# The Genetic Landscape of *NUP98*-rearranged Pediatric Leukemia

AML 283

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## Abstract

**Context:** Rearrangements involving *NUP98* are frequently found in pediatric acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia (T-ALL) and are associated with poor prognosis. Various fusion partners have been identified; however, the genetic basis of how different fusion genes and cooperating mutations contribute to the disease phenotypes and the refractory nature remains to be clarified.

**Objective:** To elucidate the transcriptional and genetic background of pediatric *NUP98*-rearranged acute leukemia.

**Design:** Transcriptomic and genetic analyses were performed on pediatric leukemia with *NUP98* rearrangements from multiple clinical studies at St. Jude Children's Research Hospital, by the Italian Association for Pediatric Hematology and Oncology, or by the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative. No blinding was done in this study.

**Patients:** RNA sequencing data from 95 patients (median age of 9.0 years) with *NUP98*-rearranged (*NUP98*r) leukemia was performed or obtained from previous studies. Of these, 82 patients were also studied by tumor–normal-paired whole-genome or whole-exome sequencing.

**Results:** We identified 17 unique *NUP98* fusion partners from RNAseq data with *NSD1* as the most frequent partner (n=44, 46.3%), followed by *KDM5A* (n=27, 28.4%) and *RAP1GDS1* (n=6, 6.3%). We also found rare fusion partners recurrent in this cohort, including *LNP1*, *HOXA13*, and *HOXD13* (n=2, 2.1%, respectively). Among cooperating mutations, *FLT3*-ITD and *WT1* were frequent in *NSD1*-AML (57.9% and 52.6%, respectively), and copy number loss of chromosome 13 harboring RB1 was found in 86.3% of *NUP98-KDM5A* AML with erythromegakaryocytic expression patterns (FAB M6-7). *NUP98-RAP1GDS1* fusions are found in both AML (n=3) and T-ALL (n=3) cases and those with T lineage differentiation have concurrent *NOTCH1* mutations, indicating that both fusions and cooperating mutations are contributing to the disease phenotypes.

We further performed transcriptome analysis of *NUP98*r AML, integrating various AML subtypes from previous studies. *NUP98*r AML expressed high *MEIS1*, *PRDM16*, and *HOXA-B* cluster genes similar to AMLs with *NPM1* or *UBTF* mutations, suggesting a molecular basis shared by these subtypes.

**Conclusions:** Genomic and transcriptional profiling of pediatric *NUP98*-rearranged leukemia identified unique associations among fusions, cooperating mutations, and disease phenotypes.

## Context & Objective

**Context:**

*NUP98* rearrangements in pediatric leukemia

- reported to involve more than 30 fusion partners showing various phenotypes including AML, T-ALL, MDS (myelodysplastic syndrome), CMML (chronic myelomonocytic leukemia), and MPAL (mixed-phenotype acute leukemia)[1]

→ How fusion partners and cooperating mutations contribute to the disease phenotypes are yet to be elucidated

Pediatric AML with *NUP98* rearrangement

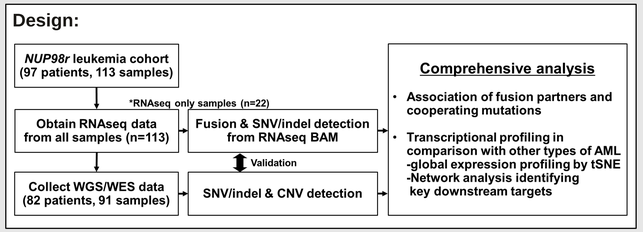
- Included in the latest WHO classification[2] as a disease entity "Acute myeloid leukaemia with *NUP98* rearrangement"
- Regarded to have poor prognosis[1,3,4]

**Objective:**

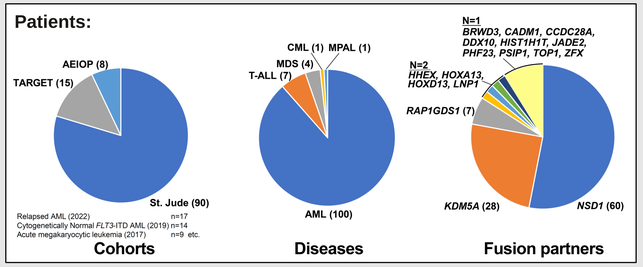
To elucidate the genetic and transcriptional background of pediatric leukemia with *NUP98* rearrangement to find a therapeutic target and improve the clinical outcomes

## Design and Patients

**Design:**




**Patients:**




## Results

### Figure 1: Genetic landscape of leukemia with *NUP98* rearrangement

Mutational and transcriptional profiling revealed strong associations with fusion partners, disease phenotypes, and cooperating mutations.

