blood advances

Optimized cytogenetic risk-group stratification of *KMT2A*-rearranged pediatric acute myeloid leukemia

Romy E. van Weelderen,[1,2] Christine J. Harrison,[3] Kim Klein,[1,2,4] Yilin Jiang,[1] Jonas Abrahamsson,[5] Todd Alonzo,[6] Richard Aplenc,[7] Nira Arad-Cohen,[8] Emmanuelle Bart-Delabesse,[9] Barbara Buldini,[10] Barbara De Moerloose,[11] Michael N. Dworzak,[12] Sarah Elitzur,[13] José M. Fernández Navarro,[14] Alan Gamis,[15] Robert B. Gerbing,[16] Bianca F. Goemans,[1] Hester A. de Groot-Kruseman,[1,17] Erin Guest,[15] Shau-Yin Ha,[18] Henrik Hasle,[19] Charikleia Kelaidi,[20] Hélène Lapillonne,[21] Guy Leverger,[21] Franco Locatelli,[22] Takako Miyamura,[23] Ulrika Norén-Nyström,[24] Sophia Polychronopoulou,[20] Mareike Rasche,[25] Jeffrey E. Rubnitz,[26] Jan Stary,[27] Anne Tierens,[28] Daisuke Tomizawa,[29] C. Michel Zwaan,[1,30] and Gertjan J. L. Kaspers[1,2]

[1]Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands; [2]Emma Children's Hospital, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; [3]Leukemia Research Cytogenetics Group, Translational and Clinical Research Institute, Newcastle University Centre for Cancer, Newcastle-upon-Tyne, United Kingdom; [4]Department of Pediatrics, Wilhelmina Children's Hospital/University Medical Center Utrecht, Utrecht, The Netherlands; [5]Department of Pediatrics, Institute of Clinical Sciences, Salgrenska University Hospital, Gothenburg, Sweden; [6]Division of Biostatistics, University of Southern California, Los Angeles, CA; [7]Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [8]Department of Pediatric Hematology-Oncology, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel; [9]Institut Universitaire du Cancer Toulouse-Oncopole, Laboratoire d'Hématologie secteur Génétique des Hémopathies, Toulouse, France; [10]Division of Pediatric Hematology, Oncology, and Stem Cell Transplant, Department of Maternal and Child Health, Padua University, Padua, Italy; [11]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium; [12]Department of Pediatrics, St. Anna Children's Hospital, Medical University of Vienna & St. Anna Children's Cancer Research Institute, Vienna, Austria; [13]Department of Pediatric Hematology and Oncology, Schneider Children's Medical Center & Tel Aviv University, Tel Aviv, Israel; [14]Department of Pediatric Oncohematology, Hospital Universitari i Politècnic la Fe, Valencia, Spain; [15]Department of Hematology and Oncology, Children's Mercy Hospital, Kansas City, MO; [16]Department of Statistics, Children's Oncology Group, Monrovia, CA; [17]DCOG, Dutch Childhood Oncology Group, Utrecht, The Netherlands; [18]Department of Pediatrics & Adolescent Medicine, Hong Kong Children's Hospital, Kowloon Bay, Hong Kong; [19]Department of Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark; [20]Department of Pediatric Hematology and Oncology, Aghia Sophia Children's Hospital, Athens, Greece; [21]Department of Pediatric Hematology and Oncology, Hôpital Armand Trousseau, Paris, France; [22]Department of Pediatric Hematology and Oncology and Cell and Gene Therapy, IRCCS Ospedale Pediatrico Bambino Gesù, Catholic University of the Sacred Heart, Rome, Italy; [23]Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Japan; [24]Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; [25]Department of Pediatric Hematology and Oncology, University Hospital Essen, Essen, Germany; [26]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN; [27]Department of Pediatric Hematology and Oncology, University Hospital Motol and Second Faculty of Medicine, Charles University, Prague, Czech Republic; [28]Department of Pathobiology and Laboratory Medicine, University Health Network, Toronto General Hospital, Toronto, ON, Canada; [29]Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan; and [30]Department of Pediatric Oncology, Erasmus Medical Center Sophia Children's Hospital, Rotterdam, The Netherlands

Key Points

- We clinically characterized 3 additional, recurring *KMT2A*-r groups and identified specific ACAs of independent prognostic significance.

- We present an optimized, fusion-based risk-group stratification of *KMT2A*-r pediatric AML.

A comprehensive international consensus on the cytogenetic risk-group stratification of *KMT2A*-rearranged (*KMT2A*-r) pediatric acute myeloid leukemia (AML) is lacking. This retrospective (2005-2016) International Berlin-Frankfurt-Münster Study Group study on 1256 children with *KMT2A*-r AML aims to validate the prognostic value of established recurring *KMT2A* fusions and additional cytogenetic aberrations (ACAs) and to define additional, recurring *KMT2A* fusions and ACAs, evaluating their prognostic relevance. Compared with our previous study, 3 additional, recurring *KMT2A*-r groups were defined: Xq24/*KMT2A*::*SEPT6*, 1p32/*KMT2A*::*EPS15*, and 17q12/t(11;17)(q23;q12). Across 13 *KMT2A*-r groups, 5-year event-free survival probabilities varied significantly (21.8%-76.2%; *P* < .01). ACAs occurred in 46.8% of 1200 patients with complete karyotypes, correlating with inferior overall survival (56.8% vs 67.9%; *P* < .01). Multivariable analyses confirmed independent associations of 4q21/*KMT2A*::*AFF1*, 6q27/*KMT2A*::*AFDN*, 10p12/*KMT2A*::*MLLT10*, 10p11.2/*KMT2A*::*ABI1*, and 19p13.3/*KMT2A*::*MLLT1* with adverse

overlap in our data set has been published in https://doi.org/10.1200/JCO.22.02120.

The full-text version of this article contains a data supplement.

outcomes, but not those of 1q21/*KMT2A*::*MLLT11* and trisomy 19 with favorable and adverse outcomes, respectively. Newly identified ACAs with independent adverse prognoses were monosomy 10, trisomies 1, 6, 16, and X, add(12p), and del(9q). Among patients with 9p22/*KMT2A*::*MLLT3*, the independent association of French-American-British–type M5 with favorable outcomes was confirmed, and those of trisomy 6 and measurable residual disease at end of induction with adverse outcomes were identified. We provide evidence to incorporate 5 adverse-risk *KMT2A* fusions into the cytogenetic risk-group stratification of *KMT2A*-r pediatric AML, to revise the favorable-risk classification of 1q21/*KMT2A*::*MLLT11* to intermediate risk, and to refine the risk-stratification of 9p22/*KMT2A*::*MLLT3* AML. Future studies should validate the associations between the newly identified ACAs and outcomes and unravel the underlying biological pathogenesis of *KMT2A* fusions and ACAs.

Introduction

KMT2A-rearranged (*KMT2A*-r) acute myeloid leukemia (AML) is a heterogeneous pediatric AML subtype involving chromosomal rearrangement of the *KMT2A* (formerly known as *MLL*) gene located at chromosome 11q23.[1] Outcome of this subtype is highly variable and related to the *KMT2A* fusion partner, as determined in our previous International Berlin-Frankfurt-Münster (I-BFM) Study Group (SG; I-BFM-SG) analysis (1993-2005).[2] Our more recent (2005-2016) I-BFM-SG cohort demonstrated that the outcome of childhood *KMT2A*-r AML is also dependent on flow cytometry–based measurable residual disease (flow-MRD) at end of induction 2 (EOI2).[3]

The most frequently occurring *KMT2A* translocation, t(9;11)(p22;q23) (9p22/*KMT2A*::*MLLT3* fusion), has been associated with an intermediate prognosis.[2,4] However, among patients with 9p22/*KMT2A*::*MLLT3*, significantly better survival was repeatedly reported for those with the French-American-British (FAB)–type M5.[2,5] The translocation t(1;11)(q21;q23) (1q21/*KMT2A*::*MLLT11* fusion) has been associated with a favorable outcome,[2] although not confirmed by others.[4] Markedly inferior outcomes have been reported for the translocations t(4;11)(q21;q23) (4q21/*KMT2A*::*AFF1* fusion), t(6;11)(q27;q23) (6q27/*KMT2A*::*AFDN* fusion), t(10;11)(p12;q23) (10p12/*KMT2A*::*MLLT10* fusion), t(10;11)(p11.2;q23) (10p11.2/*KMT2A*::*ABI1* fusion), and t(11;19)(q23;p13.3) (19p13.3/*KMT2A*::*MLLT1* fusion),[2,4] which are often considered as adverse risk. In our more recent I-BFM-SG analysis,[3] these 5 fusions were clustered into an adverse-risk fusion-based group, which was independently associated with a poor outcome, like in the study by Pollard et al.[4]

The Children's Oncology Group incorporated these 5 distinct adverse-risk *KMT2A* fusions as unfavorable prognostic markers into the treatment stratification algorithm of their ongoing AAML1831 trial (ClinicalTrials.gov identifier: NCT04293562).[6,7] However, other SGs did not consider these fusions, incorporated only few, or relied more on flow-MRD as a prognostic factor because of the lack of a comprehensive international consensus on risk-group stratification of childhood *KMT2A*-r AML.[8,9]

Additional cytogenetic aberrations (ACAs) have been reported to be of prognostic value in childhood *KMT2A*-r AML.[2,5] Trisomy 8 was associated with a favorable outcome, whereas trisomy 19 and structural aberrations were associated with adverse outcomes.[5]

The aims of this large I-BFM-SG study were to validate the prognostic value of previously defined recurring *KMT2A* fusions and reported ACAs and to define additional, recurring *KMT2A* fusions and ACAs, evaluating their prognostic relevance. The overall aim was to provide evidence to optimize the cytogenetic risk-group stratification of *KMT2A*-r pediatric AML.

