Age-specific mutation profiles and their prognostic implications in pediatric *KMT2A*-rearranged acute myeloid leukemia

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Abstract

Driver mutations in *KMT2A*-rearranged (*KMT2A*-r) have been identified in acute myeloid leukemia (AML); however, age-related differences in their frequency and prognostic factors remain unclear. In this study, we report age-specific mutation profiles and outcomes in pediatric patients with *KMT2A*-r AML. In 239 cases of *KMT2A*-r AML, infants (<1 year, N= 59) showed a significantly higher event-free survival (EFS) and overall survival (OS) compared with children (≥1 year, N=180). Conversely, in 538 cases of non-*KMT2A*-r AML, infants exhibited a significantly lower EFS and OS than children. *KMT2A::MLLT4* was only detected in children with *KMT2A*-r AML and was associated with a poor prognosis. In *KMT2A*-r AML, mutations in signaling pathway genes, such as *KRAS*, were frequently detected in infants and children. However, the frequency of non-signaling pathway mutations was significantly higher in children. Moreover, non-signaling pathway mutations had no significant effect on the prognosis in infants and children, whereas *KRAS* mutations were associated with poor prognosis

in both groups. Multivariate analysis identified older age, a high white blood cell count, *KMT2A::MLLT4*, and *KRAS* mutations as independent adverse prognostic factors for both EFS and OS. These age-specific mutation profiles suggest distinct disease mechanisms across age groups and may help to refine risk stratification and treatment strategies for pediatric *KMT2A*-r AML.

Introduction

Acute myeloid leukemia (AML) is a relatively rare but aggressive pediatric leukemia characterized by the clonal expansion of immature myeloid precursors.[1] The genetic and molecular features of AML have been revealed by advances in next-generation sequencing technology.[2,3] The accumulation of data on patients' clinical outcomes associated with genetic abnormalities has enabled risk stratification and appropriate treatment of AML.[4,5] However, relapses remain common, and patients with relapsed AML have a poor prognosis.[6] Therefore, further research is required to understand the detailed mechanisms of AML and provide better risk stratification.

One of the hallmark genetic abnormalities in AML is *KMT2A* rearrangement, in which chromosomal translocations occur between the *KMT2A* gene (located at 11q23, formerly referred to as *MLL*) and various partner genes.[7] More than 100 partner genes associated with *KMT2A* rearrangements have been identified, and the prognosis varies depending on the specific rearrangement pattern.[7-9] For example, t(9;11)(p22;q23)/*KMT2A::MLLT3*, the most common fusion pattern, is associated with intermediate risk, whereas t(6;11)(q27;q23)/*KMT2A::MLLT4* is associated with high risk. Therefore, some of the fusion patterns are currently used for the risk stratification of AML treatment.[10-12] *KMT2A*-rearranged (*KMT2A*-r) AML is known to have fewer coexisting mutations compared to other AML subtypes.[2,3] Among the coexisting mutations in *KMT2A*-r AML, RAS pathway genes are frequently mutated.[13-16] Our recent study identified mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene as poor prognostic factors in *KMT2A*-r AML.[17] In particular, *KRAS* codon 12 (G12) mutations were associated with a poorer prognosis when compared with other *KRAS* mutations.[18]

Differences in recurrent chromosomal and genetic abnormalities between adult and pediatric AML have been previously elucidated.[3,19,20] *KMT2A*-r AML is frequently observed in pediatric AML, with a prevalence of approximately 40% in infants (aged <1 year) and 15% in children (aged ≥1 year).[3,21] Several reports have shown that patients with infant AML have a more favorable prognosis compared to patients with childhood AML;[22-24] however, whether the results are applicable to all AML subtypes is unclear, as are the underlying molecular mechanisms. In this study, we focused on pediatric *KMT2A*-r AML and compared the mutation profiles and prognosis between infants and children. As several differences were identified between infants and children in terms of genetic abnormalities and prognosis, this study may lead to better risk stratification and *KMT2A*-r AML treatment outcomes.

Methods

Patients

The AML99, AML-05, and AML-12 studies were nationwide, multicenter clinical trials conducted in Japan by the Japan Children's Cancer Group (JCCG) in children (<18 years) with *de novo* AML. The treatment schedules and regimens have been previously described.[25-27] In this study, we included 11 cases of pediatric *KMT2A*-r AML from the AML99 trial, 58 cases from the AML-05 trial, and 58 cases from the AML-12 trial. In addition, 112 cases of *KMT2A*-r AML and 538 cases of non-*KMT2A*-r AML were sourced from the TARGET cohort, with the clinical data obtained from the TARGET Data Matrix.[3]

In accordance with previous studies,[7,22,23,27,28] infants were defined as <1 year of age, and children were defined as ≥1 year of age. However, as several studies have defined the threshold between infants and children as 3 years of age,[3,24] analyses were also performed with the differentiation established at 3 years of age.

All procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki and were reviewed and approved by the ethics committees of all participating institutions, including the Kyoto University Medical Ethics Committee and the JCCG Research Review Committee (approval numbers: G0361 and 106). Written informed consent was obtained from all patients and/or their parents/guardians.

Targeted sequencing

Targeted sequencing was performed, targeting 338 genes in the *KMT2A*-r AML patients' samples from the AML99 and AML-05 studies. Sample preparation, sequencing, and data analyses were conducted as described previously.[16] AML-12 samples underwent targeted sequencing of 507 genes selected for their relevance to myeloid malignancies and therapeutic potential. Target capture was performed using a SureSelect custom kit (Agilent), and sequencing was conducted on a HiSeq 2000/2500 (Illumina). Detailed information regarding mutation calling is provided in the *Online Supplementary Methods*.

