

Essential role of flow cytometry in the diagnosis of acute myeloid leukemia

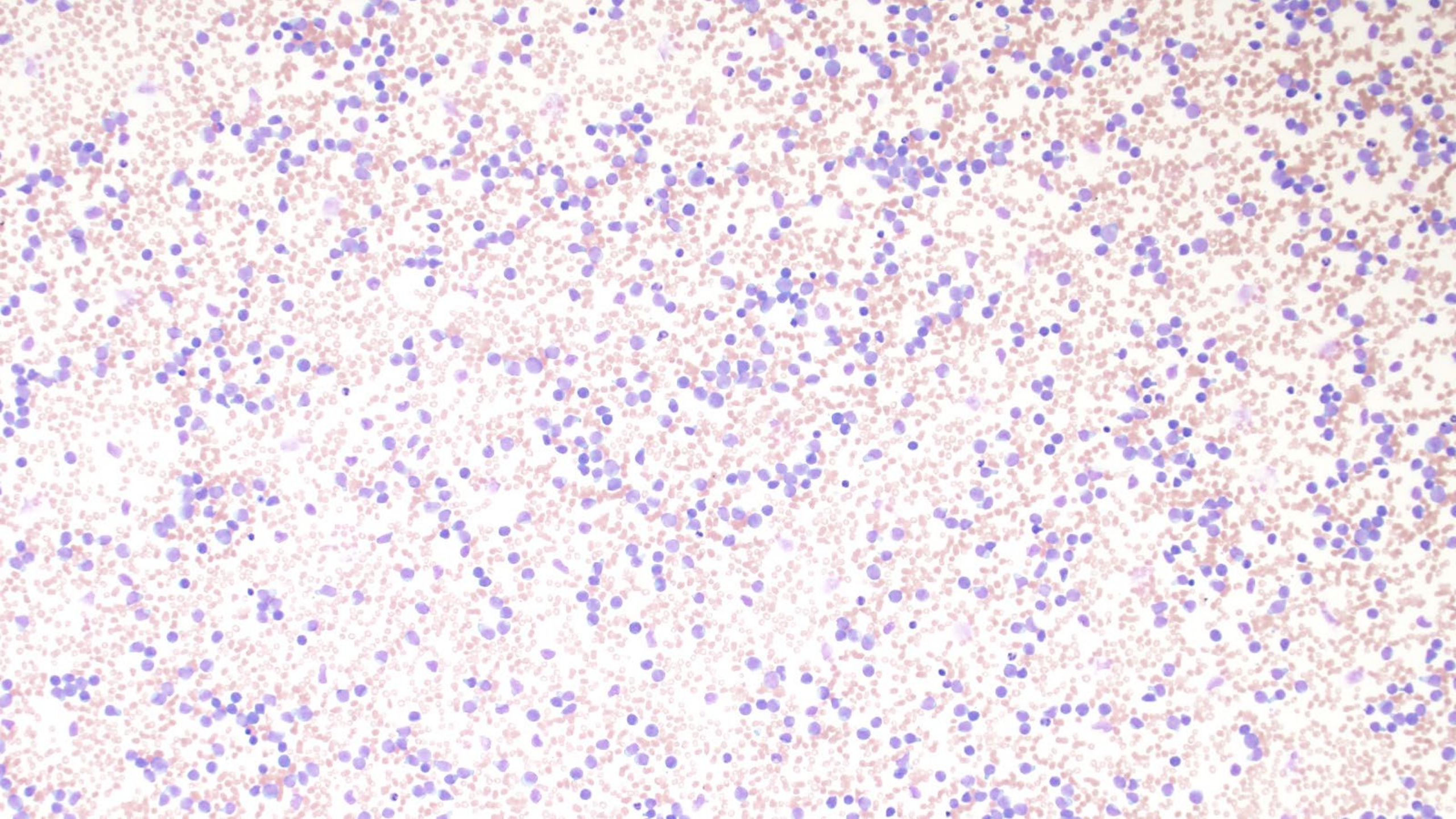
Emily Mason, MD, PhD

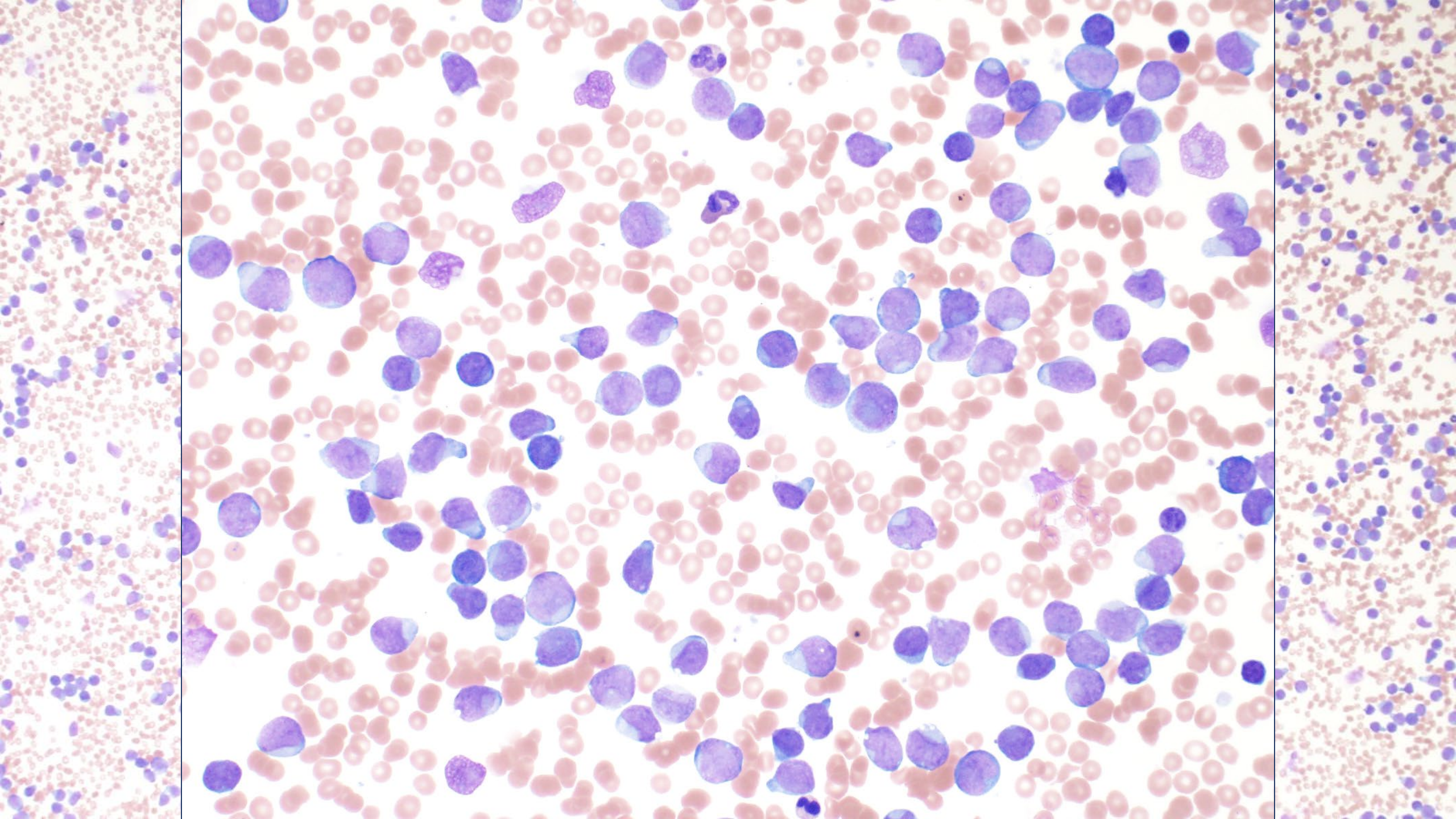
Disclosures

- I am receiving an honorarium from Sysmex for preparing and delivering today's presentation.
- **The views expressed in the case studies are those of the presenter. Results of case studies are not predictive of other cases and results may vary.**

Objectives

- Understand the work flow used in the diagnosis of acute myeloid leukemia (AML)
- Understand how to interpret flow cytometry data from specimens with increased blasts
- Understand how flow cytometry results dictate further testing in the context of acute leukemia





Acute leukemia is complicated!

Acute myeloid leukemia (AML) and related neoplasms

AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

APL with *PML-RARA*

AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*

AML with t(6;9)(p23;q34.1); *DEK-NUP214*

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*

AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*

Provisional entity: AML with BCR-ABL1

AML with mutated *NPM1*

AML with biallelic mutations of *CEBPA*

Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, NOS

AML with minimal differentiation

AML without maturation

AML with maturation

Acute myelomonocytic leukemia

Acute monoblastic/monocytic leukemia

Pure erythroid leukemia

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis (TAM)

Myeloid leukemia associated with Down syndrome

- Blast lineage
- Genetic results
- Clinical history

Acute leukemias of ambiguous lineage

Acute undifferentiated leukemia

Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*

MPAL with t(v;11q23.3); *KMT2A* rearranged

MPAL, B/myeloid, NOS

MPAL, T/myeloid, NOS

B-lymphoblastic leukemia/lymphoma

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B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities

B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); *BCR-ABL1*

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B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*

B-lymphoblastic leukemia/lymphoma with hyperdiploidy

B-lymphoblastic leukemia/lymphoma with hypodiploidy

B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*

B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*

Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like

Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21

T-lymphoblastic leukemia/lymphoma

Provisional entity: Early T-cell precursor lymphoblastic leukemia

Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

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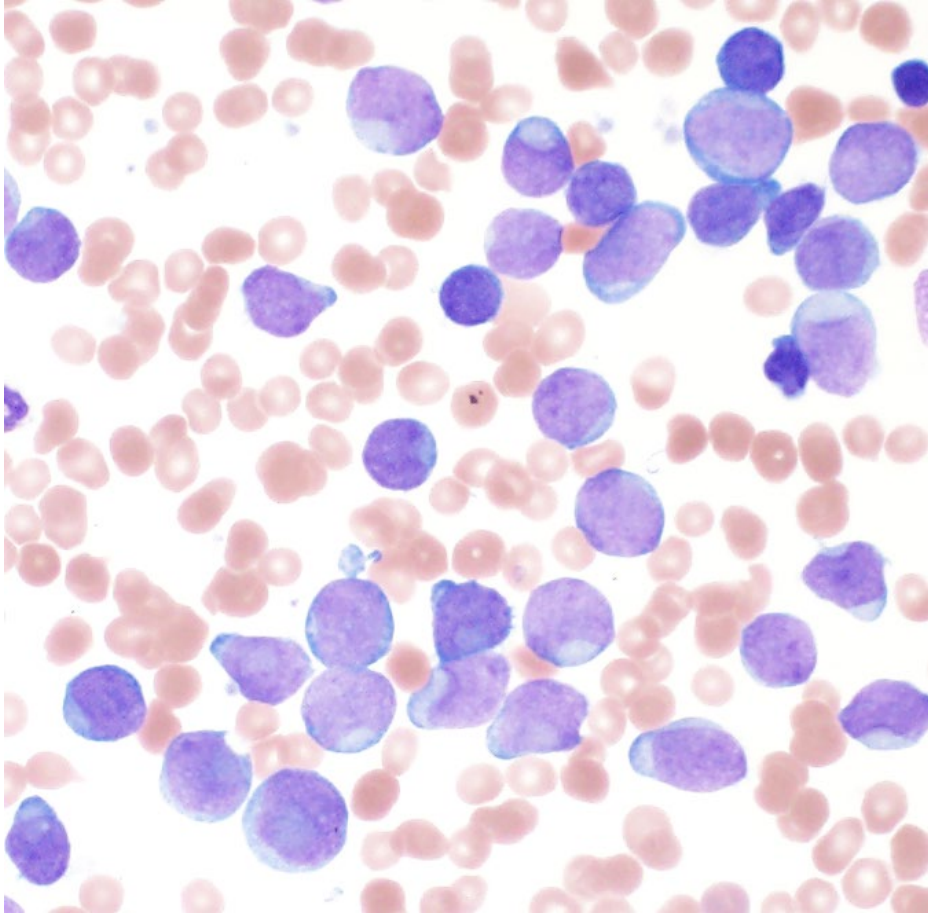
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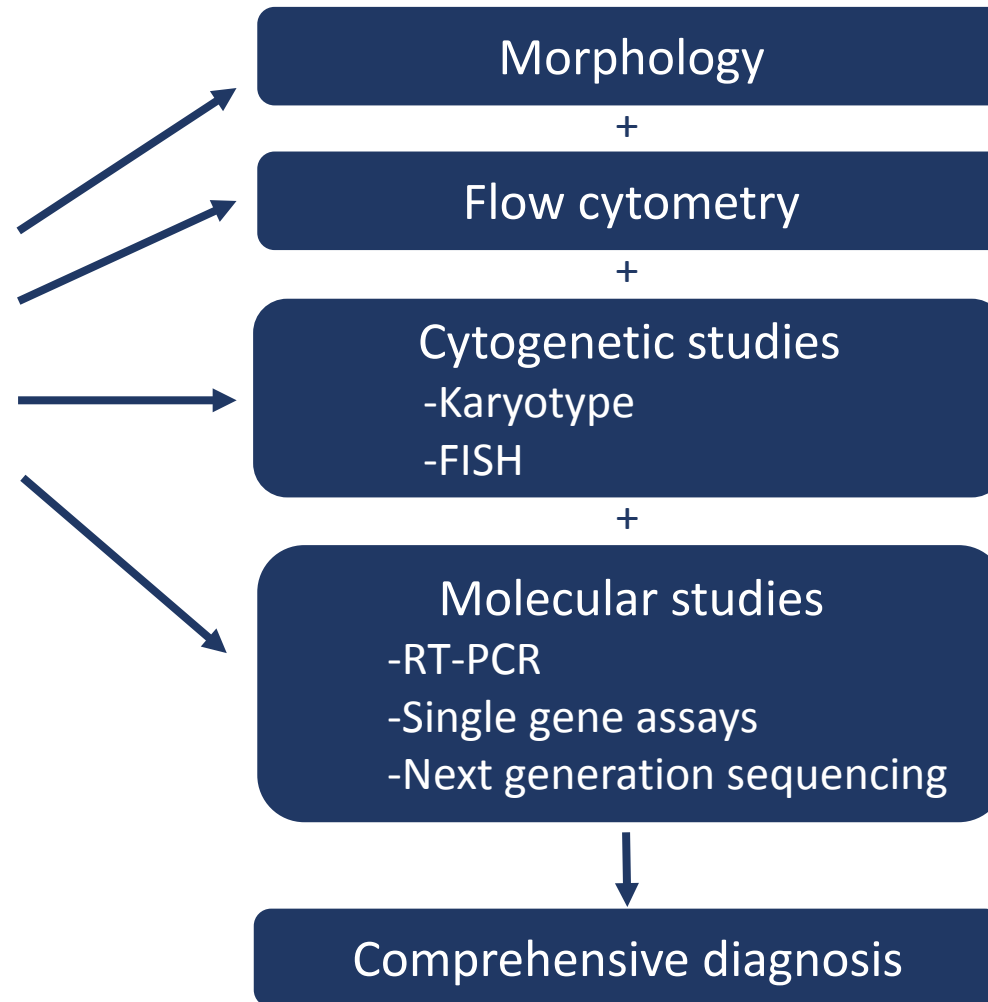
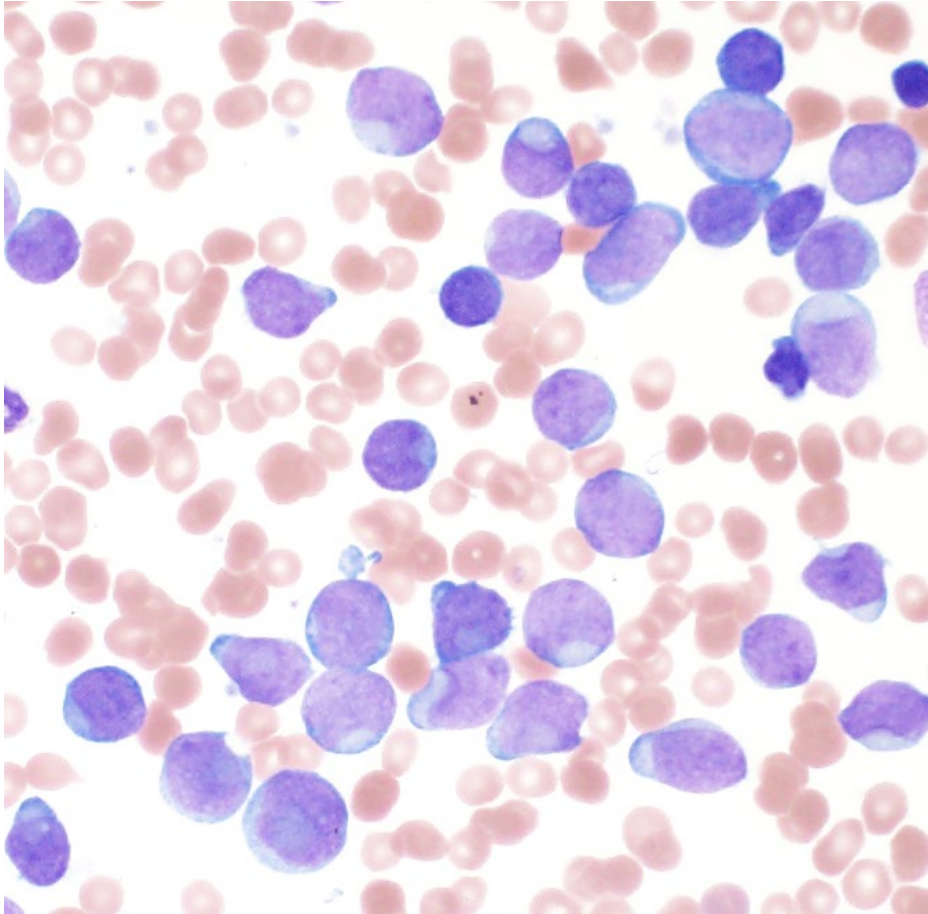
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AML diagnosis requires integrated analysis



Acute myeloid leukemia

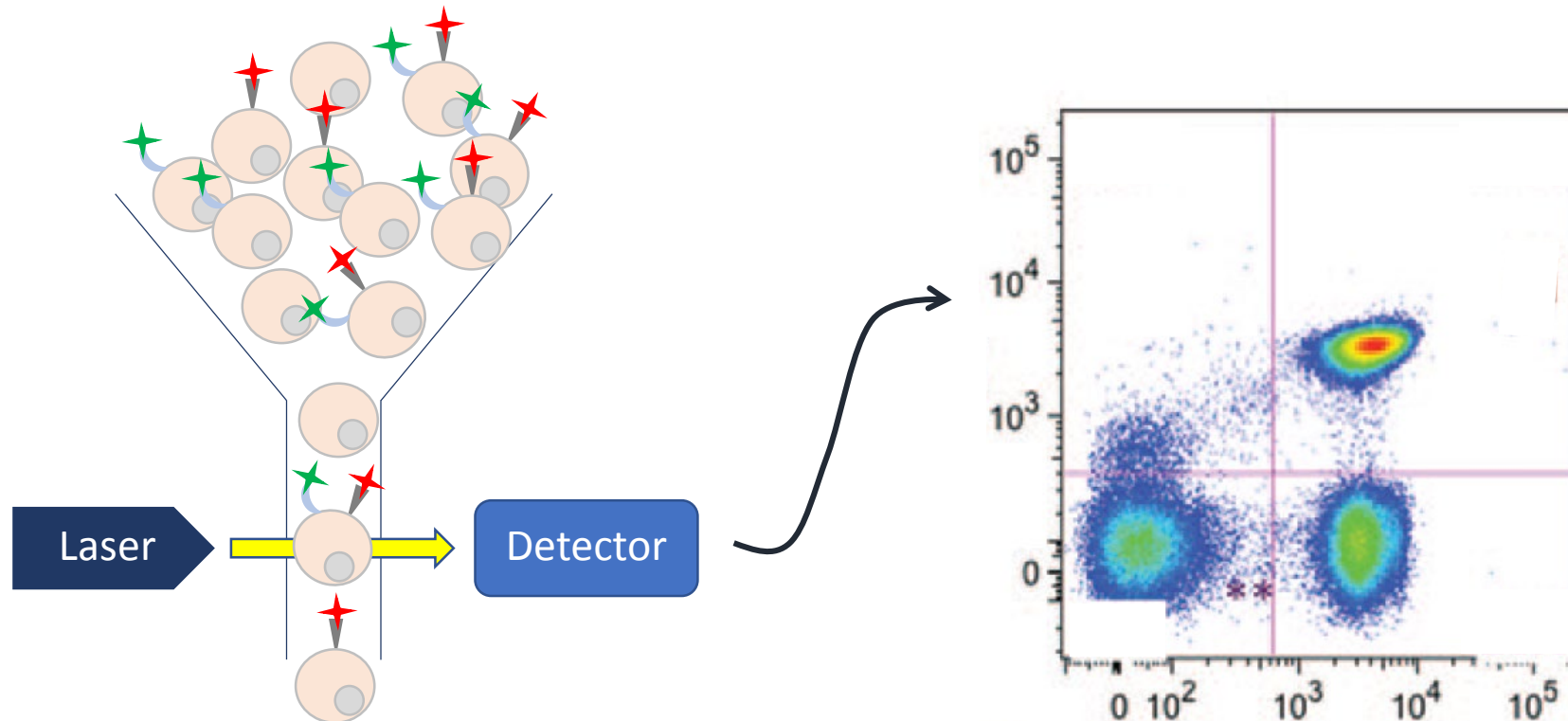
AML diagnosis requires integrated analysis



First step in evaluation of circulating blasts?

