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

SH2017-0203

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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



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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.



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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

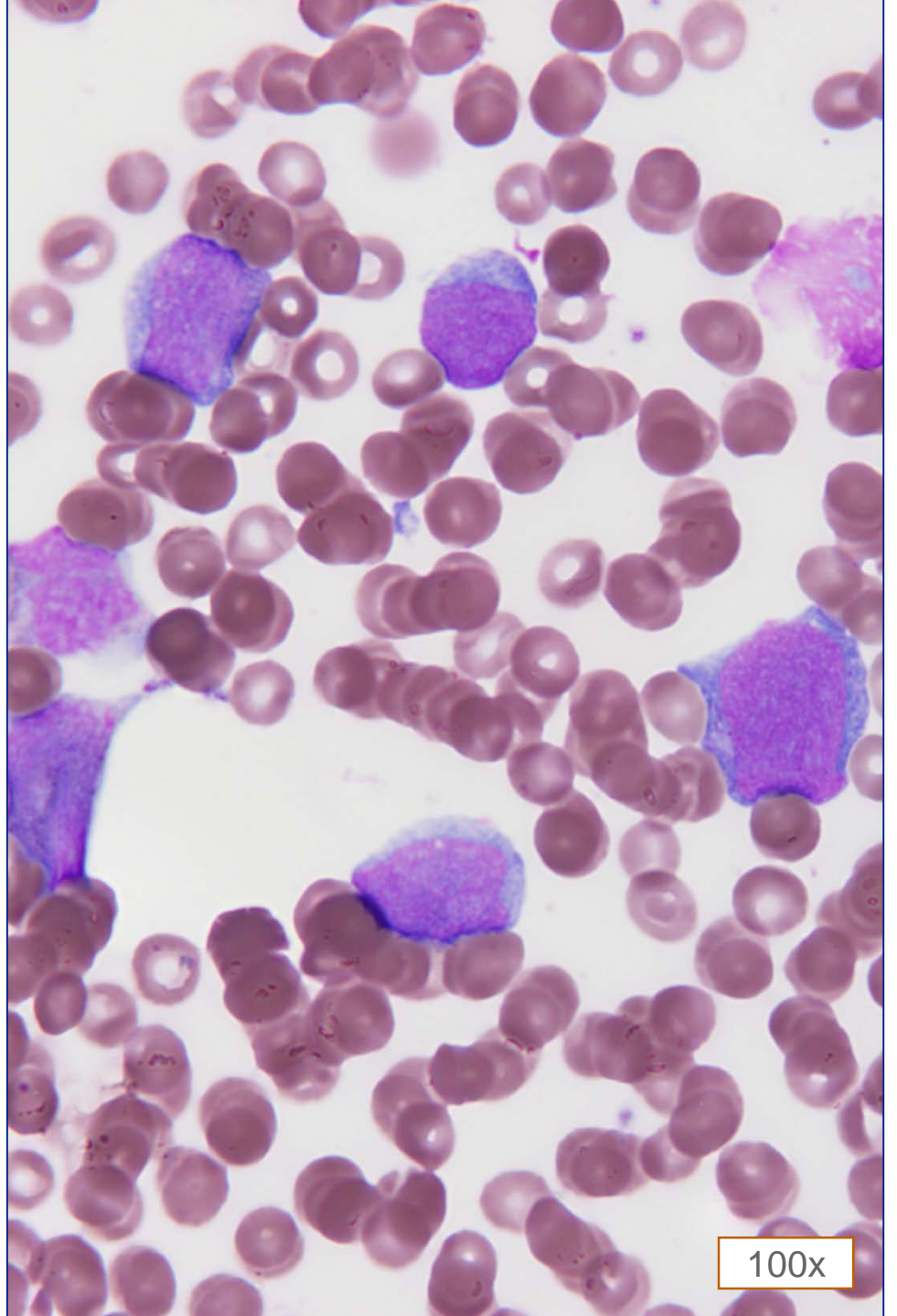
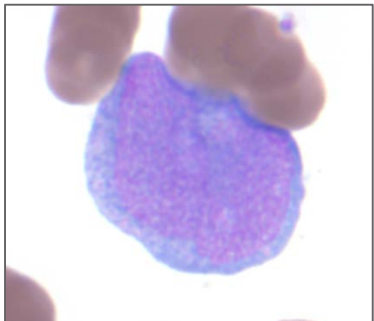
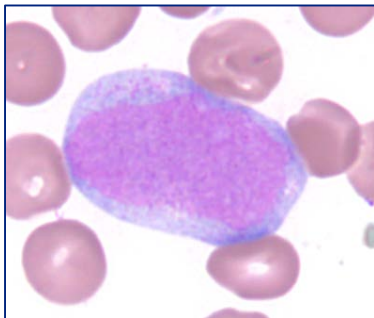
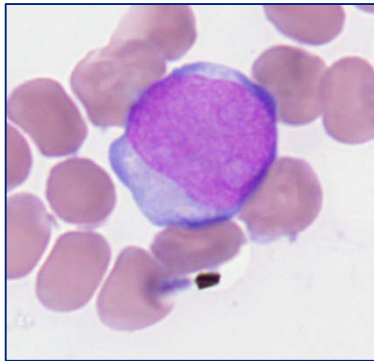
Diff: PMN 41, Band 3, Lymph 25, Mono 24, Eos 1, Meta 1, Myelo 1,
Blasts 4.



