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Juvenile Myelomonocytic Leukemia with Concurrent Somatic *K-RAS* and *PTPN11* Mutations in A Child Harboring Germline *ASXL1* Mutation

Shunyou Gong, Rachel Mariani, Sharad Salvi, Nobuko Hijiya

2017 SH/EAHP Workshop Case 171

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Clinical History

- A 3 years 11 months-old previously healthy boy presented to one of our satellite hospitals with easy bruising and leg pain.
- Physical examination revealed hepatosplenomegaly.
- CBC showed marked leukocytosis (WBC 39.7 k/ul), monocytosis (22 k/ul), anemia (Hb 7.3g/dl), and thrombocytopenia (44 k/ul).
- Marrow aspiration and biopsy were performed and sent to main hospital for further work-up.

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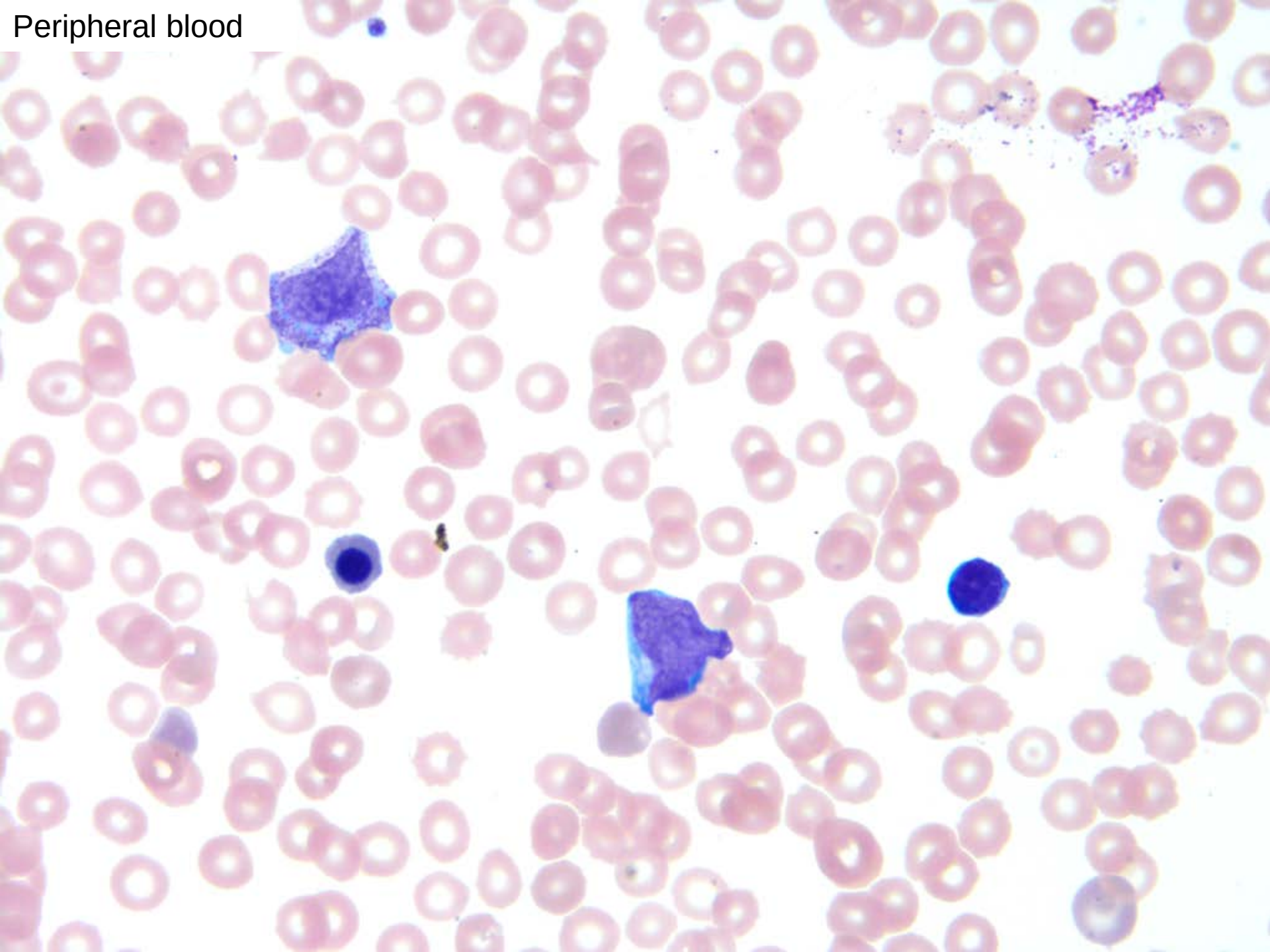
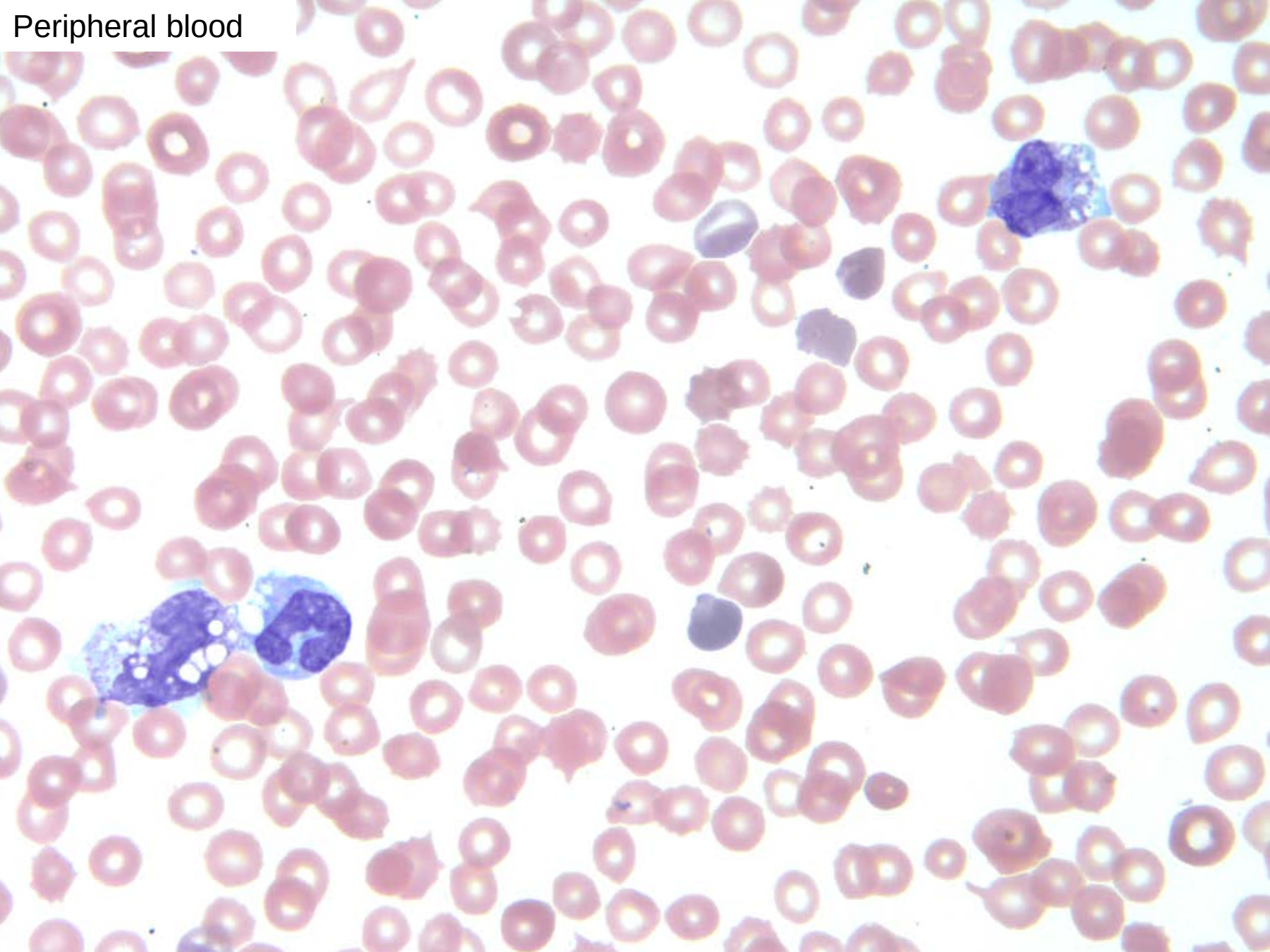
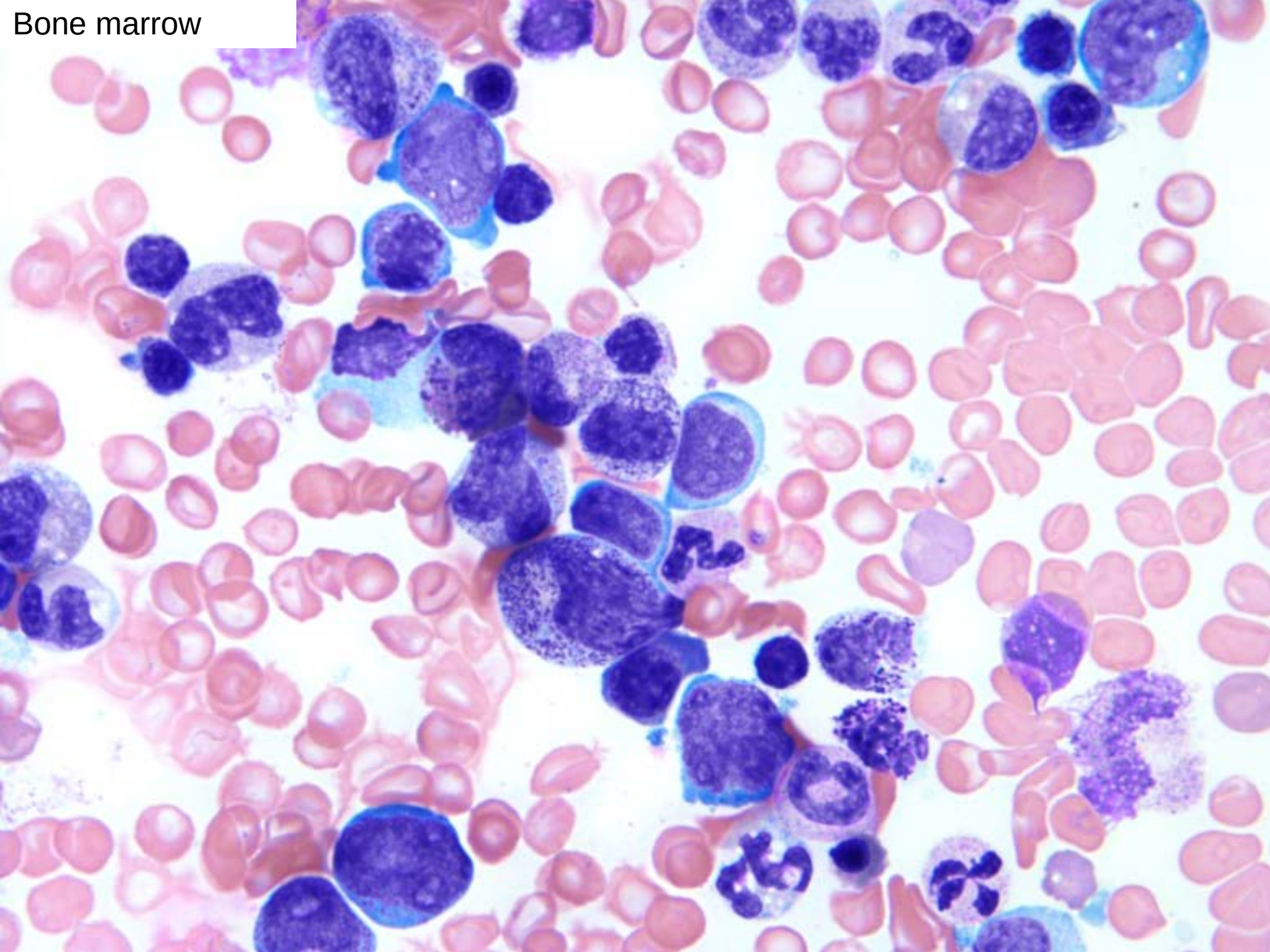
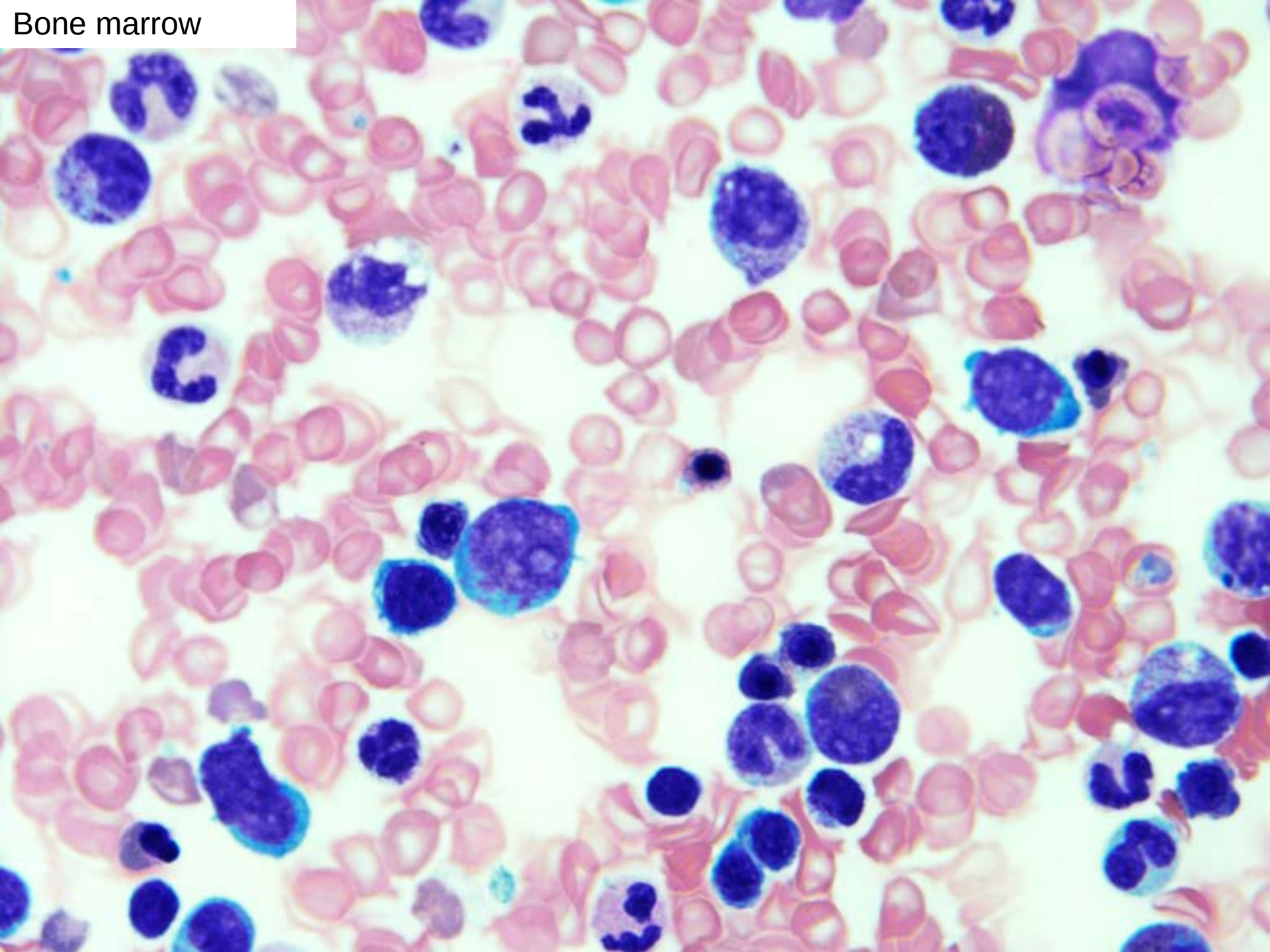
Peripheral blood