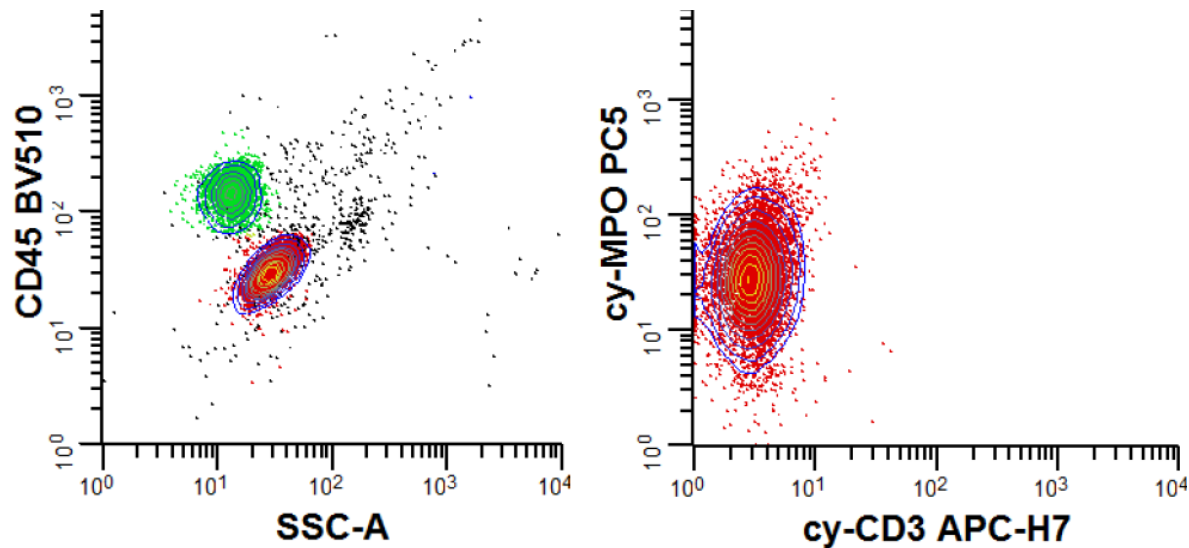
➤ Flow cytometry

- Fast results
- Best modality for assigning blast lineage
- Assess multiple markers on single cells



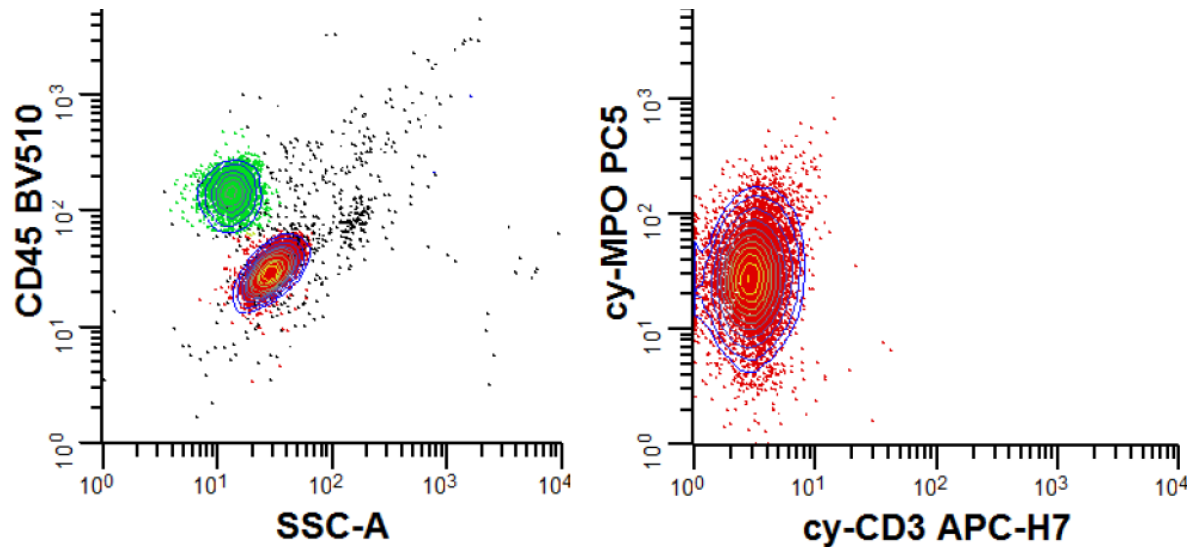
Lineage defining markers

- Myeloid: MPO
- B-lymphoid: CD19, CD79a, CD22, CD10
- T-lymphoid: CD3



Lineage defining markers

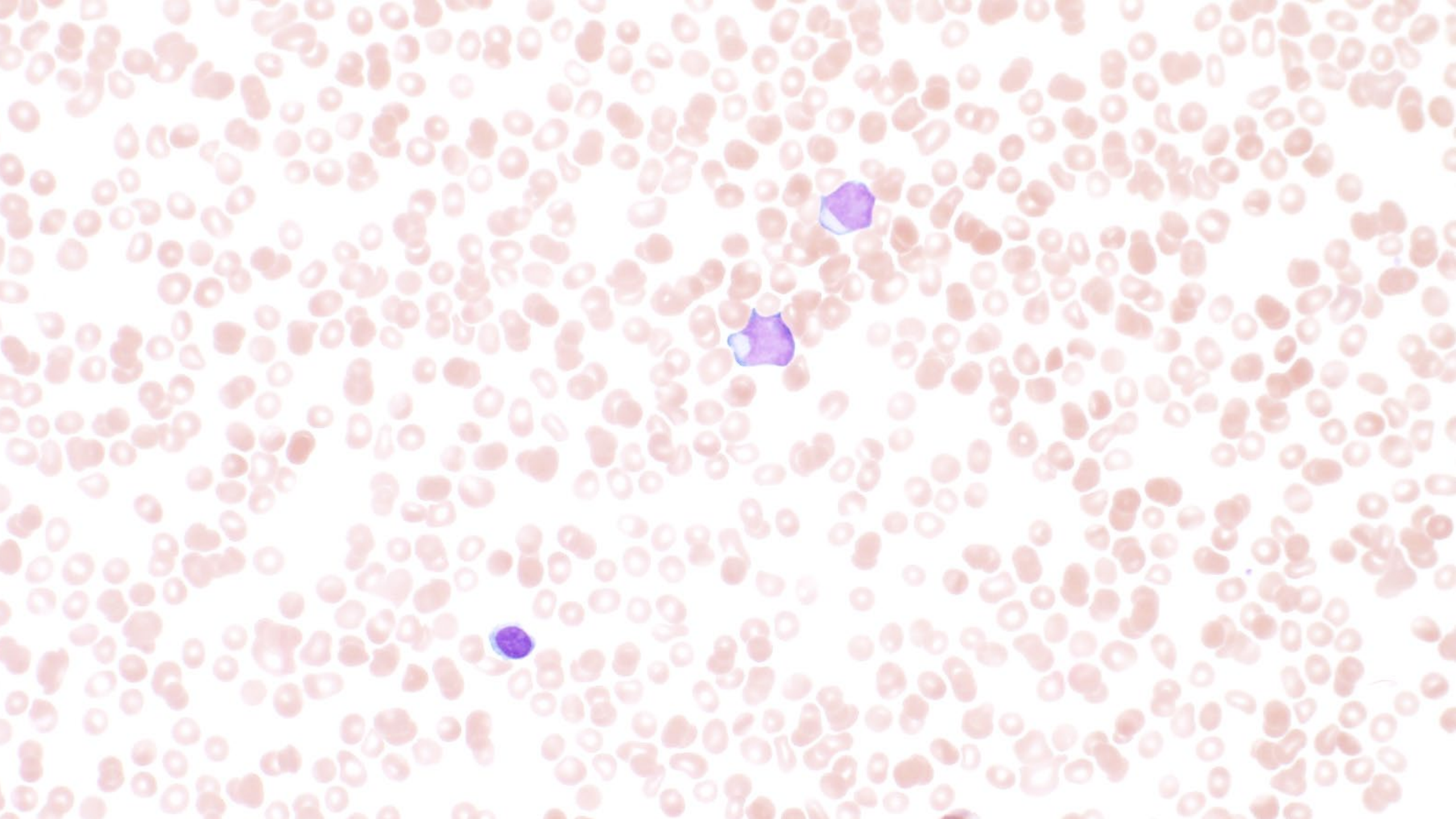
- Myeloid: MPO
 - B-lymphoid: CD19, CD79a, CD22, CD10
 - T-lymphoid: CD3
- Blast lineage dictates further ancillary testing
 - AML: Cytogenetics, Next generation sequencing
 - B-ALL: Cytogenetics, BCR-ABL testing, Philadelphia-like testing

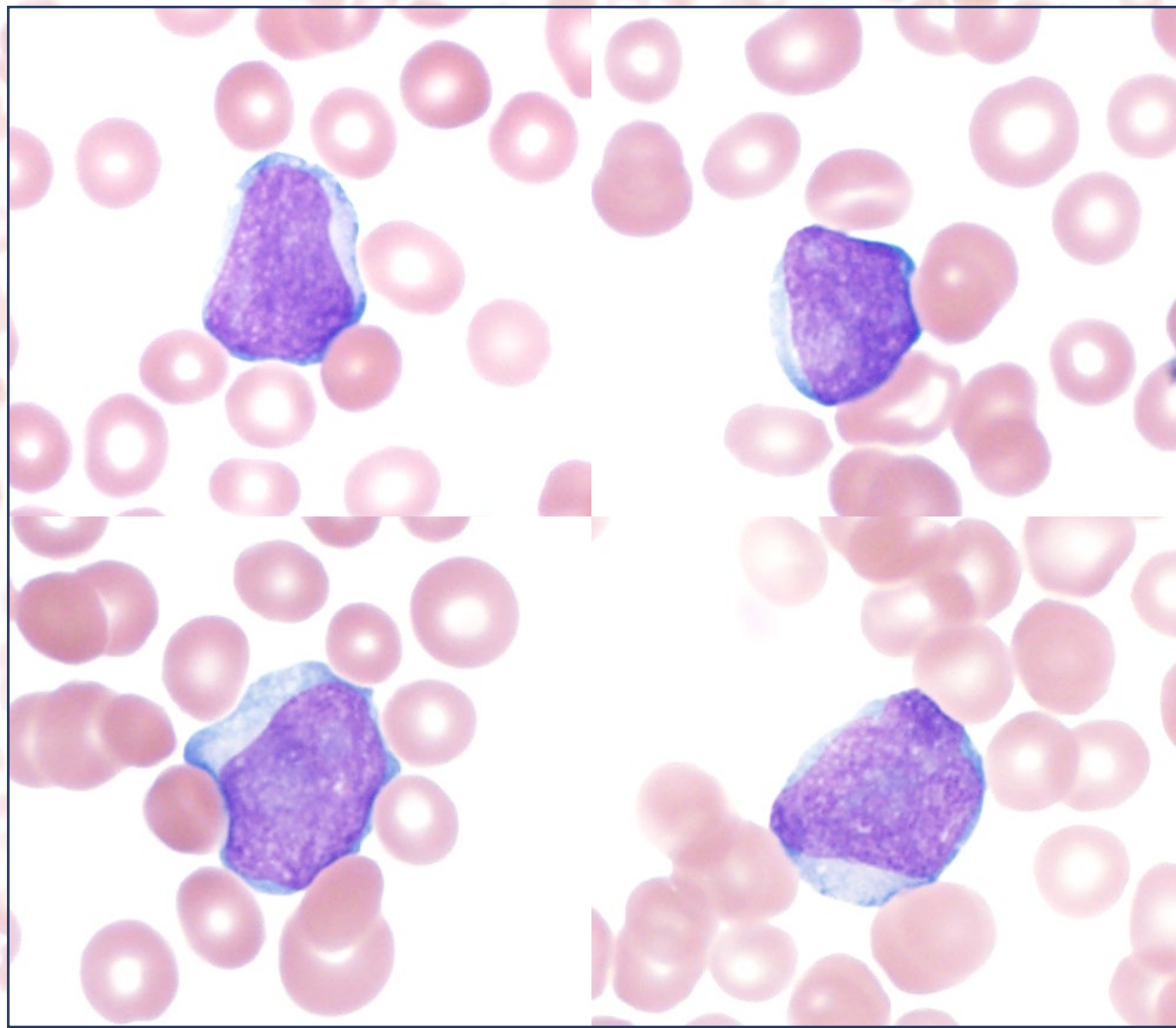


Case 1

- 48 year old female with a history of AML with inv(16) diagnosed 12 years ago, s/p 7+3 induction and 4 cycles HiDAC, in complete remission since 2006
- Presents with worsening fatigue and easy bruising

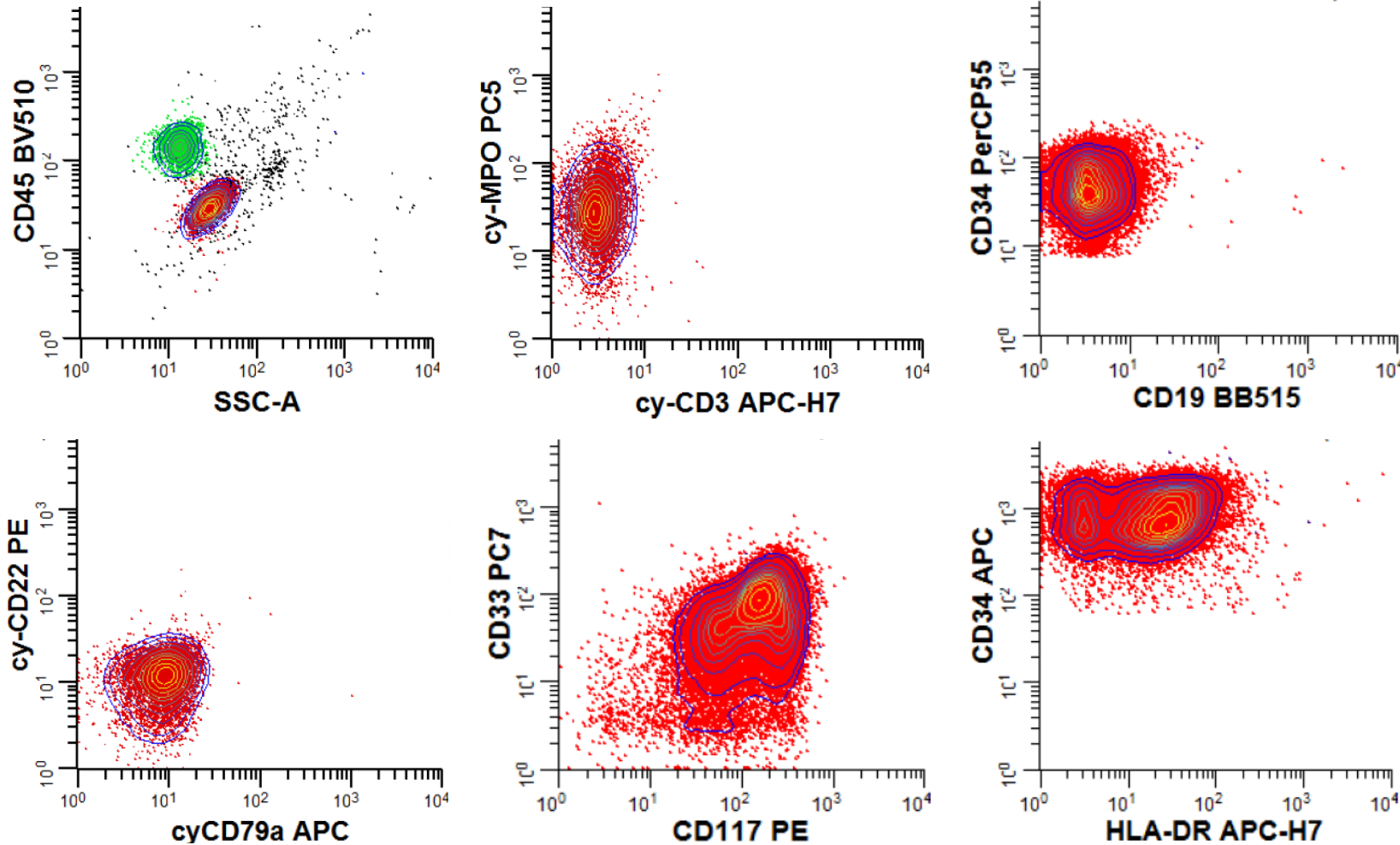
CBC	Result	Reference range
WBC	3.7	3.9-10.7 x 10 ³ /μL
WBC Differential:	Neutrophils 1.7%, Lymphocytes 46.1%, Monocytes 0.9% Blasts 51.3%	
Hgb	8.7	14.0-18.1 g/dL
HCT	24	41-49 %
Platelets	8	135-371 x 10 ³ /μL





