Table II. Clinical characteristics and detailed karyotype data in T-LBL patients with t(9;17).

	Age (years)	Sex	Tumour site	Stage	BM blast %	Karyotype
Kaneko *et al* (1988)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	15	M	Mediastinum	III	0	46,XY,-9,del(6)(q13q21),t(9;17)(q34;q23),+der(9)t(9;17)(q34;q23)
	10	M	Mediastinum	III	0	47,XY,+19,t(9;17)(q34;q23)
Shikano *et al* (1992)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	7	M	Mediastinum	III	0	49,XY-l,+der(l)t(l;?)(p36;?),t(9;17)(q34;q23),+14,+marl,+mar2
	5	F	Mediastinum	III	0	47,XX,t(9;17)(q34;q23),+der(17)t(9;17)(q34;q23)
Burkhardt *et al* (2006)	ND	ND	ND	ND	ND	46,XX,del(6)(q1?2q1?6),t(9;17)(q34;q22)
	ND	ND	ND	ND	ND	47,XX,t(9;17)(q34;q22),+20
Lones *et al* (2007)	8	M	Mediastinum	III	0	47,XY,t(9;17)(q3?4;q2?3),+20
Current study	7	M	Mediastinum	III	0	46,XY,t(9;17)(q34;q22)

ND, no data available.

study groups reported *NOTCH1* mutations in 31–62% of T-ALL patients (Weng *et al*, 2004; Breit *et al*, 2006; van Grotel *et al*, 2006; Zhu *et al*, 2006; Malyukova *et al*, 2007; Asnafi *et al*, 2009; Gedman *et al*, 2009; Park *et al*, 2009). In contrast, only two studies reported *NOTCH1* mutation analyses in T-LBL: Park *et al* (2009) reported *NOTCH1* mutations in six out of 14 paediatric T-LBL patients (43%), and Baleydier *et al* (2008) reported mutations in six out of nine paediatric T-LBL (66%), with 32 adult patients with *NOTCH1* mutations in 16 cases (54% in all patients) (Baleydier *et al*, 2008). According to these reports, the frequencies of *NOTCH1* mutation were not significantly different between T-LBL and T-ALL.

ABL1 fusion genes have been identified that provide proliferation and survival advantage to lymphoblasts. *NUP214-ABL1*, *EML1-ABL1*, *BCR-ABL1* and *ETV6-ABL1* chimeric genes have been reported. The most frequent one in T-ALL is the *NUP214-ABL*1 fusion gene, which has been identified in 6% of cases, in both children and adults (Graux *et al*, 2009). In addition, using an oligonucleotide microarray, *ABL1* overexpression was identified in 8% of cases in T-ALL (Chiaretti *et al*, 2007). Our review of these published reports indicated that the frequency of ABL1 mutation in T-LBL is unknown.

Raetz *et al* (2006) analysed the gene expression profiles of ten T-ALL BM samples and nine T-LBL samples using a microarray. They identified 133 genes for which the expression levels differed between T-LBL and T-ALL. *ZNF79* (encoding zinc finger protein 79) and *ABL1*, both located in chromosome region 9q34, were included in these genes and showed at least twofold higher overexpression in T-LBL than that in T-ALL. Additionally, *MED13* (previously termed *THRAP1*), which is located in 17q22-q23, also showed at least twofold higher overexpression in T-LBL than that in T-ALL (Raetz *et al*, 2006). Taking these findings together, it is possible that *ZNF79*, *ABL1* or *THRAP1* as well as other genes at 9q34 and 17q22–23 are involved in the 'lymphoma phenotype' such as a bulky mass in the mediastinum and minimal BM involvement. These findings need further study to determine if this linkage constitutes a unique 'lymphoma phenotype'.

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Authorship

MS designed the study, prepared the data file, performed the analysis, interpreted data and wrote the manuscript. SS is a lead principal investigator for the JPLSG ALB-NHL03 study. AN contributed to pathological diagnosis. YH contributed to chromosome analysis. YO is a principal investigator contributing a patient to this study. AMS contributed to statistical analysis. KH received a research grant from the Ministry of Health, Labour and Welfare of Japan. MT is a chairperson of JPLSG. TM is a chairperson of JPLSG lymphoma committee. SS, KH, MT and TM were primarily responsible for the study design, data analysis and interpretation of the data. All authors approved the final manuscript.

Disclosure

The authors declare no competing financial interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Respective clinical characteristics with and without karyotype data in 111 T-LBL patients in the current study.

Table S2. Comparison of cytogenetic findings in T-LBL between current study and combined data of three published reports.

Table S3. Published data of cytogenetic findings in T-LBL and T-ALL.

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ORIGINAL ARTICLE

Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/Lymphoma Study Group

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Abstract Although the antigen expression patterns of childhood acute lymphoblastic leukemia (ALL) are well known, little attention has been given to standardizing the diagnostic and classification criteria. We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL in JPLSG. T- and B-lineage ALL accounted for 13 and 87% of childhood ALL cases, respectively. Cytoplasmic CD3 and CD7 antigens were positive in all T-ALL cases. More than 80% of T-ALL cases expressed CD2, CD5 and TdT. In B-lineage ALL, the frequencies of early pre-B, pre-B, transitional pre-B and B-ALL were 81, 15.5, 0.6 and 2.9%, respectively. More than 90% of early pre-B ALL cases expressed CD19, CD79a, CD22, CD10 and TdT. CD34 was expressed in three-fourths of early pre-B ALL cases. The frequencies of TdT and CD34 expression were lower in pre-B ALL than in early pre-B ALL. B-ALL showed less frequent expression of CD22, CD10, CD34 and TdT than other B-lineage ALL cases. Expression of CD13 and CD33, aberrant myeloid antigens, was significantly more frequently associated with B-lineage ALL than with T-ALL. Based on this retrospective study of antigen expression in 1,774 de novo childhood ALL cases in JPLSG, we propose standardized clinical guidelines for the immunophenotypic criteria for diagnosis and classification of pediatric ALL.

Keywords Acute lymphoblastic leukemia · Childhood · Flow cytometry · Immunophenotype

1 Introduction

Flow cytometric immunophenotyping of childhood acute lymphoblastic leukemia (ALL) plays an important role not

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only in the diagnosis and classification of B and T cell lineages, but also in predicting the outcome [1–8].

Childhood ALL is a heterogeneous group of diseases. Therefore, leukemic cells from patients with ALL express a variety of differentiation antigens that are also found on normal lymphocyte precursors at discrete stages of maturation. With the development of monoclonal antibodies specific for relatively lineage-restricted or hematopoietic cell antigens, it has been possible to demonstrate considerable phenotypic heterogeneity in the vast majority of ALL cases by using panels of those antibodies [1, 2, 9–12].

The immunophenotypic patterns of acute leukemia, especially ALL, are well known, and classification into major immunologic categories is also accepted [1, 2, 9–12]. However, little attention has been given to standardizing the criteria for concluding which antigens are present on childhood leukemic cells, especially in Japan.

Herein, we report for the first time the results of a large, retrospective study of antigen expression in 1,774 children, older than 1 year and younger than 19 years of age, with newly diagnosed ALL, who had been enrolled between 1997 and 2007 at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Based on these results, we have formulated guidelines for use of immunologic markers and proper interpretation of the results. It should be noted that this study did not investigate possible associations of antigen expression with the clinical, hematological and biological features or their prognostic importance, because the present study included patients for whom a complete set of these information and the immunophenotypic characteristics based on flow cytometry were not available due to several limiting factors associated with the registration system.

2 Methods

2.1 Patient samples

This is a retrospective analysis of 1,774 pediatric patients with newly diagnosed and untreated ALL. It excluded acute undifferentiated leukemia and true mixed-lineage leukemia, defined as co-expression of golden markers of two different lineages, e.g., MPO^+ and $CD79a^+$, or MPO^+ and $CD3^+$ [10]. The analyzed patients had been enrolled between 1997 and 2007 at hospitals affiliated to the Japan Association of Childhood Leukemia Study (JACLS), the Tokyo Children's Cancer Study Group (TCCSG) and the Japanese Children's Cancer and Leukemia Study Group (JCCLSG). These three study groups, combined with the Kyushu Yamaguchi Children's Cancer Study Group (KY-CCSG), constitute the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). All patients were diagnosed with ALL according to the French–American–British (FAB) morphology, enzyme cytochemical analysis and immunologic phenotype based on flow cytometric analysis. Samples obtained from bone marrow or peripheral blood of patients were immediately transported in sodium heparin tubes overnight to the central reference flow cytometry laboratories of the JPLSG. Informed consent for reference laboratory studies was obtained using forms approved by the local institutional review boards.