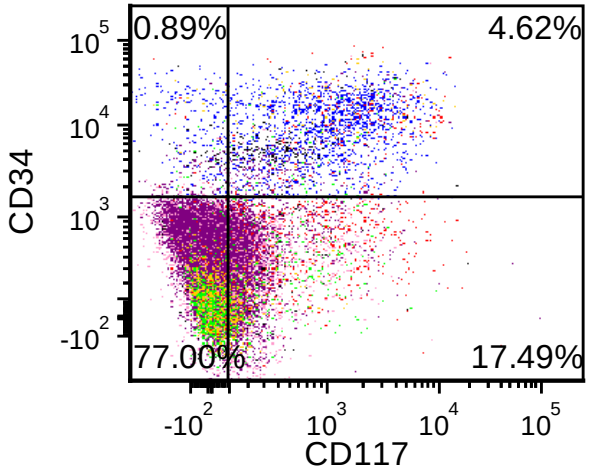
Peripheral blood

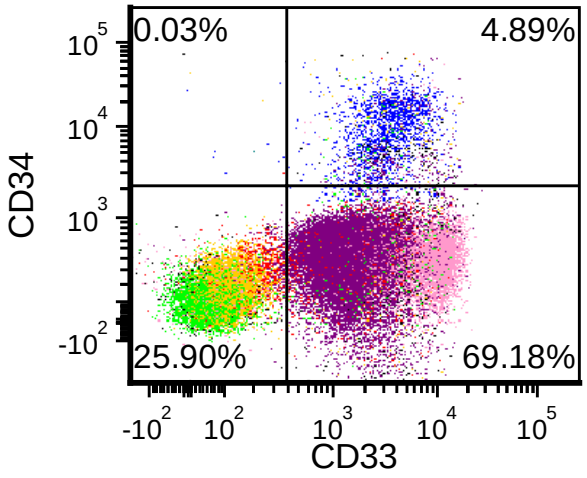


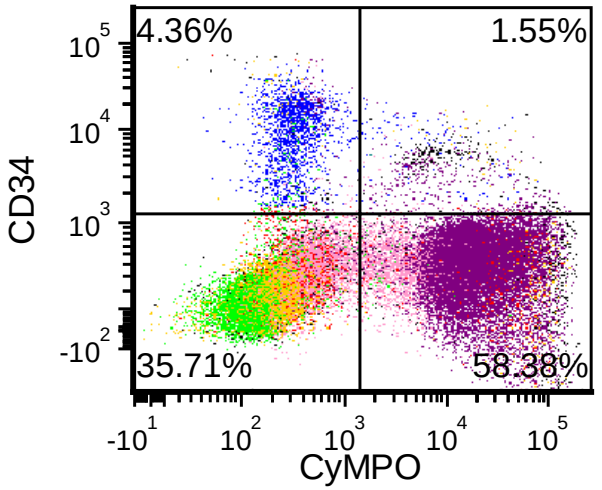

Bone marrow

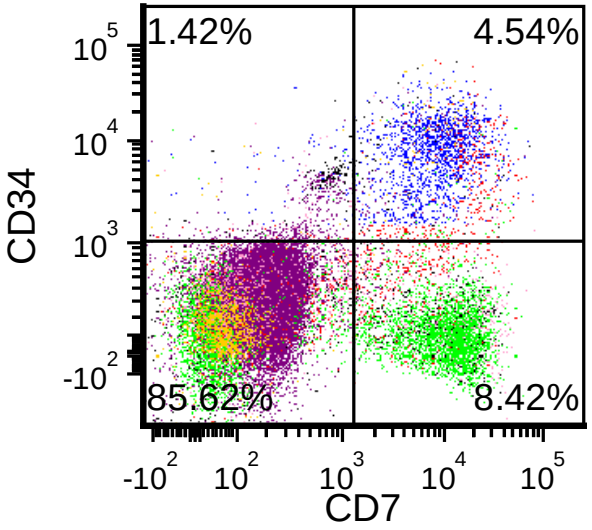

Bone marrow

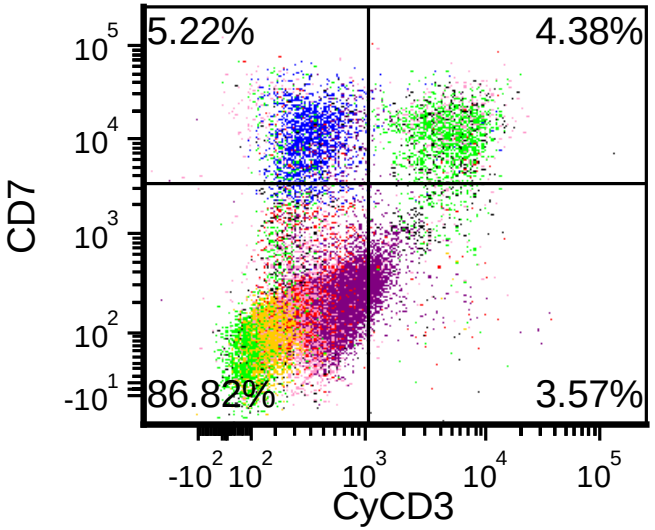
Flow cytometry of marrow aspirate

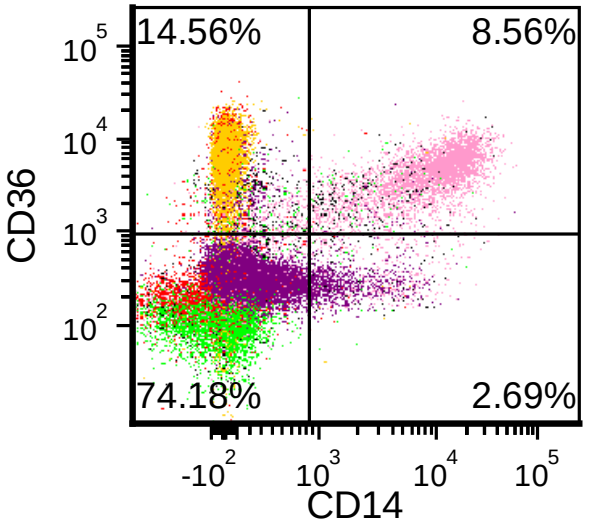




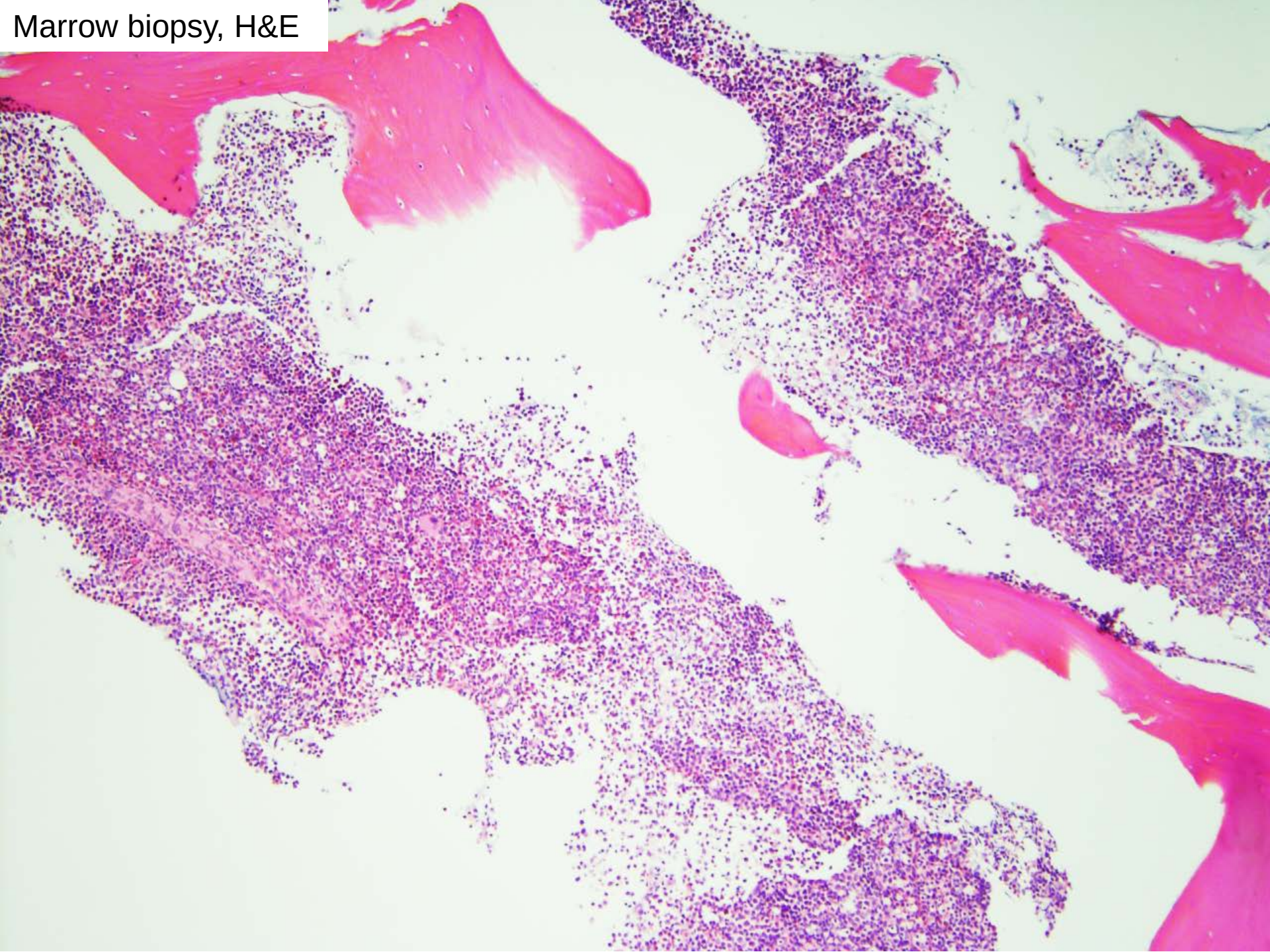


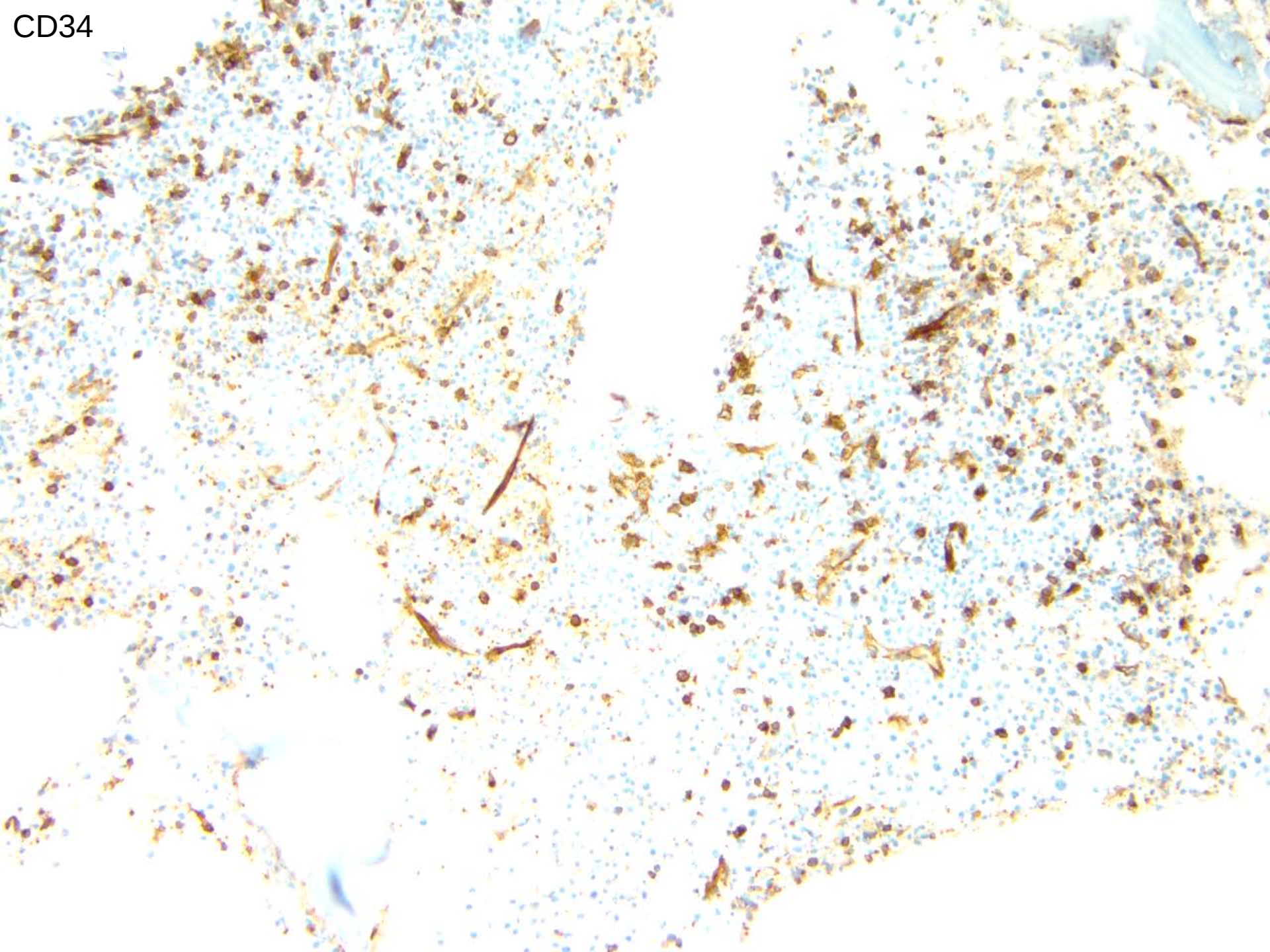


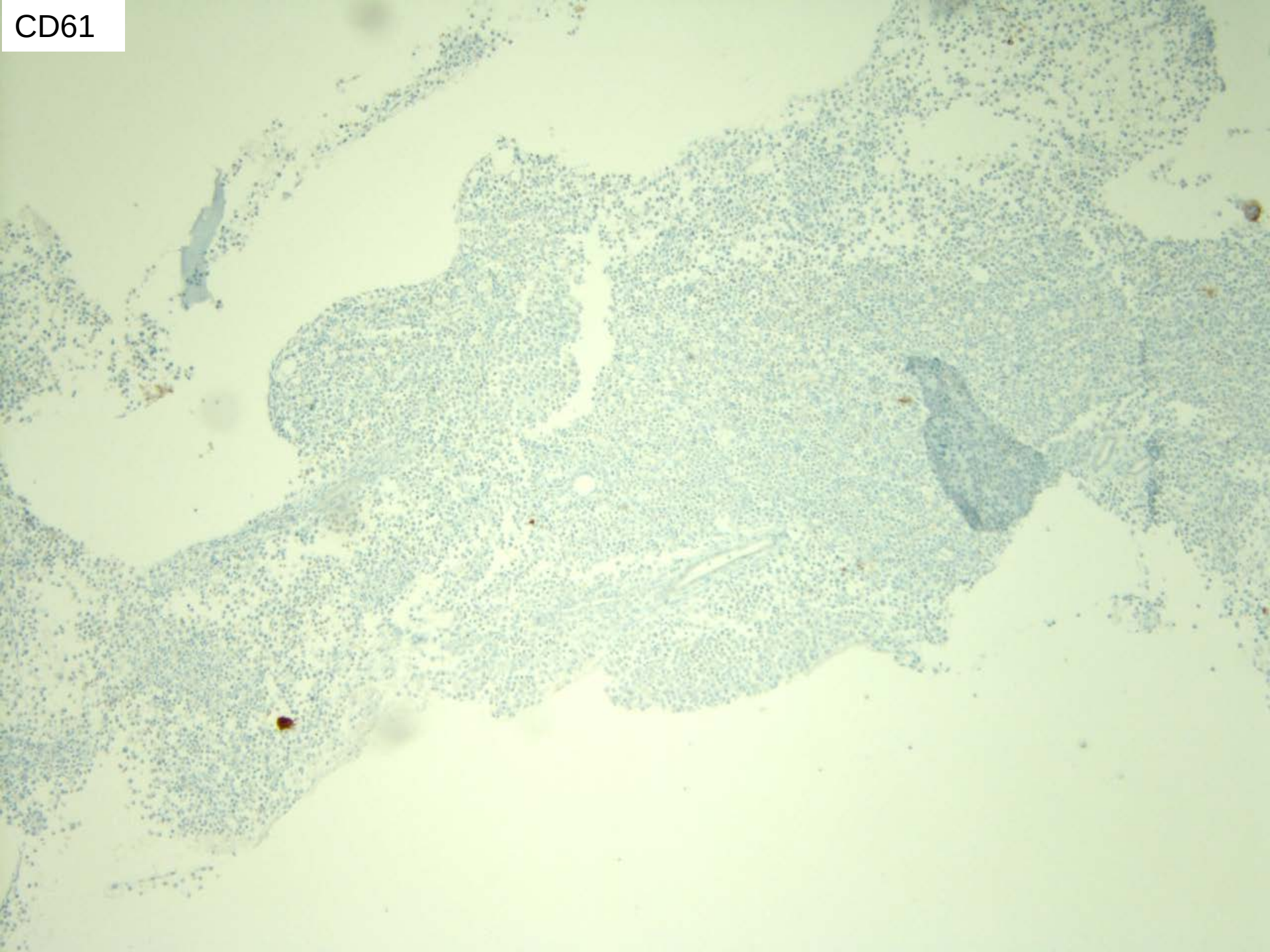




Blue: blasts; Pink: monocytes; Purple: granulocytes, Green: lymphocytes; Yellow: erythroid cells


Marrow biopsy, H&E


CD34


CD61

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Differential diagnosis

- Juvenile myelomonocytic leukemia (JMML)
