Multi-Institutional Hematopathology Interesting Case Conference
Case 1

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Hematopathology Fellow
3/11/2026

Mount Sinai

Disclosure

I have nothing to disclose

Clinical Information

History of Present Illness:

- 5-year-old female presented to ED with fatigue, easy bruising, pallor, bilateral lower extremity shin pain for the past several weeks.
- Parents also reported loss of appetite for the last several days.
- Denies any fevers, chills, rashes, upper respiratory tract infection, gastrointestinal symptoms.

- **Past Medical History:**
  - Born prematurely with a NICU admission requiring several blood transfusions for an unknown etiology.
  - The patient met developmental milestones at the expected ages.

Laboratory Findings

Test	Result	Reference Range
WBC	6.5 K/µL	5.3 – 12.5 K/µL
Hemoglobin (Hg)	6.8 g/dL	10.5 – 13.8 g/dL
MCV	90.8 fL	72.4 – 94.3 fL
Platelets (Plt)	4 K/µL	150 – 450 K/µL

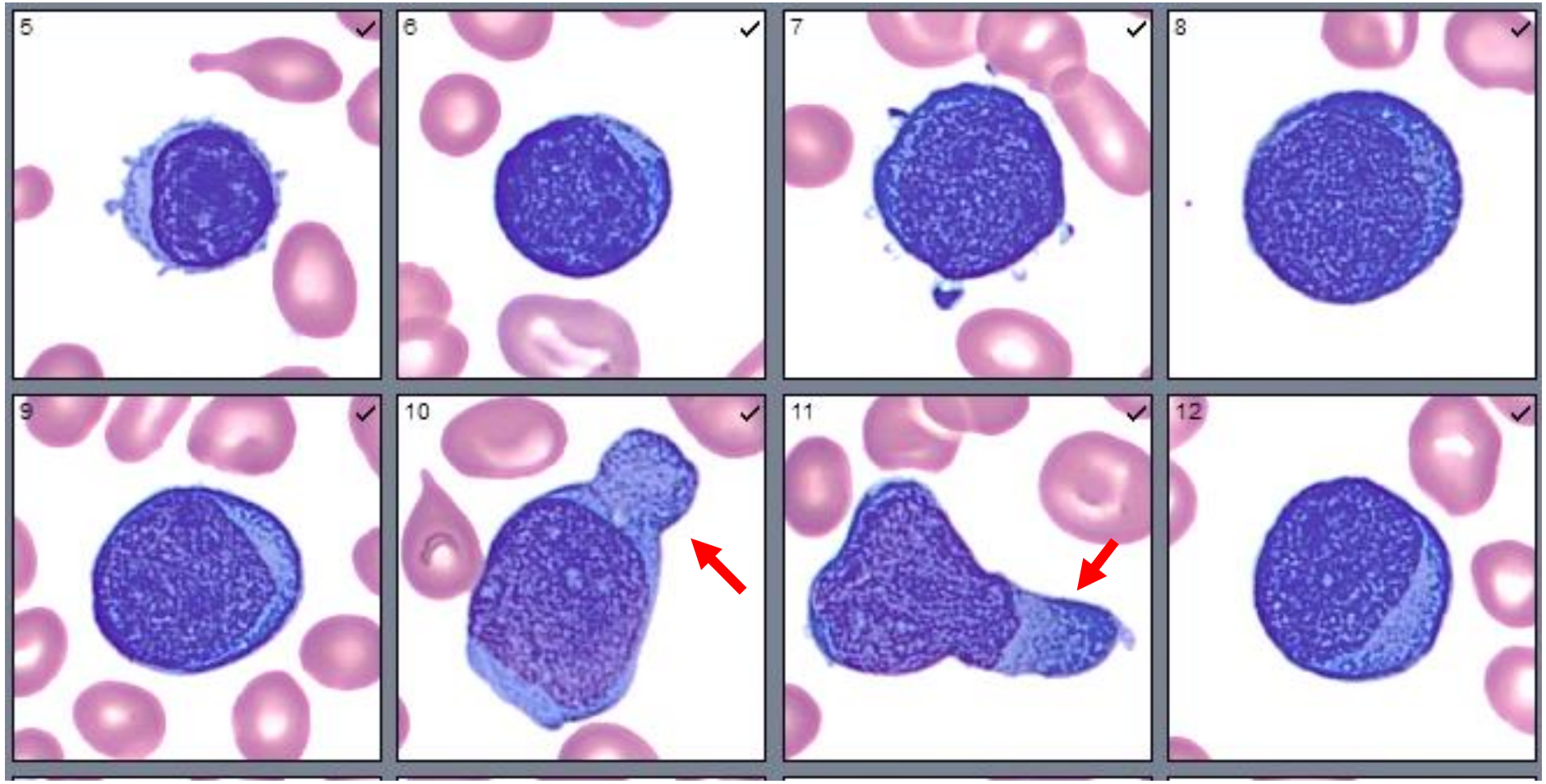
Electrolytes were done --> Normal

Coagulation studies (PT, INR, PTT, D-dimer, fibrinogen) were all normal

LDH: 630 U/L

Parameters	Value (%)
Neutrophil	52.8
Lymphocyte	39.2
Monocyte	4.4
Eosinophil	1.0
Basophil	0.0
"Atypical Lymphocytes"	"2.6"

Peripheral blood smear



Differential Diagnosis

Infectious (viral) changes

Benign nocturnal limb pains of childhood (growing pains)

Non- hematolymphoid

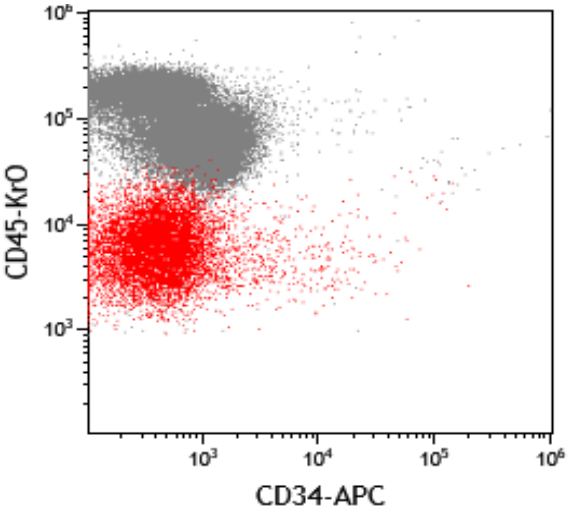
- Neuroblastoma
- Sarcoma
- Other circulating tumor cells

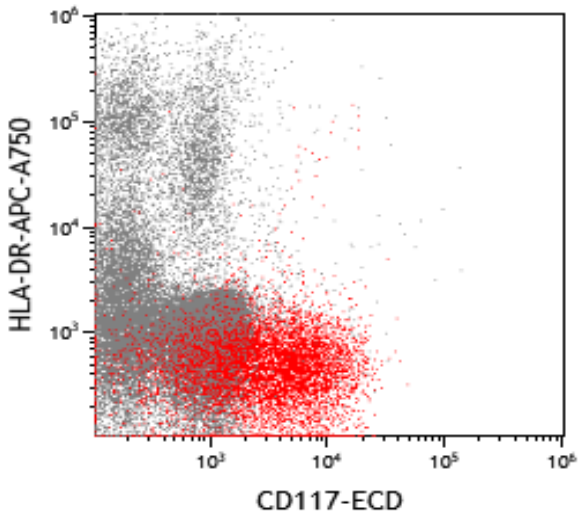
Hematolymphoid

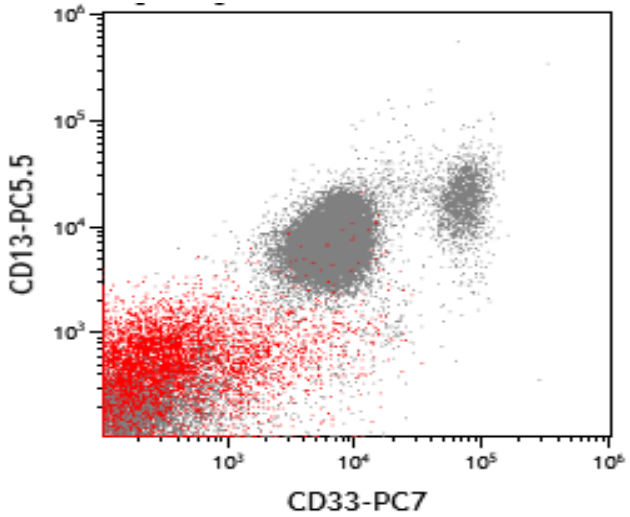
- Lymphoma (B-lineage vs T-lineage)
- Other myeloid neoplasm (MDS, MPN, MDS/MPN)
- Mast cell neoplasm
- Acute leukemia (myeloid vs lymphoid vs mixed)

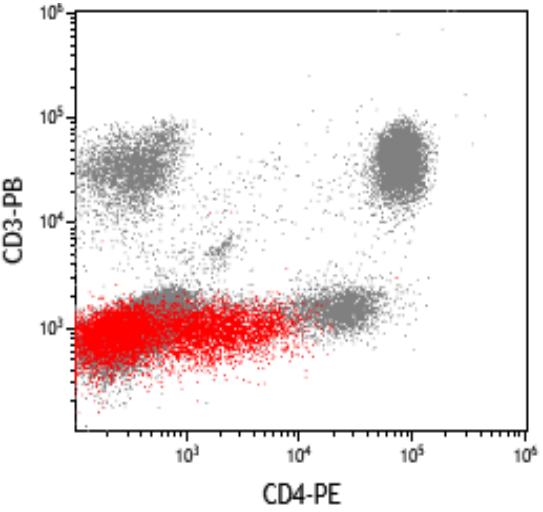
Peripheral Blood Flow Cytometry

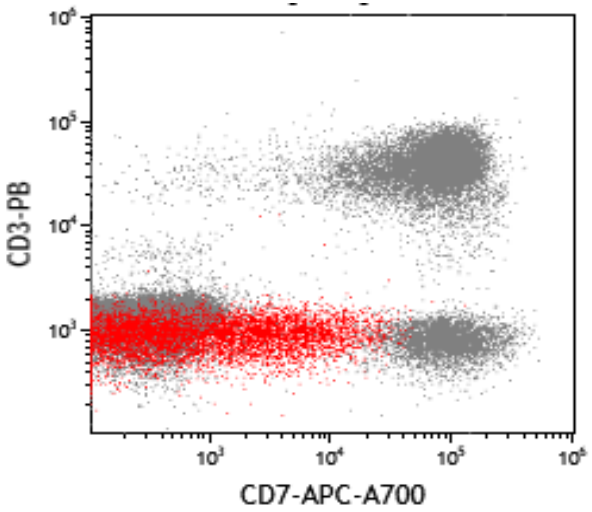
A LARGE POPULATION OF ATYPICAL CD117+CELLS, 15% OF TOTAL.

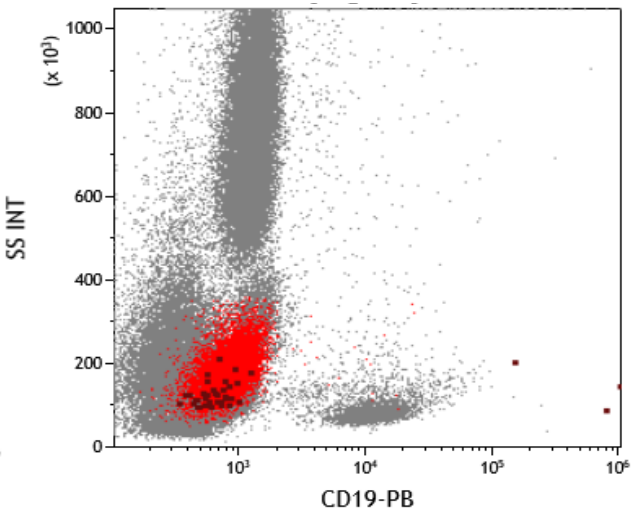












POSITIVE	NEGATIVE
CD117	CD45-/dim
CD7 partial/dim	CD34
CD4 partial/dim	HLA-DR
CD33 partial dim	CD3
	CD13
	CD56
	All other markers are Negative.

Peripheral blood flow cytometry -

A LARGE POPULATION OF ATYPICAL CD117+CELLS, 15% OF TOTAL.

The differential diagnosis of these atypical cells includes:

1) Megakaryocytic lineage
2) Erythroid lineage
3) Circulating non-hematopoietic neoplastic cells.

