



# The Genetic Landscape of Relapsed Pediatric Acute Myeloid Leukemia

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## Abstract

**Context:** Previous studies revealed that the genomic of pediatric acute myeloid leukemia (AML) is different from AML in adults, yet the genetic changes associated with relapsed disease in children have yet to be fully described.

**Objective:** To elucidate the genetic background of pediatric AML at relapse

**Design:** Transcriptomic and genetic analyses were performed on relapsed AML from multiple clinical studies in St. Jude Children's Research Hospital.

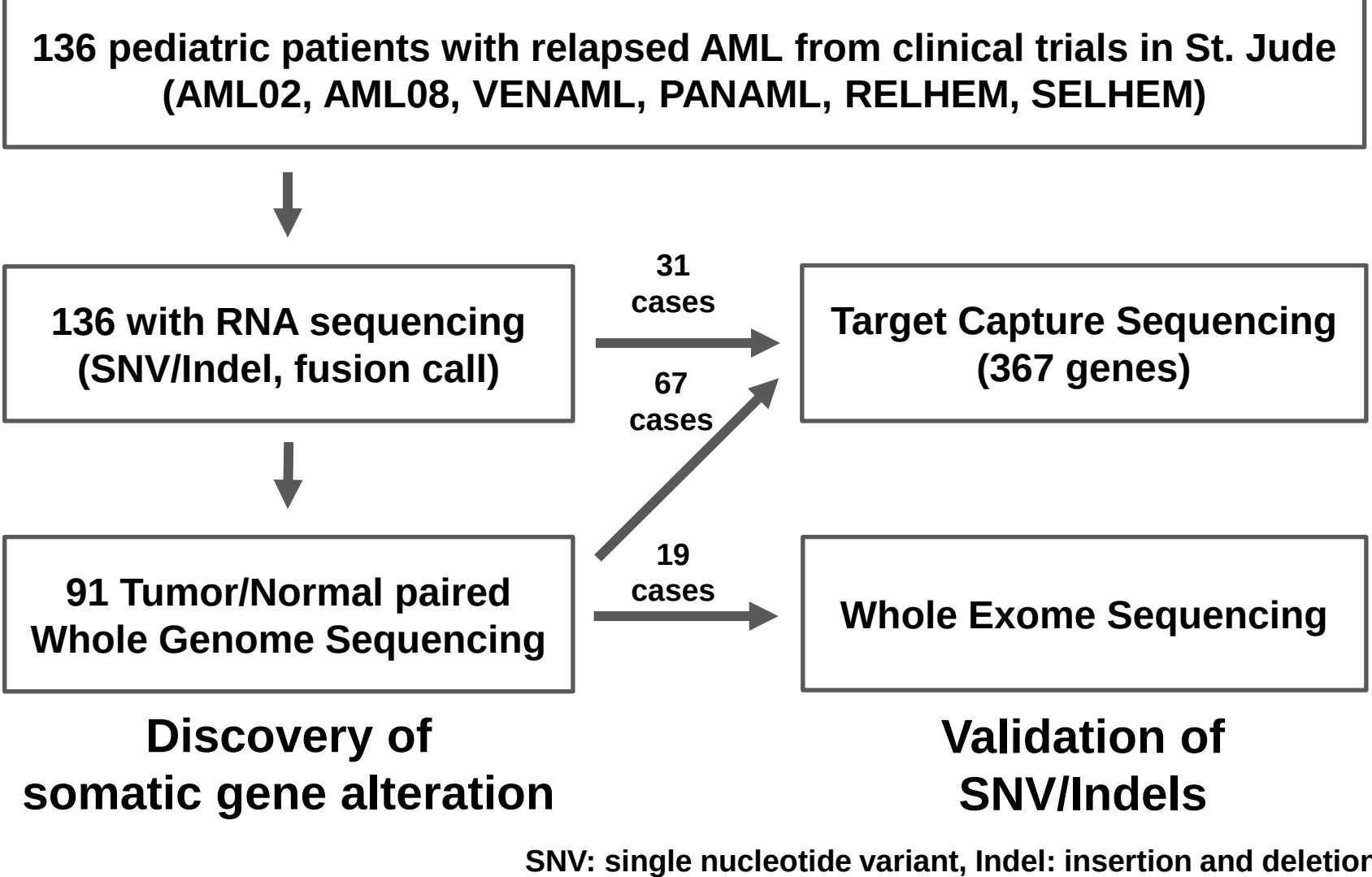
**Patients:** RNA sequencing was performed from 136 patients with relapsed pediatric AML. Additional transcriptome from pediatric AML at diagnosis (n=417) was obtained from previous reports to characterize the molecular alterations common in pediatric AML.