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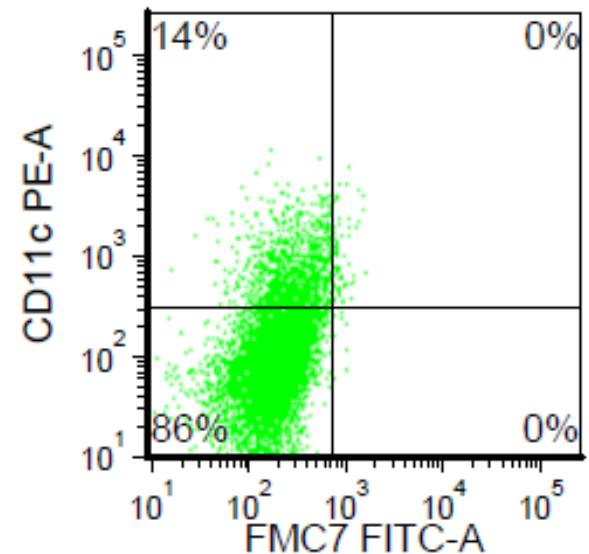
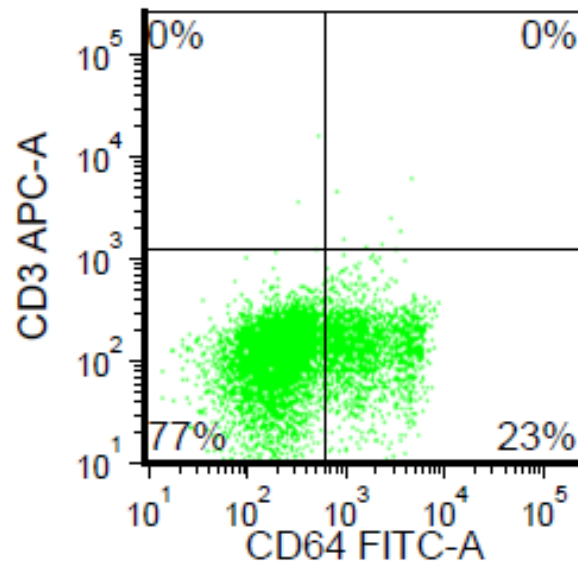
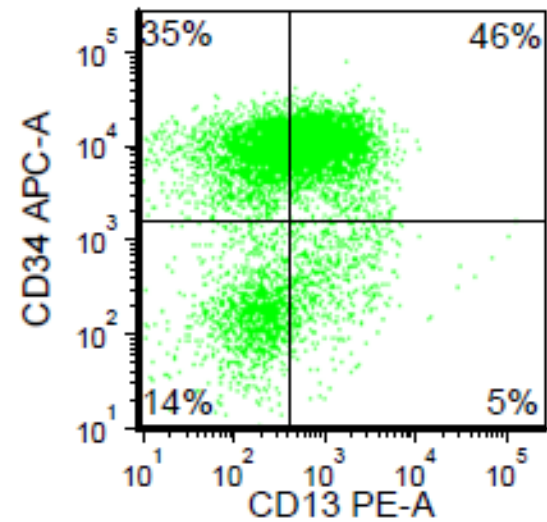
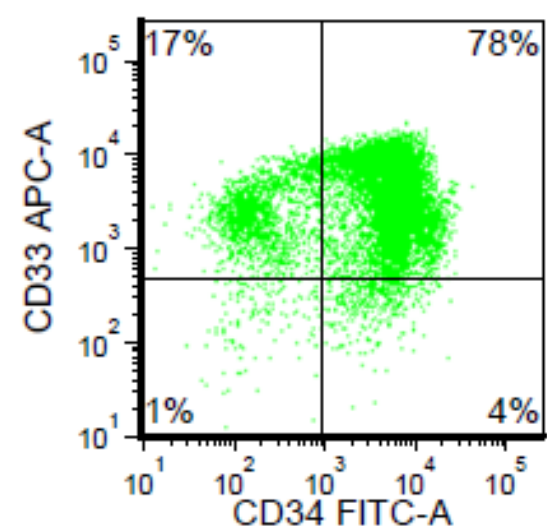
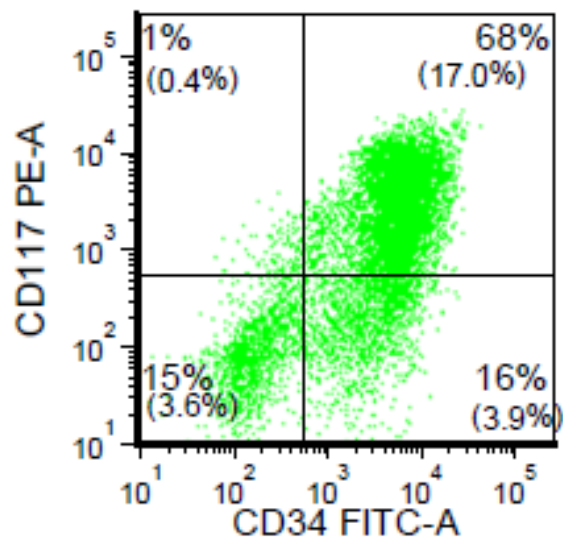
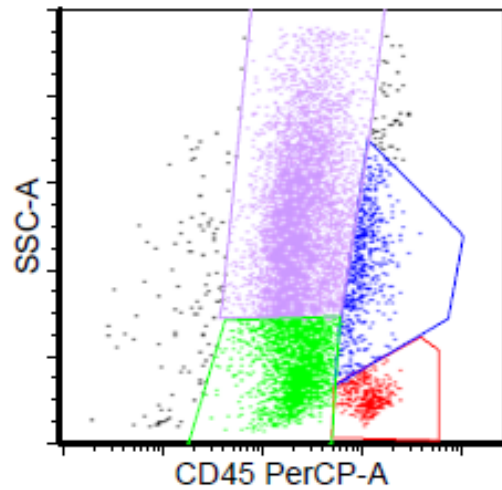
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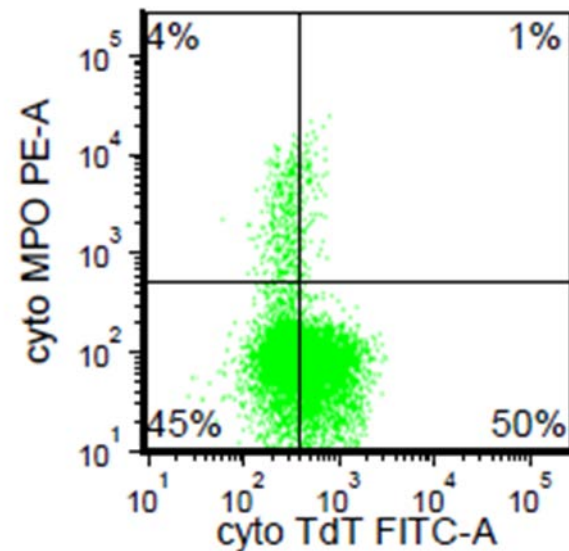
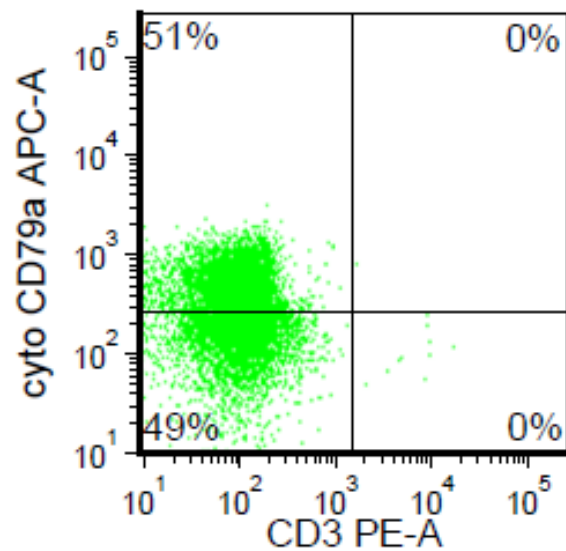
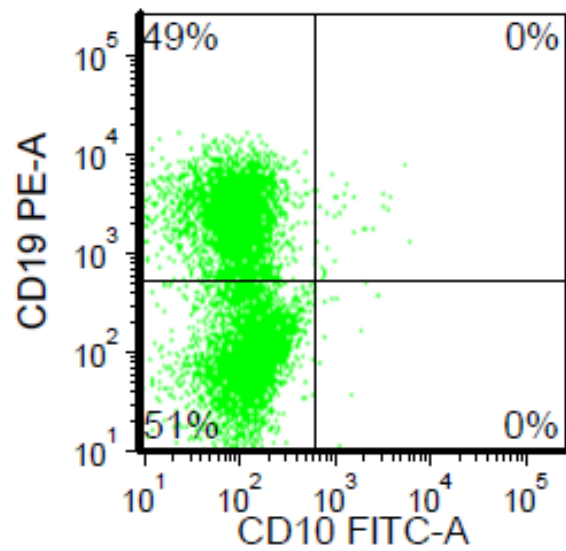
Bone marrow aspirate