Flow cytometry results

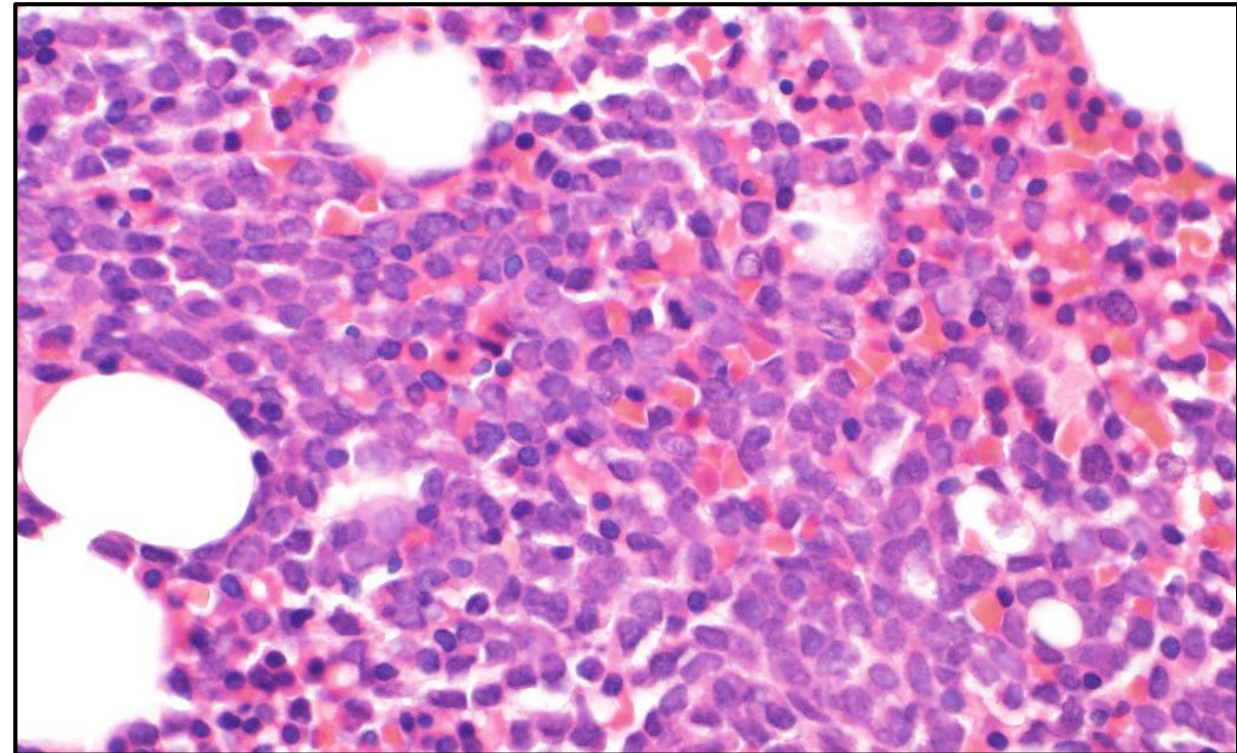
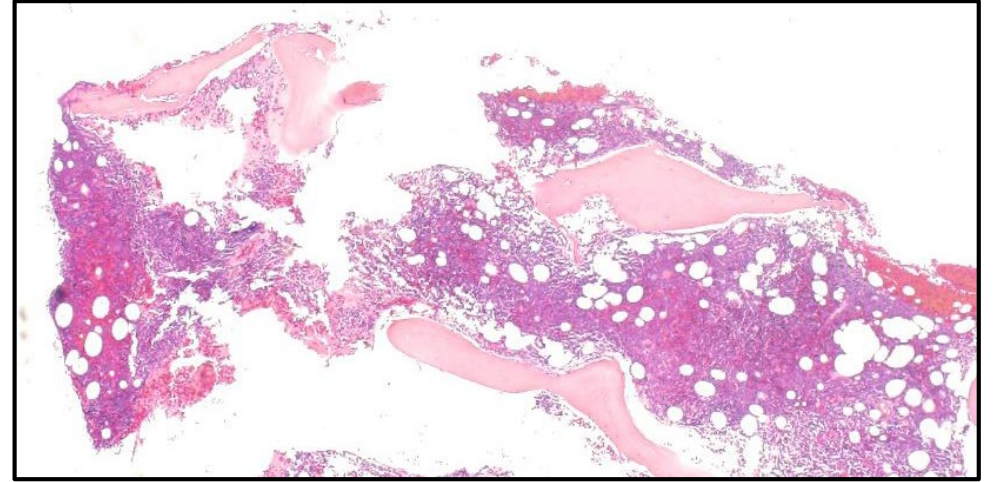
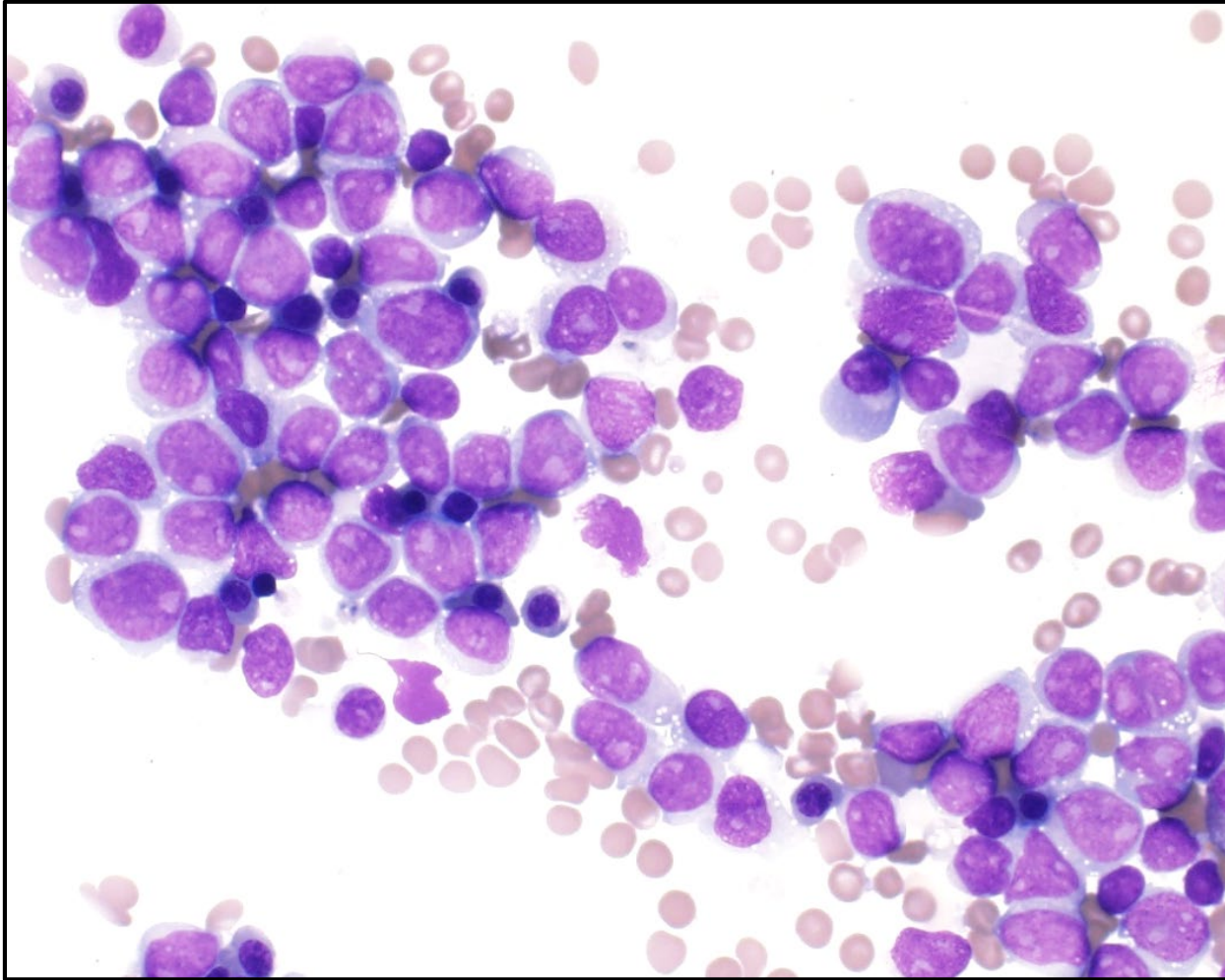
- 51% myeloid blasts in the peripheral blood → AML
- Relapsed AML with inv(16)?
- New AML?



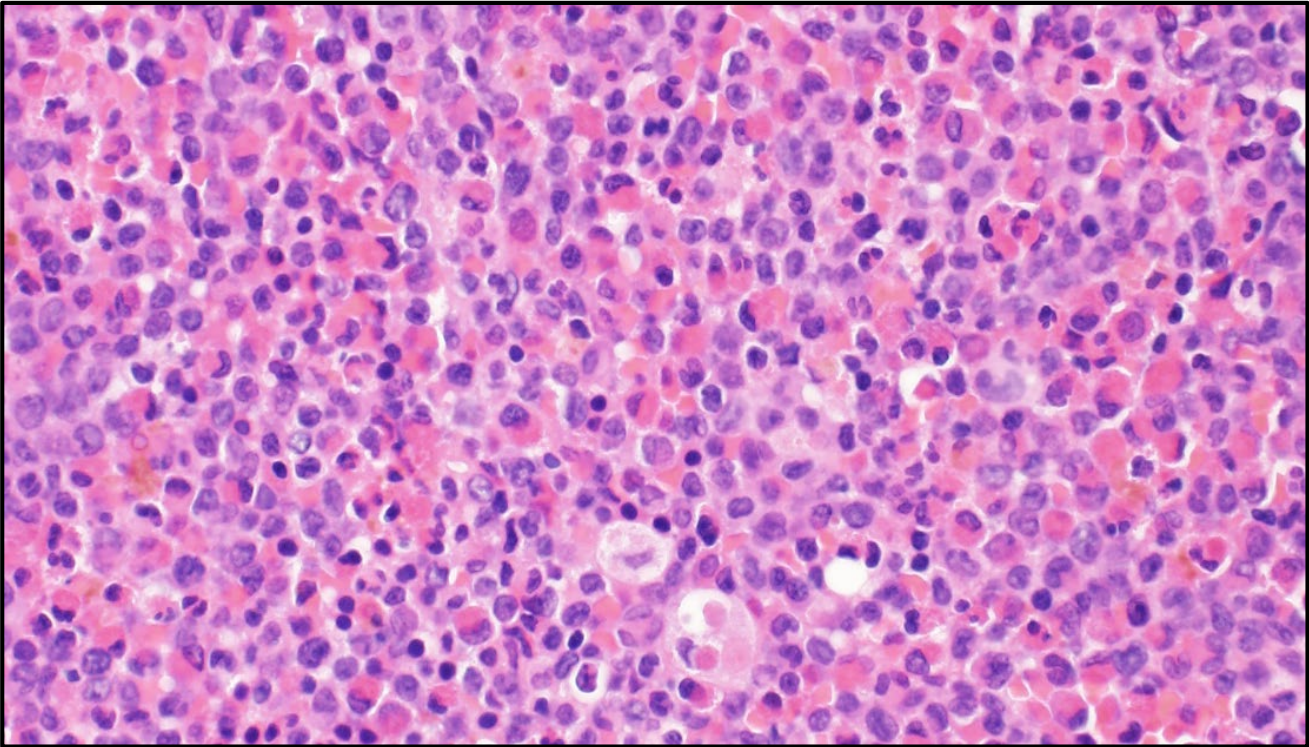
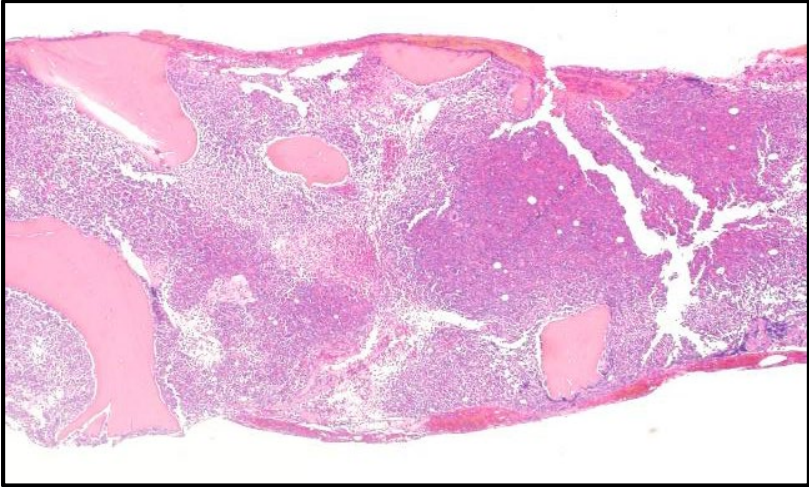
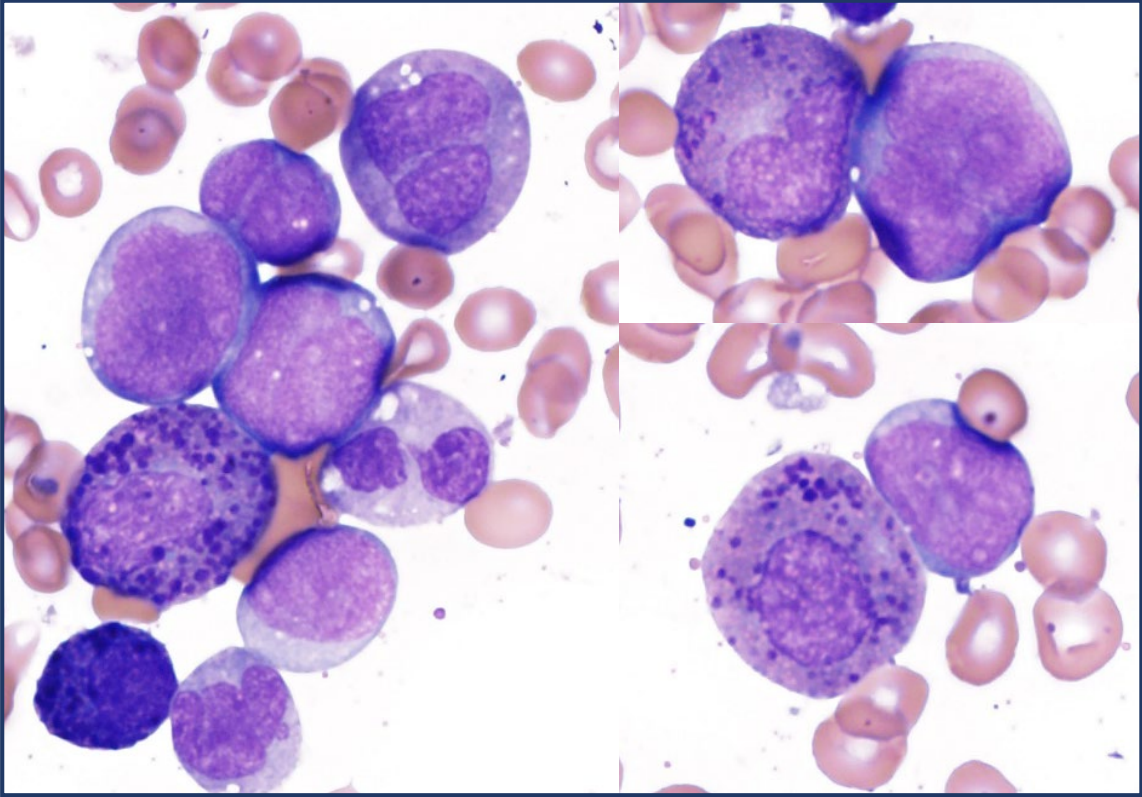
Positive: CD7 (dim), CD13, CD33, CD34 (bright), CD38, CD45 (dim), CD117, HLA-DR (het), MPO

Negative: CD2, CD4, CD11b, CD14, CD15, CD19, CD64, cCD3, cCD22, cCD79a, TdT

Bone marrow biopsy



AML with inv(16)



Ancillary testing results

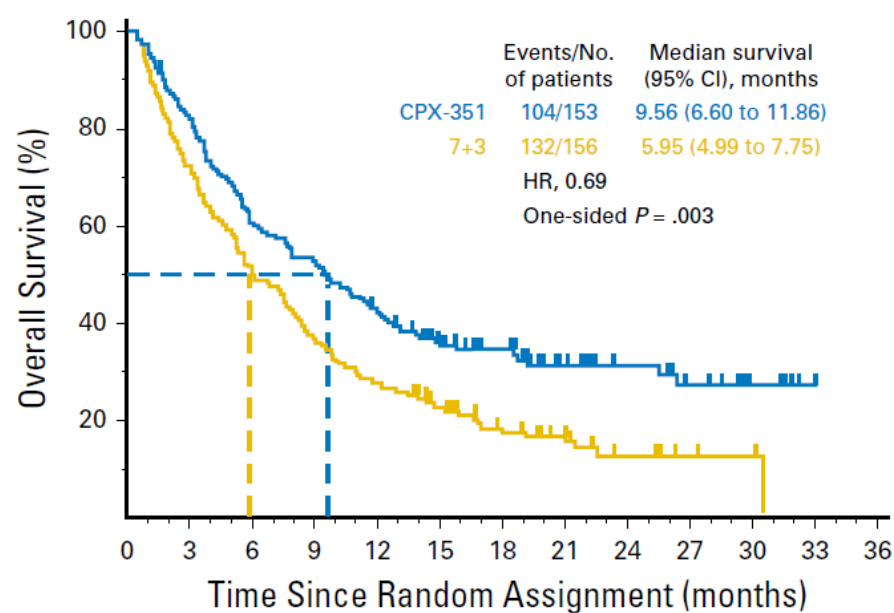
- Karyotype: 45~47,XX,-5,add(7)(q11.2),+8,+11,der(11)t(11;11)(p15;q13),add(16)(q21),psu dic(17;5)(p11.2;q22),-17,-20,+1~2mar[cp16]/46,XX[4]
 - FISH:
 - Negative for rearrangement of the CFBF locus at 16q22
 - Positive for a loss of one copy of the CFBF locus at 16q22 in 34.5% of cells
 - Positive for multiple copies of multiple probes in 1.3%~54% of cells
 - Next generation sequencing:
 - *TP53* (VAF 45%)
 - *IDH1* (VAF 34%)
- Comprehensive diagnosis: Therapy-related acute myeloid leukemia with complex karyotype and *TP53* and *IDH1* mutations

Therapy-related AML

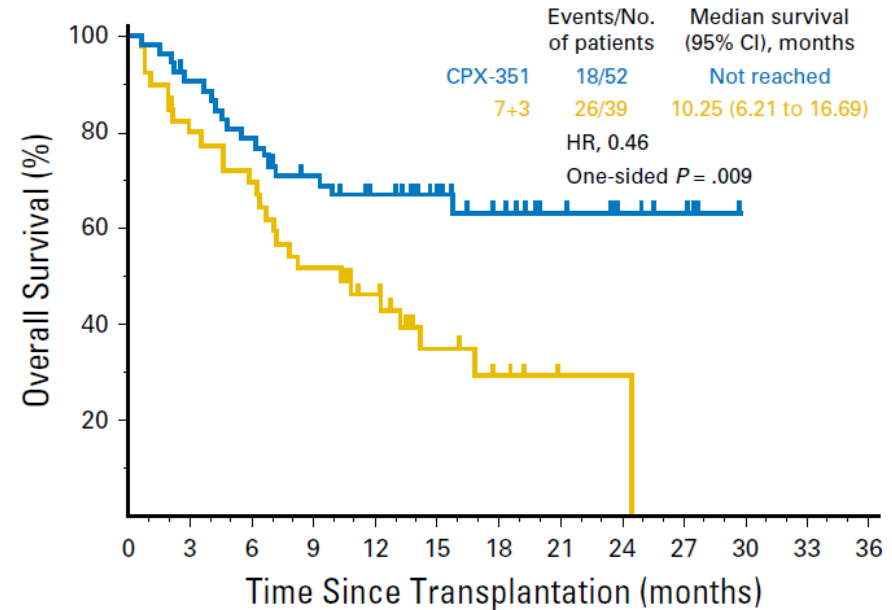
- Complication of prior cytotoxic chemotherapy and/or radiotherapy
 - Occurs in a small fraction of treated patients
- Associated with poor response to conventional therapy and poor prognosis
- Enriched for cases with deletions of 5q and 7/7q, complex karyotypes, and *TP53* mutations
 - Cytotoxic therapy may select for therapy-resistant pre-existing stem/progenitor cell clones harboring *TP53* mutations

Targeted therapy in acute myeloid leukemia

- Vyxeos: Liposomal formulation of daunorubicin and cytarabine in fixed 5:1 ratio
 - FDA approved for newly diagnosed therapy-related AML and AML with myelodysplasia-related changes



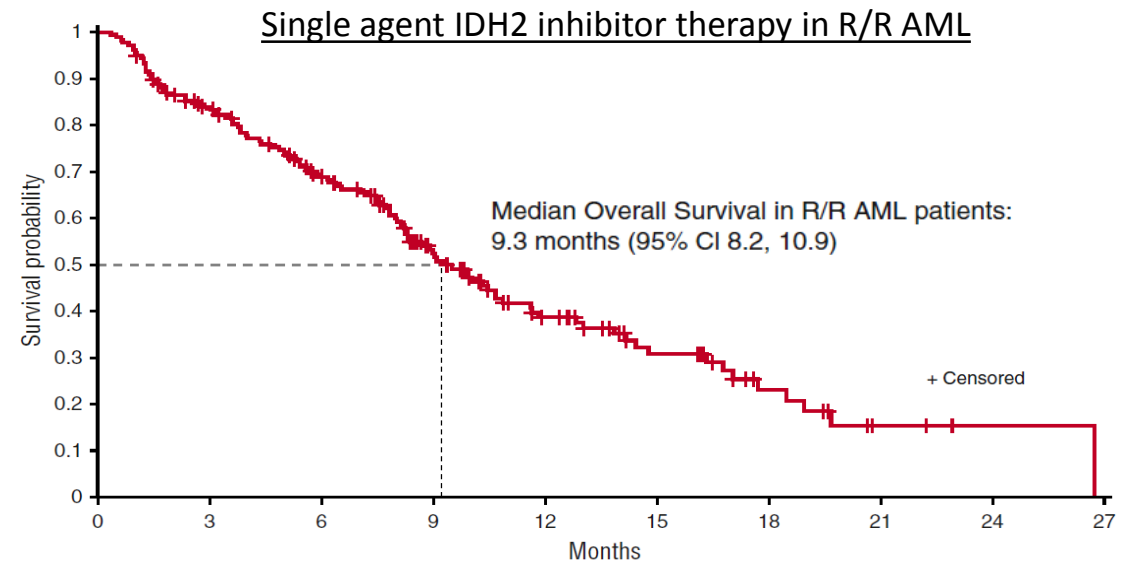
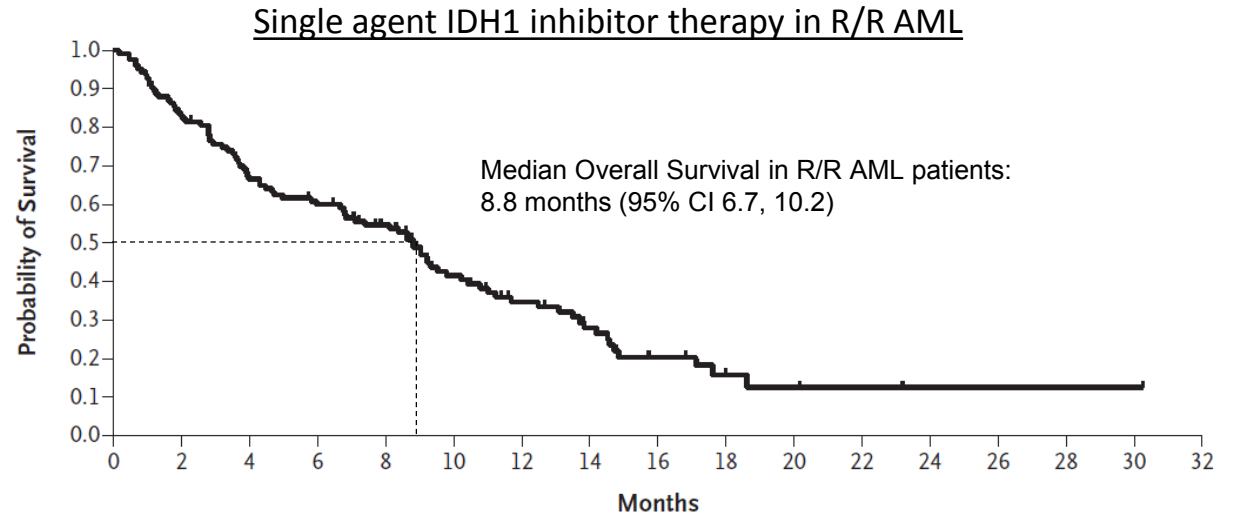
No. at risk	
CPX-351	153 122 92 79 62 46 34 21 16 11 5 1
7+3	156 110 77 56 43 31 20 12 7 3 2 0



