MYELOID NEOPLASIA

Recurrent abnormalities can be used for risk group stratification in pediatric AMKL: a retrospective intergroup study

Jasmijn D. E. de Rooij,^{1,*} Riccardo Masetti,^{2,*} Marry M. van den Heuvel-Eibrink,^{1,3} Jean-Michel Cayuela,⁴ Jan Trka,⁵ Dirk Reinhardt,⁶ Mareike Rasche,⁶ Edwin Sonneveld,⁷ Todd A. Alonzo,⁸ Maarten Fornerod,¹ Martin Zimmermann,⁹ Martina Pigazzi,¹⁰ Rob Pieters,³ Soheil Meshinchi,¹¹ C. Michel Zwaan,^{1,†} and Franco Locatelli^{12,13,†}

¹Pediatric Oncology/Hematology, Erasmus Medical Center–Sophia Children's Hospital, Rotterdam, The Netherlands; ²Pediatric Hematology/Oncology, University of Bologna, Bologna, Italy; ³Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands; ⁴Laboratory of Hematology, University Hospital Saint-Louis, Assistance Publique–Hôpitaux de Paris and EA3518, University Paris Diderot, Paris, France; ⁵Pediatric Hematology/Oncology, 2nd Medical School, Charles University, Prague, Czech Republic; ⁶Acute Myeloid Leukemia–Berlin-Frankfurt-Munster Study Group, Pediatric Hematology/ Oncology, University Hospital Essen, Essen, Germany; ⁷Dutch Childhood Oncology Group, The Hague, The Netherlands; ⁸Children's Oncology Group, Monrovia, CA; ⁹Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany; ¹⁰Onco-Hematology, Dipartimento di Salute della Donna e del Bambino, University of Padova, Padova, Italy; ¹¹Fred Hutchinson Cancer Research Center, Seattle, WA; ¹²Department of Pediatric Hematology/Oncology, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Pediatrico Bambino Gesù, Rome, Italy; and ¹³University of Pavia, Pavia, Italy

Key Points

- NUP98/KDM5A, CBFA2T3/ GLIS2, KMT2A-rearrangements, and monosomy 7 are associated with poor outcome; RBM15/ MKL1 and others fare better.
- Screening for NUP98/ KDM5A, RBM15/MKL1, CBFA2T3/GLIS2, and KMT2A rearrangements combined with conventional karyotyping is advisable.

Genetic abnormalities and early treatment response are the main prognostic factors in acute myeloid leukemia (AML). Acute megakaryoblastic leukemia (AMKL) is a rare subtype of AML. Deep sequencing has identified *CBFA2T3/GLIS2* and *NUP98/KDM5A* as recurrent aberrations, occurring in similar frequencies as *RBM15/MKL1* and *KMT2A*-rearrangements. We studied whether these cytogenetic aberrations can be used for risk group stratification. To assess frequencies and outcome parameters of recurrent cytogenetic aberrations in AMKL, samples and clinical data of patients treated by the Associazione Italiana Ematologia Oncologia Pediatrica, Berlin-Frankfurt-Munster Study Group, Children's Oncology Group, Dutch Childhood Oncology Group, and the Saint Louis Hôpital were collected, enabling us to screen 153 newly diagnosed pediatric AMKL cases for the aforementioned aberrations and to study their clinical characteristics and outcome. *CBFA2T3/GLIS2* was identified in 16% of the cases; *RBM15/MKL1*, in 12%; *NUP98/KDM5A* and *KMT2A* rearrangements, in 9% each; and monosomy 7, in 6%. These aberrations were mutually exclusive. *RBM15/MKL1*-rearranged patients were significantly younger. No significant differences in sex and white blood cell count were found.

