eha

EHA-GBMTA-AHA Hematology Tutorial: **Diagnostic work-up in acute myeloid leukemia**

Session: Acute Myeloid Leukemia

20th October 2024, Margarita Guenova

Disclosure

Prof. Margarita Guenova, MD, PhD

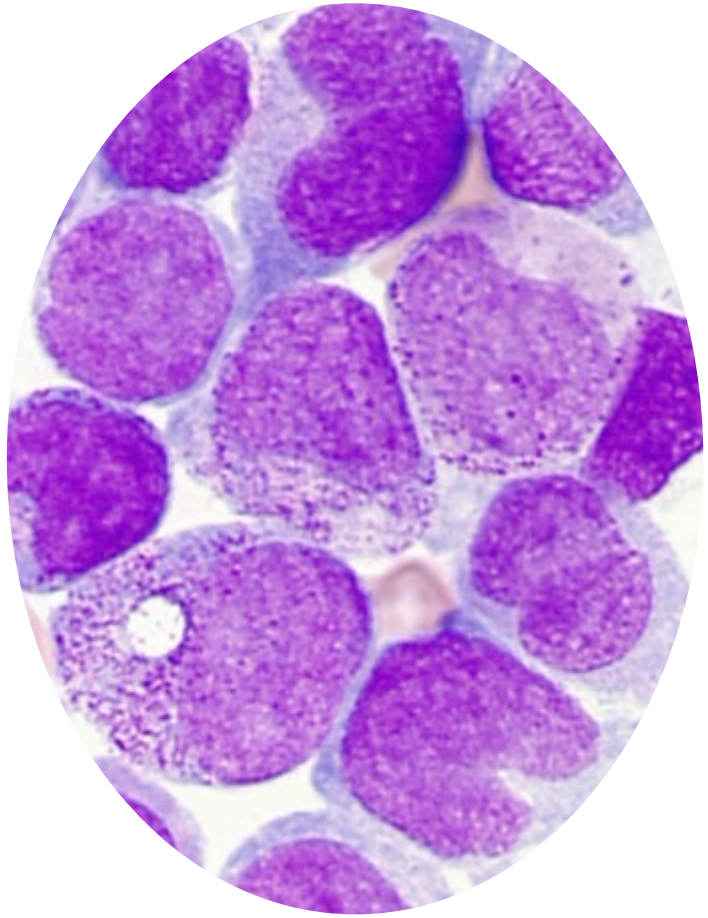
Company	Relationship
Novartis	Speakers' Bureau
AstraZeneka	Speakers' Bureau, Scientific advisory board
Abbvie	Speakers' Bureau
Swixx	Speakers' Bureau, Scientific advisory board
SOBI	Speakers' Bureau, Scientific advisory board

Learning objectives

After attending this presentation, you will be able to:

1. Understand which tests need to be performed for the diagnosis of acute myeloid leukemia.
2. Recognize how morphology supplemented by immunophenotyping can provide clues to specific cytogenetic/molecular anomalies and the diagnosis of specific AML classification categories.
3. Discuss current classification systems.

Acute myeloid leukemia



Acute myeloid leukemia (AML) is a rapidly progressing myeloid **neoplasm** characterized by the clonal expansion of immature myeloid-derived cells, known as **myeloid blasts**, in the peripheral blood and bone marrow.

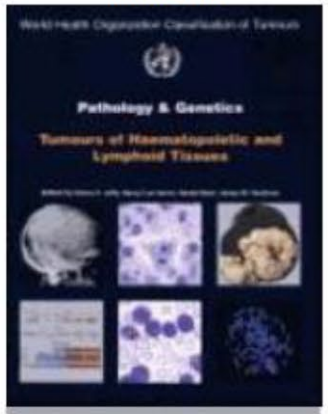
This expansion results in ineffective erythropoiesis and megakaryopoiesis, clinically manifesting as relatively **rapid bone marrow failure** compared to chronic and indolent leukemias. This leads to inadequate production of red blood cells and platelets.

Vakiti A, Reynolds SB, Mewawalla P. Acute Myeloid Leukemia. [Updated 2024 Apr 27]. In: StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507875/

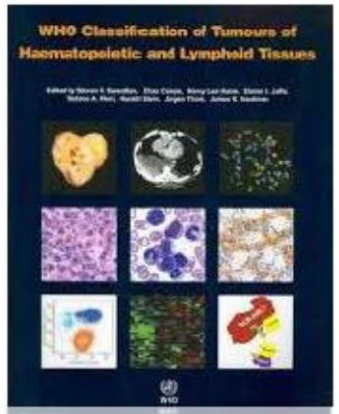
Classifications of AML

FAB 1976

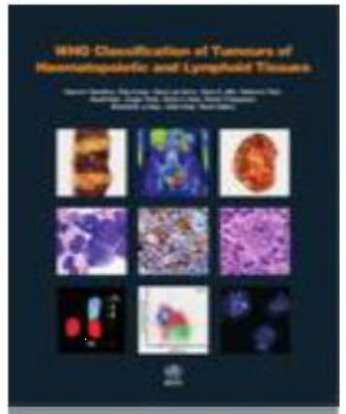
WHO 2001
HAEM3



WHO 2008
HAEM4



WHO 2016
HAEM4R



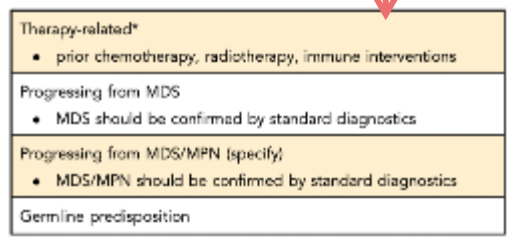
WHO 2022
HAEM5

ICC 2022

A story starting with 200 AL cases, 7 hematologists, 8 months of correspondence and 2 days in Paris.

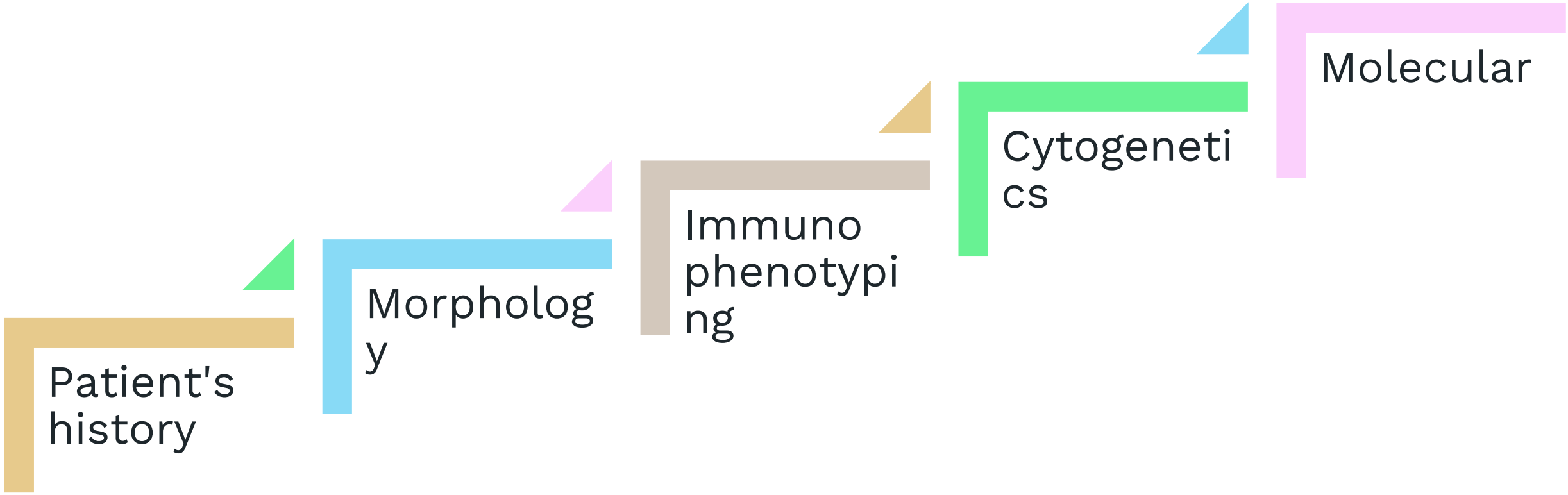
eha

WHO – HAEM5	ICC	
Acute myeloid leukaemia with defining genetic abnormalities		
Acute promyelocytic leukaemia with PML::RARA fusion	Acute promyelocytic leukemia with t(15;17)(q24.1;q21.2)/PML::RARA	≥ 10%
	APL with other RARA rearrangements*	≥ 10%
Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion	AML with t(8;21)(q22;q22.1)/RUNX1::RUNX1T1	≥ 10%
Acute myeloid leukaemia with CBFB::MYH11 fusion	AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11	≥ 10%
Acute myeloid leukaemia with KMT2A rearrangement	AML with t(9;11)(p21.3;q23.3)/MLLT3::KMT2A	≥ 10%
	AML with other KMT2A rearrangements†	≥ 10%
Acute myeloid leukaemia with DEK::NUP214 fusion	AML with t(6;9)(p22.3;q34.1)/DEK::NUP214	≥ 10%
	AML with inv(3)or t(3;3)(q21.3;q26.2)/GATA2; MECOM(EVI1)	≥ 10%
Acute myeloid leukaemia with MECOM rearrangement	AML with other MECOM rearrangements‡	≥ 10%
	AML with t(9;22)(q34.1;q11.2)/BCR::ABL1§	≥ 20%
	AML with mutated NPM1	≥ 10%
Acute myeloid leukaemia with BCR::ABL1 fusion	AML with in-frame bZIP CEBPA mutations ≥ 10%	≥ 10%
Acute myeloid leukaemia with NPM1 mutation	AML with myelodysplasia-related cytogenetic abnormalities	≥ 10%*
Acute myeloid leukaemia with CEBPA mutation	MDS/AML (10-19%) / AML (≥ 20%)	
Acute myeloid leukaemia, myelodysplasia-related	AML (≥ 20%) and MDS/AML (10-19%) with myelodysplasia-related gene mutations	≥ 10%*
	AML (≥ 20%) and MDS/AML (10-19%) with mutated TP53†	≥ 10%*
Acute myeloid leukaemia with RBM15::MRTFA fusion	AML with other rare recurring translocations	≥ 10%
Acute myeloid leukaemia with NUP98 rearrangement		
AML with other defined genetic alterations		
Acute myeloid leukaemia, defined by differentiation	**AML not otherwise specified (NOS) 10-19% (MDS/AML) (AML)**	
Acute myeloid leukaemia with minimal differentiation	MDS/AML, NOS	10-19%
	AML, NOS	≥ 20%
Acute myeloid leukaemia without maturation		



eha

Stairway to diagnosis



01

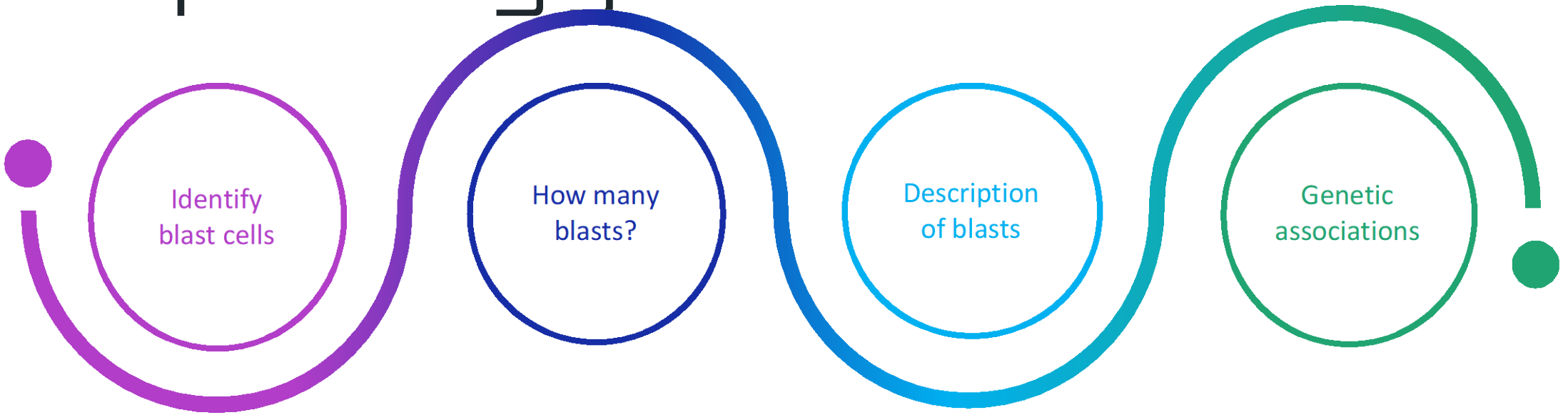
Medical history

- Demographics and medical history
  - prior exposure to toxic agents
  - prior malignancy
  - therapy for prior malignancy
- Detailed family history
  - potential germline predisposition
- Patient bleeding history
- Analysis of comorbidities