No. at risk	
CPX-351	52 46 40 34 27 20 15 9 6 3 0 0
7+3	39 31 27 20 15 7 4 1 1 0 0 0

Targeted therapy in acute myeloid leukemia

- Targeted IDH inhibitors
 - Selectively inhibit mutant IDH proteins
 - FDA approved for newly diagnosed AML with *IDH1* mutation and for relapsed/refractory AML with *IDH1* or *IDH2* mutation
 - With single agent therapy in R/R AML patients, median OS of 8.8 months and 9.3 months
 - Historical control of 3.3 month median OS in R/R AML patients receiving non-targeted therapy



Targeted therapy in acute myeloid leukemia

Currently recruiting trials registered at Clinicaltrials.gov for IDH inhibitor therapy in AML

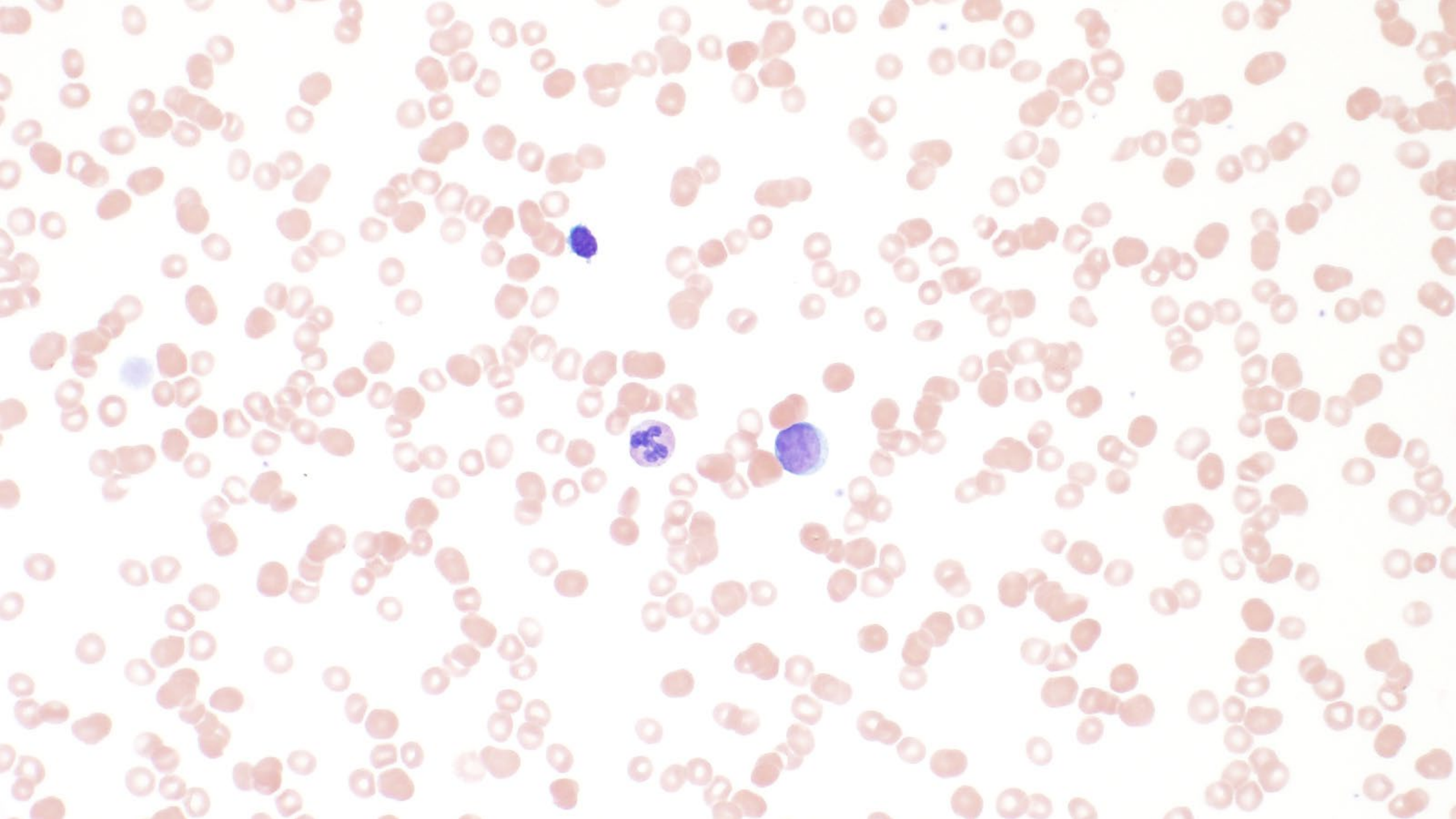
Clinical trial	Study Title	Phase
NCT03683433	Enasidenib and Azacitidine in Treating Patients With Recurrent or Refractory Acute Myeloid Leukemia and IDH2 Gene Mutation	II
NCT03825796	CPX-351 and Enasidenib in Treating Patients With Relapsed Acute Myeloid Leukemia Characterized by IDH2 Mutation	II
NCT03515512	IDH2 Inhibition Using Enasidenib as Maintenance Therapy for IDH2-mutant Myeloid Neoplasms Following Allogeneic Stem Cell Transplantation	I
NCT02577406	An Efficacy and Safety Study of AG-221 (CC-90007) Versus Conventional Care Regimens in Older Subjects With Late Stage Acute Myeloid Leukemia Harboring an Isocitrate Dehydrogenase 2 Mutation	III
NCT03173248	Study of AG-120 (Ivosidenib) vs. Placebo in Combination With Azacitidine in Patients With Previously Untreated Acute Myeloid Leukemia With an IDH1 Mutation	III
NCT03471260	Ivosidenib and Venetoclax With or Without Azacitidine in Treating Participants With IDH1 Mutated Hematologic Malignancies	I/II

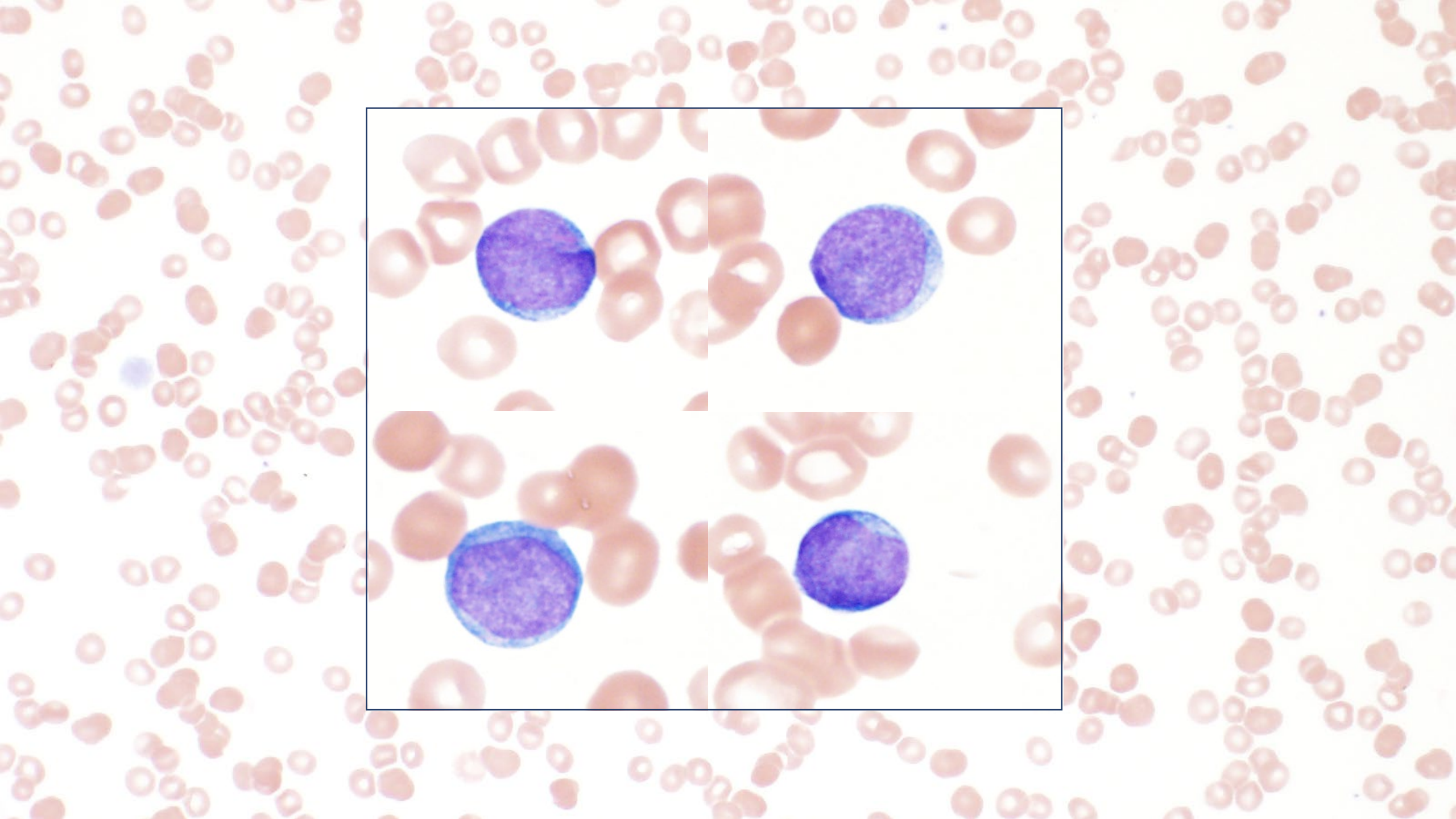
➤ Blast lineage assignment by flow cytometry → appropriate ancillary testing → identification of therapeutic targets

Case #2

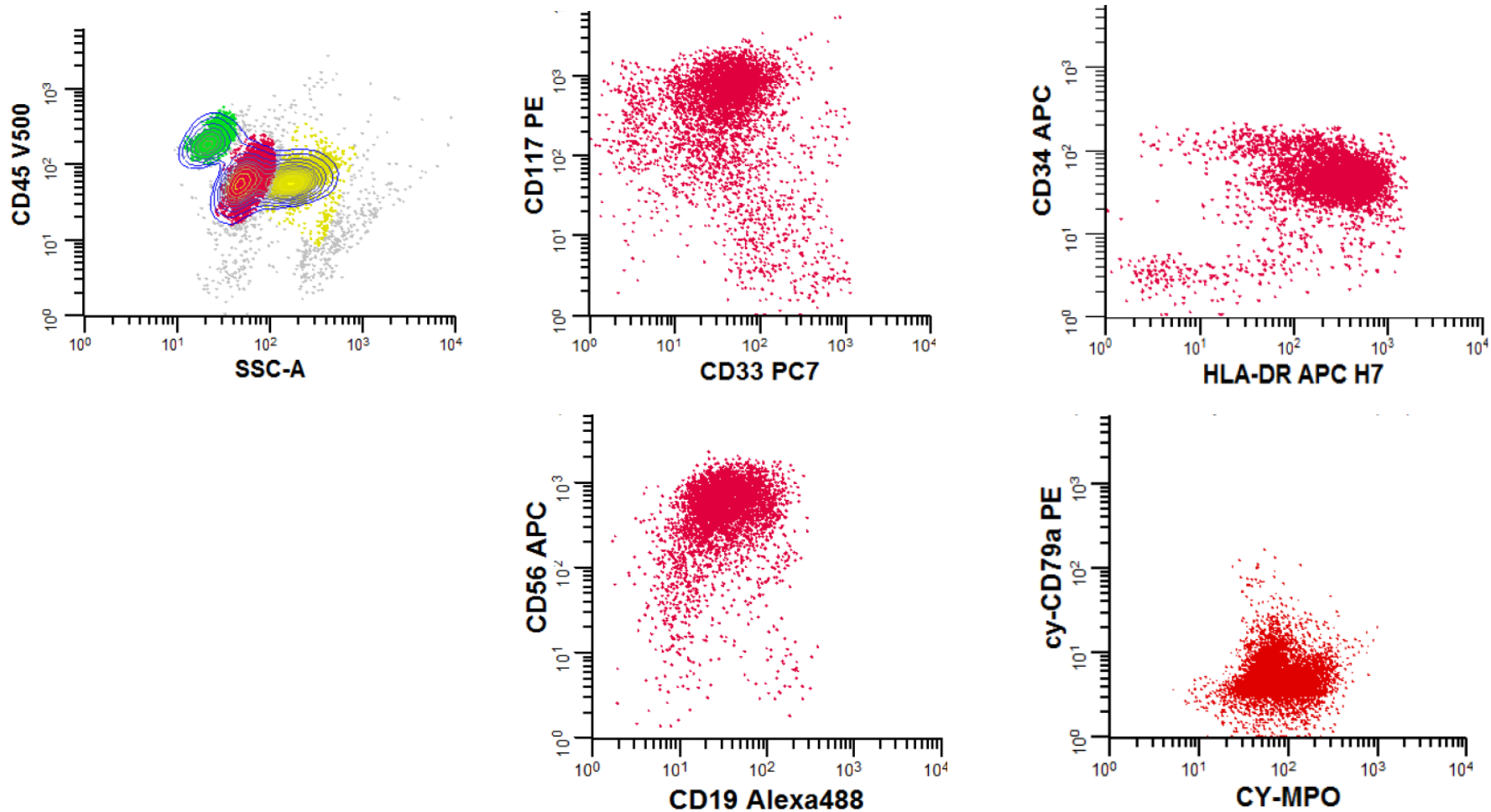
- 71 year old female with a history of hypertension presented to her PCP with 3 weeks of fatigue
- CBC at the PCPs office showed pancytopenia with 16% circulating blasts
- The patient was instructed to present to the emergency room

CBC	Result	Reference range
WBC	2.9	3.9-10.7 x 10 ³ /μL
WBC Differential:	Neutrophils 26.3%, Lymphocytes 45.8%, Metamyelocytes 2.5% Myelocytes 1.7% Promyelocytes 1.7% Blasts 22%	
Hgb	6.0	14.0-18.1 g/dL
HCT	18	41-49 %
Platelets	21	135-371 x 10 ³ /μL





Flow cytometry results

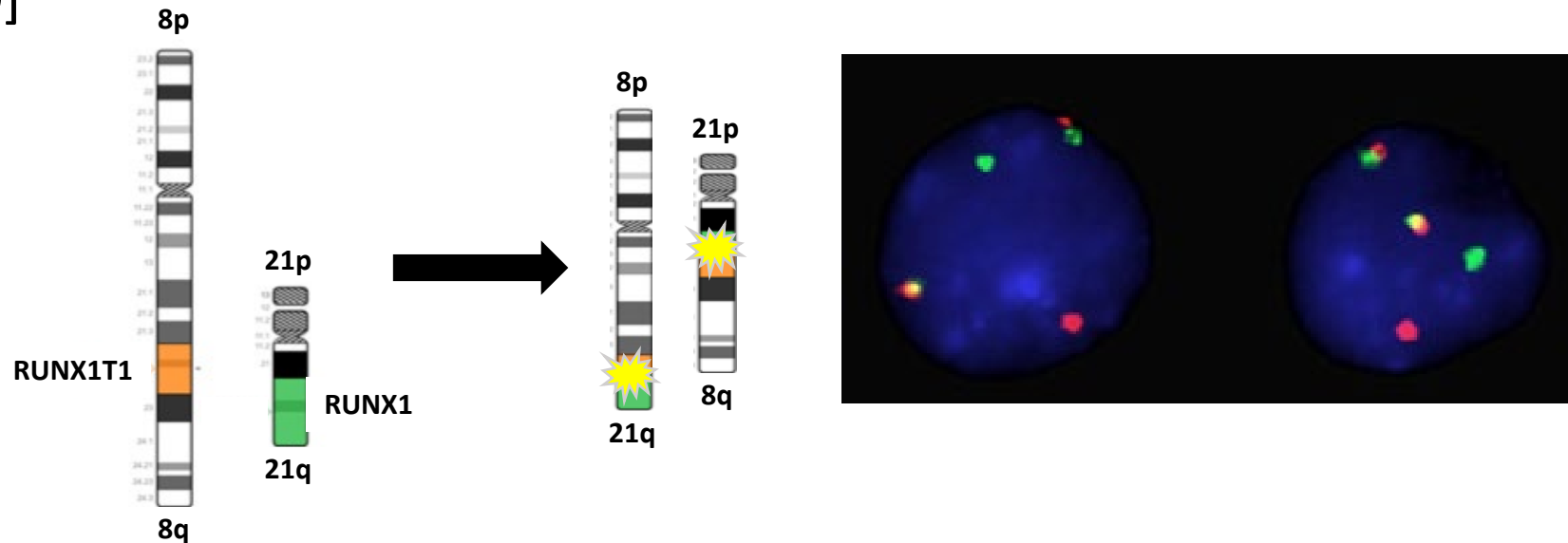


Positive: CD13, CD19, CD33, CD34, CD45 (dim), CD56 (bright) CD117 (bright), HLA-DR, MPO

Negative: CD2, CD4, CD7, CD11b, CD14, CD15, CD64, cCD3, cCD79a, TdT

Additional ancillary testing results

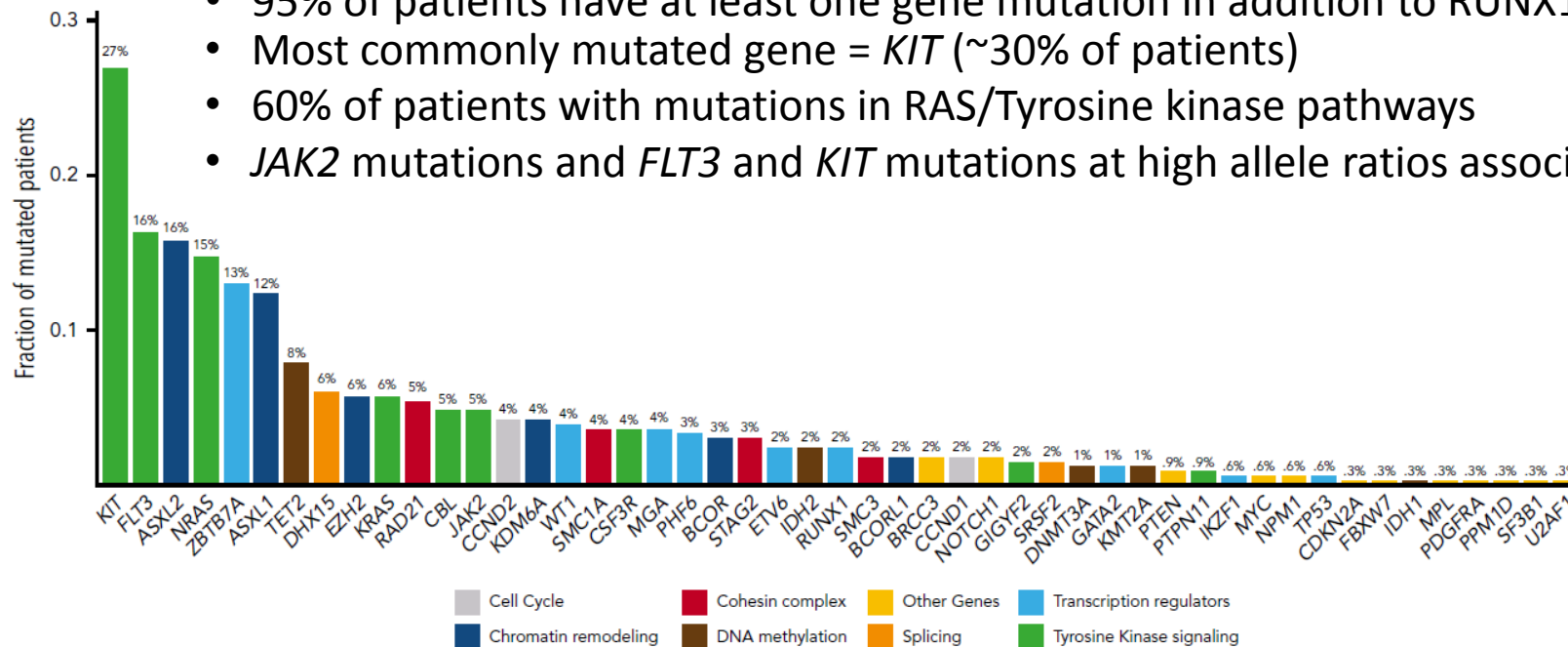
- Karyotype: 47,XX,t(8;21)(q22;q22),+15[20]
- FISH: nuc ish 8q22(RUNX1T1x3),21q22(RUNX1x3),(**RUNX1T1 con RUNX1x2**) [172/200]