NUP98/KDM5A, CBFA2T3/GLIS2, KMT2A-rearranged lesions and monosomy 7 (NCK-7) independently predicted a poor outcome, compared with *RBM15/MKL1*-rearranged patients and those with AMKL not carrying these molecular lesions. NCK-7-patients (n = 61) showed a 4-year probability of overall survival of $35 \pm 6\%$ vs $70 \pm 5\%$ in the *RBM15/MKL1*-other groups (n = 92, *P* < .0001) and 4-year probability of event-free survival of $33 \pm 6\%$ vs $62 \pm 5\%$ (*P* = .0013), the 4-year cumulative incidence of relapse being $42 \pm 7\%$ and $19 \pm 4\%$ (*P* = .003), respectively. We conclude that these genetic aberrations may be used for risk group stratification of pediatric AMKL and for treatment tailoring. (*Blood*. 2016;127(26):3424-3430)

Introduction

Pediatric acute myeloid leukemia (AML) is a heterogeneous disease characterized by recurrent genetic aberrations. The most important factors predicting clinical outcome are presence of (molecular) genetic aberrations and early response to treatment.^{1,2} Acute megakaryoblastic leukemia (AMKL) is a rare subtype of AML and is diagnosed mostly in myeloid leukemia of Down syndrome (ML-DS) patients, where it is associated with mutations in the transcription factor *GATA1*.³ In pediatric non-Down syndrome AML, AMKL accounts for ~10% of the cases and has been reported to be associated with poor outcome.⁴⁻⁷

Most study groups consider pediatric AMKL as high-risk AML, in contrast to the outcome of ML-DS, and some consider it to have the indication for allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1).^{4,8-10} However, risk group stratification and treatment protocols are not yet optimized for this subtype of pediatric AML.¹¹

Translocation t(1;22)(p13;q13), resulting in a chimeric fusion of *RBM15* and *MKL1*, formerly known as *OTT/MAL*, until recently was the only recurrent aberration described in pediatric AMKL, occurring

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^{*}J.D.E.d.R. and R.M. contributed equally to this study.

[†]C.M.Z. and F.L. contributed equally to this study.

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There is an Inside Blood Commentary on this article in this issue.

in $\sim 10\%$ of the patients.¹²⁻¹⁴ Conflicting results on the prognostic relevance of RBM15/MKL1 have been reported, as some series suggested the outcome to be poor and others favorable.^{5,6,13,15} Recently, the cytogenetically cryptic translocation t(11;12)(p15;p13), resulting in the chimeric fusion gene NUP98/KDM5A, was identified as a recurrent aberration found in $\sim 10\%$ of AMKL cases.^{5,7,16} Moreover, another cytogenetically cryptic event, inv(16)(p13q24), resulting in a fusion between CBFA2T3 and GLIS2 and accounting for $\sim 15\%$ of the cases, was identified as a recurrent aberration in this specific subtype of AML.5,7,16,17 Both NUP98/KDM5A and CBFA2T3/GLIS2 fusion transcripts were reported to confer poor outcome, but numbers of patients analyzed to support this conclusion were small. Moreover, CBFA2T3/GLIS2 was also reported to behave as an aggressive leukemia in mouse models.^{7,16} Another recurrent abnormality consists of KMT2A rearrangements, which are seen in $\sim 10\%$ of the pediatric AMKL cases.⁵ The clinical outcome of KMT2A-rearranged leukemia is dependent on the fusion-partner gene.^{18,19} NUP98/KDM5A, CBFA2T3/GLIS2, and RBM15/MKL1 are rarely or never seen in other pediatric AML subtypes, with CBFA2T3/GLIS2 being the least specific for AMKL.^{5,17,20} In addition, Inaba et al described AMKL risk groups based on cytogenetic data obtained with conventional karyotyping, proposing 3 groups: good risk including patients carrying 7p abnormalities; poor risk including cases with monosomy 7, or 9p abnormalities, including KMT2A/MLLT3, -13/13q-, and -15, and intermediate risk, which encompassed all other AKML patients.¹¹

In this study, we present the clinical, cell-biological, and genetic characteristics of 153 pediatric AMKL patients from the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP; Italian Association of Pediatric Hematology and Oncology, Italy), the Berlin-Frankfurt-Munster-Study Group (BFM-SG; Essen, Germany), the Dutch Childhood Oncology Group (DCOG; The Hague, The Netherlands), the Saint Louis Hôspital (SLH; Paris, France), and the Children's Oncology Group (COG; Philadelphia, PA), which were fully characterized for the aforementioned genetic aberrations, and karyotypes were analyzed, We also investigated the frequencies and prognostic relevance of these recurrent aberrations.