Döhner H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

02

Morphology



MILESTONE 1.

Identification of myeloid blast-equivalents.
Differentiation of myeloid from lymphoid blasts.

MILESTONE 2.

Quantification of blast equivalents in bone marrow and peripheral blood

MILESTONE 3.

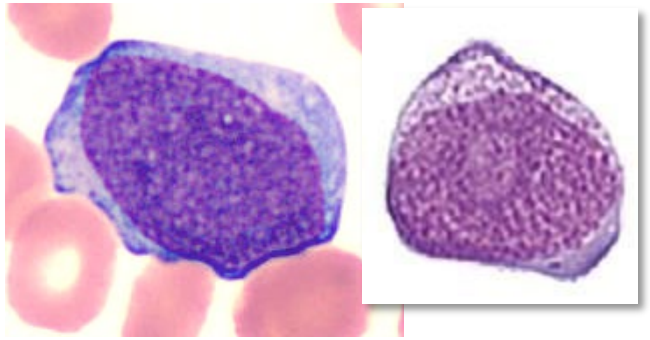
Description of blast morphology and concomitant hematopoiesis

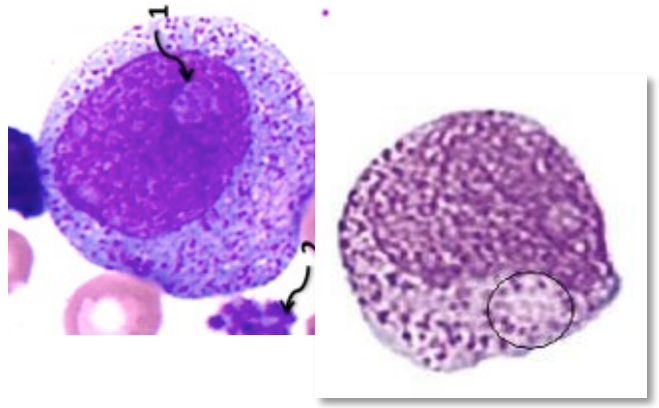
MILESTONE 4.

Morphological features pointing to specific genetic aberrations/ nosological entities

Blast equivalents in AML

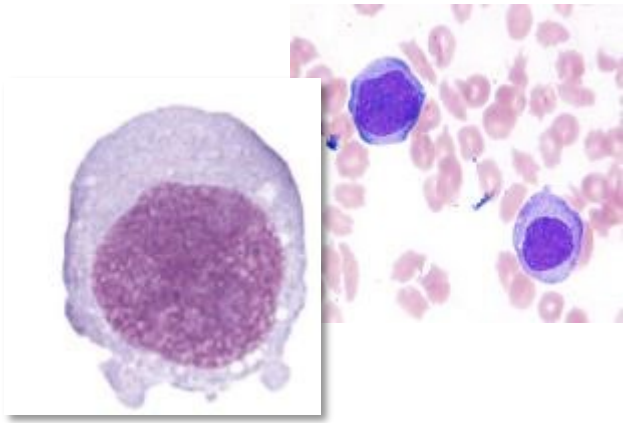
Myeloblast & Promyelocyte

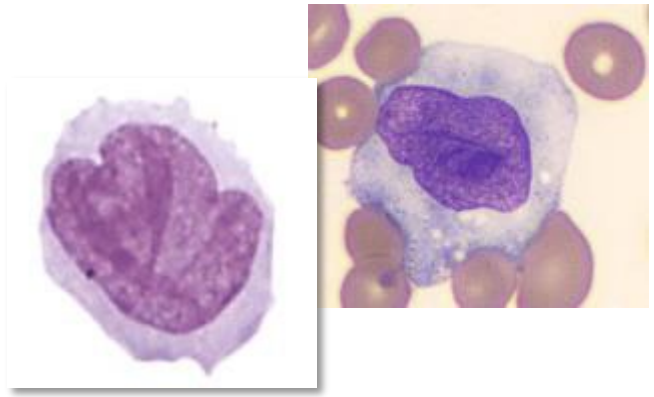




Mufti, G. J. et al. Haematologica 2008;93:1712-1717;
Palmer L et al, Int Journal Lab Hematology 2015; 37:287-303

Monoblast & Promonocyte



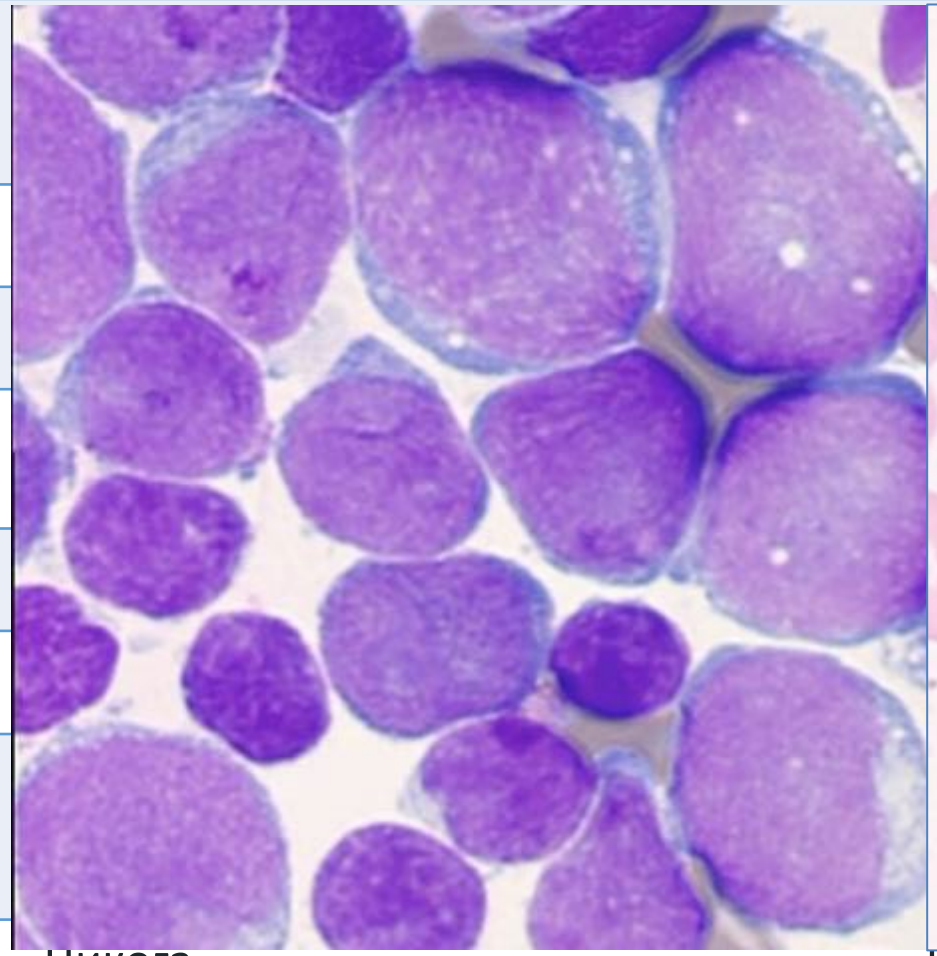
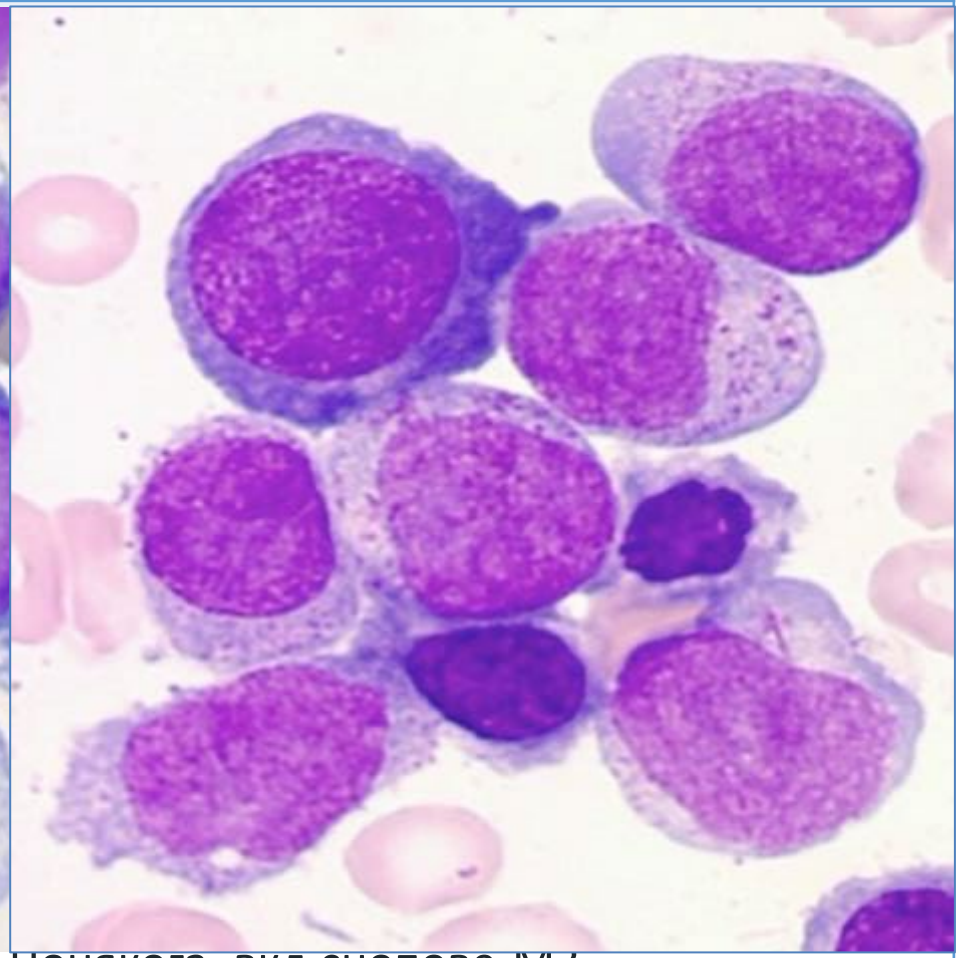


Goasguen et al, Morphological evaluation of monocytes and their precursors. Haematologica. 2009 Jul;94(7):994-7.