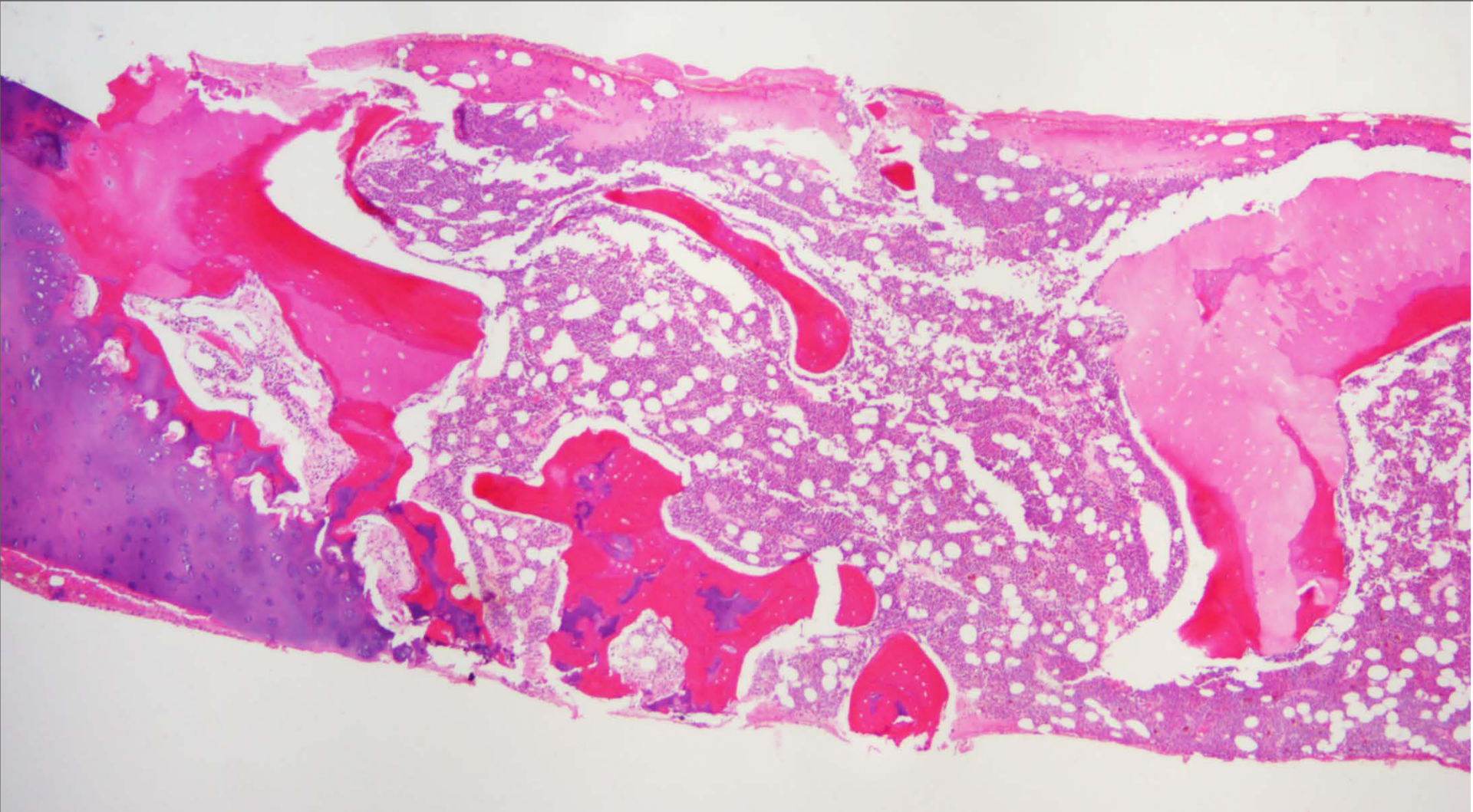


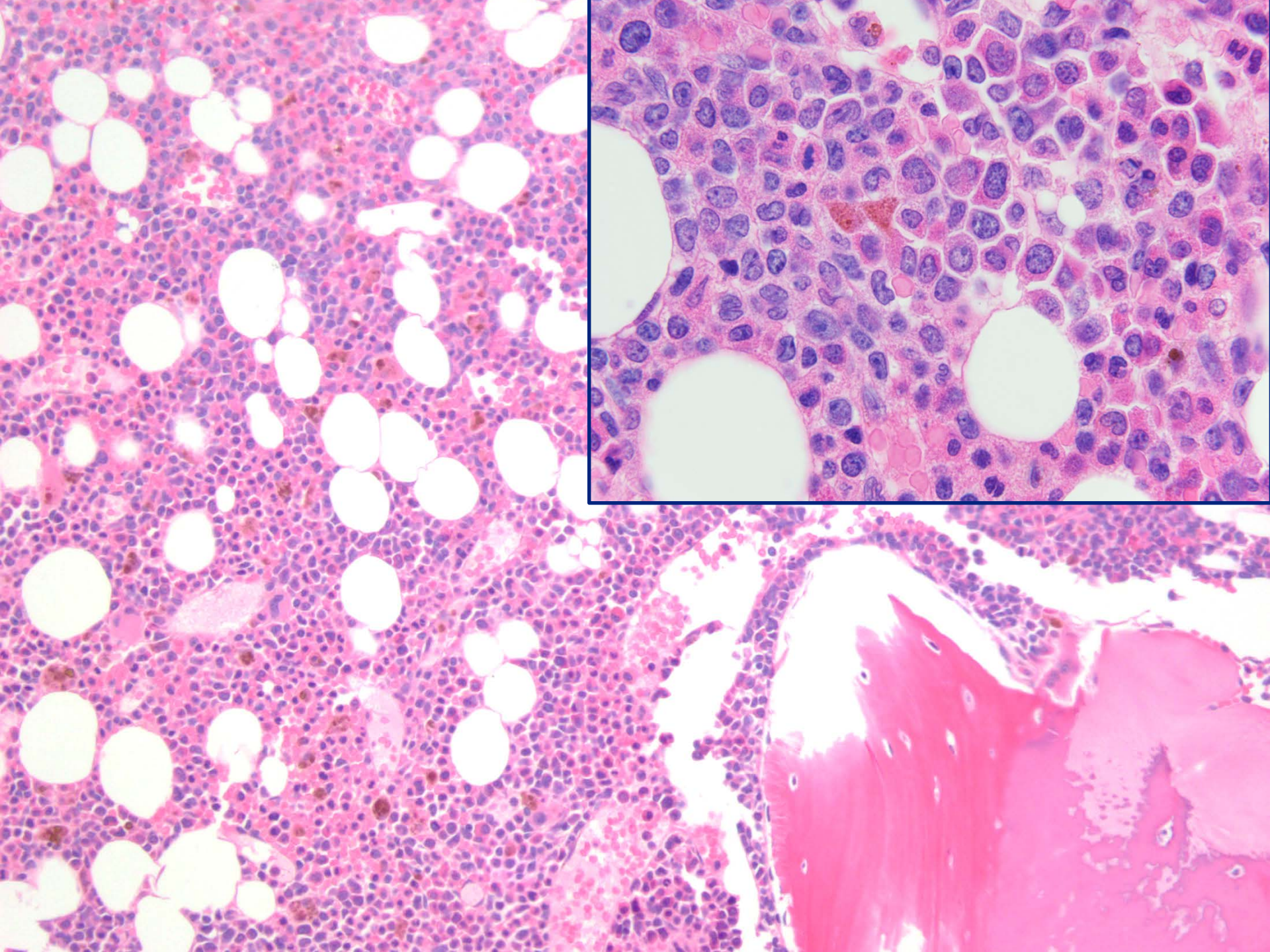
100x

Flow cytometry at our institution

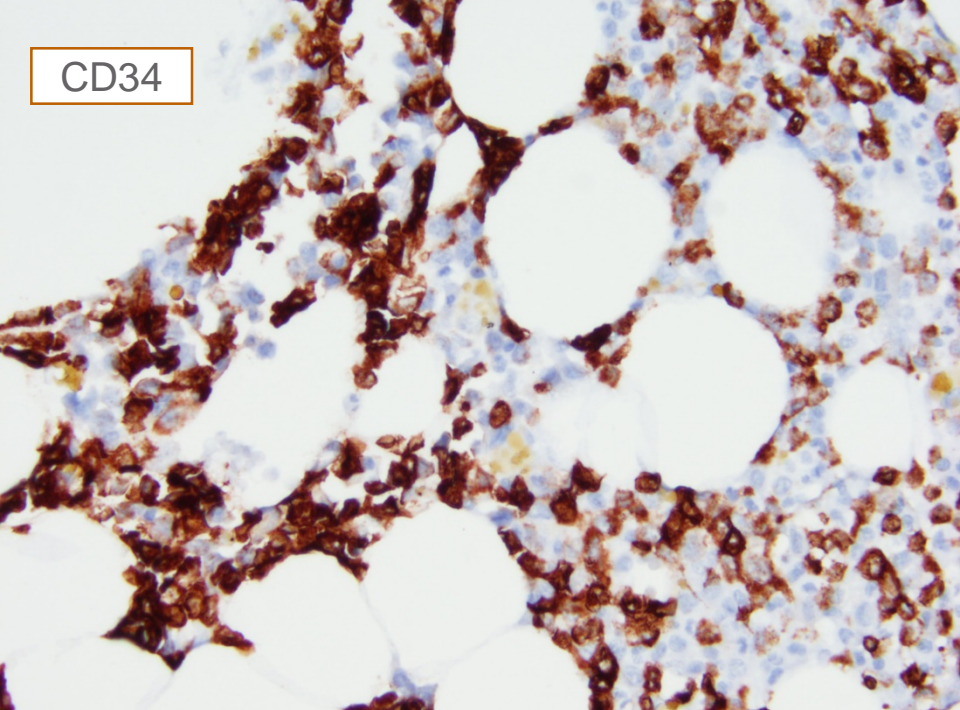




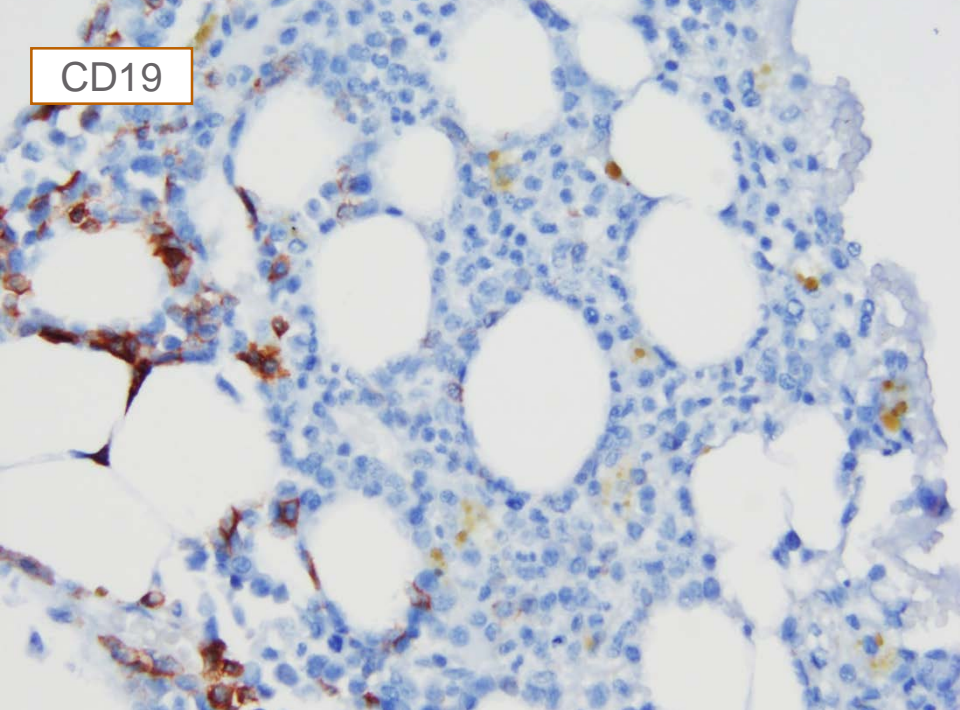




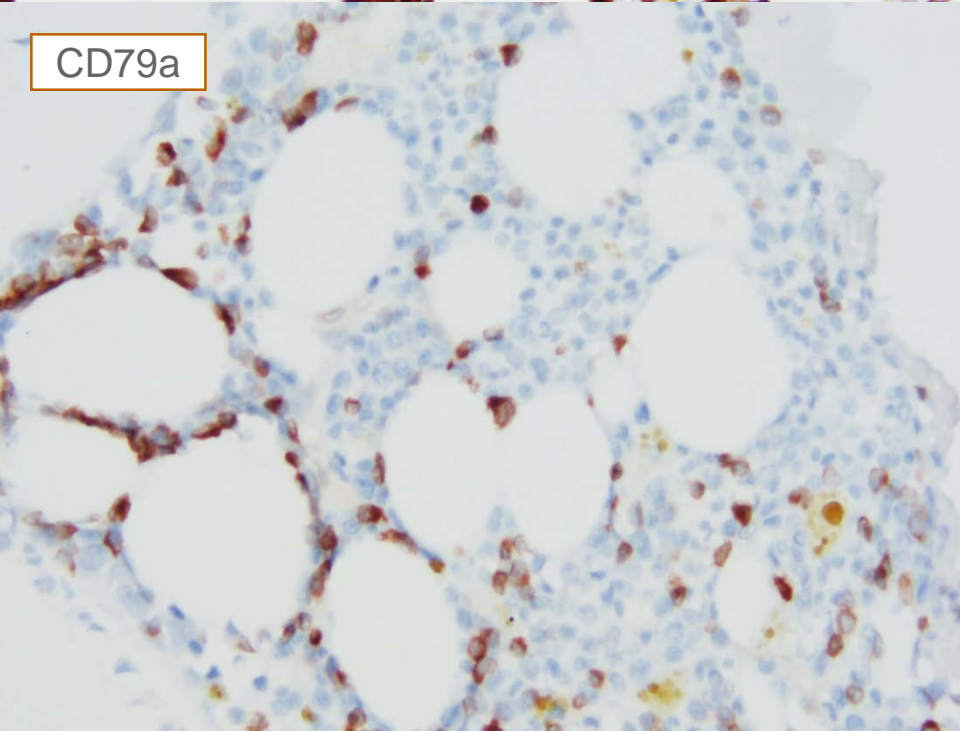
CD34



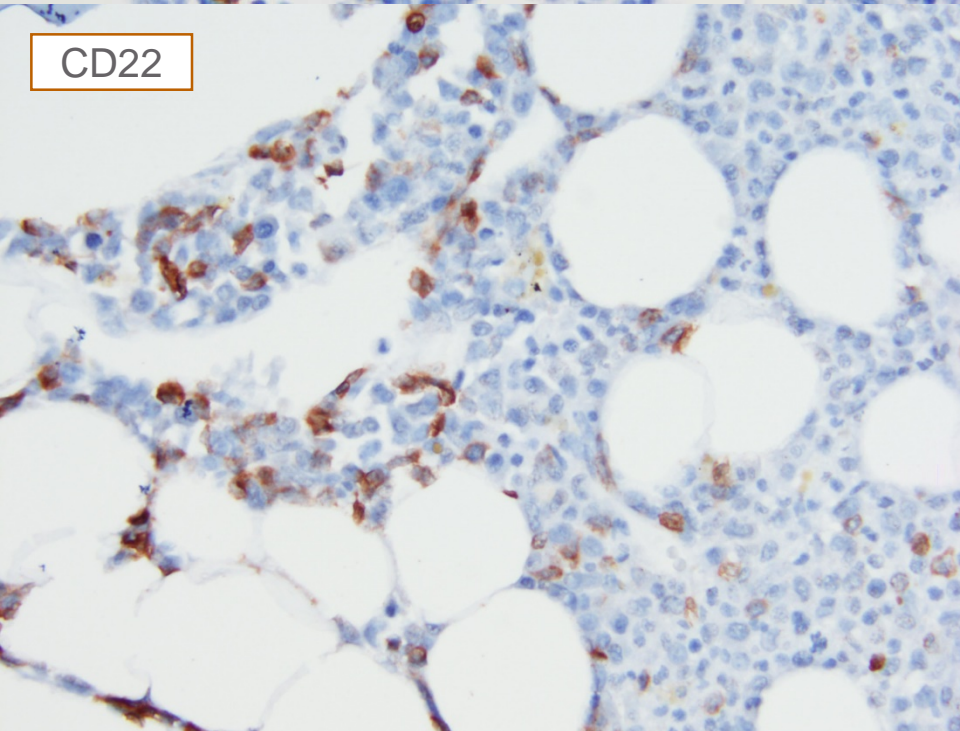
CD19