Methods

Study design and patients

This retrospective study included patient data from 1256 children with *KMT2A*-r AML, assembled from 15 pediatric AML SGs/countries affiliated with the I-BFM-SG (supplemental Table 1). This same cohort was used in our previous study, analyzing the impact of flow-MRD and use of allogeneic stem cell transplantation (allo-SCT) in first complete remission (CR1) on the outcome of patients with *KMT2A*-r AML.[3] Patients were treated according to national or SG pediatric AML clinical trials,[10-23] approved by the institutional ethics committees of all collaborating centers. The study included patients aged<19 years with de novo *KMT2A*-r AML, diagnosed between 1 January 2005 and 31 December 2016. Patients with a diagnosis of acute promyelocytic leukemia, isolated myeloid sarcoma, myeloid leukemia of Down syndrome, and/or who had received previous anticancer treatment for diseases other than AML for >1 week were excluded a priori. Data were validated for accuracy and correctness.

Cytogenetic analysis

KMT2A rearrangements were detected by cytogenetics (G-, Q-, or R-banding according to local practice), with some confirmed by fluorescence in situ hybridization, or reverse transcription polymerase chain reaction within the local centers. Karyotypes were reviewed by 2 authors (R.E.v.W. and C.J.H.) and written according to the International System for Human Cytogenetic Nomenclature 2020.[24] Patients were assigned to 1 of 10 individual, recurring fusion-based *KMT2A*-r groups or the *KMT2A*-other group, as

previously described by Balgobind et al.[2] Two authors (R.E.v.W. & C.J.H.) validated the group assignments and defined additional, recurring KMT2A-r groups when the same fusion was observed in at least 10 patients. Unknown fusions and those present in <10 patients remained assigned to the KMT2A-other group.

An ACA was defined as the presence of an acquired chromosomal abnormality in addition to the KMT2A rearrangement. Constitutional abnormalities did not constitute ACA. Patients with incomplete karyotypes were excluded from the ACA analysis. Ploidy changes were regarded as a single ACA. Patients with ACAs were categorized into having numerical, structural, or both numerical and structural aberrations. Numerical aberrations were defined as the loss or gain of whole chromosomes, whereas structural aberrations involved changes to chromosome short (p) or long (q) arms. Within the category of both numerical and structural aberrations, patients with separate numerical, and structural aberrations were included, as well as gains of structurally abnormal chromosomes, for example, +der(9p) and +i(8q). The gain of a marker chromosome was classified as a numerical aberration.

Statistical analyses

CR was defined as <5% blasts in the bone marrow after 2 induction courses, with regeneration of peripheral blood cells and the absence of extramedullary disease and cells with Auer rods. Patients who did not achieve CR after induction therapy were considered refractory to treatment. Relapse was defined as ≥5% blasts in the bone marrow, reappearance of leukemic blasts in the peripheral blood, or the presence of extramedullary disease after initial CR. Event-free survival (EFS) was calculated from the date of diagnosis to the date of the first event or last follow-up. Events included induction failure (ie, death before the start of intended treatment, death within 42 days after the start of treatment [early death], death after >42 days but before CR assessment, or refractory disease), death in CR, relapse, and secondary malignancy. Induction failure was considered an event at time zero. Cumulative incidence of relapse (CIR) was defined as the time from end of induction 1 until relapse for patients in CR, with deaths without a relapse considered competing events. Overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow-up.

The χ^2 test was used to compare differences in proportions of clinical characteristics and groups. The Mann-Whitney U and Kruskal-Wallis tests were used to compare differences in medians of 2 or >2 groups, respectively. The prognostic impact of recurring ACAs, occurring in at least 10 patients, was explored. Probabilities of EFS and OS with 95% confidence intervals (CIs) were estimated with the Kaplan-Meier method and compared using the log-rank test. CIR estimates with 95% CIs were compared using the Gray test for competing risks. Cox proportional hazards models were used to calculate hazard ratios (HRs) with 95% CIs. Variables with a 2-sided P-value < .05 in univariable Cox regression analyses were included in multivariable Cox regression models. Subsequently, variables with a 2-sided P-value > .10 were omitted from the final multivariable models by stepwise backward elimination. To correct for multiple testing, 2-sided P-values ≤ .01 were considered statistically significant. SPSS version 28 and R version 4.1.2 were used.

Results

Patient characteristics and ACA distribution

A total of 1256 children with KMT2A-r AML were included, of whom 1130 (90.0%) were assigned to 1 of 13 KMT2A-r groups and 126 (10.0%) to the KMT2A-other group (Figure 1). 9p22/KMT2A::MLLT3 (n = 544, 43.3%), 10p12/KMT2A::MLLT10 (n = 218, 17.4%), 6q27/KMT2A::AFDN (n = 92, 7.3%), and t(11;19)(q23;p13.1) (19p13.1/KMT2A::ELL fusion) (n = 75, 6.0%) were most frequent, together accounting for around 75% of cases. As compared with Balgobind et al,[2] 3 additional, recurring KMT2A-r groups were defined: t(X;11)(q24;q23) (Xq24/KMT2A::SEPT6 fusion) (n = 22, 1.8%), t(1;11)(p32;q23) (1p32/KMT2A::EPS15 fusion) (n = 13, 1.0%), and t(11;17)(q23;q12) (17q12, variable at the molecular level) (n = 10, 0.8%).

Among the 1256 patients, only 56 (4.5%) had incomplete karyotypes. There were no statistically significant differences between patients with and without complete karyotypes in terms of sex, median age, white blood cell (WBC) count at diagnosis, central nervous system involvement, FAB-type, KMT2A-r groups, and clinical outcome (supplemental Table 2). Of the 1200 patients with complete karyotypes, 562 (46.8%) had ACAs (Figure 1). The number of ACAs ranged from 0 to 17. The median number of ACAs among patients with ACAs was 2 (interquartile range, 1.0-3.0).

Table 1 shows the clinical characteristics and outcomes of the total cohort, stratified by KMT2A-r group. There were statistically significant differences in median age and WBC count at diagnosis and in the proportions of FAB-type and type of ACAs between these groups (Table 1). In all KMT2A-r groups, the median age at diagnosis was <4 years except for the 19p13.3/KMT2A::MLLT1, t(11;19)(q23;p13) (19p13, subband unknown), and 6q27/KMT2A::AFDN groups, in which the median ages were 6.8, 9.2, and 10.5 years (P < .01), respectively. The median WBC count at diagnosis was higher in patients with 19p13 (50.8 × 10⁹/L), 6q27/KMT2A::AFDN (65.8 × 10⁹/L), and 4q21/KMT2A::AFF1 (114.3 × 10⁹/L) than in other KMT2A-r groups (P < .01). Most patients were classified as FAB-M5 (n = 619, 71.5%) or FAB-M4 (n = 121, 14.0%), but these proportions were not similar across all groups (P < .01). For example, most children with 1q21/KMT2A::MLLT11 were classified as FAB-M4.

Supplemental Tables 3 and 4 show the frequency distributions of all numerical and structural ACAs, respectively, in total and stratified by KMT2A-r group. There were 28 recurring ACAs (supplemental Table 5), of which trisomy 8 (n = 210, 37.4% of all ACA cases) was the most common. Analyses to test for associations between specific ACAs and KMT2A-r groups were not possible because of the small numbers.

The type of ACAs differed significantly across KMT2A-r groups (P < .01; Table 1). For example, numerical aberrations were more common in patients with 9p22/KMT2A::MLLT3 (44.9%), 17q12 (50%), 19p13 (63.6%), Xq24/KMT2A::SEPT6 (66.7%), and t(11;17)(q23;q21) (17q21, fusion unknown) (83.3%) than in other KMT2A-r groups. Associations between the presence and type of ACAs and clinical characteristics are shown in supplemental Table 6. The median WBC count at diagnosis was lower in patients with ACAs than in patients without ACAs, whereas ACAs
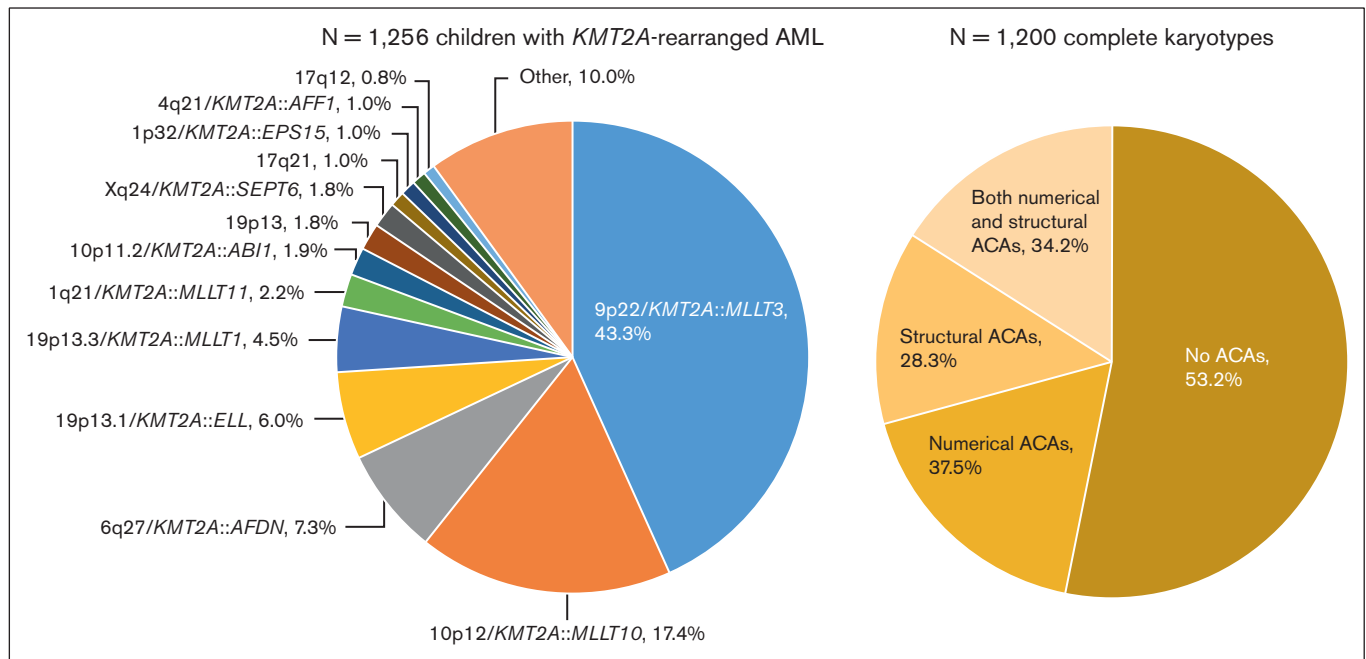
Figure 1. Distribution of the fusion-based groups and the presence and type of ACAs in our cohort of childhood *KMT2A*-r AML. 9p22/*KMT2A*::*MLLT3* refers to t(9;11)(p22;q23) (n = 544), 10p12/*KMT2A*::*MLLT10* to t(10;11)(p12;q23) (n = 218), 6q27/*KMT2A*::*AFDN* to t(6;11)(q27;q23) (n = 92), 19p13.1/*KMT2A*::*ELL* to t(11;19)(q23;p13.1) (n = 75), 19p13.3/*KMT2A*::*MLLT1* to t(11;19)(q23;p13.3) (n = 56), 1q21/*KMT2A*::*MLLT11* to t(1;11)(q21;q23) (n = 28), 10p11.2/*KMT2A*::*ABI1* to t(10;11)(p11.2;q23) (n = 24), 19p13 to t(11;19)(q23;p13) without ascertained subband (n = 23), Xq24/*KMT2A*::*SEPT6* to t(X;11)(q24;q23) (n = 22), 17q21 to t(11;17)(q23;q21) (n = 13), 1p32/*KMT2A*::*EPS15* to t(1;11)(p32;q23) (n = 13), 4q21/*KMT2A*::*AFF1* to t(4;11)(q21;q23) (n = 12), and 17q12 t(11;17)(q23;q12) (n = 10). Of the 1200 patients with complete karyotypes, 638 had no ACAs and 562 had ACAs, of whom 211 had solely numerical ACAs, 159 solely structural aberrations, and 192 both numerical and structural ACAs. Figure created with BioRender.com.

