Molecular Profiling Defines Distinct Prognostic Subgroups in Childhood AML: A Report From the French ELAM02 Study Group

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Abstract

Despite major treatment improvements over the past decades, pediatric acute myeloid leukemia (AML) is still a life-threatening malignancy with relapse rates up to 30% and survival rates below 75%. A better description of the pattern of molecular aberrations in childhood AML is needed to refine prognostication in such patients. We report here the comprehensive molecular landscape using both high-throughput sequencing focused on 36 genes and ligation-dependent RT-PCR in 385 children with de novo AML enrolled in the prospective ELAM02 trial and we evaluated their prognostic significance. Seventy-six percent of patients had at least 1 mutation among the genes we screened. The most common class of mutations involved genes that control kinase signaling (61%) followed by transcription factors (16%), tumor suppressors (14%), chromatin modifiers (9%), DNA methylation controllers (8%), cohesin genes (5%),...
and spliceosome (3%). Moreover, a recurrent transcript fusion was detected in about a half of pediatric patients. Overall, CBF rearrangements, NPM1 and double CEBPA mutations represented 37% of the cohort and defined a favorable molecular subgroup (3 years OS: 92.1%) while NUP98 fusions, WT1, RUNX1, and PHF6 mutations (15% of the cohort) segregated into a poor molecular subgroup (3 years OS: 46.1%). KMT2A-rearrangements (21% of the cohort) were associated with an intermediate risk. Despite some overlaps, the spectrum of molecular aberrations and their prognostic significance differ between childhood and adult AML. These data have important implications to contribute in refining risk stratification of pediatric AML and show the need for further validations in independent pediatric cohorts.

**Introduction**

Approximately 20% of childhood acute leukemia is of myeloid origin. Acute myeloid leukemia (AML) is defined as a clonal disorder caused by stepwise accumulation of successive genetic defects. In recent years, the use of genetic data to inform disease classification and clinical practice has been an active field of research. Improvements in identifying such molecular and cytogenetic aberrations have revealed the heterogeneity of this group of diseases. Recurrent mutations and gene fusions have been shown to affect a wide range of genes that have been classified into 8 functional categories: kinase signaling, transcription factors, tumor suppressors, DNA methylation, chromatin modifiers, cohesin, spliceosome, and the NPM1 gene. Consequently, some genetic alterations with major prognostic significance - such as inv(16)(p13;q22)/CBFB–MYH11, t(8;21)(q22;q22)/RUNX1–RUNXIT1, single NPM1 mutations and CEBPA double mutations (CEBPAdm) - have been implemented into the World Health Organization (WHO) classification of AML. Nevertheless, most of the investigations are based on the study of large cohorts of adult AML patients while genetic profiles are known to be quite different between adults and children with AML. Moreover, despite major treatment improvements over the past decades, pediatric AML is still associated with relapse rates up to 30% and survival rates below 75%. In this context, a better description of the pattern of molecular aberrations in childhood AML remains a great challenge to refine prognostication and improve outcome in such patients.

We report here the comprehensive molecular landscape of a large and well-annotated cohort of de novo pediatric AML enrolled in the prospective ELAM02 trial and propose a new prognostic molecular classifier in this particular group of patients.

**Methods**

**Patients**

The present study focuses on 385 patients of the 438 children treated in the ELAM02 trial (Treating Patients with Childhood Acute Myeloid Leukemia with Interleukin-2; ClinicalTrials.gov NCT00149162). Patient selection was based on the availability of genomic DNA at AML diagnosis. Children aged 0 to 18 years with newly diagnosed AML were enrolled between March 2005 and December 2011. Acute promyelocytic leukemia, therapy-related AML and Down syndrome were excluded from the ELAM02 trial. The study was approved by the Ethics Committee of Saint-Antoine Paris University Hospital (Assistance Publique-Hôpitaux de Paris) and by the Institutional Review Board of the French Regulatory Agency and was conducted in accordance with the Declaration of Helsinki.