Material and methods

Patient inclusion

We collected data on clinical characteristics and outcome of newly diagnosed pediatric (younger than 18 years at disease onset) patients with non-Down syndrome AMKL. These patients were diagnosed between 1990 and 2014 and had to have available RNA or complementary DNA for molecular studies and a blast percentage >20%. They were from 3 consortia: (1) AIEOP; (2) BFM-SG, DCOG, and SLH; and (3) COG. Approval for this study had been obtained from the institutional review board of the participating centers. Morphological classification and karyotyping, including the presence of *KMT2A* rearrangements, monosomy 7, or a hyperdiploid karyotype, were centrally reviewed by each study group.

Screening for recurrent translocations

Screening for molecular aberrations was performed in the Erasmus Medical Center–Sophia Children's Hospital for samples provided by the AML BFM-SG, COG, DCOG, and the SLH, whereas samples provided by the AIEOP group were screened at the University of Padova and the University of Bologna. All patients were screened for *NUP98/KDM5A*, *CBFA2T3/GLIS2*, and *RBM15/MKL1* with reverse transcription polymerase chain reaction (RT-PCR), using specific primers for each fusion gene, as previously described (Primers; supplemental Table 1, available on the *Blood* Web site).^{5,17} In case of a positive sample, purified PCR product was directly sequenced on a PRISM 3100 genetic

analyzer (Applied Biosystems) and analyzed using CLCWorkbench (Version 3.5.1; CLC Bio) to confirm the translocation. A higher incidence of AMKL is seen in Down syndrome patients, and these children are usually characterized by a *GATA1* mutation.²¹⁻²³ All 153 samples, including the positive *KMT2A*-rearranged cases (diagnosis being based on either *KMT2A* split signal fluorescence in situ hybridization or cytogenetic analysis as performed by each study group), were screened for *KMT2A* rearrangements for selected partner genes using specific primers for *KMT2A/MLLT3*, *KMT2A/MLLT10*, *KMT2A/MLLT4*, *MLLT4*(*MLL/AF6*), *KMT2A/MLLT1*(*MLL/ENL*), *KMT2A/ELL*, and *KMT2A/MLLT6*(*MLL/AF17*), and the purified PCR product was directly sequenced (Primers; supplemental Table 1).^{18,19}

Statistical analyses

Statistical analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY). To assess the outcome, the following parameters were used: (1) probability of achieving CR1, (2) probability of event-free survival (pEFS), (3) probability of overall survival (pOS), and (4) cumulative incidence of relapse (pCIR). CR was defined as <5% blasts in the bone marrow, with regeneration of trilineage hematopoiesis, and absence of leukemic cells elsewhere. pEFS was defined as the time between diagnosis and first event, including relapse, death because of any cause, and second malignancy, whichever occurred first. For calculation of EFS, lack of achieving CR was considered an event occurring on day 0. pOS was defined as the time between diagnosis and death. Both pEFS and pOS were estimated by the Kaplan-Meier method, and groups were compared with the logrank test. pCIR was defined as time between diagnosis and relapse, with nonresponders being attributed an event on day 0, and was analyzed by the Kalbfleisch and Prentice method taking into account death and second malignancy as competing event; groups were compared with the Gray's test. A Cox regression analysis was performed for EFS, OS, and for the probability of relapse-free survival (RFS) considering the following covariables: cytogenetic subgroup, age, sex, white blood cell (WBC) count at diagnosis, and HSCT (as time-dependent variable), as well as the previously described poor prognostic factors for pediatric AMKL "normal karyotype" and monosomy 7.11,24,25 Furthermore, we included a hyperdiploid karyotype and 7p-abnormalities in the Cox regression analysis, based on the association with pediatric AMKL.^{11,26,27} Statistical analysis was restricted to groups with >5 cases. Statistical significance was considered if P values were <.05.