in general were more likely to occur in patients with FAB-M7 and FAB-M0.

Outcome

Of the 1219 patients known to have commenced chemotherapy, 1066 (87.5%) achieved CR, with no significant differences in CR rates among *KMT2A*-r groups (*P* = .02; Table 1). However, EFS, CIR, and OS estimates of the *KMT2A*-r groups differed significantly (all *P* < .01; Table 1; Figure 2). Good outcomes were observed in patients with Xq24/*KMT2A*::*SEPT6* and 1p32/*KMT2A*::*EPS15*, with EFS and OS rates exceeding 75% and 90%, respectively, and CIR rates below 20%. Most patients that achieved CR in these 2 groups were treated with chemotherapy only and did not receive allo-SCT in CR1 (16/21 patients with Xq24/*KMT2A*::*SEPT6*; 9/10 patients with 1p32/*KMT2A*::*EPS15*). Relapses in these groups occurred only in patients who did not undergo transplantation, and all but 1 patient were salvaged. Patients with 10p11.2/*KMT2A*::*ABI1*, 6q27/*KMT2A*::*AFDN*, and 4q21/*KMT2A*::*AFF1* had very poor outcomes with EFS rates of 21.8% (95% CI, 4.9-38.7), 23.3% (95% CI, 14.3-32.3), and 25.0% (95% CI, 0.5-49.5), respectively. Patients with 10p12/*KMT2A*::*MLLT10* and 19p13.3/*KMT2A*::*MLLT1* also had poor outcomes, with EFS rates <40%. In these 5 *KMT2A*-r groups with poor outcomes, CIR rates were ≥50% (Table 1).

Patients with ACAs had inferior OS compared with patients without ACAs (56.8% [95% CI, 52.5-61.1] vs 67.9% [95% CI, 64.2-71.6]; *P* < .01), but EFS and CIR rates were not statistically

significantly different (supplemental Table 7; supplemental Figure 1). Patients with numerical ACAs showed better outcomes than patients with structural or both numerical and structural ACAs (EFS, 52.0% [95% CI, 44.9-59.1] vs 33.7% [95% CI, 25.9-41.5] vs 37.3% [95% CI, 30.1-44.6]; *P* < .01; CIR, 34.5% [95% CI, 27.5-41.6] vs 57.0% [95% CI, 47.5-65.4] vs 51.7% [95% CI, 43.2-59.6]; *P* < .01; OS, 64.0% [95% CI, 57.1-70.9] vs 53.9% [95% CI, 47.7-62.1] vs 50.8% [95% CI, 43.0-58.6]; *P* < .01; supplemental Table 7). EFS curves for patients with and without recurring ACAs that were included in multivariable Cox regression analyses on the basis of entry criterion 2-sided *P* value < .05 in univariable Cox regression analyses (supplemental Table 5) are shown in Figure 3, and the CIR and OS curves of these patients are shown in supplemental Figure 2. Patients with trisomy 8 had statistically significantly superior EFS and a lower CIR than patients without trisomy 8. Patients with monosomy 10 and del(9q) had statistically significantly inferior EFS and OS and a higher CIR than patients without these ACAs. Patients with trisomies 4, 6, 12, 16, and X, and add(12p) had statistically significantly inferior EFS and/or higher CIR than patients without these ACAs. Patients with trisomy 1 had a statistically significantly inferior OS than patients without trisomy 1.

Multivariable analyses

In multivariable analyses (Table 2), age >10 years was independently associated with inferior OS (HR, 1.8; *P* < .01), and WBC count >100 × 10^9/L with inferior EFS (HR, 1.3; *P* < .01) and OS (HR, 1.5; *P* < .01). The 10p12/*KMT2A*::*MLLT10*, 6q27/*KMT2A*::*AFDN*, and

Table 1. Clinical characteristics and outcomes of 1256 children with *KMT2A*-r AML and stratified by fusion-based group

	Total	9p22/ *KMT2A::MLLT3*	10p12/ *KMT2A::MLLT10*	6q27/ *KMT2A::AFDN*	19p13.1/ *KMT2A::ELL*	19p13.3/ *KMT2A::MLLT1*	1q21/ *KMT2A::MLLT11*	10p11.2/ *KMT2A::ABI1*
No. (%)	1256 (100)	544 (43.3)	218 (17.4)	92 (7.3)	75 (6.0)	56 (4.5)	28 (2.2)	24 (1.9)
Sex, no. (%) (n = 1235)								
Male	638 (51.7)	276 (51.9)	127 (58.5)	44 (47.8)	39 (52.7)	30 (54.5)	9 (32.1)	14 (63.6)
Female	597 (48.3)	256 (48.1)	90 (41.5)	48 (52.2)	35 (47.3)	25 (45.5)	19 (67.9)	8 (36.4)
Age at diagnosis, y (n = 1256)								
Median (IQR)	2.5 (1.0-10.0)	3.0 (1.1-9.1)	1.6 (0.7-7.7)	10.5 (5.4-15.2)	3.6 (0.6-12.3)	6.8 (1.6-13.2)	1.2 (0.5-3.0)	1.5 (0.9-5.1)
WBC count, ×10^9/L, (n = 1186)								
Median (IQR)	21.4 (5.7-87.8)	12.3 (4.4-74.2)	15.1 (5.2-57.7)	65.8 (20.4-120.8)	33.2 (11.0-93.7)	28.1 (12.1-92.3)	30.4 (9.0-63.0)	30.9 (7.5-45.9)
CNS involvement, No. (%) (n = 722)								
Negative	574 (79.5)	274 (83.3)	105 (80.8)	41 (83.7)	25 (69.4)	19 (65.5)	12 (80)	7 (63.6)
Positive	148 (20.5)	55 (16.7)	25 (19.2)	8 (16.3)	11 (30.6)	10 (34.5)	3 (20)	4 (36.4)
FAB-type, no. (%) (n = 866)								
FAB-M0	18 (2.1)	7 (1.8)	0 (0)	3 (5.2)	0 (0)	2 (6.1)	1 (5.3)	0 (0)
FAB-M1	33 (3.8)	9 (2.3)	1 (0.7)	12 (20.7)	3 (6.5)	0 (0)	1 (5.3)	0 (0)
FAB-M2	19 (2.2)	6 (1.5)	1 (0.7)	1 (1.7)	4 (8.7)	1 (3.0)	4 (21.1)	0 (0)
FAB-M4	121 (14.0)	29 (7.4)	11 (7.2)	15 (25.9)	17 (37.0)	6 (18.2)	9 (47.4)	1 (6.7)
FAB-M5	619 (71.5)	308 (78.6)	132 (86.3)	26 (44.8)	20 (43.5)	22 (66.7)	3 (15.8)	13 (86.7)
FAB-M7	31 (3.6)	21 (5.4)	6 (3.9)	0 (0)	0 (0)	2 (6.1)	0 (0)	0 (0)
FAB unspecified	25 (2.9)	12 (3.1)	2 (1.3)	1 (1.7)	2 (4.3)	0 (0)	1 (5.3)	1 (6.7)
ACA, no. (%) (n = 1200)								
No	638 (53.2)	271 (52.5)	100 (47.6)	60 (67.4)	44 (61.1)	26 (47.3)	20 (71.4)	14 (58.3)
Yes	562 (46.8)	245 (47.5)	110 (52.4)	29 (32.6)	28 (38.9)	29 (52.7)	8 (28.6)	10 (41.7)
ACA type, no. (%) (n = 562)								
Structural	159 (28.3)	53 (21.6)	44 (40.0)	6 (20.7)	4 (14.3)	9 (31.0)	3 (37.5)	7 (70)
Numerical	211 (37.5)	110 (44.9)	30 (27.3)	9 (31.0)	11 (39.3)	11 (37.9)	2 (25)	2 (20)
Both*	192 (34.2)	82 (33.5)	36 (32.7)	14 (48.3)	13 (46.4)	9 (31.0)	3 (37.5)	1 (10)
CR, no. (%) (n = 1219)	1066 (87.5)	464 (89.9)	187 (87.8)	77 (85.6)	62 (87.3)	50 (90.9)	22 (81.5)	22 (91.7)