**Cytogenetic analyses and extensive fusion transcripts detection**

Cytogenetic analyses were locally performed on bone marrow samples using R- or G-banding. Results were centrally reviewed and described in accordance with the International System for Human Cytogenetic Nomenclature. Karyotypes were classified as follows: CBF-rearranged [i.e., inv(16)(p13;q22)/t(16;16)(p13; q22) and t(8;21)(q22;q22)], KMT2A-rearranged, normal karyotype, adverse [i.e., monosomy 7, t(6;9)(p23;q34), inv(3) (q11q26)t(3;3)(q11;q26) and complex karyotype] and other aberrations. A complex karyotype was defined by the presence of 3 or more unrelated chromosome abnormalities. Furthermore, all diagnosis samples were screened for more than 50 recurrent gene rearrangements and KMT2A-partial tandem duplication (KMT2A-PTD) using ligation-dependent RT-PCR amplification assay (LD-RT-PCR) as previously described by Runyon et al.

**Mutational analysis**

Genomic DNA from bone marrow aspirates at diagnostic was studied by high-throughput sequencing (HTS) of 36 genes recurrently mutated in myeloid malignancies. The studied panel included genes encoding proteins involved in kinase signaling [CBL (exons 8–9), FLT3 (exon 20), JAK2 (exons 12, 14, 16), KIT (exons 8–13, 17), KRAS (exons 2–3), MPL (exon 10), NRAS (exons 2–3), PTPN11 (exons 3, 13), SETBP1 (exon 4)], transcription factors [CEBPA (exon 1), ETV6 (exons 1–8), GATA1 (exon 2), GATA2 (exons 2–6), RUNX1 (exons 1–6)], tumor suppressors [PHF6 (exons 2–10), PTEN (exons 5–7), TP53 (exons 2–11), WT1 (exons 7, 9)], chromatin modifiers [ASXL1 (exons 11–12), BCOR (exons 2–15), BCORL1 (exons 1–12), EZH2 (exons 2–20)], DNA methylation [DNMT3A (exons 2–23), IDH1 (exon 4), IDH2 (exon 4), TET2 (exons 3–11)], cohesin complex [NIPBL (exons 2–47), RAD21 (exons 2–14), SMC1A (exons 1–25), SMC3 (exons 1–29), STAG2 (exons 3–35)], RNA splicing [SF3B1 (exons 13–18), SRSF2 (exon 1), U2AF1 (exons 2, 6), ZRSR2 (exons 1–11)] and NPM1 (exon 11), Two distinct HTS technologies were used to allow direct cross validation. Firstly, libraries were prepared using the Ampiseq System according to the manufacturer’s instructions and run on Ion Proton (Thermofisher, Waltham, MA, USA). Raw data were analyzed with both Torrent Browser (Thermofisher) and SeqNext (JSI Medical System, Los Angeles, CA, USA). Secondly, libraries were also prepared using the Haloplex Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA) and run on MiSeq (Illumina, San Diego, CA, USA). Raw data were processed by SureCall (Agilent Technologies) and SeqNext (JSI Medical System). A high depth of coverage (>1300×) was obtained for all genes with both HTS technologies, allowing detection of mutations with a variant allele frequency (VAF) until 1%. Frameshift and nonsense variants were always considered as relevant mutations. Single nucleotide variants were retained in the absence of description into public databases of human polymorphisms, and effects on protein function were predicted with 6 established prediction tools: SIFT (Sorting Intolerant From Tolerant), PolyPhen-1, PolyPhen-2, MAPP (Multivariate Analysis of Protein Polymorphism), PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphism), and SNAP.
The presence of the FLT3-internal tandem duplication (ITD) was performed for all patients by fragment analysis as previously described.

**Statistical methods**

Event-free survival (EFS) and overall survival (OS) were estimated by the Kaplan–Meier method and compared by cause-specific hazard Cox models. EFS was measured from the date of diagnosis to the date of the first event (induction failure, relapse, or death) or to the date of last follow-up. Patients who failed to achieve complete remission (CR) were considered as failures at day 60. OS was measured from the date of diagnosis to the date of death from any cause or last follow-up. Data were analyzed and compared without censor at transplant for patients who received allogeneic stem cell transplantation in first CR. Comparisons between patient subgroups were performed by the Mann–Whitney test for continuous variables and by Chi-square or Fisher exact test for categorical variables. Hazard ratios (HRs) are given with 95% confidence interval (CI). Multivariate analyses assessing the independent effect of the covariates were performed using Cox proportional hazard model. Variables associated with the outcome and a P-value < 0.10 in univariate analysis or known as validated factors were included in the multivariable models. Then a backward and forward stepwise selection was performed. All P-values were 2-sided and values < 0.05 were considered statistically significant. All statistical tests were performed with the SPSS 22.0 (IBM Corp., Armonk, NY) and R3.2.3 software packages (R Development Core Team, Vienna, Austria).