➤ Comprehensive diagnosis: AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

AML with t(8;21) (q22;q22); *RUNX1-RUNX1T1*

- Frequent B lineage marker (CD19, PAX5) expression on myeloid blasts
- Associated with high complete remission rates and long term disease-free survival
- *RUNX1-RUNX1T1* fusion = leukemia-initiating event, but insufficient to induce leukemia
 - 95% of patients have at least one gene mutation in addition to *RUNX1-RUNX1T1* fusion
 - Most commonly mutated gene = *KIT* (~30% of patients)
 - 60% of patients with mutations in RAS/Tyrosine kinase pathways
 - *JAK2* mutations and *FLT3* and *KIT* mutations at high allele ratios associated with shorter overall survival

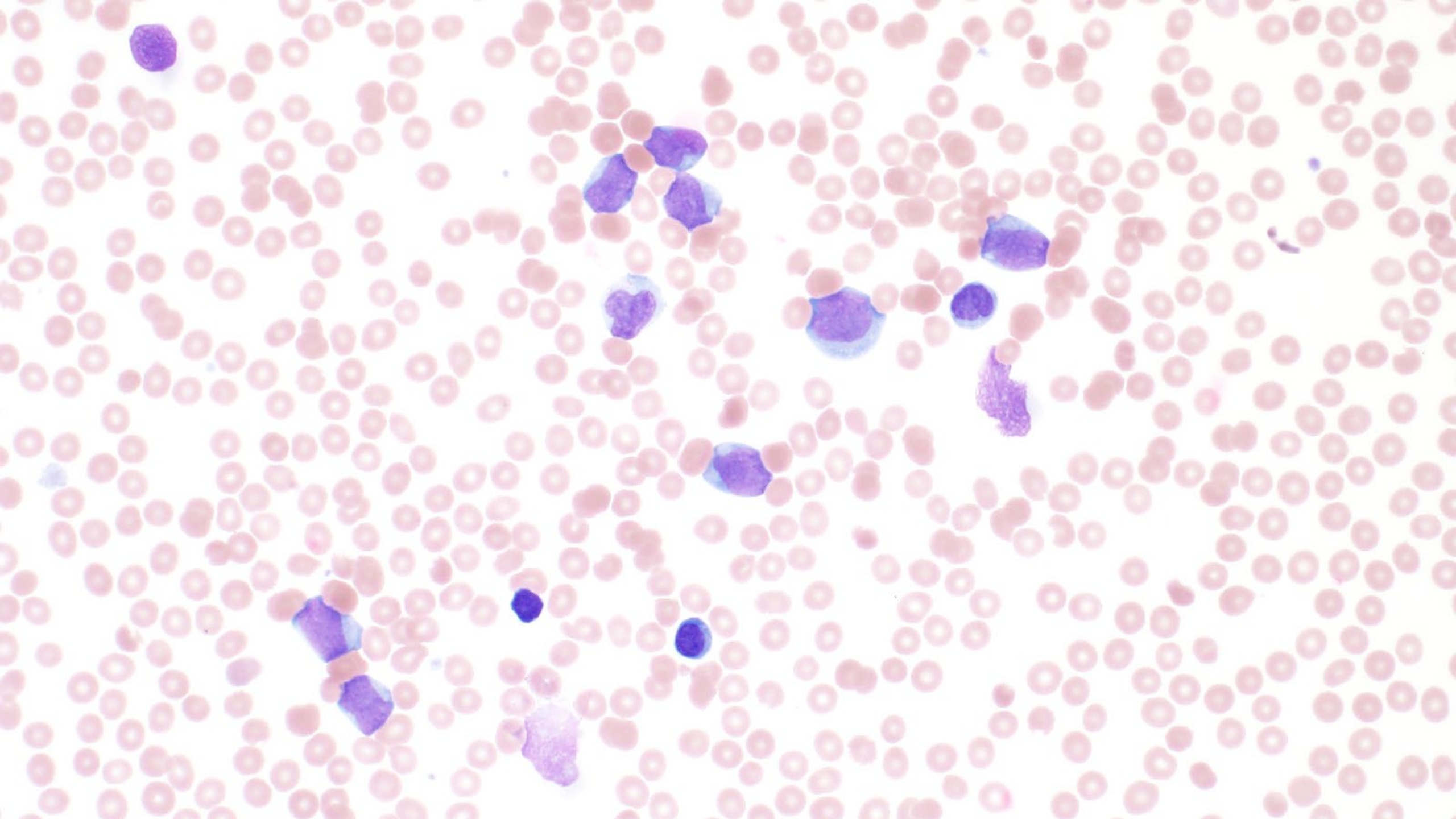


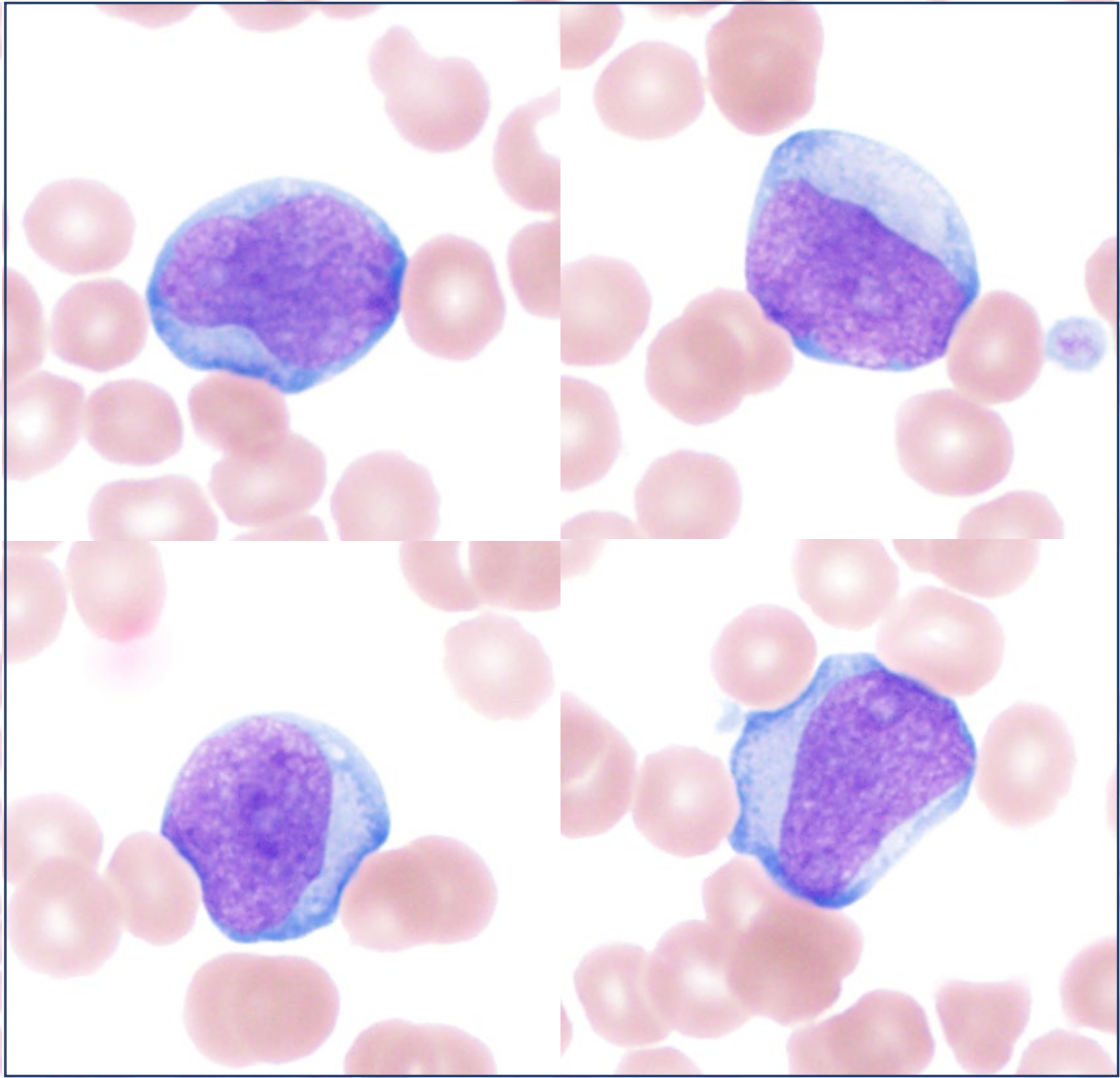
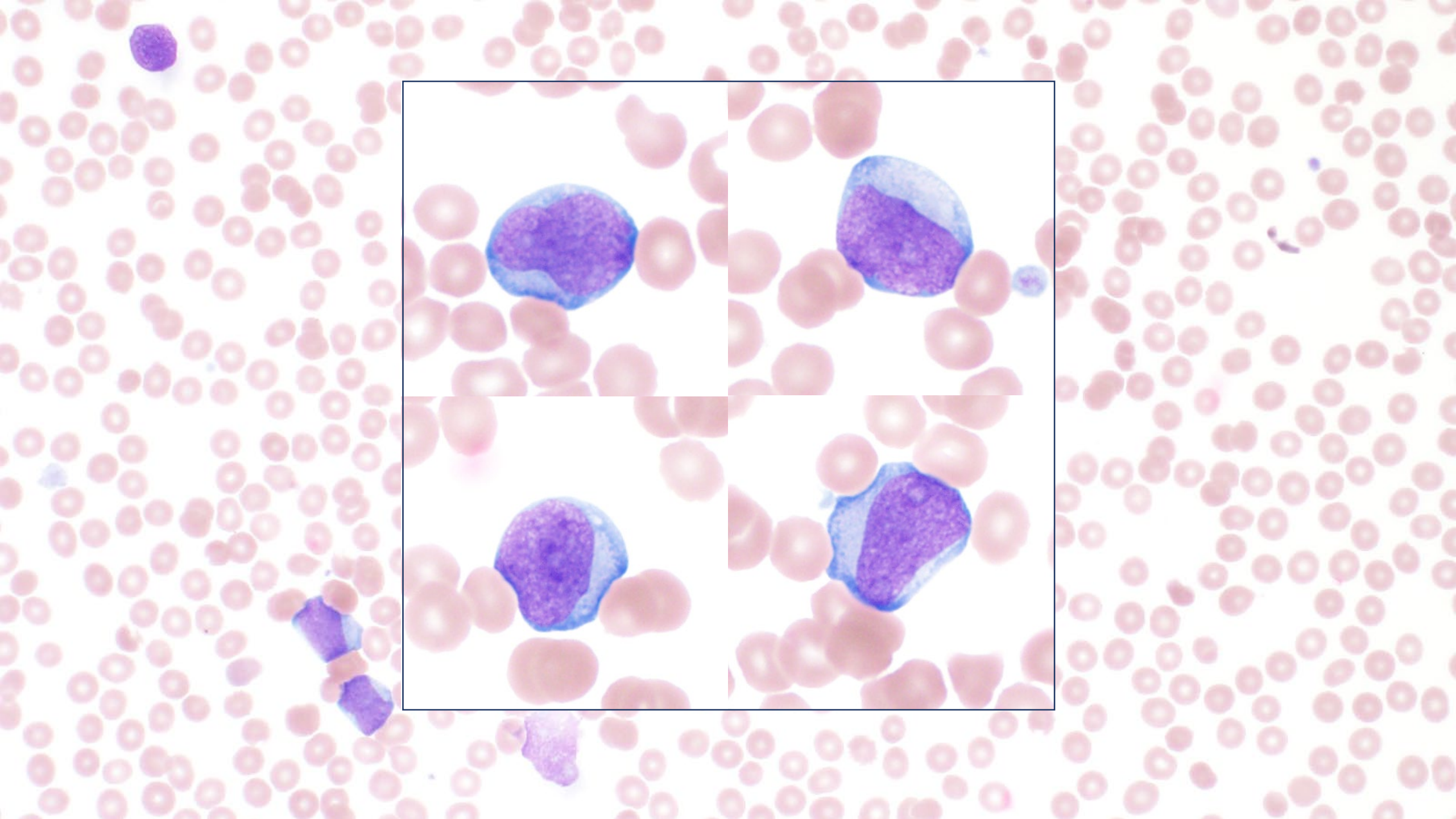
- Gemtuzumab ozogamicin (anti-CD33 antibody-drug conjugate) improves overall survival

Do we need flow cytometry to diagnose acute leukemia?

- Case #2a
 - 70 year old male presenting with several weeks of severe fatigue, found to have anemia and leukocytosis

CBC	Result	Reference range
WBC	20.1	3.9-10.7 x 10 ³ /μL
WBC Differential:	Neutrophils 1.8%, Lymphocytes 21.2%, Monocytes 16.8% Metamyelocytes 0.9% Others 59.3%	
Hgb	9.0	14.0-18.1 g/dL
HCT	28	41-49 %
Platelets	116	135-371 x 10 ³ /μL





Cytogenetic results

- Karyotype: 46,XY,t(9;22)(q34;q11.2)[20]
- Acute leukemia with a *BCR-ABL1* translocation: what's the diagnosis?

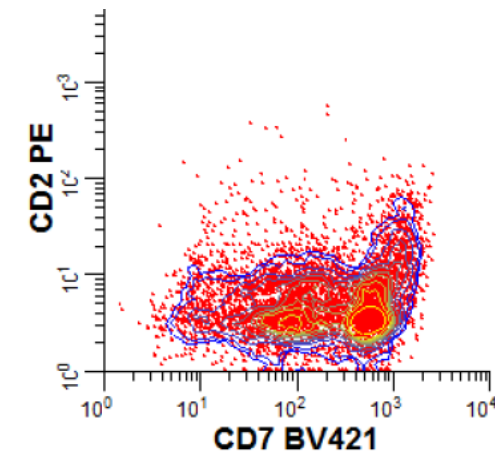
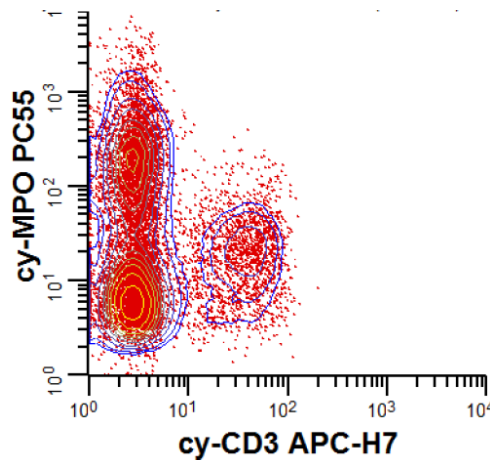
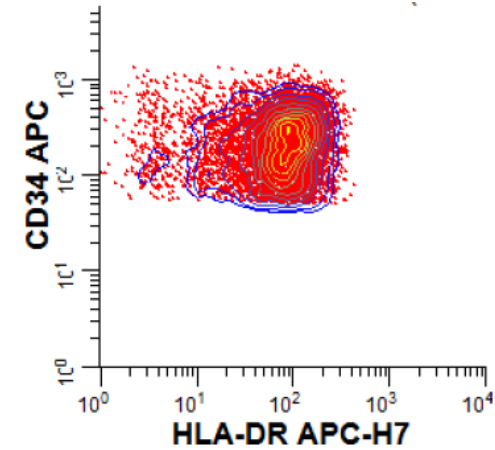
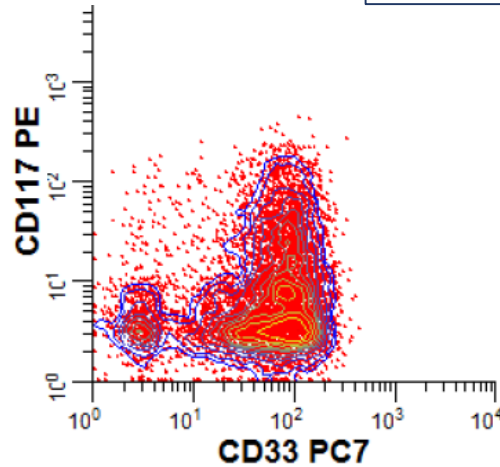
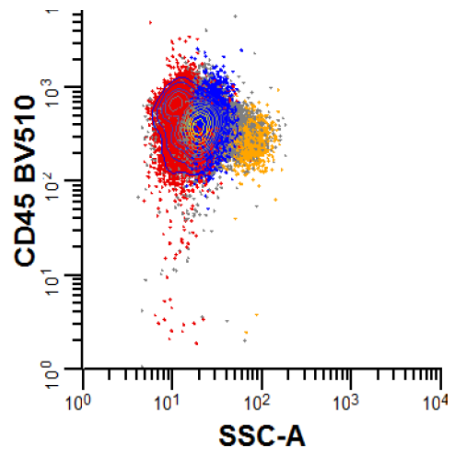
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>
Provisional entity: Acute myeloid leukemia with <i>BCR-ABL1</i>
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>

➤ Blast immunophenotype required for accurate diagnosis

Flow cytometry results

➤ Comprehensive diagnosis:

Mixed phenotype acute leukemia with
 $t(9;22)(q34;q11.2);BCR-ABL1$, T/myeloid



Positive: CD4 (dim), CD7, CD13, CD15, CD33, CD34, CD45, CD117, HLA-DR, cCD3, MPO, TdT

Negative: CD2, CD19, CD56, cCD22, cCD79a

Acute leukemia diagnosis requires integration of flow cytometric and genetic results

- Multiple categories of acute leukemia can show both myeloid and lymphoid marker expression
 - AML with t(8;21); *RUNX1;RUNX1T1* – AML with B lymphoid marker expression
 - Mixed phenotype acute leukemia (MPAL)
- Multiple categories can show the same genetic abnormality
 - t(9;22); *BCR-ABL1* in AML, B-ALL and MPAL
 - *KMT2A (MLL)* rearrangements in AML, B-ALL, and MPAL
 - *FLT3, DNMT3A, and IDH1/2* mutations in AML and ETP-ALL

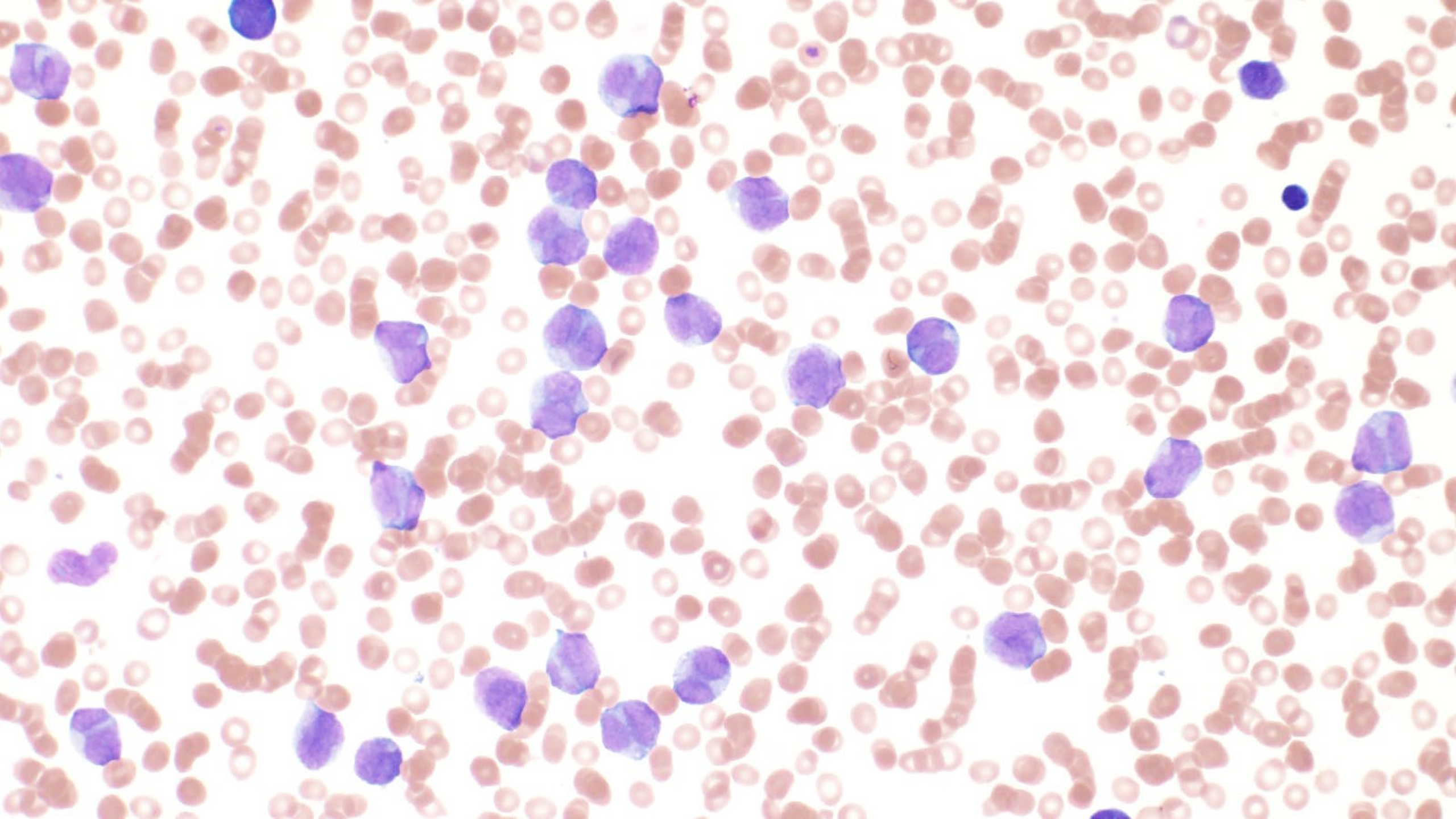
➤ *Accurate diagnosis requires integration of flow cytometric and genetic results*