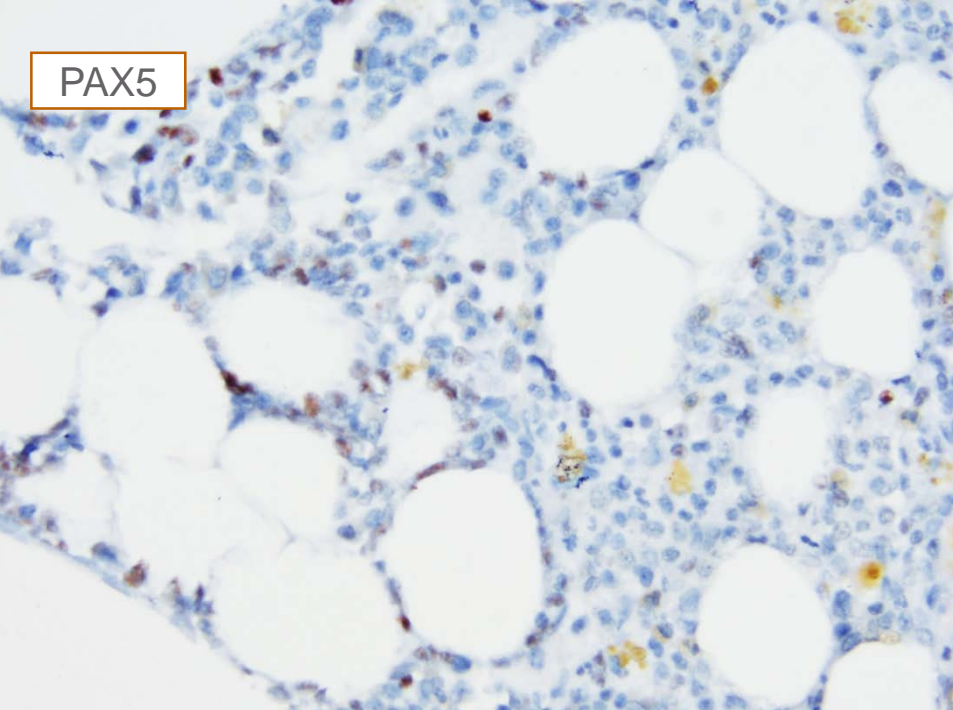
CD79a



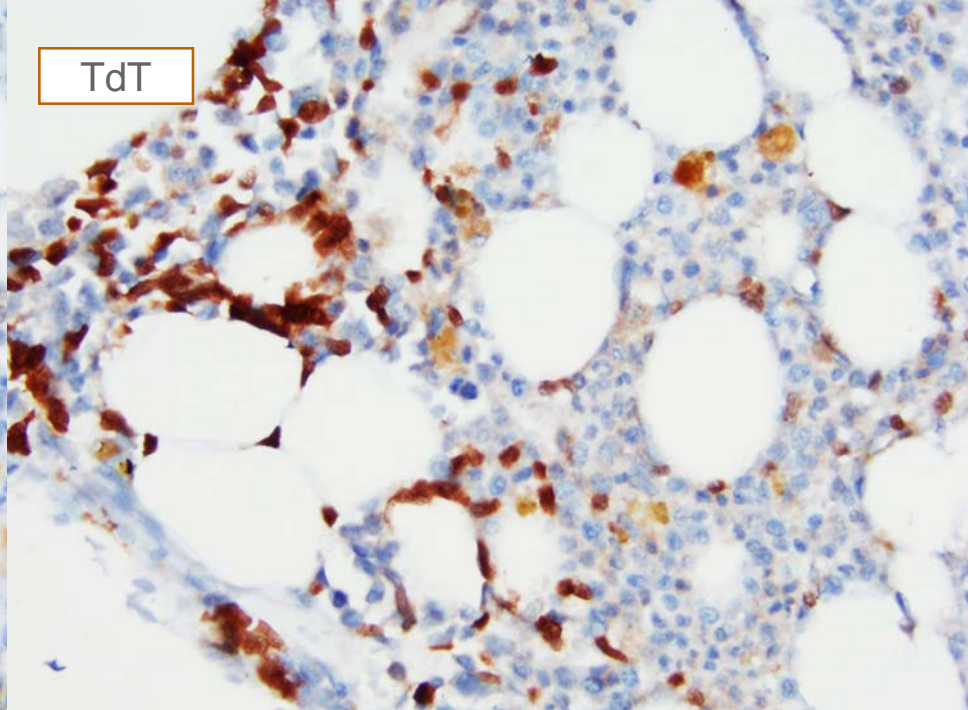
CD22



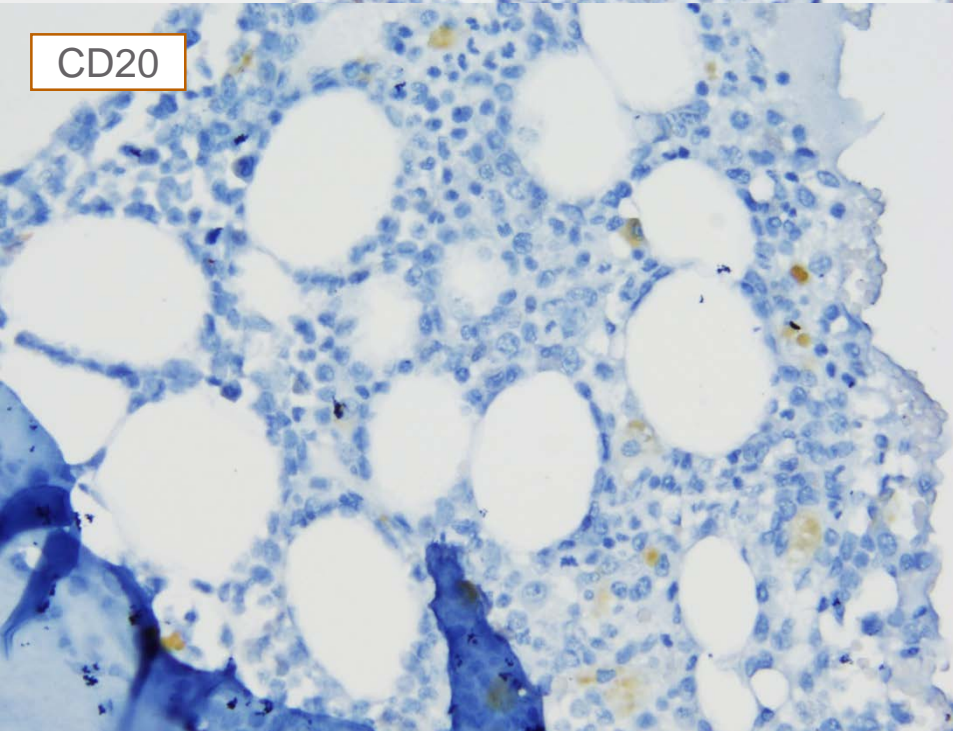
PAX5



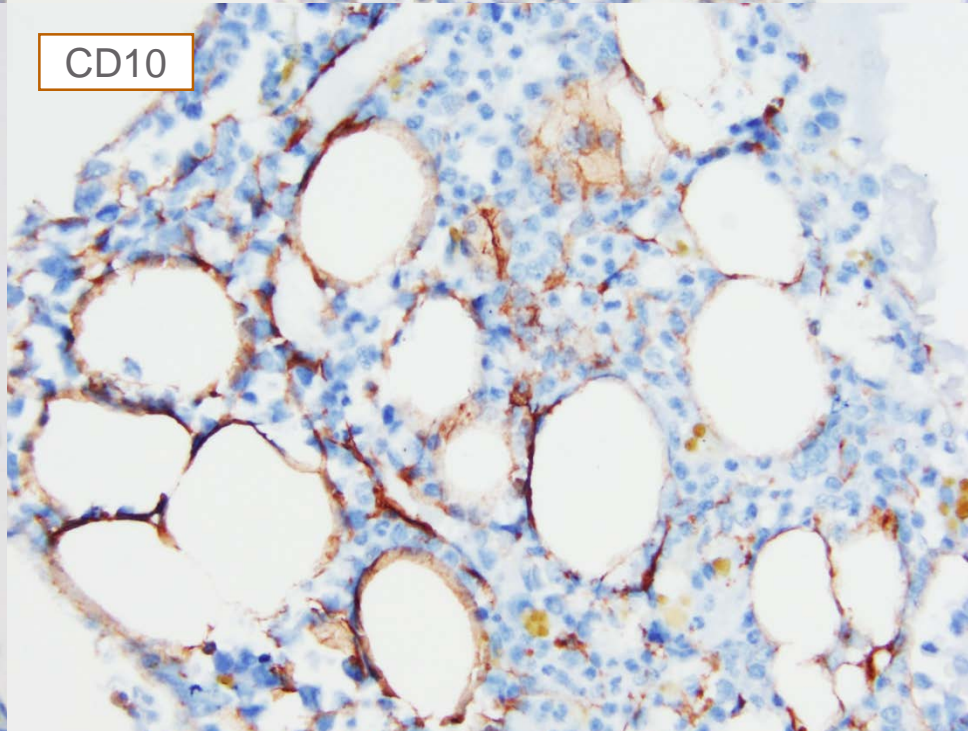
TdT



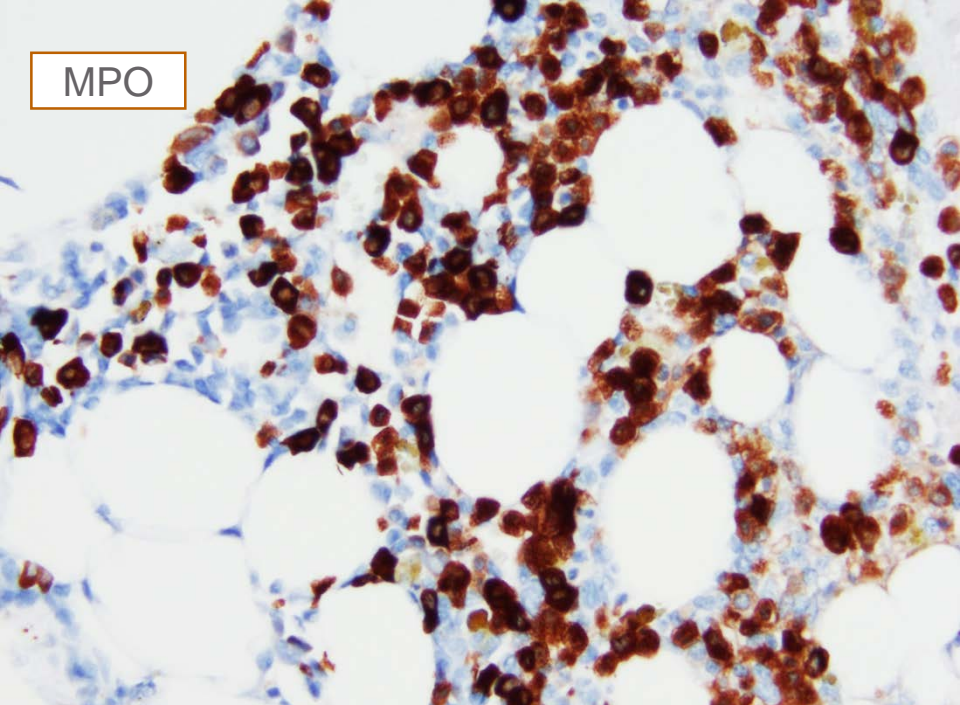
CD20



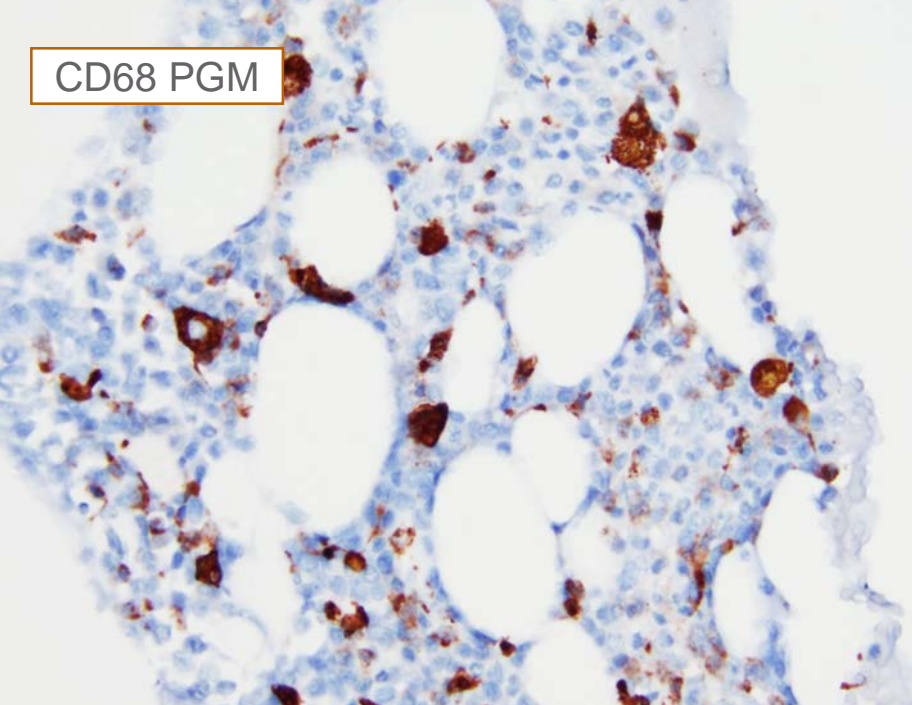
CD10



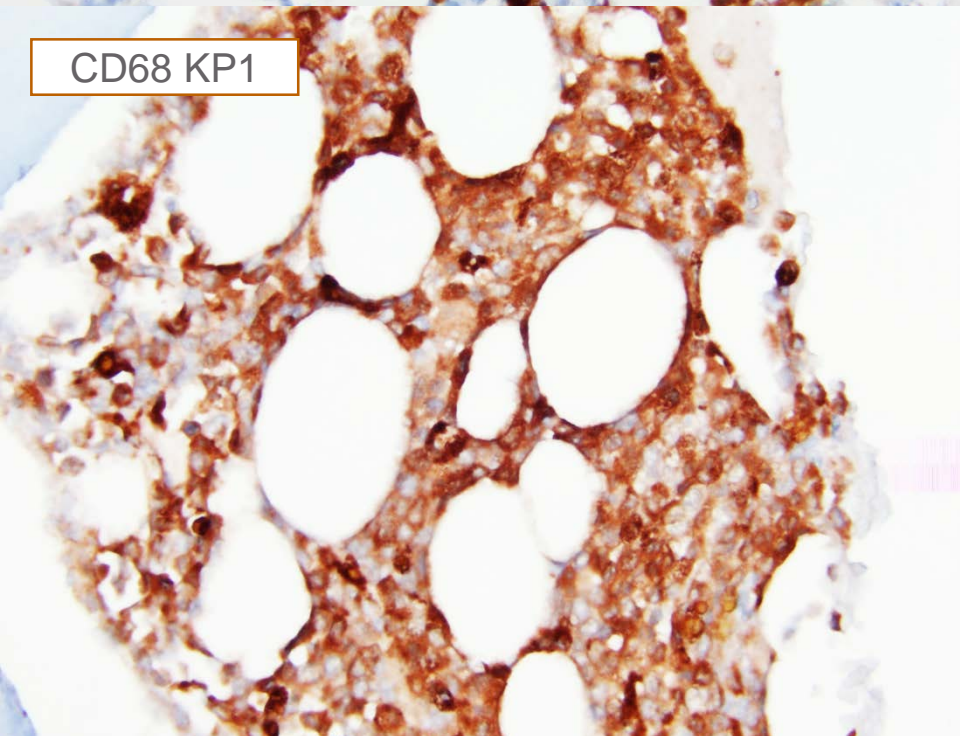