**Results**

**Patients’ characteristics at diagnosis**

Among the 385 patients in this study, 210 were male and 175 were female. The median age at AML diagnosis was 8.6 years (range, 0–18) and the median white blood cell (WBC) count was $16.6 \times 10^3/L$ (range, 0.40–575). The present cohort was not different from the entire ELAM02 cohort (Supplemental Table S1, Supplemental Digital Content, http://links.lww.com/HS/A1). The distribution in the cytogenetic subgroups was as follows: normal karyotype ($n=101$, 26.2%), CBF-rearranged ($n=92$, 24% including $t(8;21)$: $n=57$ and inv(16)/$t(16;16)$: $n=35$), KMT2A (MLL)-rearranged ($n=79$, 21%), adverse karyotype ($n=40$, 10% including complex karyotype: $n=27$, monosomy 7: $n=9$ and $t(6;9): n=4$), and other aberrations (named “others” hereafter) ($n=73$, 19%) (Supplemental Table S1, Supplemental Digital Content, http://links.lww.com/HS/A1).

![Figure 1. Distribution of the cytogenetic subgroups in the studied cohort and according to age classes.](image-url)
HS/A1, and Fig. 1). Inv(3) or t(3;3) was not identified in the present study. Cytogenetic appeared significantly different according to age with younger children harboring more KMT2A-rearrangements while other cytogenetic subgroups increased with age, especially for CBF-rearrangements and normal karyotypes (Fig. 1).

Molecular profiling in childhood AML and association with cytogenetic aberrations

Molecular analyses with HTS and LD-RT-PCR allowed the identification of 579 mutations involving 35 different genes as well as 191 fusion transcripts (23 different fusion genes) among 385 pediatric patients.

Twenty-eight genes were mutated in more than 1% of our cohort but only 5 genes (NRAS, FLT3, KIT, KRAS, and WT1) were mutated in more than 10% (Fig. 2). The most common class of mutations involved genes that control kinase signaling (61% of the whole cohort) followed by transcription factors (16%), tumor suppressors (14%), chromatin modifiers (9%), DNA methylation controllers (8%), cohesin genes (5%), and spliceosome (3%). Overall, 76% of patients (292/385) had at least one mutation among the genes we examined. The mean number of mutated genes was 1.5 per patient (range, 0–5) with the highest rate of mutations in normal karyotype AML (mean 2.2; range, 0–5) and the lowest rate in KMT2A-rearranged AML (mean 0.7; range, 0–3). The mean number of mutated genes increased with age (means of 0.7, 1.5, and 1.9 for 0–2, 2–10, and 10–18 years, respectively, \( P < 0.001 \)) mostly due to the different distribution of cytogenetic subgroups (Supplemental Figs. S1 and S2, Supplemental Digital Content, http://links.lww.com/HS/A1).

The most frequent identified fusion transcripts were RUNX1–RUNX1T1 (15%), KMT2A–MLLT3 (9%), and CBFB–MYH11 (9%). All other fusion transcripts were found in less than 5% of

![Figure 2. Gene mutations and fusion transcripts frequencies in childhood AML. Only aberrations detected with a frequency higher than 1% are shown.](image-url)

* including 4.2% and 2.3% of biallelic and monoallelic CEBPA mutations respectively.
patients (Fig. 2 and Table S2, Supplemental Digital Content, http://links.lww.com/HS/A1). KMT2A were found to be rearranged in 79 AML (21%) with 13 different partners among which MLLT3 was by far the most common (n = 36, 46% of KMT2A-rearranged AML) followed by MLLT10 (n = 13, 16%), ELL (n = 6, 8%), MLLT1 (n = 5, 6%), and MLLT4 (n = 5, 6%). Only 3 patients with KMT2A-rearrangement (identified by fluorescent in situ hybridization) had no identified partner. The cryptic NUP98–NSD1 fusion was found in 9 patients (2.3% of the whole cohort) in which 5 had a normal karyotype.