Clinical outcome	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
5-y pEFS	1199	44.8 (41.9-47.7)	512	54.0 (49.5-58.5)	212	33.4 (26.7-40.1)	89	23.3 (14.3-32.3)	71	44.8 (33.0-56.6)	55	34.7 (21.6-47.8)	27	55.0 (36.0-74.0)	24	21.8 (4.9-38.7)
5-y pCIR	1057	44.8 (41.7-47.9)	459	36.2 (31.6-40.7)	186	57.4 (49.6-64.4)	77	66.3 (54.0-76.0)	61	41.9 (29.2-54.2)	50	54.1 (39.1-66.9)	22	27.9 (10.9-47.9)	22	71.4 (45.4-86.6)
5-y pOS	1217	62.6 (59.9-65.3)	522	70.2 (66.1-74.3)	214	54.0 (47.1-60.9)	90	39.0 (27.8-50.2)	72	67.8 (56.6-79.0)	55	51.7 (37.6-65.8)	27	73.3 (56.3-90.4)	24	50.9 (29.9-71.9)

Values in boldface indicate statistical significance. Fusion-based groups are displayed in descending order of occurrence. 9p22/*KMT2A::MLLT3* refers to t(9;11)(p22;q23), 10p12/*KMT2A::MLLT10* to t(10;11)(p12;q23), 6q27/*KMT2A::AFDN* to t(6;11)(q27;q23), 19p13 to t(11;19)(q23;p13) without ascertained subband, 19p13.1/*KMT2A::ELL* to t(11;19)(q23;p13.1), 19p13.3/*KMT2A::MLLT1* to t(11;19)(q23;p13.3), 1q21/*KMT2A::MLLT11* to t(1;11)(q21;q23), 10p11.2/*KMT2A::ABI1* to t(10;11)(p11.2;q23), Xq24/*KMT2A::SEPT6* to t(X;11)(q24;q23), 17q21 to t(11;17)(q23;q21), 4q21/*KMT2A::AFF1* to t(4;11)(q21;q23), 1p32/*KMT2A::EPS15* to t(1;11)(p32;q23), and 17q12 to t(11;17)(q23;q12).

CNS, central nervous system; No., number of patients; pCIR, probability of CIR; pEFS, probability of EFS; pOS, probability of OS.

*Including patients who had separate numerical and structural aberrations, as well as patients with gain of a chromosome with a structural aberration, for example, +der(9p) and +i(8q).

Table 1 (continued)

	19p13	Xq24/*KMT2A::SEPT6*	17q21	1p32/*KMT2A::EPS15*	4q21/*KMT2A::AFF1*	17q12	Other	P value
No. (%)	23 (1.8)	22 (1.8)	13 (1.0)	13 (1.0)	12 (1.0)	10 (0.8)	126 (10.0)	
Sex, no. (%) (n = 1235)								
Male	8 (38.1)	9 (42.9)	7 (53.8)	8 (61.5)	5 (41.7)	6 (60)	56 (44.8)	.24
Female	13 (61.9)	12 (57.1)	6 (46.2)	5 (38.5)	7 (58.3)	4 (40)	69 (55.2)	
Age at diagnosis, y (n = 1256)								
Median (IQR)	9.2 (1.0-13.0)	1.4 (0.9-5.7)	1.0 (0.3-3.8)	0.6 (0.3-1.6)	0.6 (0.1-1.9)	1.7 (0.6-13.9)	1.5 (0.6-7.7)	**<.01**
WBC count, ×10^9/L, (n = 1186)								
Median (IQR)	50.8 (11.4-103.0)	18.0 (5.4-48.1)	39.2 (8.4-127.4)	39.4 (13.5-56.9)	114.3 (24.3-401.0)	41.0 (8.8-102.3)	30.0 (8.2-145.8)	**<.01**
CNS involvement, no. (%) (n = 722)								
Negative	17 (94.4)	9 (75)	8 (72.7)	8 (80)	4 (66.7)	3 (75)	42 (67.7)	.10
Positive	1 (5.6)	3 (25)	3 (27.3)	2 (20)	2 (33.3)	1 (25)	20 (32.3)	
FAB-type, no. (%) (n = 866)								
FAB-M0	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	4 (4.5)	**<.01**
FAB-M1	0 (0)	2 (15.4)	0 (0)	0 (0)	2 (22.2)	0 (0)	3 (3.4)	
FAB-M2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (1.1)	
FAB-M4	4 (28.6)	3 (23.1)	6 (60)	1 (11.1)	0 (0)	2 (33.3)	17 (19.1)	
FAB-M5	10 (71.4)	8 (61.5)	4 (40)	8 (88.9)	4 (44.4)	3 (50)	58 (65.2)	
FAB-M7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.2)	
FAB unspecified	0 (0)	0 (0)	0 (0)	0 (0)	2 (22.2)	0 (0)	4 (4.5)	
ACA, no. (%) (n = 1200)								
No	12 (52.2)	16 (72.7)	5 (45.5)	7 (53.8)	3 (25)	4 (40)	56 (48.7)	.02
Yes	11 (47.8)	6 (27.3)	6 (54.5)	6 (46.2)	9 (75)	6 (60)	59 (51.3)	
ACA type, no. (%) (n = 562)								
Structural	0 (0)	0 (0)	0 (0)	3 (50)	3 (33.3)	2 (33.3)	25 (42.4)	**<.01**
Numerical	7 (63.6)	4 (66.7)	5 (83.3)	2 (33.3)	2 (22.2)	3 (50)	13 (22.0)	
Both*	4 (36.4)	2 (33.3)	1 (16.7)	1 (16.7)	4 (44.4)	1 (16.7)	21 (35.6)	
CR, no. (%) (n = 1219)	20 (95.2)	21 (100)	12 (92.3)	10 (83.3)	6 (50)	9 (90)	104 (85.2)	.02

Clinical outcome	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
5-y pEFS	20	41.8 (18.9-64.7)	21	76.2 (58.0-94.4)	12	66.7 (40.0-93.4)	12	75.0 (50.5-99.5)	12	25.0 (0.5-49.5)	10	56.3 (24.0-88.6)	122	39.9 (30.9-48.9)	**<.01**
5-y pCIR	19	43.4 (20.1-64.8)	21	19.0 (5.7-38.3)	11	9.1 (0.4-35.0)	10	10.0 (0.5-37.4)	6	50.0 (7.7-82.9)	9	37.5 (7.2-69.4)	104	52.2 (41.8-61.7)	**<.01**
5-y pOS	20	62.4 (39.9-84.9)	21	90.5 (78.0-100)	13	69.2 (44.1-94.3)	13	92.3 (78.0-100)	12	25.0 (0.5-49.5)	10	50.0 (13.5-86.5)	124	60.8 (52.0-69.6)	**<.01**

Values in boldface indicate statistical significance. Fusion-based groups are displayed in descending order of occurrence. 9p22/*KMT2A::MLLT3* refers to t(9;11)(p22;q23), 10p12/*KMT2A::MLLT10* to t(10;11)(p12;q23), 6q27/*KMT2A::AFDN* to t(6;11)(q27;q23), 19p13 to t(11;19)(q23;p13) without ascertained subband, 19p13.1/*KMT2A::ELL* to t(11;19)(q23;p13.1), 19p13.3/*KMT2A::MLLT1* to t(11;19)(q23;p13.3), 1q21/*KMT2A::MLLT11* to t(1;11)(q21;q23), 10p11.2/*KMT2A::ABI1* to t(10;11)(p11.2;q23), Xq24/*KMT2A::SEPT6* to t(X;11)(q24;q23), 17q21 to t(11;17)(q23;q21), 4q21/*KMT2A::AFF1* to t(4;11)(q21;q23), 1p32/*KMT2A::EPS15* to t(1;11)(p32;q23), and 17q12 to t(11;17)(q23;q12).

CNS, central nervous system; No., number of patients; pCIR, probability of CIR; pEFS, probability of EFS; pOS, probability of OS.