Mutation data for samples from 650 TARGET cohort patients were collected as previously described.[3]

Statistical analysis

In this study, survival analysis was performed using the Kaplan-Meier method, and log-rank tests were performed to compare the survival distributions between groups. Categorical variables were compared using the Fisher exact test. The Mann-Whitney U test was applied to compare the data distributions between two independent groups. In addition, multivariate analysis was carried out on patients with *KMT2A*-r AML. All statistical analyses were performed using R software with $P<0.05$ considered statistically significant.

Results

Age distribution and prognosis of *KMT2A*-rearranged and non-*KMT2A*-rearranged acute myeloid leukemia

The distribution of the infant and childhood cases of *KMT2A*-r AML and non-*KMT2A*-r AML is illustrated in Figure 1A and B, respectively. The age distribution of pediatric patients with *KMT2A*-r AML showed that, among a total of 239 cases, 59 patients (24.7%) were infants and 180 (75.3%) were children. In contrast, among the 538 cases of pediatric AML without *KMT2A* rearrangements, 13 patients (2.4%) were infants and 525 (97.6%) were children. The proportion of infant cases was significantly higher in *KMT2A*-r AML compared to non-*KMT2A*-r AML ($P<0.001$). Furthermore, the patients were categorized into groups of either infants or children, and Kaplan-Meier curves for event-free survival (EFS) and overall survival (OS) were generated and compared (Figure 1C, D). In *KMT2A*-r AML, infant cases showed a significantly better prognosis compared to that of children (EFS: $P=0.049$, OS: $P=0.032$). In contrast, in non-*KMT2A*-r AML, infant cases exhibited a significantly worse prognosis compared to that of children (EFS: $P<0.001$, OS: $P=0.015$).

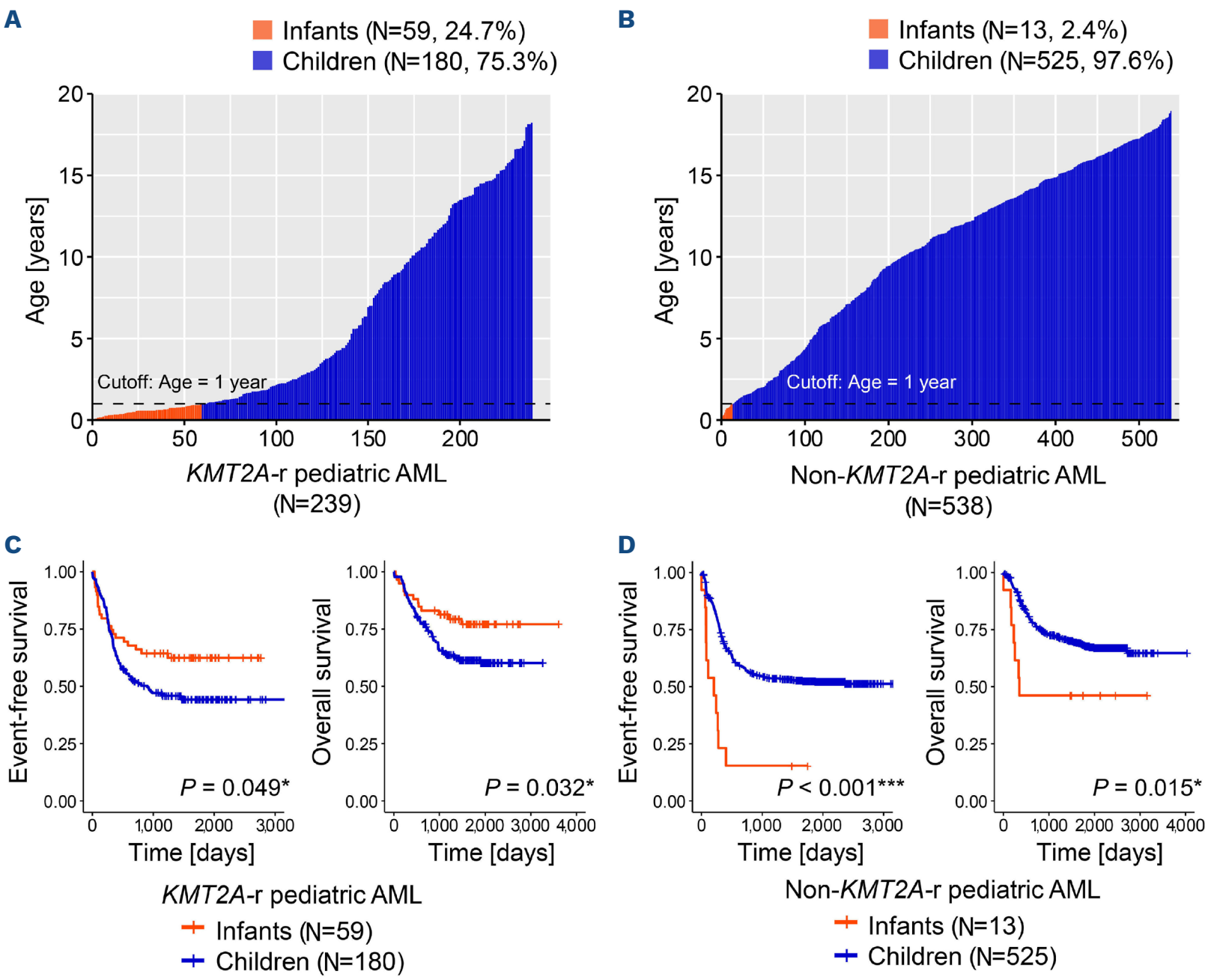

Figure 1. Comparison of *KMT2A*-rearranged and non-*KMT2A*-rearranged acute myeloid leukemia cases. (A, B) Age distribution of *KMT2A*-rearranged acute myeloid leukemia (*KMT2A*-r AML) (N=239) and non-*KMT2A*-r AML cases (N=538). Infants are defined as <1 year old, and children are defined as ≥1 year old. (C) Event-free survival (EFS) and overall survival (OS) in *KMT2A*-r AML based on the age group (infants and children). (D) EFS and OS in non-*KMT2A*-r AML based on age group (infants and children). *$P<0.05$; ***$P<0.001$