Taken together, we identified at least 1 molecular aberration (mutations or fusion transcripts) in 344 (89%) out of 385 patients. Cytogenetics in the 41 remaining patients was distributed as follows: normal karyotype (n = 12), complex karyotype (n = 9), isolated monosomy 7 (n = 2), and other karyotype aberrations (n = 18).

Figure 3 depicts the interrelationship among the various mutations in cytogenetic subgroups. The mutational spectrum for the different age groups is provided in Supplemental Fig. S3 (Supplemental Digital Content, http://links.lww.com/HS/A1). As expected, NPM1 mutations, FLT3–ITD and CEBPA biallelic mutations (CEBPAdm) were associated with normal cytogenetics (P < 0.001 for each comparison) whereas WT1 mutations were linked with the “other” subgroup (P < 0.001) (Fig. 4). CBF rearrangements were closely associated with KIT (P < 0.001), RAS (P = 0.012) and cohesin mutations (P = 0.024). Notably, mutations involving epigenetic regulators and cohesin genes were restricted to patients with (821) AML while they were nearly absent in inv(16)(16;16) AML, as we described previously in a larger cohort of CBF AML including both pediatric and adult patients.10 On the other hand, no association was found in adverse cytogenetics and KMT2A-rearranged subgroups. We also investigated mutation cooccurrences showing that NPM1 mutations were strongly associated with FLT3–ITD (P = 0.009), FLT3–TKD (P = 0.001) and mutations in epigenetic controllers (P < 0.001). GATA2 mutations were significantly associated with CEBPAdm (P < 0.001), as previously described11,12 and WT1 mutations appeared associated with FLT3–ITD (P < 0.001). RUNX1 mutations were significantly associated with mutations in epigenetic controllers (P = 0.001) (Fig. 5). Considering that the NUP98–NSD1 fusion has been associated with specific findings,13–15 the 9 positive patients were grouped together. We failed to identify a unique entity whatever karyotype aberrations for subsequent analyses. Consequently, a strong association was found between NUP98–NSD1 fusion and FLT3–ITD (P < 0.001) and WT1 mutations (P = 0.002).

Impact of molecular abnormalities on complete remission rate and clinical outcome

Among the 385 patients included in this study, 350 (91%) achieved CR after 2 courses of intensive induction chemotherapy. In univariate analysis, FLT3–ITD, WT1 mutations, WBC count higher than 30 × 10^9/L, “other” cytogenetics and NUP98 fusions were associated with more induction failures (Supplemental Table S3, Supplemental Digital Content, http://links.lww.com/HS/A1). Despite the small number of cases, only the presence of a NUP98 fusion remained associated with induction failure in multivariate analysis (P = 0.038) (Table 1). Characteristics of NUP98-rearranged cases are detailed below.

At 3 years, EFS and OS for the whole cohort were estimated at 58.9% (95% CI: 54–63.9) and 76.1% (95% CI: 71.8–80.4) respectively with a median follow-up of 59 months. EFS and OS according to cytogenetic subgroups are presented in Supplemental Fig. S4 (Supplemental Digital Content, http://links.lww.com/HS/A1). In univariate analysis, NPM1 mutations, CEBPAdm and KIT mutations were associated with significant or a trend of higher OS and/or EFS (Supplemental Fig. S5A-F, Supplemental Digital Content, http://links.lww.com/HS/A1). By contrast, FLT3–ITD, WT1, RUNX1, PHF6, and NUP98-rearrangements were associated with poorer OS and/or EFS (Supplemental Fig. S5G-P, Supplemental Digital Content, http://links.lww.com/HS/A1). Multivariate prognostic analyses for the HR are indicated in Table 2. Co-tested factors included NPM1, CEBPAdm, FLT3–ITD, RUNX1, WT1, and PHF6 mutations as well as WBC count, cytogenetic subgroups, and NUP98 fusions. KIT mutations were excluded because of a strong association with CBF rearrangements. Five factors were demonstrated to be significantly associated with a higher risk of event by cause-specific hazard Cox models: WBC count higher than 30 × 10^9/L (P = 0.005); NUP98 fusions (P < 0.001); FLT3–ITD (P = 0.01); WT1 mutations (P = 0.018) and adverse cytogenetics (P = 0.009). On the other hand, 4 factors were significantly associated with a lower risk of event: NPM1 mutations (P = 0.009); CEBPAdm (P = 0.027); CBF rearrangements (P = 0.066); and KMT2A rearrangements (P = 0.021). A similar analysis for OS revealed 5 factors that have a negative impact: WBC count higher than 30 × 10^9/L (P = 0.01); WT1 mutations (P = 0.027); RUNX1 mutations (P = 0.043); PHF6 mutations (P = 0.038); and adverse cytogenetics (P < 0.001). On the other hand, 3 factors were shown to positively impact OS: NPM1 mutations (P = 0.004), CEBPAdm (P = 0.042), and CBF rearrangements (P < 0.001).

