%). The pOS and pEFS improved significantly during the study period (Appendix Fig A2, online only).

Clinical Characteristics and Aberrations

In multivariable analysis, patient-specific factors or the number of additional aberrations were not associated with pCR; however, the presence of additional del(9q) was associated with lower pCR ($P = .010$; Fig 2A). *cKIT* mutations or RAS mutations were not significantly associated with pCR. Table 2 shows the results of univariable and multivariable analyses for pCR.

WBCs greater than $20 \times 10^9/L$ were associated with inferior outcomes on all end points ($P < .01$). CNS involvement was associated with a higher CIR ($P = .001$) but did not affect pOS or pEFS. Although the number of additional chromosomal aberrations was not associated with survival or CIR, the presence of +4

was associated with a higher CIR ($P = .005$), inferior pEFS ($P = .005$), and inferior pOS ($P = .001$; Fig 2B, 2C, and 2D).

In explorative analysis, age 12 years or older and complex karyotype did not comply with the proportional hazards of assumption, which resulted in a time-dependent statistical correction for the first 2 years of follow-up.

Appendix Tables A7 and A8 (online only) show the results of univariable and explorative multivariable analyses, respectively. There were no significant survival differences in patients with or without *cKIT* or RAS mutations (Appendix Fig A3, online only).

Induction Regimen

Use of anthracycline doses greater than 150 mg/m² during the first induction course was associated with superior pCR ($P = .039$), pEFS ($P = .033$), and pOS ($P = .031$). High-dose (HD) cytarabine 3 g/m² during induction was associated with better pCR ($P = .015$) and higher pEFS ($P = .034$). Etoposide doses greater than 500 mg/m² during the first induction course were associated with higher pEFS ($P = .009$) and pOS ($P = .022$). None of the determined cutoff doses for induction was significantly associated with a lower CIR. Figure 3 shows Kaplan-Meier curves of induction regimens.

Overall Treatment

Use of cumulative cytarabine doses greater than 30 g/m² was associated with a lower CIR ($P = .029$) and better pEFS ($P = .007$). Cumulative etoposide doses greater than 1,500 mg/m² were associated with a lower CIR ($P = .020$) and better pEFS ($P = .012$). Maintenance therapy after the last consolidation course was not significantly associated with better outcome. Allogenic SCT in CR1 was significantly associated with a lower CIR but not with inferior pOS or pEFS. The time-varying effect of SCT was not significant (data not shown).

Tables 3 and 4 show the results of the multivariable models for the induction and overall regimens, respectively. When the cumulative models were applied for patients in CR1 only, results were similar (data not shown). Mortality rates for patients in CR were not significantly higher in any of the HD groups (Appendix Table A9, online only).

Fig 3. Kaplan-Meier curves of event-free survival with regard to induction therapy. (A) Event-free survival with anthracyclines ≤ 150 mg/m² ($n = 594$) or greater than 150 mg/m² ($n = 244$) in first induction course. (B) Event-free survival with etoposide ≤ 500 mg/m² ($n = 568$) or greater than 500 mg/m² ($n = 270$) in first induction course. (C) Event-free survival with high-dose cytarabine (3 g/m²; $n = 236$) or without high-dose cytarabine or less than 3 g/m² ($n = 599$) during induction. HD-AraC, high-dose arabinoside cytosine (cytarabine).

DISCUSSION

Although the previously reported mediocre EFS and high relapse rate were confirmed in this cohort, the 5-year OS of 74% was relatively good, especially given the number of patients who were diagnosed many years ago. Novel potential prognostic factors were

identified, and several known ones, like high WBC count^{8,17} and CNS involvement,¹⁸ were confirmed. However, in contrast to a previous report by Rubnitz et al,¹¹ our results suggest superior OS for women compared with men.

Consistent with previous findings, the prevalence of additional cytogenetic aberrations was high (68%), and the most frequently occurring cytogenetic aberrations were LOS and del(9q).^{1,4,11-13,19} Although the number of additional aberrations or complex karyotype (≥ 3) did not affect outcome in our or other cohorts,⁴ specific additional aberrations did. Del(9q) was significantly associated with refractory disease. Conversely, Rege et al¹⁰ suggested, with borderline significance, a favorable outcome for patients with t(8;21) plus del(9q), but that study had low patient numbers and mainly involved adults. Other studies reported inferior outcome in adults²⁰ and children⁴ with the additional del(9q). To our knowledge, this is the first study to report lower pCR in children. Although we did not identify significant associations with survival probabilities, these patients might represent a subgroup that could benefit from adaptation or intensification of induction regimen.

Although the occurrence of t(8;21) with +4 was low (3%), the significant association with inferior outcome in these patients may be an important novel finding. These results are consistent with previous findings in adults, in whom +4 is reported as a rare sole karyotypic aberration that is associated with relatively low CR rates and a poor prognosis.²¹ On the basis of differences in morphologic features, phenotypic findings, and clinical features, Nishii et al²² suggested that t(8;21) with +4 constitutes a distinctive subtype of t(8;21)-AML. Therefore, patients with +4 may represent a high-risk subgroup that warrants additional research at the molecular and clinical levels, with consideration for adaptation or intensification of conventional treatment.

Borderline higher pOS (univariately) for patients with LOS was not confirmed in the multivariable analysis. Other study groups have reported prognostic benefits for additional LOS in adults⁹ and children^{3,4} with t(8;21)-AML, however, these results were based mainly on univariable analyses and/or small patient numbers. Conversely, inferior outcome for these patients has been reported as well,¹⁰ so the prognostic impact remains uncertain.

We did not identify associations between *cKIT* or *N-RAS/K-RAS* mutations and outcome. Previous studies on *cKIT* mutations are conflicting and report both inferior²³⁻²⁵ and similar outcomes.^{3,26-28} Although we do not have an explanation for these contradictions, we hypothesize that some form of ethnic/genetic susceptibility or treatment-related influence occurs in specific subgroups. Alternatively, the effect of *cKIT* mutations may have ceased because of various treatment regimens. In addition, patients with *cKIT* mutations received significantly more cytarabine in our cohort, so one could speculate that this difference resulted in similar outcomes for patients with the mutations compared with patients without *cKIT* mutations. However, we could not confirm this after multivariable testing was performed on the subgroup of patients who had available molecular data (data not shown). Both groups were treated as (in)frequent according to high-risk protocols, and both achieved CR equally often.

Interestingly, Beghini et al²⁹ reported that trisomy 4 can lead to duplication of the *cKIT* mutated allele, consistent with the significant correlation between trisomy 4 and *cKIT* mutations in our cohort. Shimada et al²³ reported a poor outcome for *cKIT*-mutated pediatric patients with t(8;21)-AML but also reported an association between

Table 3. Multivariable Model of Induction Therapy for t(8;21)-Positive Patients With AML and Full Karyotype

Variable	Induction Model					
	CIR		pEFS		pOS	
	SHR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Year of diagnosis						
1988-1997			Ref		Ref	
1998-2010*			0.76 (0.58 to 1.00)	.046	0.57 (0.43 to 0.76)	< .001
Sex						
Male			Ref		Ref	
Female*			0.82 (0.63 to 1.07)	.140	0.76 (0.58 to 1.00)	.050
Age, years†						
< 12	NA		NA		Ref	
≥ 12						
Follow-up in first 2 years					1.71 (1.25 to 2.33)	.001
Follow-up after 2 years					0.56 (0.30 to 1.05)	.072
WBC count at diagnosis, × 10 ⁹ /L						
≤ 20	Ref		Ref		Ref	
> 20	1.67 (1.21 to 2.31)	.002	1.52 (1.18 to 1.97)	.001	1.58 (1.21 to 2.05)	.001
Blasts in BM, %	NA				NA	
< 60			Ref			
≥ 60			0.81 (0.63 to 1.04)	.099		
CNS involvement	2.02 (1.23 to 3.32)	.005	1.37 (0.91 to 2.06)	.133	NA	
Additional cytogenetic aberrations						
Loss of sex chromosome*			0.80 (0.62 to 1.03)	.083	NA	
Gain of chromosome 4	2.72 (1.31 to 5.65)	.007	2.36 (1.30 to 4.28)	.005	2.56 (1.44 to 4.55)	.002
Gain of chromosome 8†	NA				0.56 (0.28 to 1.14)	.112
Complex karyotype (≥ 3)	NA		NA		NA	
Complex karyotype (≥ 5)‡	NA		NA			
Follow-up in first 2 years					1.01 (0.41 to 2.51)	.967
Follow-up after 2 years					4.77 (1.39 to 16.38)	.013
Induction regimen						
Course 1 anthracycline, mg/m ²						
≤ 150	Ref		Ref		Ref	
> 150	0.84 (0.59 to 1.18)	.314	0.74 (0.57 to 0.98)	.033	0.71 (0.52 to 0.97)	.031
Course 1 etoposide, mg/m ²						
≤ 500	Ref		Ref		Ref	
> 500	0.77 (0.54 to 1.10)	.137	0.67 (0.50 to 0.90)	.009	0.69 (0.50 to 0.96)	.026
Courses 1-2 anthracycline*			NA		NA	
Courses 1-2 HD cytarabine*						
No			Ref		Ref	
Yes			0.71 (0.51 to 0.97)	.034	1.00 (0.75 to 1.36)	.969
Courses 1-2 etoposide§						