We also performed the same analysis by categorizing patients into age groups using a threshold of 3 years (*Online Supplementary Figure S1A-D*). The proportion of patients under the age of 3 years was significantly higher in the *KMT2A*-r AML group than in the non-*KMT2A*-r AML group (*P*<0.001). Among *KMT2A*-r AML patients, those younger than 3 years had a significantly better OS (*P*=0.0032), although the EFS difference was not statistically significant (*P*=0.062). In contrast, the prognostic difference nearly disappeared in the non-*KMT2A*-r AML group (EFS: *P*=0.67, OS: *P*=0.96). We also conducted a similar analysis using 0.5 years as the cutoff (*Online Supplementary Figure S2A-D*). Patients younger than 0.5 years were significantly more common in the *KMT2A*-r AML group than in the non-*KMT2A*-r AML group (*P*<0.001). When stratified into three age groups (<0.5 years, 0.5 to <1 year, and ≥1 year), no significant differences in EFS or OS were observed between the <0.5 years and 0.5 to <1-year groups for *KMT2A*-r AML (EFS: *P*=0.057, OS: *P*=0.39) or non-*KMT2A*-r AML (EFS: *P*=0.94, OS: *P*=0.92). These findings indicate that the most notable prognostic differences occur when patients are stratified using 1 year of age as the cutoff. In this study, infants accounted for a larger proportion of *KMT2A*-r AML cases, and the prognostic impact of age differed significantly between *KMT2A*-r and non-*KMT2A*-r AML.

Differences in patients' characteristics between infants and children

To integrate patients' characteristics across cohorts, we summarized the clinical features of the AML99, AML-05, AML-12, and TARGET AML cohorts (*Online Supplementary Table S1*). No major differences in baseline characteristics were observed among the cohorts, and the proportions of infants and children were nearly identical. Prognostic comparisons within each cohort consistently demonstrated better outcomes in infants than in children (*Online Supplementary Figure S3A-E*). These findings indicated that there were no substantial biases in patients' characteristics or prognoses between infants and children across cohorts, and that the influence of treatment protocols on prognosis appeared limited. Therefore, subsequent analyses were conducted using the integrated cohort.

The characteristics of infants and children with *KMT2A*-r AML and non-*KMT2A*-r AML at diagnosis are summarized in *Online Supplementary Tables S2* and *S3*. In *KMT2A*-r AML, there were no significant differences between infants and children with regard to gender, white blood cell (WBC) count, or French-American-British (FAB) classification (*Online Supplementary Table S2*). To examine the relationship between age and *KMT2A* rearrangement subtypes, we assessed both the age distribution for each subtype and their frequencies by age group (infants *vs*. children) (Figure 2A, B; *Online Supplementary Table S2*). *KMT2A::MLLT4* was associated with a higher age distribution, whereas other minor *KMT2A* fusion subtypes were predominantly observed in younger patients (Figure 2A). When stratified by age, both *KMT2A::MLLT3* and *KMT2A::MLLT4* were significantly more frequent in children than in infants (*P*=0.031 and *P*=0.0085, respectively) (Figure 2B, *Online Supplementary Table S2*), while *KMT2A::MLLT10*, *KMT2A::ELL*, and *KMT2A::MLLT1* showed no significant age-related differences. In contrast, other *KMT2A* fusions were significantly more common in infants than in children (*P*<0.001).

In non-*KMT2A*-r AML, there was no significant difference between infants and children in terms of gender (*Online Supplementary Table S3*). The WBC count at diagnosis was significantly higher in infants than in children (*P*=0.019). Likewise, the percentage of FAB M0 was significantly higher in infants than in children (*P*=0.0035), whereas the percentage of FAB M2 was significantly higher in children than in infants (*P*=0.024). To investigate the mechanism of underlying poor prognosis in infant non-*KMT2A*-r AML, we further characterized these 13 cases (*Online Supplementary Figure S4*). Among them, four harbored the *CBFB::MYH11* fusion, and all survived. In contrast, among the nine cases without *CBFB::MYH11*, seven deaths occurred. One case each harbored *KAT6A::EP300* and *CBFA2T3::GLIS2* fusions. Fatal cases also showed mutations unrelated to activated signaling pathways, such as *WT1* mutations, and chromosomal abnormalities, including del(7q).

These results suggest that there are significant differences in the characteristics of patients between infants and children in both *KMT2A*-r AML and non-*KMT2A*-r AML. The most notable difference was the absence of *KMT2A::MLLT4* rearrangements in infant *KMT2A*-r AML cases.