MPO



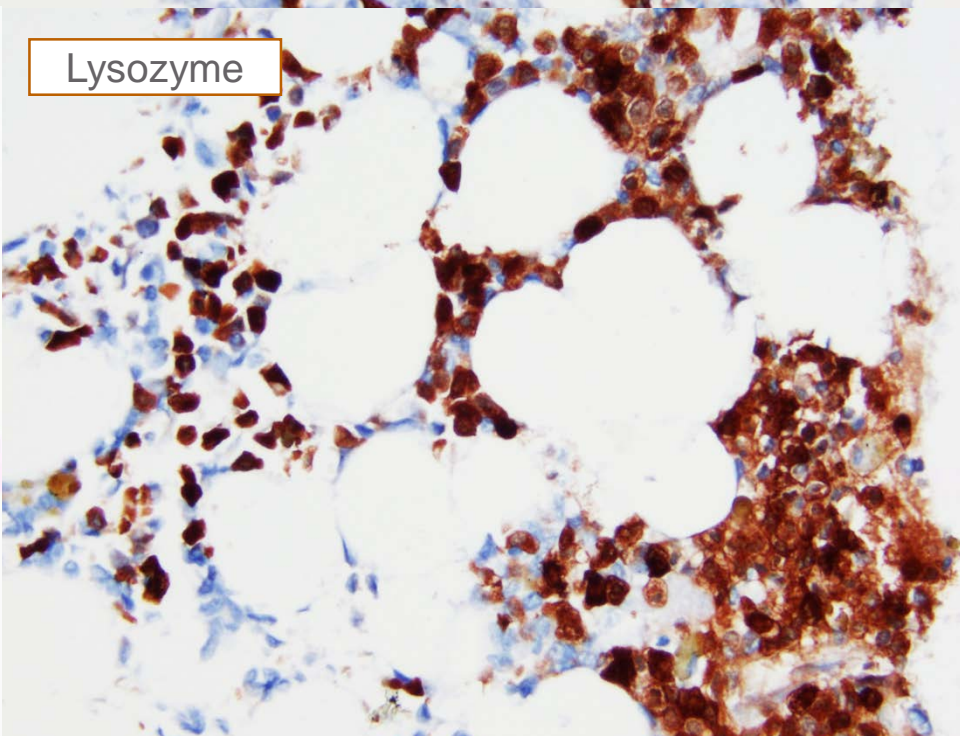
CD68 PGM



CD68 KP1



Lysozyme



Immunophenotype

	Diagnosis	Relapse
<u>Myeloid:</u> CD33 CD64 CD11c CD13 MPO CD14 Lysozyme CD68 PGM1 CD68 KP1	+ partially + + - - - - - - - - - -	+ partially + partially + partially + - - - - - - +