NOTE. The results of the multivariable models for assessment of induction elements are shown. In this model, the most important thresholds of the three agents used in induction (first two courses) were entered into this model. Variables significant at $P < .10$ in univariable analyses were entered into an explorative multivariable regression model. Next, a backward elimination procedure was applied, and the remaining important covariates were used in a multivariable model to assess the effects of induction therapy (induction model). To overcome multicollinearity among agents in different courses, the most relevant courses were entered into the induction model. Data were analyzed by using the intention-to-treat principle. Significant variables with (S)HR > 1 were associated with inferior outcome, whereas significant variables with (S)HR < 1 were associated with superior outcome. Significant treatment elements were tested for interaction terms, but no relevant interactions terms were identified (data not shown).

Abbreviations: allo-SCT, allogeneic stem-cell transplantation; BM, bone marrow; CIR, cumulative incidence of relapse, with death while in CR and secondary malignancy as competing events, CR, complete remission; CR1, first CR; HD, high-dose (ie, 3 g/m²); HR, hazard ratio; NA, not applied (not significant variables in univariable analyses); pEFS, probability of event-free survival; pOS, probability of overall survival; Ref, reference category; SHR, subdistributional HR.

*In explorative analysis, omitted from the CIR model.

†In explorative analysis, omitted from the event-free survival model.

‡Statistical correction for violation of proportional hazard assumption, because hazard rates were not proportional for the complete follow-up time; an HR for the first 2 years of follow-up and an HR for follow-up after the first 2 years were estimated.

§Etoposide > 1,000 mg/m² correlated highly with etoposide > 500 mg/m² and therefore was omitted from the model.

cKIT mutations and +4, so it would be interesting to evaluate the prognostic relevance of this combination. Because of the low number of patients in our study, we were unable to do so.

Present *RAS* mutations were not associated with impact on outcome, in line with previous reports about patients with

CBF-AML.^{24,26} However, despite the lack of significant survival differences for patients with *cKIT* and/or *RAS* mutations in our study, in vitro sensitivity profiles suggest that patients with these mutations may still benefit from treatment with [tyrosine kinase inhibitors](#).³⁰

Table 4. Multivariable Model of Overall Therapy for t(8;21)-Positive Patients With AML and Full Karyotype

Variable	Overall Treatment Model					
	CIR		pEFS		pOS	
	SHR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Year of diagnosis*						
1988-1997			Ref		Ref	
1998-2010			0.89 (0.63 to 1.24)	.479	0.52 (0.38 to 0.71)	< .001
Sex*						
Male			Ref		Ref	
Female			0.86 (0.64 to 1.15)	.301	0.80 (0.59 to 1.08)	.140
Age, years	NA		NA			
< 12					Ref	
≥ 12					1.31 (0.97 to 1.77)	.080
WBC count at diagnosis, × 10 ⁹ /L						
≤ 20	Ref		Ref		Ref	
> 20	1.65 (1.20 to 2.29)	.002	1.74 (1.30 to 2.32)	< .001	1.56 (1.17 to 2.08)	.003
Blasts in BM, %	NA				NA	
< 60			Ref			
≥ 60			0.87 (0.65 to 1.15)	.327		
CNS involvement	1.83 (1.12 to 2.99)	.015	1.33 (0.84 to 2.11)	.224	NA	
Additional cytogenetic aberrations						
Loss of sex chromosome*			0.77 (0.58 to 1.04)	.084	NA	
Gain of chromosome 4	2.80 (1.40 to 5.61)	.004	2.89 (1.54 to 5.42)	.001	2.88 (1.57 to 5.28)	.001
Gain of chromosome 8†	NA				0.63 (0.30 to 1.34)	.232
Complex karyotype (≥ 5)‡	NA		NA			
Follow-up first 2 years					0.93 (0.34 to 2.57)	.892
Follow-up after 2 years					4.93 (1.33 to 18.33)	.017
Overall therapy						
Cumulative anthracycline, mg/m ²						
≤ 400	Ref		Ref		Ref	
> 400	1.11 (0.81 to 1.54)	.490	1.03 (0.77 to 1.38)	.851	0.82 (0.61 to 1.11)	.196
Cumulative cytarabine, g/m ²						
≤ 30	Ref		Ref		Ref	
> 30	0.69 (0.50 to 0.96)	.029	0.64 (0.46 to 0.88)	.007	1.00 (0.73 to 1.38)	.990
Cumulative etoposide, mg/m ²						
≤ 1,500	Ref		Ref		Ref	
> 1,500	0.68 (0.50 to 0.94)	.020	0.67 (0.49 to 0.92)	.012	0.97 (0.72 to 1.32)	.865
Allo-SCT in CR1	0.53 (0.32 to 0.88)	.014	NA		NA	
Maintenance†§	NA					

NOTE. The results of the multivariable model for overall treatment are shown. In this model, the most important thresholds of the cumulative doses were entered into this model. Variables significant at $P < .10$ in univariable analyses were entered into an explorative multivariable regression model. Next, a backward elimination procedure was applied, and the remaining important covariates were used in a multivariable model to assess the effects of cumulative therapy (overall treatment model). Data were analyzed using the intention-to-treat principle. Significant variables with (S)HR > 1 were associated with inferior outcome, whereas significant variables with (S)HR < 1 were associated with superior outcome. Significant treatment elements were tested for interaction terms, but no relevant interaction terms were identified (data not shown). Both models (ie, induction model [Table 3] and overall model [Table 4]) involved all treated patients; however, when the overall treatment model was tested for patients in CR1 only, results were similar (data not shown).

Abbreviations: allo-SCT, allogeneic stem-cell transplantation; CIR, cumulative incidence of relapse, with death while in CR and secondary malignancy as competing events; CR, complete remission; CR1, first CR; HD, high-dose (ie, 3 g/m²); HR, hazard ratio; NA, not applied (not significant variables in univariable analyses); pEFS, probability of event-free survival; pOS, probability of overall survival; Ref, reference category; SHR, subdistributional HR.

*In explorative analysis, omitted from the CIR model.

†In explorative analysis, omitted from the event-free survival model.

‡Statistical correction for violation of proportional hazard assumption, because hazard rates were not proportional for the complete follow-up time; an HR for the first 2 years of follow-up and an HR for follow-up after the first 2 years were estimated.

§In explorative analysis, omitted from the OS model.

Previous studies about adults with AML demonstrated a benefit for intensive induction regimens with anthracyclines, etoposide, and intermediate-dose cytarabine³¹ and for intensive use of HD cytarabine in both induction and intensification regimens.^{18,32,33} Pediatric study groups reported superior outcomes for patients with t(8;21)-AML who received higher doses of cytarabine as well, in line with our results.^{3,34,35} Although the role of HD cytarabine during consolidation was not specifically evaluated in this study, cumulative cytarabine

doses greater than 30 g/m² were met only in protocols that comprised repetitive cycles of intermediate-dose or HD (ie, ≥ 1 g/m²) cytarabine. In our study and previous studies, the timing, doses, and number of courses of HD cytarabine and the cumulative doses cytarabine differed substantially among protocols.

Few studies have reported about the individual role of anthracyclines in pediatric t(8;21)-AML. Creutzig et al³ suggested beneficial effects of anthracyclines during reinduction as part of courses

comprising HD cytarabine and mitoxantrone; however it is not clear whether these patients benefited the most from either agent or from their combination. Our results support the use of HD anthracyclines during induction, but the role of its approach during consolidation remains unclarified. Furthermore, the use of HD anthracyclines may be limited by potential (cardio)toxicity, which could have been insufficiently considered in this study. To decrease severe toxicity as a result of high doses of anthracyclines, patients with t(8;21)-AML might benefit from liposomal daunorubicin in future protocols,¹³ because it can be administered in higher doses with equal or less toxicity than the nonliposomal form.³⁶

To our knowledge, this is the first study to report an association between the cumulative dose of etoposide and improved outcome in children with t(8;21)-AML, although a beneficial effect of etoposide has been described for other AML subgroups.³⁷ Otherwise, the effect in our cohort might be explained by synergistic effects of combination therapy, which we could not confirm after testing for interaction terms (data not shown).