Results

Clinical characteristics

The AIEOP group included 47 patients; the BFM-SG, DCOG, and SLH consortium included 45 patients; and COG provided 61 patients. All 153 patients were eligible for screening of the 4 molecular aberrations, and 119 patients for cytogenetics. Median age was 1.6 years (range 0.1-17.1), median WBC count at diagnosis was 13.7×10^9 /L (range 1.1×10^9 /L to 378.5×10^9 /L), and 46% of the included patients were males (Table 1). Patients were treated with different treatment protocols according to their respective study group. All protocols consisted of intensive chemotherapy using an anthracy-cline and cytarabine backbone for both induction and consolidation; in addition, 41% of patients received HSCT in CR1. HSCT was performed in 66% of the patients reported by the AIEOP group, in 44% of the COG cases, and in 11% of the cases provided by DCOG, BFM, and SLH. Patient characteristics are detailed in Table 1.

Twenty-four (16%) samples were positive for *CBFA2T3/GLIS2*, 18 (12%) samples harbored the *RBM15/MKL1* fusion gene, *NUP98/KDM5A* was present in 14 (9%) of cases, and 14 samples were positive for a *KMT2A* rearrangement, all diagnosed with fluorescence in situ hybridization. Three *KMT2A*-positive cases had no karyotype available, and in the other 11, the *KMT2A* rearrangement was also detected through karyotype analysis. Different fusion partners were identified;

Table 1	. Presenting	clinical	characteristics	of	pediatric	AMKL	patients	(n=	153)
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Characteristics	<i>CBFA2T3/GLIS2</i> (n = 24)	<i>NUP98/KDM5A</i> (n = 14)	<i>KMT2A-</i> r (n = 14)	<i>RBM15/MKL1</i> (n = 18)	Monosomy 7 (n = 9)	Other (n = 74)	All (n = 153)
Median age, y (range)	1.5 (0.5-4.0)	1.9 (0.8-8.5)	1.9 (0.7-12.0)	0.7* (0.1-2.7)	1.5 (0.5-17.1)	1.6 (0.1-15.1)	1.6 (0.1-17.1)
Median WBC count (range)	$17.3 imes10^9/L$	$14.0 imes10^9$ /L	$7.4 imes10^9$ /L	$13.8 imes10^9$ /L	$14.5 imes10^9$ /L	$14.4 imes10^9$ /L	$13.7 imes10^9$ /L
	(7.5-300.1)	(5.8-188.0)	(1.1-31.0)	(5.6-32.7)	(8.7-90.2)	(1.1-378.5)	(1.1-378.5)
Male, %	25	43	71	33	22	51	46
Origin							
AIEOP, %	42	21	36	17	22	32	31
DCOG/BFM/SLH, %	17	50	36	28	33	29	29
COG, %	42	29	29	56	45	39	40
HSCT	11 (46%)	4 (29%)	9 (64%)	9 (50%)	5 (56%)	30 (36%)	63 (41%)
4-y pEFS, %	33 ± 10	36 ± 13	36 ± 13	59 ± 12	33 ± 16	62 ± 6	51 ± 4
4-y pOS, %	38 ± 10	36 ± 13	36 ± 13	70 ± 11	33 ± 16	70 ± 5	56 ± 4
4-y pCIR, %	42 ± 10	36 ± 14	50 ± 14	6 ± 6	33 ± 18	22 ± 5	29 ± 4

KMT2A-r, KMT2A rearrangement.

*Significantly lower age at diagnosis (P = .038).

9/14 were positive for *KMT2A/MLLT3*, 3 were identified with fusion *KMT2A/MLLT10*, and 1 case each harbored either *KMT2A/MLLT1* or *KMT2A/MLLT6* (supplemental Table 2).

Detailed data on karyotype were lacking in 34/153 cases. Twenty cases were cytogenetically normal. Cytogenetics showed 30 cases with an acquired trisomy 21, another 9 cases were identified to carry monosomy 7, 33 cases were hyperdiploid with 49 to 84 chromosomes, and 31 showed a complex karyotype (defined as 3 or more aberrations excluding trisomy's). A 7p-abnormality was identified in 13 cases. Some of these cases belonged to >1 of the cytogenetic or molecular subgroups (see also Figure 1 and supplemental Table 3 for details).