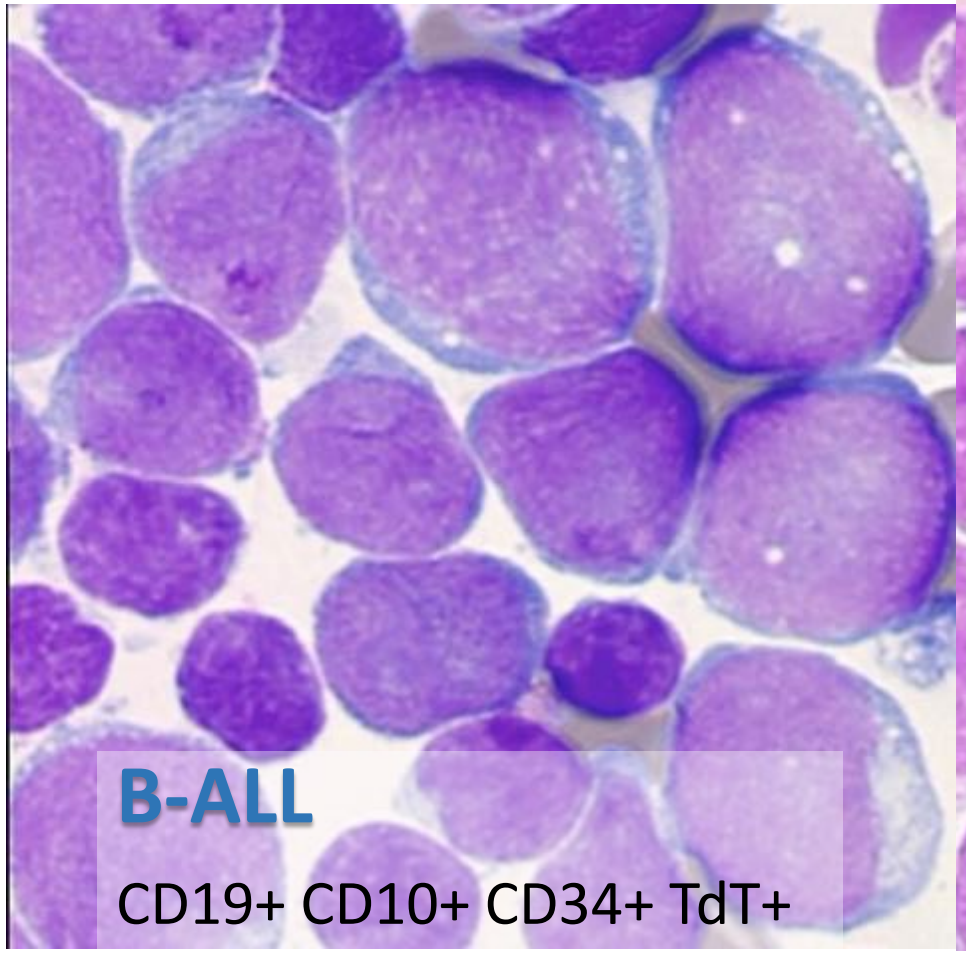
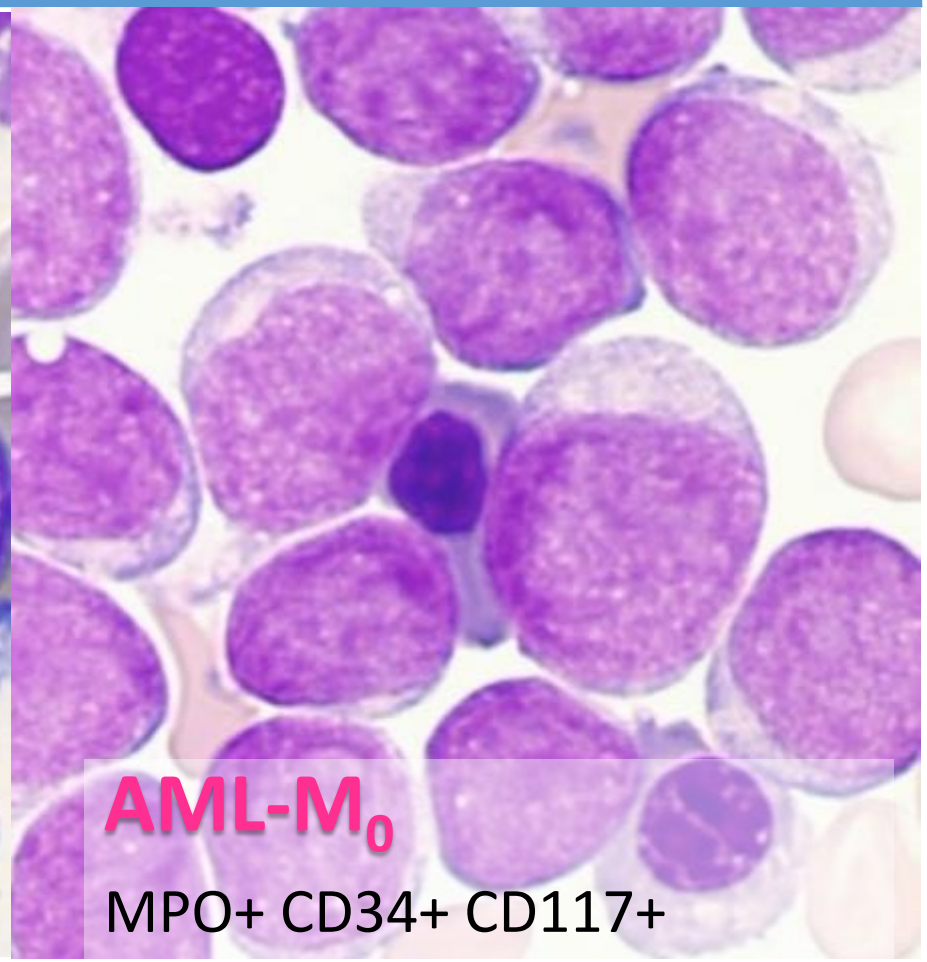
Arber et al The 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia. Blood. **2016**;127(20)
Khoury et al, The 5th edition of the WHO of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. **2022**;36(7)
Arber et al. ICC of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. **2022**; 140(11)

eha

Myeloblasts vs Lymphoblasts

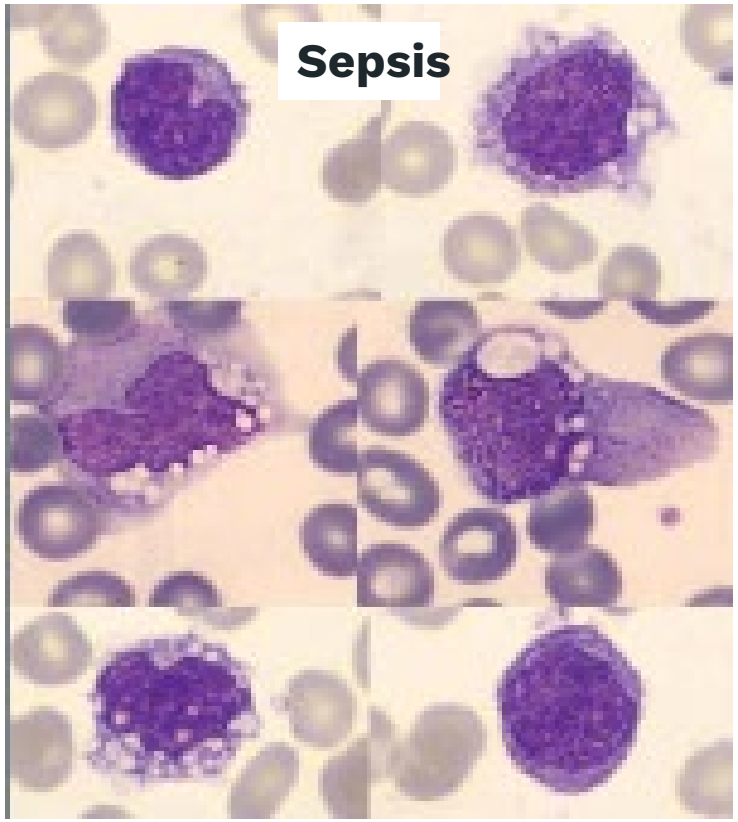
	Lymphoblasts	Myeloblasts
Heterogeneity		
Cell size		
Nucleus		
Chromatin		
Nucleoili		
Cytoplasm		
Granulations		
Auer rods	Никога	Понякога, вкл.снопове-М3
Vacuoles	Понякога	Понякога, особено моноцитните