Although SCT in CR1 was associated with lower CIR in this cohort, pEFS or pOS were not affected. Mortality rates in CR1 were nonsignificantly higher in the allogeneic SCT group (27%) than in the nontransplantation group (24%). Unfortunately, data on SCT-related toxicity were not available for the majority of patients. An international expert panel no longer recommends SCT in pediatric patients with favorable risk who are in CR1, because morbidity rates are high and there is not a survival advantage.¹⁸

The most important limitation of this study is the retrospective design, which leads to missing data and potential unknown confounders (eg, supportive care regimens, other molecular aberrations). Moreover, multivariable analyses were complex because of the heterogeneity of the study population, the divergent treatment protocols (Appendix Table A10, online only), and the wide study period.

The pOS and the pEFS were better for patients diagnosed in 1998 or later than for those diagnosed before 1998. This year was chosen, because several new protocols were initiated then, and 1998 appeared to be the most important year of influence among several tested cutoff points (data not shown). Nevertheless, dichotomization of time, instead of control for time as a continuous factor, may be inaccurate.

Application of intention-to-treat analyses hampers interpretation, because it is unknown whether patients actually received the treatment. However, results for the cumulative models were similar for all of the study population and for only patients in CR1 (data not shown), which demonstrates the low influence of the potential remaining lead-time bias.

Our results support the use of anthracycline doses greater than 150 mg/m² and etoposide doses greater than 500 mg/m² in the first induction course, HD cytarabine 3 g/m² during induction, as well as the use of cumulative cytarabine doses greater than 30g/m² and of

cumulative etoposide doses greater than 1,500 mg/m² in future pediatric t(8;21)-AML protocols. However, evaluation of single agents without adequate consideration of the potential synergistic effects of combination therapy could not be performed accurately in this retrospective setting. Furthermore, treatment evolves, and these data were obtained almost completely before novel agents, such as gemtuzumab, ozogamicin or tyrosine kinase inhibitors, were introduced; therefore, these novel agents were not taken into account.

Multicenter, randomized trials are required to validate our results, establish combination therapy, define doses with acceptable toxicity, and identify the impact of different treatment aspects among cytogenetic subgroups. Nevertheless, this is, to our knowledge, the largest pediatric t(8;21)-AML cohort evaluated for clinical, cytogenetic, and treatment-related prognostic factors. Therefore, it provides valuable information about risk stratification for this AML subgroup.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

AML-ETO (t[8;21]-AML): the t(8;21)(q22;q22) translocation replaces the C terminus of acute myeloid leukemia, including its transactivation domain, with ETO, a nuclear protein of unknown function. The fusion protein acts as a transcriptional repressor.

anthracyclines: a class of antineoplastic agents derived from *Streptomyces* bacterium used to treat a variety of hematologic and solid malignancies. Anthracyclines have a well-established dose-related risk of cardiomyopathy and congestive heart failure. Anthracyclines include agents like daunorubicin, doxorubicin, epirubicin, and idarubicin.

c-KIT: a tyrosine kinase receptor that dimerizes after ligand binding and is autophosphorylated on intracellular tyrosine residues.

cumulative incidence of relapse (CIR): the use of competing risk analyses indicated in the presence of competing events (such as death and relapse); the Gray's test is a recommended method to estimate cumulative incidence of relapse.

event-free survival: calculated from the date of diagnosis to the date of the first event, which is resistance, relapse, death, or second malignant neoplasm.

karyotype: an organized chromosomal profile defining chromosomal arrangement and number. In a karyotype, chromosomes are photographically arranged and displayed in pairs, ordered by size. Chromosomal size, banding pattern, and centromere position are typically used as guides to determine chromosomal abnormalities, but improved resolution may be obtained by combining traditional banding techniques with genome-wide molecular cytogenetics such as multicolor fluorescent in situ hybridization (FISH) and locus-specific FISH.

overall survival: the duration between random assignment and death.

RAS: gene family consisting of *H-RAS*, *N-RAS*, and *K-RAS*. The RAS proteins are typically small triphosphate-binding proteins and are the common upstream molecules of several signaling pathways that play a key role in signal transduction, which results in cellular proliferation and transformation.

tyrosine kinase inhibitors: molecules that inhibit the activity of tyrosine kinase receptors. Tyrosine kinase inhibitors are small molecules developed to inhibit the binding of ATP to the cytoplasmic region of the receptor (eg, gefitinib), thus further blocking the cascade of reactions that is activated by the pathway.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical Impact of Additional Cytogenetic Aberrations, *cKIT* and *RAS* Mutations, and Treatment Elements in Pediatric t(8;21)-AML: Results From an International Retrospective Study by the International Berlin-Frankfurt-Münster Study Group

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Appendix

Table A1. Cutoff Values of Evaluated Chemotherapeutic Agents

Treatment Element	Cutoff Value	Other
Anthracycline		Conversion factors*: daunorubicin:mitoxantrone ratio, 1:5; daunorubicin:idarubicin ratio, 1:5; and daunorubicin:doxorubicin, ratio 1:1
First induction course	≤/≥ 150 mg/m ²	
First two courses	≤/≥ 300 mg/m ²	
Cumulative	≤/≥ 400 mg/m ²	
Cytarabine		The potentially beneficial dose of 3 g/m ² was chosen to define HD cytarabine. ¹⁸
First induction course	≤/≥ 1,400 mg/m ²	
First two courses	≤/≥ 5,000 mg/m ²	
HD cytarabine, first two courses	3 g/m ²	
Cumulative dose	≤/≥ 30 g/m ²	
Etoposide		
First induction course	≤/≥ 500 mg/m ²	
First two courses	≤/≥ 1,000 mg/m ²	
Cumulative dose	≤/≥ 1,500 mg/m ²	
Allogeneic stem-cell transplantation in first complete remission	Yes or no	
Maintenance	Yes or no	

Abbreviation: HD, high dose.
*Kaspers GJ, et al: Leukemia 19:2025-2029, 2005.

Table A2. Definitions of End Points

End Point	Abbreviation	Definition
Complete remission	CR	Morphologically, < 5% blasts in the bone marrow with signs of regeneration of normal hematopoiesis, in the absence of leukemia elsewhere after 60 days.* Patients with early death during induction were considered CR failures at t = 0.
Cumulative incidence of relapse	CIR	The cumulative probability of relapse during the follow-up time, with death in CR and development of a secondary malignancy as competing events
Overall survival (time)	OS	Survival, measured from the date of diagnosis to the date of last follow-up or death
Event-free survival (time)	EFS	Survival without experiencing an event, measured from the date of diagnosis to the date of last follow-up or an event
Event		
Relapse		The reoccurrence of leukemic blasts in the bone marrow or peripheral blood or leukemic infiltrates elsewhere after patients achieved CR1
Refractory disease		Morphologically, ≥ 5% leukemic blasts in the bone marrow, peripheral blood, or extramedullary leukemia after the second induction course
Induction death/early death		Death before achieving CR; considered CR failure at t = 0
Death		
Secondary malignancy		

Abbreviations: CR1, first complete remission; t, time point.
*Cheson BD, et al: J Clin Oncol 8:813-819, 1990.

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Table A3. Demographic and Clinical Characteristics by the Number of Additional Cytogenetic Aberrations in Patients With Cytogenetic Confirmation of t(8;21) and Full Karyotype

Characteristic	No. (%) With Aberrations				
	t(8;21) Only (n = 265)	+1 (n = 366)	+2 (n = 154)	+3 (n = 31)	+4 or More (n = 22)
Year of diagnosis					
1988-1997	109 (41.1)	154 (42.1)	68 (44.2)	14 (45.2)	7 (31.8)
1998-2010	156 (58.9)	212 (57.9)	86 (55.8)	17 (54.8)	15 (68.2)
Male sex	127 (47.9)	243 (66.4)	94 (61.0)	20 (64.5)	11 (50.0)
Median (IQR) age, years	9 (6-13)	9 (6-9)	9 (6-13)	7 (4-14)	11 (5-14)
< 12	169 (63.8)	244 (66.7)	107 (69.5)	20 (64.6)	12 (54.6)
≥ 12	96 (36.2)	122 (33.3)	47 (30.5)	11 (35.4)	10 (45.4)
Median (IQR) WBC count, × 10 ⁹ /L	14.9 (6.6-32.8)	13.8 (7.4-26.2)	12.6 (7.0-27.6)	11.9 (4.3-29.8)	20.1 (10.1-55.9)
≤ 20	161 (61.0)	236 (64.8)	97 (63.8)	20 (66.7)	11 (50.0)
> 20	103 (39.0)	128 (35.2)	55 (36.2)	10 (33.3)	11 (50.0)
Median (IQR) BM blasts,* %	62 (41-79)	55 (39-71)	58 (39-75)	48 (35-62)	56 (32-83)
FAB classification					
M2	199 (77.4)	298 (84.4)	127 (83.6)	22 (73.3)	14 (63.6)
M other	58 (22.6)	55 (15.6)	25 (16.4)	8 (26.7)	8 (36.4)
CNS involvement	15 (7.4)	23 (7.7)	10 (7.9)	1 (4.0)	3 (16.7)
Additional cytogenetic aberration					
Loss of sex chromosome	—	243 (66.4)	109 (70.8)	22 (71.0)	7 (31.8)
Deletion chromosome 9q	—	42 (11.5)	45 (29.2)	13 (41.9)	4 (18.2)
Abnormalities chromosome 7q	—	15 (4.1)	17 (11.0)	5 (16.1)	4 (18.2)
Gain of chromosome 8	—	14 (3.8)	25 (16.2)	7 (22.6)	3 (13.6)
Gain of chromosome 4	—	9 (2.5)	7 (4.6)	1 (3.2)	4 (18.2)

NOTE. Percentages may not add up to 100% because of rounding.