Age at diagnosis was significantly lower in the *RBM15/MKL1*positive group, compared with the other pediatric AMKL cases (0.7 years, range 0.1-2.7, P = .035). Seventy-four patients were characterized as "other," as they were negative for any of the above-mentioned translocations and monosomy 7. Other karyotypes identified were t(1:22)(p13;q13), but this case was negative for *RBM15/MKL1* using RT-PCR, and t(16;21)(q24;q22.1), which was positive for *RUNX1/ CBFA2T3* (supplemental Table 3). The "other" subgroup had a median age of 1.6 years (range 0.1-15.1), and a median WBC count of 14.4×10^9 /L (range 1.1×10^9 /L to 378.5×10^9 /L; Table 1).

Survival analyses

The median follow-up of surviving patients was 67 months (range 2.7-259.0). The 4-year pOS of the entire pediatric AMKL cohort was $56 \pm 4\%$, the 4-year pEFS was $51 \pm 4\%$, and the 4-year pCIR was



Figure 1. Overview of cytogenetic aberrations and aberrations found in the karyotypes of pediatric AMKL (n = 153). Each column represents a single case. A colored cells means positive for the aberration corresponding with that particular row. X indicates that the karyotype is not available, the only information given is hyperdiploid, or complex karyotype. -13/13q, monosomy 13 or del(13q); NA, not available.



Figure 2. Survival curves of pediatric AMKL patients. The 4-year pEFS (A), 4-year pOS (B), and 4-year pCIR (C) comparing the outcome of patients of the different enrolled study groups. There is no significant difference in outcome among the study groups. The 4-year pEFS (D), 4-year pOS (E), and 4-year pCIR (F) comparing the described cytogenetic subgroups as identified in pediatric non-Down syndrome AMKL. *RBM15/MKL1*-positive cases and other pediatric AMKL have a favorable outcome compared with *NUP98/KDM5A*, *CBFA2T3/GLIS*, *KMT2A* rearrangements and monosomy 7 cases. The 4-year pEFS (G), 4-year pOS (H), and 4-year pCIR (I) of the *NUP98/ KDM5A*, *CBFA2T3/GLIS*, *KMT2A* rearrangements and monosomy 7 cases compared with the *RBM15/MKL1* and other pediatric AMKL cases. Harboring *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A* rearrangements or monosomy 7 confers a poor outcome.

 $29 \pm 4\%$. To test whether our cohort (n = 146, excluding the 7 cases present in both this study and the study published by Inaba et al¹¹) had a selection bias, our patients were compared for outcome with all AMKL cases in the BFM cohort recently published by Inaba et al (n = 97), and outcome was comparable for pOS (56 ± 4% vs 60 ± 5%, P = .51), pEFS (51 ± 4% vs 47 ± 5%, P = .62) and pCIR (29 ± 4% vs 32 ± 5%, P = .62).

Outcome did not differ significantly between the different collaborative groups. The 4-year pEFS of all non-Down syndrome AMKL cases was 57 \pm 7%, 45 \pm 8%, and 49 \pm 6% for the AIEOP, BFM/DCOG/SLH, and COG cohorts, respectively (P = .32; Figure 2A). The 4-year pOS was 60 \pm 7%, 48 \pm 8%, and 59 \pm 6%

(P = .32; Figure 2B), and 4-year pCIR was $28 \pm 7\%$, $36 \pm 8\%$, and $23 \pm 5\%$ (P = .29; Figure 2C).

When we analyzed the outcome of patients according to the specific recurrent molecular lesions, we found that *RBM15/MKL1* and pediatric AMKL without the aforementioned translocations showed a 4-year pOS of 70 \pm 11% and 66 \pm 5%, respectively, vs a poorer outcome in *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A*–rearranged patients (4-year pOS 36 \pm 13%, 38 \pm 10%, and 33 \pm 13%, respectively; *P* = .0036) with a similar trend for pEFS (*P* = .060; Figure 2D-E). The difference in outcome was mainly because of relapsed and refractory disease, as 22 and 9 of the 52 *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A*–rearranged patients either relapsed or did not achieve CR,

respectively (supplemental Table 2). The pCIR of *RBM15/MKL1* translocated cases was $6 \pm 6\%$, compared with $36 \pm 14\%$, $42 \pm 10\%$, and $51 \pm 15\%$ for the cases positive for *NUP98/KDM5A*, *CBFA2T3/ GLIS2*, or *KMT2A* rearrangements, respectively (P = .03; Figure 2F). Next, we analyzed the poor prognostic cytogenetic aberrations as proposed by Inaba et al.¹¹ The monosomy 7 cases also showed a poor outcome, confirming the previously found results. The 4-year pOS, pEFS, and pCIR were $33\pm\%$, $33\pm 16\%$, and $33\pm 18\%$, respectively (Figure 2D-F).