Not always easy to decide

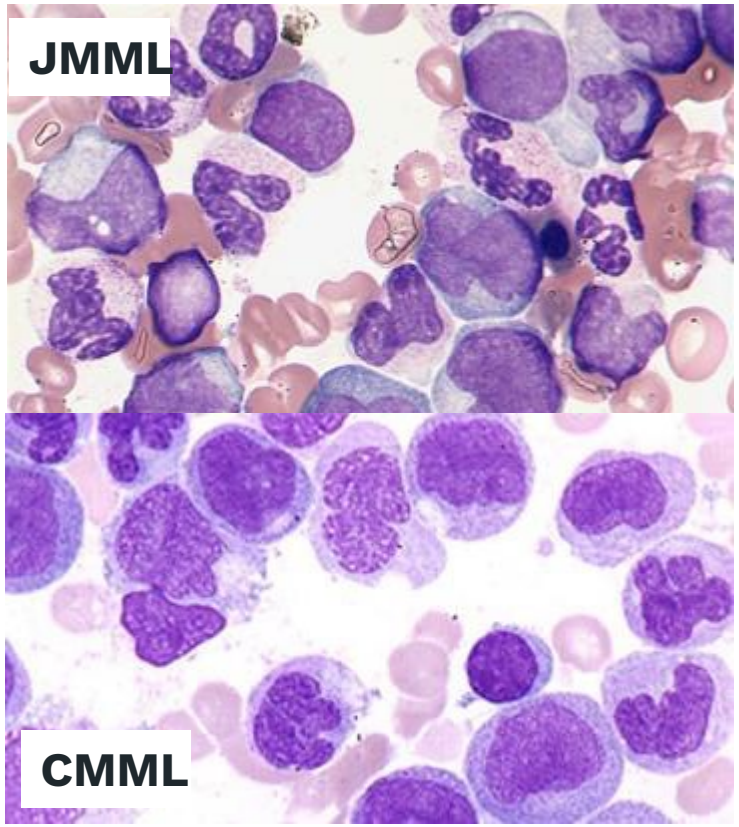
Lymphoblasts	Myeloblasts
	

The monocytic challenge

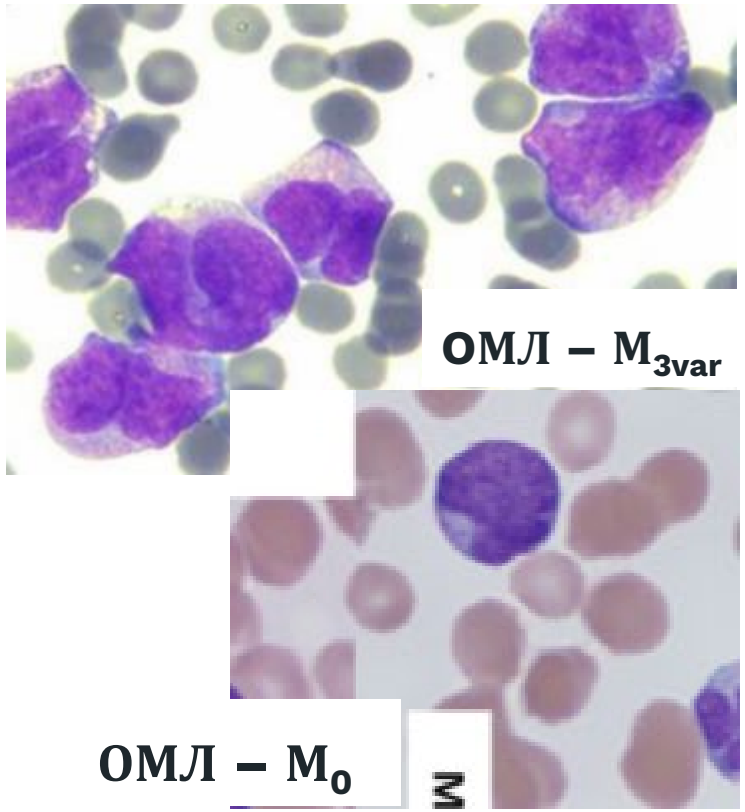
AMoL vs reactive



Acute vs Chronic



AMoL vs other AML

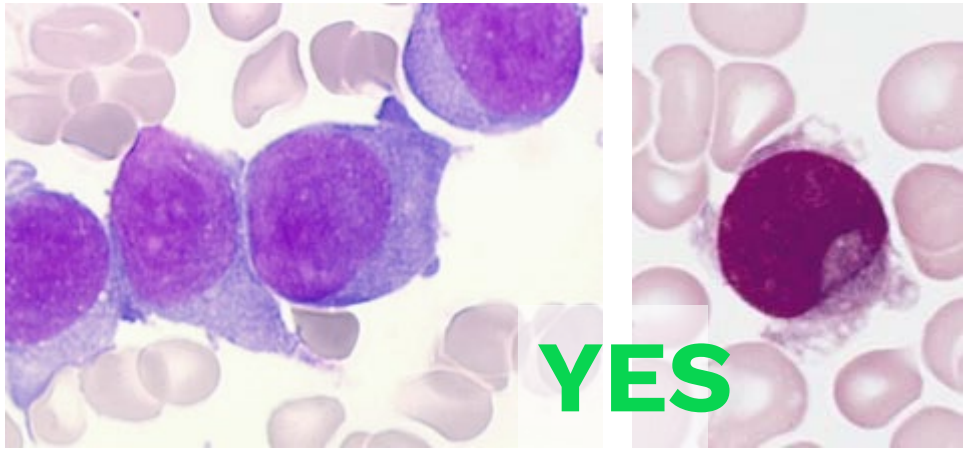


Varotto et al. Diagnostic challenges in acute monoblastic/monocytic leukemia in children. Front Pediatr. 2022;10:911093.; Sukhacheva, https://clinlabint.com/the-role-of-monocytes-in-the-progression-of-sepsis/; Zini, *Int J Lab Hematol.* 2021;43:346–353. ; Lynch et al, How I investigate monocytosis. Int J Lab Hem. 2018;40:107–114

eha

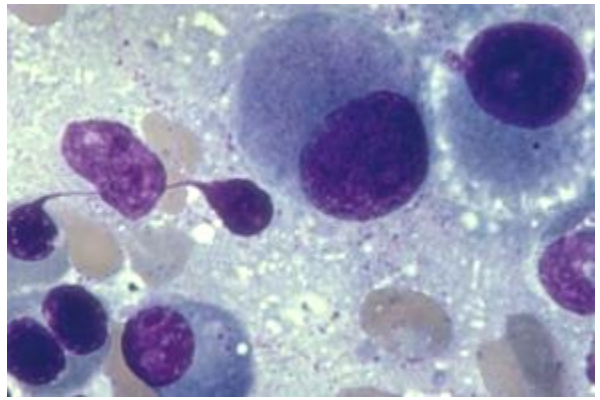
Blast equivalents in specific AML

Megakaryoblasts ≥ 20%



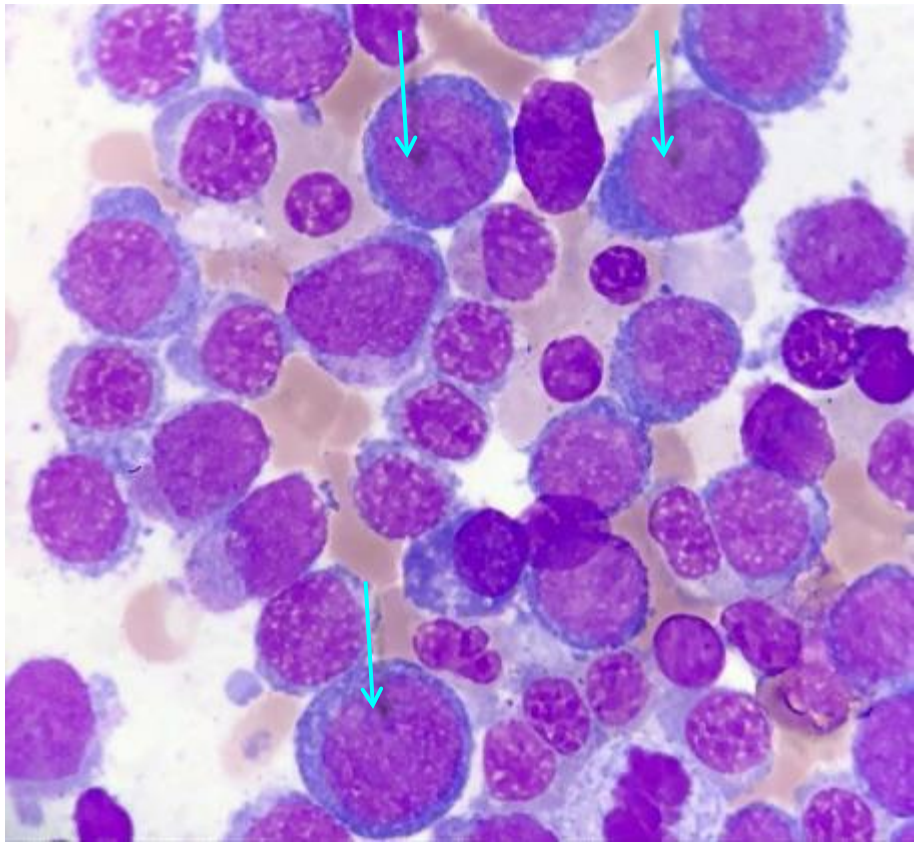
No

microMe



Erythroblasts ≥ 80%

Proerythroblasts ≥ 30%



ASH Image bank


QUA
LI
TY
NTI
TY

How many blasts are needed for AML?

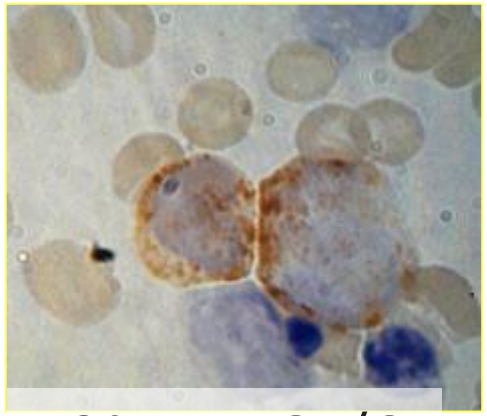
FAB, 1976		**≥ 30%**
WHO, 2001-17	AML with recurrent genetic aberrations All other AMLs	NA ≥20%
WHO, 2022	AML with defining genetic abnormalities AML defined by differentiation, AML-MR	NA ≥20%
ICC, 2022	AML with defining genetic abnormalities MDS/AML with TP53^{m} with myelodysplasia-related cytogenetic abnormalities with myelodysplasia-related mutations Not otherwise specified (NOS) AML with TP53^{m} with myelodysplasia-related cytogenetic abnormalities with myelodysplasia-related mutations Not otherwise specified (NOS) AML with t(9;22)(q34.1;q11.2)/BCR::ABL1	≥10% 10-19% 10-19% 10-19% 10-19% ≥20% ≥20% ≥20% ≥20% ≥20% ≥20%

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

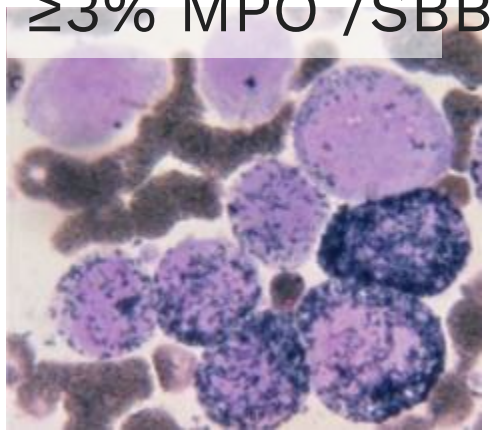
AML without maturation vs with maturation