Abbreviations: BM, bone marrow; FAB, French-American-British classification of acute myeloid leukemia; IQR, interquartile range.

*Overall BM blasts were normally distributed; however, in one of these subgroups it was not; therefore, median (IQR) is presented.

Table A4. Demographic and Clinical Characteristics by Specific Additional Cytogenetic Aberration in Patients With Cytogenetic Confirmation of t(8;21) and Full Karyotype

Characteristic	No. (%) of Patients by Aberration														
	LOS			Del(9q)			Abn(7q)			+8			+4		
	Absent (n = 457)	Present (n = 381)	P	Absent (n = 734)	Present (n = 104)	P	Absent (n = 797)	Present (n = 41)	P	Absent (n = 789)	Present (n = 49)	P	Absent (n = 817)	Present (n = 21)	P
Year of diagnosis			NS			NS			NS			NS			NS
1988-1997	199 (44)	153 (40)		310 (42)	42 (40)		333 (42)	19 (46)		332 (42)	20 (41)		342 (42)	10 (48)	
1998-2010	258 (56)	228 (60)		424 (58)	62 (60)		464 (58)	22 (54)		457 (58)	29 (59)		475 (58)	11 (52)	
Sex			< .001			NS			NS			NS			NS
Male	228 (50)	267 (70)		434 (59)	61 (59)		476 (60)	19 (46)		467 (59)	28 (57)		481 (59)	14 (67)	
Female	229 (50)	114 (30)		300 (41)	43 (41)		321 (40)	22 (54)		322 (41)	21 (43)		336 (41)	7 (33)	
Age, years			NS			NS			NS			NS			NS
< 12	292 (64)	260 (68)		486 (66)	66 (63)		521 (65)	31 (76)		517 (66)	35 (71)		539 (66)	13 (62)	
≥ 12	165 (36)	121 (32)		248 (34)	38 (37)		276 (35)	10 (24)		272 (34)	14 (29)		278 (34)	8 (38)	
WBC count, × 10 ⁹ /L			.010			NS			.042			NS			.001
≤ 20	268 (59)	257 (68)		458 (63)	67 (66)		493 (62)	32 (78)		489 (62)	36 (73)		519 (64)	6 (29)	
> 20	185 (41)	122 (32)		272 (37)	35 (34)		298 (38)	9 (22)		294 (38)	13 (27)		292 (36)	15 (71)	
BM blasts, %*			.019			.035			NS			.015			NS
< 60	194 (48)	200 (57)		336 (51)	58 (62)		375 (52)	19 (49)		378 (53)	16 (35)		388 (53)	6 (33)	
≥ 60	209 (52)	153 (43)		327 (49)	35 (38)		342 (48)	20 (51)		332 (47)	30 (65)		350 (47)	12 (67)	
FAB classification*			.005			NS			NS			< .001			NS
M2	338 (86)	325 (92)		583 (89)	80 (86)		631 (89)	32 (91)		631 (90)	32 (73)		648 (89)	15 (94)	
M other	56 (14)	27 (8)		70 (11)	13 (14)		80 (11)	3 (9)		71 (10)	12 (27)		82 (11)	1 (6)	
CNS involvement*			NS			NS			NS			NS			NS
No	326 (92)	291 (93)		540 (92)	77 (95)		589 (92)	28 (93)		579 (92)	38 (95)		603 (92)	14 (93)	
Yes	29 (8)	23 (7)		48 (8)	4 (5)		50 (8)	2 (7)		50 (8)	2 (5)		51 (8)	1 (7)	
Additional cytogenetic aberrations†															
LOS present			NA	338 (46)	43 (41)	NS	369 (46)	12 (29)	.033	355 (45)	26 (53)	NS	377 (46)	4 (19)	.014
del(9q) present	61 (13)	43 (11)	NS			NA	98 (12)	6 (15)	NS	99 (13)	5 (10)	NS	102 (13)	2 (10)	NS
+8 present	23 (5)	26 (7)	NS	44 (6)	5 (5)	NS	49 (6)	0 (0)	NS			NA	47 (6)	2 (10)	NS
abn(7q) present	29 (6)	12 (3)	.033	35 (5)	6 (6)	NS			NA	41 (5)	0 (0)	NS	39 (5)	2 (10)	NS
+4 present	17 (4)	4 (1)	.014	19 (3)	2 (2)	NS	19 (2)	2 (5)	NS	19 (2)	2 (4)	NS			NA
Complex karyotype (≥ 3) present	69 (15)	138 (36)	< .001	145 (20)	62 (60)	< .001	181 (23)	26 (63)	< .001	172 (22)	35 (71)	< .001	195 (24)	12 (57)	< .001
Complex karyotype (≥ 5) present	15 (3)	7 (2)	NS	18 (3)	4 (4)	NS	18 (2)	4 (10)	.019	19 (2)	3 (6)	NS	18 (2)	4 (19)	.002
Molecular aberrations*															
cKIT mutation															
Present	34 (26)	19 (20)	NS	48 (25)	5 (17)	NS	50 (24)	3 (25)	NS	52 (25)	1 (6)	NS	46 (22)	7 (70)	.002
Absent	95 (74)	74 (80)	NS	144 (75)	25 (83)	NS	160 (76)	9 (75)	NS	154 (75)	15 (94)	NS	166 (78)	3 (30)	NS
RAS mutation															
Present	21 (19)	8 (10)	NS	26 (16)	3 (10)	NS	29 (16)	0 (0)	NS	28 (16)	1 (11)	NS	28 (16)	1 (14)	NS
Absent	87 (81)	72 (90)	NS	133 (84)	26 (90)	NS	148 (84)	11 (100)	NS	151 (84)	8 (89)	NS	153 (85)	6 (86)	NS
Induction therapy†															
Course 1															
Anthracycline > 150 mg/m ²	130 (28)	114 (30)	NS	215 (29)	29 (28)	NS	238 (30)	5 (15)	.036	231 (29)	13 (27)	NS	239 (29)	5 (24)	NS
Cytarabine > 1,400 mg/m ²	129 (28)	114 (30)	NS	205 (28)	38 (37)	NS	230 (29)	13 (32)	NS	226 (29)	17 (35)	NS	233 (29)	10 (48)	NS
Etoposide > 500 mg/m ²	145 (32)	125 (33)	NS	233 (32)	37 (36)	NS	259 (33)	11 (27)	NS	253 (32)	17 (35)	NS	261 (32)	9 (43)	NS
Courses 1 and 2															
Anthracycline > 300 mg/m ²	48 (11)	51 (13)	NS	84 (11)	15 (14)	NS	96 (12)	3 (7)	NS	90 (11)	9 (18)	NS	97 (12)	2 (10)	NS
Cytarabine > 5,000 mg/m ²	150 (54)	126 (46)	NS	246 (34)	30 (29)	NS	260 (33)	16 (39)	NS	259 (33)	17 (35)	NS	271 (33)	5 (24)	NS
HD cytarabine > 30 g/m ²	126 (28)	110 (29)	NS	211 (29)	25 (24)	NS	225 (28)	11 (27)	NS	224 (29)	12 (25)	NS	233 (29)	3 (14)	NS
Etoposide > 1,000 mg/m ²	142 (31)	119 (31)	NS	225 (31)	36 (35)	NS	250 (31)	11 (27)	NS	244 (31)	17 (35)	NS	252 (31)	9 (43)	NS
Overall treatment†															
Anthracycline > 400 mg/m ²	197 (43)	174 (46)	NS	323 (44)	48 (46)	NS	353 (44)	18 (44)	NS	344 (44)	27 (55)	NS	360 (44)	11 (52)	NS
Cytarabine > 30 g/m ²	177 (39)	146 (38)	NS	288 (39)	35 (34)	NS	307 (39)	16 (39)	NS	302 (38)	21 (43)	NS	316 (39)	7 (33)	NS
Etoposide > 1,500 mg/m ²	212 (46)	177 (47)	NS	336 (46)	53 (51)	NS	369 (46)	20 (49)	NS	369 (47)	20 (41)	NS	377 (46)	12 (57)	NS
Allo-SCT in first complete remission	47 (10)	61 (16)	.013	93 (13)	15 (15)	NS	101 (13)	7 (18)	NS	100 (13)	8 (16)	NS	107 (13)	1 (5)	NS

NOTE. Percentages may not add up to 100% because of rounding.