2.2 Flow cytometry

Ficoll–Hypaque-enriched blasts were stained by two-color immunofluorescence using various combinations of monoclonal antibodies, conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC), against the following antigens: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD42b, CD45, CD56, CD58, CD66c, CD117, glycophorin A, HLA-DR, immunoglobulin kappa (Igκ) and lambda (Igλ) light chains, T cell receptors ($\alpha\beta$ and $\gamma\delta$) on the surface of leukemic cells and cytoplasmic Igμ chain, CD3, CD22, CD79a and myeloperoxidase antigens, as well as nuclear TdT. For detection of cytoplasmic (cCD3, cCD22, CD79a and MPO) and nuclear TdT antigens, antibodies were added after permeabilization using an Intraprep Permeabilization reagent kit (Beckman Coulter Immunotech, Miami, FL, USA). Isotypical immunoglobulins were used as negative controls. Two-color flow cytometric immunophenotyping was performed on an FACScan (Becton–Dickinson, San Jose, CA, USA) or EPICS flow cytometer (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's directions. The analysis gate was set in the forward and side light-scattering positions with lymphoid morphology. Data were recorded by an observer blinded to the patient's clinical status and diagnostic features, except for the immunophenotype. An antigen was rated as "positive" if more than 20% of the gated cells showed specific labeling above that of controls, or if a positive subpopulation was distinctively identified even in less than 20% positive cases. In principle, the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others [1, 9, 10] were used for immunophenotypic classification.

2.3 Statistical analysis

Statistical analysis was performed by taking into account gender, age and the presence or absence of myeloid antigens, i.e., CD13 and CD33. Differences in the distributions of variables between groups of patients were analyzed by Mann–Whitney's U test, Kruskal–Wallis test or the χ^2 test.

3 Results

3.1 Clinical features and FAB morphology

The clinical presenting features, which include gender and age, and the FAB morphology, are summarized in Table 1. The boys-to-girls ratio of the incidence and the median age in cases of T-lineage ALL were significantly higher than in cases of B-lineage ALL ($p < 0.001$). Among patients with B-lineage ALL, these clinical characteristics were statistically more frequent in cases of mature B-ALL than in other types of B-lineage ALL ($p < 0.05$). In FAB morphology,

Table 1 Characteristics and immunophenotypic profile of 1,774 de novo cases of acute lymphoblastic leukemia

	T-ALL	B-lineage ALL		
		Early pre-B	Pre-B[a]	Mature B
Number of cases	231	1250	248	45
Frequency (%)	13.0	70.5	14.0	2.5
Clinical features				
Gender (boy/girl) (%)	74/26	55/45	51/49	74/26
Median age (range)	8 (1–16)	4 (1–18)	5 (1–15)	10 (1–15)
FAB morphology				
L1/L2/L3 (%)	72/28/0	82/17.5/0.5	84/16/0	0/0/100
T-lineage markers				
CD1a	53.7	0.3	1.5	0.0
CD2	83.5	4.1	4.0	2.2
cCD3	100	0.0	0.0	0.0
sCD3	49.3	0.0	0.0	0.0
CD4	54.8	0.8	0.0	0.0
CD5	94.2	0.5	10.1	0.0
CD7	100	3.2	6.9	2.2
CD8	68.3	1.1	0.0	0.0
TCR$\alpha\beta$	29.4	6.3	8.5	0.0
TCR$\gamma\delta$	10.9	0.0	0.0	0.0
B-lineage markers				
CD19	0.0	99.6	98.8	100
CD20	0.0	19.2	23.6	88.9
cCD22	2.9	90.1	97.3	77.8
sCD22	1.8	70.3	87.6	60.5
CD79a	21.8	99.2	100	100
cIgμ	0.0	0.0	100	88.9
sIgμ	0.0	2.1	9.0	83.3
sIgκ or λ	0.0	0.0	0.0	100
Non-lineage specific markers				
TdT	84.4	97.0	83.8	13.0
CD10	31.6	91.2	93.5	77.8
CD34	37.3	74.6	44.5	7.0
HLA-DR	16.7	99.3	94.7	97.7
Myeloid markers				
MPO	0.0	0.0	0.0	0.0
CD13	20.7	36.0	22.7	14.3
CD14	0.0	0.6	0.0	0.0
CD33	15.2	31.6	15.0	2.2
CD41	0.0	0.8	3.3	0.0
CD66c	0.5	43.5	25.9	0.0
CD117	15.6	10.1	13.4	11.5
GlyA	0.0	0.0	0.0	0.0

Values indicate the proportion of positive cases (%)

c cytoplasmic, *s* surface

[a] Pre-B cases include transitional pre-B cases

3 Results

3.1 Clinical features and FAB morphology

The clinical presenting features, which include gender and age, and the FAB morphology, are summarized in Table 1. The boys-to-girls ratio of the incidence and the median age in cases of T-lineage ALL were significantly higher than in cases of B-lineage ALL ($p < 0.001$). Among patients with B-lineage ALL, these clinical characteristics were statistically more frequent in cases of mature B-ALL than in other types of B-lineage ALL ($p < 0.05$). In FAB morphology,

Table 1 Characteristics and immunophenotypic profile of 1,774 de novo cases of acute lymphoblastic leukemia

	T-ALL	B-lineage ALL		
		Early pre-B	Pre-B[a]	Mature B
Number of cases	231	1250	248	45
Frequency (%)	13.0	70.5	14.0	2.5
Clinical features				
Gender (boy/girl) (%)	74/26	55/45	51/49	74/26
Median age (range)	8 (1–16)	4 (1–18)	5 (1–15)	10 (1–15)
FAB morphology				
L1/L2/L3 (%)	72/28/0	82/17.5/0.5	84/16/0	0/0/100
T-lineage markers				
CD1a	53.7	0.3	1.5	0.0
CD2	83.5	4.1	4.0	2.2
cCD3	100	0.0	0.0	0.0
sCD3	49.3	0.0	0.0	0.0
CD4	54.8	0.8	0.0	0.0
CD5	94.2	0.5	10.1	0.0
CD7	100	3.2	6.9	2.2
CD8	68.3	1.1	0.0	0.0
TCR$\alpha\beta$	29.4	6.3	8.5	0.0
TCR$\gamma\delta$	10.9	0.0	0.0	0.0
B-lineage markers				
CD19	0.0	99.6	98.8	100
CD20	0.0	19.2	23.6	88.9
cCD22	2.9	90.1	97.3	77.8
sCD22	1.8	70.3	87.6	60.5
CD79a	21.8	99.2	100	100
cIgμ	0.0	0.0	100	88.9
sIgμ	0.0	2.1	9.0	83.3
sIgκ or λ	0.0	0.0	0.0	100
Non-lineage specific markers				
TdT	84.4	97.0	83.8	13.0
CD10	31.6	91.2	93.5	77.8
CD34	37.3	74.6	44.5	7.0
HLA-DR	16.7	99.3	94.7	97.7
Myeloid markers				
MPO	0.0	0.0	0.0	0.0
CD13	20.7	36.0	22.7	14.3
CD14	0.0	0.6	0.0	0.0
CD33	15.2	31.6	15.0	2.2
CD41	0.0	0.8	3.3	0.0
CD66c	0.5	43.5	25.9	0.0
CD117	15.6	10.1	13.4	11.5
GlyA	0.0	0.0	0.0	0.0

Values indicate the proportion of positive cases (%)

c cytoplasmic, *s* surface

[a] Pre-B cases include transitional pre-B cases

ALL cases, and more than 90% expressed CD10 and HLA-DR. However, the frequencies of TdT and CD34 expression were 83.8 and 44.5%, respectively, which are lower than for early pre-B ALL cells. The expression frequencies of CD13 and CD33 were also lower than in the early pre-B ALL cases, at 22.7 and 15.0% ($p < 0.001$) (Fig. 1).

3.5 B cell ALL

B-ALL cells are characterized by L3 morphology, as defined in the FAB classification, and by surface membrane expression of immunoglobulin μ heavy chains (sIg) plus monotypic light chain [1, 9, 10]. In our present study, B-ALL cases accounted for 2.5% (45/1,774) of our de novo ALL cases (Table 1). The blasts of the B-ALL cases also expressed CD19, cCD79a, CD20 and HLA-DR. Both CD22 and CD10 were less frequently expressed in these cases than in other B-lineage ALL cases, including early pre-B and pre-B ALL. Although B-ALL cells are generally negative for expression of TdT and CD34, a few B-ALL cases with blasts that expressed TdT and/or CD34 have been reported [10, 16–19]. Moreover, Gluck et al. [20] diagnosed a B-ALL case that was L3 in the FAB classification with typical Burkitt's type translocation, but lacking sIg. In fact, we also identified a few cases with expression of TdT and/or CD34 and one case without sIg expression (positive for monotypic light chain) in this series. CD13 and CD33 antigens were expressed in some cases: 14.3 and 2.2%, respectively (Fig. 1).