*Including patients who had separate numerical and structural aberrations, as well as patients with gain of a chromosome with a structural aberration, for example, +der(9p) and +i(8q).
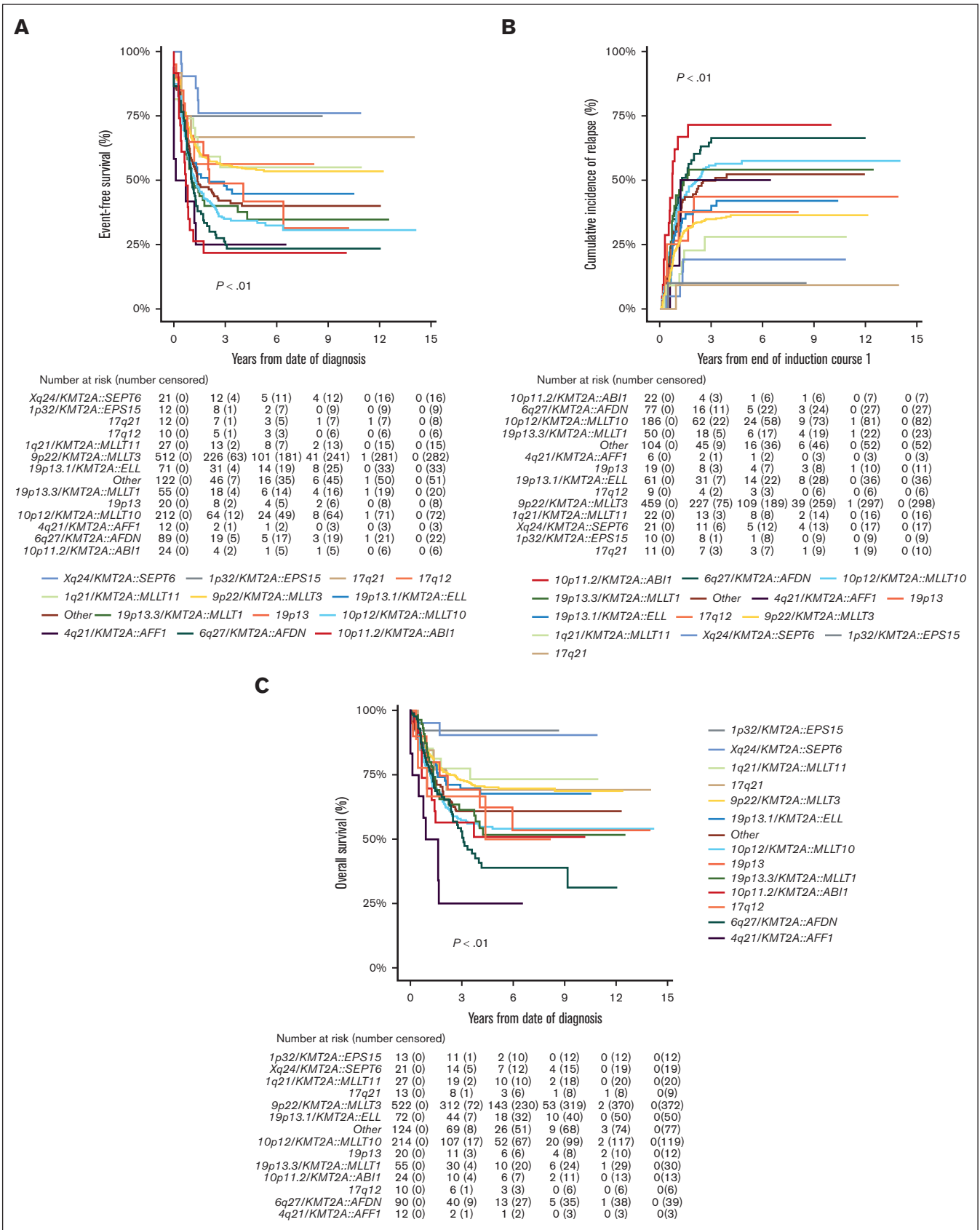
Figure 2.

10p11.2/KMT2A::ABI1 groups were independently associated with inferior EFS ([HR, 1.7; $P < .01$], [HR, 1.8; $P < .01$], and [HR, 2.6; $P < .01$], respectively) and OS ([HR, 1.8; $P < .01$], [HR, 1.8; $P < .01$], and [HR, 2.4; $P < .01$], respectively), and higher CIR ([HR, 1.7; $P < .01$], [HR, 2.0; $P < .01$], and [HR, 3.0; $P < .01$], respectively). The 19p13.3/KMT2A::MLLT1 group was independently associated with inferior EFS (HR, 1.6; $P = .01$) and higher CIR (HR, 1.8; $P < .01$). The 4q21/KMT2A::AFF1 group was independently associated with inferior EFS (HR, 2.7; $P < .01$) and OS (HR, 4.5; $P < .01$). Among ACAs, trisomy 6 was independently associated with inferior EFS (HR, 1.6; $P = .01$) and OS (HR, 1.7; $P = .01$) and higher CIR (HR, 2.0; $P < .01$). Add(12p) was independently associated with inferior EFS (HR, 2.2; $P = .01$) and trisomy 16 with inferior EFS (HR, 2.5; $P < .01$) and higher CIR (HR, 4.5; $P < .01$). Monosomy 10, trisomy 1, trisomy X, and del(9q) were independently associated with inferior OS ([HR, 2.8; $P < .01$], [HR, 3.9; $P < .01$], [HR, 2.8; $P < .01$], and [HR, 2.8; $P < .01$], respectively).

The 9p22/KMT2A::MLLT3 group

Within this group, patients with FAB-M5 had superior EFS (65.9% [95% CI, 60.2-71.6] vs 37.9% [95% CI, 26.9-48.9]; $P < .01$) and OS (80.3% [95% CI, 75.6-85.0] vs 52.3% [95% CI, 42.0-63.5]; $P < .01$), and lower CIR (24.5% [95% CI, 19.4-29.9] vs 48.6% [95% CI, 35.9-60.1]; $P < .01$) than patients with non–FAB-M5 (supplemental Table 7). In multivariable analyses (supplemental Table 8), FAB-M5 was independently associated with superior EFS (HR, 0.5; $P < .01$) and OS (HR, 0.4; $P < .01$) and lower CIR (HR, 0.5; $P < .01$), whereas trisomy 6 was independently associated with inferior EFS (HR, 2.2; $P < .01$) and OS (HR, 2.3; $P = .01$) and higher CIR (HR, 2.7; $P < .01$). Subsequently, multivariable analyses were performed including a combination variable of FAB-type and trisomy 6, showing that, in reference to FAB-M5/no trisomy 6, the combinations of FAB-M5/trisomy 6, non–FAB-M5/no trisomy 6, or non–FAB-M5/trisomy 6 were all independently associated with inferior EFS ([HR, 3.9; $P < .01$], [HR, 2.3; $P < .01$], and [HR, 3.1; $P < .01$], respectively) and OS ([HR, 4.3; $P < .01$], HR, 2.8; $P < .01$], and [HR, 4.1; $P < .01$], respectively) and higher CIR ([HR, 3.3; $P = .02$], [HR, 2.1; $P < .01$], and [HR, 5.0; $P < .01$], respectively). The prognostic value of recurring ACAs within other KMT2A-r groups was explored, but none were significantly associated with EFS, CIR, or OS (data not shown).

Having previously demonstrated the independent prognostic significance of flow-MRD at EOI2 in childhood KMT2A-r AML (with detailed analysis described previously),[3] we explored its prognostic significance in the 9p22/KMT2A::MLLT3 group within the context of FAB-type (M5 vs non-M5). It was not feasible to assess this in both the context of FAB-type and recurring ACAs because of the lack of flow-MRD at EOI2 data in 60% of patients with 9p22/KMT2A::MLLT3. Patients with EOI2 MRD negativity had superior EFS (54.2% [95% CI, 47.1-61.3] vs 35.3% [95% CI, 12.6-58.0]; $P = .02$) and OS (73.6% [95% CI, 67.3-79.9] vs 47.1% [95% CI, 23.4-70.8]; $P < .01$), but CIR was not statistically significantly different. Among patients with FAB-M5, as well as among

patients with non–FAB-M5, EFS did not statistically significantly differ between patients with EOI2 MRD negativity and MRD positivity, but subgroup numbers were small (supplemental Table 7). In multivariable analyses (supplemental Table 8), FAB-M5 was independently associated with superior EFS (HR, 0.5; $P = .02$), whereas flow-MRD at EOI2 was independently associated with inferior EFS (HR, 2.4; $P = .04$) and OS (HR, 2.5; $P = .05$). The addition of the variable flow-MRD at EOI2 did not change the effect of FAB-type on survival, and vice versa, as confirmed by the nonsignificant interaction terms ($P = .52$ for EFS; $P = .63$ for OS).

Discussion

This largest study on childhood KMT2A-r AML confirmed the independent adverse prognostic significance of the previously defined recurring, adverse-risk KMT2A-r groups (ie, 4q21/KMT2A::AFF1, 6q27/KMT2A::AFDN, 10p12/KMT2A::MLLT10, 4q21/KMT2A::ABI1, and 19p13.3/KMT2A::MLLT1; together representing about 30% of pediatric KMT2A-r AML cases), defined and clinically characterized 3 additional, recurring KMT2A-r groups (ie, Xq24/KMT2A::SEPT6, 1p32/KMT2A::EPS15, and 17q12), the former 2 with good outcomes, and refined risk-group stratification of the most frequently occurring KMT2A-r group, 9p22/KMT2A::MLLT3, based on FAB-type and the presence/absence of trisomy 6. Flow-MRD at EOI2 was also identified as an independent adverse prognosticator in this group. Furthermore, we identified novel ACAs that were independently associated with inferior EFS (ie, add(12p) and trisomies 6 and 16), higher CIR (ie, trisomies 6 and 16), or inferior OS (ie, monosomy 10, trisomies 1, 6, and X, and del(9q)).

This and our previous studies[2,3] provide strong evidence for the inclusion of the previously defined adverse-risk KMT2A-r groups into the cytogenetic risk-group stratification algorithm of childhood KMT2A-r AML. With EFS rates <40%, patients with adverse-risk KMT2A fusions may benefit from high-risk–adapted treatment. However, we and others have previously shown that high-risk–adapted treatment approaches superior to allo-SCT in CR1 are urgently needed in this disease.[2-4] In this regard, studies to elucidate the biological role of KMT2A fusions and identify novel therapeutic targets are needed to improve survival. Ongoing phase 1/2 studies including patients with KMT2A-r acute leukemia have shown encouraging clinical responses with the menin inhibitors SNDX-5613 (revumenib)[25] and KO-539 (ziftomenib),[26] which target and disrupt the KMT2A fusion protein complex. These inhibitors constitute a novel, promising class of targeted therapeutics for this disease. However, a recent study suggests that mutations in menin are acquired with the use of SNDX-5613, thereby mediating clinical resistance.[27] Hopefully, such mutations can be avoided when menin inhibitors are combined with chemotherapy. Furthermore, the incorporation of the CD33-targeting immunoconjugate gemtuzumab ozogamicin into induction therapy in the AAML0531 trial improved EFS and reduced relapse risk in children with KMT2A-r AML.[4]