Abbreviations: +4, gain of chromosome 4; +8, gain of chromosome 8; abn(7q), abnormalities or a deletion in the long arm of chromosome 7; allo-SCT, allogeneic stem-cell transplantation; BM, bone marrow; del(9q), deletion in the long arm of chromosome 9; FAB, French-American-British classification of acute myeloid leukemia; HD, high dose; LOS, loss of sex chromosome; NA, not applicable; NS, nonsignificant at $P < .05$.

*Data on BM blast percentages, FAB classification, CNS involvement, and molecular aberrations were not complete for all patients.

†Dichotomous covariates: present (> mg/m² or g/m²) compared with absent (≤ mg/m² or g/m²).

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Table A5. Demographic and Clinical Characteristics From Pediatric t(8;21)-Patients With Full Karyotype, From Whom Samples Were or Were Not Screened for *cKIT* and *RAS* Mutations

Characteristic	No./Total Not Screened for <i>cKIT/RAS</i> Mutations (n = 610)	No. (%) of Patients Screened for <i>cKIT/RAS</i> Mutations (n = 228)	P
Year of diagnosis			
1988-1997	263/610 (43.1)	89/228 (39.0)	.287
1998-2010	347/610 (56.9)	139/228 (61.0)	
Sex			
Male	367/610 (60.2)	128/228 (56.1)	.292
Female	243/610 (39.8)	100/228 (43.9)	
Mean $\pm$ SD age, years			
< 12	412/610 (67.5)	140/228 (61.4)	.095
$\geq$ 12	198/610 (32.5)	88/228 (38.6)	
Median (IQR) WBC count at diagnosis, $\times 10^9/L$	13.6 (6.5-27.9)	13.9 (7.9-28.9)	
≤ 20	385/605 (63.6)	140/227 (61.7)	.601
> 20	220/605 (36.4)	87/228 (38.2)	
Mean $\pm$ SD hemoglobin, g/dL	7.81 $\pm$ 2.61	7.54 $\pm$ 2.48	.184
Median (IQR) platelets, $\times 10^9/L$	37 (19-69)	35 (18-70)	.457
Mean $\pm$ SD BM blasts, %	58 $\pm$ 22	54 $\pm$ 23	
< 60	278/548 (50.7)	116/208 (55.8)	.215
≥ 60	270/548 (49.3)	92/208 (44.2)	
FAB classification			
M2	486/550 (88.4)	177/196 (90.3)	.458
other	64/550 (11.6)	19/196 (9.7)	
CNS involvement	37/472 (7.8)	15/197 (7.6)	.921
Additional cytogenetic aberrations			
≥ 1 additional aberration			
Loss of sex chromosome	284/610 (46.6)	131/228 (57.5)	.299
Deletion chromosome 9q	73/610 (12.0)	31/228 (1.4)	.524
Gain of chromosome 8	33/610 (5.4)	16/228 (7.0)	.377
Abnormalities in chromosome 7q	28/610 (4.6)	13/228 (5.7)	.507
Gain of chromosome 4	11/610 (1.8)	10/228 (4.4)	.033
Complex karyotype (≥ 3 aberrations)	151/610 (24.8)	56/228 (24.6)	.954
Complex karyotype (≥ 5 aberrations)	14/610 (2.3)	8/228 (3.5)	.328
Induction regimen*			
Course 1			
Anthracycline > 150 mg/m ²	185/610 (30.3)	59/228 (25.9)	.207
Cytarabine > 1,400 mg/m ²	135/610 (22.1)	108/228 (47.4)	< .001
Etoposide > 500 mg/m ²	187/610 (30.7)	83/228 (36.4)	.113
Courses 1 and 2			
Anthracycline > 300 mg/m ²	71/610 (11.6)	28/228 (12.3)	.798
Cytarabine > 5,000 mg/m ²	240/610 (39.3)	36/228 (15.8)	< .001
HD cytarabine > 3 g/m ²	210/607 (34.6)	26/228 (11.4)	< .001
Etoposide > 1,000 mg/m ²	178/610 (29.2)	83/228 (36.4)	.044
Overall treatment*			
Anthracycline > 400 mg/m ²	258/610 (42.2)	113/228 (49.6)	.059
Cytarabine > 30 g/m ²	242/610 (39.7)	81/228 (35.5)	.272
Etoposide > 1,500 mg/m ²	228/610 (37.4)	161/228 (70.6)	< .001
Allo-SCT in first complete remission	78/600 (13.0)	30/228 (13.2)	.952
Maintenance	175/610 (28.7)	23/228 (10.1)	< .001

NOTE. Percentages may not make 100% because of rounding. Normally distributed data are represented with mean $\pm$ SD instead of with median and IQR. The differences between patients of whom samples were or were not screened for presence of *cKIT* and/or *RAS* mutations are shown. Differences in treatment regimen are explained by the fact that only a part of the participating study groups provided samples.

Abbreviations: allo-SCT, allogeneic stem-cell transplantation; BM, bone marrow; FAB classification, French-American-British classification of acute myeloid leukemia; HD, high dose (ie, 3 g/m²); IQR, interquartile range; SD, standard deviation.

*Dichotomous covariates: present (> mg/m² or g/m²) compared with absent ($\leq$ mg/m² or g/m²).

Table A6. Demographic and Clinical Characteristics by Specific Additional Molecular Aberration in Patients With Cytogenetic Confirmation of t(8;21)

Characteristic	No. (%) of Patients by Aberration					
	<i>cKIT</i> Mutation (n = 222)			<i>RAS</i> Mutation (n = 188)		
	Absent	Present	<i>P</i>	Absent	Present	<i>P</i>
Year of diagnosis						
1988-1997	61 (27.5)	25 (11.3)	NS	56 (35)	15 (52)	NS
1998-2010	108 (48.6)	28 (12.6)		103 (65)	14 (48)	
Sex						
Male	92 (41.4)	31 (14.0)	NS	86 (54)	17 (59)	NS
Female	77 (34.7)	22 (9.9)		73 (46)	12 (41)	
Age, years						
< 12	103 (46.4)	32 (14.4)	NS	98 (62)	21 (72)	NS
≥ 12	66 (29.7)	21 (9.5)		61 (38)	8 (28)	
WBC count, × 10 ⁹ /L						
≤ 20	106 (47.8)	30 (13.5)	NS	107 (68)	12 (41)	.007
> 20	62 (27.9)	23 (5.9)		51 (32)	17 (59)	
BM blasts, %*						
< 60	91 (41.0)	22 (9.9)	NS	79 (55)	20 (83)	.009
≥ 60	64 (28.8)	25 (11.3)		65 (45)	4 (17)	
FAB classification*						
M2	133 (59.9)	40 (18.0)	NS	124 (79)	23 (89)	NS
M other	14 (6.3)	4 (1.8)		33 (21)	3 (12)	
CNS involvement*						
No	138 (62.2)	40 (18.0)	NS	126 (91)	27 (96)	NS
Yes	8 (3.6)	6 (2.7)		13 (9)	1 (4)	
Additional cytogenetic aberrations†						
LOS present	74 (44)	19 (36)	NS	72 (45)	8 (28)	NS
Del(9q) present	25 (15)	5 (9)	NS	26 (16)	3 (10)	NS
+8 present	15 (9)	1 (2)	NS	8 (5)	1 (3)	NS
Abn(7q) present	9 (5)	3 (6)	NS	11 (7)	0 (0)	NS
+4 present	3 (2)	7 (13)	.002	6 (4)	1 (3)	NS
Complex karyotype (≥ 3) present	43 (25)	10 (19)	NS	41 (26)	4 (14)	NS
Complex karyotype (≥ 5) present	6 (4)	2 (4)	NS	6 (4)	1 (3)	NS
Induction therapy†						
Course 1						
Anthracycline > 150 mg/m ²	37 (22)	20 (38)	.021	47 (30)	12 (41)	NS
Cytarabine > 1,400 mg/m ²	78 (46)	28 (53)	NS	69 (43)	10 (35)	NS
Etoposide > 500 mg/m ²	61 (36)	21 (40)	NS	55 (35)	9 (31)	NS
Courses 1 and 2						
Anthracycline > 300 mg/m ²	20 (12)	7 (13)	NS	24 (15)	4 (14)	NS
Cytarabine > 5,000 mg/m ²	21 (12)	14 (26)	.015	21 (13)	5 (17)	NS
HD cytarabine > 3 g/m ²	13 (8)	12 (23)	.003	21 (13)	5 (17)	NS
Etoposide > 1,000 mg/m ²	61 (36)	21 (40)	NS	55 (35)	9 (31)	NS
Overall treatment†						
Anthracycline > 400 mg/m ²	80 (47)	31 (59)	NS	75 (47)	14 (48)	NS
Cytarabine > 30 g/m ²	52 (31)	27 (51)	.007	55 (35)	12 (41)	NS
Etoposide > 1,500 mg/m ²	123 (73)	34 (64)	NS	120 (76)	19 (66)	NS
Allo-SCT in first complete remission	23 (14)	7 (13)	NS	16 (10)	4 (14)	NS

NOTE. Percentages may not add up to 100% because of rounding.