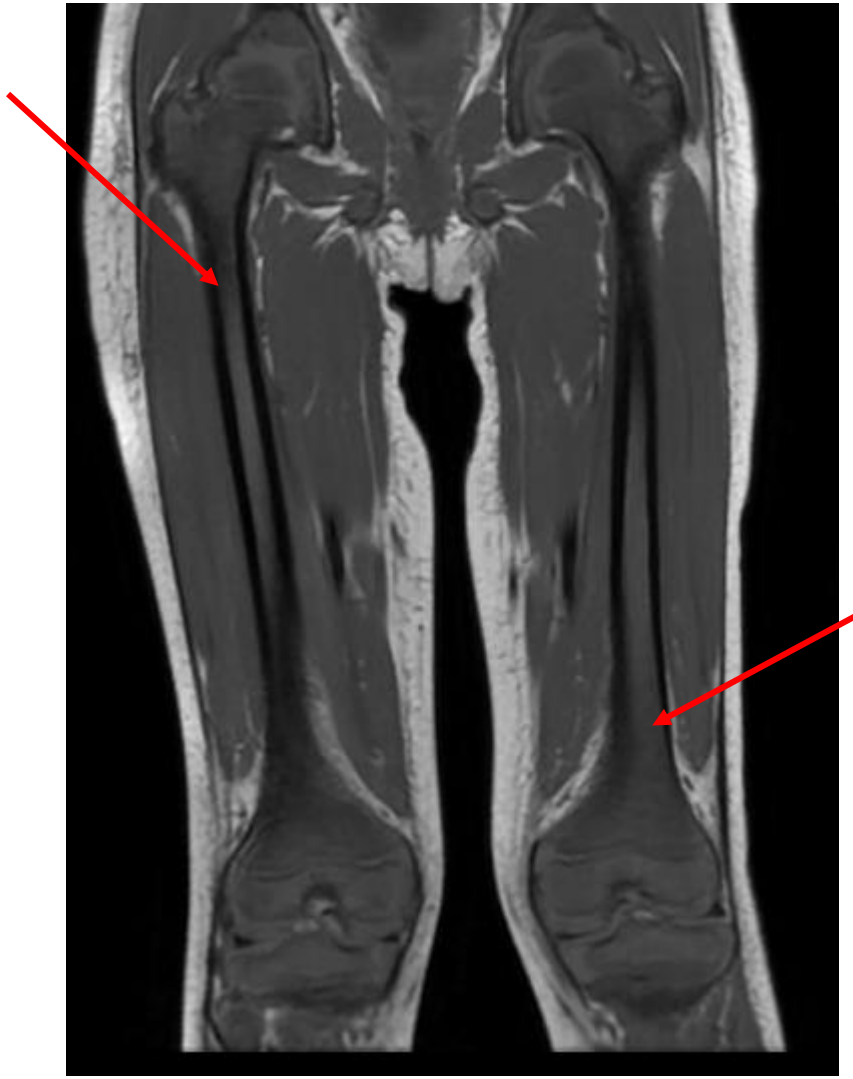
POSITIVE	NEGATIVE
CD117	CD45-/dim
CD7 partial/dim	CD34
	HLA-DR
CD4 partial/dim	CD3
	CD13
	CD56
	All other markers are Negative.

CD42b, CD61 and CD71 markers are not performed on Flow Cytometry

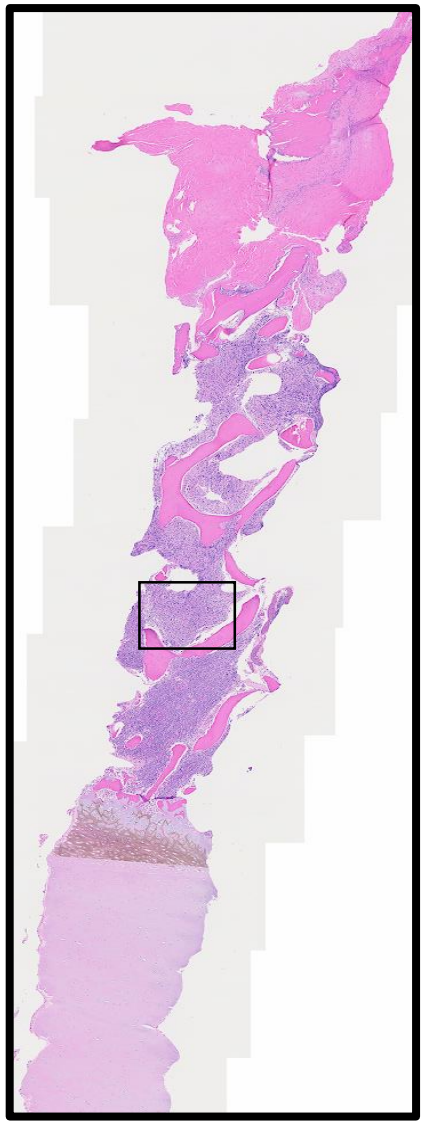
Radiology/Imaging

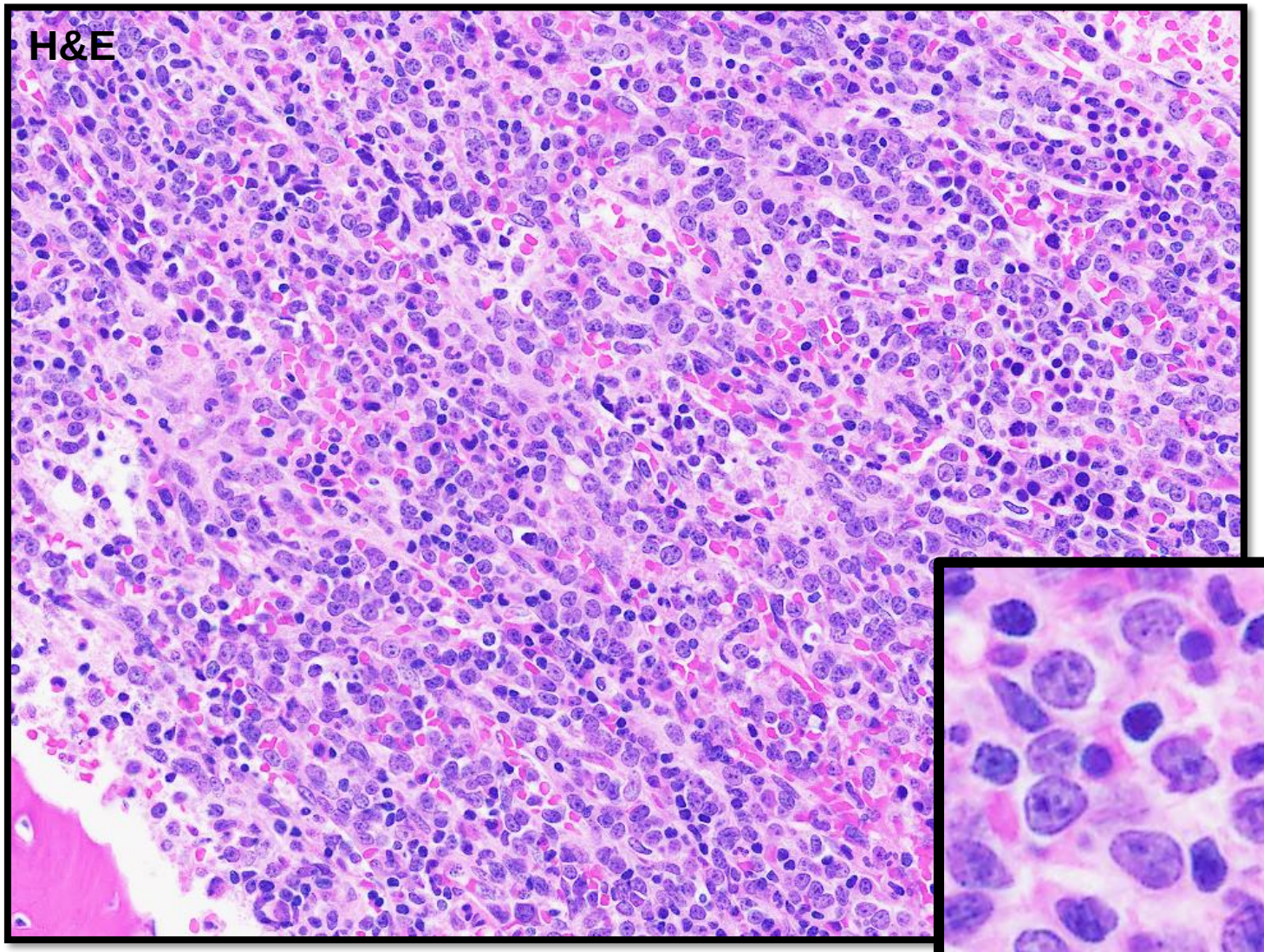
- Chest X-ray --> Negative for Mediastinal Mass
- X-ray Tibia/ Fibula → Negative
- MRI of Lower Limbs

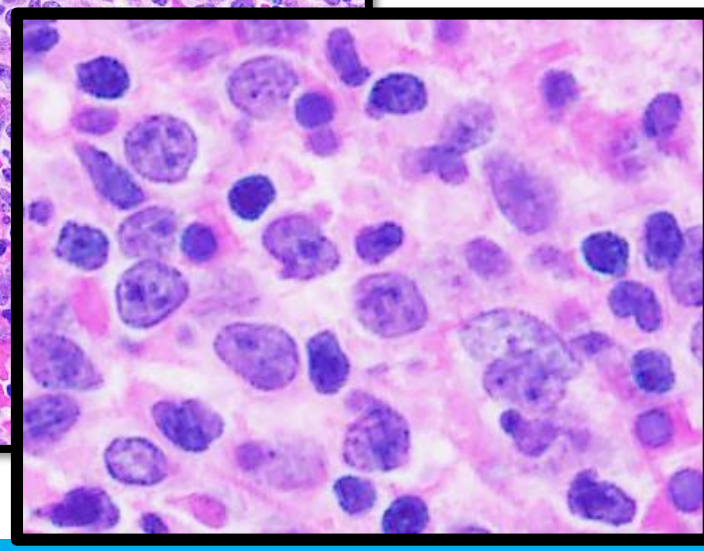
Diffuse abnormal marrow signal in the lower extremities with enhancement, concerning for diffuse marrow replacing process.