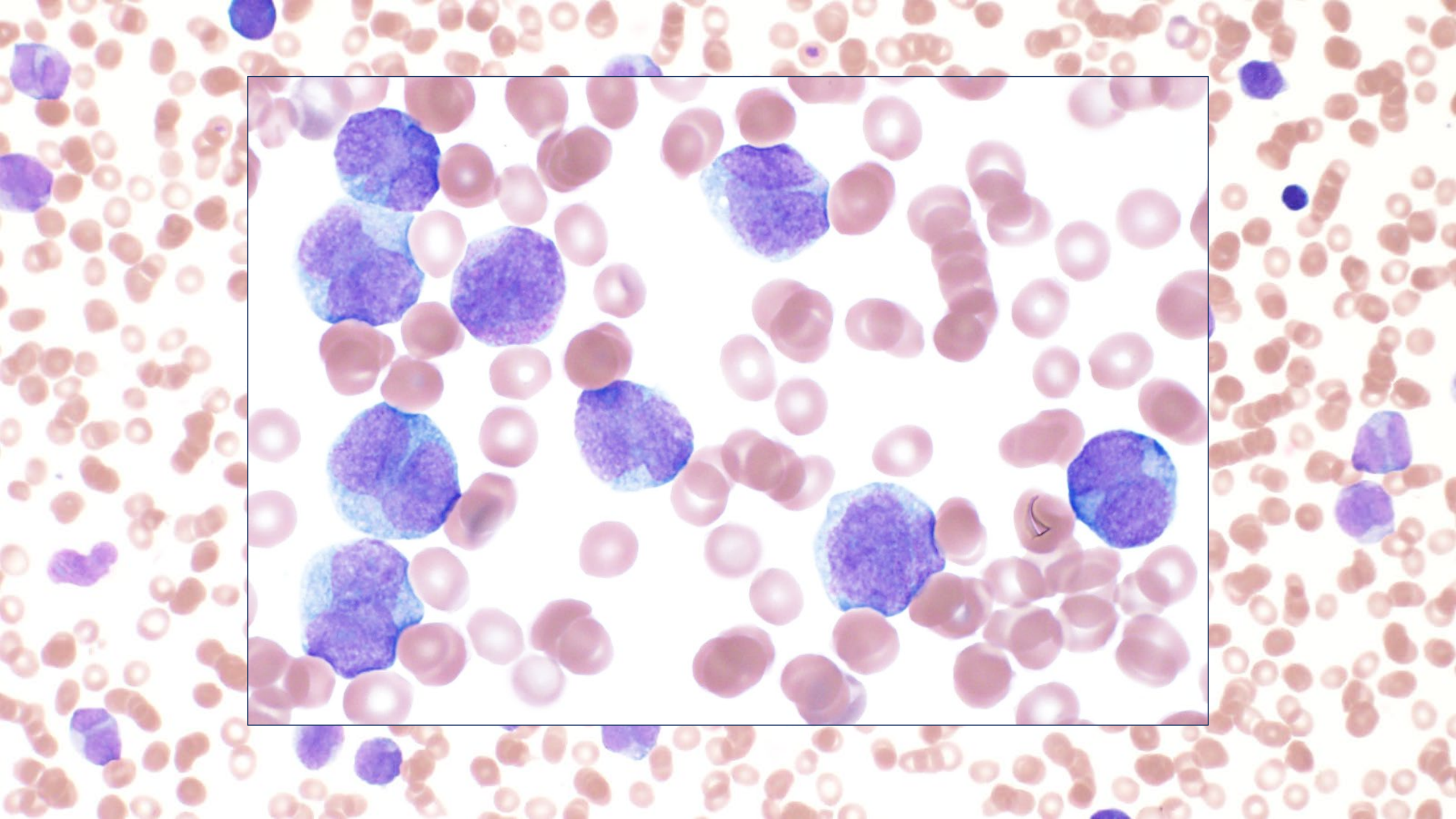
Case #3

- 43 year old male with no significant past medical history presented to the emergency room with worsening headache, dizziness, shortness of breath, and fatigue

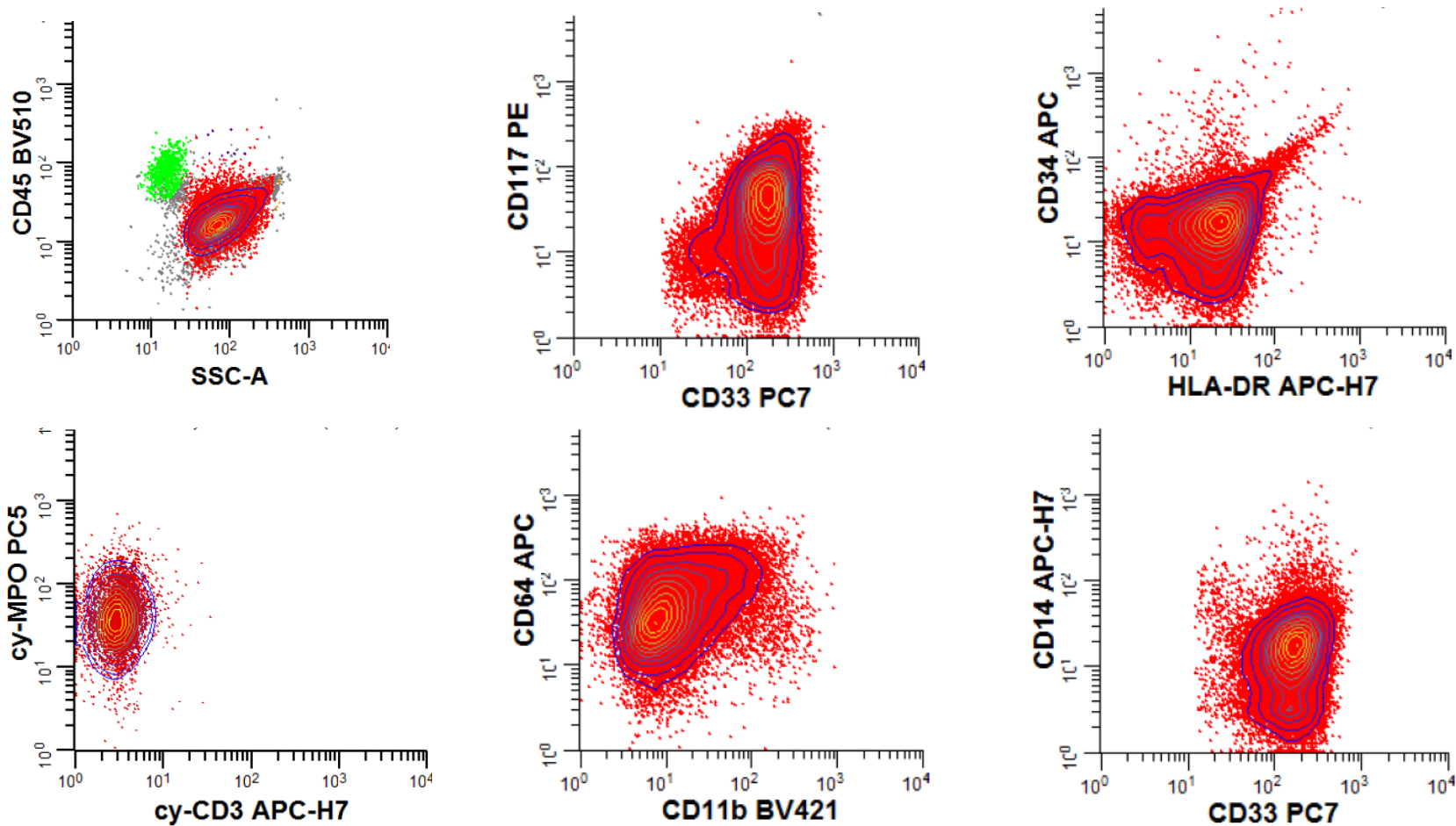
CBC	Result	Reference range
WBC	92.6	3.9-10.7 x 10 ³ /μL
WBC Differential:	Neutrophils 0.8%, Lymphocytes 0.8%, Monocytes 5.8% Myelocytes 10.8% Others 81.8%	
Hgb	9.5	14.0-18.1 g/dL
HCT	27	41-49 %
Platelets	82	135-371 x 10 ³ /μL

	Result	Reference range
PT	19.8	11.0-14.6 sec
PTT	34.7	23.8-32 sec
Fibrinogen	86	188-450 mg/dL





Flow cytometric results



Positive: CD13, CD33, CD45 (dim), CD64 (dim), CD117, MPO

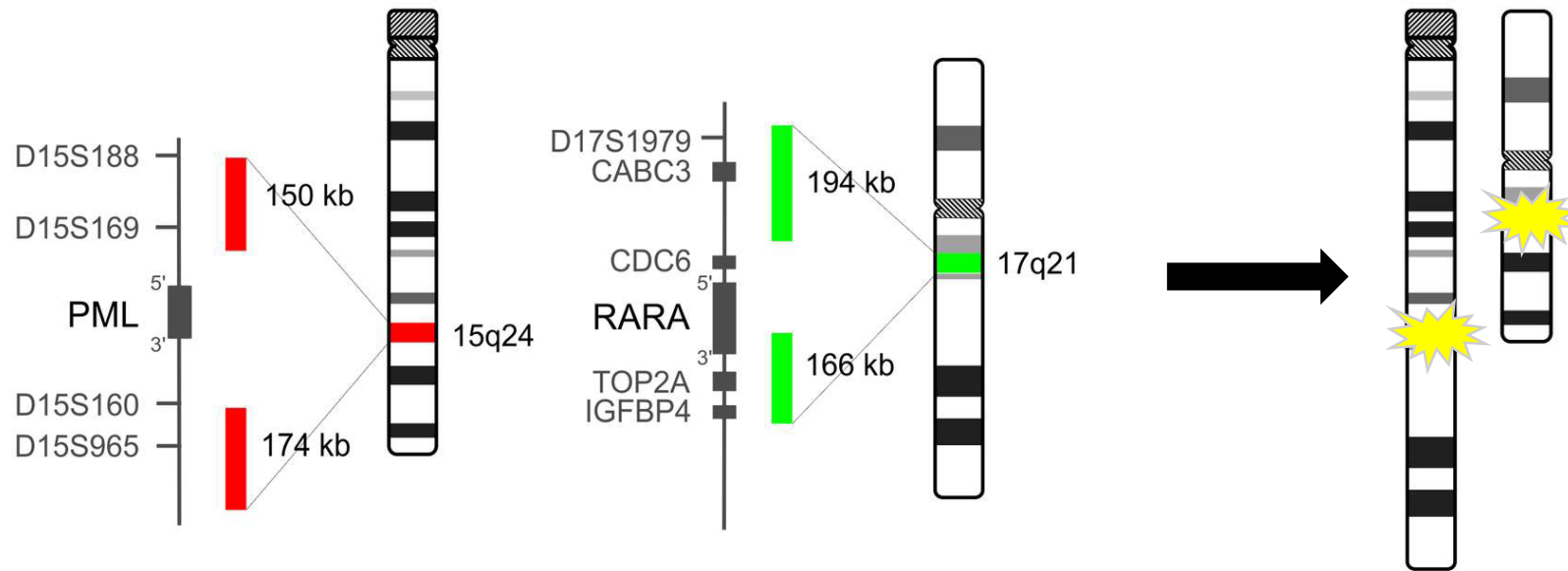
Negative: CD2, CD4, CD7, CD11b, CD14, CD15, CD19, CD56, HLA-DR, cCD3, cCD22, cCD79a, TdT



- Possible acute promyelocytic leukemia (APL)
- Additional (STAT) ancillary testing required:
 - t(15;17) FISH
 - PML-RARA RT-PCR

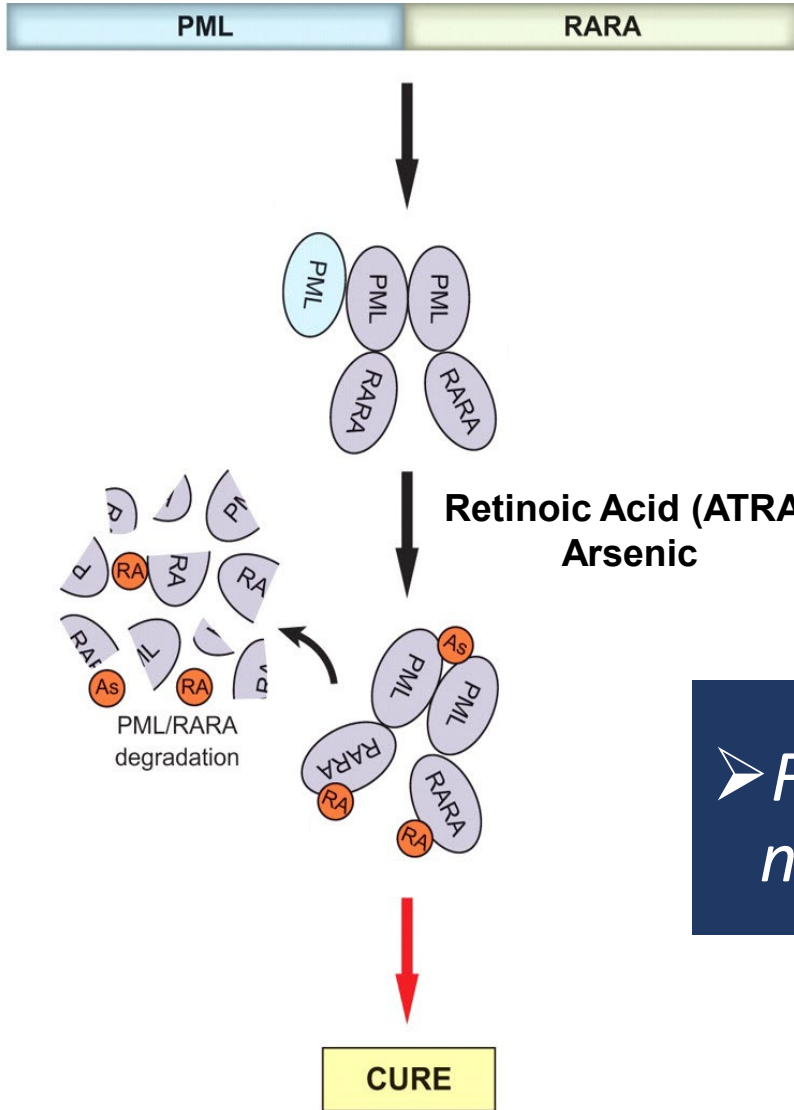
STAT FISH results

- nuc ish 15q22~24(PMLx3),17q21(RARAx3)(PML con RARAx2)[186/200]



➤ Comprehensive diagnosis: Acute promyelocytic leukemia

Acute promyelocytic leukemia



➤ *Flow cytometric results identify the need for STAT testing to direct treatment*