<u>B lineage:</u> CD19 CD79a TdT CD10 CD20 sCD22 FMC7 cCD22 PAX5	partially + - + - - - - - -	partially + partially + partially + - - - - - partially + partially +



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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).



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FISH

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.



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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%)

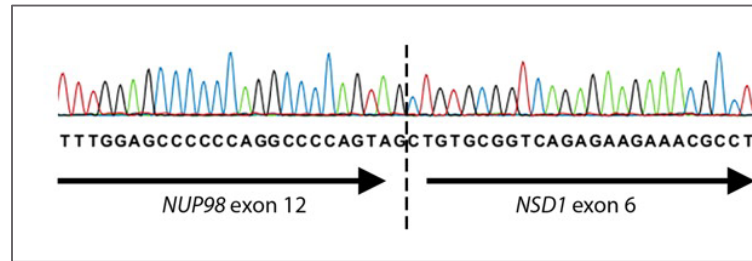


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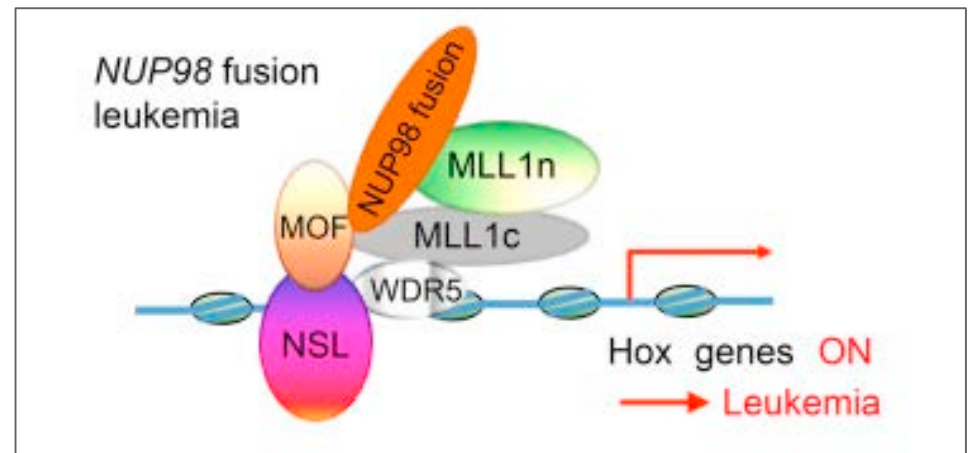
NUP98-NSD1