4 Discussion

Immunophenotypic analysis of acute leukemia by flow cytometry has been used clinically as an indispensable tool for identification of the lineage association of leukemic cells and evaluation of the response to treatment [1, 2, 10–12, 21]. Recently, panels of monoclonal antibodies specific for lineage-associated antigens have been expanded. As a result, immunophenotyping of ALL has been applied to distinguish it from acute myeloid leukemia (AML) and to achieve more accurate phenotyping within ALL.

We retrospectively analyzed the flow cytometric data from a large study of antigen expression in 1,774 children with newly diagnosed ALL who were enrolled at hospitals affiliated to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) between 1997 and 2007. Each central reference flow cytometry laboratory of the JPLSG made immunophenotypic diagnoses based on the criteria recommended by the European Group for the Immunological Characterization of Leukemias and others for childhood acute leukemia [1, 9, 10]. Although these criteria are actually similar to each other and standardized, they advocate some different subclasses in T- or B-lineage ALL. Additionally, ALL with myeloid antigen expression might be observed frequently in cases with mixed-lineage leukemia. However, the criteria for myeloid marker-positive childhood ALL and the clinical significance of these antigens also vary. We then formulated guidelines for the use of immunomarkers and proper interpretation of the results in childhood ALL, as summarized in Table 2.

T-lineage ALL, according to our analytical findings, is characterized by cytoplasmic or surface membrane expression of CD3 together with CD2, CD5, CD7 or CD8 (Table 2). Some of our T-ALL cells expressed CD79a or CD22 as a marker for B-lineage ALL. Although such T-ALL cases have been reported by other investigators [22, 23], none of our T-ALL cases satisfied the diagnostic criteria for B-lineage ALL described below. Recently, Campana et al. [13] reported diagnosis of early T cell precursor (ETP)-ALL, as a subgroup with a poor prognosis,

Table 2 Proposed immunophenotypic criteria for de novo cases of acute lymphoblastic leukemia

T-lineage ALL
1. $CD3^+$
2. Express CD2, CD5, CD7 or CD8
B-lineage ALL
Early pre-B ALL
Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
Pre-B ALL[a]
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Negative for surface membrane immunoglobulin κ or λ light chains
3. Express cytoplasmic and/or surface immunoglobulin μ heavy chains
B-ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. Express surface membrane immunoglobulin κ or λ light chains
ALL with aberrant myeloid-associated antigen expression
My Ag^+ T-lineage ALL
1. $CD3^+$ and express CD2, CD5, CD7 or CD8
2. $CD79a^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)
My Ag^+ B-lineage ALL
1. Express at least two B-lineage markers (CD19, CD20, CD22 or CD79a)
2. $CD3^-$
3. MPO^- and express myeloid-associated markers (CD13, CD15, CD33 or CD65)

[a] Pre-B ALL cases include transitional pre-B cases

characterized by absence of CD1a and CD8 expression and weak CD5 expression. At least 25% of ETP-ALL cells also express one or more of the following myeloid or stem-cell markers: CD117, CD34, HLA-DR, CD13, CD33, CD11b and CD65. Interestingly, they also pointed out that for patients with T-ALL, a diagnosis of ETP-ALL should be a stronger predictor of the outcome than is flow cytometric-based minimal residual disease [13]. We also found some ETP-ALL cases in our present study. The exact number of these immunophenotypic cases could not be indicated because not all of the myeloid or stem-cell markers reviewed above were used to diagnose our de novo ALL cases. However, six of 164 cases diagnosed using all these markers met the criteria for ETP-ALL. This frequency, 3.7%, was much less than the 12.6% reported by Campana et al. [13]. The difference in its frequency and correlation with the outcome should be ascertained in a future study.

Next, we classified B-lineage ALL into three categories, i.e., early pre-B ALL, pre-B ALL and mature B-ALL, according to the degree of B lymphoid differentiation of leukemic cells. Most cases of early pre-B ALL were positive for the common ALL antigen (CD10), CD34, HLA-DR and TdT. However, these antigens are not lineage specific. Although the immunoglobulin heavy chains are usually rearranged in these leukemic blasts, immunoglobulins were not detected. Early pre-B ALL can be conclusively defined as expression of at least two of the following four early B cell markers: CD19, CD20, CD22 and CD79a (Table 2). Pre-B ALL can be generally distinguished from transitional pre-B ALL based on their respective immunophenotypic characteristics [1, 10, 15]. However, in this study, we combined these two phenotypes as pre-B ALL, because discrimination of them might not be so important in the clinic [15, 21]. Pre-B ALL, including transitional pre-B ALL, can be defined as expression of cytoplasmic immunoglobulin μ heavy chains without κ or λ light chains and the presence of at least two of the following markers: CD19, CD20, CD22 and CD79a (Table 2). Additionally, B-ALL can be defined as expression of surface membrane immunoglobulin κ or λ light chains and at least two of the following markers: CD19, CD20, CD22 and CD79a (Table 2). Since, in rare instances, surface immunoglobulin μ heavy chains are absent in B-ALL cases, these markers are excluded from the definition of this immunophenotype [20].

Aberrant expression of one or more immunologic markers of another lineage might be observed in cases with mixed-lineage leukemia, which include myeloid antigen-positive ALL (B-lineage or T-lineage), lymphoid antigen-positive AML and true mixed-lineage leukemia [10]. Although our study included myeloid antigen-positive ALL, we did not find either biclonal or oligoclonal leukemias, which consist of two or more morphologically or immunophenotypically distinct leukemic cell populations. Expression of aberrant myeloid antigens (MyAgs) reportedly occurs in 5–22% of pediatric patients with de novo ALL [24–29]. We chose CD13 and CD33 as MyAgs, because they have been the most common antigens in MyAg-positive ALL. In our study, CD13 and CD33 were expressed in 31.7 and 26.5%, respectively, of de novo childhood ALL cases. Moreover, the frequency of CD13 expression was 33.3% in B-lineage ALL compared with 20.7% in T-ALL, while CD33 expression was 28.1% in B-lineage ALL versus 15.2% in T-ALL. These MyAgs were significantly more frequently associated with B-lineage ALL than with T-ALL ($p < 0.001$). In addition, the expression of these MyAgs was more frequent in early pre-B ALL cases than in pre-B ALL cases ($p < 0.001$). These incidences of MyAg expression in our study are in line with the data reported in the literature [24–29].

Recently, several notable studies investigated differences of race and ethnicity in the immunophenotypic subsets of childhood ALL [30–32]. Bhatia et al. [30] analyzed 8,762 children with de novo ALL who were categorized according to five groups: white, black, Hispanic, Asian and others. They showed that there was a significantly greater incidence of black children (25%) with T-ALL compared with Asian (19%), white (15%) and Hispanic (13%) children. In comparison, the frequency of T-ALL in our present report (the largest scale report in Japan to date), as representative data of East Asian children with ALL, was 13% of all cases, which is less than the 19% reported by Bhatia et al. [30]. This disparity cannot be readily explained. However, Kandan-Lottick et al. [32] pointed out that the reason might be that the Asian children analyzed by Bhatia et al. [30] were not Japanese, but from the Indian subcontinent and South Asia because they had been enrolled in the Children's Cancer Group Study.

In conclusion, based on the results of our large, retrospective study of antigen expression in 1,774 children with newly diagnosed ALL enrolled between 1997 and 2007, we have formulated clinically useful guidelines for flow cytometric immunophenotypic criteria for the diagnosis and classification of pediatric ALL in the JPLSG. The JPLSG was established in 2003 to create a research base for multi-center clinical trials for promotion of evidence-based medicine in pediatric hematologic malignancies. The JPLSG unifies several pediatric leukemia study groups, including the Japan Association of Childhood Leukemia Study (JACLS), the Tokyo Children's Cancer Study Group (TCCSG), the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) and the Kyushu Yamaguchi Children's Cancer Study Group (KYCCSG), which had been functioning in Japan since the 1970s. The patients analyzed in this study have been treated according to different clinical protocols in each study group, and some of

them have not been clinically observed long enough. In addition, the central reference flow cytometry laboratories of the JPLSG received samples and made immunophenotypic diagnoses even during the intervals between clinical studies. Therefore, in this study we did not concern ourselves with possible associations of antigen expression with the clinical, hematological or biological features, or attempt to determine the prognostic importance of antigen expression for the decision of treatments. Nevertheless, flow cytometric data generated by extensive use of our newly proposed immunological criteria together with common diagnostic panels developed according to the present analysis may be valuable for achieving more precise characterization of the leukemic blasts in each individual patient. This information, combined with the molecular and clinical features presented in the next standard clinical protocol for childhood ALL that will be issued by the JPLSG, will also contribute to the development of personalized medicine, the so-called tailor-made therapy, for each patient.