- Chronic myelogenous leukemia (CML)
- Ras-associated autoimmune leukoproliferative disorder (RALD)

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Axillary Studies

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- Fetal hemoglobin was markedly elevated (17.6%)
- Negative for *BCR-ABL* translocation
- Normal karyotyping
- MDS/MPN NGS panel:

HGNC gene name	HGNC cDNA change (c.)	HGNC amino acid change (p.)	VAF (diagnostic)	VAF (Post 7 wks chemo, 0.1% MRD by flow)	VAF (end of chemo, neg MRD by flow)	Germline or somatic	Pathogenic
ASXL1	NM_015338.5: c.3976C>G	NP_056153.2: p.Pro1326Ala	49%	49%	51%	Germline	Unknown
KRAS	NM_033360.2: c.35G>T	NP_203424.1: p.Gly12Val	17%	0	0	Somatic	Yes
PTPN11	NM_002834.3: c.227A>T	NP_002825.3: p.Glu76Val	49%	4.52%	0	Somatic	Yes

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Final diagnosis

Bone marrow aspirate, biopsy, site not specified, and peripheral blood smear:

- **MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASM, CONSISTENT WITH JUVENILE MYELOMONOCYTIC LEUKEMIA.**

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Treatments and follow-up

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- Low-dose cytarabine/6MP x 1 week as per AALL1131 (demonstrated response with CBC, organomegaly and respiratory status); FLA regimen without cis-retinoic acid x 6 weeks based on COG JMML study AAML0122; 2 weeks of consolidation per AALL1131.