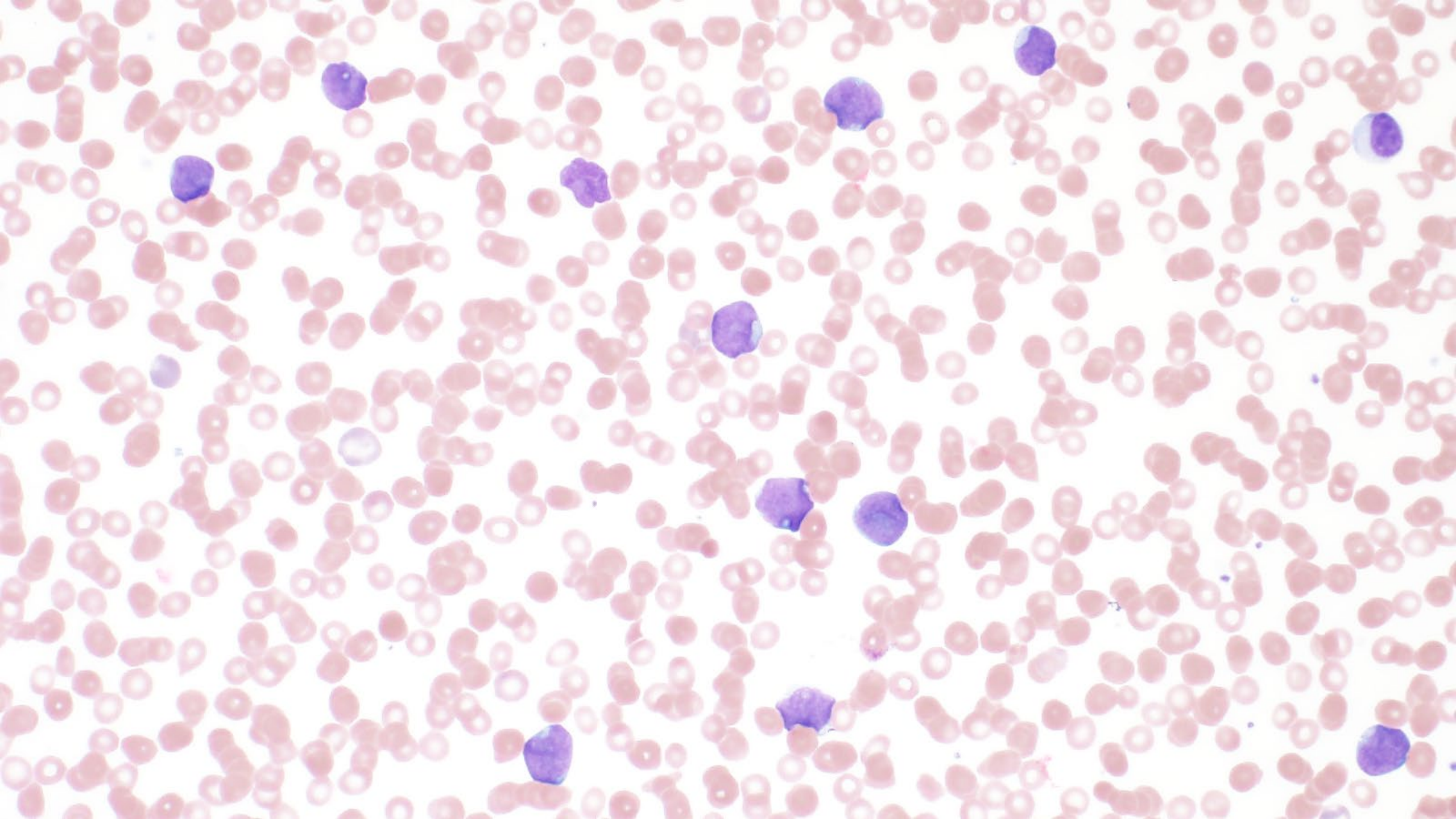
Case #4

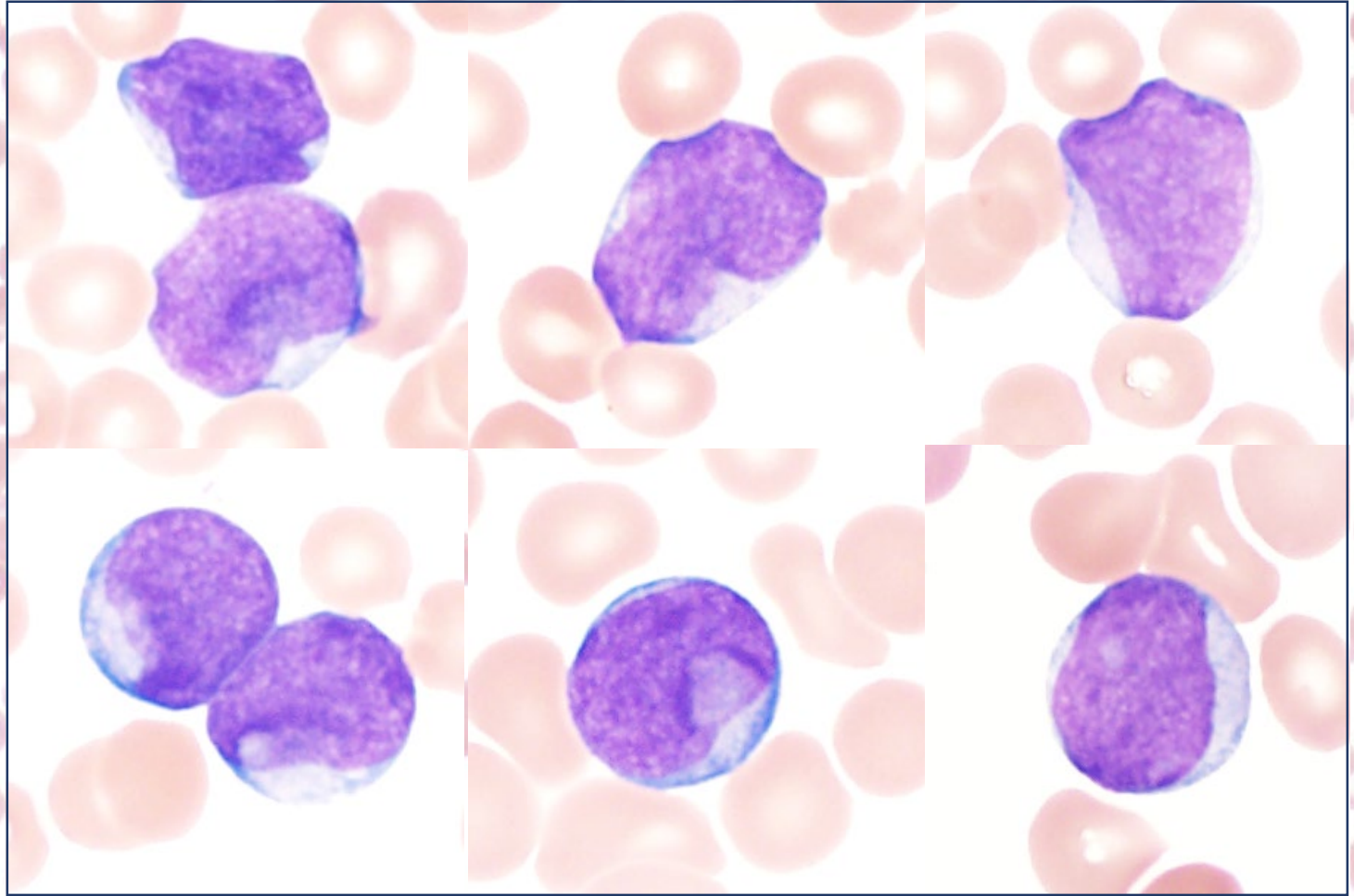
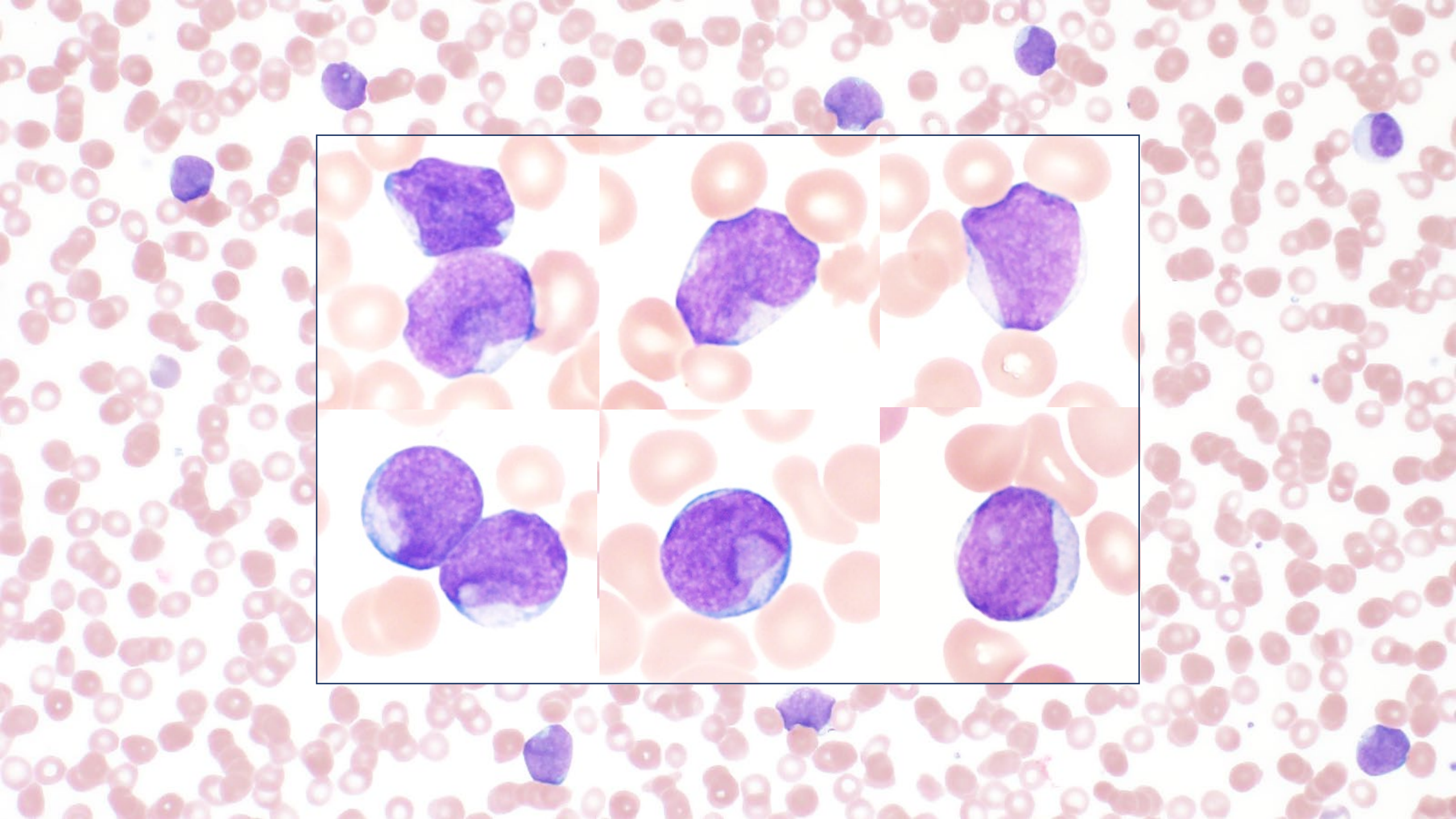
- 76 year old female with no significant past medical history presents with 3 to 4 weeks of severe fatigue and shortness of breath

CBC	Result	Reference range
WBC	38.4	3.9-10.7 x 10 ³ /μL
WBC Differential:	Neutrophils 3.1%, Lymphocytes 4.6%, Blasts 92.3%	
Hgb	10.9	14.0-18.1 g/dL
HCT	33	41-49 %
Platelets	31	135-371 x 10 ³ /μL

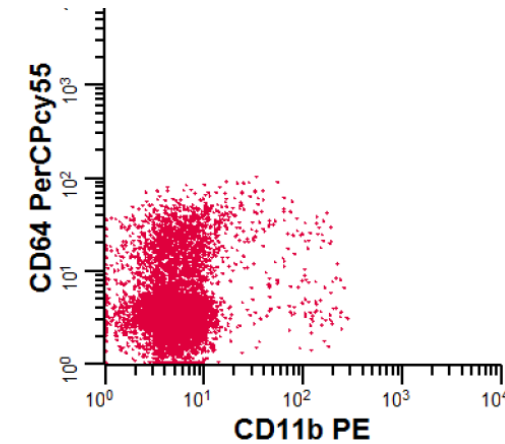
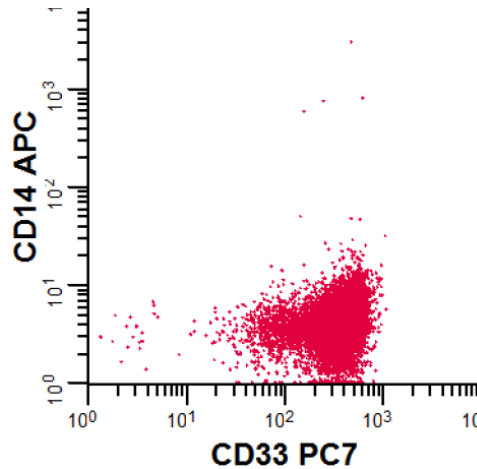
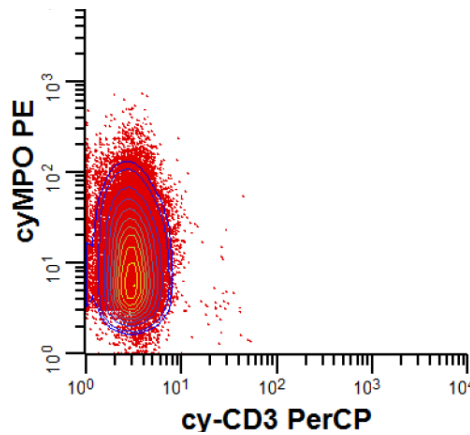
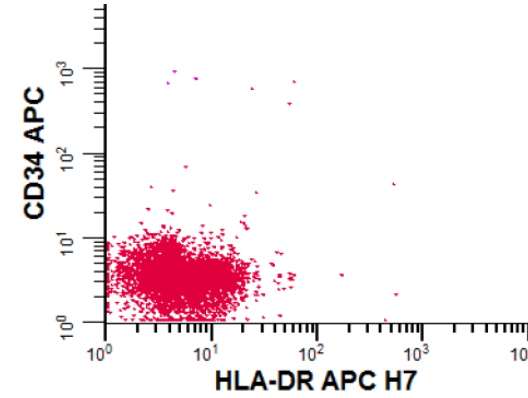
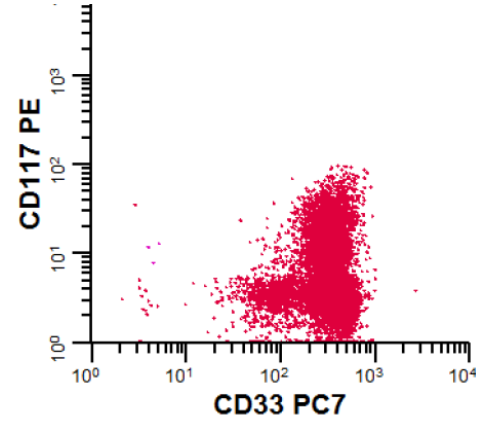
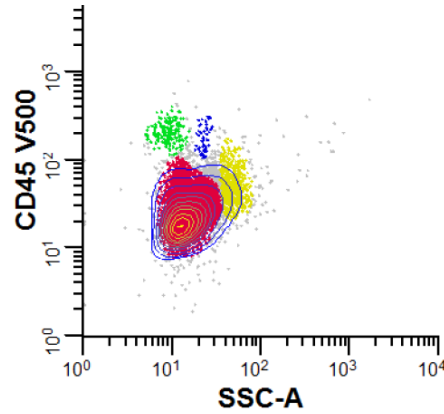
	Result	Reference range
PT	16.0	11.0-14.6 sec
PTT	38.8	23.8-32 sec
Fibrinogen	136	188-450 mg/dL
D-Dimer	19.19	0.27-0.49 μg/mL

- Concern for APL with DIC – patient given a dose of ATRA in the emergency room





Flow cytometric results



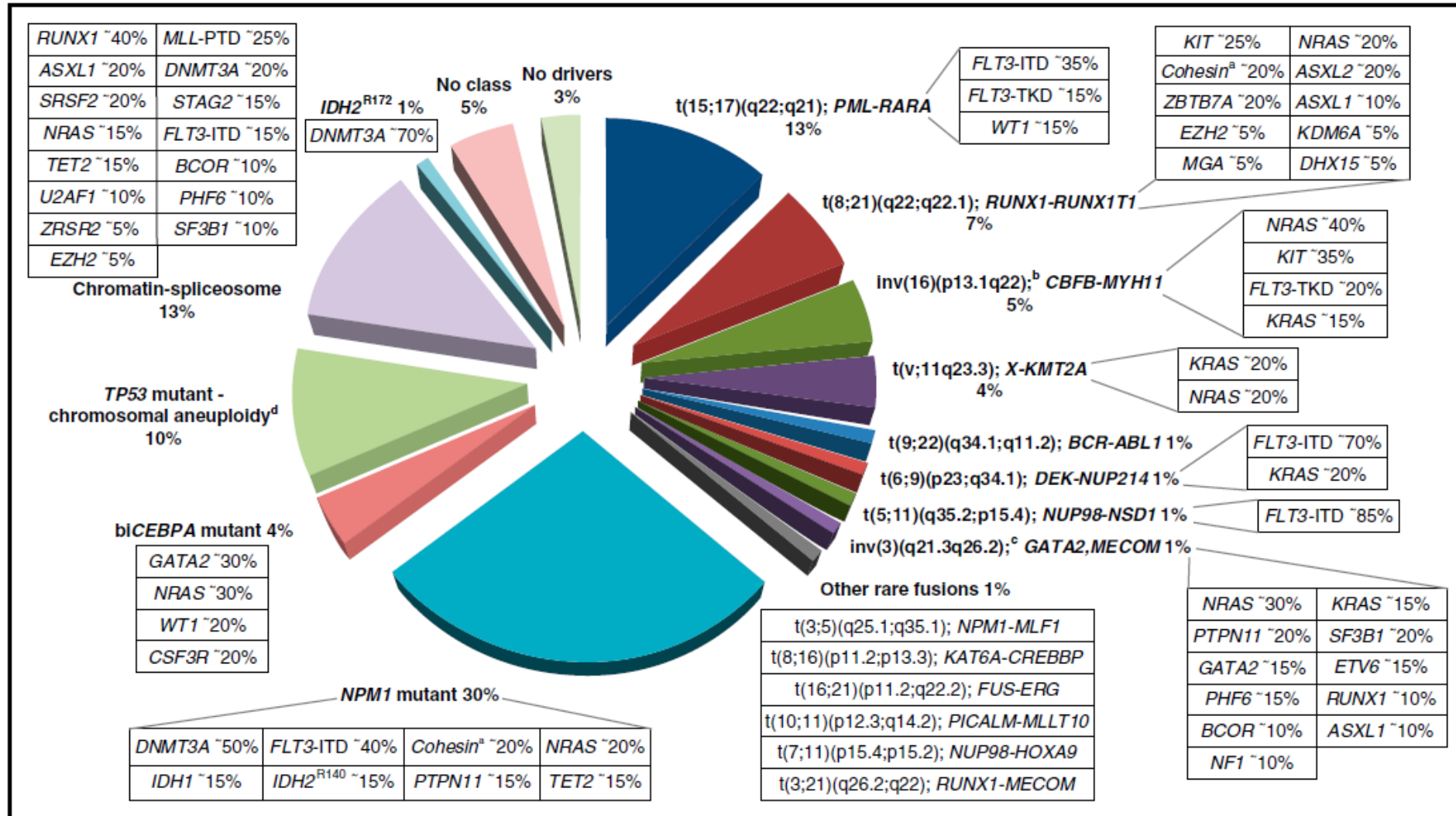
Positive: CD13, CD33, CD45 (dim), CD56, CD64 (small subset), CD117, MPO

Negative: CD2, CD4, CD7, CD11b, CD14, CD15, CD19, CD34, HLA-DR, cCD3, cCD22, cCD79a, TdT

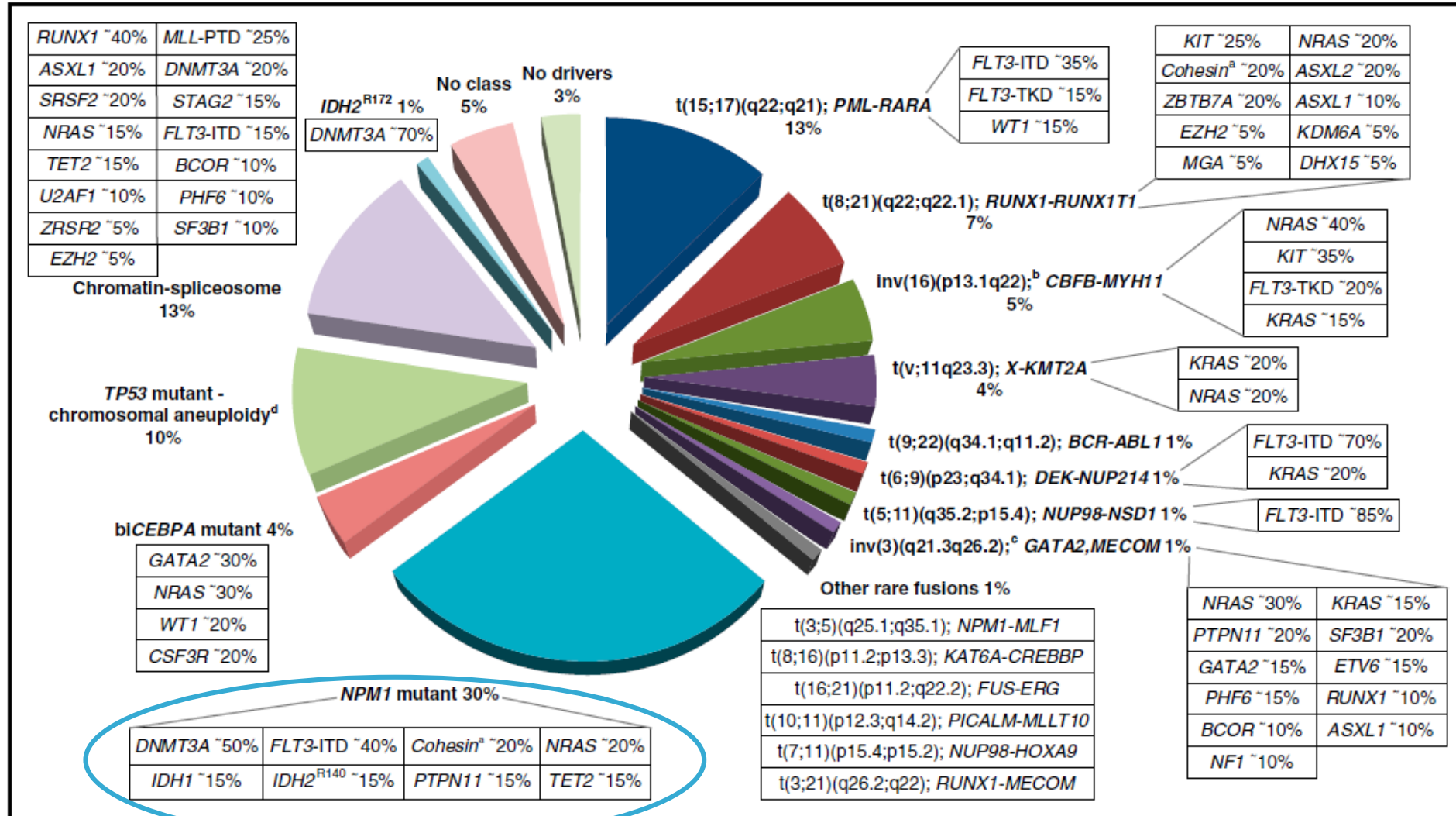
Ancillary testing results

- STAT FISH: Negative for PML-RARA translocation
- Karyotype: 46,XX[20]
- Next generation sequencing:
 - NPM1* (VAF 35%)
 - IDH2* (VAF 48%)
 - FLT3-ITD* (VAF 26%)
- Comprehensive diagnosis: Acute myeloid leukemia with mutated *NPM1*