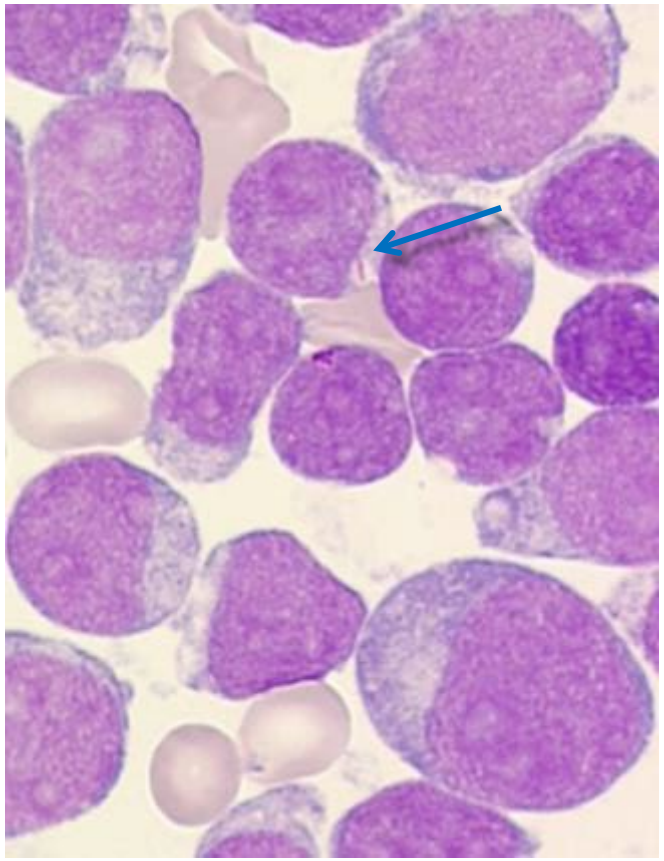
M1: <10% maturing cells of Gr lineage

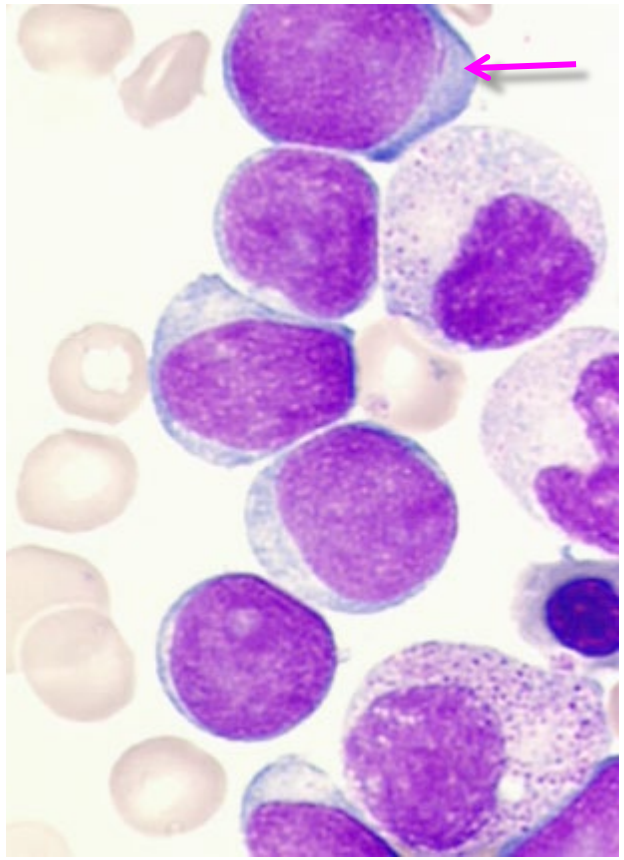
M2: ≥ 10% maturing cells of Gr lineage
< 20% monocytoid cells

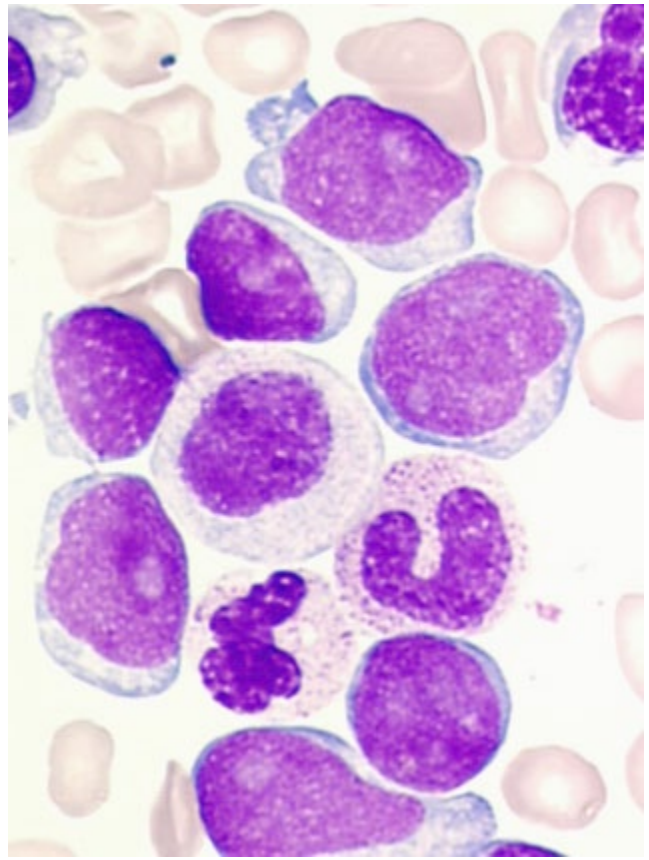


≥3% MPO /SBB







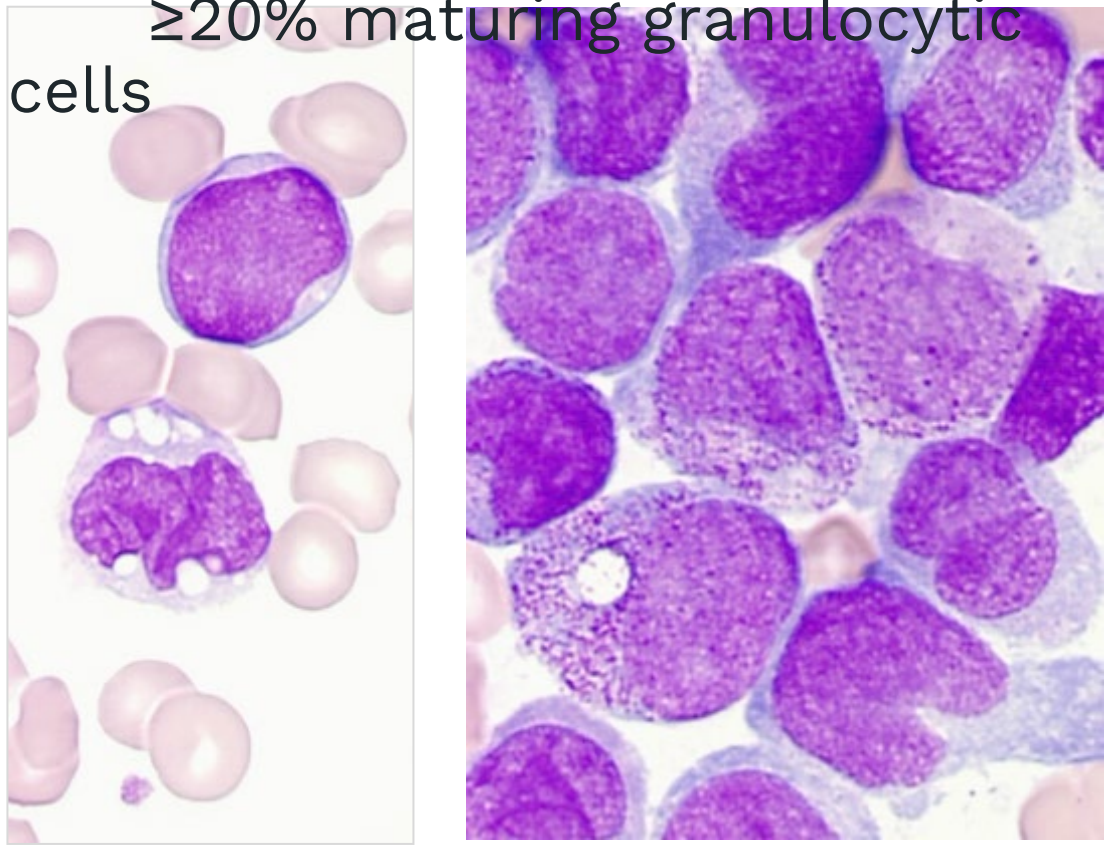


Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719.