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Prognostic significance of additional cytogenetic aberrations in 733 de novo pediatric 11q23/*MLL*-rearranged AML patients: results of an international study

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We previously demonstrated that outcome of pediatric 11q23/*MLL*-rearranged AML depends on the translocation partner (TP). In this multicenter international study on 733 children with 11q23/*MLL*-rearranged AML, we further analyzed which additional cytogenetic aberrations (ACA) had prognostic significance. ACAs occurred in 344 (47%) of 733 and were associated with unfavorable outcome (5-year overall survival [OS] 47% vs 62%, $P < .001$). Trisomy 8, the most frequent specific ACA (n = 130/344, 38%), independently predicted favorable outcome within the ACAs group (OS 61% vs 39%, $P = .003$; Cox model for OS hazard ratio (HR) 0.54, $P = .03$), on the basis of reduced relapse rate (26% vs 49%, $P < .001$). Trisomy 19 (n = 37/344, 11%) independently predicted poor prognosis in ACAs cases, which was partly caused by refractory disease (remission rate 74% vs 89%, $P = .04$; OS 24% vs 50%, $P < .001$; HR 1.77, $P = .01$). Structural ACAs had independent adverse prognostic value for event-free survival (HR 1.36, $P = .01$). Complex karyotype, defined as ≥ 3 abnormalities, was present in 26% (n = 192/733) and showed worse outcome than those without complex karyotype (OS 45% vs 59%, $P = .003$) in univariate analysis only. In conclusion, like TP, specific ACAs have independent prognostic significance in pediatric 11q23/*MLL*-rearranged AML, and the mechanism underlying these prognostic differences should be studied. (*Blood*. 2011;117(26):7102-7111)

Introduction

Pediatric acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease. In addition to the patient's initial response to treatment, its prognosis is largely determined by the presence of cytogenetic abnormalities and genetic lesions.[1-6] Several recurrent cytogenetic abnormalities, such as 11q23/*MLL*-rearrangements, predict outcome in myeloid neoplasms and acute leukemia.[7] So far, > 60 different translocation partners (TPs) have been identified, and new partners are still being reported to add to the diversity of *MLL*-rearranged leukemia.[8,9] The authors of a recent international study[10] highlighted the heterogeneity of 11q23/*MLL*-rearranged pediatric AML by demonstrating that outcome is dependent on TPs. This study also revealed that additional

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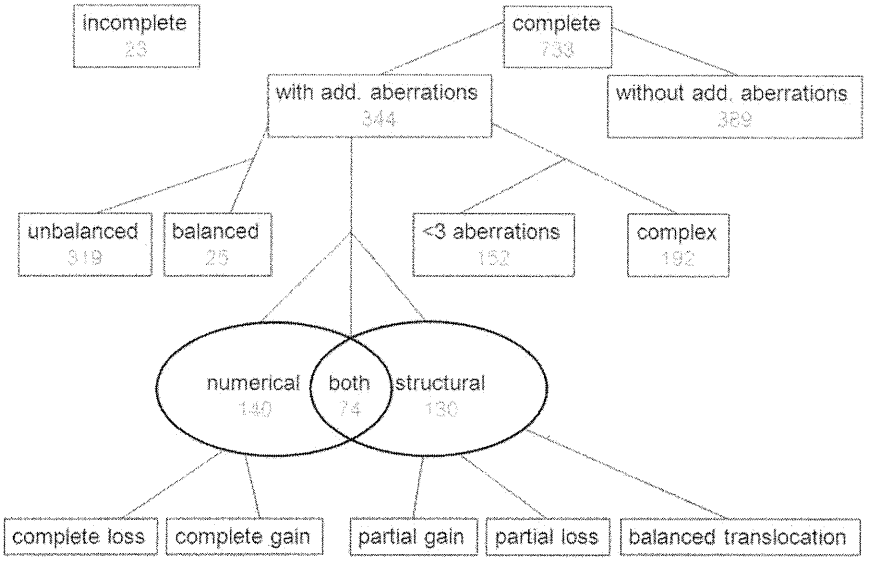

Figure 1. Flow chart showing the presence and type of ACAs in 756 pediatric patients with 11q23/MLL-rearranged AML. Complete karyotypes were not available for 23 patients, and they were therefore excluded from analyses. The presence or absence of ACAs was determined for 733 patients for whom complete karyotypes were available. In the cohort having ACAs balanced karyotype was coded for 25 patients; the remaining had an unbalanced karyotype. The types of aberrations were coded as numerical, structural, or both, and the number of aberrations was also coded. Losses and gains are further coded in other figures.

cytogenetic aberrations (ACAs) were an independent adverse prognostic factor,[10] but so far, it is unknown which additional aberration(s) determine this unfavorable outcome signature.

The authors of a recent large study in an adult AML cohort[11] showed that additional cytogenetic abnormalities in t(9;11)(p22;q23) AML did not affect outcome. However, the Berlin-Frankfurt-Münster group showed that children with t(9;11)(p22;q23) with additional aberrations had lower rates of overall survival (OS) than those with other subgroups of AML.[6]

To date, no large studies have been undertaken to study the prognostic relevance of specific ACAs in pediatric *MLL*-rearranged AML. In this multicenter international study, we retrospectively analyzed data from a large cohort (n = 733) to determine which ACAs contribute to the prognostic effect in pediatric *MLL*-rearranged AML.

Patients and methods

Patients

Patients' data collected in the retrospective international study by Balgobind et al[10] were included in this study. In summary, data from 756 patients with 11q23/*MLL*-rearranged pediatric AML were collected from 11 collaborative study groups—the Berlin-Frankfurt-Münster Study Group (Germany and Austria); the Japanese Pediatric Leukemia/Lymphoma Study Group (Japan); the Leucémies Aiguës Myéloblastiques de l'Enfant Cooperative Group (France); the Czech Pediatric Hematology Working Group (Czech Republic); the St Jude Children's Research Hospital (United States); the Associazione Italiana Ematologia Oncologia Pediatrica (Italy); Research Center for Pediatric Oncology and Hematology (Belarus); the Children's Oncology Group (United States); the Nordic Society for Pediatric Hematology and Oncology (Denmark, Finland, Iceland, Norway, and Sweden); the Dutch Children's Oncology Group (The Netherlands); and 2 centers of the Medical Research Council (United Kingdom). Patients were treated by national/collaborative group AML trials.[12-22] The treatment protocols were approved according to local law and guidelines and by the institutional review boards of each participating center, with informed consent obtained from the patients' parents or legal guardians in accordance with the Declaration of Helsinki.

Inclusion criteria for the current analyses were diagnosis between January 1, 1993, and January 1, 2005; younger than 18 years of age at diagnosis; and involvement of 11q23 or *MLL* as determined by G-, Q-, or R-banded karyotyping; FISH; or RT-PCR. Exclusion criteria were secondary AML after congenital BM failure disorders, aplastic anemia, previous chemotherapy or radiotherapy for other diseases, and previous myelodysplastic syndrome (MDS). Patients with Down syndrome were included if they met the other inclusion criteria. All clinical data obtained at initial diagnosis, data on treatment (therapy protocol, including HSCT), and all events during follow-up were checked for consistency and completeness.[10]

Cytogenetic analysis

All karyotypes were centrally reviewed by 2 cytogeneticists (J.H., S.C.R.) and assigned to 11q23/*MLL*-rearranged groups on the basis of TP.[10] All karyotypes were designated according to the International System for Human Cytogenetic Nomenclature 2005.[23]

To analyze ACAs, data from all patients with incomplete karyotypes were excluded. For all cases included in the analysis, the number of aberrations was counted. Each aberration separated from the rest of the karyotype by a comma was counted as one abnormality (regardless of its complexity), every aberration was counted only once (if present in multiple clones), and constitutional aberrations were excluded. Triploidy and tetraploidy were counted as 1 aberration (1 event). In this cohort of 11q23/*MLL*-rearranged cases, ACAs cases were defined as having 2 or more aberrations, including the 11q23/*MLL*-rearrangement (n = 344). All cases with 3 or more aberrations were considered having a complex karyotype, consistent with previously used definitions.[24,25] Numerical aberrations were defined as loss or gain of a full chromosome. Balanced translocations were defined as translocations in which no material seemed to be gained or lost as determined by conventional karyotyping. Structural aberrations were defined as aberrations resulting from breakpoints within a chromosome. In all unbalanced translocations we described which material was lost and gained and also whether 11q23 was involved. The presence of a balanced overall karyotype was defined as a karyotype with 2 complete copies of all autosomes and complete copies of sex chromosomes without any additional material (2n). Definitions used for cytogenetic classification are summarized in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Statistical analyses

Complete remission (CR) was defined as < 5% blasts in the BM, with regeneration of trilineage hematopoiesis plus absence of extramedullary disease.[26] Early death was defined as any death within the first 6 weeks of treatment. Treatment of patients who did not obtain CR within the specified time in the protocol was considered a failure on day 0. OS was measured from the date of diagnosis to the date of last follow-up or death from any

Table 1. Distribution of ACAs by translocation partner and clinically relevant parameters