NUP98-rearranged cases

NUP98-rearranged cases represented 2.6% of this cohort (10/385) with the fusion of NUP98–NSD1 being found in 9 patients. The karyotype was normal in 5 patients and complex for the sole patient with NUP98–JARID1A fusion transcript. The 4 remaining patients belonged to the “other” cytogenetic subgroup. The median age was 9.9 years (range, 1.3–16.8) and median WBC count was 179.8 × 10^9/L (range, 12.2–436). The most frequent mutations associated with this specific subgroup were FLT3–ITD (7/10), WT1 (5/10), CEBPA (monoallelic mutation; 2/10), and RUNX1 (2/10). Overall, NUP98-rearranged cases showed poor prognosis with a half of patients who did not achieve CR. At 3 years, EFS and OS in NUP98-rearranged cases were 10% (95% CI: 0–28.6) and 23% (95% CI: 0–54), respectively compared with 60.5% (95% CI: 55.5–65.5) and 77.3% (95% CI: 73.1–81.6) in NUP98-negative cases (Supplemental Fig. S5O-P, Supplemental Digital Content, http://links.lww.com/HS/A1).

Molecular classifier in childhood AML

Considering results from multivariate analysis and strong molecular markers validated among studies6,18–20 (i.e., NPM1 mutations and CEBPAdm), we defined a molecular classifier, refining the prognosis in childhood AML. The molecular classifier was based on OS predictions and segregate AML into 3 groups (Supplemental Table S4, Supplemental Digital Content, http://links.lww.com/HS/A1, and Fig. 6A): favorable molecular risk (RUNX1–RUNXIT1 or CBF–MYH11 or NPM1 mutation...
or CEBPAdm, n = 142); poor molecular risk (NUP98 fusion or RUNX1 or WT1 or PHF6 mutation, n = 59); intermediate molecular risk (all others, n = 184). Patients who harbored both a CBF rearrangement and WT1, RUNX1, or PHF6 mutations were included in the favorable subgroup. Neither karyotype nor other gene mutations were able to discriminate within patients in the intermediate molecular risk subgroup. At 3 years, OS was 92.1% (95% CI: 87.6–96.6, median not reached) for the favorable
molecular risk subgroup, 73.2% (95% CI: 66.7–79.6, median not reached) for the intermediate molecular risk subgroup and 46.1% (95% CI: 33.1–59.2, median 2.33 years) for the poor molecular risk subgroup. Although KMT2A-rearrangements were associated with a trend of better outcome compared with non-KMT2A-rearranged cases from the intermediate subgroup, it did not reach statistical significance ($P = 0.15$). Consequently, KMT2A-rearrangements were not included in the classifier. The same results were observed when separating KMT2A–MLLT3 rearrangements and other KMT2A-rearrangements. While FLT3–ITD was not retained as an independent prognostic factor for molecular classification, its cooccurrence in patients with poor molecular risk defined a subgroup of patients with the worst prognosis (3 years OS: 23.8% vs 58.8%; $P = 0.024$) (Supplemental Fig. S6, Supplemental Digital Content, http://links.lww.com/HS/A1). By contrast, FLT3–ITD had no impact in the intermediate molecular risk group ($P = 0.75$) or in NPM1-mutated patients ($P = 0.72$).