AML myelomonocytic vs monocytic

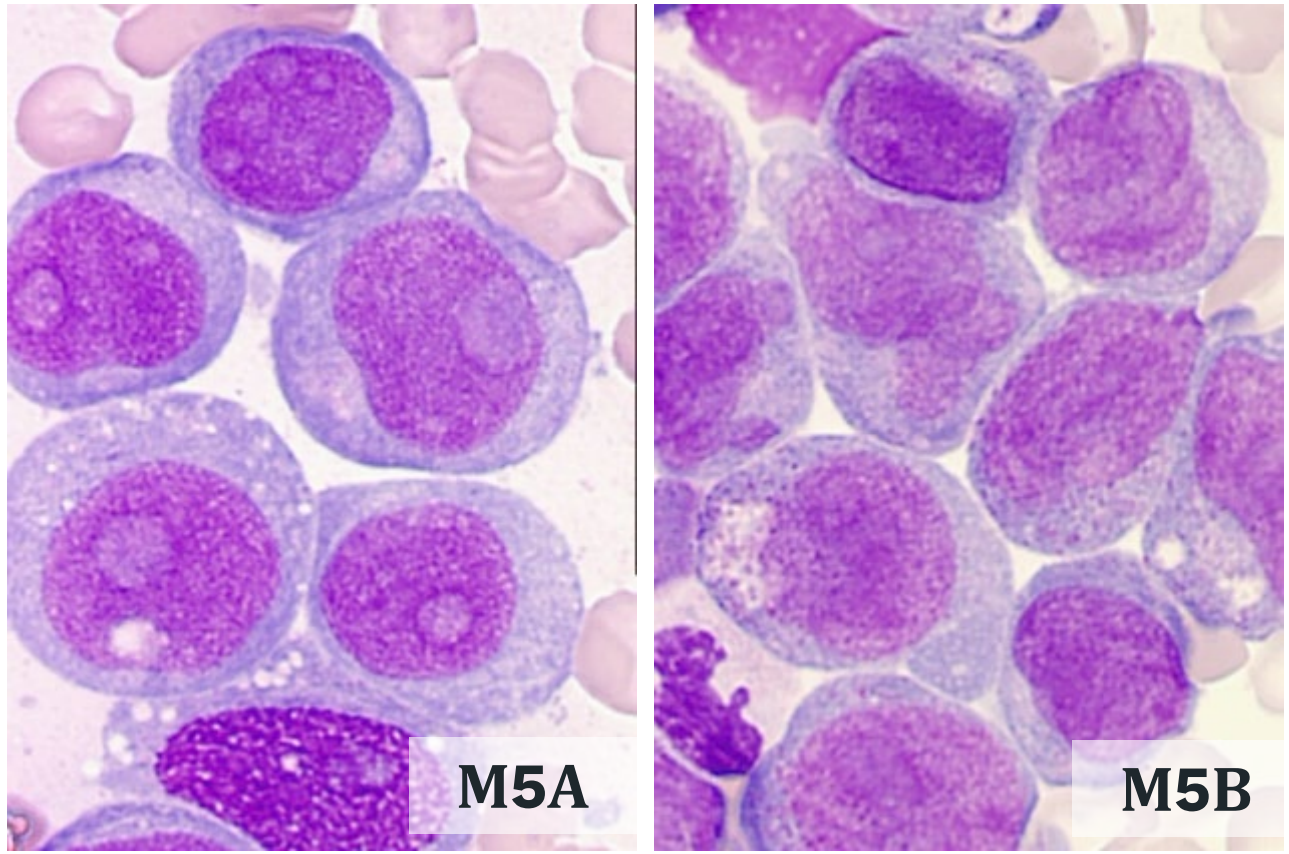
M4: ≥20% monocytes and Mo precursors

≥20% maturing granulocytic cells



M5: ≥ 80% monocytes and Mo precursors

< 20% maturing granulocytic cells

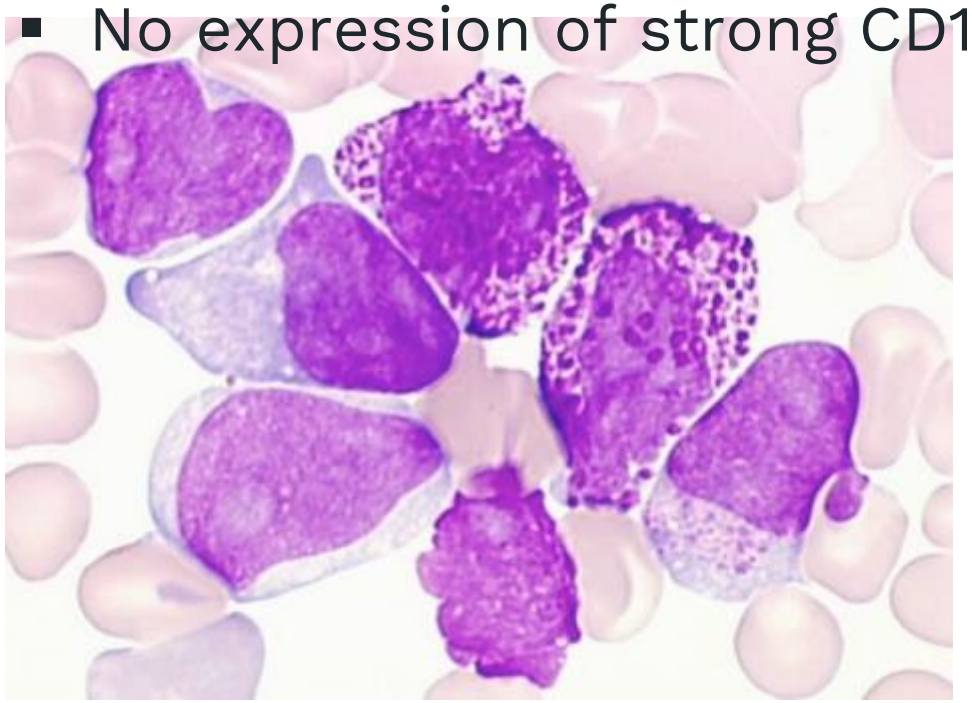


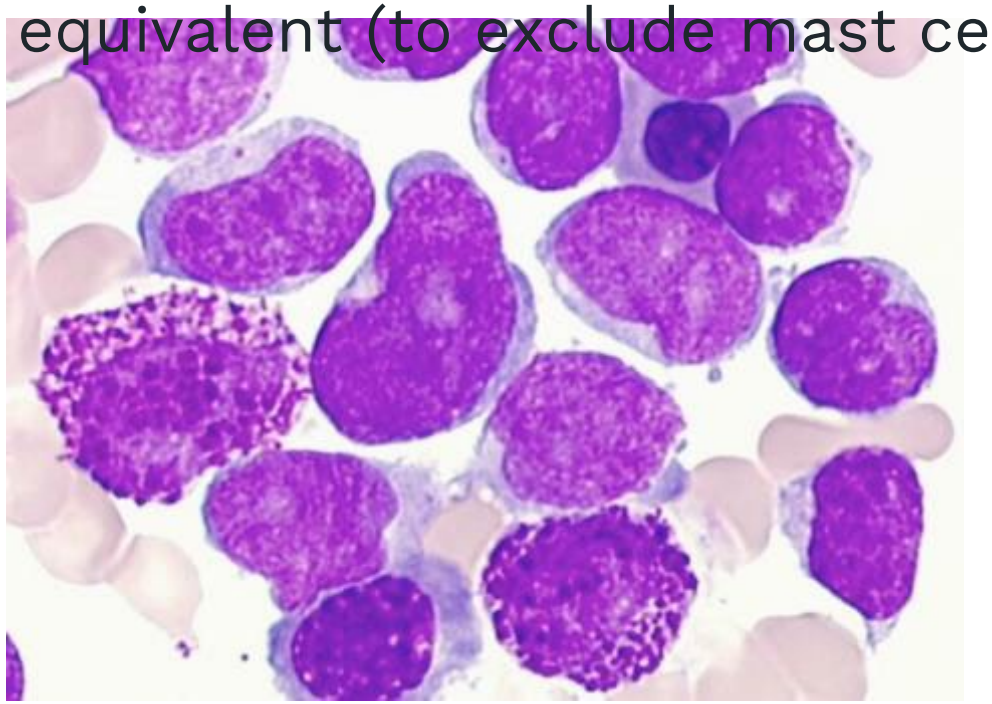
Courtesy Marie-Therese Daniel

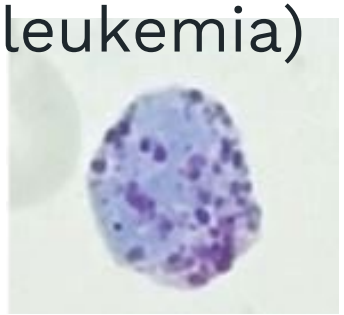
Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719.

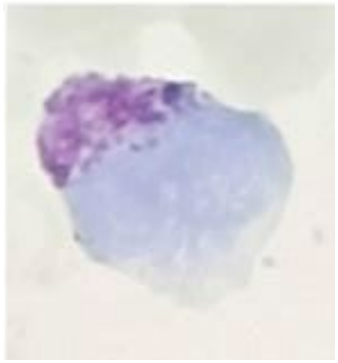
Acute basophilic leukemia

- Blasts & immature/mature basophils with metachromasia on toluidine blue staining
- Blasts are negative for cytochemical MPO, SBB, and NSE
- No expression of strong CD117 equivalent (to exclude mast cell leukemia)









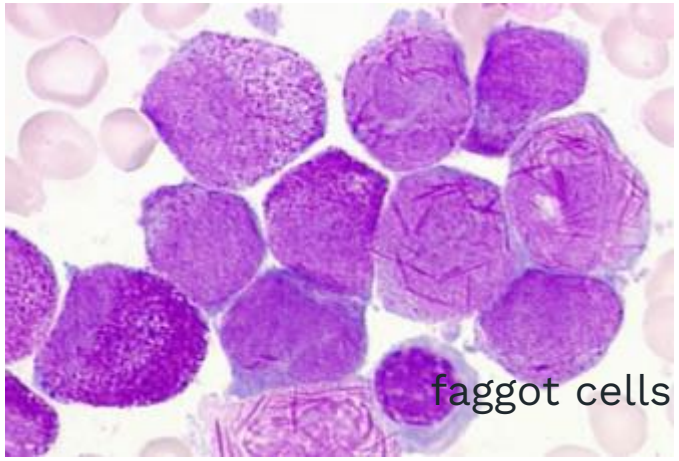
Courtesy Marie-Therese Daniel

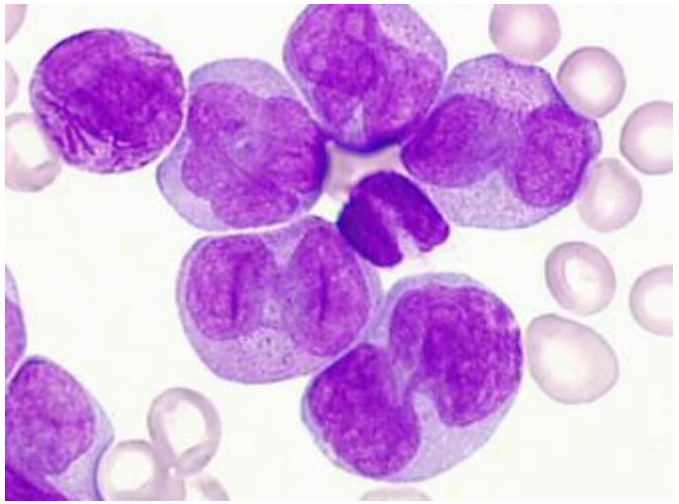
Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719.

What genetic disorders are behind a certain morphology?