			ACAs type		
	n	ACAs, n (%)	Numerical, n (%)	Structural, n (%)	Both, n (%)
TP group					
9p22	316	148 (47)	84 (57)*	40 (27)*	24 (16)*
10p12	96	48 (50)	13 (27)*	26 (54)*	9 (19)*
6q27	35	17 (49)	8 (47)*	7 (41)*	2 (12)*
19p13	30	10 (33)	6 (60)*	1 (10)*	3 (30)*
19p13.1	34	13 (38)	5 (38)*	4 (31)*	4 (31)*
19p13.3	25	13 (52)	5 (38)*	4 (31)*	4 (31)*
1q21	24	6 (25)	2 (33)*	3 (50)*	1 (17)*
4q21	13	8 (62)	2 (25)*	4 (50)*	2 (25)*
10p11.2	12	7 (58)	0*	5 (71)*	2 (29)*
17q21	12	3 (25)	1 (33)*	1 (33)*	1 (33)*
other	136	71 (52)	14 (20)*	35 (49)*	22 (31)*
	733				
Sex					
Male	358	171 (48)	78 (46)	63 (37)	30 (18)
Female	375	173 (46)	62 (36)	67 (39)	44 (25)
	733				
Age, y					
< 2	344	143 (42)	45 (31)†	68 (48)†	30 (21)†
2-9	219	115 (53)	57 (50)†	35 (30)†	23 (20)†
≥ 10	170	86 (51)	38 (44)†	27 (31)†	21 (24)†
	733				
WBC, $\times 10^9$/L					
< 20	339	175 (52)	84 (49)†	51 (29)†	40 (23)†
20-99	203	87 (43)	28 (32)†	40 (46)†	19 (22)†
≥ 100	171	73 (43)	25 (34)†	35 (48)†	13 (18)†
	713				
FAB					
M0	23	12 (52)	6 (50)	3 (25)	3 (25)
M1	39	20 (51)	9 (45)	7 (35)	4 (20)
M2	32	12 (38)	7 (58)	4 (33)	1 (8)
M4	134	49 (37)	21 (43)	21 (43)	7 (14)
M5	446	217 (49)	88 (41)	83 (38)	46 (21)
M7	19	15 (79)	7 (47)	2 (13)	6 (4)
n.d.	7	5 (71)	0	2 (40)	3 (60)
	700				
Overall		344 (47)	140 (41)	130 (38)	74 (22)

ACAs (%) indicates number of cases with additional aberrations and percentage within this group; Numerical (%), number of cases with only numerical additional aberrations and percentage of specific group (row); Structural (%), number of cases with only structural additional aberrations and percentage of specific group (row); Both (%), number of cases with both numerical and structural additional aberrations and percentage of specific group (row); and TP group, site of translocation on partner chromosome

ACA indicates additional cytogenetic aberrations; dx, diagnosis; FAB, French American British morphology classification subtype; n.d., not determined; TP, translocation partner; and WBC, white blood cell count.

*Values significantly different at the $P < .01$ level (χ^2).

†Values significantly different at the †$P < .05$ level (χ^2).

cause. Event-free survival (EFS) was calculated from the date of diagnosis to the first event or to the date of last follow-up. Events included nonremittance, relapse, secondary malignancy, or death from any cause. Cumulative incidence of relapse (CIR) was calculated from the date of CR to the first relapse. Refractory disease was included in the EFS and CIR analyses by arbitrarily setting the event date on day 0. For OS, EFS, and CIR analyses, patients who did not experience an event were censored at the time of last follow-up.

The Kaplan-Meier method was used to estimate the 5-year probabilities of OS and EFS, and survival estimates were compared by the log-rank test. The Gray test for competing risks was used for CIR analysis. Multivariate analyses were performed with the Cox proportional hazards model. Continuous variables known to be of prognostic value in AML were categorized according to cutoff points (eg, > 2 or 10 years of age, white blood cell [WBC] count $< 20 \times 10^9$/L or $> 100 \times 10^9$/L]). The χ^2 or Fisher exact test was used to compare differences in proportions of variables among groups; the Mann-Whitney *U* test was used for continuous variables. All *P* values are descriptive and explorative and were considered significant if ≤ .05. All statistical data were analyzed by the use of SAS-PC, Version 9.1 (SAS Institute Inc).

Results

Distribution of ACAs

Of the 756 patients, 733 (97%) had complete karyotypes, and their data were included in the study (see flowchart in Figure 1). There were no significant differences in the patients included (n = 733) and not included (n = 23) in this study with respect to sex, age, WBC count, and TP group (data not shown). ACAs were found in 344 (47%) of 733 cases (Figure 1). The number of additional aberrations ranged from 0 to 15 (mean, 1.2 additional aberrations; supplemental Figure 1).

There were 3 or more aberrations (including the 11q23/*MLL*-rearrangement) in 192 of 733 (26%) cases, which were therefore

Table 2. Number of aberrations by 11q23 translocation partner and clinically relevant parameters

	Number of aberrations							
	0	1	2	3	4	5	> 5	All
TP group								
9p22		168 (44)*	75 (49)*	33 (41)*	19 (43)*	7 (28)*	14 (33)*	316 (43)
10p12	1 (14)*	47 (12)*	19 (13)*	12 (15)*	7 (16)*	5 (20)*	5 (12)*	96 (13)
6q27	1 (14)*	17 (4)*	7 (5)*	1 (1)*			9 (21)*	35 (5)
19p13		20 (5)*	3 (2)*	4 (5)*	1 (2)*	1 (4)*	1 (2)*	30 (4)
19p13.1	1 (14)*	20 (5)*	7 (5)*	2 (3)*	1 (2)*	3 (12)*		34 (5)
19p13.3		12 (3)*	7 (5)*		3 (7)*	2 (8)*	1 (2)*	25 (3)
1q21		18 (5)*	3 (2)*	1 (1)*	1 (2)*		1 (2)*	24 (3)
4q21		5 (1)*	2 (1)*	4 (5)*	1 (2)*	1 (4)*		13 (2)
10p11.2		5 (1)*	2 (1)*	3 (4)*		2 (8)*		12 (2)
17q21		9 (2)*	1 (1)*	1 (1)*			1 (2)*	12 (2)
Other	4 (57)*	61 (16)*	26 (17)*	19 (24)*	11 (25)*	4 (16)*	11 (26)*	136 (19)
								733
Sex								
Male		187 (49)†	89 (59)†	35 (44)†	13 (30)†	11 (44)†	23 (53)†	358 (49)
Female	7 (100)†	195 (51)†	63 (41)†	45 (56)†	31 (70)†	14 (56)†	20 (47)†	375 (51)
								733
Age, y								
< 2	4 (57)*	197 (52)*	61 (40)*	39 (49)*	12 (27)*	16 (64)*	15 (35)*	344 (47)
2-9		104 (27)*	49 (32)*	29 (36)*	22 (50)*	5 (20)*	10 (23)*	219 (30)
≥ 10	3 (43)*	81 (21)*	42 (28)*	12 (15)*	10 (23)*	4 (16)*	18 (42)*	170 (23)
								733
WBC, $\times 10^9$/L								
< 20	5 (71)	159 (42)	76 (50)	39 (49)	23 (52)	16 (64)	21 (49)	339 (46)
20-99	1 (14)	115 (30)	38 (25)	20 (25)	14 (32)	5 (20)	10 (23)	203 (28)
≥ 100	1 (14)	97 (25)	34 (22)	19 (24)	7 (16)	2 (8)	11 (26)	171 (23)
								713 (97)
FAB								
M0		11 (3)*	4 (3)*	2 (3)*	3 (7)*	1 (4)*	2 (5)*	23 (3)
M1		19 (5)*	12 (8)*	4 (5)*	1 (2)*		3 (7)*	39 (5)
M2	1 (14)*	19 (5)*	7 (5)*	2 (3)*	3 (7)*			32 (4)
M4	2 (29)*	83 (22)*	25 (16)*	12 (15)*	5 (11)*	2 (6)*	5 (12)*	134 (18)
M5	4 (57)*	225 (59)*	97 (64)*	54 (68)*	24 (55)*	17 (68)*	25 (58)*	446 (61)
M7		4 (1)*	3 (2)*	2 (3)*	4 (9)*		6 (14)*	19 (3)
n.d.		2 (1)*	1 (1)*	1 (1)*	1 (2)*	1 (4)*	1 (2)*	7 (1)
								700 (95)
Overall	7 (1)	382 (52)	152 (21)	80 (11)	44 (6)	25 (3)	43 (6)	733

The number of aberrations indicates total number of aberrations in the karyotype, including 11q23/MLL-rearrangement, percentages per group shown in parentheses (per column).

dx indicates diagnosis; FAB, French American British morphology classification subtype; n.d., not determined; TP, translocation partner; and WBC, white blood cell count.

*Significantly different at the $P < .01$ level (χ^2).