Genetic mutation patterns in infants and children

The genetic mutation landscape of infants and children with *KMT2A*-r AML is depicted in Figure 3A. To investigate the distribution of genetic variants, genes were categorized into the following pathways: activated signaling pathway (*FLT3, KRAS, NRAS, PTPN11, CBL,* and *BRAF*), epigenetic regulators (*SETD2, ASXL1, ASXL2, BCOR, CREBBP, EP300,* and *KDM6A*), transcription factors (*WT1, SPI1, GATA2, and RUNX1*), cohesion complex (*STAG2* and *SMC3*), and other pathways (*CCND3, U2AF1, TET2,* and trisomy 8). A comparison of the number of mutated genes between infants and children revealed that the average number of mutated genes was 0.92 (range, 0-3) in infants and 1.36 (range, 0-7) in children, with significantly more mutations identified in children (*P*=0.0061) (*Online Supplementary Figure S5*). The number of patients with at least one mutation was counted and plotted in each pathway, relative to the total number of patients (Figure 3B, C). Mutations in the activated signaling pathway were common in both infants and children, with no significant difference. In contrast, non-signaling pathway mutations were significantly more frequent in children than in infants (infants: 15.3% *vs*. children: 43.9%, *P*<0.001) (Figure 3B). Among non-signaling mutations, a significant difference was observed in epigenetic regulation (infants:

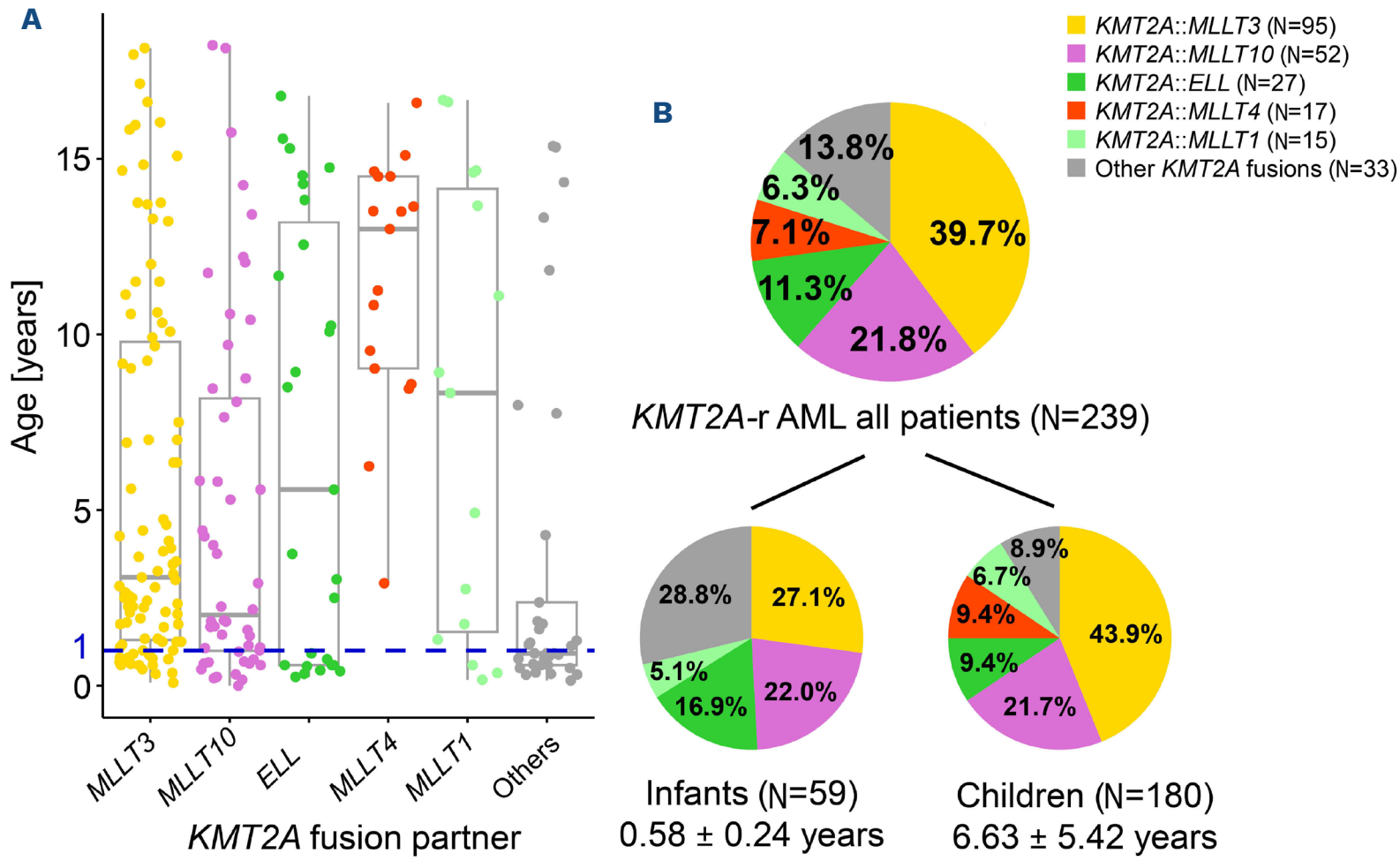

Figure 2. Comparison of *KMT2A* rearrangement patterns in infants and children with *KMT2A*-rearranged acute myeloid leukemia. (A) Scatter and box plots showing the age distribution for each *KMT2A* rearrangement pattern. (B) Pie charts showing the proportions of *KMT2A* rearrangement patterns in infants, children, and the combined cohort, along with mean age and standard deviation. The legend is shared across all panels. *KMT2A*-r AML: *KMT2A*-rearranged acute myeloid leukemia.

3.4% *vs*. children: 17.8%, *P*=0.0046). While no significant differences were found in transcription factors, cohesin complex, or other pathways, these mutations were frequent in children. At the individual gene level, *NRAS* mutations were more frequent in infants than in children (infants: 27.1% *vs*. children: 16.1%), although the difference was not statistically significant (*P*=0.083). In contrast, *SETD2* mutations were significantly more common in children than in infants (infants: 0% *vs*. children: 11.1%, *P*=0.005) (Figure 3A). Similarly, a comparison was made for non-KMT2A-r cases categorized by pathways, but no significant differences were observed (*Online Supplementary Figure 6A, B*). These results suggest that there are significant differences in mutation patterns between infants and children in *KMT2A*-r AML, with the frequency of non-signaling mutations being significantly higher in children with *KMT2A*-r AML.