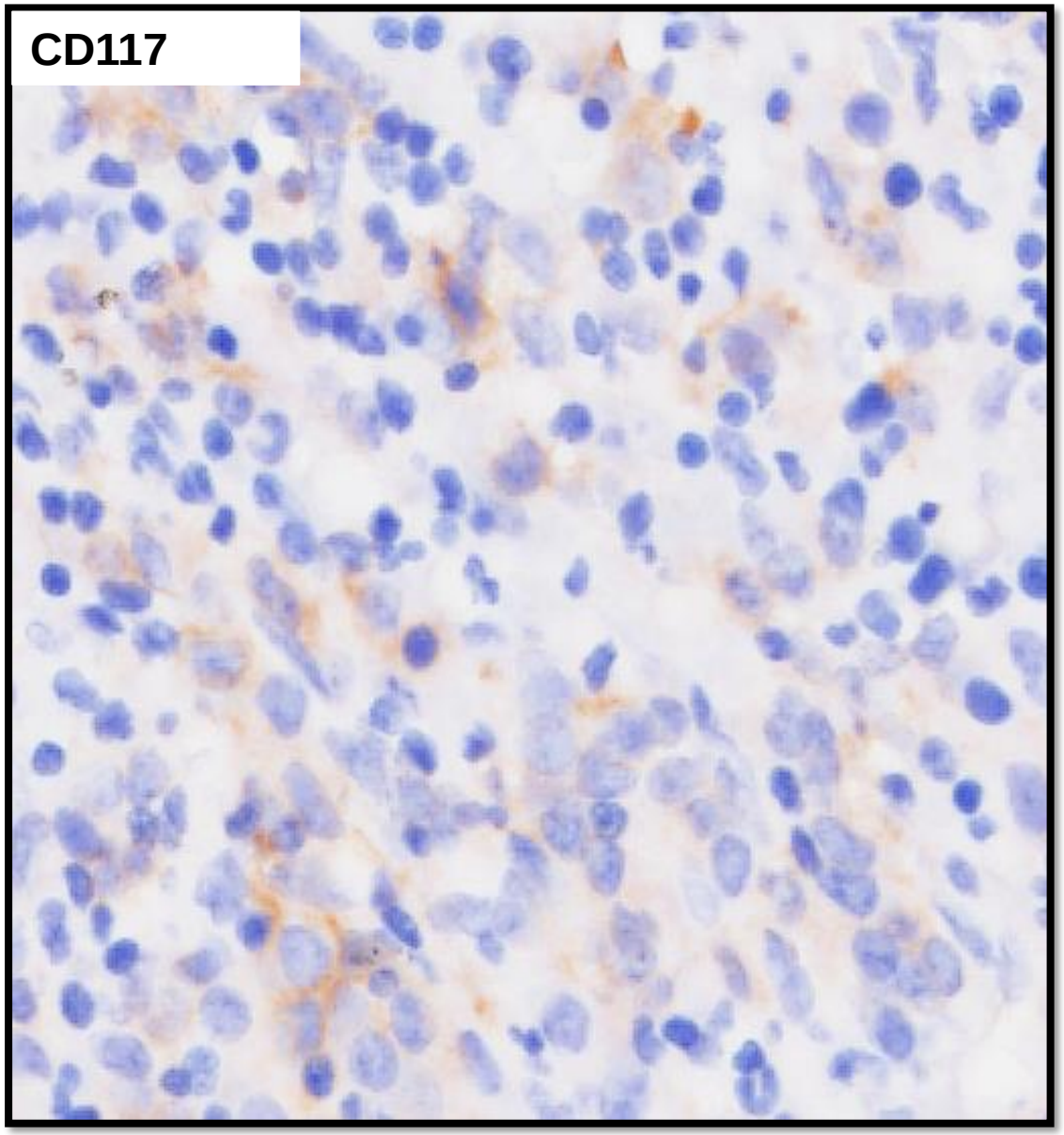
Bone Marrow Biopsy

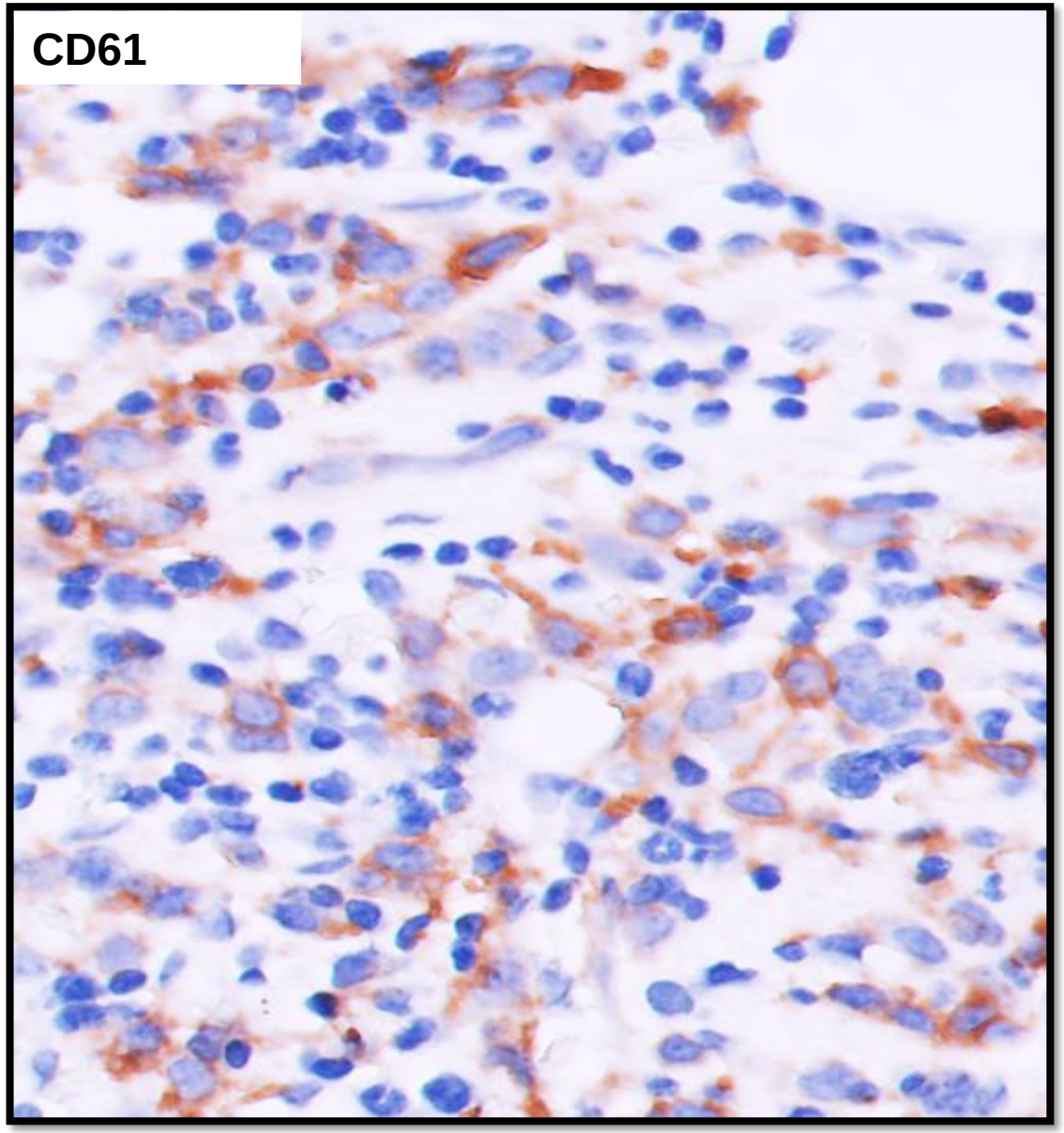




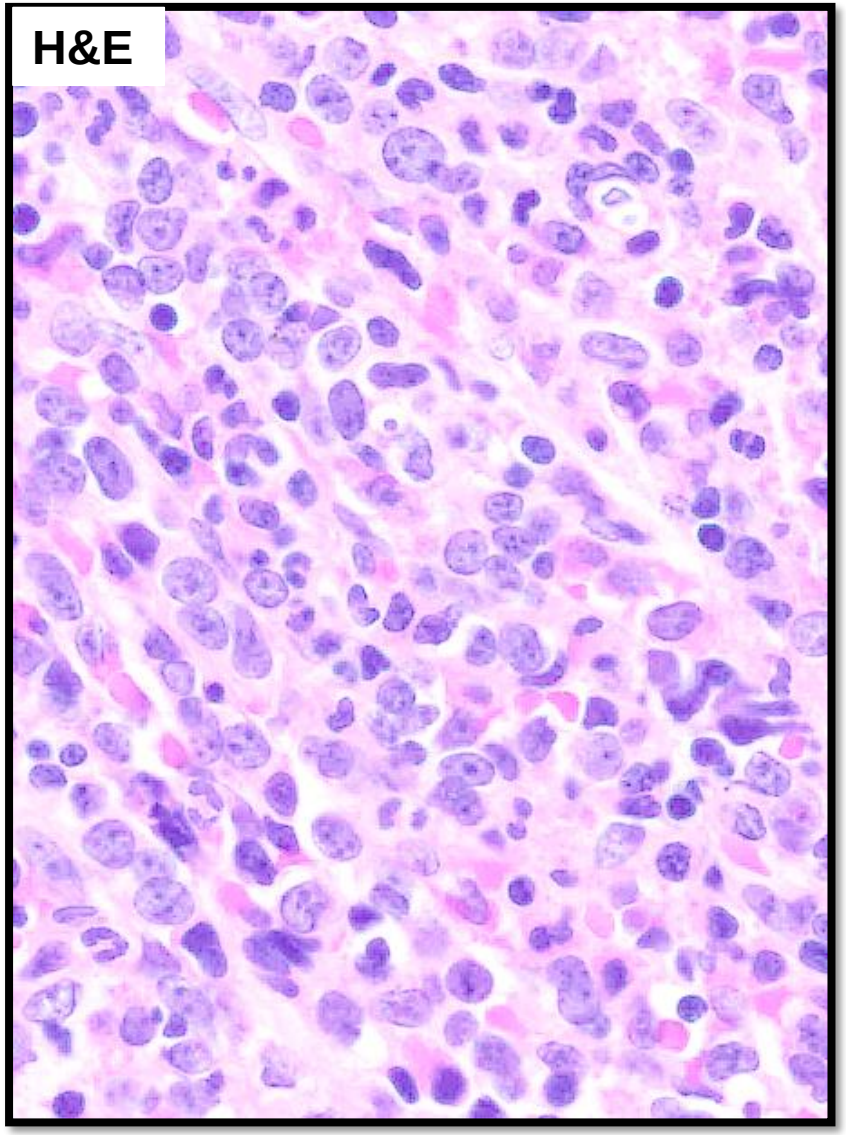


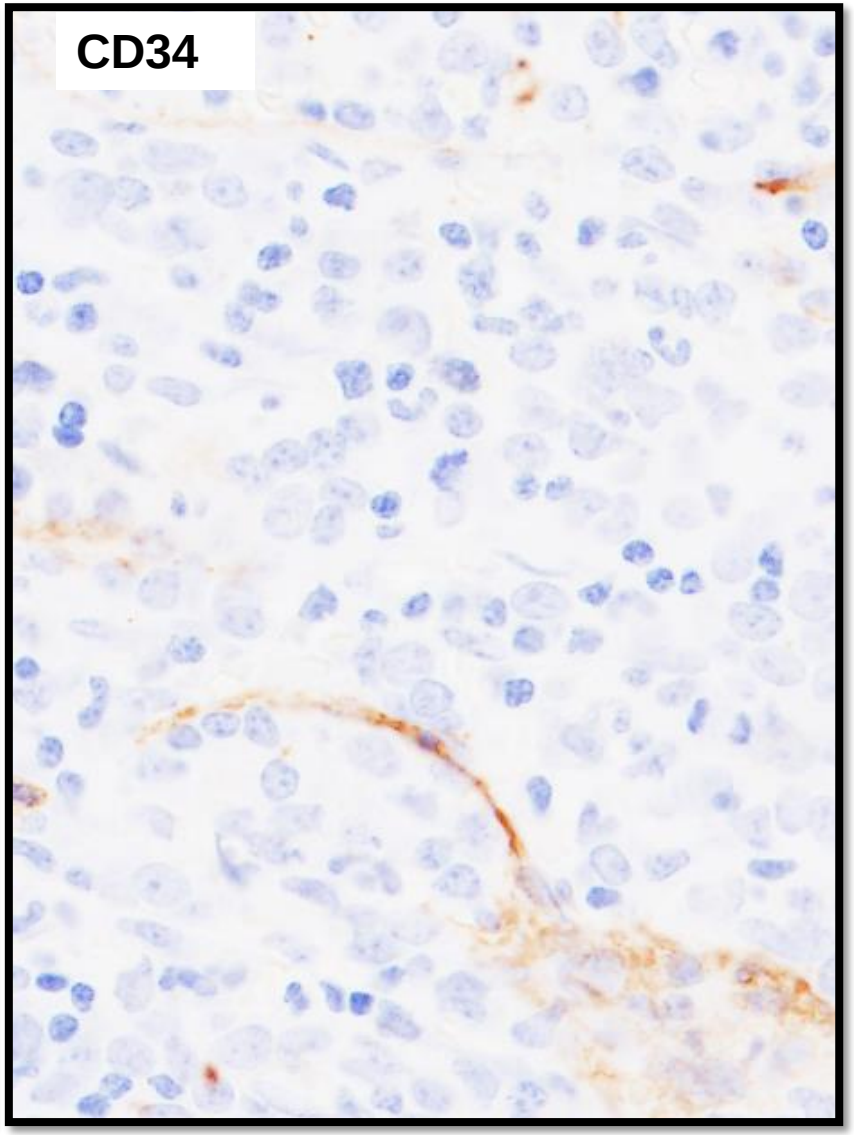
Bone Marrow Biopsy - IHC

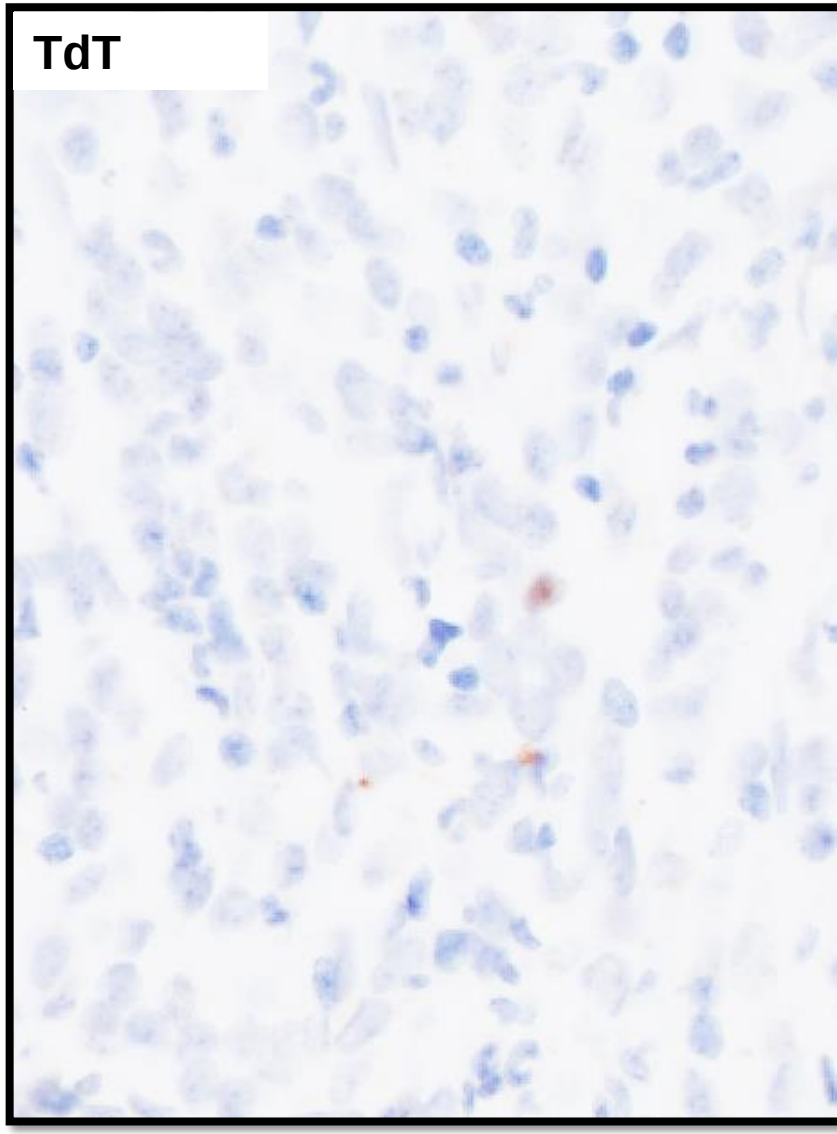




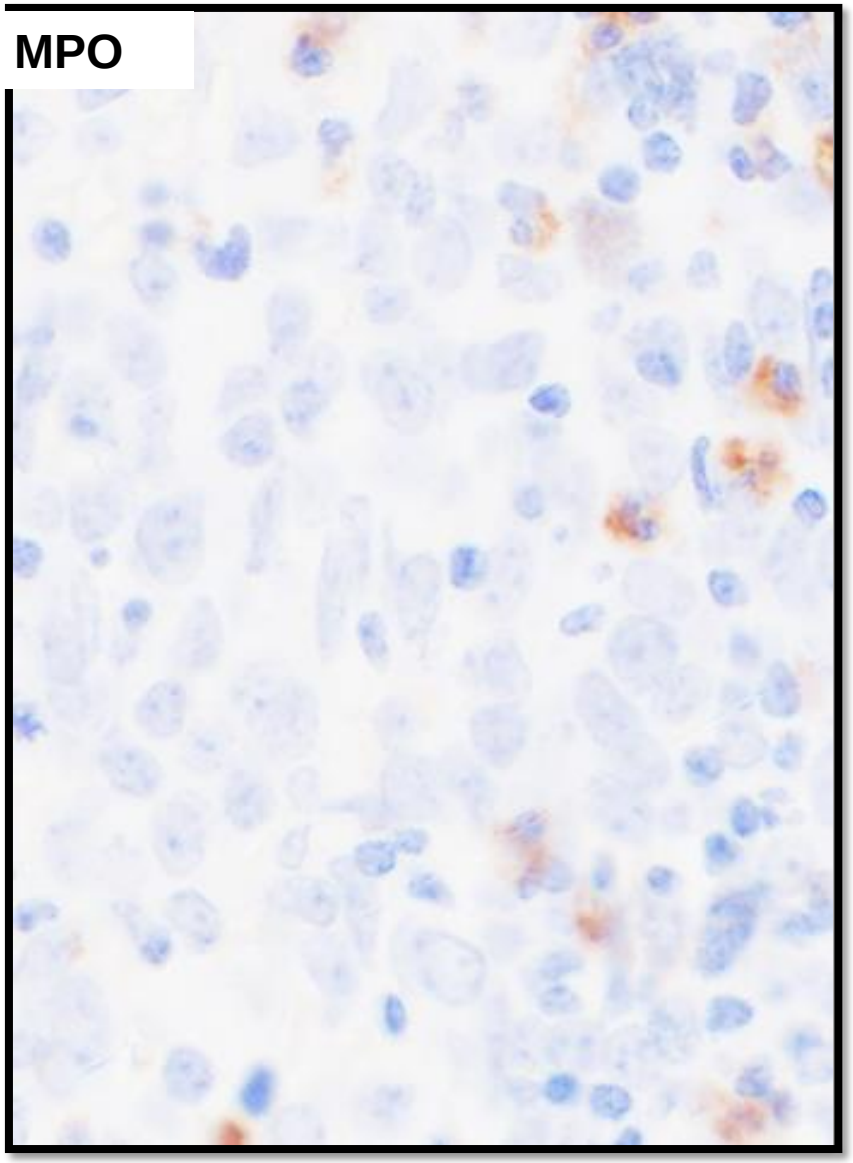
Bone Marrow Biopsy - IHC

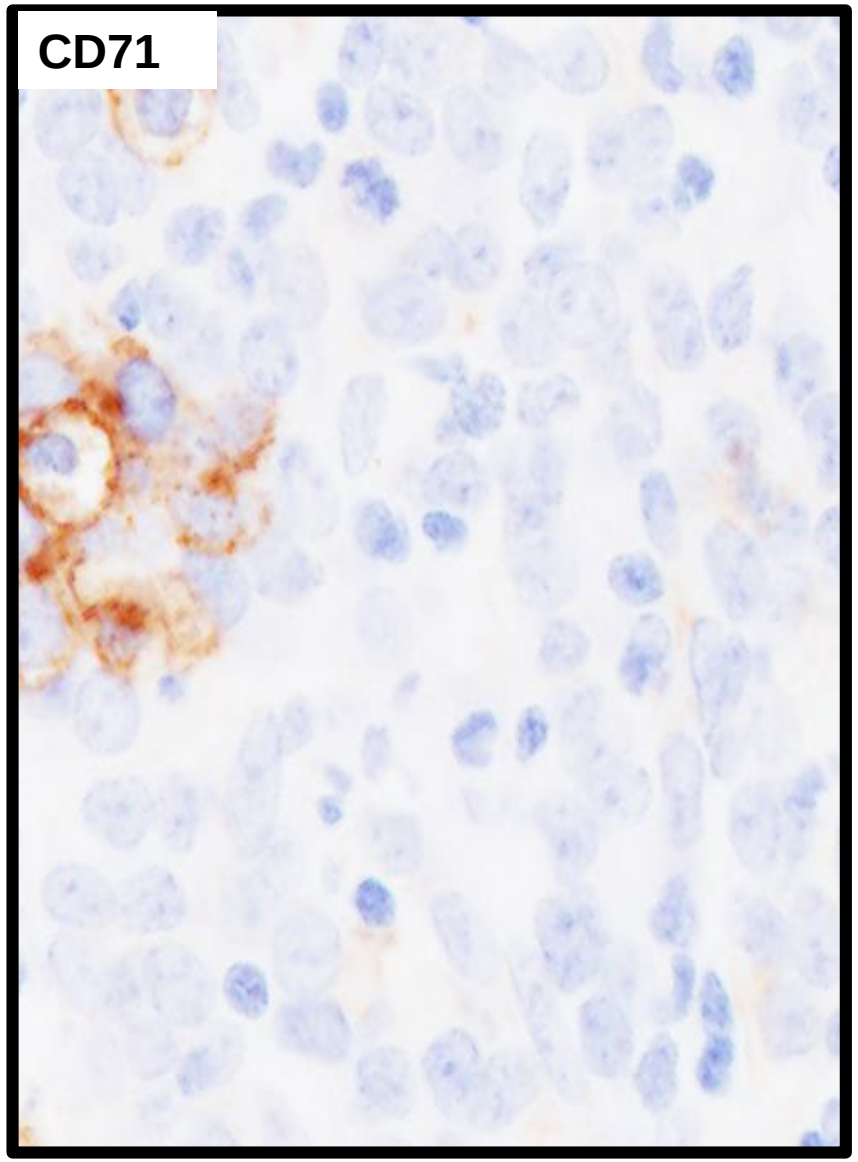