*NUP98::NSD1*

AML with myelomonocytic phenotypes with high frequencies of *FLT3*-ITD and *WT1* mutations

*NUP98::KDM5A*

Erythromegakaryocytic AML with *RB1* loss

*NUP98::RAP1GDS1*

T-ALL with *NOTCH1* mutations

However, *NUP98::KDM5A*, *::RAP1GDS1*, and other fusion partners with different cooperating mutations showed various disease phenotypes, suggesting that fusion partners and cooperating mutations are both contributing to the disease phenotypes.

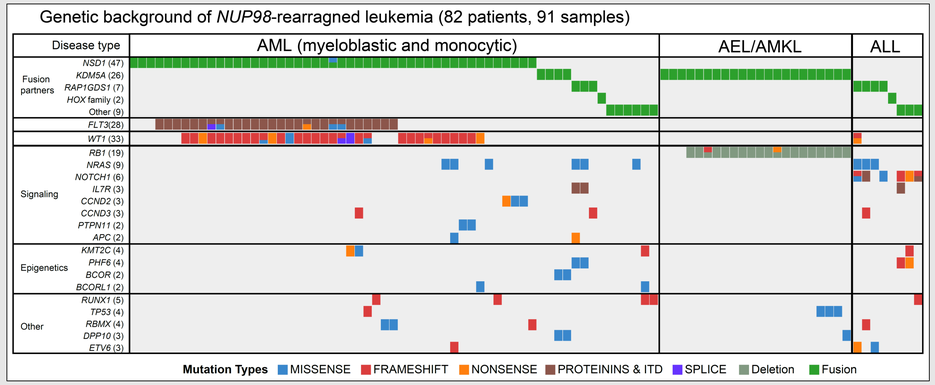



**Figure.1** Mutational landscape of *NUP98*-rearranged leukemia with WGS data (81 patients, 92 samples). Disease types were inferred from clinical diagnosis and expression profiling. AEL: acute erythroid leukemia, AMKL: acute megakaryocytic leukemia

### Figure 2: Transcriptional profiling of leukemia with *NUP98* rearrangement

tSNE analysis showed global similarity of *NUP98-NSD1* AMLs with AMLs with *NPM1* or *UBTF* mutations with high *HOXA-B* cluster gene expression, and *NUP98-KDM5A* with other AMKL/AELs showing high expression of megakaryocytic gene expression. However, remaining *NUP98*-rearranged leukemia are also found in monocytic AML cluster or undifferentiated/T-ALL clusters, showing the heterogeneity of the *NUP98*-rearranged leukemia.

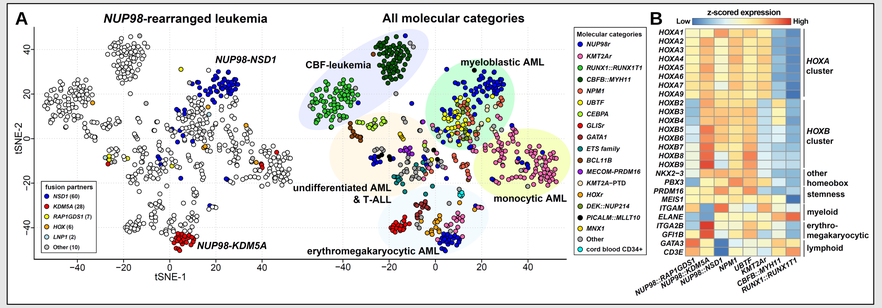



**Figure 2.** Transcriptional analysis using *NUP98*-rearranged leukemia (N=113) and AMLs from published cohort[4] (n=501)
**A.** tSNE analysis. Dots represent samples, and colors represent *NUP98* fusion partners (left) or molecular categories (right).
**B.** Heatmap showing averaged expression of representative *HOX* cluster genes, other homeobox genes, and differentiation-relate genes.

## Discussion

Although each *NUP98* fusion partner has an association with phenotypes (e.g., *NSD1* and AML, *KDM5A* and AMKL), highly frequent mutations (*FLT3*-ITD or *RB1* loss) suggest that these cooperating mutations are also critical for leukemogenesis, chemoresistance, or lineage choice in each leukemia. We are studying the relationship between cooperating mutations, disease phenotypes, and relapse using multiple diagnosis-relapse paired samples.

At the transcriptional level, *NUP98* leukemias share *MEIS1* or the *HOXA* cluster gene expressions, whereas these leukemias show various expressions of the *HOXB* cluster genes or differentiation-related genes. We are applying a network analysis to identify core gene regulatory networks in *NUP98* leukemia which can be its genetic dependencies.

References: 1. Michmerhuizen NL, et al. Blood. 2020 Nov 12;136(20):2275-2289 2. Khoury JD, et al. Leukemia. 2022 Jul;36(7):1703-171 3. Struski S, et al. Leukemia. 2017 Mar;31(3):565-572 4. Umeda, et al. Blood Cancer Discov. 2022 May 5;3(3):194-207 Funding: NIH U54CA243124