Prognostic impact of *KMT2A* fusion subtypes and concurrent non-signaling mutations in *KMT2A*-rearranged acute myeloid leukemia

KMT2A rearrangement patterns are known prognostic indicators. In our cohort, differences in prognosis were observed among the various *KMT2A* rearrangement subtypes (*Online Supplementary Figure S7*). Notably, the *KMT2A::MLLT4* subtype was associated with a particularly poor prognosis. Based on the observed differences in genetic abnormalities between infants and children, we hypothesized that *KMT2A::MLLT4* and non-signaling mutations contribute to the poor prognosis of children with *KMT2A*-r AML compared with that of infants. First, we divided the patients into two groups based on the presence or absence of *KMT2A::MLLT4*, which was exclusively detected in cases of children with AML, and compared their prognosis (Figure 4A). We found that children with *KMT2A::MLLT4* had a significantly poorer prognosis than children without *KMT2A::MLLT4* (EFS: *P*<0.001, OS: *P*<0.001). When the prognosis of children without *KMT2A::MLLT4* was compared with that of infants, no significant differences were detected (EFS: *P*=0.14, OS: *P*=0.10). Next, we examined the prognostic impact of non-signaling mutations; however, no significant differences were detected in the prognosis between patients with and without non-signaling mutations (Figure 4B). These results suggest that *KMT2A::MLLT4* is associated with a poor prognosis in childhood *KMT2A*-r AML, while non-signaling mutations have no prognostic impact.

We then evaluated prognosis in groups of patients stratified by age – infants (<1 year) and children (≥1 year) – for each *KMT2A* rearrangement subtype (*Online Supplementary Figure S8A-E*). In the more common subtypes, *KMT2A::MLLT3* and *KMT2A::MLLT10*, infants had a better prognosis than children, although the differences were not statistically significant. We also conducted similar analyses dividing

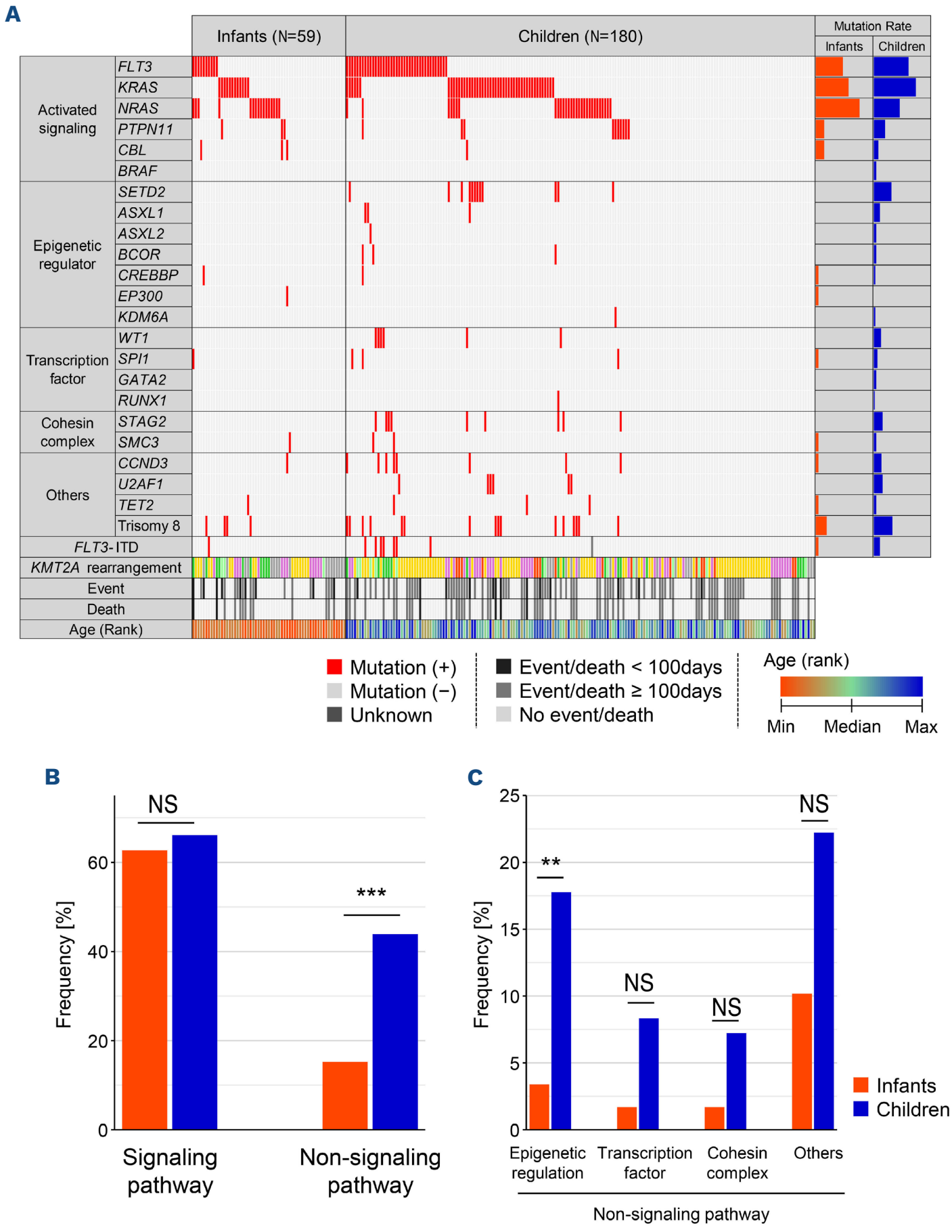

Figure 3. Comparison of gene mutations in infants and children with *KMT2A*-rearranged acute myeloid leukemia. (A) Gene mutation landscape, *KMT2A* rearrangement patterns, age, event status, and death status in infants and children with acute myeloid leukemia (AML). The cohort (N=239) is ranked by age, with the youngest (rank 1) shown in red, the oldest (rank 239) shown in blue, and the median (rank 120) shown in green. Events and deaths that occurred within 100 days are color-coded differently from those that occurred later. The color legend for *KMT2A* fusion patterns corresponds to Figure 2. The bar graph on the right summarizes the proportions of each category. (B, C) Comparison of the positive rates of gene mutations classified by function between infants and children with *KMT2A*-rearranged AML. ***P*<0.01; ****P*<0.001.