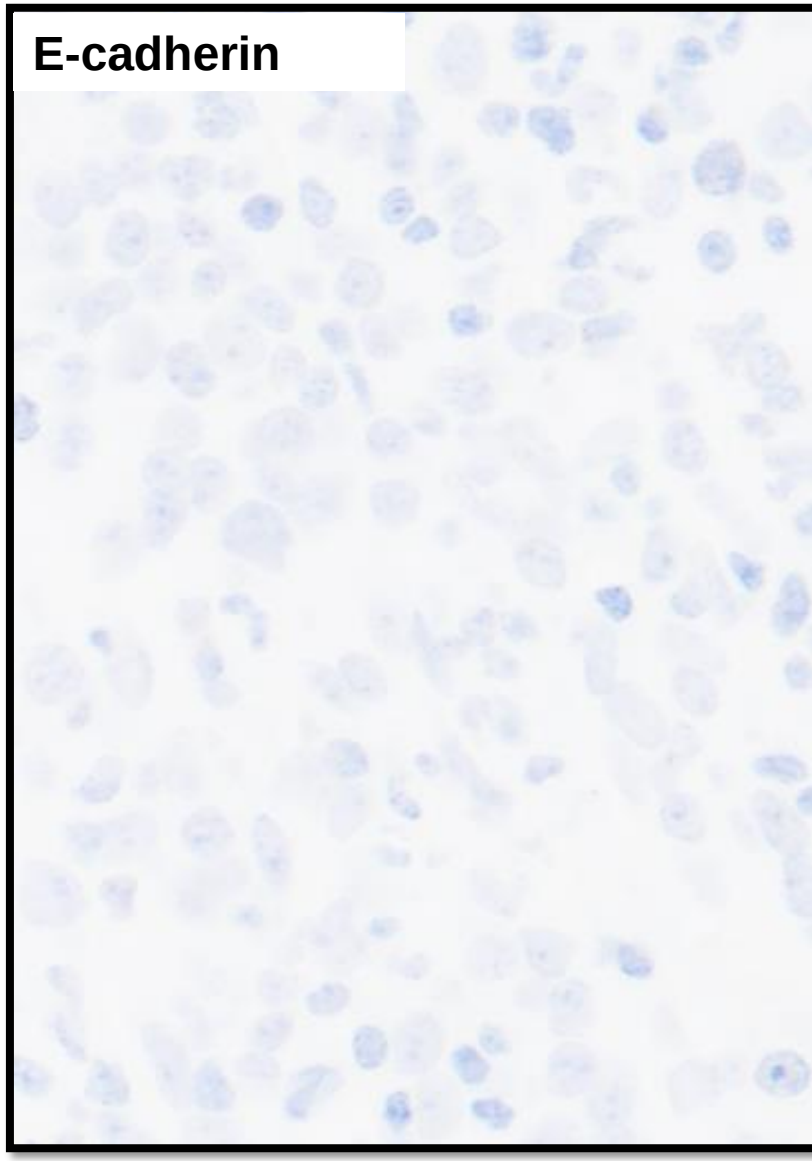




Bone Marrow Biopsy - IHC





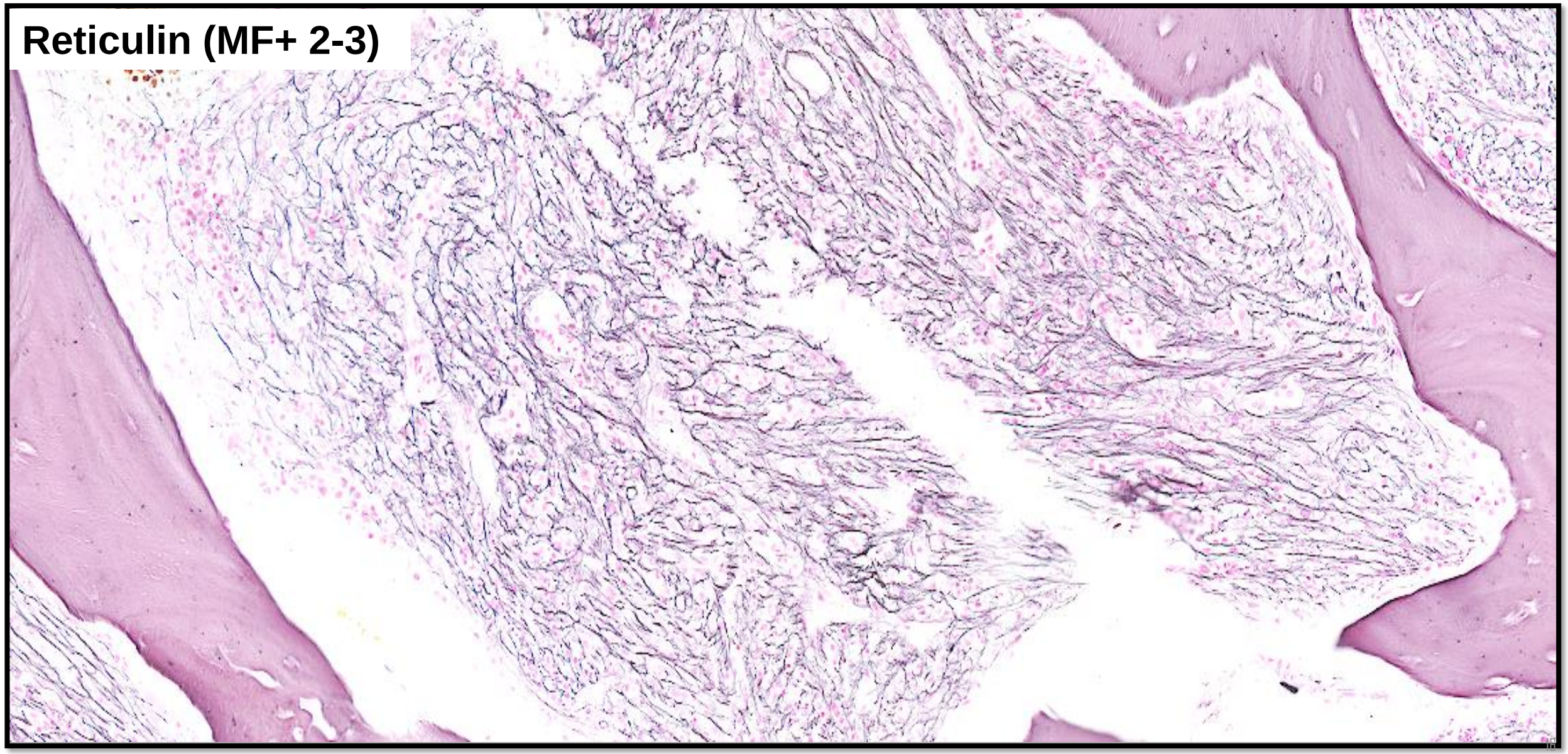


Summary of IHC Performed

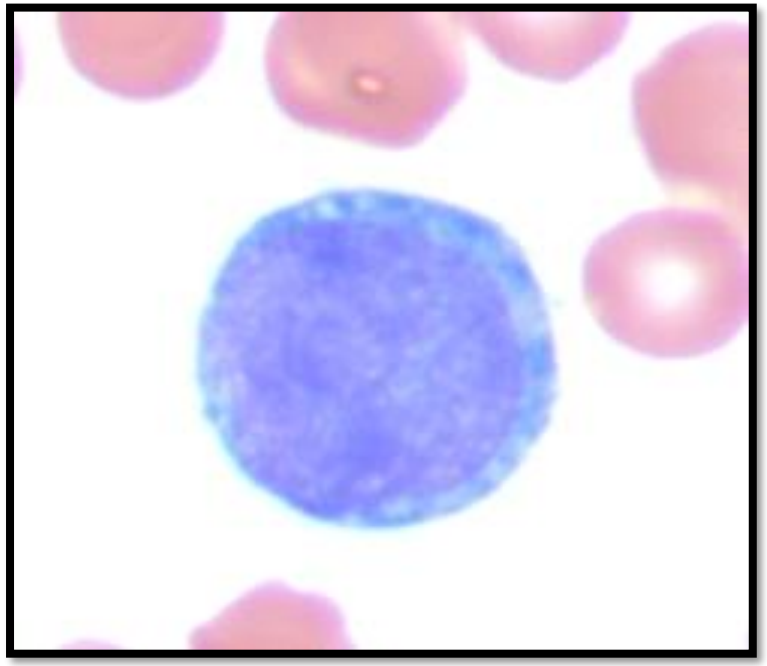
POSITIVE	NEGATIVE
CD117 CD61 Lysozyme	CD34 TdT CD3 CD19 PAX-5 CD71 E-Cadherin Myeloperoxidase CD33 Synaptophysin Chromogranin CAM5.2 DESMIN, MYOD1,INI-1, ACTIN