Altogether we concluded that *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A* rearrangements and monosomy 7 (collectively referred to as NCK-7 positive) should be considered as poor risk, and *RBM15/MKL1* and others as standard risk. Comparing the NCK-7-positive cases vs the *RBM15/MKL1* and other cases, the pCIR differed significantly (4-year pCIR of NCK-7 cases being $42 \pm 7\%$ vs $19 \pm 4\%$ for *RBM15/MKL1* and other cases, P = .003). This lower pCIR translated into a worse EFS ($33 \pm 6\%$ for NCK-7 cases vs $62 \pm 5\%$ for *RBM15/MKL1* and other cases, P = .0013) and a lower probability of OS ($35 \pm 6\%$ vs $70 \pm 5\%$, P < .0001; Figure 2G-I).

Apart from relapsed and refractory cases, other events leading to mortality among the patients with one of the identified fusions consisted of infections (n = 6; 3 *CBFA2T3/GLIS2* positive cases and 3 other cases), toxicity (n = 3; a *NUP98/KDM5A*-positive, a monosomy 7, and an *RBM15/MKL1*-positive case), graft-versus-host disease after HSCT (n = 3; 1 *KMT2A* rearranged, an *RBM15/MKL1*-positive case, and 1 classified as other), hemorrhage (n = 3; a *RBM15/MKL1* translocated case, a monosomy 7 case, and 1 "other"), and acute respiratory distress syndrome (n = 1) in a *KMT2A*-rearranged case, supplemental Table 2).

Multivariate analysis

Harboring *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A* rearrangement or monosomy 7 was an independent risk factor for EFS, RFS, and OS (hazard ratio [HR] 1.69; 95% confidence interval [CI], 1.04-2.76, P = .035; HR 2.26, 95% CI, 1.15-4.48, P = .019; and HR 2.33, 95% CI, 1.36-3.99, P = .002, respectively; Table 2), when other risk factors such as sex, age at diagnosis, WBC count, HSCT, normal karyotype, 7p-aberrations, and hyperdiploid karyotype were included in the model.

Harboring a hyperdiploid karyotype or normal karyotype did have an overlap among the different cytogenetic subgroups (Figure 1). Presence of a hyperdiploid karyotype, including the cases harboring another cytogenetic or molecular aberration, was an independent good prognostic factor for EFS (HR 0.43, 95% CI, 0.21-0.90, P = .026), but not for RFS or OS (P = .09 and P = .053, respectively; Table 2). We could not confirm favorable outcome for cases harboring 7p-abnormalities (pOS $69 \pm 13\%$ vs $55 \pm 4\%$, respectively, P = .45), neither did harboring a normal karyotype influence outcome (pOS $53 \pm 11\%$ vs $57 \pm 4\%$ in all other cases, P = .75; Table 2).

In the NCK-7 groups, 47% of the patients received HSCT, compared with 37% in the *RBM15/MKL1* and other cases. HSCT did not influence the RFS (HR 0.95, 95% CI, 0.47-1.92, P = .0.882; Table 2).

Discussion

We report the results of an international collaborative study on the prognostic value of the recently identified, recurrent (cyto-) genetic aberrations found in pediatric AMKL. Pediatric AMKL is a rare entity, which comprises 5% to 10% of the pediatric AML cases and is considered to be characterized by poor outcome, in contrast to the

Table 2. HRs of cytogenetic and clinical markers for event-free, relapse-free, and overall survival in multivariate analysis