patients into three age groups: <1 year, 1 to <3 years, and ≥3 years (*Online Supplementary Figure S9A-F*). In the most common subtype, *KMT2A::MLLT3*, younger age groups appeared to have better prognosis; however, these differences did not reach statistical significance. In the remaining *KMT2A* fusion subtypes, the prognostic ranking of the age groups varied, and none showed statistically significant differences.

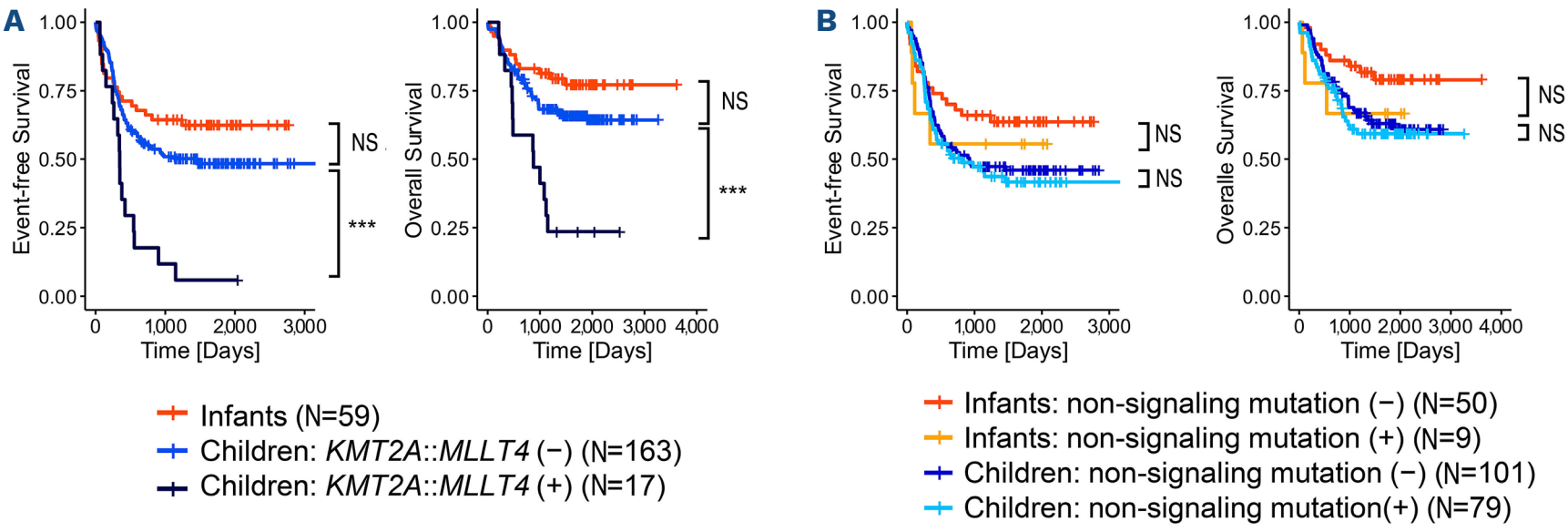

Figure 4. Impact of *KMT2A::MLLT4* and non-signaling mutations on prognosis. (A) Event-free survival (EFS) and overall survival (OS) in *KMT2A*-rearranged acute myeloid leukemia (*KMT2A*-r AML) based on the presence or absence of *KMT2A::MLLT4* and age group (infants and children). (B) EFS and OS in *KMT2A*-r AML based on the presence or absence of non-signaling mutation and age group (infants and children). ***P<0.001.