Figure 2. Survival curves for pediatric patients with KMT2A-rearranged AML, stratified by fusion-based group. Kaplan-Meier estimates of (A) EFS, (B) CIR, and (C) OS of KMT2A fusion-based groups. KMT2A::MLLT3 refers to t(9;11)(p22;q23) (n = 544), KMT2A::MLLT10 to t(10;11)(p12;q23) (n = 218), KMT2A::AFDN to t(6;11)(q27;q23) (n = 92), KMT2A::ELL to t(11;19)(q23;p13.1) (n = 75), KMT2A::MLLT1 to t(11;19)(q23;p13.3) (n = 56), KMT2A::MLLT11 to t(1;11)(q21;q23) (n = 28), KMT2A::ABI1 to t(10;11)(p11.2;q23) (n = 24), 19p13 to t(11;19)(q23;p13) without ascertained subband (n = 23), KMT2A::SEPT6 to t(X;11)(q24;q23) (n = 22), 17q21 to t(11;17)(q23;q21) (n = 13), KMT2A::EPS15 to t(1;11)(p32;q23) (n = 13), KMT2A::AFF1 to t(4;11)(q21;q23) (n = 12), and 17q12 to t(11;17)(q23;q12) (n = 10).
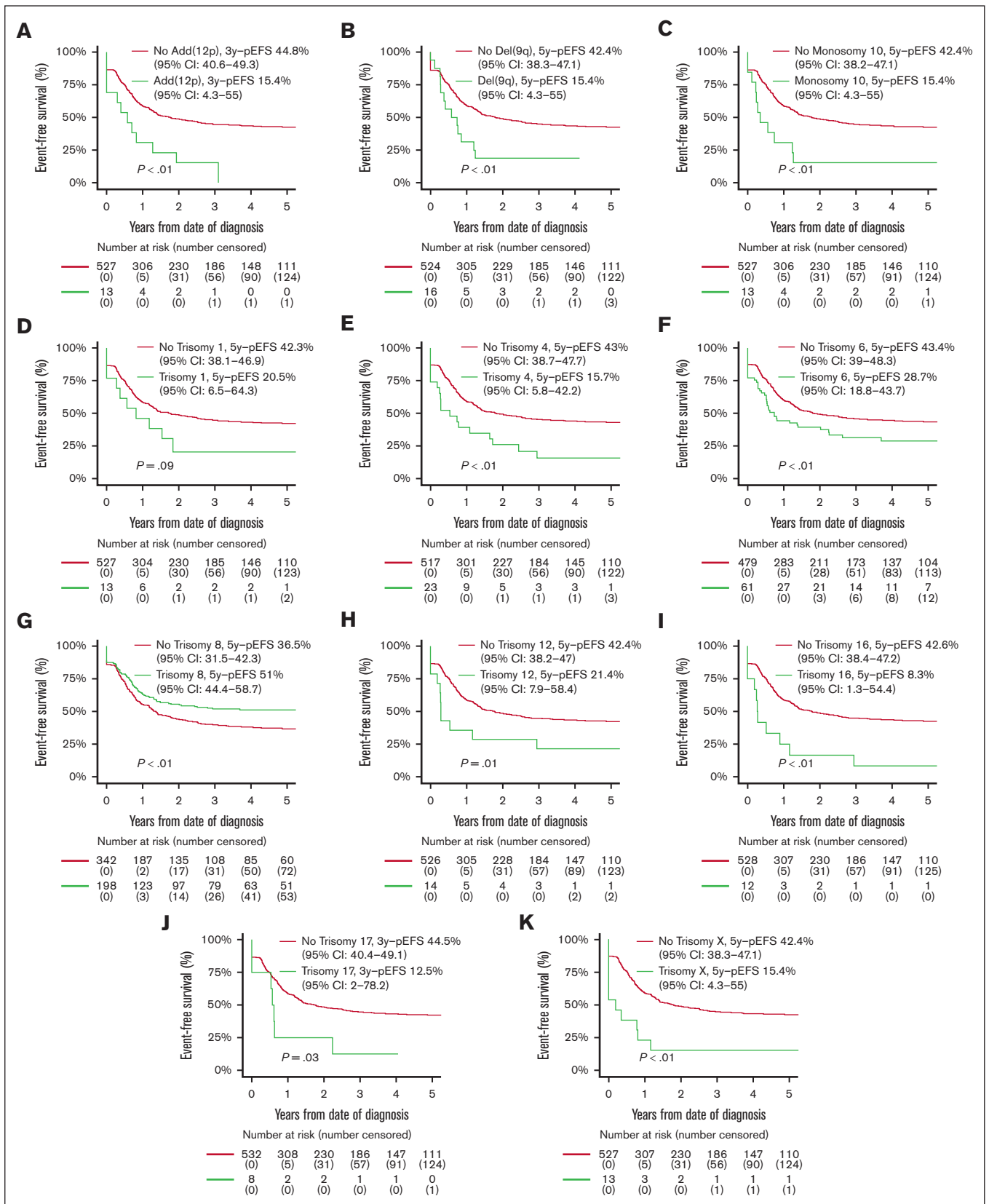
Figure 3. Survival curves for pediatric patients with KMT2A-rearranged AML with and without specific, recurring ACAs. Kaplan-Meier estimates of EFS of patients with and without (A) add (12p), (B) del(9q), (C) monosomy 10, (D) trisomy 1, (E) trisomy 4, (F) trisomy 6, (G) trisomy 8, (H) trisomy 12, (I) trisomy 16, (J) trisomy 17, and (K) trisomy X. Patients with specific ACAs are compared with patients with other ACAs.

Table 2. Multivariable analyses of EFS, CIR, and OS in childhood *KMT2A*-r AML

	pEFS				pCIR				pOS			
	No.	HR	95% CI	*P* value	No.	HR	95% CI	*P* value	No.	HR	95% CI	*P* value
Age at diagnosis, y												
≤10	837	1.0			751	1.0			845	1.0		
>10	277	1.3	1.0-1.5	.02	262	1.3	1.0-1.6	.03	279	1.8	1.4-2.2	**<.01**
WBC count, ×10⁹/L												
≤100	865	1.0			NA				872	1.0		
>100	249	1.3	1.1-1.6	**<.01**	NA	NA	NA	NA	252	1.5	1.2-1.8	**<.01**
Fusion-based group												
9p22/*KMT2A*::*MLLT3*	479	1.0			440	1.0			484	1.0		
Xq24/*KMT2A*::*SEPT6*	21	0.5	0.2-1.1	.08	21	0.5	0.2-1.3	.14	21	0.3	0.1-1.4	.13
1p32/*KMT2A*::*EPS15*	12	0.5	0.2-1.5	.22	10	0.2	0.0-1.6	.13	13	0.3	0.0-1.9	.19
17q21	10	0.6	0.2-1.8	.40	10	0.2	0.0-1.5	.12	10	0.9	0.3-2.6	.84
17q12	10	0.9	0.4-2.5	.90	9	1.0	0.3-3.3	.94	10	1.3	0.5-3.6	.61
1q21/*KMT2A*::*MLLT11*	26	1.1	0.6-2.0	.78	22	0.7	0.3-1.6	.40	26	1.1	0.5-2.4	.76
19p13.1/*KMT2A*::*ELL*	67	1.3	0.9-1.9	.13	58	1.3	0.8-2.0	.24	67	1.1	0.7-1.8	.65
19p13	14	1.1	0.5-2.3	.85	19	1.3	0.6-2.6	.51	14	1.4	0.6-3.3	.38
19p13.3/*KMT2A*::*MLLT1*	52	1.6	1.1-2.3	.01	49	1.8	1.2-2.8	**<.01**	52	1.5	0.9-2.3	.10
10p12/*KMT2A*::*MLLT10*	198	1.7	1.3-2.1	**<.01**	178	1.7	1.3-2.2	**<.01**	200	1.8	1.4-2.4	**<.01**
4q21/*KMT2A*::*AFF1*	11	2.7	1.3-5.5	**<.01**	6	1.6	0.5-5.1	.40	11	4.5	2.2-9.4	**<.01**
6q27/*KMT2A*::*AFDN*	81	1.8	1.3-2.4	**<.01**	74	2.0	1.4-2.8	**<.01**	82	1.8	1.3-2.5	**<.01**
10p11.2/*KMT2A*::*ABI1*	22	2.6	1.6-4.4	**<.01**	22	3.0	1.7-5.2	**<.01**	22	2.4	1.3-4.5	**<.01**
Other	111	1.3	1.0-1.8	.06	95	1.5	1.1-2.1	.02	112	1.3	0.9-1.9	.11
Recurring ACAs	**Yes/no**				**Yes/no**				**Yes/no**			
Monosomy 10	13/1101	2.0	1.1-3.8	.03	10/1003	2.1	1.0-4.5	.04	13/1111	2.8	1.5-5.5	**<.01**
Trisomy 1	NA	NA	NA	NA	NA	NA	NA	NA	13/1111	3.9	2.0-7.4	**<.01**
Trisomy 4	*	*	*	*	*	*	*	*	*	*	*	*
Trisomy 6	60/1054	1.6	1.1-2.3	.01	47/966	2.0	1.4-3.0	**<.01**	60/1064	1.7	1.1-2.5	.01
Trisomy 8	194/920	0.8	0.6-1.0	.05	172/841	0.7	0.5-1.0	.03	*	*	*	*
Trisomy 12	*	*	*	*	*	*	*	*	*	*	*	*
Trisomy 16	12/1102	2.5	1.3-4.8	**<.01**	9/1004	4.5	2.1-9.5	**<.01**	NA	NA	NA	NA
Trisomy 17	8/1106	2.1	0.9-4.7	.07	*	*	*	*	8/1116	2.2	0.9-5.1	.08
Trisomy X	12/1102	2.2	1.1-4.3	.03	NA	NA	NA	NA	12/1112	2.8	1.3-5.9	**<.01**
Add(12p)	13/1101	2.2	1.2-3.9	**.01**	*	*	*	*	NA	NA	NA	NA
Del(9q)	16/1098	2.0	1.1-3.5	.03	14/999	2.2	1.1-4.5	.02	16/1108	2.8	1.5-5.1	**<.01**

Values in boldface indicate statistical significance. ACA type was excluded from multivariable analyses, as the specific ACAs and the ACA type variables are related. 9p22/*KMT2A*::*MLLT3* refers to t(9;11)(p22;q23), 10p12/*KMT2A*::*MLLT10* to t(10;11)(p12;q23), 6q27/*KMT2A*::*AFDN* to t(6;11)(q27;q23), 19p13 to t(11;19)(q23;p13) without ascertained subband, 19p13.1/*KMT2A*::*ELL* to t(11;19)(q23;p13.1), 19p13.3/*KMT2A*::*MLLT1* to t(11;19)(q23;p13.3), 1q21/*KMT2A*::*MLLT11* to t(1;11)(q21;q23), 10p11.2/*KMT2A*::*ABI1* to t(10;11)(p11.2;q23), Xq24/*KMT2A*::*SEPT6* to t(X;11)(q24;q23), 17q21 to t(11;17)(q23;q21), 4q21/*KMT2A*::*AFF1* to t(4;11)(q21;q23), 1p32/*KMT2A*::*EPS15* to t(1;11)(p32;q23), and 17q12 to t(11;17)(q23;q12).