	HR	95% CI	$P(\chi^2)$
NCK-7 positive (n = 61)			
EFS	1.69	1.04-2.76	.035
RFS	2.26	1.15-4.48	.019
OS	2.33	1.36-3.99	.002
Hyperdiploidy (n = 33)			
EFS	0.43	0.21-0.90	.026
RFS	0.43	0.16-1.14	.090
OS	0.44	0.20-1.01	.053
7p-abnormality (n = 13)			
EFS	1.06	0.41-2.73	.909
RFS	1.35	0.39-4.70	.639
OS	1.10	0.38-3.17	.860
Normal karyotype (n = 20)			
EFS	0.85	0.43-1.70	.651
RFS	0.98	0.40-2.43	.966
OS	0.97	0.47-2.01	.928
Sex (male, $n = 70$)			
EFS	1.32	0.86-2.23	.186
RFS	1.14	0.60-2.16	.688
OS	1.41	0.84-2.38	.193
Age <2 y (n = 97)			
EFS	0.71	0.43-1.15	.159
RFS	0.57	0.30-1.10	.093
OS	0.82	0.49-1.38	.453
WBC count >20.0 \times 10 ⁹ /L (n = 38)			
EFS	1.32	0.80-2.20	.276
RFS	1.67	0.86-3.25	.129
OS	1.62	0.95-2.76	.079
HSCT (n = 63)			
EFS	1.14	0.61-2.11	.680
RFS	0.95	0.47-1.92	.882
OS	1.18	0.67-2.08	.575

outcome found in ML-DS cases. The high incidence of myelofibrosis may complicate cytogenetic diagnosis based on conventional karyotyping, and robust prognostic markers are still to be identified.^{1,4-6,8,11} In our cohort, the translocations *CBFA2T3/GLIS2*, *NUP98/KDM5A*, *RBM15/MKL1* and rearrangements of *KMT2A* accounted for 46% of the pediatric AMKL cases, and harboring translocation *CBFA2T3/GLIS2*, *NUP98/KDM5A*, *KMT2A* rearrangement or monosomy 7 appeared to be an independent prognostic factor for poor outcome. This poor outcome was mainly because of a high incidence of nonresponse and relapse in these patients, as documented in 42% of these cases in our cohort. Our data confirm the poor prognosis for overall survival of monosomy 7 cases, which was previously described in pediatric AML and pediatric AMKL.^{11,24,25,28}

Previous studies showed the association between a hyperdiploid karyotype and AMKL.^{26,27} In adult and pediatric AML hyperdiploidy was reported to result in intermediate or poor outcome, and other studies showed that hyperdiploid cases should be assessed for the presence of specific abnormalities to predict outcome.^{26,29,30} Based on our results a hyperdiploid karyotype should not be considered as risk factor in pediatric AMKL.

The outcome of *RBM15/MKL1* positive cases is not uniform in different studies because of the nature of the events. The studies of Inaba et al and Schweitzer et al describe an intermediate overall survival and a poor EFS.^{6,11} However, Inaba et al reported that most events were early deaths, and Schweitzer et al reported a high nonremitter rate.^{6,11} In our study, only 1/18 patients harboring *RBM15/MKL1* suffered from early death, 2 cases died because of treatment-related mortality, and 2 cases died because of leukemia progression. This better outcome may be the result of differences in supportive care, as hypothesized by Inaba et al.¹¹

Pediatric AMKL arises in very young patients, the median age at diagnosis being below 2 years. Our study shows that neither age, sex, nor WBC count at diagnosis is an independent prognostic factor in pediatric AMKL. In addition, there were no differences in presenting characteristics among the various genetic groups, with the exception of *RBM15/MKL1*-positive cases, which had a significant younger age at diagnosis.

An important difference between the treatment protocols of the various collaborative groups in this study was the inclusion of HSCT in CR1 in case of an available donor. However, in multivariate analysis, HSCT did not influence the RFS. The added value of HSCT for pediatric AML in general is under discussion. There is clear evidence that allogeneic HSCT has a greater antileukemic potential than chemotherapy as postremissional treatment, but this favorable effect may be blunted by a higher risk of treatment-related mortality.^{9,10,31} Nevertheless, HSCT in CR1 is recommended in many AML treatment protocols for high-risk cases as defined by presence of genetic aberrations or early response to treatment (ie, high levels of minimal residual disease after 1 or 2 courses of induction chemotherapy). Based on the design of the current study, a benefit of HSCT could not be demonstrated in this retrospective analysis of pediatric AMKL.