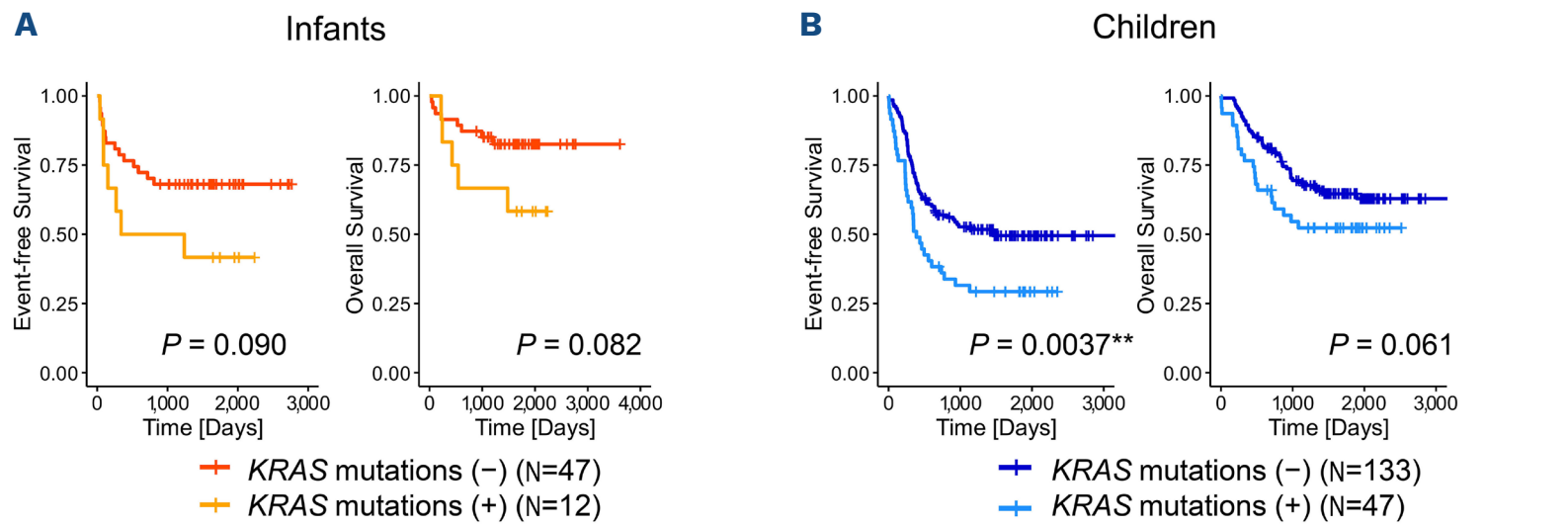

Figure 5. Impact of *KRAS* mutations on prognosis. (A) Event-free survival (EFS) and overall survival (OS) in infants with *KMT2A*-rearranged acute myeloid leukemia (*KMT2A*-r AML) based on the presence or absence of *KRAS* mutations. (B) EFS and OS in children with *KMT2A*-r AML based on the presence or absence of *KRAS* mutations. **P<0.01.

Prognostic impact of *KRAS* mutations in *KMT2A*-rearranged acute myeloid leukemia

In addition, we investigated the prognostic impact of *KRAS* mutations stratified in *KMT2A*-r AML. In infants, 12 patients (20.3%) were *KRAS* mutation-positive, and despite these cases having poorer outcomes, the differences were not statistically significant (EFS: P=0.090, OS: P=0.082) (Figure 5A). Among the children, 47 patients (26.1%) were *KRAS* mutation-positive, and these cases had significantly worse outcomes in terms of EFS but not OS (EFS: P=0.0037, OS: P=0.061) (Figure 4B).

The prognostic impact of *KRAS* mutations according to each codon was also examined because we previously showed that *KRAS* G12 mutations are particularly associated with adverse prognostic factors in pediatric *KMT2A*-r AML[18] (*Online Supplementary Figure S10A-F*). *KRAS* G12 mutations were observed in 3.4% of infants and 9.4% of children, *KRAS* G13 mutations in 10.2% of infants and 10.6% of children, and other *KRAS* mutations in 6.8% of infants and 6.7% of children (*Online Supplementary Table S2*). Children with *KRAS* G12 mutations had significantly poorer outcomes (EFS: P<0.001, OS: P<0.001); however, no significant differences were detected in infants. Infants with *KRAS* G13 mutations also had poor outcomes, but the differences were not statistically significant (EFS: P=0.062, OS: P=0.075). These results suggest that *KRAS* mutations are associated with a poor prognosis in childhood *KMT2A*-r AML.

Multivariate analysis

To comprehensively assess the prognostic impact of each variable, we performed a multivariate analysis including the following factors: age (+1 year), WBC count (+10^4/μL), *KMT2A::MLLT3, KMT2A::MLLT10, KMT2A::ELL, KMT2A::MLLT4, KMT2A::MLLT1*, other *KMT2A* fusions, non-signaling mutations, *KRAS* mutations, and *FLT3*-ITD (Table 1). Both EFS

Table 1. Results of multivariate analysis for event-free survival and overall survival in *KMT2A*-rearranged acute myeloid leukemia.

Characteristic	Event-free survival			Overall survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (+1 year)	1.04	1.00-1.08	0.032*	1.07	1.03-1.12	< 0.001 ***
WBC (+10^4/μL)	1.02	1.00-1.03	0.035*	1.02	1.00-1.04	0.032*
KMT2A::MLLT3	0.77	0.41-1.45	0.42	1.01	0.43-2.38	0.99
KMT2A::MLLT10	1.63	0.85-3.11	0.14	1.92	0.80-4.62	0.14
KMT2A::ELL	1.14	0.53-2.46	0.73	1.80	0.67-4.83	0.24
KMT2A::MLLT4	2.48	1.12-5.46	0.025*	2.85	1.07-7.62	0.037*
KMT2A::MLLT1	1.19	0.48-2.90	0.71	0.78	0.22-2.73	0.70
Other *KMT2A* fusions	1.47	0.82-2.63	0.20	2.64	1.26-5.51	0.0097**
Non-signaling mutations	0.89	0.54-1.48	0.66	0.59	0.30-1.18	0.14
KRAS mutations	1.74	1.17-2.59	0.0062**	1.75	1.07-2.87	0.027*
FLT3-ITD	1.82	0.70-4.76	0.22	2.60	0.85-7.94	0.095

*P<0.05; **P<0.01; ***P<0.001. HR: hazard ratio; 95% CI: 95% confidence interval; WBC: white blood cells; *FLT3*-ITD: *FLT3* internal tandem duplications.

and OS analyses identified age (+1 year), WBC count (+10^4/μL), *KMT2A::MLLT4*, and *KRAS* mutations as independent adverse prognostic factors.

We further subdivided *KRAS* mutations into *KRAS* G12, *KRAS* G13, and other *KRAS* mutations (*Online Supplementary Table S4*). The results were consistent with the overall analysis; however, only *KRAS* G12 mutations emerged as independent adverse prognostic factors.