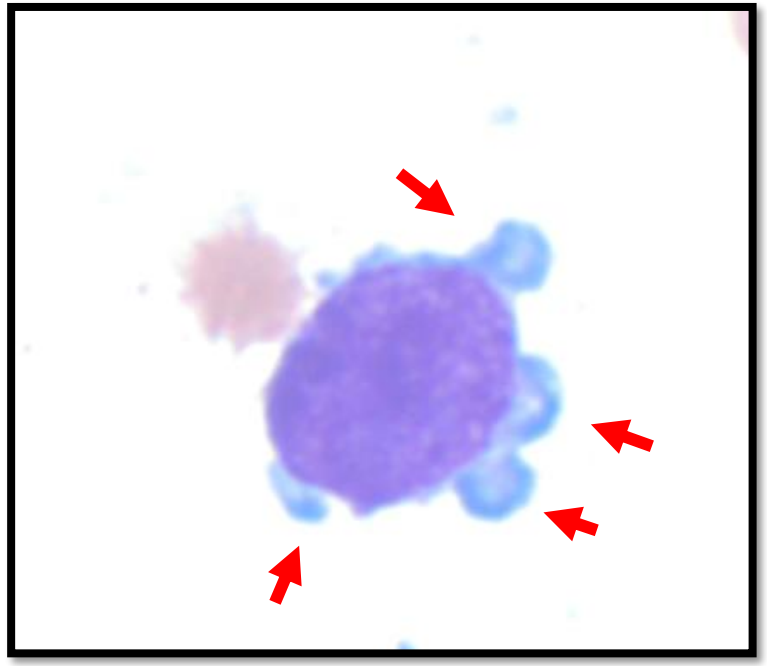
Markers for Neuronal and Muscle origin

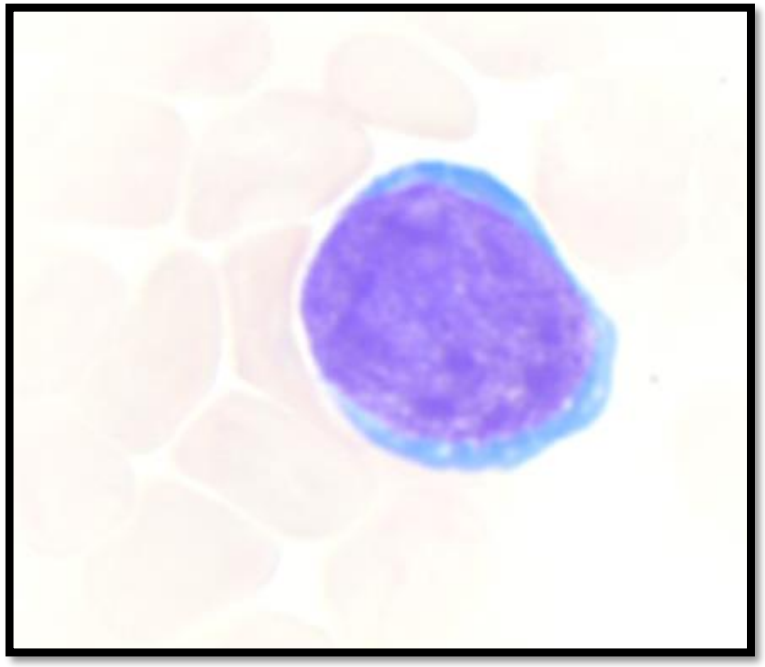
Bone Marrow Biopsy – Special Stains



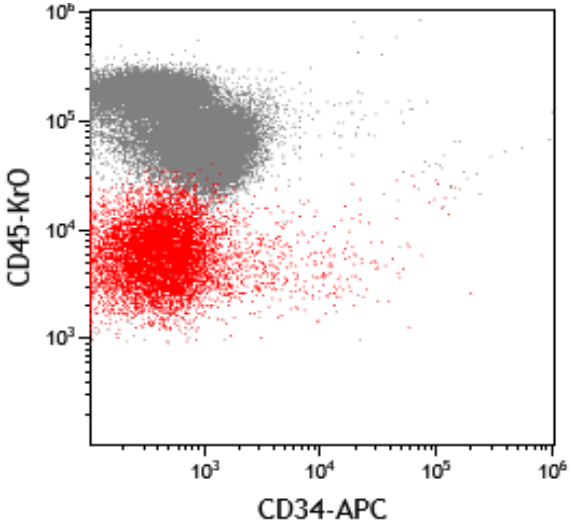
Bone Marrow Aspirate Smears ~3% blasts

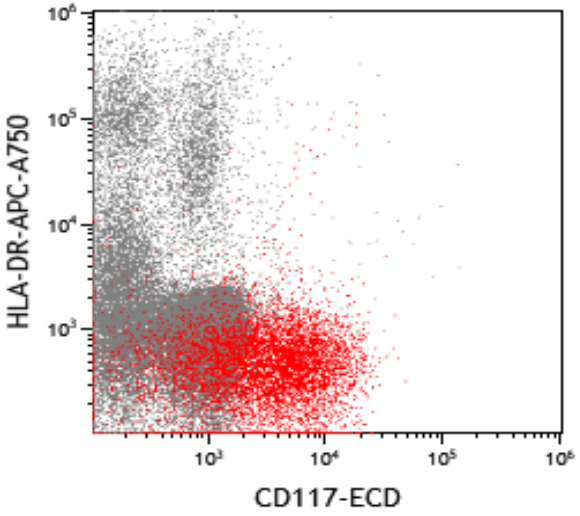


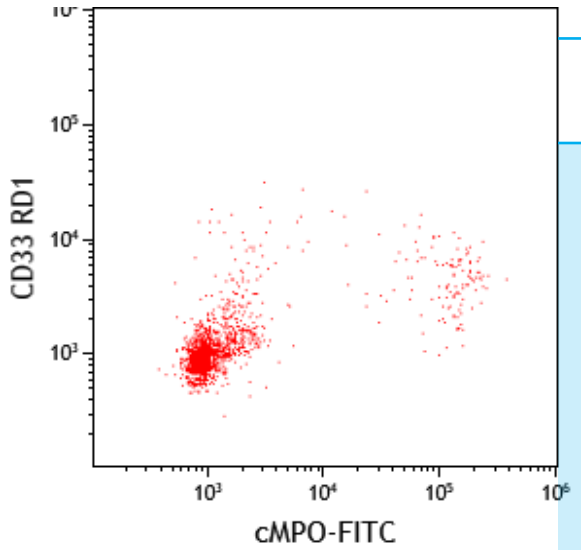


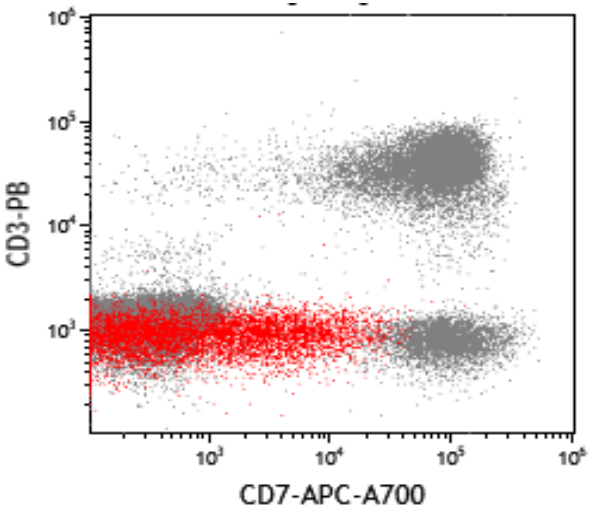


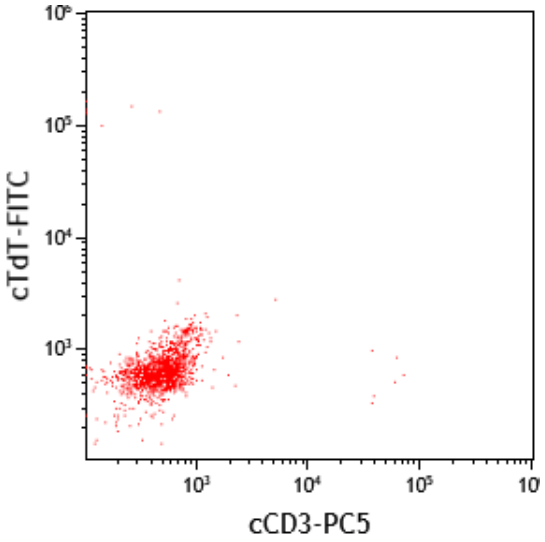
Bone Marrow Flow Cytometry

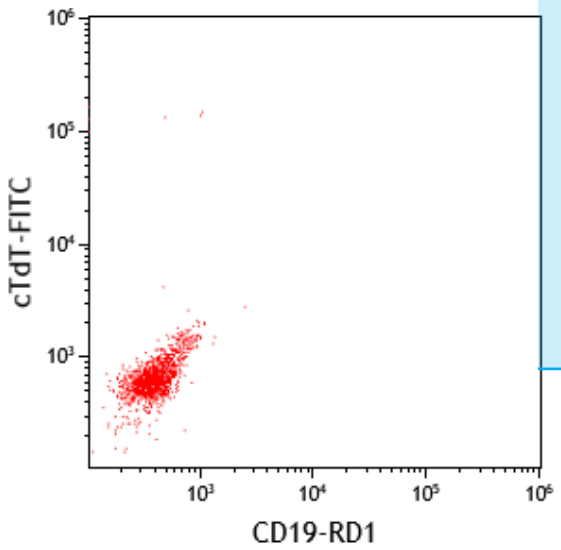












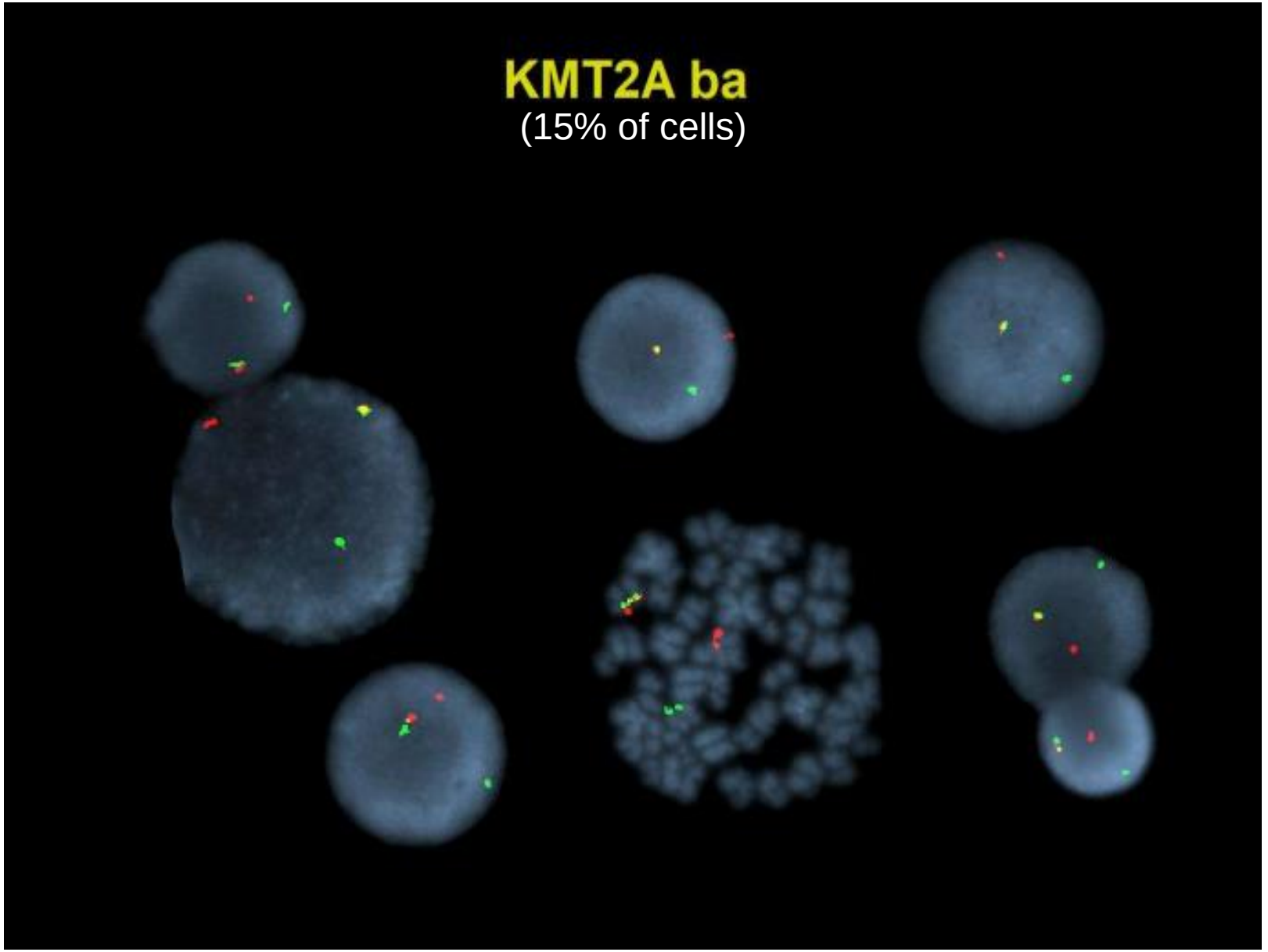
POSITIVE	NEGATIVE
CD117	CD45-/dim
CD7	CD34
partial/dim	HLA-DR