AMKL is seen in >70% of ML-DS patients.^{6,11,32} ML-DS AMKL cases are characterized by mutations in the hematopoietic transcription factor GATA1, and these patients have a good prognosis when treated with reduced-intensity chemotherapy because of the unique sensitivity of leukemia cells to chemotherapeutic agents.^{21,22,32-36} Our group previously showed that RBM15/MKL1, CBFA2T3/GLIS2, NUP98/ KDM5A, and KMT2A rearrangements were not present in ML-DS.⁵ GATA1 mutations have been described in non-Down syndrome AMKL, often seen with an acquired trisomy 21, and it was suggested that these cases fare well in terms of prognosis, although numbers were small.^{6,23,37-39} Cases of pediatric AMKL that we could not classify based on the 4 studied translocations may harbor a GATA1 mutation, because we were lacking DNA material of a large number of patients in this cohort. Next-generation sequencing techniques may be instrumental to unravel other recurrent aberrations in the near future in this relatively large group of AMKL cases, negative for any of the aforementioned aberrations.

Recently, Inaba et al described AMKL risk groups based on cytogenetic data obtained with conventional karyotyping, proposing 3 groups: good risk including patients carrying 7p abnormalities; poor risk including cases with monosomy 7, or 9p abnormalities, including KMT2A/MLLT3, -13/13q-, and -15; and intermediate risk, which encompasses all other AKML patients.¹¹ This risk classification may be difficult to use, because AMKL is frequently associated with myelofibrosis, which contributes to difficulties in obtaining sufficient material for cytogenetic analysis. In our study, 34/153 (22%) patients lacked data on karyotype and could therefore not be included in any of the risk groups as proposed by Inaba et al. The percentage of missing karyotypes in our cohort is comparable to the findings of Inaba et al, although this is higher than that reported in pediatric AML including all morphology subtypes (3% to 13%).^{6,25,38} Moreover, conventional karyotyping will not reveal cryptic translocations, such as CBFA2T3/ GLIS2 or NUP98/KDM5A. Through screening by RT-PCR, we were able to classify 38 cases with these cryptic events, of which 10 did not have an available karyotype, and the remaining cases did not show the occurrence of these events. Therefore, screening for the most frequent recurrent aberrations will provide additional information with respect to a risk stratification based on conventional karyotyping, revealing additional groups with poor outcome.

With international collaborations novel prognostic subgroups may be identified, which may lead to improved risk group stratification. Although the overall survival is poor for pediatric AMKL in general, with a 4-year pOS of 56% in this study, *NUP98/KDM5A*, *CBFA2T3/ GLIS2*, *KMT2A* rearrangements and monosomy 7 cases fare worse. We suggest that *NUP98/KDM5A*, *CBFA2T3/GLIS2*, *KMT2A* rearrangements and monosomy 7 cases in pediatric AMKL identify a high-risk patient subgroup, whereas children belonging to either *RBM15/MKL1* or other pediatric AMKL should be considered as a standard risk subgroup.

Altogether, our results indicate that pediatric AMKL is a heterogeneous disease, and that presence of *NUP98/KDM5A*, *CBFA2T3/ GLIS2*, or *KMT2A* rearrangements confers a poor clinical outcome, as well as cases with monosomy 7. Screening for the aforementioned translocations combined with conventional karyotyping in pediatric AMKL is advisable in future studies for improving risk group stratification and tailoring treatment intensity according to the biological characteristics of the leukemic clone.

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Authorship

Contribution: J.D.E.d.R., R.M., M.M.v.d.H.-E., R.P., C.M.Z., and F.L. designed the study; M.M.v.d.H.-E., J.-M.C., J.T., M.R., D.R., E.S., T.A.A., M.P., S.M., C.M.Z., and F.L. provided samples and clinical data; J.D.E.d.R. and R.M. performed the research; M.Z. and T.A.A. performed statistical analyses; J.D.E.d.R., R.M., M.M.v.d.H.-E., C.M.Z., and F.L. analyzed the data; J.D.E.d.R., C.M.Z., and F.L. wrote the manuscript; and all authors critically reviewed the manuscript.

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Correspondence: C. Michel Zwaan, Department of Pediatric Oncology/Hematology, Erasmus MC–Sophia Children's Hospital, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands; e-mail: c.m. zwaan@erasmusmc.nl.

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