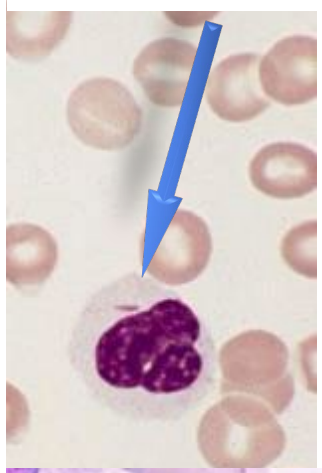
AML with defining genetic abnormalities

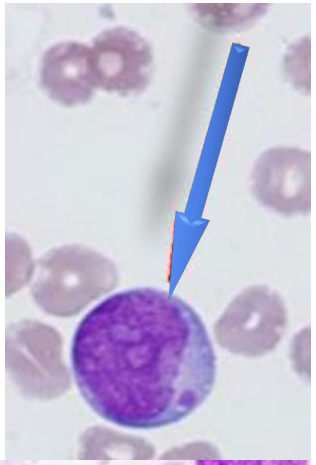
PML::RARA fusion

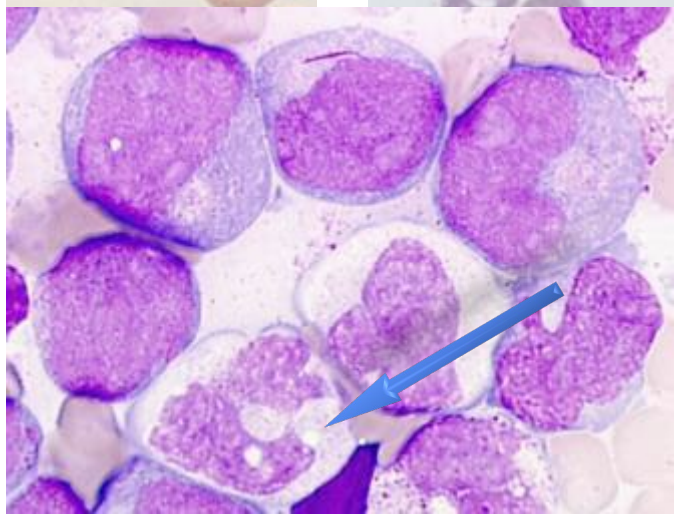




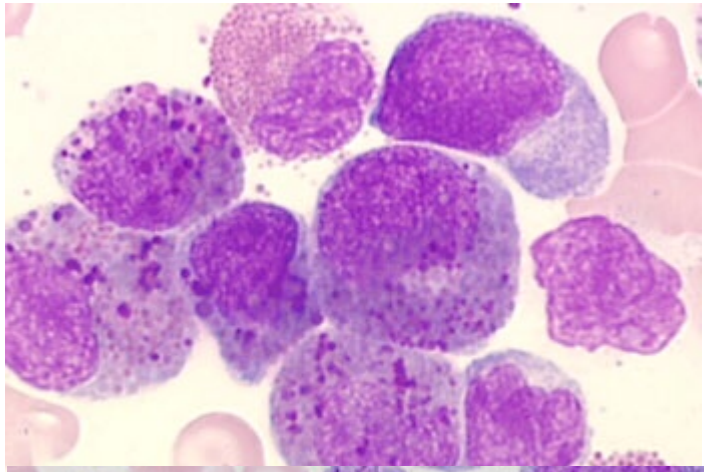
RUNX1::RUNX1T1

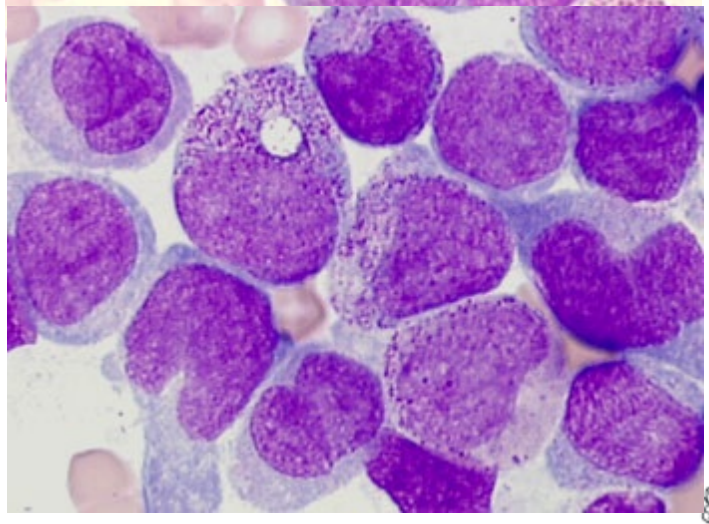




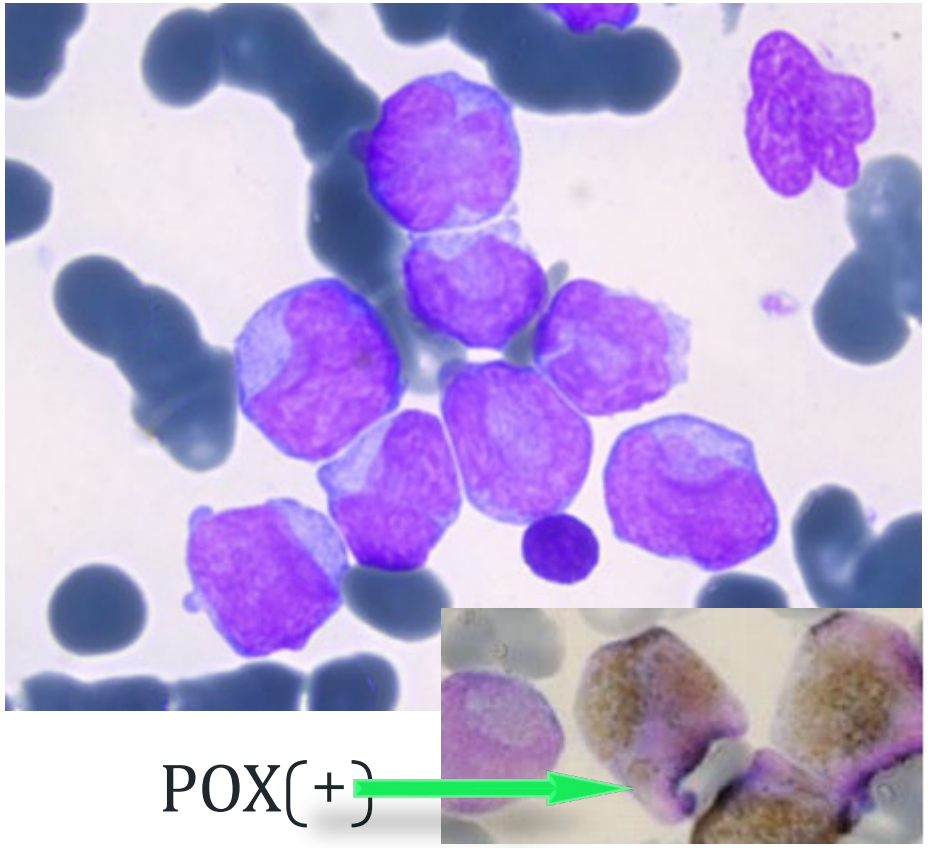


CBFB::MYH11



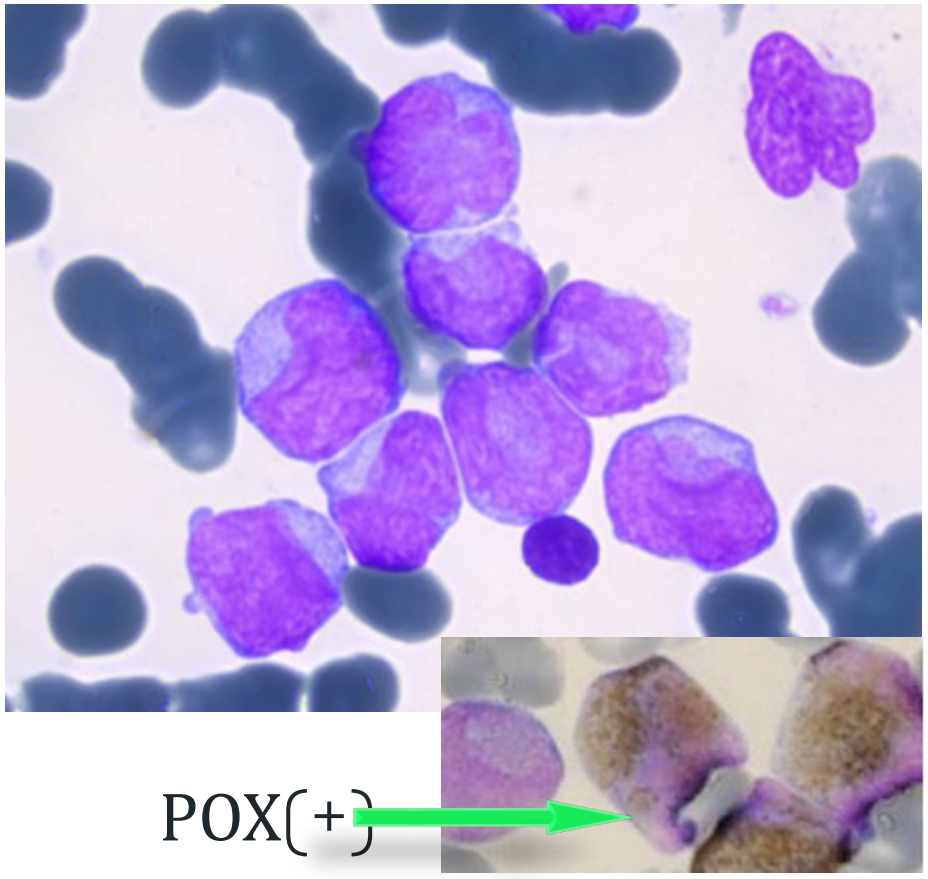


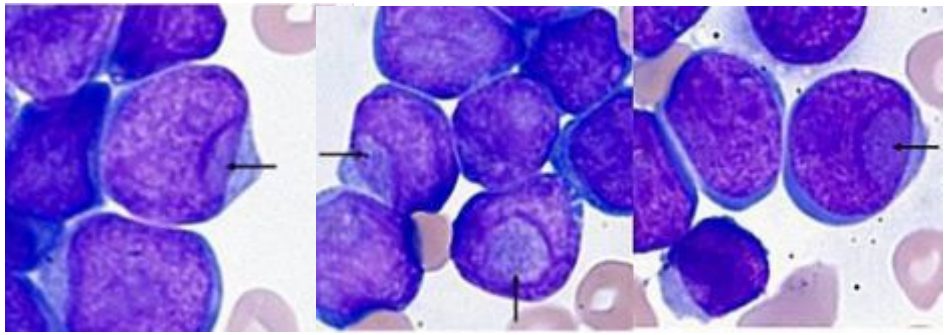
Cup-like morphology is associated with $NPM1^{mut}$ and/or $FLT3^{mut}$



Kroschinsky FP, et al. Cup-like acute myeloid leukemia: new disease or artificial phenomenon? Haematologica. 2008; 93: 283; Park BG, et al.. Ann Hematol. 2013;92(4):451.

Cup-like morphology surp  s

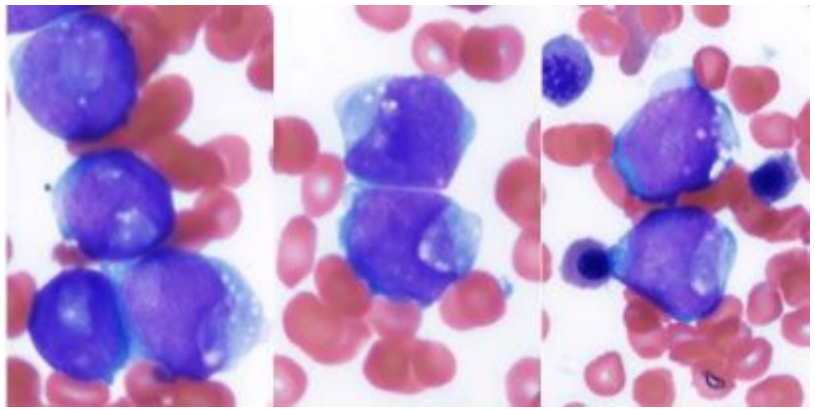




B-ALL **Genetics:** t(4;11)(q21;q23) ; KMT2A-AFF1

NGS: KRAS mutation (G13D, VAF 49%)

Rieu et al, eJHaem. 2020;1:589–592.



B-ALL **Genetics:** 72~80, XXY [5]/XY[12]

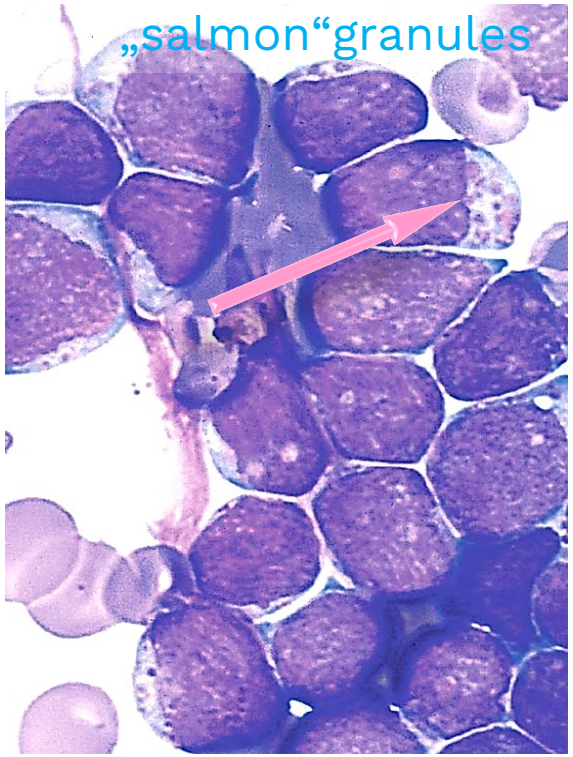
NGS: mutations in TP53 and DNMT3A

Wang et al. BJH. 2015;170(5):596.

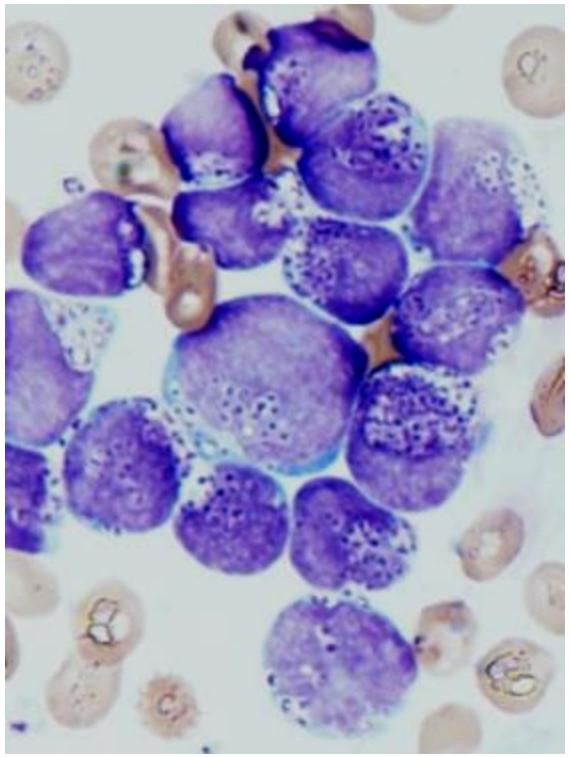
Kroschinsky FP, et al. Cup-like acute myeloid leukemia: new disease or artificial phenomenon? Haematologica. 2008; 93: 283; Park BG, et al.. Ann Hematol. 2013;92(4):451.