These findings suggest that in *KMT2A*-r AML, older age, elevated WBC count, *KMT2A::MLLT4* and *KRAS* mutations independently predict poor prognosis.

Discussion

In this study, we examined mutation profiles and their prognostic implications in *KMT2A*-r AML (N=239) and non-*KMT2A*-r AML (N=538) and compared these between infants (<1 year) and children (≥1 year). To our knowledge, this is one of the largest studies to date focusing on age-specific mutation profiles and their prognostic implications in pediatric AML. The proportion of infants was high in the *KMT2A*-r AML group, which is consistent with previous studies.[3,21] Interestingly, infants exhibited a better prognosis in *KMT2A*-r AML and a worse prognosis in non-*KMT2A*-r AML compared with that of children. The present study may be the first to uncover data on the reversal of prognosis between infants and children according to AML subtype.

To explore the reasons for these prognostic differences in *KMT2A*-r AML, we compared *KMT2A* fusion patterns between the two age groups and evaluated their impact on outcomes. *KMT2A::MLLT4* was detected exclusively in children and was associated with poor prognosis. This finding aligns with previous reports showing that *KMT2A::MLLT4* is more frequent in older patients with *KMT2A*-r AML and is linked to inferior survival.[8,9,29] *KMT2A::MLLT3* was also significantly more frequent in children, whereas other minor *KMT2A* fusions predominated in infants. As these age-related differences are rarely reported, validation in independent cohorts and further investigation are warranted.

We also assessed prognostic differences by *KMT2A* fusion subtypes other than *KMT2A::MLLT4*; no significant associations were observed. However, small subgroup sizes limit these analyses, and larger cohorts will be needed. Multivariate analysis confirmed that both older age and *KMT2A::MLLT4* were independent adverse prognostic factors, indicating that the poorer prognosis in children relative to infants is attributable to both. Given prior evidence of age-related differences in gene expression and DNA methylation in AML,[3,30,31] elucidating the age-dependent mechanisms driving poor outcomes in *KMT2A*-r AML may reveal novel therapeutic targets and improve survival.

We also compared genetic abnormalities between infants and children within the *KMT2A*-r AML cohort. Mutations in activated signaling pathways were detected in over 60% of patients in both groups. These mutations were also reported to promote the development of *KMT2A*-r AML in a mouse model,[32] which suggests that signaling mutations are important in the development of *KMT2A*-r AML, regardless of age. In contrast, non-signaling mutations were less frequent in infants (<20%) and more frequent in children (>40%). Generally, hematopoietic cells are known to accumulate mutations with increasing age, and cells from the founding clone can acquire additional cooperative mutations, yielding subclones that can contribute to leukemogenesis.[33] The

higher frequency of non-signaling mutations in children likely reflects this age-related accumulation; however, their prognostic impact appeared limited.

We have previously demonstrated that *KRAS* mutations are adverse prognostic factors in *KMT2A*-r AML;[17,18] therefore, we examined their prognostic significance in this study. Multivariate analysis confirmed *KRAS* mutations as independent adverse prognostic factors for both EFS and OS. Unlike *KMT2A::MLLT4*, which was confined to children, *KRAS* mutations were present in both infants and children, suggesting that *KRAS* may represent a broadly relevant adverse prognostic factor. This finding aligns with recent studies that have also identified *KRAS* mutations as predictors of poor prognosis in *KMT2A*-r AML.[34-37] It would be valuable to determine whether this adverse effect persists across age groups in other cohorts.

In stark contrast, the non-*KMT2A*-r AML cohort showed the opposite age-related trend, with infants exhibiting significantly poorer outcomes than children. Notably, all infant patients harboring *CBFB::MYH11* in this cohort survived, consistent with this translocation's well-established role as a favorable prognostic marker.[11] Thus, infants with *CBFB::MYH11* can be considered to have a favorable prognosis even within cases of non-*KMT2A*-r AML. Fatal infant cases included patients with chromosomal translocations such as *KAT6A::EP300* and *CBFA2T3::GLIS2*, mutations not related to activated signaling pathways such as *WT1*, and chromosomal abnormalities including del(7q), all of which have been associated with adverse outcomes.[38-43] However, some fatal cases lacked identifiable high-risk genetic lesions, highlighting the need for larger cohorts and comprehensive approaches such as multi-omics analyses to clarify the underlying mechanisms.

In conclusion, this study clarified prognostic differences between infants and children with *KMT2A*-r and non-*KMT2A*-r AML in a large cohort, highlighting the prognostic relevance of age, *KMT2A* fusion patterns, and gene mutations, as well as their interrelationships. These findings may advance understanding of subtype-specific AML characteristics, support the development of improved treatment stratification strategies, and help elucidate age-specific pathogenic mechanisms in *KMT2A*-r AML with potential therapeutic implications.

Disclosures

SO holds a leadership position/advisory role with Eisai Co., Ltd. and Chordia Therapeutics Inc.; is a stockholder in Asahi Genomics Co., Ltd.; and has received grants/research funding from Chordia Therapeutics Inc., Otsuka Pharmaceutical Co., Ltd., and Eisai Co., Ltd. The other authors have no conflicts of interest to disclose.

Contributions

KS, KY and HMa analyzed the clinical and sequencing data. SI, MI, MT, MNo, NSa and YShin assisted with the analysis. KY, YN, GY, ST, NSh and YH performed sequencing. YShio, YShir, KC, AO, HT and SM developed the sequence data processing pipelines. YK, HG, KT, EI, NK, DT, TT, HMo, AT, JT and SA collected the clinical samples. KY, MNi, SA, SO and HMa supervised the project. KS, KY and HMa wrote the manuscript.

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Data-sharing statement

The datasets used in this study are available from the corresponding author upon reasonable request.

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