Abbreviations: +4, gain of chromosome 4; +8, gain of chromosome 8; abn(7q), abnormalities or a deletion in the long arm of chromosome 7; BM, bone marrow; del(9q), deletion in the long arm of chromosome 9; FAB, French-American-British classification of acute myeloid leukemia; LOS, loss of sex chromosome; NS, nonsignificant at *P* < .05.

*Data on BM blast percentage, FAB classification, CNS involvement, and molecular aberrations were not complete for all patients.

†Dichotomous covariates: present (> mg/m² or g/m²) compared with absent (≤ mg/m² or g/m²).

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Table A7. Five-Year Outcomes and Univariable Analyses for Probabilities of EFS and OS for t(8;21)-Positive Patients With AML and Full Karyotype

Characteristic	No. of Patients	5-Year pEFS (SE)	P (pEFS log-rank)	5-Year pOS (SE)	P (pOS log-rank)
Year of diagnosis					
1988-1997	352	0.49 (0.03)	< .001	0.65 (0.03)	< .001
1998-2010	486	0.65 (0.02)		0.80 (0.02)	
Sex					
Male	495	0.55 (0.02)	.033	0.71 (0.02)	.049
Female	343	0.63 (0.03)		0.77 (.02)	
Age, years					
< 12	552	0.60 (0.02)	.170	0.76 (0.02)	.014
≥ 12	286	0.55 (0.03)		0.69 (0.03)	
WBC count at diagnosis, × 10 ⁹ /L					
≤ 20	525	0.63 (0.02)	< .001	0.78 (0.02)	< .001
> 20	307	0.50 (0.03)		0.65 (0.03)	
≤ 50	737	0.58 (0.02)	.885	0.73 (0.02)	.385
> 50	95	0.60 (0.05)		0.76 (0.04)	
≤ 100	804	0.58 (0.02)	.802	0.73 (0.02)	.177
> 100	28	0.64 (0.09)		0.86 (0.07)	
Blasts in BM, %					
< 50	291	0.57 (0.03)	.726	0.72 (0.03)	.767
≥ 50	464	0.58 (0.02)		0.73 (0.02)	
< 60	394	0.55 (0.03)	.076	0.70 (0.02)	.143
≥ 60	362	0.61 (0.03)		0.75 (0.02)	
FAB type*					
M2	663	0.59 (0.02)	.487	0.74 (0.02)	.410
M other	83	0.55 (0.06)		0.72 (0.05)	
CNS involvement					
Yes	617	0.45 (0.07)	.020	0.75 (0.06)	.720
No	52	0.61 (0.02)		0.75 (0.02)	
Additional cytogenetic aberrations					
t(8;21) alone	265	0.57 (0.03)	.363	0.72 (0.03)	.164
+ 1 cytogenetic aberration	366	0.62 (0.03)		0.76 (0.02)	
+ 2	154	0.54 (0.04)		0.72 (0.04)	
+ 3	31	0.61 (0.09)		0.80 (0.07)	
+ 4 or more	22	0.40 (0.11)		0.58 (0.11)	
Loss of sex chromosome					
Present	381	0.61 (0.03)	.051	0.76 (0.02)	.173
Absent	457	0.56 (0.02)		0.72 (0.02)	
Deletion chromosome 9q					
Present	104	0.55 (0.05)	.433	0.79 (0.04)	.293
Absent	734	0.59 (0.02)		0.73 (0.02)	
Abnormalities chromosome 7q					
Present	41	0.54 (0.08)	.420	0.71 (0.07)	.312
Absent	797	0.59 (0.02)		0.74 (0.02)	
Gain of chromosome 4					
Present	21	0.18 (0.09)	< .001	0.33 (0.11)	< .001
Absent	817	0.59 (0.02)		0.75 (0.02)	
Gain of chromosome 8					
Present	49	0.71 (0.07)	.063	0.85 (0.05)	.092
Absent	789	0.57 (0.02)		0.73 (0.02)	
Complex karyotype (≥ 3)					
Present	207	0.54 (0.04)	.192	0.72 (0.03)	.431
Absent	631	0.60 (0.02)		0.74 (0.02)	
Complex karyotype (≥ 5)					
Present	22	0.59 (0.02)	.226	0.58 (0.11)	.027
Absent	816	0.40 (0.11)		0.74 (0.02)	
Molecular aberration					
<i>cKIT</i> mutation					
Present	53	0.60 (0.07)	.884	0.70 (0.06)	.365
Absent	169	0.60 (0.04)		0.77 (0.03)	
<i>RAS</i> mutation					
Present	29	0.72 (0.08)	.327	0.72 (0.08)	.250
Absent	159	0.56 (0.04)		0.76 (0.03)	

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Table A7. Five-Year Outcomes and Univariable Analyses for Probabilities of EFS and OS for t(8;21)-Positive Patients With AML and Full Karyotype (continued)

Characteristic	No. of Patients	5-Year pEFS (SE)	P (pEFS log-rank)	5-Year pOS (SE)	P (pOS log-rank)
Induction regimen					
Course 1 anthracycline, mg/m ²					
≤ 150	594	0.56 (0.02)	.011	0.72 (0.02)	.039
> 150	244	0.65 (0.03)		0.79 (0.03)	
Course 1 cytarabine, mg/m ²					
≤ 1,400	595	0.58 (0.02)	.597	0.74 (0.02)	.950
> 1,400	243	0.60 (0.03)		0.73 (0.03)	
Course 1 etoposide, mg/m ²					
≤ 500	568	0.54 (0.02)	.001	0.71 (0.02)	.003
> 500	270	0.67 (0.03)		0.80 (0.03)	
Courses 1-2 anthracycline, mg/m ²					
≤ 300	739	0.58 (0.02)	.974	0.73 (0.02)	.459
> 300	99	0.60 (0.05)		0.77 (0.04)	
Courses 1-2 cytarabine, mg/m ²					
≤ 5,000	562	0.56 (0.02)	.134	0.73 (0.02)	.739
> 5,000	276	0.62 (0.03)		0.75 (0.03)	
Courses 1-2 HD cytarabine					
No	599	0.56 (0.02)	.025	0.73 (0.02)	.465
Yes	236	0.64 (0.03)		0.76 (0.03)	
Courses 1-2 etoposide, mg/m ²					
≤ 1,000	577	0.55 (0.02)	.002	0.71 (0.02)	.003
> 1,000	261	0.66 (0.03)		0.80 (0.03)	
Overall treatment					
Cumulative anthracycline, mg/m ²					
≤ 400	467	0.56 (0.02)	.152	0.71 (0.02)	.012
> 400	371	0.61 (0.03)		0.78 (0.02)	
Cumulative cytarabine, g/m ²					
≤ 30	515	0.54 (0.02)	.005	0.72 (0.02)	.082
> 30	323	0.65 (0.03)		0.77 (0.02)	
Cumulative etoposide, mg/m ²					
≤ 1,500	449	0.57 (0.02)	.480	0.73 (0.02)	.207
> 1,500	389	0.60 (0.03)		0.75 (0.02)	
Allo-SCT in CR1					
Yes	104	0.62 (0.05)	.270	0.75 (0.04)	.925
No	734	0.58 (0.02)		0.74 (0.02)	
Maintenance					
Yes	198	0.65 (0.03)	.022	0.80 (0.03)	.046
No	640	0.56 (0.02)		0.72 (0.02)	

Abbreviations: allo-SCT in CR1, allogeneic stem-cell transplantation in first complete remission; BM, bone marrow; EFS, event-free survival; FAB type, French-American-British classification system of acute myeloid leukemia; HD (ie, 3 g/m²); , OR, odds ratio; OS, overall survival; pEFS, probability of EFS; pOS, probability of OS.