- Cytoreduction w busulfan, cyclophosphamide, melphalan and rATGA, followed by allogenic stem cell transplant (unrelated matched same sex donor).

- Follow-up: 3 months post SCT, he was clinically well, had normal CBC and no splenomegaly; marrow revealed normal cellularity, progressive multilineage hematopoiesis with no evidence of residual disease by flow and sequencing.

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2016 WHO Diagnostic Criteria of JMML

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I. Clinical and hematologic features (all 4 features mandatory)
• PB monocyte count ≥1 × 10^9/L
• Blast percentage in PB and BM <20%
• Splenomegaly
• Absence of Philadelphia chromosome (*BCR/ABL1* rearrangement)
II. Genetic studies (1 finding sufficient)
• Somatic mutation in *PTPN11** or *KRAS** or *NRAS**
• Clinical diagnosis of NF1 or *NF1* mutation
• Germ line *CBL* mutation and loss of heterozygosity of CBL†
III. For patients without genetic features, besides the clinical and hematologic features listed under I, the following criteria must be fulfilled:
• Monosomy 7 or any other chromosomal abnormality or at least 2 of the following criteria:
• Hemoglobin F increased for age
• Myeloid or erythroid precursors on PB smear
• GM-CSF hypersensitivity in colony assay
• Hyperphosphorylation of STAT5

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Differential Diagnosis: RALD

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- Ras-associated autoimmune leukoproliferative disorder is a chronic, nonmalignant condition with clinical and laboratory features overlapping those of JMML. Distinguishing RALD from JMML has implications for clinical care and prognosis.

- RALD demonstrates characteristic circulating activated monocytes (CD14+ CD16+), CD14 expression on granulocytes, and polyclonal CD10+ B cells.

- RALD is driven by somatic mutations in KRAS or NRAS genes, and always show normal karyotyping. PTPN11 gene mutation should not be present.

Calvo, K.R., et al. Blood. 2015;125(18):2753-2758

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Mutations in JMML

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- JMML may be triggered by mutations of genes encoding Ras-signaling pathway proteins including *KRAS, NRAS, PTPN11, NF1*, and *CBL*. These mutations are generally thought to be mutually exclusive.

- The 2016 revision of WHO classification excludes germline *KRAS, NRAS, and PTPN11* mutations from qualifying molecular evidence for a diagnosis of JMML, as these mutations are often associated with Noonan syndrome and such cases may not be real JMML.

- JMMLs are clinically heterogeneous. JMML cases with germline *CBL* mutations demonstrated spontaneous regression. Therefore, genetic studies for JMML are mandatory and JMMLs with various gene mutations may warrant different managements ranging from stem cell transplant to watchful waiting.

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The Genomic Landscape of Juvenile Myelomonocytic Leukemia

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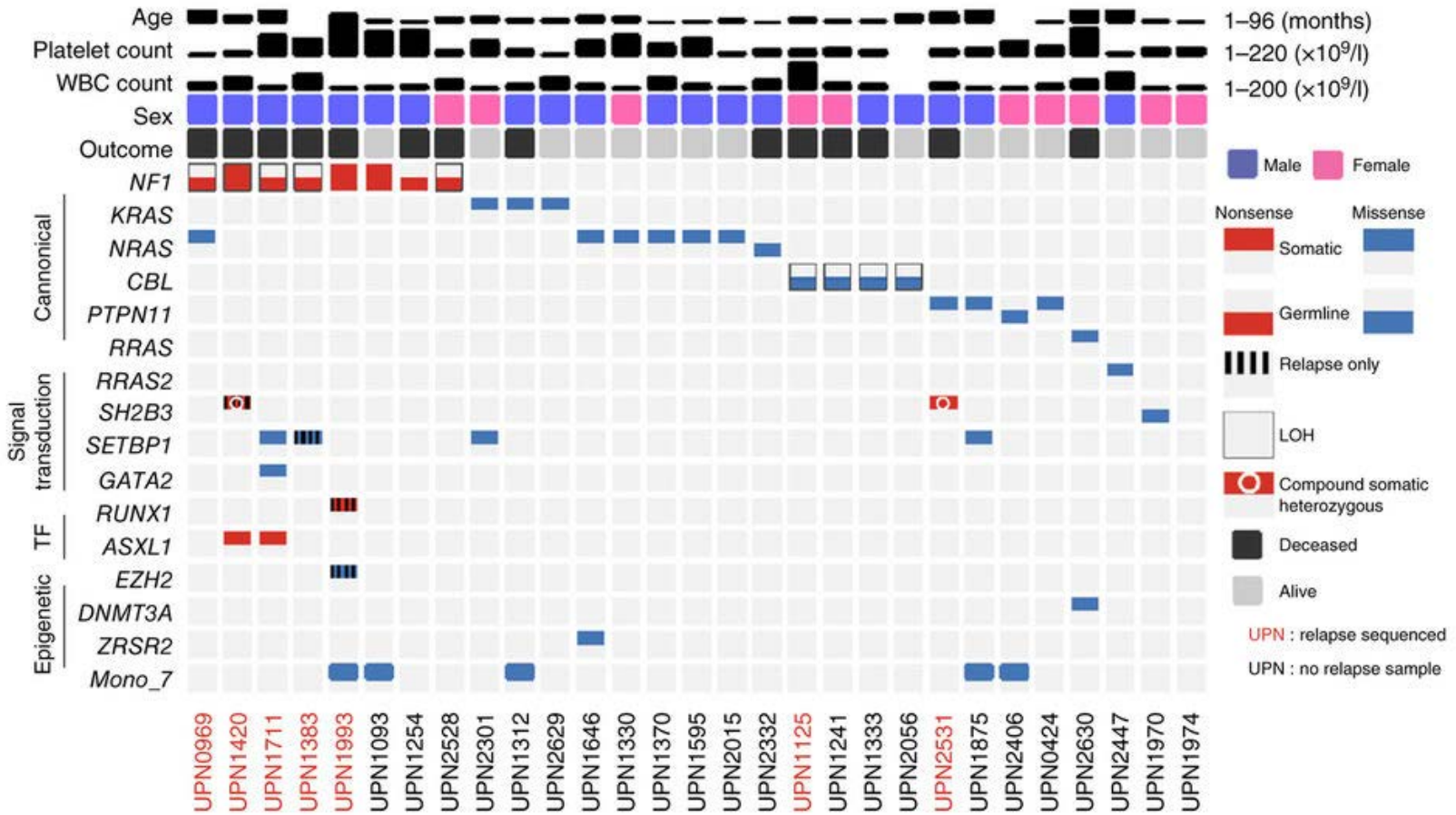