CD4	CD13
partial/dim	Myeloperoxidase
	TdT
CD41	CD19
CD42b	cCD3
CD61	CD56
	All other markers are Negative.

Differential Diagnosis

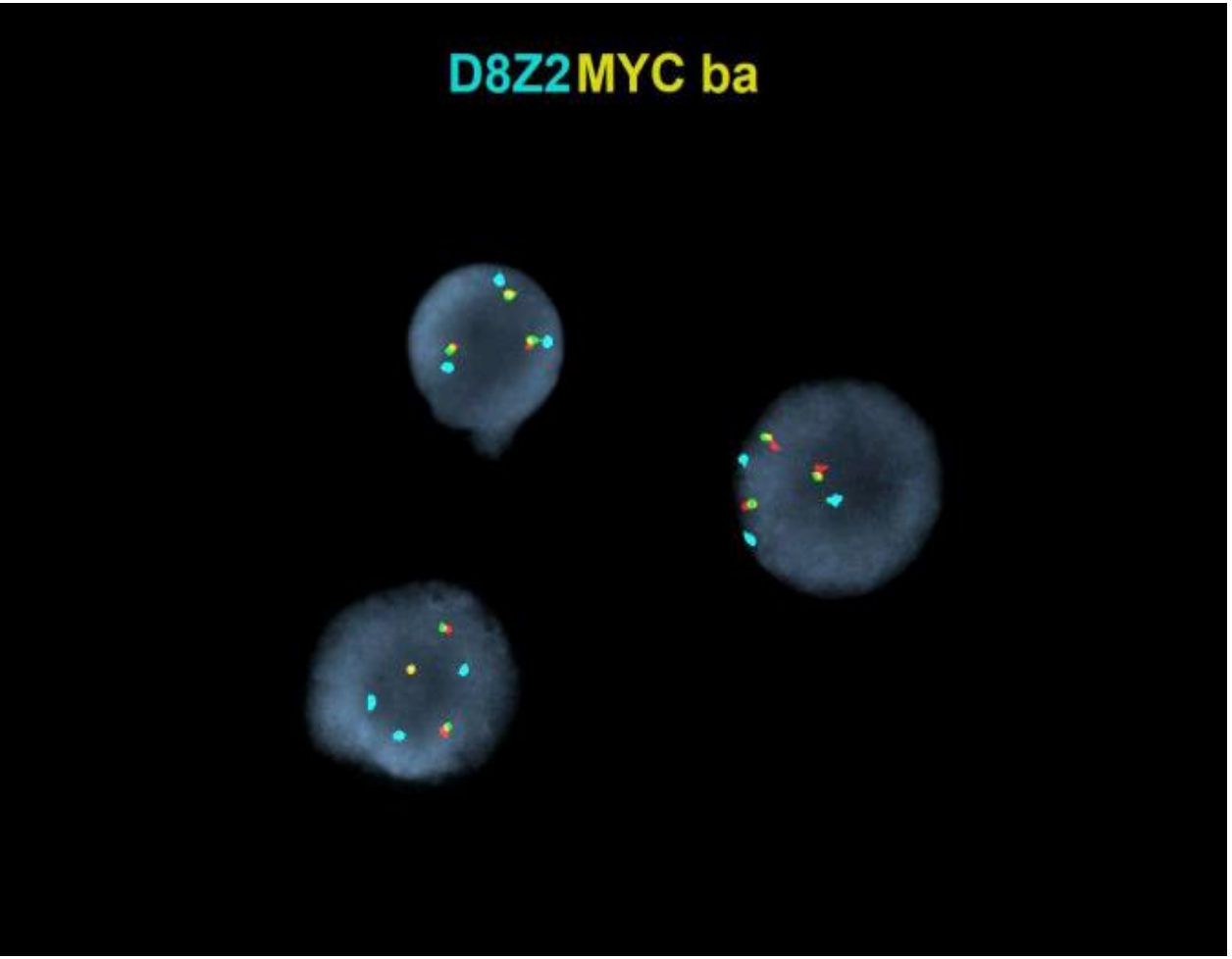
Acute Megakaryoblastic Leukemia

Acute Myeloid Leukemia with defining genetic abnormalities

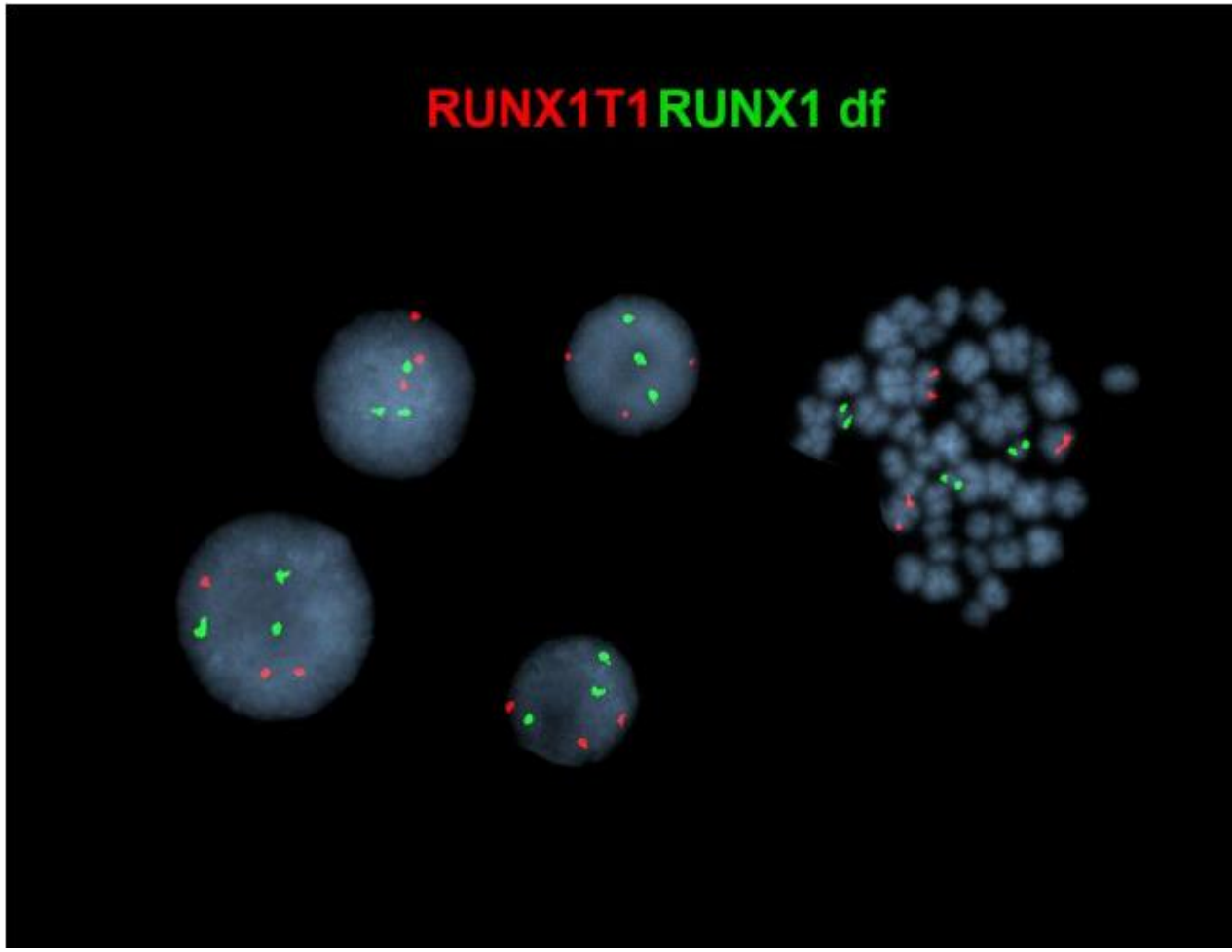
Fluorescent In Situ Hybridization (FISH) - *KMT2A* (11q23) gene rearrangement (15% of cells)



Fluorescent In Situ Hybridization (FISH)- Trisomy 8 and Trisomy 21



3 copies of D8Z2 (centromere 8), *MYC* (8q24.21)



3 copies of *RUNX1* (21q22)

Fluorescent In Situ Hybridization (FISH)