Finally, the molecular classifier was compared to the 2017 European LeukemiaNet (ELN) classification which is currently used to stratify adult patients with AML. A total of 139 patients were classified in the favorable subgroup with both classifications. Only 3 NPM1-mutated-AML were classified as favorable according to the molecular classifier and as intermediate or adverse according to the ELN classification because of high FLT3–ITD ratio ($n = 2$) or complex karyotype ($n = 1$). Interestingly, the ELN classification fails to separate intermediate and adverse subgroups in our pediatric cohort (Fig. 6B, Supplemental Table S5, Supplemental Digital Content, http://links.lww.com/HS/A1). Together, these data show that the ELN classification lacks of prognostic significance in childhood AML, especially in unfavorable AML and the use of the present molecular classification could improve risk stratification in pediatric patients.

Discussion

The better knowledge of molecular aberrations in AML has greatly improved the management of AML patients over the past decades. However, most of reported studies have focused on adult cohorts. The ELAM02 trial gave us the opportunity to investigate incidences and prognostic significances of molecular aberrations in childhood AML which currently remains a life-threatening malignancy with poor outcome compared to acute lymphoblastic leukemia.

The most common mutations involved genes controlling kinase signaling (especially NRAS/KRAS, FLT3–ITD, KIT mutations). These mutations concerned 61% of the whole cohort and were

| Variables       | SHR  | 95% CI   | $P$   |
|-----------------|------|----------|-------|
| WBC $>30 \times 10^9$/L | 0.550 | 0.260–1.165 | 0.119 |
| NUP98 fusions   | 0.215 | 0.050–0.922 | 0.038 |
| FLT3–ITD       | 0.569 | 0.234–1.390 | 0.212 |
| W7T mutations  | 0.419 | 0.164–1.073 | 0.070 |
found in all cytogenetic subgroups. All but FLT3–ITD had no independent impact on outcome. To date, the prognostic significance of FLT3–ITD in pediatric AML remains controversial. In the present study, FLT3–ITD was associated with reduced EFS but did not influence OS in multivariate analysis in the whole cohort. Importantly, FLT3–ITD were found in heterogeneous diseases including NPM1-mutated or CBF AML which have shown to have a highly favorable outcome but also in NUP98-rearranged and WT1-mutated AML which are associated with poor prognosis. Among NPM1-mutated childhood AML, FLT3–ITD did not impact outcome in line with a previous study. Transcription factors were the second most common class of mutations (16% of the whole cohort). CEBPA dm and RUNX1 mutations defined independent molecular subgroups of patients (4.2% and 6.2% respectively) associated with highly favorable and poor outcome respectively. Both mutations were mutual exclusive with NPM1 mutations and occurred almost exclusively in normal karyotype-AML. By contrast, all other classes of mutations were found in less than 10% of patients. Importantly, while mutations within DNA-methylation-related genes (DNMT3A, TET2, IDH1/2) are highly prevalent in adult AML (together higher than 50%), only 8% of children with AML harbor such mutations, especially in normal karyotype-AML. Among normal karyotype-AML (n = 101/385), 3 patients harbored DNMT3A mutations (all at codon R882), 12 had IDH1 mutations (codon R132), 4 had IDH2 mutations (codon R140), and 3 had TET2 mutations. These results are in line with a previous report from the Children’s Oncology Group.

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**Table 2**

| Variables | EFS | | OS | |
| --- | --- | --- | --- | --- |
| | SHR | 95% CI | P | SHR | 95% CI | P |
| WBC > 30 x 10⁹/L | 1.58 | 1.15–2.17 | 0.005* | 1.93 | 1.30–2.86 | 0.001* |
| Cytogenetics | | | | | | |
| CBF | 0.47 | 0.27–0.80 | 0.006* | 0.16 | 0.07–0.37 | <0.001* |
| KMT2A-rearranged | 0.52 | 0.30–0.91 | 0.021* | 0.53 | 0.27–1.04 | 0.066 |
| Normal | 0.96 | 0.56–1.62 | 0.868 | 0.91 | 0.47–1.73 | 0.762 |
| Others | 0.83 | 0.48–1.42 | 0.490 | 0.83 | 0.44–1.58 | 0.571 |
| Adverse | — | — | 0.009* | — | — | <0.001 |
| NUP98 fusions | 3.79 | 1.81–7.92 | — | — | — | — |
| Gene mutations | | | | | | |
| NPM1 | 0.21 | 0.09–0.46 | <0.001* | 0.12 | 0.03–0.51 | 0.004* |
| CEBPA dm | 0.35 | 0.14–0.89 | 0.027* | 0.22 | 0.05–0.95 | 0.042* |
| FLT3–ITD | 1.71 | 1.14–2.57 | 0.010* | — | — | — |
| WT1 | 1.71 | 1.10–2.66 | 0.018* | 1.83 | 1.07–3.15 | 0.027* |
| RUNX1 | — | — | — | 1.88 | 1.02–3.44 | 0.043* |
| PHF6 | — | — | 2.33 | 1.04–5.17 | 0.038* |