**Results:** Genomic profiling of relapsed pediatric AML demonstrated an increase in *KMT2A* (n=36, 26.5%) and *NUP98* rearrangements (n=18, 13.2%) when compared to de novo pediatric AML, as well as an increase in *WT1* mutations (n=33, 24.3%). Comprehensive characterization of pediatric AML with extension RNA transcriptome data revealed 18 molecular categories with unique expression patterns and mutually exclusive gene alteration patterns. These molecular categories included rare categories with structural variants involving oncogenes of *MECOM* (n=3, 2.2%) or *BCL11B* (n=2, 1.5%), which lead to the upregulation and allele-specific expression (ASE) of each gene.

**Conclusions:** Integrated genomic profiling of pediatric AML identified unique molecular categories associated with relapsed diseases.

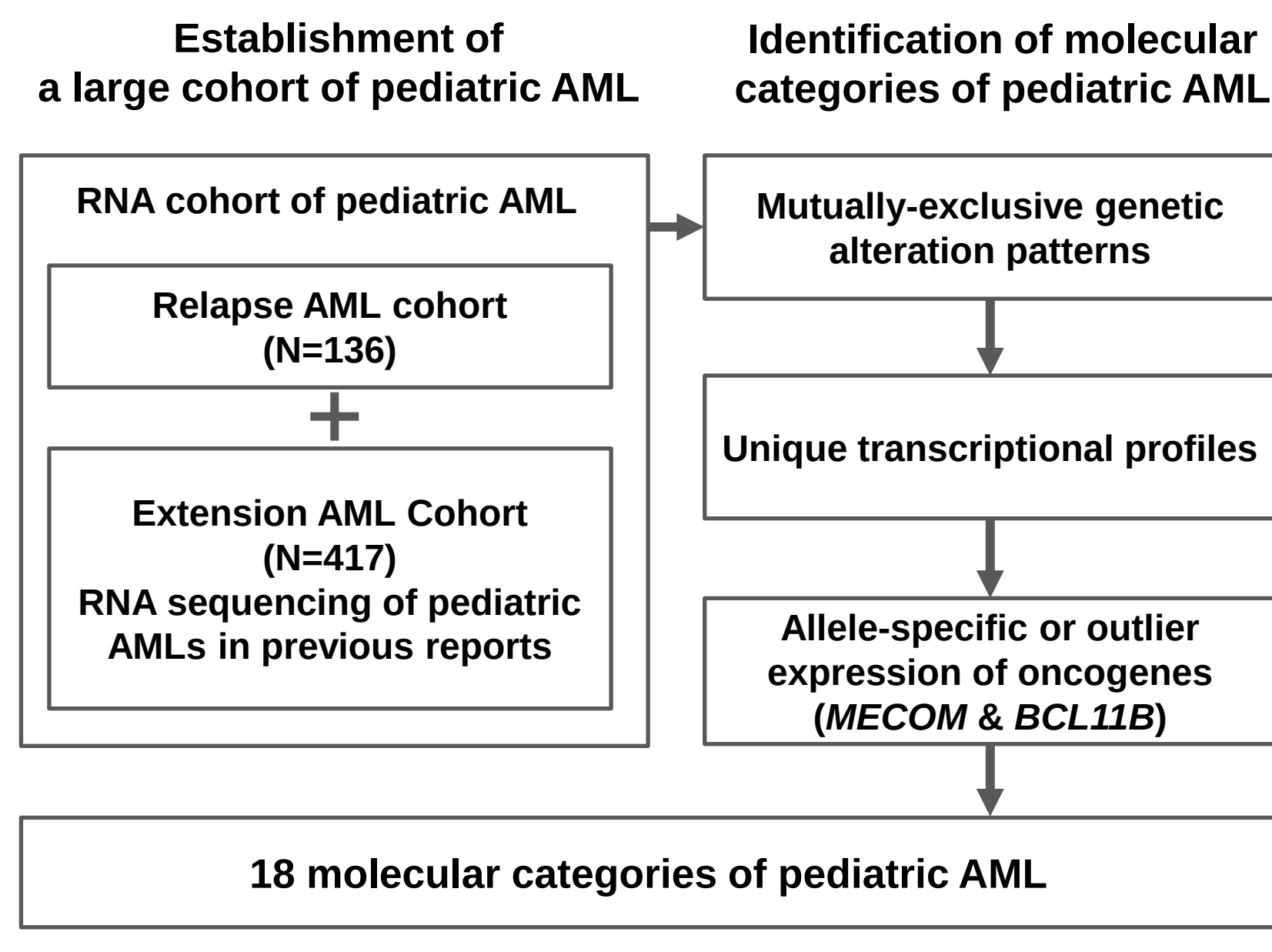
## Materials & Methods

### Characterization of relapsed pediatric AML



A total of 136 patients with relapsed AML were enrolled from multiple clinical studies in St. Jude Children's Research Hospital. Genetic alterations were investigated using both whole genome sequencing and RNA sequencing data. Single nucleotide variants (SNVs) and insertions and deletions (Indels) were validated by multiple modalities.

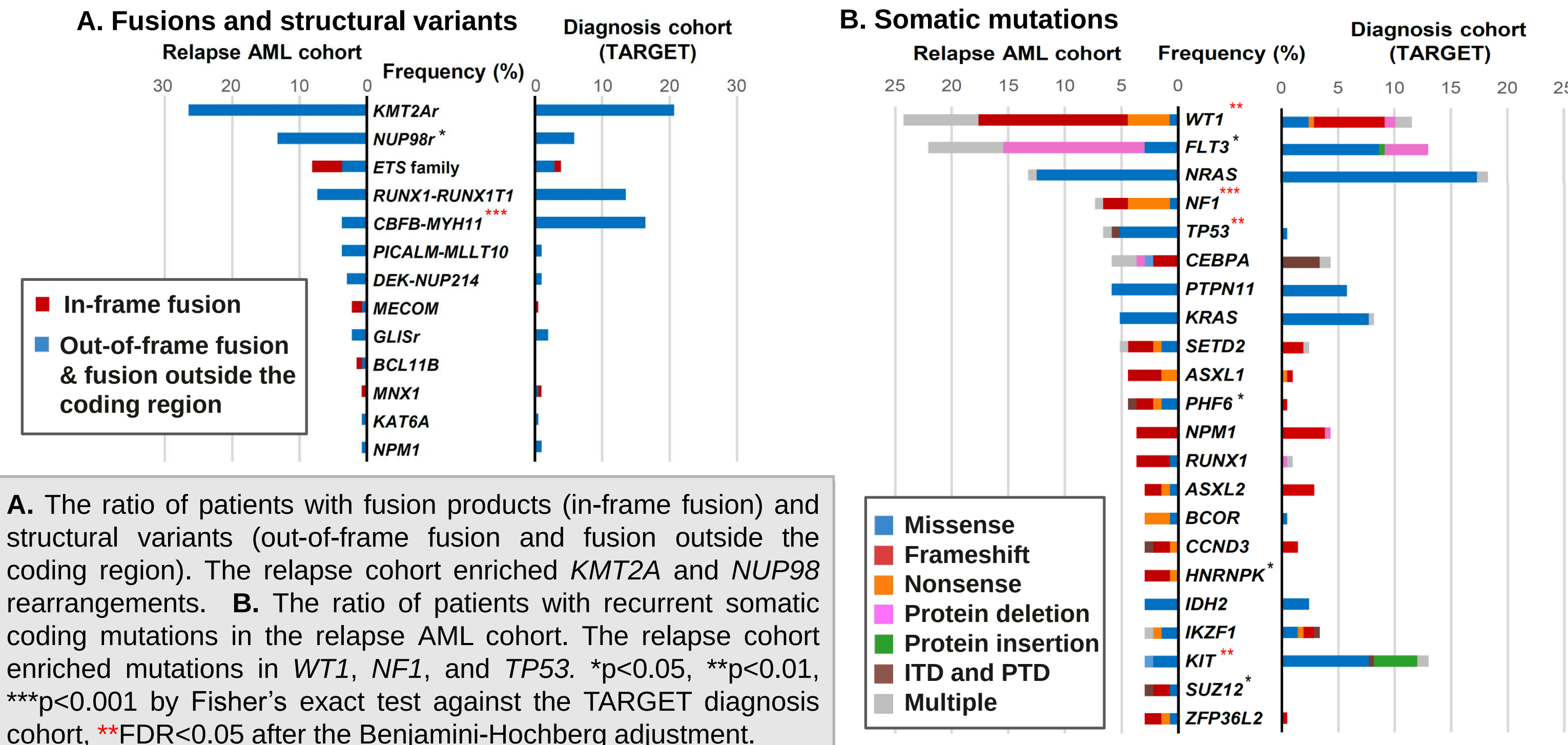
### Molecular categorization of pediatric AML