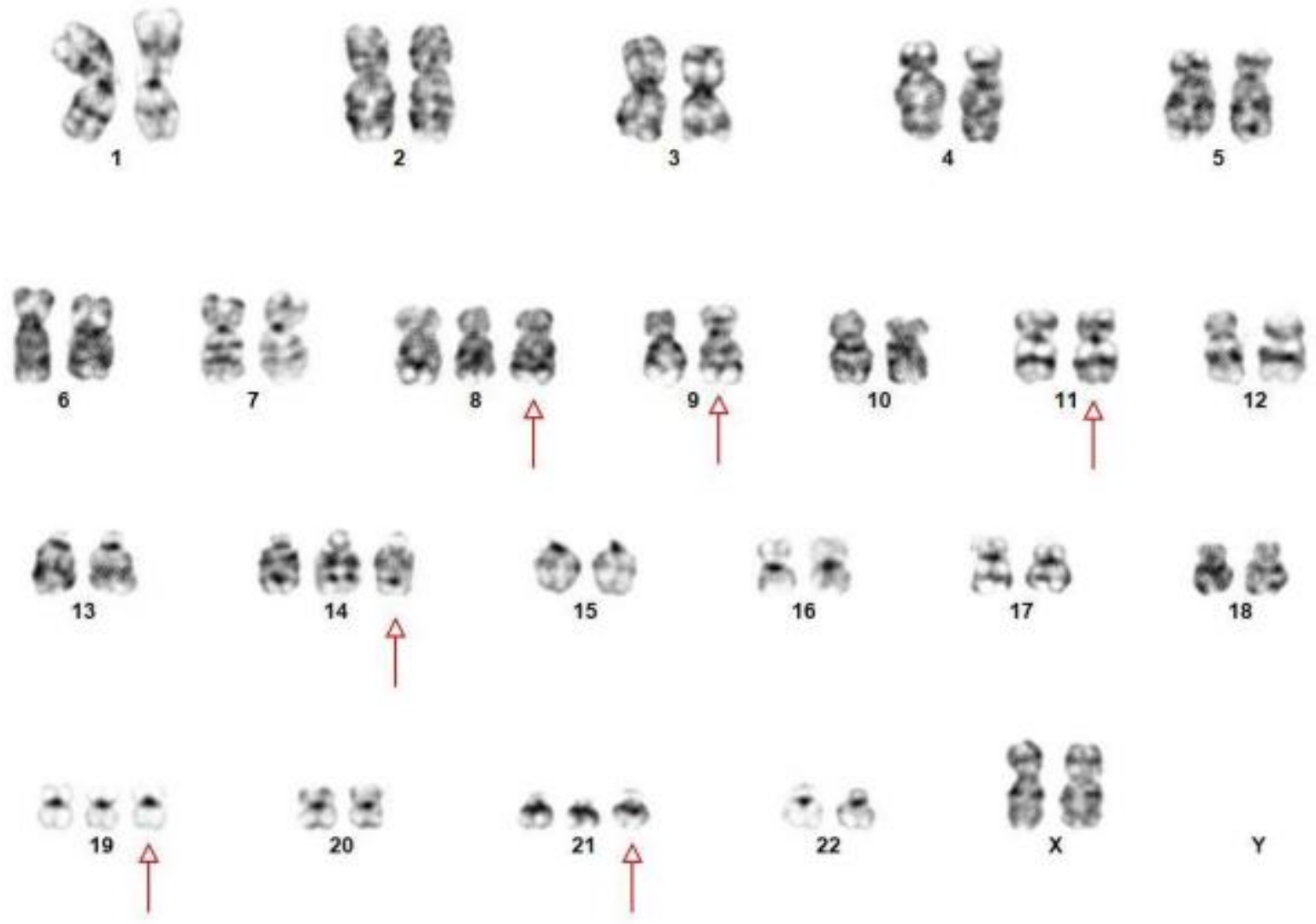
AML FISH PANEL	
***KMT2A* (11q23) gene rearrangement**	15%
Centromere 8 (D8Z2)	3 Copies (15-16%)
***MYC* (8q24.21)**	3 copies (15-16%)
***RUNX1* (21q22)**	3 copies (15-16%)
***BCR::ABL* t(9;22)**	Negative
***RUNX1::RUNX1T1* [t(8;21)]**	Negative
***PML::RARA* t(15;17)]**	Negative
***MYC* (8q24.21), *FOXO1* (13q14.11) and *CBFB* (16q22) rearrangements**	Negative
***TP53* (17p13.1) deletion**	Negative

Trisomy 8

Trisomy 21

Cytogenetics –

t(9,11) in the Setting of a Complex Karyotype



50,XX,+8,t(9;11)(p21;q23),+14,+19,+21[8]/46,XX[2]

Molecular analysis (Foundation One)

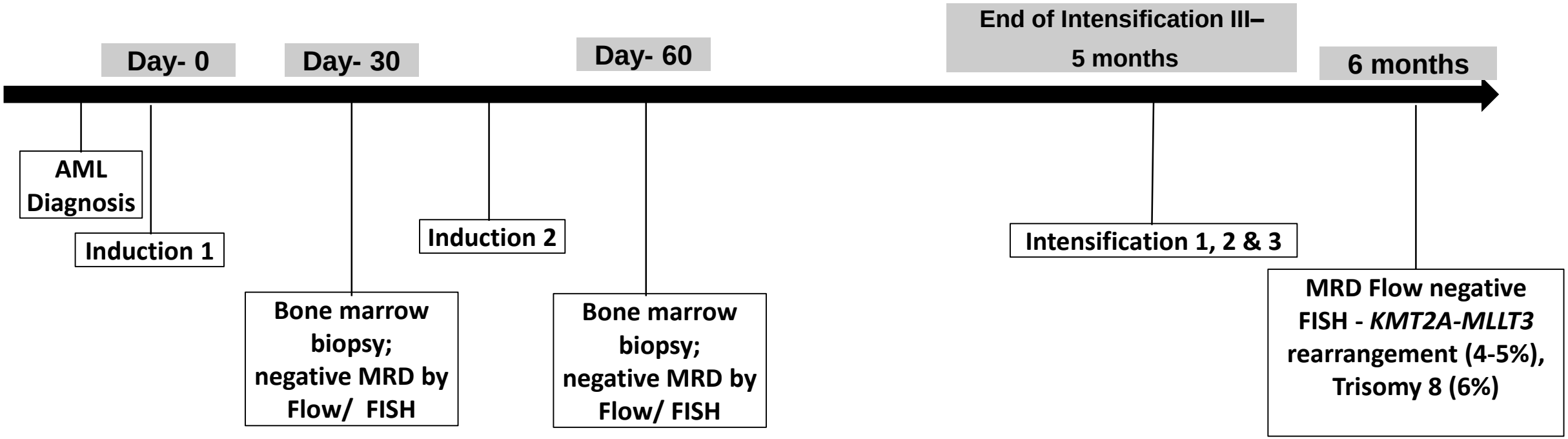
KMT2A::MLLT3 fusion - t(9;11)(p21;q23)

Gene	Mutation/ Rearrangement	Variant Allele Frequency
MPL	p.Trp515Arg (W515R)	VAF 1.5%
TSC2	p.Asp1690Glyfs*27	VAF 34.3%

Final Diagnosis

ACUTE MYELOID LEUKEMIA, MEGAKARYOBLASTIC, WITH *KMT2A* REARRANGEMENT

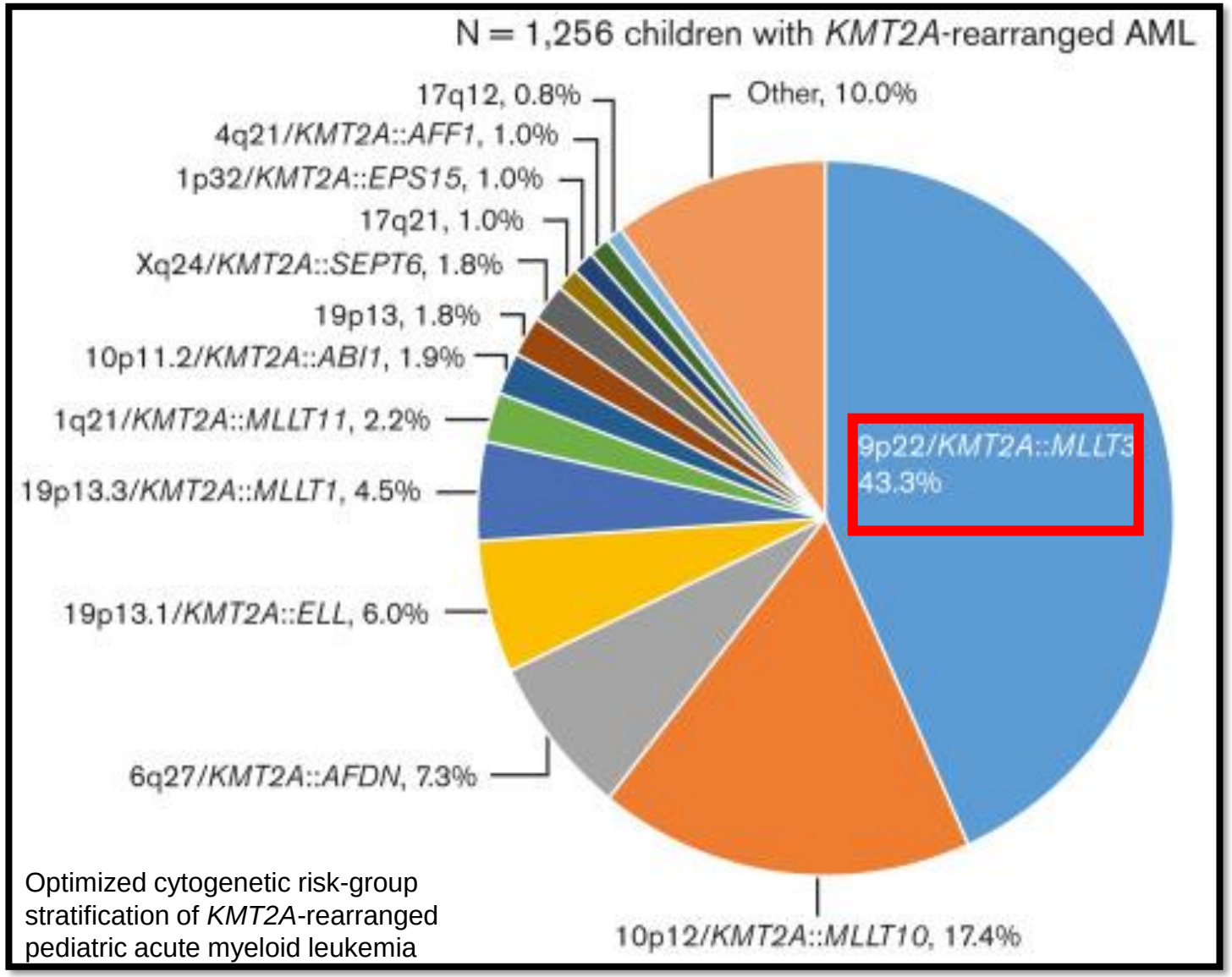
Clinical course – Protocol AAML1831



She was then referred to outside institution to undergo allogeneic hematopoietic stem cell transplantation

KMT2A Rearrangement in Pediatric AML

- In children, *KMT2A* rearrangement is the most common genetic abnormality of all AML cases (20-25%) .
- Most cases show monocytic, monoblastic, or myelomonocytic morphology.
- In children, especially *KMT2A::MLLT3* and *KMT2A::MLLT10* can present as Acute Megakaryoblastic Leukemia (AMKL)



Optimized cytogenetic risk-group stratification of *KMT2A*-rearranged pediatric acute myeloid leukemia

Pathogenesis and Risk Category >18 years old

AML Risk Stratification

Risk Category	Genetic Abnormality
Favorable	t (8;21) (q22;q22.1); *RUNX1-RUNX1T1* inv (16) (p13.1q22) or t (16;16) (p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD bZIP in-frame mutated *CEBPA*
Intermediate	Mutated *NPM1* with *FLT3*-ITD Wild-type *NPM1* with *FLT3*-ITD t (9;11) (p21.3;q23.3); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t (6;9) (p23;q34.1); *DEK-NUP214* t (v;11q23.3); *KMT2A rearranged* t (9;22) (q34.1;q11.2); *BCR-ABL1* inv(3) (q21.3q26.2) or t (3;3) (q21.3;q26.2); *GATA2, MECOM(EVI1)* t (3q26.2;v); *MECOM (EVI1)*-rearranged −5 or del (5q); −7; −17/abn (17p) Complex karyotype, monosomal karyotype Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1*, or *ZRSR2* Mutated *TP53*

European Leukemia Network 2022 guidelines

Pathogenesis and Risk Category – Conflicting Studies

Favorable impact of the t(9;11) in childhood acute myeloid leukemia

May 2002

Jeffrey E Rubnitz [1], Susana C Raimondi, Xin Tong, Deo Kumar Srivastava, Bassem I Razzouk,

Analysis of 42 pediatric A rearrangements showed that t more favorabl

Novel prognostic sub leukemia:

Brian V. Balgobind, Susana C. Rain

Analysis o n

According to the Children's Oncology Group, in children, AML with **KMT2A::MLLT3 fusion** has been associated with a **standard-risk category**.

Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to trial AML-BFM 98

May 2010

Christine von Neuhoff [1], Dirk Reinhardt, Annette Sander, Martin Zimmermann, Jutta Bradtke,

KMT2A rearrangements – including t(9;11) were associated with inferior prognosis

Risk stratification of Pediatric AML with *KMT2A* rearrangements (*KTM2A*-r)

Pediatric AML with *KMT2A* Rearrangement
n= 733 patients

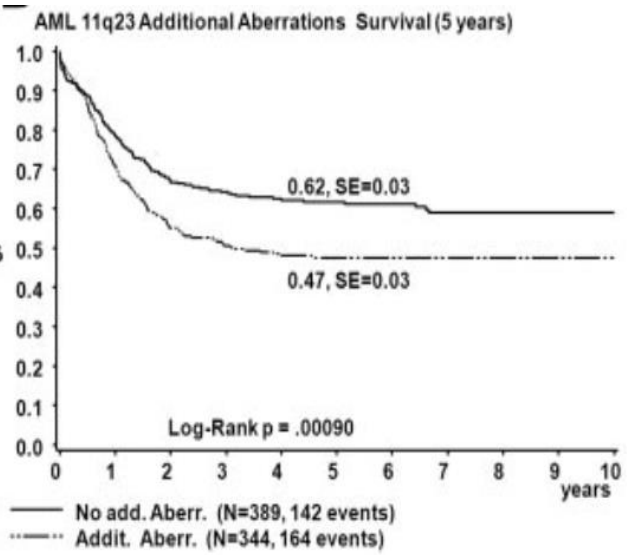
No additional cytogenetic abnormalities (ACAs)
n= 389 (53%)

Additional cytogenetic abnormalities (ACAs)
n= 344 (47%)

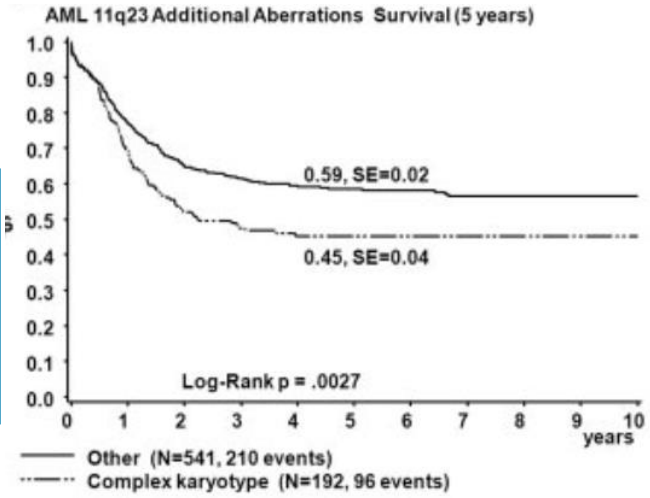
<3 Total abnormalities
N =152 (44%)

Complex Karyotype
>3 Total abnormalities
N= 192 (56%)

ACA vs non-ACA



Complex Karyotype vs others



Trisomy 8, the most common ACA, was associated with **better survival and lower relapse**.

Trisomy 19 was less common but had **strong negative prognostic significance**.

Coenen EA. Blood 2011; 117(26):7102-7111

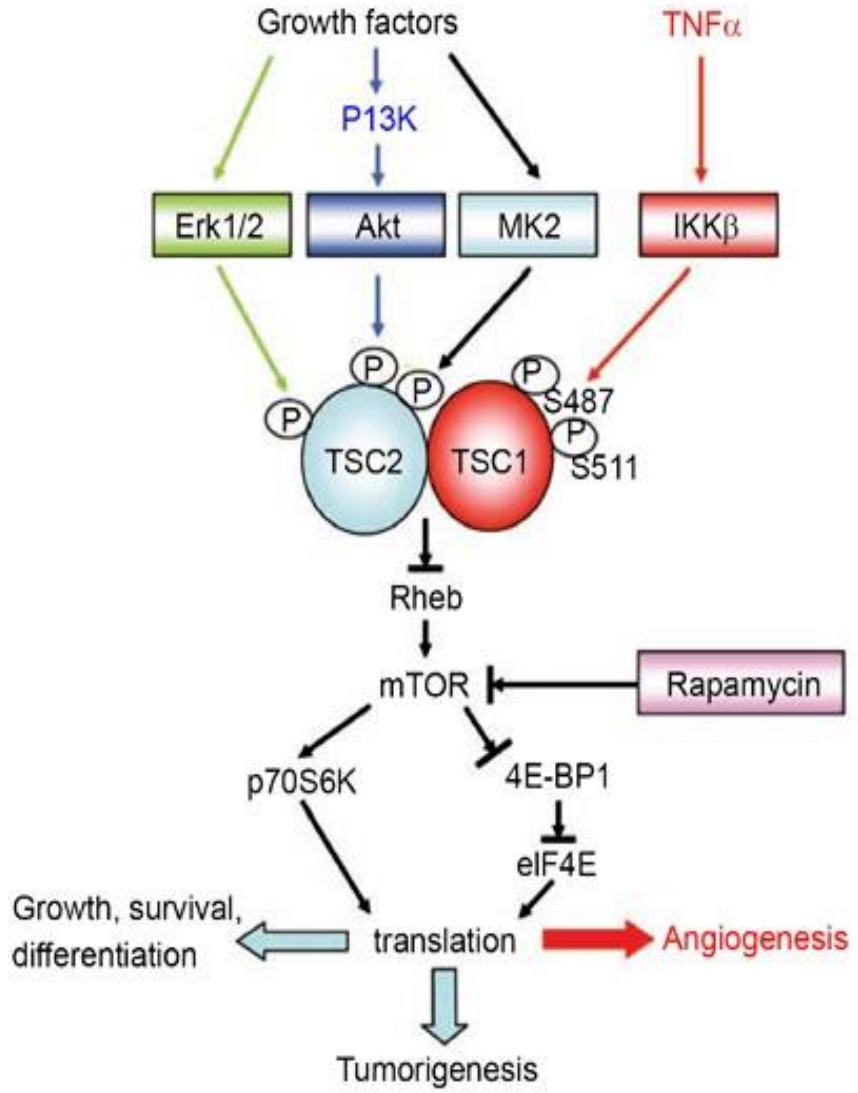
Impact of *KMT2A* and Additional Mutations

- *KMT2A* (*MLL*)::*MLLT3* (AF9) fusion
- *TSC2* alteration D1690fs*27 (VAF 34.3%)
- *MPL* p.W515R mutation (VAF 1.5%)

TSC2 p.Asp1690Glyfs*27

- *TSC1* (hamartin) and *TSC2* (tuberin) genes function as tumor suppressor proteins

- Form an intracellular heterodimer and participate in signaling pathway of the mTOR



Zhifang X et al. Leuk Res.2009

TSC2 mRNA expression is significantly reduced in acute leukemia compared with normal controls

Aberrant expression of TSC2 gene in the newly diagnosed acute leukemia

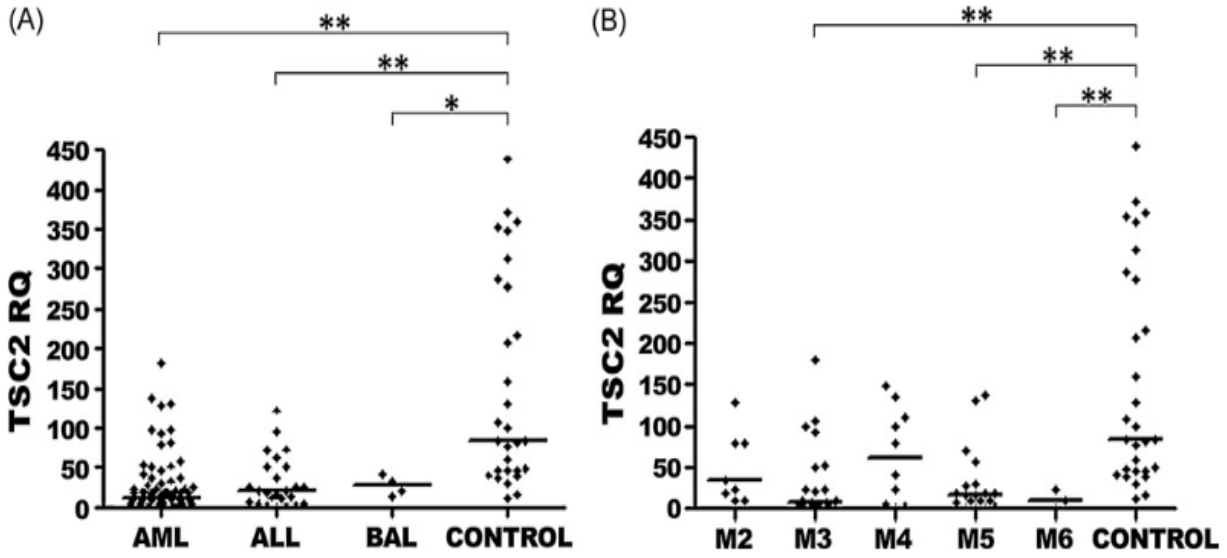
Zhifang Xu, Min Wang, Lin Wang, Yang Wang, Xin Zhao, Qing Rao, Jianxiang Wang*

State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences

Available online 27 February 2009

104 patients newly diagnosed acute leukemia (AML- ALL –Bi-phenotypic AL)

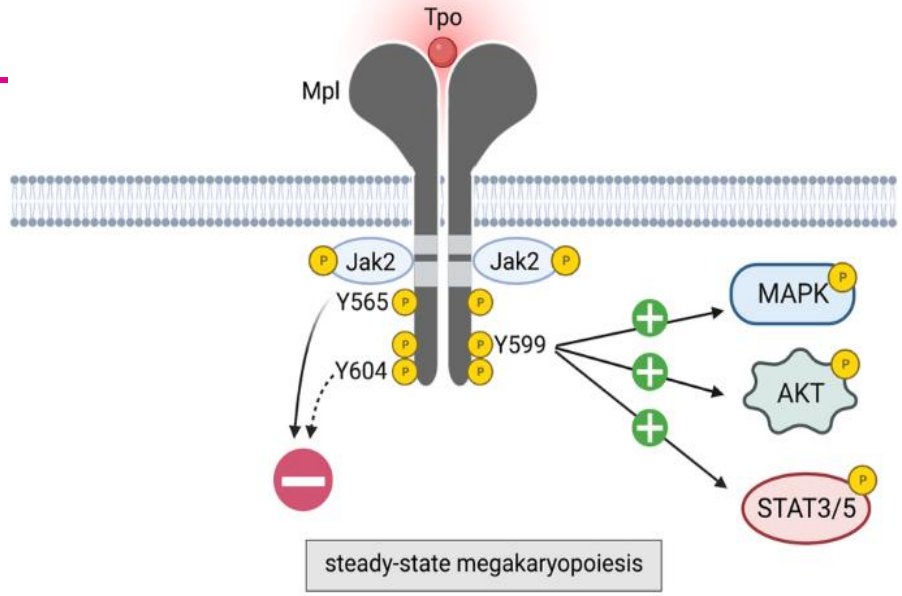
29 healthy controls



The reduction is most pronounced in **AML subtypes M3, M5, and M6**

MPL W515R mutation in AMKL

- *MPL* encodes the thrombopoietin receptor (TpoR) gene expressed in megakaryocytes lineage cells and hematopoietic stem cells
- Gain of function point mutation W515 K/L induce receptor dimerization without requiring TPO triggering JAK-STAT pathway (occurs in Myeloproliferative neoplasm)



12 cases with AMKL with myelofibrosis

3 patients had *MPL* W515L mutation

- Small Leukemia Subclone
- Clonal hematopoiesis of unknown significance

Deep mutational studies show that W515L/K/R/A are canonical *MPL* mutation 10 driver that activate JAK-STAT pathway

Hussein K et al. Leukemia 2009: 23, 852-855. Novel drivers and modifiers of MPL-dependent oncogenic transformation identified by deep mutational scanning. *Blood* 2020; 135 (4): 287–2

TAKE-HOME POINTS

- Acute myeloid leukemia with megakaryoblastic differentiation is diagnostically challenging.
- Accurate diagnosis requires integration of morphology, immunophenotyping, immunohistochemistry, cytogenetics, and molecular testing
- In this case, the KMT2A::MLLT3 rearrangement represents the primary driver, while additional alterations such as complex Karyotype, TSC2 and low-VAF MPL variants likely reflect secondary or subclonal events contributing to the complex molecular landscape of pediatric AML.

Thank You/Any Questions?

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Acknowledgement

Amy Duffield, MD PhD
Shafinaz Hussein, MD
Bruce Petersen, MD
David Gajzer, MD
Alina Dulau-Florea, MD
Dalia Abdel Azim, MD PhD
Christian Salib, MD
Mathew Shapiro, MD

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