CI = confidence interval. EFS = event-free survival. OS = overall survival. SHR = specific hazard ratio. WBC = white blood cell.

The P values that are statistically significant are indicated in bold.

*Statistically significant (Cox proportional hazard model).

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**Figure 6.** Childhood AML outcome. (A) Childhood AML outcome according to the molecular classifier. Favorable molecular risk: RUNX1–RUNX1T1 or CBF–MYH11 or NPM1 mutation or CEBPA dm; poor molecular risk: NUP98 fusion or RUNX1 or WT1 or PHF6 mutation; intermediate molecular risk (all others). (B) Childhood AML outcome according to the 2017 European LeukemiaNet (ELN) classification.
Moreover, the systematic use of LD-RT-PCR allowed the detection of recurrent transcript fusions in about a half of pediatric patients. Fusions involving 1 of the 2 CBF subunits or the KMT2A gene were found in 24% and 21% of patients respectively. Among KMT2A-rearranged cases, the KMT2A–MLLT3 fusion was by far the most common, representing nearly the half of KMT2A fusions. While CBF rearrangements were associated with a favorable prognosis, KMT2A rearrangements were associated with an intermediate outcome in the present study. KMT2A–MLLT3 fusion did not show a better prognosis than other KMT2A rearrangements in line with a recent large retrospective study of KMT2A-rearranged pediatric AML.

In accordance with previous reports, CBF rearrangements, NPM1 mutations and CEBPAAdm defined a particular subgroup with good prognosis. Together, these aberrations were found in more than one third of childhood AML. By contrast, NUP98 fusions were associated with the worst prognosis, mostly due to induction failures. Other aberrations associated with poor outcome included RUNX1, PHF6, and WT1 mutations. In a previous report by the Children’s Oncology Group, WT1 mutations were shown to be an independent factor of poor prognosis both on EFS and OS. Interestingly, AML with RUNX1 mutations has been added to the last WHO classification as a provisional entity, considering they represent a biologically distinct group with a possibly worse prognosis in adults AML. Our results show that RUNX1 mutations also define a distinct subgroup with poor outcome in childhood AML. Finally, PHF6 mutations are a rare event in childhood AML and to our knowledge, their prognostic impact has not been reported in a large series. Importantly, by contrast to the adult-based-ELN classification, the present molecular classification identified a group of pediatric patients with particular poor prognosis. Moreover, the cooccurrence of FLT3–ITD in this subgroup identified patients with the worst outcome. This result remains of great interest in the context of FLT3 inhibitors use.

In conclusion, we reported the comprehensive genomic landscape of a large cohort of pediatric de novo AML enrolled in the ELAM02 trial and proposed a prognostic classification based on gene mutations and fusions in this particular group of patients. Despite some overlaps between childhood and adult AML, pediatric patients harbored a different pattern of molecular aberrations, especially with fewer mutations within epigenetic-related genes. We confirmed the favorable-risk group including CBF fusions, NPM1 mutations, and CEBPA biallelic mutations and refined the poor-risk group including RUNX1, WT1, and PHF6 mutations as well as NUP98 fusions. KMT2A-rearranged AML were included in the intermediate-risk group with no difference between KMT2A–MLLT3 and other KMT2A fusions in this study. Overall, these results have important implications to contribute in refining risk stratification of pediatric AML and show the need for further validations in independent pediatric cohorts.

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