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Stieglitz, E. Nat Genet. 2015 November ; 47(11): 1326–1333.

Two or more gene mutations are common in JMML

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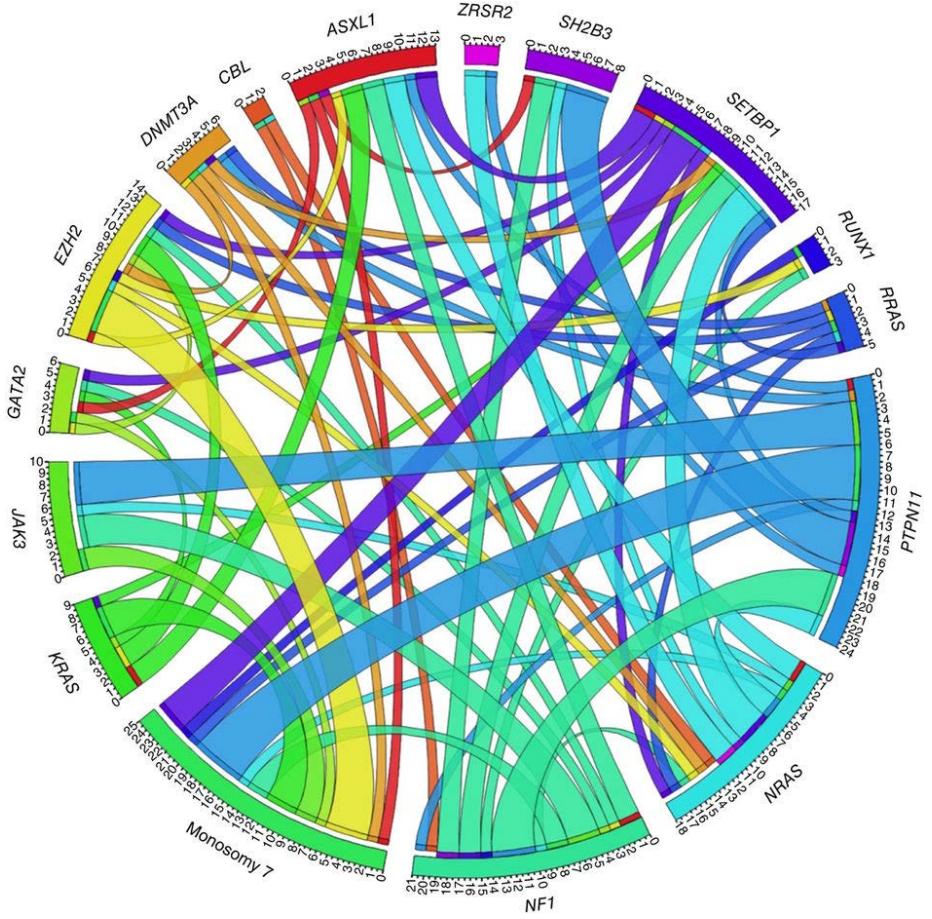

- Largest cohort: Among 98 children with JMML, 2 or more gene alterations were found in 34 (34.7%) patients.
- Co-existing mutations in NRAS, KRAS, PTPN11, CBL, and NF1 were found in 11 (11.0%) of patients. PTPN11 and NF1 lesions were the most frequent of these cooperative events.
- Somatic alterations at diagnosis predicts outcome.

Stieglitz, E. Nat Genet. 2015 November ; 47(11): 1326–1333.

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Take home messages

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- Our case showed germline *ASXL1* (Additional Sex Combs-Like 1) missense mutation with uncertain significance, and somatic gain-of-function mutations of two Ras-pathway genes *PTPN11 and KRAS*, which has not been reported in literature.

- The significance of germline *ASXL1* missense mutation in this case is unclear. We could not find report in literature or record in COSMIC. This is possibly a single-nucleotide polymorphism (SNP) of *ASXL1* gene but we are not 100% sure.

- While somatic mutations of *ASXL1* are well known to be associated with myeloid malignancies, germline heterozygous mutations of *ASXL1* gene leads to a dysmorphic syndrome called Bohring-Opitz syndrome (OMIM). Our patient did not seem to have it. Parents declined testing of *ASXL1* gene mutation.

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Panel diagnosis

- **JUVENILE MYELOMONOCYTIC LEUKEMIA (WITH PROBABLE GERMLINE ASXL1 SNP AND SOMATIC PTPN11 MUTATION)**

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