Using RNA-seq data of additional 417 pediatric AMLs from previous reports, pediatric AML were categorized into 18 molecular categories with mutually exclusive gene alterations and unique expression profiles. Allele-specific of *MECOM*<sup>6,7</sup> or *BCL11B*<sup>8,9</sup> were evaluated by cis-X<sup>10</sup>.

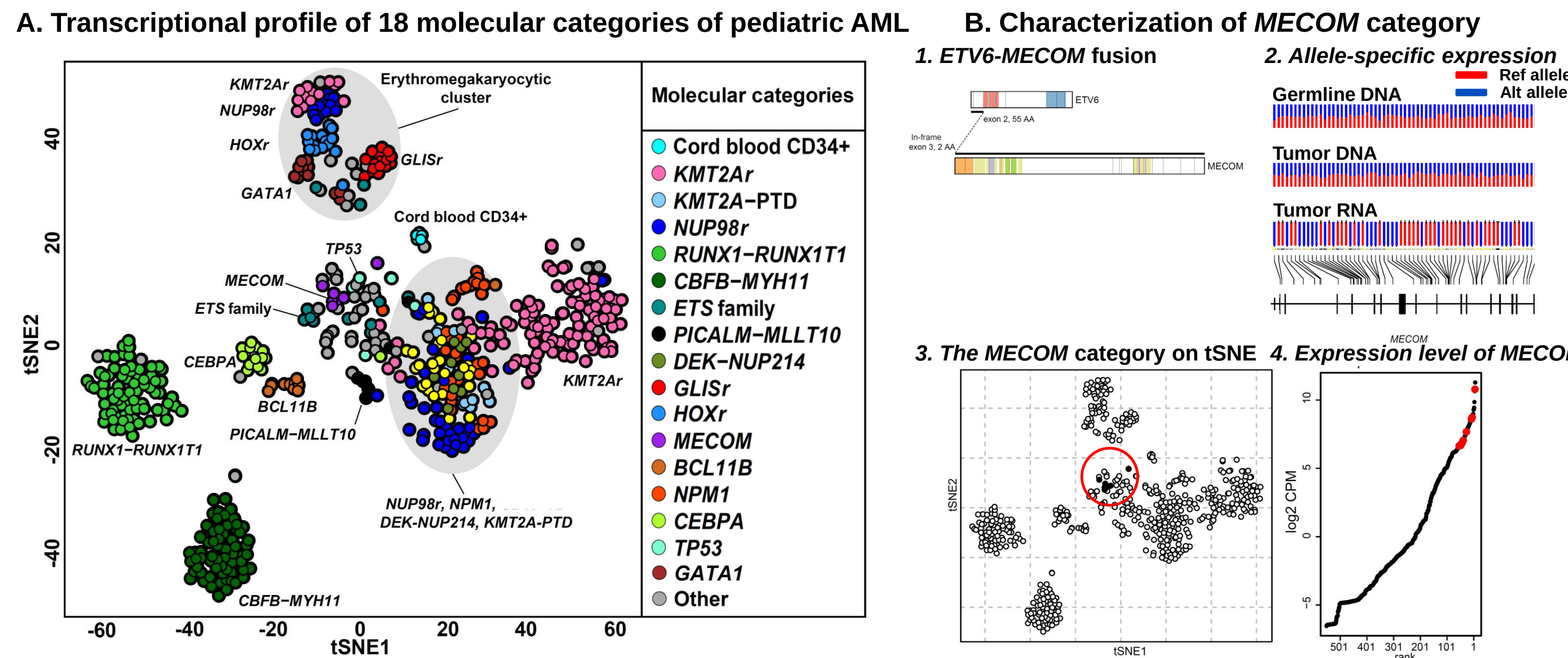
## Results

**Figure 1: Genetic landscape of relapsed pediatric AMLs**



**A.** The ratio of patients with fusion products (in-frame fusion) and structural variants (out-of-frame fusion and fusion outside the coding region). The relapse cohort enriched *KMT2A* and *NUP98* rearrangements. **B.** The ratio of patients with recurrent somatic coding mutations in the relapse AML cohort. The relapse cohort enriched mutations in *WT1*, *NF1*, and *TP53*. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by Fisher's exact test against the TARGET diagnosis cohort, \*\*FDR<0.05 after the Benjamini-Hochberg adjustment.

**Figure 2: Molecular categories of pediatric AML**



**A.** tSNE plot of transcriptome of pediatric AMLs (n=553). Dots represent each AML sample, and the color indicates its molecular category defined by genetic alteration and transcriptional profile. **B1.** A representative fusion involving *MECOM*. **B2.** Allele-specific expression of *MECOM* identified by SNPs on the gene. **B3.** AMLs in *MECOM* category (black dots) clustered on the tSNE plot. **B4.** Outlier expression of *MECOM* in the category (red dots).

## Summary

Integrated genomic profiling revealed a unique molecular landscape of pediatric AML at relapse compared to the diagnosis cohort of the TARGET study.

The relapse AML cohort enriched *NUP98* rearrangement, *WT1*, *NF1*, and *TP53* mutations with statistical significance, while the diagnosis TARGET cohort enriched *CBFB-MYH11* fusions and *KIT* mutations.