†Significantly different at the $P < .05$ level (χ^2).

defined as complex karyotypes. Of the 344 cases with ACAs, 140 (41%) had numerical ACAs only, 130 (38%) had structural ACAs only, and 74 (22%) had both numerical and structural ACAs (Figure 1). There were 25 (7%) cases of ACA that had only balanced structural abnormalities in their karyotypes (Figure 1).

Distribution of ACAs in clinically relevant groups

Tables 1 and 2 show the distribution of ACAs by TP group and clinically relevant parameters (sex, age, WBC count, and FAB [ie, French-American-British] subtype). TP groups 9p22 and 19p13 were characterized by a relatively high frequency of numerical ACAs, whereas groups 10p12, 10p11.2, and 4q21 showed greater prevalence of structural ACAs ($P < .001$; Table 1). Also, there were significant differences in the number of aberrations among TP groups: the 6q27 group had a relatively high number of ACAs ($P = .002$), whereas groups 9p22, 19p13, and 1q21 had a lower number of ACAs (Table 2).

ACAs were less likely to occur in young children (< 2 years of age) than in children 2-9 years of age or 10 years or older (42% vs 53% vs 51%, $P = .02$; Table 1). However, structural ACAs were more frequent in children < 2 years of age than in children 2-9 years of age or 10-18 years of age (48% vs 30% vs 31%, $P < .01$; Table 1). There was a greater prevalence of highly complex karyotypes (> 5 aberrations) in children 10-18 years of age than those younger than 2 years or 2-9 years of age (11% vs 4% vs 5%, $P = .02$, Table 2).

Although the number of patients with FAB M7 was small, ACAs were more likely to occur in patients with AML FAB M7 compared with those with other FAB types (79% vs 46%, $P = .008$), whereas patients with AML FAB M2 and M4 had the lowest occurrence of ACAs (Table 1). Also, patients with AML FAB M7 seem to have a higher number of aberrations than those with other FAB morphologies ($P = .003$; Table 2).

Specific recurrent aberrations

Trisomy 8 was the most frequently occurring numerical abnormality (130/733, 18% of all cases and 38% of ACA cases, Figure 2A). In addition, trisomy 4, 6, 13, 19, and 21 were recurrent ACAs (at least 15 cases each). Two cases with Down syndrome were included in this study. However, because constitutional aberrations

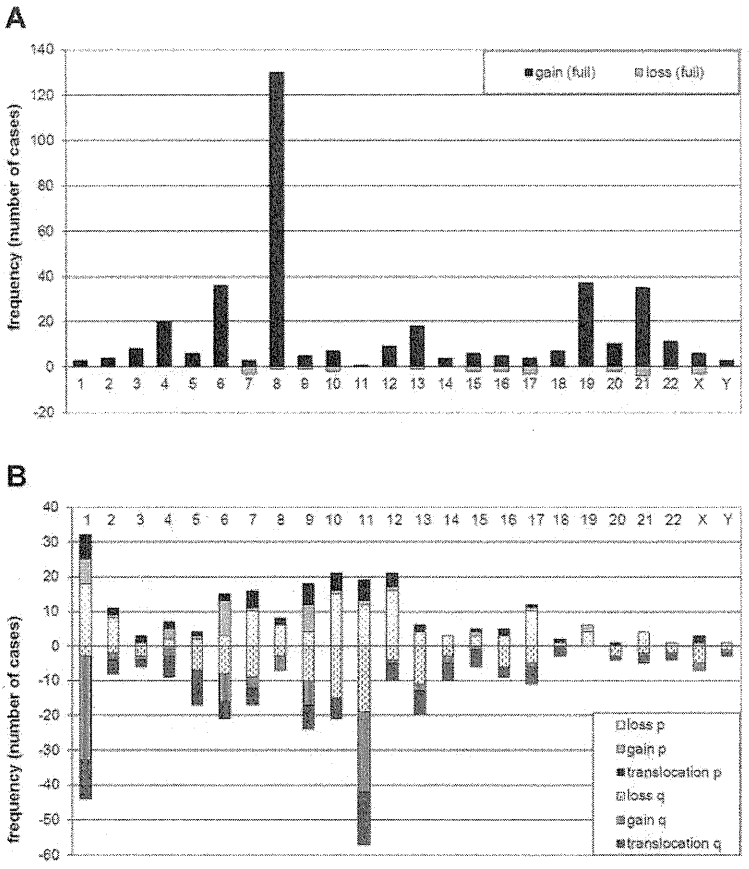

Figure 2. Frequency (number of cases) of numerical and structural ACAs. (A) Numerical ACAs. Gains are shown on the positive y-axis, and losses are shown on the negative y-axis. Chromosomes are on the x-axis. (B) Structural ACAs The short arms (p) of the chromosomes are shown on the positive y-axis and the long arms (q) on the negative y-axis. Lightest shades are used for losses, medium-shaded colors are used for gains, and the darkest-shaded colors for breakpoints of balanced translocations. Chromosomes are on the x-axis. Balanced 11q23 translocations are not included in the figure.

were not included in the additional aberrations, they were not included in the trisomy 21 group. Only 11 patients had losses of full chromosomes, collectively accounting for 25 monosomies (Figure 2A).

Figure 2B shows the collective analysis of structural ACAs per chromosome arm but does not include breakpoints involved in balanced 11q23/*MLL*-translocations. However, the figure includes unbalanced 11q23/*MLL*-translocations in which chromosomal

Table 3. Univariate survival analysis of the complete cohort (n = 733)

	Complete cohort						
	n	EFS	*P* (log-rank)	OS	*P* (log-rank)	CIR	*P* (Gray)
Additional aberrations			.002†		< .001†		< .001†
Absent	389	0.48		0.62		0.38	
Present	344	0.38		0.47		0.52	
No. of aberrations			< .001†		< .001†		.001†
2	152	0.39		0.50		0.50	
3	80	0.45		0.53		0.48	
≥ 3	192	0.37	.018*	0.45	.003†	0.53	< .001†
4	44	0.40		0.50		0.53	
5	25	0.36		0.43		0.60	
> 5	43	0.18	< .001†	0.25		0.61	
Type			.001†		.003†		< .001†
Numerical	140	0.47		0.56		0.41	
Structural	130	0.32		0.43		0.59	
Both	74	0.31		0.40		0.59	
Trisomy							
4	20	0.43	.72	0.52	.87	0.52	.93
6	36	0.35	.43	0.35	.029*	0.54	.65
8	130	0.53	< .001†	0.61	.003†	0.35	< .001†
13	18	0.49	.52	0.64	.41	0.40	.37
19	37	0.17	.003†	0.24	< .001†	0.54	.88
21	35	0.19	.007†	0.28	.015*	0.69	.014*

CIR, indicates 5-year cumulative incidence of relapse; EFS, 5-year event-free survival estimates; n, number of patients; OS, 5-year overall survival estimate; *P* (Gray), *P* value from the Gray test; and *P* (log-rank), *P* value from log-rank test.

*Significant at $P < .05$ level.

†Significant at $P < .01$ level.

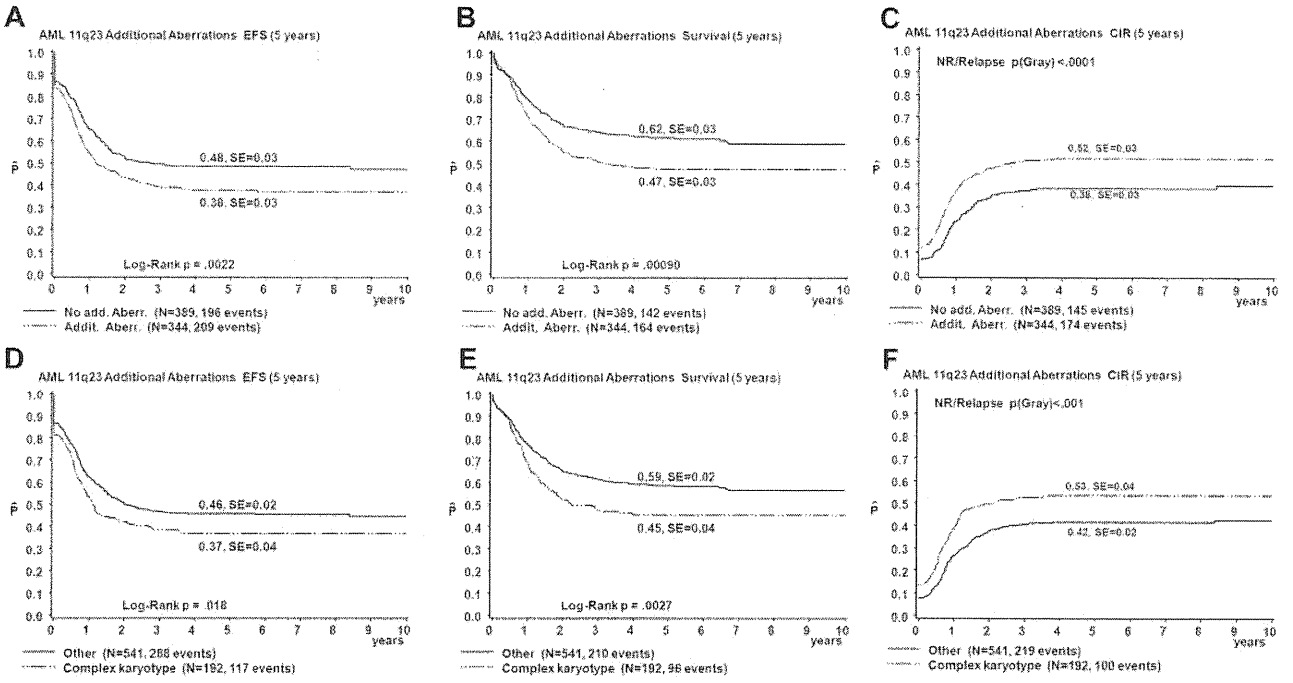

Figure 3. Survival curves obtained from univariate analysis comparing patients with ACAs to patients without ACAs and comparing patients with complex karyotype with all patients with < 3 aberrations. (A-C) Patients with ACAs are compared to patients without ACAs. (D-F) Patients with complex karyotype are compared to patients with < 3 aberrations. EFS (A,D), OS (Survival; B,E), and CIR (C,F).