AML with mutated *NPM1*

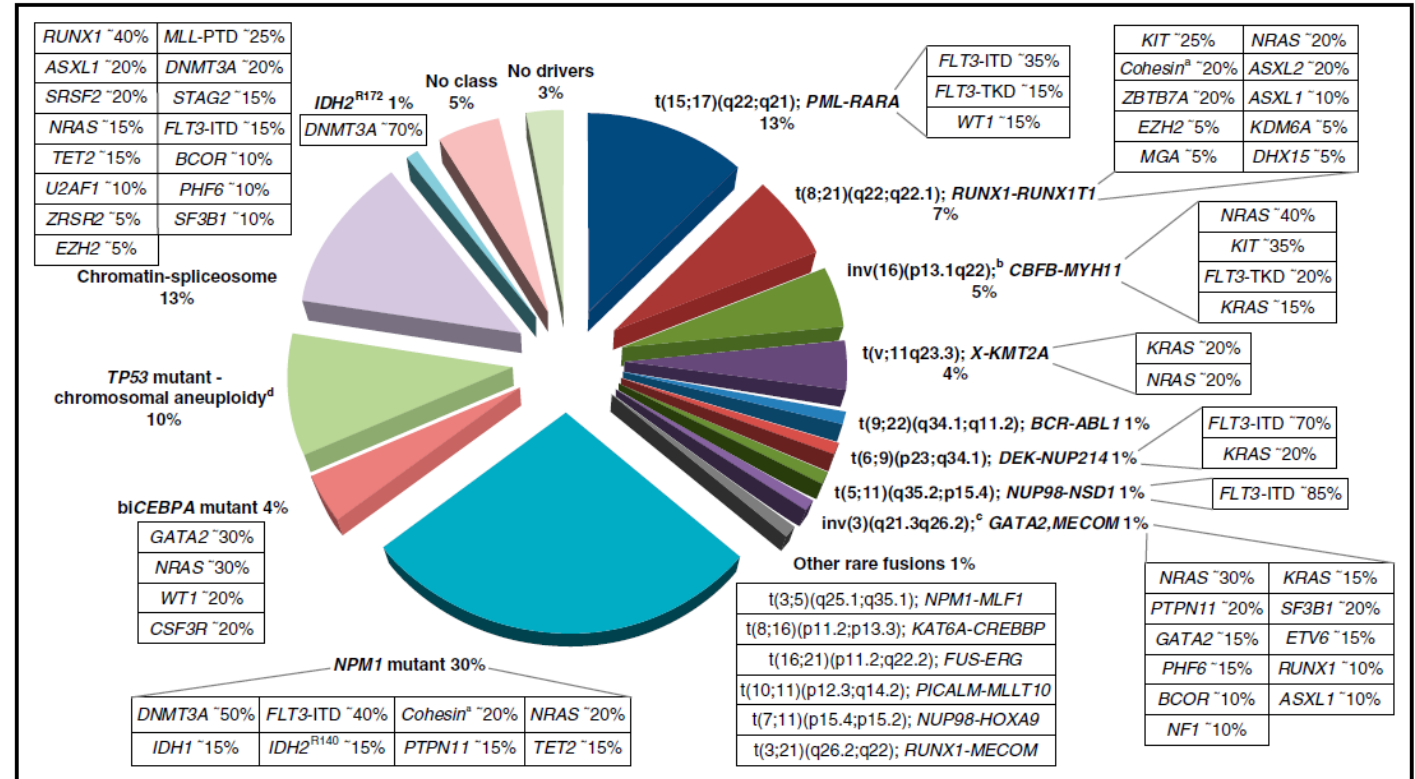


AML with mutated *NPM1*

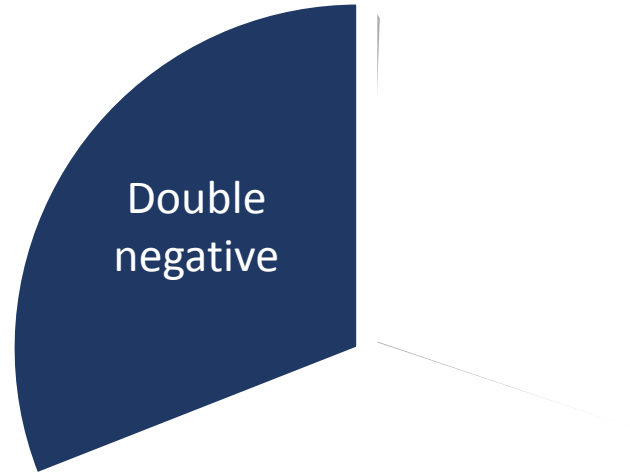
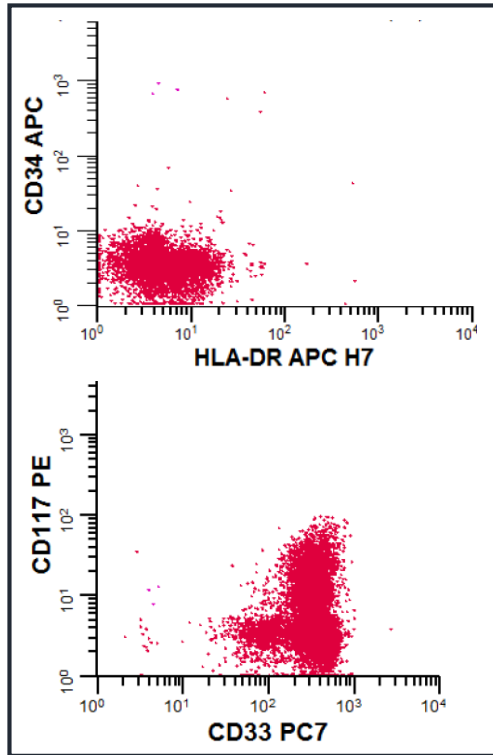


AML with mutated *NPM1*

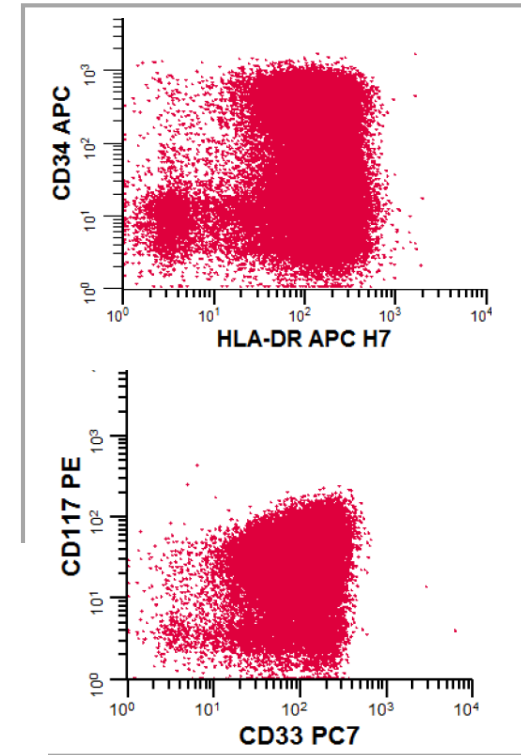
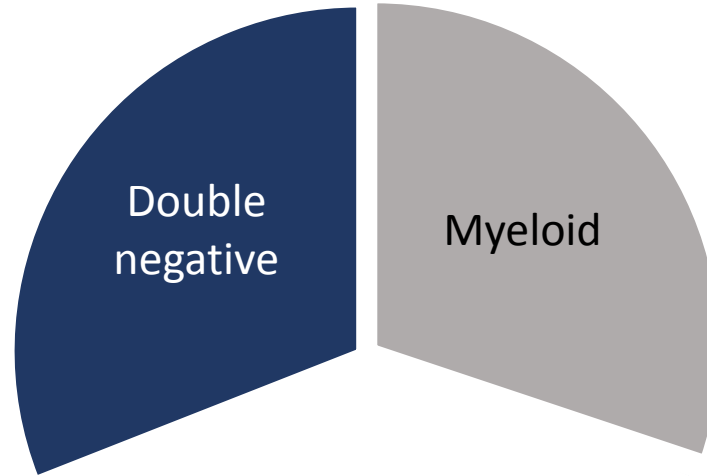
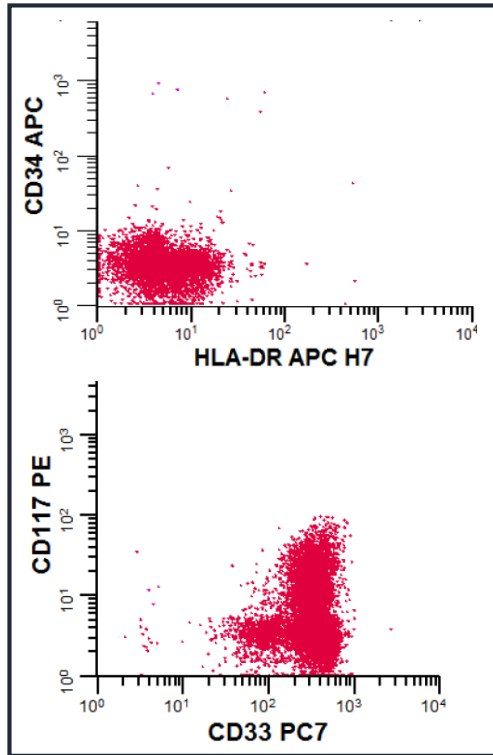
- *De novo* AML
- Normal karyotype
- Typically other co-mutations present
- Good prognosis if no *FLT3*-ITD



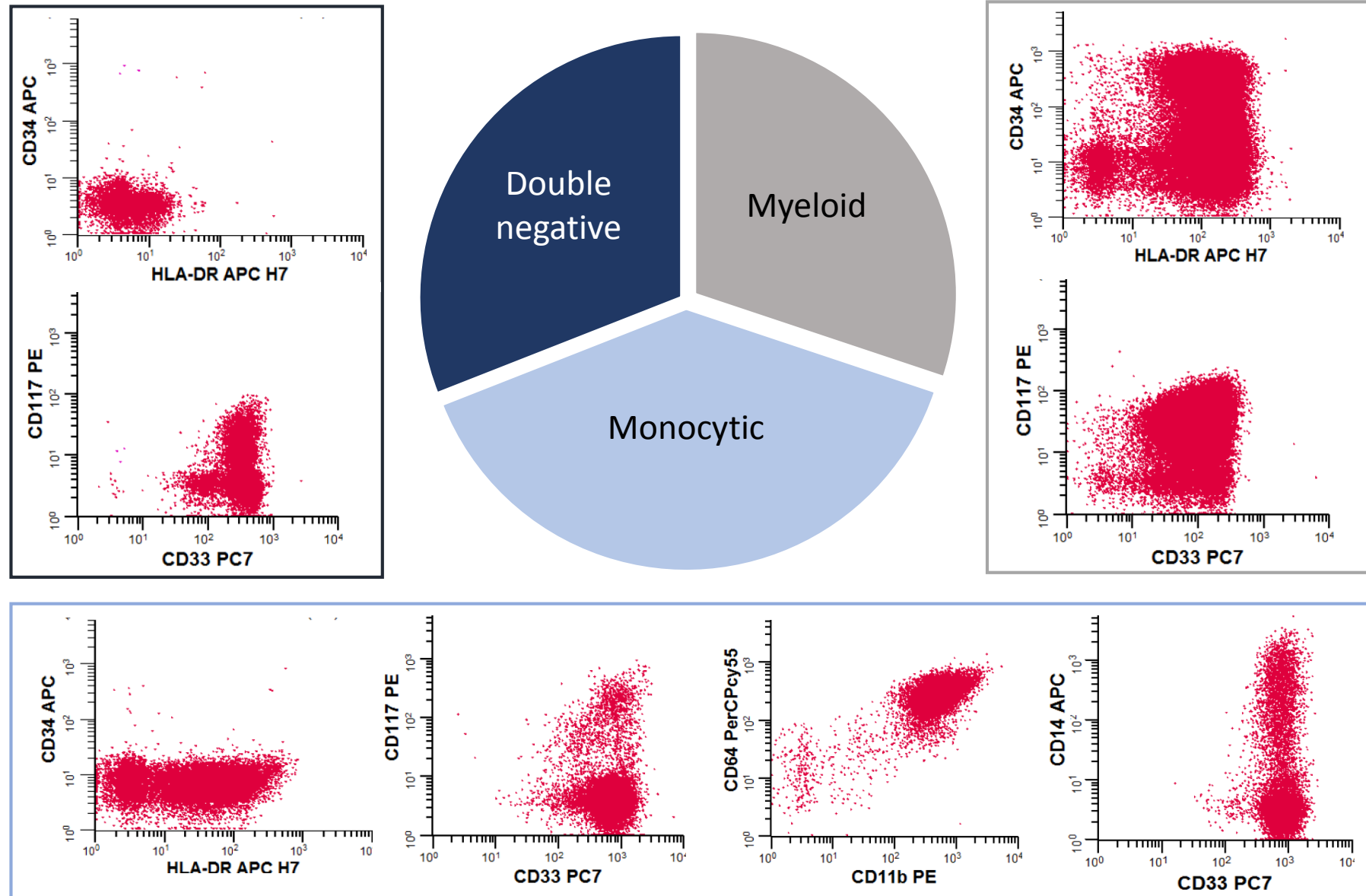
Blast phenotypes in AML with mutated *NPM1*



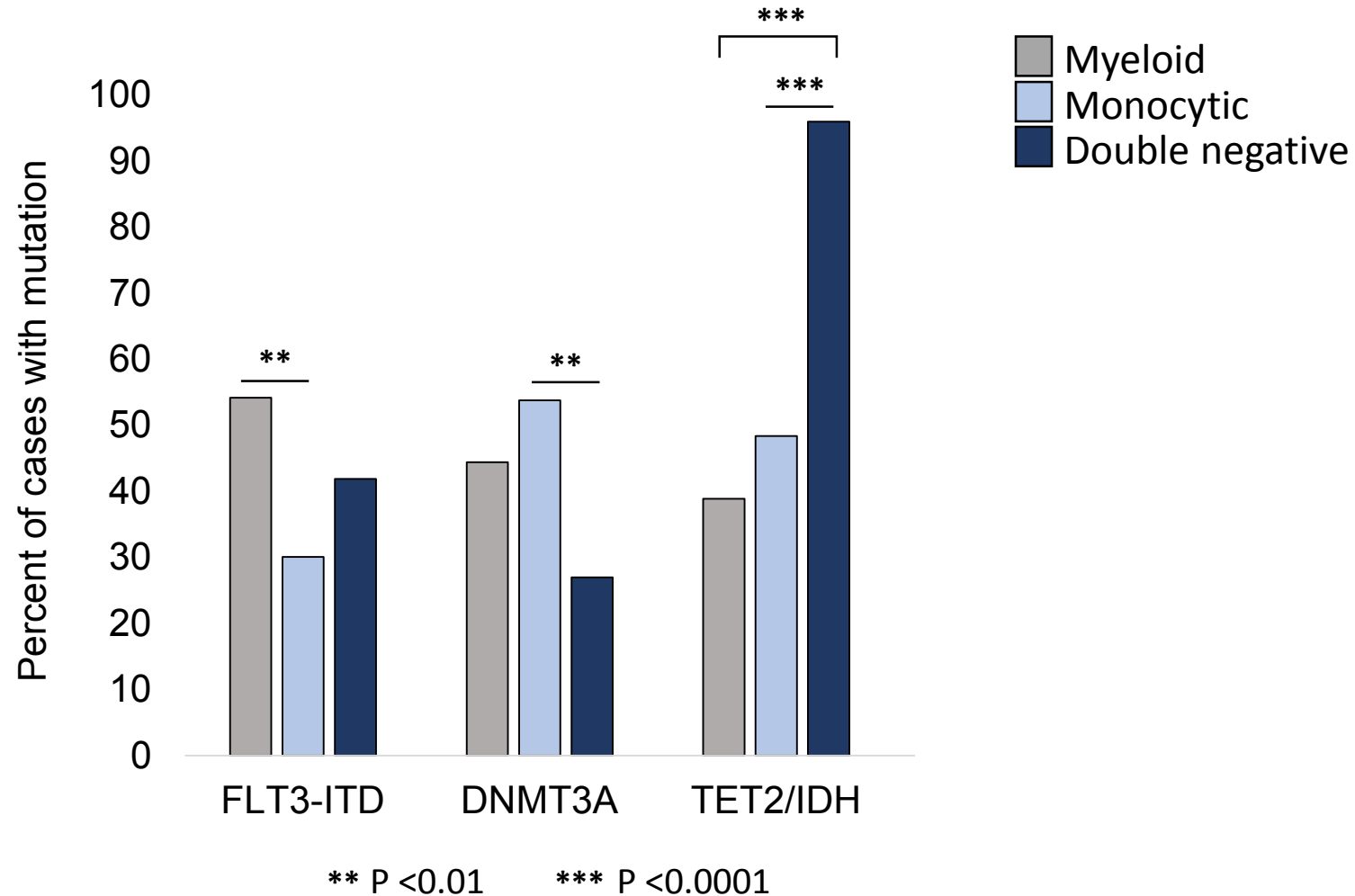
Blast phenotypes in AML with mutated *NPM1*



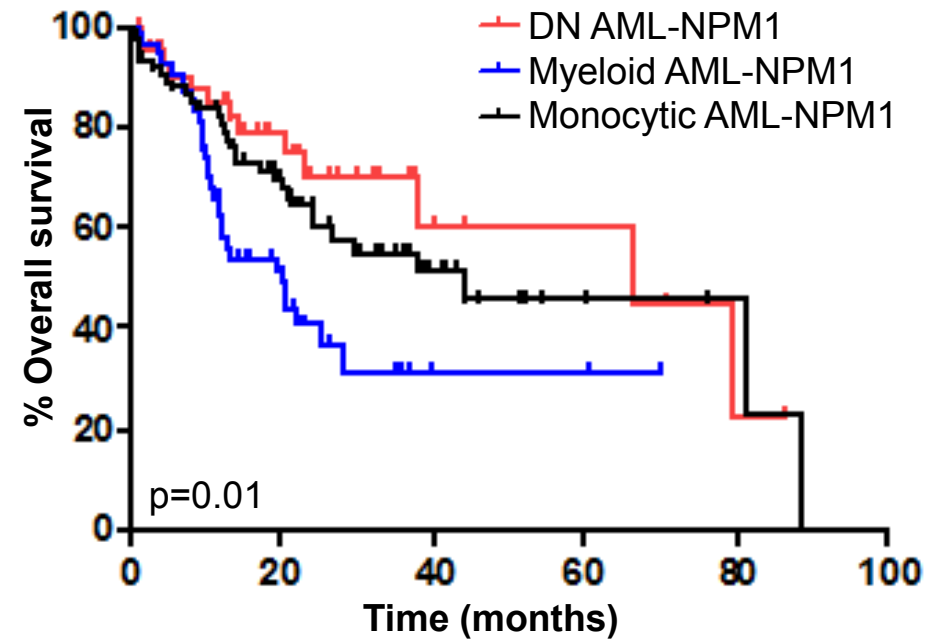
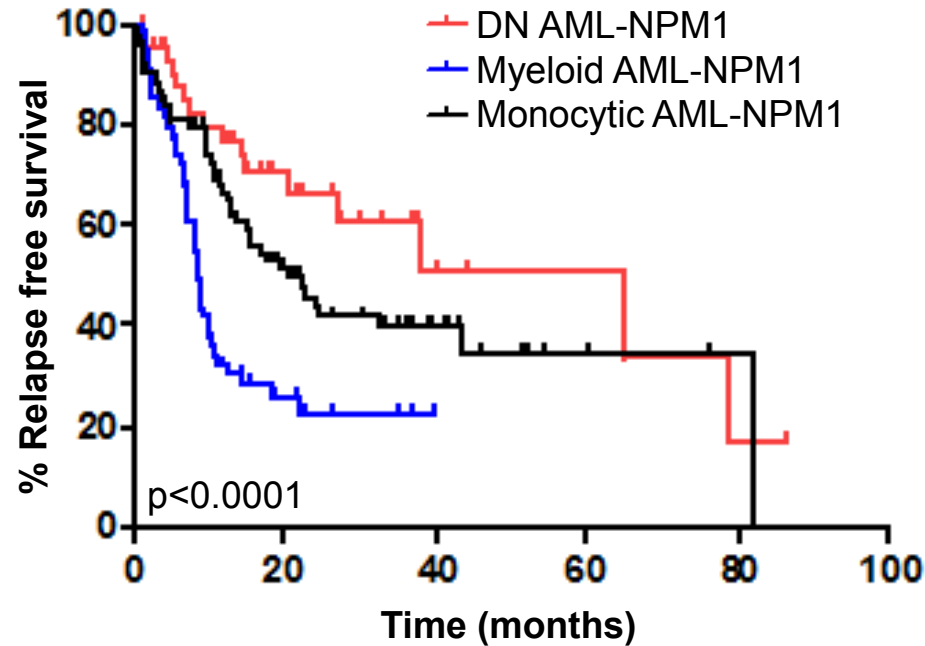
Blast phenotypes in AML with mutated *NPM1*



Phenotypic and genetic heterogeneity in AML with mutated *NPM1*



Blast phenotype impacts prognosis in AML with mutated *NPM1*



➤ *Blast immunophenotype influences outcome in AML with mutated *NPM1**

Flow cytometry plays an essential role in the diagnosis of AML

- *Optimal assay to characterize of blast lineage, which dictates ancillary testing and options for targeted therapy*
- *Accurate diagnosis requires integration of flow cytometric and genetic results*
- *Flow cytometric results identify the need for STAT testing to direct treatment*
- *Blast immunophenotype impacts outcome in AML with mutated NPM1*

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