Genetic and transcriptional profiling of pediatric AML in the RNA cohort identified 18 unique molecular categories with specific gene alterations mutually exclusive with each other and unique expression profiles.

The molecular categories included well-defined subtypes of pediatric AML such as *RUNX1-RUNX1T1* and *KMT2A* rearranged AML, as well as novel molecular categories of *MECOM* or *BCL11B* AMLs.

AMLs in the *MECOM* category showed outlier and allele-specific expression of *MECOM* gene under the control of promoters of genes located upstream *MECOM* as a result of structural variants involving chromosome 3.

## Discussion

The genetic landscape of relapsed pediatric AMLs

Q: Are the gene alterations present at diagnosis or acquired under the selective stress of chemotherapy and during disease progress?

→ Genetic profiling of diagnosis-relapse paired AMLs will show the mutational architecture of clonal evolution.

Q: Are these alterations risk factors at diagnosis?

→ Retrospective study of previous clinical trials focusing on the presence of the gene alteration should be indispensable for future prospective studies.

Molecular categories of pediatric AML

Q: Are the gene alterations driver?

→ To prove that they are driving gene alteration, an in vitro leukemia model to show the effect of the expression of these genes on hematopoietic cells and induction of serially transplantable leukemia will be required.

Q: Can all pediatric AML be categorized into 18 categories?

→ "Other" category includes candidate molecular categories with small sample sizes in this cohort (e.g., *MXN1*, *KAT6A*) and with no apparent driver alteration. Further collective studies will characterize these AML more.

## References

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