*FAB type M2 included three occurrences that were classified as FAB type M1/M2.

Prognostic Factors and Treatment in Pediatric t(8;21)-AML

Table A8. Explorative Multivariable Analyses in t(8;21)-Positive Patients With AML and Full Karyotype

Characteristic	Multivariable Analyses					
	CIR		pEFS		pOS	
	SHR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Year of diagnosis						
1988-1997			Ref		Ref	
1998-2010*			0.76 (0.58 to 1.00)	.046	0.57 (0.43 to 0.76)	< .001
Sex						
Male			Ref		Ref	
Female*			0.82 (0.63 to 1.07)	.140	0.76 (0.58 to 1.00)	.048
Age, years						
< 12	NA		NA		Ref	
≥ 12						
Follow-up in first 2 years					1.71 (1.25 to 2.33)	.001
Follow-up after 2 years					0.56 (0.30 to 1.05)	.072
WBC count at diagnosis, × 10 ⁹ /L						
≤ 20	Ref		Ref		Ref	
> 20	1.66 (1.20 to 2.28)	.002	1.52 (1.18 to 1.97)	.001	1.58 (1.21 to 2.05)	.001
Blasts in BM, %	NA				NA	
< 60			Ref			
≥ 60			0.81 (0.63 to 1.04)	.099		
CNS involvement	1.96 (1.21 to 3.18)	.007	1.37 (0.91 to 2.06)	.133	NA	
Additional cytogenetic aberrations						
Loss of sex chromosome*			0.80 (0.62 to 1.03)	.083	NA	
Gain of chromosome 4	2.82 (1.34 to 5.91)	.006	2.36 (1.30 to 4.28)	.005	2.56 (1.44 to 4.55)	.001
Gain of chromosome 8†	NA				0.56 (0.27 to 1.13)	.106
Complex karyotype (≥ 3)	NA		NA		NA	
Complex karyotype (≥ 5)	NA		NA			
Follow-up in first 2 years					1.02 (0.41 to 2.53)	.967
Follow-up after 2 years					4.76 (1.39 to 16.33)	.013
Molecular aberration						
<i>cKIT</i> mutation‡			NA		NA	
<i>RAS</i> mutation‡			NA		NA	
Induction regimen						
Course 1 anthracycline, mg/m ²	NA					
≤ 150			Ref		Ref	
> 150			0.74 (0.57 to 0.98)	.033	0.71 (0.52 to 0.97)	.031
Course 1 cytarabine	NA		NA		NA	
Course 1 etoposide, mg/m ²						
≤ 500	Ref		Ref		Ref	
> 500	0.70 (0.50 to 1.00)	.047	0.67 (0.50 to 0.90)	.009	0.69 (0.49 to 0.95)	.022
Courses 1-2 anthracycline	NA		NA		NA	
Courses 1-2 cytarabine	NA		NA		NA	
Courses 1-2 HD cytarabine					NA	
No			Ref			
Yes*			0.71 (0.51 to 0.97)	.034		
Courses 1-2 etoposide§	NA					
Overall therapy						
Cumulative anthracycline, mg/m ²	NA		NA			
≤ 400						
> 400						
Cumulative cytarabine, g/m ²	NA					
≤ 30						
> 30†						
Cumulative etoposide	NA		NA		NA	
Allo-SCT in CR1	0.55 (0.32 to 0.94)	.030	NA		NA	
Maintenance†	NA					

NOTE. The results of the explorative analyses, which were performed to determine variables relevant for the assessment of treatment elements in the final induction model and the overall treatment model (Tables 3 and 4), are shown. Significant variables with (S)HR > 1 were associated with inferior outcome, whereas significant variables with (S)HR < 1 were associated with superior outcome. Data were analyzed according to the intention-to-treat principle. Statistical correction was made for violation of proportional hazard assumption, because hazard rates were not proportional for the complete follow-up time; an HR for the first 2 years of follow-up and an HR for follow-up after the first 2 years were estimated.

Abbreviations: allo-SCT in CR1, allogeneic stem-cell transplantation in first complete remission; BM, bone marrow; CR, complete remission; CIR, cumulative incidence of relapse, with death during CR and secondary malignancy as competing events; EFS, event-free survival; HD, high dose (ie, 3 g/m²); HR, hazard ratio; NA, not applicable, because these variables were not significant in univariable analyses; OS, overall survival; pEFS, probability of EFS; pOS, probability of overall survival; Ref, reference category; SHR, subdistributional hazard ratio.

*By stepwise backward elimination, variables with *P* > .2 were omitted from the competing risk model.

†By stepwise backward elimination, variables with *P* > .2 were omitted from the EFS model.

‡Not included as variables in the Fine and Grey model for CIR because of the large amount of missing molecular data.

§Etoposide > 1,000 mg/m² correlated highly with etoposide > 500 mg/m² and therefore was omitted from the models of pEFS and pOS.

||By stepwise backward elimination variables with *P* > .2 were omitted from the OS model.

Table A9. Distribution of Incidence of Death for Patients With t(8;21) in CR1 Among Different Treatment Groups

Treatment Group	No. of Patients in CR1	No. (%) of Patients		<i>P</i>
		Alive	Dead	
Cumulative anthracycline, mg/m ²				
≤ 400	408	307 (73)	111 (27)	.125
> 400	349	273 (78)	76 (22)	
Cumulative cytarabine, g/m ²				
≤ 30	464	341 (74)	123 (27)	.089
> 30	303	239 (79)	64 (21)	
Cumulative etoposide, mg/m ²				
≤ 1,500	417	310 (74)	107 (26)	.368
> 1,500	350	270 (77)	80 (23)	
No allo-SCT in CR1	656	499 (76)	157 (24)	.539
Allo-SCT in CR1	104	76 (73)	28 (27)	

Abbreviations: allo-SCT, allogeneic stem-cell transplantation; CR1, first complete remission.

Table A10. Overview of Treatment Protocols

Study Group	Protocol	No. of courses	Course 1				Courses 1 and 2			
			Cytarabine (mg/m ²)	HD Cytarabine (g/m ²)	Anthracycline (mg/m ²)	Etoposide (mg/m ²)	Cytarabine (mg/m ²)	HD Cytarabine (g/m ²)	Anthracycline (mg/m ²)	Etoposide (mg/m ²)
AIEOP BFM (no HR in patients with t(8;21))	AML2002/01	3	1,400	No	150 ida	500	2,800	No	300	1,000
	AML-BFM1987	4 + MT	1400	No	180	450	3,200	No	300	450
	AML-BFM1993 (R: ADE or AIE and 6-week consolidation)	3 + MT	1400	No	180	450	3,200	No	300	450
	AML-BFM1998	5 + MT	1400	No	180	450	19,400	Yes: 3 g	300	450
	AML-BFM2004 (R: ADx E or AIE)	4 + MT	1400	No	180 if AIE 240 if ADx E	450	SR 3,400	No	300 if AIE, 360 if ADx E	450
EORTC	EORTC-CLG 58,872	4+MT	1,400	No	150	450	5,000	No	350	450
	EORTC-CLG 58,921 (R: mitox or ida)	4+MT	1,400	No	150	450	25,400 if no SCT; 19,400 if allo-SCT	Yes: 3 g	300	450
COG	COG2891	5	800	No	80	400	1,600	No	160	800
	COG2961 R1: DCTEI or DCTER, R2: DCTEI/R or FAMP	If DCTEI/R, 5; if FAMP, 4	800	No	100	400	1,600	No	180	800
	AAML03P1	5	2,000	No	150	500	3,600	No	300	1,000
CPH	AML-BFM protocols (AML-BFM93, -98, -98interim, and -2004)									
DCOG	ANLL87	4	1,600	No	180	450	1,400	No	300	450
	ANLL92/94	4	1,600	No	180	450	13,600	Yes: 3 g	280	450
GATLA	ANLL97	= MRC12								
	No HAM	4	1,400	No	150	450	2,600	No	270	450
HongKong	With HAM	5	1,400	No	150	450	19,400	Yes: 3 g	250	450
	= MRC10									
JPLSG (only few patients had Ida (Induction B))	AML99	6	1400	No	125	750	19,400	Yes: 3 g	175	1,250
CCLSG 9805 (pirarubicin)	FAB M1 or M2	8	1,120	No	80	0	2,240	No	160	0
	Other									
FRALLE	LAME89 and LAME91	3 + MT	1,400	No	300	0	1,800	No	460	400
	LAME97	4	800	No	180	0	1,400	No	300	0
MRC	MRC-AML10 (RDAT or ADE)	4	2000	No	150	0 if DAT; 500 if ADE	3,600	No	300	0 v 1,000
	MRC-AML12 (R: ADE or MAE and with/without CLASP)	4-5	2,000	no	150 (ADE) or 180 (MAE)	500	36,000	No	300 (ADE) or 360 (MAE)	1,000
NOPHO	NOPHO-AML 1988	7	800	No	75	400	1,300	No	225	400
	NOPHO-AML 1993	SR 6	800	No	75	400	1,600	No	150	800
	NOPHO-AML 2004	PH 6 or 7	800	No	180	400	1,300	No	225	400
POG	NOPHO-AML 2004	6	800	No	180	400	1,300	No	330	400
	POG8821 R: auto-SCT or chemotherapy	9	700	no	135	0	18,700	Yes: 3 g	135	0
PPLSG	POG9421 R: DAT/HDAT	5	700 v 14,000	No/yes: 1 g	135	0	10,700 or 24,000	Yes: 1 g	135	0
	Adapted BFM protocol(s)									
Pinda/Chile	AML-BFM protocols									
Russia	Unknown	4	1,600	No	180	500	2,600; 1 patient received HAM	No (1 patient, HAM)	300-320	500
St Jude	AML87	6	2,000	No, 500 mg	0	2,000	4,500	No, 500 mg	100	2,000
	AML91 2-CDA 2x	4	0	No	0	0	0	No	90	400
	AML97 Based on AML91; induction 2-CDA plus cytarabine	3	2,500	No, 500 mg	0	0	3,750	No, 500 mg	90	400
	AML02 random assignment: HD-cytarabine for course 1; t(8;21), no HD cytarabine	5	2000 v 18,000	No; yes: 3 g if HD cytarabine	150	400	4,000 or 20,000	No, Yes: 3 g if HD cytarabine	300	800
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Table A10. Overview of Treatment Protocols (continued)