NA, not applied (not significant variables in univariable Cox regression analyses, see supplemental Table 5); abbreviations are explained in Table 1.

*Omitted from the final multivariable model of EFS/CIR/OS because of a *P*-value > .10 in stepwise backward elimination.

The outcome of the 1q21/*KMT2A*::*MLLT11* group was not statistically significantly superior compared with that of the 9p22/*KMT2A*::*MLLT3* group, which is in contrast to Balgobind et al[2] but in agreement with Pollard et al.[4] Therefore, we recommend revision of the previous favorable-risk classification of 1q21/*KMT2A*::*MLLT11* to intermediate risk. The underlying biological function of 1q21/*KMT2A*::*MLLT11* remains unclear, although high *MLLT11* expression has been shown to be an independent adverse prognosticator in pediatric AML.[28] Another study showed that *MIR29B* directly regulates *MLLT11* expression in vitro and that

low *MIR29B* expression corresponded to high *MLLT11* expression in patients with AML, resulting in poor survival.[29] In pediatric patients with 1q21/*KMT2A*::*MLLT11* AML, it remains unknown how *MLLT11* expression is regulated.

We defined and clinically characterized Xq24/*KMT2A*::*SEPT6* (n = 22) and 1p32/*KMT2A*::*EPS15* (*n* = 13) as 2 additional, recurring *KMT2A*-r groups with good outcomes, although not statistically significantly superior. Our findings need to be validated in future large cohort studies, which may include more patients with

Table 3. Evolution in risk of fusion-based groups over time and our proposed cytogenetic risk-group stratification of childhood *KMT2A*-r AML

Fusion-based group	Balgobind et al 2009[2]			Pollard et al 2021[4]			van Weelderen et al 2024		
	No.	5-y pEFS (%)	Risk-group	No.	5-y pEFS (%)	Risk-group	No.	5-y pEFS (%)	Risk-group
1q21/*KMT2A::MLLT11*	24	92	Favorable*	5	60	Intermediate	27	55	Intermediate
9p22/*KMT2A::MLLT3*	321	50	Intermediate	82	49	Intermediate	512	54	Intermediate
Non–FAB-M5	59	31	Adverse	ND	ND	ND	81	38	Adverse
FAB-M5	254	59	Intermediate†	ND	ND	ND	298	66	Intermediate†
FAB-M5/no trisomy 6	ND	ND	ND	ND	ND	ND	273	68	Intermediate
FAB-M5/trisomy 6	ND	ND	ND	ND	ND	ND	11	29‡	Adverse§
Non–FAB-M5/no trisomy 6	ND	ND	ND	ND	ND	ND	66	39	Adverse§
Non–FAB-M5/trisomy 6	ND	ND	ND	ND	ND	ND	10	27	Adverse§
19p13	31	49	Intermediate	ND	ND	ND	20	42	Intermediate
19p13.1/*KMT2A::ELL*	33	46	Intermediate	15	65	Intermediate	71	45	Intermediate
19p13.3/*KMT2A::MLLT1*	23	46	Intermediate	7	14	Adverse‖	55	35	Adverse*
17q21	12	42	Intermediate	ND	ND	ND	12	67	Intermediate
10p12/*KMT2A::MLLT10*	97	31	Adverse*	40	20	Adverse‖	212	33	Adverse*
4q21/*KMT2A::AFF1*	13	29	Adverse	2	0	Adverse‖	12	25	Adverse*
10p11.2/*KMT2A::ABI1*	12	17	Adverse*	6	17	Adverse‖	24	22	Adverse*
6q27/*KMT2A::AFDN*	35	11	Adverse*	15	15	Adverse‖	89	23	Adverse*
Xq24/*KMT2A::SEPT6*	ND	ND	ND	5	80	Intermediate	21	76	Intermediate
1p32/*KMT2A::EPS15*	ND	ND	ND	ND	ND	ND	12	75	Intermediate
17q12	ND	ND	ND	ND	ND	ND	10	56	Intermediate

Risk-group assignment was determined arbitrarily according to the EFS rate. Fusion-based groups with an EFS rate of <40% were classified as adverse-risk, whereas those with an EFS rate >40% were designated at intermediate risk. Furthermore, fusion-based groups with an EFS rate >75%, which demonstrated an independent association with superior EFS compared with patients with 9p22/*KMT2A::MLLT3*, were considered favorable risk. 9p22/*KMT2A::MLLT3* refers to t(9;11)(p22;q23), 10p12/*KMT2A::MLLT10* to t(10;11)(p12;q23), 6q27/*KMT2A::AFDN* to t(6;11)(q27;q23), 19p13.1/*KMT2A::ELL* to t(11;19)(q23;p13.1), 19p13.3/*KMT2A::MLLT1* to t(11;19)(q23;p13.3), 1q21/*KMT2A::MLLT11* to t(1;11)(q21;q23), 10p11.2/*KMT2A::ABI1* to t(10;11)(p11.2;q23), 19p13 to t(11;19)(q23;p13) without ascertained subband, Xq24/*KMT2A::SEPT6* to t(X;11)(q24;q23), 17q21 to t(11;17)(q23;q21), 1p32/*KMT2A::EPS15* to t(1;11)(p32;q23), 4q21/*KMT2A::AFF1* to t(4;11)(q21;q23), and 17q12 to t(11;17)(q23;q12).

*Independently associated with superior/inferior EFS compared with patients with 9p22/*KMT2A::MLLT3*.

†Independently associated with superior EFS compared with patients with 9p22/*KMT2A::MLLT3* with non–FAB-M5 morphology.

‡For patients with FAB-M5 morphology with trisomy 6, the 3-year pEFS is shown because this estimate could not be extrapolated to 5 years.

§Independently associated with inferior EFS compared with patients with 9p22/*KMT2A::MLLT3* with FAB-M5 morphology and without trisomy 6.

‖In the study by Pollard et al,[4] these 5 fusion-based groups were clustered into a high-risk cohort, which was independently associated with inferior EFS compared with the non–high-risk cohort.

---

these fusions as screening methods have improved over the years. For now, it seems justified to consider these fusions as intermediate risk.

Our study confirms the independent favorable prognostic significance of FAB-M5 in the most common *KMT2A*-r group, 9p22/*KMT2A::MLLT3*, which is a notable repeated finding.[2,5] It remains to be determined how FAB-M5 is associated with favorable outcomes in these children. Potentially, high sensitivity to chemotherapy,[30] or overexpression of specific genes, such as *IGSF4*, may be related to differences in outcome. *IGSF4* has been identified as a discriminative, epigenetically, upregulated gene in children with 9p22/*KMT2A::MLLT3* AML with FAB-M5 morphology.[31] We propose to consider patients who are non–FAB-M5 as adverse risk, as their EFS rate was <40% (supplemental Table 7). Although the use of FAB morphology has dwindled in clinical pediatric AML practice, the determination of FAB-type in these patients thus remains relevant. Furthermore, although the number of cases in specific subgroups may be limited, we propose to consider children with 9p22/*KMT2A::MLLT3* AML with FAB-M5/no trisomy 6 as intermediate risk and those with FAB-M5/trisomy 6, non–FAB-M5/no trisomy 6, or non–FAB-M5/trisomy 6 as adverse risk, as

their EFS rates were <40% and on par with those of adverse-risk *KMT2A* fusions (supplemental Table 7).

Compared with Coenen et al (supplemental Table 9),[5] our study identified different recurring ACAs to have a prognostic impact. This may be explained by the larger number of patients, allowing us to identify ACAs of greater independent prognostic significance, conduction of our study over a different period with the application of different treatment protocols, the co-occurrence of ACAs, or concurrent gene mutation profiles. Trisomy 8 has been previously reported to be an independent prognosticator for improved survival among children with *KMT2A*-r AML.[5] In our study, trisomy 8 was significantly associated with a superior outcome in univariable analyses only. Regarding structural ACAs in general, they have been previously reported as independent adverse prognostic indicators of EFS in childhood *KMT2A*-r AML.[5] To our knowledge, we are the first to specifically identify add(12p) and del(9q) to be independently associated with inferior EFS and OS, respectively. Abnormalities of 12p have also been associated with an adverse outcome in pediatric AML in general,[32-34] and del(9q) has been previously reported to be associated with lower CR rates among children with t(8;21)(q22;q22)/*RUNX1::RUNX1T1* AML.[35]

Regarding numerical ACAs, an independent association of trisomy 6 with higher CIR was found in our study, whereas in the study of Coenen et al,[5] it was associated with inferior OS in univariable analysis only. Furthermore, independent associations of monosomy 10 and trisomies 1, 16, and X with inferior outcomes were found, whereas the former 3 ACAs had not been previously described as recurring ACAs in pediatric AML. To date, biological indicators of the prognostic relevance of the ACAs identified in our study remain to be elucidated.