material was lost or gained. Chromosomes 1 and 11 were most frequently involved in structural ACAs. Analysis of specific breakpoints showed that 11q23 was the only breakpoint found more than 10 times (data not shown).

Univariate analysis of the prognostic impact of ACAs on survival

Table 3 summarizes results of the univariate analysis of survival parameters. The EFS and OS estimates of patients with ACAs were significantly lower than those without ACAs (EFS 38% vs 48%, $P = .002$; OS 47% vs 62%, $P < .001$; Figure 3). CIR estimates of patients with ACAs were significantly greater than for those without ACAs (52% vs 38%, $P < .001$; Figure 3). Patients with complex karyotypes had significantly worse outcomes than those without complex karyotypes (EFS 37% vs 46%, $P = .02$; OS 45% vs 59%, $P = .003$; CIR 53% vs 42%, $P < .001$; Figure 3).

The presence of trisomy 8 (n = 130) was a favorable prognostic factor (EFS 53% vs 29% for patients without trisomy 8, $P < .001$; OS 61% vs 39% for patients without trisomy 8, $P = .003$; CIR 35% vs 62% for patients without trisomy 8, $P < .001$; Figure 4). Survival differences are mainly explained by reduced relapse rate in trisomy 8 patients (relapse rate 26% vs 49% for patients without trisomy 8, $P < .001$; Figure 4). The presence of trisomy 19 (n = 37) and trisomy 21 (n = 36) was an unfavorable prognostic factor (EFS 17% vs 40% for patients without trisomy 19, $P = .003$; OS 24% vs 50% for patients without trisomy 19, $P < .001$; CIR 54% vs 51% for patients without trisomy 19, $P = .88$; and EFS 19% vs 40% for patients without trisomy 21, $P = .007$; OS 28% vs 50% for patients without trisomy 21, $P = .02$; CIR 69% vs 50% for patients without trisomy 21, $P = .01$; Figure 4). Both trisomies 19 and 21 were present in 15 patients. Survival curves for patients with either trisomy 19 or 21 were not different from those for patients with both trisomies 19 and 21 (Figure 4). Combined trisomy 19 and trisomy 8 was present in 23 patients. These patients showed a survival curve intermediate to that of trisomy 8 and trisomy 19 cases (EFS 30%, data not shown). The survival disadvantage of patients with trisomy 19 seems to be determined by refractory disease (probability of CR 74% for patients with trisomy 19 vs 89% for patients with other ACAs, as calculated over the fraction of patients who survive beyond the first 6 weeks after diagnosis, $P = .04$) rather than relapse. In addition, patients with trisomy 19 had a significantly greater incidence of early death (16% vs 3.3% in other ACA cases, $P = .004$), which could not be explained by adverse clinical prognostic factors such as greater WBC or age. Structural aberrations were diverse and randomly distributed among TP groups and survival analysis of patients with specific breakpoints was not feasible because none of the breakpoints was involved > 10 times.

Multivariate analyses of the prognostic impact of ACAs on survival

Table 4 summarizes results of the multivariate survival analysis. Cox proportional hazards model for EFS, OS, and relapse incidence of the full cohort (n = 733) showed that trisomy 8 and trisomy 19 were independent prognostic factors at $P < .05$ for EFS (hazard ratio [HR] 0.57, $P = .02$; and HR 1.77, $P = .01$) and OS (HR 0.54, $P = .03$; and HR 2.11, $P = .002$; Table 4). Structural aberrations as a general finding predicted EFS (HR 1.39, $P = .01$; Table 4). The TPs identified by Balgobind et al[10] (10p12, 6q27, 1q21, and 10p11.2) remained significant independent prognostic factors in these models. Trisomy 8, 19, and 21 were not significant factors in the model for the prediction of relapse incidence. Complexity of the karyotype, tested by different cutoff values (≥ 2 aberrations, ≥ 3 aberrations, and > 5 aberrations), was not a significant factor for outcome in all models and was therefore excluded from the final model. A separate analysis of t(9;11)(p22;q23) cases showed that they did not differ considerably from the complete cohort (supplemental Figure 2 and supplemental Table 2).

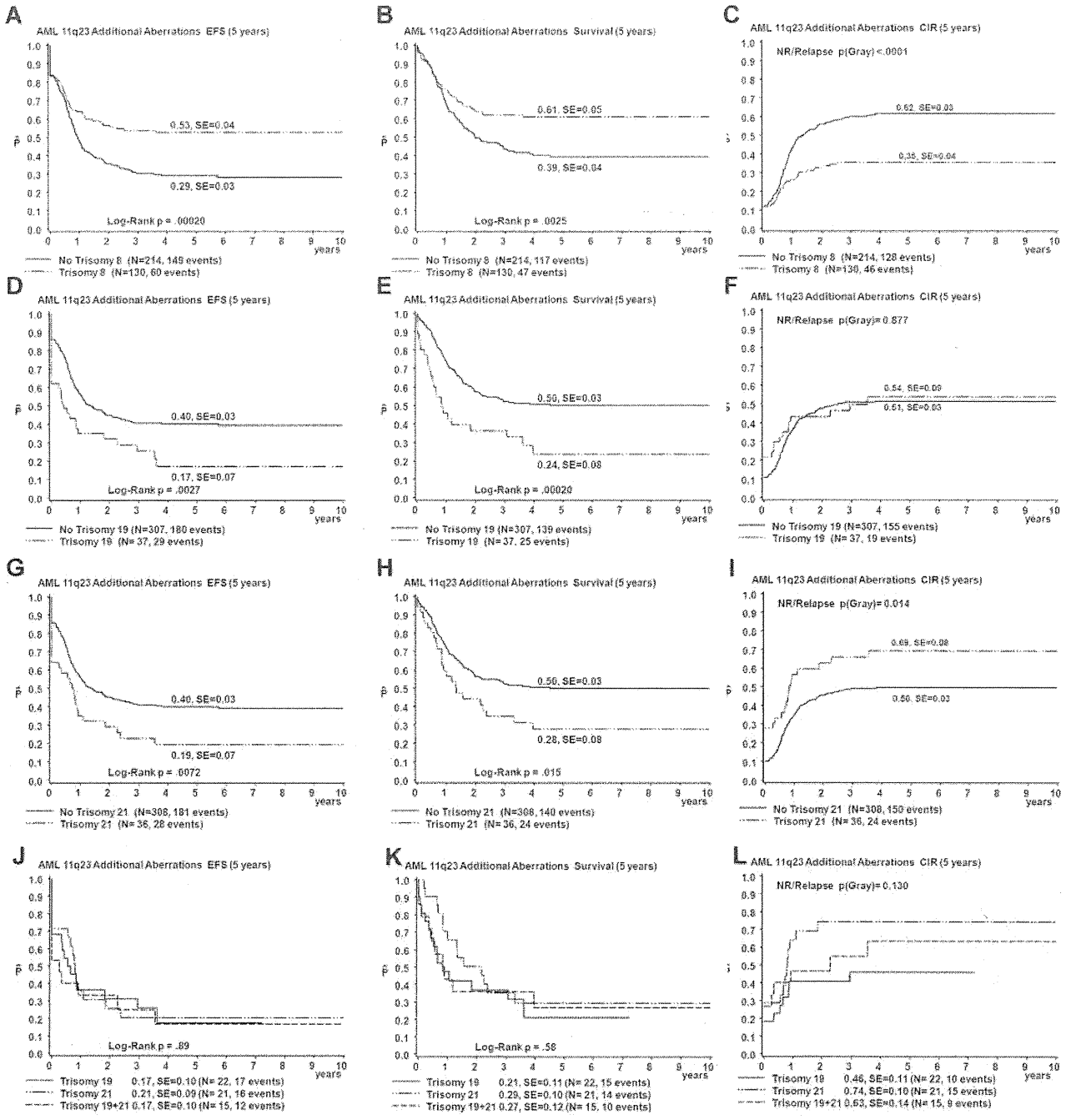

Figure 4. Comparison of survival curves obtained from univariate analysis for patients with trisomy 8, trisomy 19, and those with trisomy 21 and defined by strata of occurrence of trisomy 19 and trisomy 21. For curves A-I, patients with a specific trisomy are compared with patients with other ACAs. Patients with trisomy 8 are shown in panels A-C, patients with trisomy 19 in panels D-F, and patients with trisomy 21 in panels G-I. The strata of occurrence of trisomy 19 and trisomy 21 are shown in panels J-L. EFS (A,D,G,J), OS (Survival; B,E,H,K), and CIR (C,F,I,L).