Study Group	SCT	Cumulative Treatment				CNS	Maintenance	Additional SCT	Author
		Cytarabine (g/m ²)	Anthracycline (mg/m ²)	Etoposide (mg/m ²)					
AIEOP		20.8	400	1,000	No				Pession et al ⁶
BFM (no HR in patients with t(8;21))	No (HR only)	41	300	1,450	Yes		Yes, tg and cytarabine SC	Allo or auto for all patients	Creutzig et al ^a
	No (HR only)	SR 21.2	SR 300	950	Yes		Yes, tg and cytarabine SC	HR only	Creutzig et al ^a
		39.2 if consolidation; 45.4 if AI+HAM	420 if consol; 450 if AI+HAM	950	Yes		Yes, tg and cytarabine SC	HR only	Creutzig et al ^b
		SR 27.4	SR, 350 if AIE 410 if ADXE	950	Yes		Yes, tg and cytarabine SC	HR only	Creutzig et al ¹³ (via personal comment at time of analyses)
EORTC		29 if no SCT; 5 if SCT	500	950	Yes		Yes		Entz-Werle et al ^c
		38.2; 19.4 if SCT	380; 300 if allo-SCT	1,350	Yes		Yes	HR only	Entz-Werle et al ^c
COG	Allo-SCT if donor, R auto-SCT, or consolidation	27.2	320	1,600	Yes		No	Yes if donor, otherwise R auto-SCT or consolidation	Smith et al ^d
	SCT or HD cytarabine	If 2 nd DCTE/IR, 27.2; if FAMP, 33.1	360	800	Yes		R IL-2 or no maintenance	chemotherapy	Lange et al ^e
	SCT after 3rd course or not	69.6	540	1,750	Yes		No	Related donor if available	Cooper et al ^f
CPH		27.4 39.2 45.4	Protocols 93 and 98; 420, 350 SR; protocol 98	825	Yes		No		Received data per patient were slightly different from BFM protocols
			Interim SR, and protocol 2004; 350						
DCOG		38.2	300	1,450	Yes		No	HR only	Kardos et al ^g
		32.2	400	950	No		No	Allo if donor, auto-SCT otherwise	Hählen et al ^h (from DCOG archive)
GATLA		21.2	270	950	Yes		Yes	No	Armendariz et al ⁱ
		39.2	370	950	Yes		Yes	No	Armendariz et al ⁱ
HongKong JPLSG (only few patients had Ido (Induction B))		59.4-78.4	300-375	3150-3,200	Yes		No	Allo-SCT if donor and intermediate risk; allo-SCT for all HR	Tsukimoto et al ³⁴
CCLSG 9805 (pirarubicin)		25.4	560	600	Yes		No	Allo-SCT if no CR after induction 1	Taga et al ^j
		21.4	560	1,200					
FRALLE	Allo-SCT if donor, otherwise chemotherapy	9.8; 13.4 if MT	460 + amsacrine, 450	400	Yes for M4/M5 or WBC > 50 × 10 ⁹ /L at diagnosis		R MP and cytarabine SC or none	Allo-SCT	Perel et al ^k
	Allo-SCT if donor, otherwise chemotherapy	9.8;	460 + 450, amsacrine	400	Yes, for M4/M5 or WBC > 50 × 10 ⁹ /L at diagnosis		No	Allo-SCT if donor	Perel et al ^k
		13.4 if MT (not given from 1996)							
MRC	Allo-SCT if donor, otherwise R between auto-SCT or no additional therapy	10.6	550 + 500, amsacrine	500-1,500	Yes		No	Allo-SCT or auto-SCT	Hann et al ^l
	if GR, SR, or HR and without donor without CLASP	10.6	550 or 610 if MAE; 500, amsacrine if with CLASP	1,500	Yes		No	If donor available and SR or HR	Gibson et al ^m
	Allo-SCT = no midac	34.6 if with CLASP	500, amsacrine if with CLASP						
		4.6/28.6 if CLASP	300 or 360 if MAE						

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Abbreviations: ADE, cytarabine, daunorubicin, etoposide; ADXe, cytarabine, daunoxome, etoposide; AIE, cytarabine, idarubicin; AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; allo-SCT, allogeneic SCT; AML, acute myeloid leukemia; ANLL, acute nonlymphoblastic leukemia; Ara-C, arabinofuranosyl cytidine (cytarabine); auto-SCT, autologous SCT; BFM, Berlin-Frankfurt-Münster; CCLSG, Children's Cancer and Leukemia Study Group; CDA, chlorodeoxyadenosine; CLASP, cytarabine, fludarabine, hasparaginase; COG, Children's Oncology Group; CR, complete remission; DAT, Ara-C, daunorubicin, thioguanine; DCOG, Dutch Childhood Oncology Group; DCTE1/R, dexamethasone cytarabine, thioguanine, etoposide, idarubicin/daunorubicin; EORTC-CLG, European Organisation for Research and Treatment of Cancer–Children's Leukemia Group; FAB, French-American-British; FAMP, fludarabine, cytarabine, idarubicin; FRALLE, French Acute (Lymphoblastic) Leukemia Study Group; GATLA, Grupo Argentino de Tratamiento de la Leucemia Aguda; GO, gemtuzumab ozogamicin; GR, good response; HAZE, HD cytarabine 2 g/m², etoposide; HAE, HD cytarabine, etoposide; HAT, HD cytarabine, thioguanine; HD, high dose; HDAT, HD cytarabine, daunorubicin, thioguanine; HR, high risk; Ida, idarubicin; IL-2, interleukin 2; JPLSG, Japanese Paediatric Leukemia/Lymphoma Study Group; LAME, Leucémies Aigües Myéloblastiques de l'Enfant Cooperative Group; MAE, mitoxantrone, cytarabine, etoposide; midac, mitoxantrone, Ara-C; mitox, mitoxantrone; MRC, Medical Research Council; MT, maintenance; NOPHO, Nordic Society of Paediatric Haematology and Oncology; POG, Pediatric Oncology Group; PPLLSG, Polish Pediatric Leukemia Lymphoma Study Group; PR, poor response; R, random assignment; R1, first randomization; R2, second randomization; SC, subcutaneous; SCT, stem-cell transplantation; SR, standard risk; tg, thioguanine.

Fig A1. Distribution of additional cytogenetic aberrations in patients with t(8;21) and full karyotype (n = 838). +4, gain of chromosome 4 (n = 21); +8, gain of chromosome 8 (n = 49); abn(7q), abnormalities of chromosome 7q (n = 41); del(9q), deletion of the long arm of chromosome 9 (n = 104); LOS, loss of sex chromosome (n = 381).

Fig A2. (A) Kaplan-Meier curves of event-free survival time: year of diagnosis. (B) Kaplan-Meier curves of overall survival: year of diagnosis.

Fig A3. (A) Kaplan-Meier curves of event-free survival for *cKIT* mutation. (B) Kaplan-Meier curves of event-free survival for *RAS* mutation.