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



Hollink et al, *Blood*, 2011

- 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



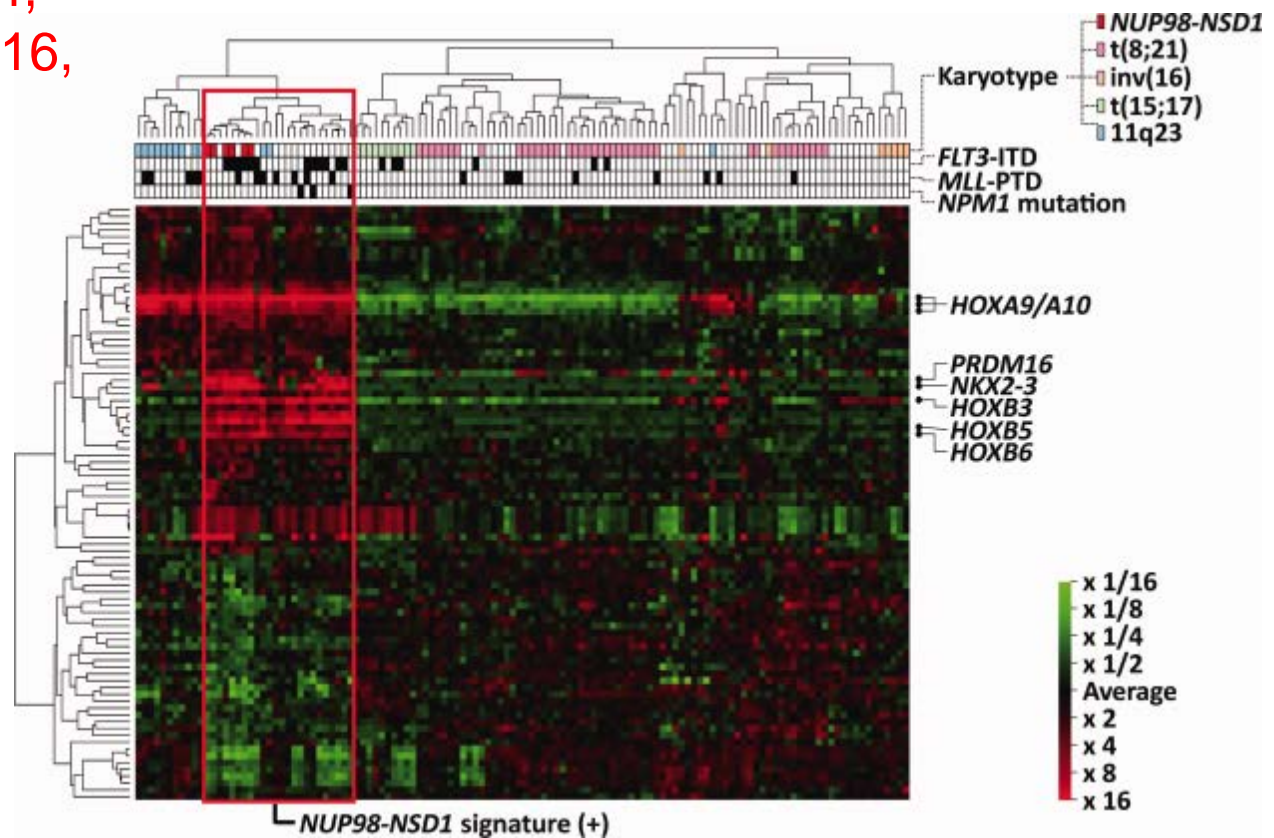
Xu et al, *Cancer Cell*, 2016

NUP98-NSD1 gene expression signature

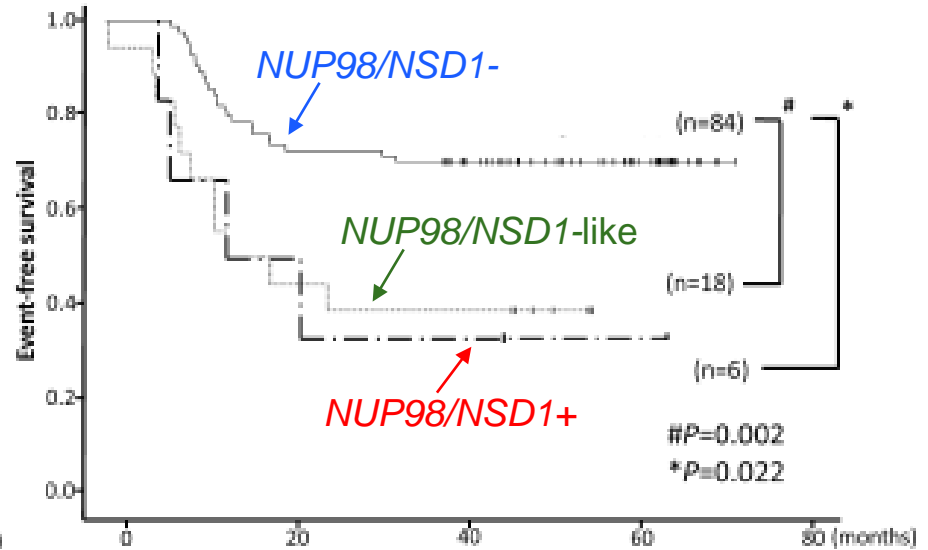
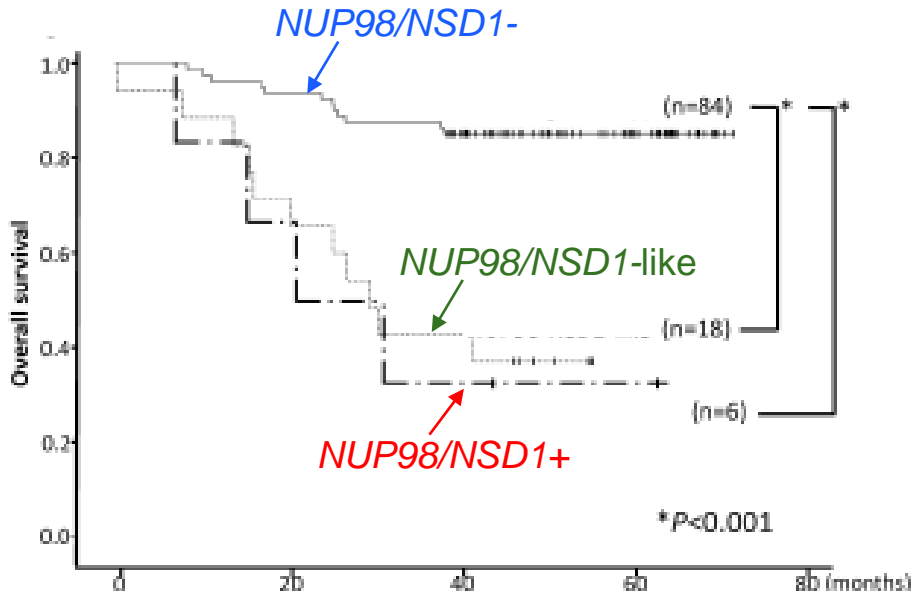
Transcriptome sequencing:

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



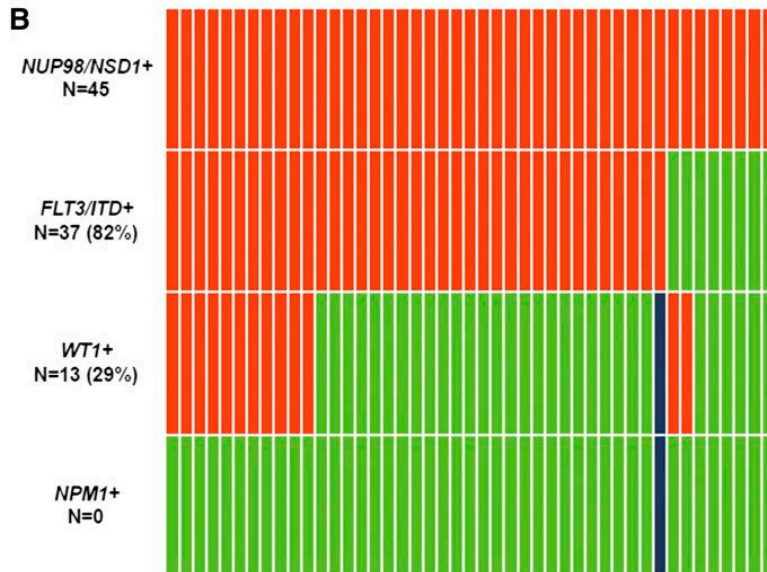
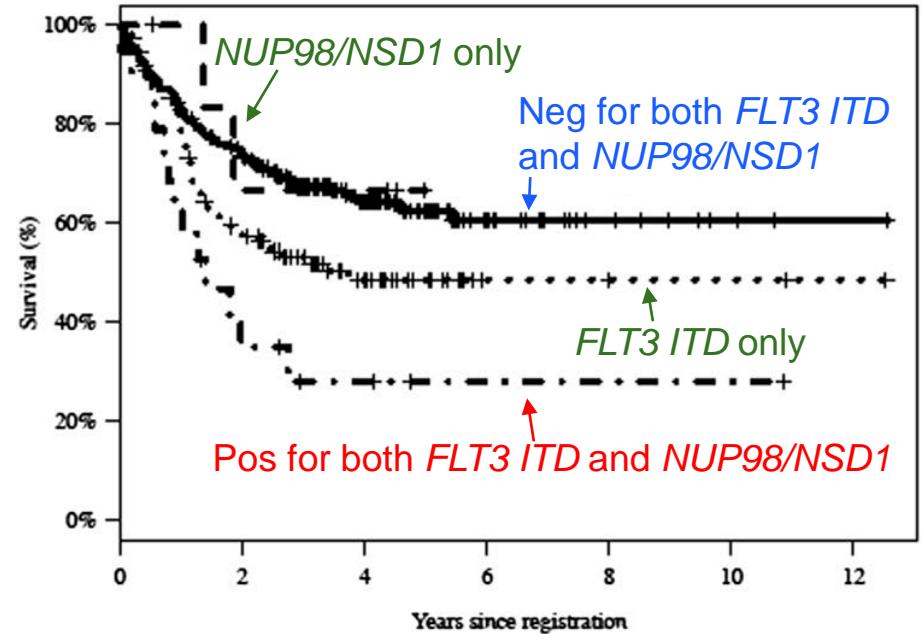
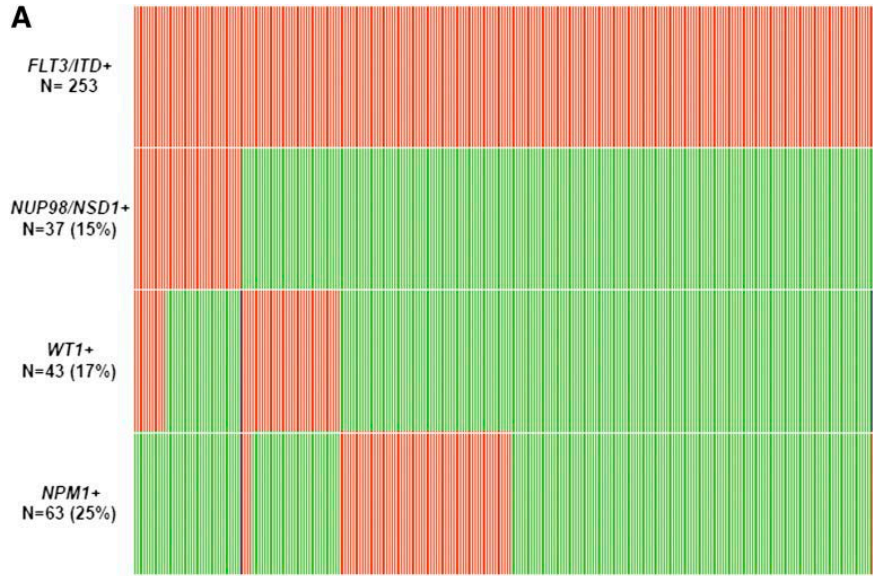
Shiba et al, *Genes Chr Cancer*, 2013



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NUP98-NSD1 and FLT3/ITD



Ostronoff et al, *Blood*, 2014

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.



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Final Panel Diagnosis:

Acute leukemia of ambiguous lineage
at relapse (with *NUP98-NSD1*)



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Interesting Features

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



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Thank you!