Discussion

The heterogeneity of pediatric AML is mainly determined by specific karyotypes and molecular aberrations, which have become important prognosticators.[1,3-6,8,11,27-33] In addition, within distinct groups such as 11q23/*MLL*-rearranged AML, we have reported that additional cytogenetic aberrations are of prognostic relevance.[10] In the present exploratory study, we identified trisomy 8, trisomy 19, and trisomy 21 to be recurrent ACAs of prognostic significance in pediatric 11q23/*MLL*-rearranged AML. Multivariate analysis showed that only trisomy 8 and trisomy 19 as additional aberrations were of independent prognostic value. Notably, the adverse outcome for 11q23/MLL-rearranged AML patients harboring trisomy 19 was because of refractory disease and early death rather than an increased rate of relapse. Complex karyotype was a frequent finding (26%) and a negative prognostic factor in univariate analysis only.

Trisomy 19 in AML is an aberration that is rarely found as the sole aberration.[34] In infants with AML it is associated with t(7;12)(q36;p13) and t(7;12)(q32;p13).[35] In most of such cases it can seem to be the sole aberration because of the cryptic t(7;12).[35]

Table 4. Multivariate survival analysis of the complete cohort by use of the Cox proportional hazards model

	Cox proportional hazards model								
	EFS			OS			Relapse incidence		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
TP									
9p22	1	reference		1	reference		1	reference	
other	1.15	(0.87-1.51)	.328	1.13	(0.82-1.57)	.461	1.17	(0.92-1.47)	.195
10p12	1.36	(1.01-1.83)	.042*	1.62	(1.16-2.27)	.005†	1.76	(1.36-2.29)	.000†
6q27	2.29	(1.54-3.39)	.000†	2.72	(1.77-4.19)	.000†	2.79	(1.80-4.33)	.000†
19p13	1.06	(0.62-1.80)	.832	1.44	(0.82-2.51)	.204	0.88	(0.57-1.37)	.579
19p13.1	1.11	(0.69-1.79)	.667	0.97	(0.53-1.77)	.931	1.04	(0.71-1.53)	.841
19p13.3	1.06	(0.60-1.88)	.832	1.64	(0.90-3.00)	.105	1.18	(0.71-1.94)	.522
1q21	0.12	(0.03-0.49)	.003†	0.00			0.68	(0.44-1.05)	.080
4q21	1.46	(0.74-2.88)	.276	2.04	(1.02-4.09)	.043*	1.84	(0.99-3.43)	.054
10p11.2	2.12	(1.10-4.06)	.024*	2.56	(1.24-5.32)	.011*	1.37	(0.67-2.78)	.384
17q21	1.14	(0.53-2.43)	.743	1.15	(0.47-2.82)	.763	1.28	(0.68-2.42)	.446
Trisomy									
No trisomy	1	reference		1	reference		1	reference	
8	0.57	(0.36-0.92)	.022*	0.54	(0.32-0.94)	.028*	0.79	(0.56-1.12)	.188
19	1.77	(1.13-2.78)	.012*	2.11	(1.31-3.42)	.002†	1.15	(0.68-1.94)	.596
21	1.35	(0.85-2.13)	.198	1.25	(0.76-2.03)	.377	0.98	(0.60-1.60)	.926
Type									
No ACAs	1	reference		1	reference		1	reference	
numerical	1.16	(0.83-1.63)	.376	1.17	(0.84-1.62)	.353	1.09	(0.81-1.47)	.588
structural	1.39	(1.07-1.80)	.013*	1.27	(0.98-1.63)	.068	1.13	(0.90-1.43)	.288

Results are of 3 independent analyses.
ACA indicates additional cytogenetic aberrations; CI, 95% confidence interval; EFS, event-free survival; HR, hazard ratio; and OS, overall survival.
*Significant at $P < .05$ level.
†Significant at $P < .01$ level.

Trisomy 19 has been described as an additional aberration with adverse prognostic significance in adult AML.[11] It has been postulated that a gene dosage effect of the DNA methyltransferase 1 located on 19p13.2 contributes to the hypermethylation found in patients with MDS and thereby to prognosis.[36] Future studies may reveal whether this mechanism also contributes to aberrant methylation found in pediatric 11q23/*MLL*-rearranged AML.[37]

In our study, trisomy 8 was found to be an independent favorable prognostic factor. Kok et al[38] identified a gene expression signature with high *HOX*A gene expression in adult AML patients with AML with trisomy 8 as the sole abnormality, which clustered together with patents with *MLL*-rearranged AML. This finding may suggest similarities in the biology of these diseases. In contrast, in pediatric MDS, trisomy 8 is recognized as a positive prognostic factor, possibly because of differences in apoptosis regulation between cells with trisomy 8 and cells with other abnormalities.[39,40] To date, it is not clear how trisomy 8 influences the biology of *MLL*-rearranged AML.

Interestingly, in our study, although 26% of all cases of 11q23/*MLL*-rearranged had complex karyotypes, this ACA was not an independent prognostic factor. Although the use of definitions on complex karyotypes is not uniform, the occurrence of complex karyotypes in pediatric AML cohorts has been reported to range from 7% to 15%.[2,6,14,41] A Cancer and Leukemia Group B study on adult de novo AML showed that patients with increased number of aberrations had significantly worse outcome than those with normal karyotypes.[42] Recently, Göhring et al[43] used a new definition of "structural complex karyotype," defined as a karyotype with ≥ 3 chromosomal aberrations including at least one structural aberration. This specific karyotype independently predicted very poor survival in a cohort of 192 children with advanced MDS.[43]

Although all the cases of complex karyotype in our study fit their definition, we did not find the presence of such karyotype to be associated with the poor prognosis that was reported in pediatric advanced MDS.[43] Only some studies have specifically shown a correlation between complexity of the karyotype and outcome in pediatric AML.[2,6,14,33,44] EFS rates for patients with complex karyotype have ranged from 29% to 42% in these studies, which is comparable with the EFS obtained in our study. Alternatively, a strong negative association between monosomal karyotype, defined as a karyotype with at least 2 monosomies or 1 monosomy combined with at least 1 structural aberration, and outcome was described in adult AML.[45] This monosomal karyotype was only present in 1.5% (n = 11) of our cases and therefore it was not possible to evaluate the predictive value in our pediatric 11q23/*MLL*-rearranged AML cohort.

Although we have added additional prognostic factors in our study, the multivariate models still point out that previously determined risk factors (among which the TPs) retain their independent prognostic significance irrespective of ACA status.

A limitation of our study is the variety of treatment regimens, although all protocols had a similar backbone, including intensive chemotherapy with cytarabine/anthracycline. Unfortunately, numbers were too small to do specific analyses for different protocols, or to draw any meaningful conclusion regarding provided treatment and outcome.

In separate analysis of t(9;11)(p22;q23) cases, we confirmed most of the findings from the complete cohort, regarding frequent recurrent aberrations and predictive factors. In addition, FAB M5 morphology was still recognized as independent favorable prognostic factor in this group of patients.

In conclusion, in this exploratory study we have identified specific ACAs as novel independent prognostic variables in pediatric 11q23/*MLL*-rearranged AML, which can be identified by conventional karyotyping. Future studies should be aimed to test the associations found in this study in different patient cohorts. Our findings may also guide further studies that unravel the biologic differences that determine outcome differences in 11q23/MLL-rearranged AML as well as future treatment stratification.

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Authorship

Contribution: E.A.C., S.C.R., J.H., R.P., C.M.Z., and M.M.v.d.H.-E. conveyed and planned the study, analyzed the data, and wrote the paper; M.Z. performed the statistical analyses and wrote the paper; and T.A.A., A.A., H.B.B., M.C., U.C., M.N.D., E.F., B.G., H.H., C.J.H., N.A.H., G.J.L.K., A.L., N.L., L.L.N., A.M., C.P., D.R., J.E.R., F.O.S., J.S., I.S., S.S., T.T., D.T., D.W., and Z.Z. participated in data collection and in critical review and final approval of the paper.

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