A pediatric patient with acute leukemia of ambiguous lineage with a NUP98-NSD1 rearrangement

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Clinical History

- 11 year old girl presented (to outside facility) with pharyngitis, fatigue and decreased appetite.
- WBC 169, Hgb 9.3, Plt 50
Workup at outside institution

- Peripheral blood flow cytometry reportedly showed a blast population (71% of events) with the following phenotype:

  CD34+, CD117+, CD33+, CD13-, CD11c+, CD64(subset)+, HLA-DR+, TdT+, MPO- and small subset CD19+.

  Abnormal granulocytic maturation: downregulation of CD10, CD11b, and CD16

- Karyotype: 46,XX; Negative FISH using probes for 5q33 (CSF1R, RPS14), 7q31 (MDFICx2), RUNX1T1/RUNX1, 11q23 (KMT2Ax2), PML/RARA, 16q22 (CBFBx2)

- Positive for FLT3-ITD (allelic ratio reportedly 0.519).

- Diagnosis: AML WITH TDT EXPRESSION

- Therapy was initiated per COG AML protocol

- Following therapy, patient was found to have low level MRD.
Bone marrow transplant

- Allogeneic stem cell transplantation (from fraternal twin sister).
- Bone marrow evaluation on day 35 after BMT showed hypocellular marrow with patchy maturing trilineage hematopoiesis and no overt evidence of disease.
- Persistent cytopenias 5 months after transplant:

  CBC: 0.59>10.3/29.1<19 MCV 81.3

  Diff: PMN 41, Band 3, Lymph 25, Mono 24, Eos 1, Meta 1, Myelo 1, Blasts 4.
Bone marrow aspirate
Flow cytometry at our institution
## Immunophenotype

<table>
<thead>
<tr>
<th></th>
<th>Diagnosis</th>
<th>Relapse</th>
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<tbody>
<tr>
<td><strong>Myeloid:</strong></td>
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</tr>
<tr>
<td>CD33</td>
<td>partial +</td>
<td>partial +</td>
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<tr>
<td>CD64</td>
<td>+</td>
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<tr>
<td>CD11c</td>
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<tr>
<td>CD13</td>
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<td>MPO</td>
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<tr>
<td>CD14</td>
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<tr>
<td>Lysozyme</td>
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<tr>
<td>CD68 PGM1</td>
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<tr>
<td>CD68 KP1</td>
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<tr>
<td><strong>B lineage:</strong></td>
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<tr>
<td>CD19</td>
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<tr>
<td>CD79a</td>
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<tr>
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<tr>
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<td>PAX5</td>
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Cytogenetic analysis

G-band karyotype:
46,XX,del(5)(q22q34)[1]/46,idem,t(8;17)(q21;q22),der(14)t(1;14)(q21;q24)[1]/46,idem,t(8;17)(q21;q22),der(14)t(1;14)(q21;q24),add(22)(q13)[15]/46,XX,t(7;12)(p12;p13)[2]/46,XX,t(7;17)(p12;q25)[3]/46,XX,t(7;17)(p12;q25),t(2;8)(p13;q21)[1]/46,XX,del(9)(p22)[1]/46,XX,t(8;11)(p21;q22)[1]/46,XX,complex[1]/46,XX[2]

The stemline showed deletion 5q in one metaphase.

One sub-clone showed an additional unbalanced translocation between 1q and 14q (resulting in gain of 1q and partial deletion of 14q) and a balanced translocation between 8q and 17q in one metaphase.

Second sub-clone showed additional material added on 22q (in addition to the changes noted in sub-clone 1) in fifteen metaphases.

Distinct clone #1 showed a balanced translocation between 7p and 12p in two metaphases.

Distinct clone #2 showed a balanced translocation between 7p and 17q in three metaphases.

Other distinct clones showed non-clonal abnormalities (one metaphase each).
FISH analysis using EGR1/5p15 probes showed 5q31 deletion in 15.5% cells.

FISH analysis using p16/CEP 9 and ETV6 break-apart probes showed no evidence of clonal abnormality.
Whole exome and transcriptome sequencing:

Fusion of *NUP98* with *NSD1* was detected. Both, *NUP98-NSD1* and *NSD1-NUP98* transcripts were seen.

**Other tier 2 changes included:**

Loss of function frameshift mutation of *WT1* (R370fs; VAF 40%)

Gain of function *PTPN11* missense mutation (E76K; VAF 54%)
NUP98-NSD1

• Sometimes cytogenetically cryptic translocation involving nucleoporin 98kD (NUP98) on chromosome 11p15 and nuclear receptor-binding SET domain protein 1 (NSD1) on chromosome 5q35.

  ![Gene expression profile](image)

  **NUP98 exon 12**  **NSD1 exon 6**  
  TTTGGAGCCTCCCAGGCCCCAGTAGCCTGTCGGTCAGAAGAAAACGCTT


• Interacts with MLL1 and NSL histone-modifying complexes at Hox gene promoters. Oncogenic activity relies on MLL1 (KMT2A).

• Identified in 2-5% of AML

• Associated with poor prognosis

• Enriched in FLT3/ITD+ cases

[Diagram of molecular interactions](image)

**Xu et al, *Cancer Cell*, 2016**
NUP98-NSD1 gene expression signature

Transcriptome sequencing:

High to moderate expression of HOXA9, HOXA10, HOXB3, HOXB2, HOXB4, HOXB5, HOXB6, PRDM16, and MEIS1

→ NUP98-NSD1 gene expression signature associated with poor prognosis

NUP98-NSD1 gene expression signature

Shiba et al, Genes Chr Cancer, 2013
**NUP98-NSD1** and **FLT3/ITD**


Younger age, higher WBC, higher platelet count

High rate of induction failure—requirement of alternative therapy

Recommendation: Screening, at diagnosis of AML, for cytogenetically cryptic **NUP98-NSD1** (by FISH or PCR).
Follow up:

- Entered clinical trial at outside facility (out of state).
- Patient has since passed away.
Final Panel Diagnosis:

Acute leukemia of ambiguous lineage at relapse (with \textit{NUP98-NSD1})
Interesting Features

- Example of NUP98/NSD1 plus FLT3/ITD in refractory acute leukemia.
- Ambiguous lineage.
- Cytogenetic evolution.
Thank you!