Limitations of our study include its retrospective nature, the diverse treatment regimens used across SGs/countries, although with similar chemotherapeutic backbones, and no inclusion of data on allo-SCT in CR1 and flow-MRD response at EOI2 in the entire cohort analysis. Inclusion of the latter was precluded because subgroups became too small because of the large number of KMT2A-r groups and the low number of patients with specific ACAs, as well as the overall low transplantation rate and lack of flow-MRD data, which was also discussed in our previous study analyzing the impact of flow-MRD and use of allo-SCT in CR1 on outcome in this disease.[3] Furthermore, 10% of the cohort were assigned to the KMT2A-other group, and molecular genetic data were not available to analyze the mutational landscape. With the increasing use of flow-MRD assays, quantitative polymerase chain reaction, and next-generation sequencing, future studies will likely allow for accurate detection of MRD in all patients and identification of all (cryptic) fusion genes, as well as gene mutations, which may affect the prognosis of this disease.

In conclusion, from this study, we can propose an optimized cytogenetic risk-group stratification of KMT2A-r pediatric AML in the pre-menin inhibitor era. Table 3 highlights how the risk of fusion-based groups has evolved over time and shows our proposal. Children with KMT2A-r AML may be stratified into an intermediate-risk or adverse-risk group based on cytogenetics, or both cytogenetics and FAB morphology in 9p22/KMT2A::MLLT3 cases. Regarding the 3 additional, recurring KMT2A-r groups and newly identified ACAs, future studies should validate the associations found in this study. In addition, once a large cohort becomes available with more flow-MRD data, it would be of great interest to evaluate whether individual KMT2A-r groups and ACAs retain their independent prognostic value if flow-MRD response is considered in multivariable analyses. Moreover, future studies should validate the role of allo-SCT in CR1, especially among the adverse-risk KMT2A-r groups, elucidate the mutational landscape, and further unravel the underlying biological pathogenesis of KMT2A fusions and ACAs. Together with ongoing discoveries and encouraging results from new targeted therapeutics, it will likely lead to improved risk-group stratification and risk-adapted treatment, as well as enhanced survival of childhood KMT2A-r AML.

Acknowledgments

Authorship

ORCID profiles: R.E.v.W., 0000-0001-8485-5126; B.D.M., 0000-0002-2449-539X; A.G., 0000-0003-1513-2893; B.F.G., 0000-0003-4030-3228; E.G., 0000-0003-2482-5608; H.H., 0000-0003-3976-9231; H.L., 0000-0002-6900-8313; T.M., 0000-0002-3226-556X; U.N.-N., 0000-0001-5606-5442; S.P., 0000-0001-9716-9264; M.R., 0000-0002-7687-3655; J.E.R., 0000-0001-9885-3527; J.S., 0000-0002-6818-7743; A.T., 0000-0002-5350-8313; D.T., 0000-0003-1520-7007; G.J.L.K., 0000-0001-7716-8475.

Correspondence: Gertjan J. L. Kaspers, Academy & Outreach, Princess Máxima Center for Pediatric Oncology, Heidelberglaan 25, 3584 CS, Utrecht, The Netherlands; email: g.j.l.kaspers@prinsesmaximacentrum.nl.

References

1. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, et al. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. *Blood*. 2012;120(16):3187-3205.

2. Balgobind BV, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009;114(12):2489-2496.

3. van Weelderen RE, Klein K, Harrison CJ, et al. Measurable residual disease and fusion partner independently predict survival and relapse risk in childhood KMT2A-rearranged acute myeloid leukemia: a study by the International Berlin-Frankfurt-Munster Study Group. *J Clin Oncol*. 2023;41(16):2963-2974.

4. Pollard JA, Guest E, Alonzo TA, et al. Gemtuzumab ozogamicin improves event-free survival and reduces relapse in pediatric KMT2A-rearranged AML: results from the phase III Children's Oncology Group Trial AAML0531. *J Clin Oncol*. 2021;39(28):3149-3160.

5. Coenen EA, Raimondi SC, Harbott J, et al. Prognostic significance of additional cytogenetic aberrations in 733 de novo pediatric 11q23/MLL-rearranged AML patients: results of an international study. *Blood*. 2011;117(26):7102-7111.

6. Cooper TM, Ries RE, Alonzo TA, et al. Revised risk stratification criteria for children with newly diagnosed acute myeloid leukemia: a report from the Children's Oncology Group. *Blood*. 2017;130(suppl 1):407.

7. Lamble AJ, Tasian SK. Opportunities for immunotherapy in childhood acute myeloid leukemia. *Blood Adv*. 2019;3(22):3750-3758.

8. Klein K, de Haas V, Kaspers GJL. Clinical challenges in de novo pediatric acute myeloid leukemia. *Expert Rev Anticancer Ther*. 2018;18(3):277-293.

9. Rubnitz JE, Kaspers GJL. How I treat pediatric acute myeloid leukemia. *Blood*. 2021;138(12):1009-1018.

10. Rubnitz JE, Inaba H, Dahl G, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *Lancet Oncol*. 2010;11(6):543-552.

11. Rubnitz JE, Lacayo NJ, Inaba H, et al. Clofarabine can replace anthracyclines and etoposide in remission induction therapy for childhood acute myeloid leukemia: the AML08 multicenter, randomized phase III trial. *J Clin Oncol*. 2019;37(23):2072-2081.

12. Tierens A, Bjørklund E, Siitonen S, et al. Residual disease detected by flow cytometry is an independent predictor of survival in childhood acute myeloid leukaemia; results of the NOPHO-AML 2004 study. *Br J Haematol*. 2016;174(4):600-609.

13. Pession A, Masetti R, Rizzari C, et al. Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. *Blood*. 2013;122(2):170-178.

14. Creutzig U, Zimmermann M, Bourquin JP, et al. Randomized trial comparing liposomal daunorubicin with idarubicin as induction for pediatric acute myeloid leukemia: results from study AML-BFM 2004. *Blood*. 2013;122(1):37-43.

15. De Moerloose B, Reedijk A, de Bock GH, et al. Response-guided chemotherapy for pediatric acute myeloid leukemia without hematopoietic stem cell transplantation in first complete remission: results from protocol DB AML-01. *Pediatr Blood Cancer*. 2019;66(5):e27605.

16. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol*. 2014;32(27):3021-3032.

17. Aplenc R, Meshinchi S, Sung L, et al. Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children's Oncology Group. *Haematologica*. 2020;105(7):1879-1886.

18. Creutzig U, Zimmermann M, Lehrnbecher T, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. *J Clin Oncol*. 2006;24(27):4499-4506.

19. Petit A, Ducassou S, Leblanc T, et al. Maintenance therapy with interleukin-2 for childhood AML: results of ELAM02 phase III randomized trial. *Hemasphere*. 2018;2(6):e159.

20. van der Velden VH, van der Sluijs-Geling A, Gibson BE, et al. Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. *Leukemia*. 2010;24(9):1599-1606.

21. Waack K, Schneider M, Walter C, et al. Improved outcome in pediatric AML - the AML-BFM 2012 study. *Blood*. 2020;136(suppl 1):12-14.

22. Tomizawa D, Tawa A, Watanabe T, et al. Excess treatment reduction including anthracyclines results in higher incidence of relapse in core binding factor acute myeloid leukemia in children. *Leukemia*. 2013;27(12):2413-2416.

23. Burnett AK, Russell NH, Hills RK, et al. Optimization of chemotherapy for younger patients with acute myeloid leukemia: results of the medical research council AML15 trial. *J Clin Oncol*. 2013;31(27):3360-3368.

24. McGowan-Jordan J, Hastings RJ, Moore S. *ISCN 2020: An International System for human Cytogenomic Nomenclature*. Karger; 2020.

25. Issa GC, Aldoss I, DiPersio J, et al. The menin inhibitor revumenib in KMT2A-rearranged or NPM1-mutant leukaemia. *Nature*. 2023;615(7954):920-924.

26. Erba HP, Fathi AT, Issa GC, et al. Update on a phase 1/2 first-in-human study of the menin-KMT2A (MLL) inhibitor ziftomenib (KO-539) in patients with relapsed or refractory acute myeloid leukemia. *Blood*. 2022;140(suppl 1):153-156.

27. Perner F, Stein EM, Wenge DV, et al. MEN1 mutations mediate clinical resistance to menin inhibition. *Nature*. 2023;615(7954):913-919.

28. Tse W, Meshinchi S, Alonzo TA, et al. Elevated expression of the AF1q gene, an MLL fusion partner, is an independent adverse prognostic factor in pediatric acute myeloid leukemia. *Blood*. 2004;104(10):3058-3063.

29. Xiong Y, Li Z, Ji M, et al. MIR29B regulates expression of MLLT11 (AF1Q), an MLL fusion partner, and low MIR29B expression associates with adverse cytogenetics and poor overall survival in AML. *Br J Haematol*. 2011;153(6):753-757.

30. Zwaan CM, Kaspers GJ, Pieters R, et al. Cellular drug resistance profiles in childhood acute myeloid leukemia: differences between FAB types and comparison with acute lymphoblastic leukemia. *Blood*. 2000;96(8):2879-2886.

31. Kuipers JE, Coenen EA, Balgobind BV, et al. High IGSF4 expression in pediatric M5 acute myeloid leukemia with t(9;11)(p22;q23). *Blood*. 2011;117(3):928-935.

32. Harrison CJ, Hills RK, Moorman AV, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *J Clin Oncol*. 2010;28(16):2674-2681.

33. Creutzig U, Zimmermann M, Reinhardt D, et al. Changes in cytogenetics and molecular genetics in acute myeloid leukemia from childhood to adult age groups. *Cancer*. 2016;122(24):3821-3830.

34. Quessada J, Cuccuini W, Saultier P, Loosveld M, Harrison CJ, Lafage-Pochitaloff M. Cytogenetics of pediatric acute myeloid leukemia: a review of the current knowledge. *Genes (Basel)*. 2021;12(6):924.

35. Klein K, Kaspers G, Harrison CJ, et al. 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-Munster Study Group. *J Clin Oncol*. 2015;33(36):4247-4258.