Other surprises

Granules are not AML®

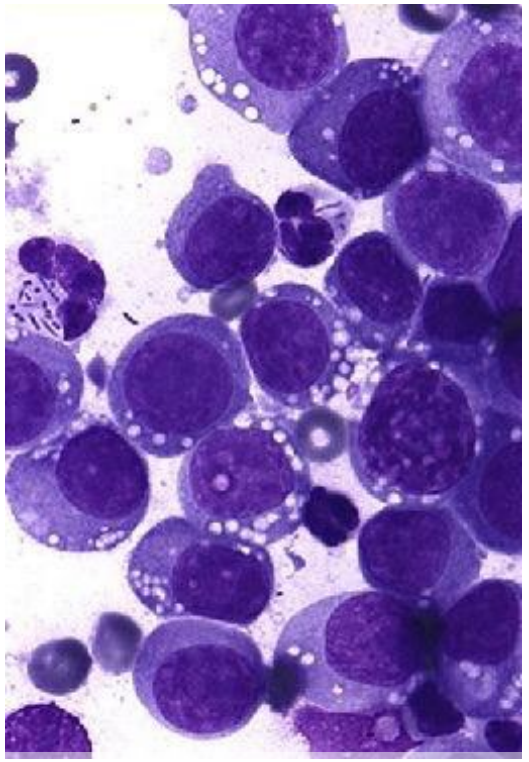


B-ALL



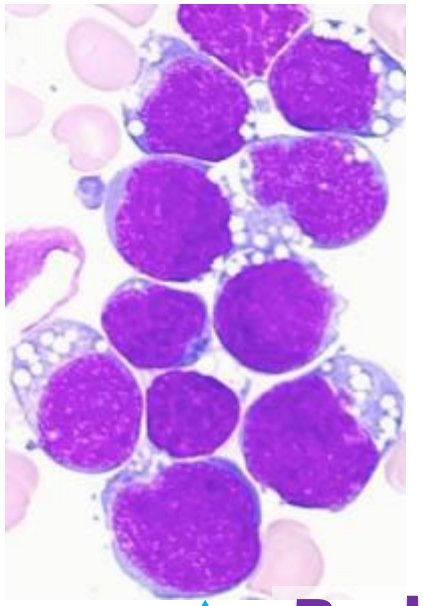
Ph’(+) B-ALL

Looks like Burkitt®



AML-M5

Lyzozym+; CD56+

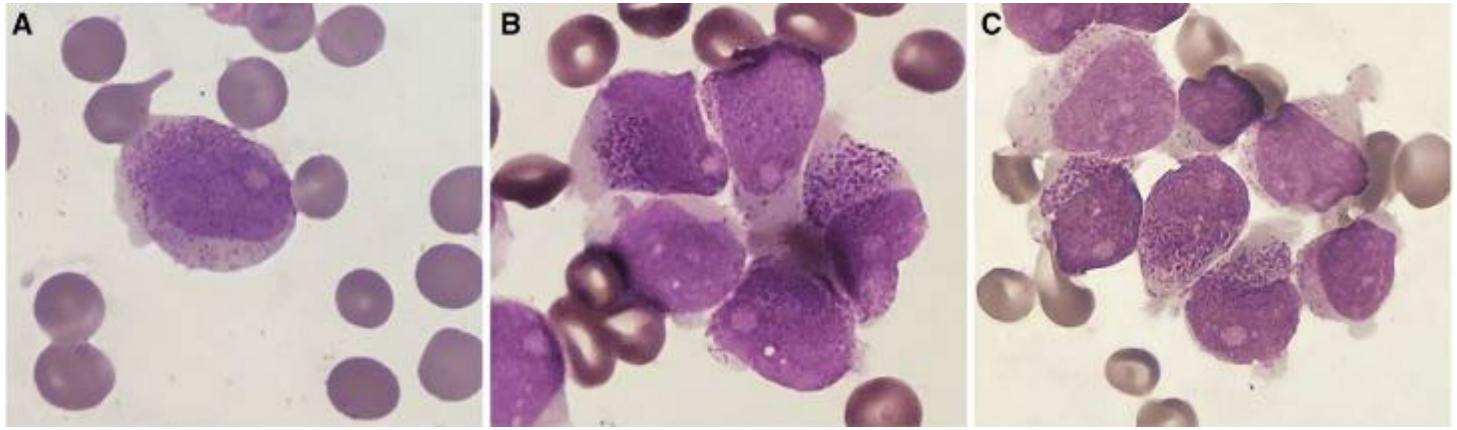


Burkitt

Literature: Song JY, et al. B lymphoblastic leukemia with granules mimicking acute myeloid leukemia. Int J Hematol. 2015;102(3):251-2.

Other misleading images

Promyelocytic disguise

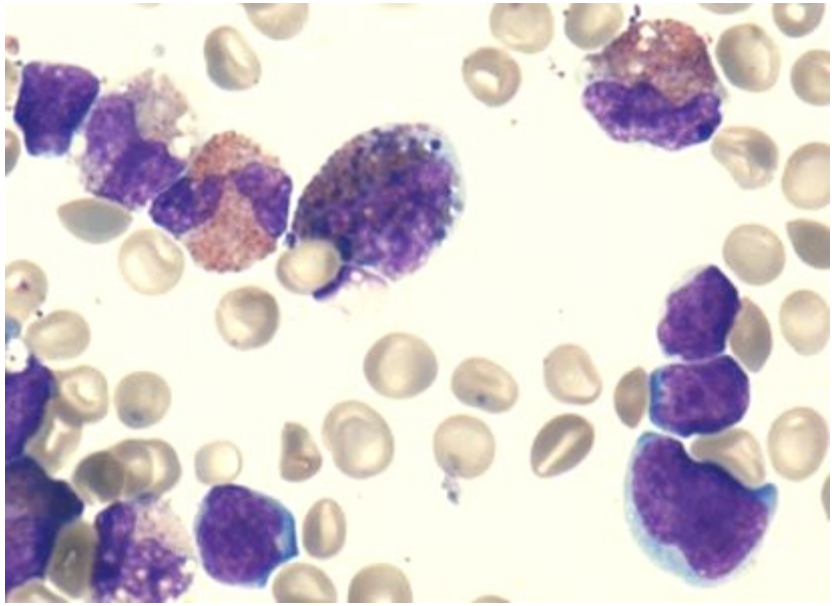


B-ALL

CD19+ CD10+ CD34+ TdT+

Ma Y et al. IJH. 2019;110(6):645-646.

Eosinophillic clue



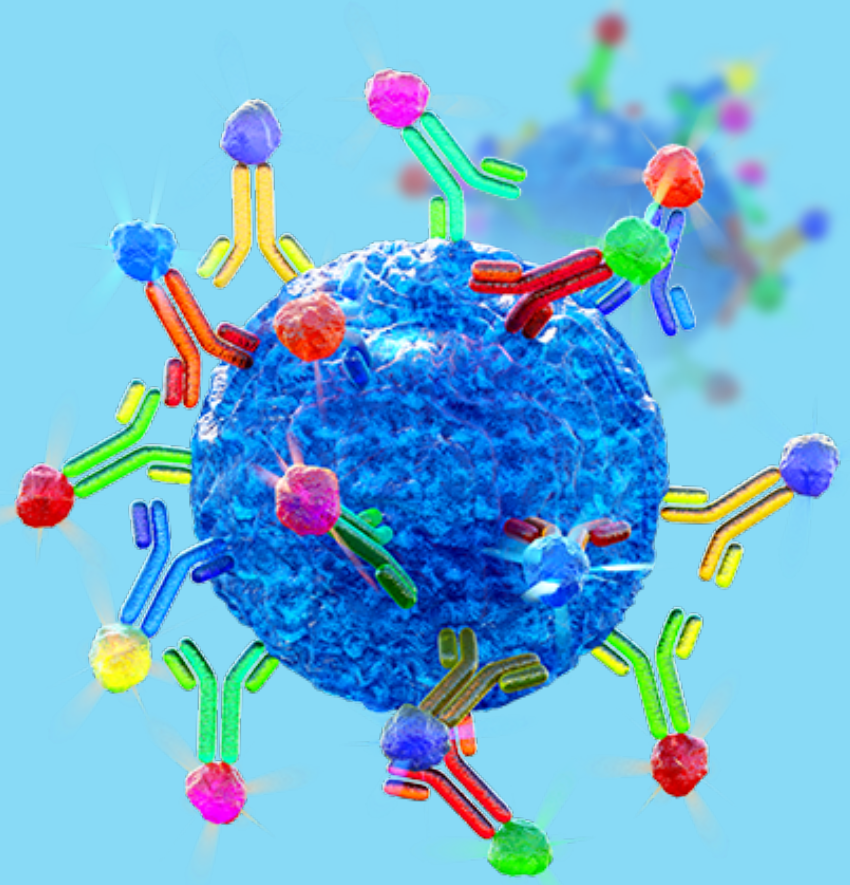
B-ALL

5q33.2 rarrangement

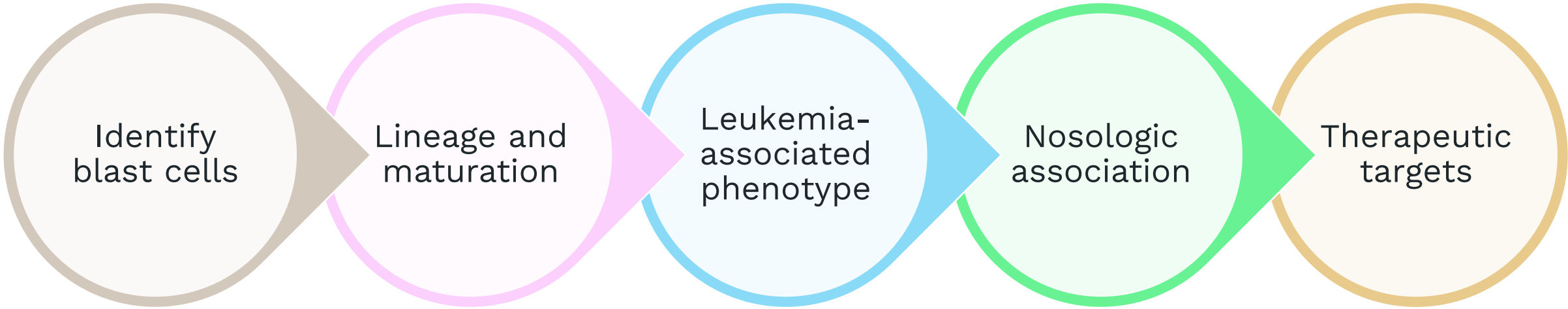
Ma Y, et al. Promyelocyte-like blasts in B-lymphoblastic leukemia of a 67-year-old male patient. Int J Hematol. 2019 Dec;110(6):645-646.
Gujral S et al. B-lymphoblastic leukemia/lymphoma with IGH::IL3 fusion. In: In: WHO Classification of Tumours Editorial Board. Haematolymphoid tumours. Lyon (France): IARC; 2024. . (WHO classification of tumours series, 5th ed.; vol. 11). https://publications.iarc.who.int/637.

03

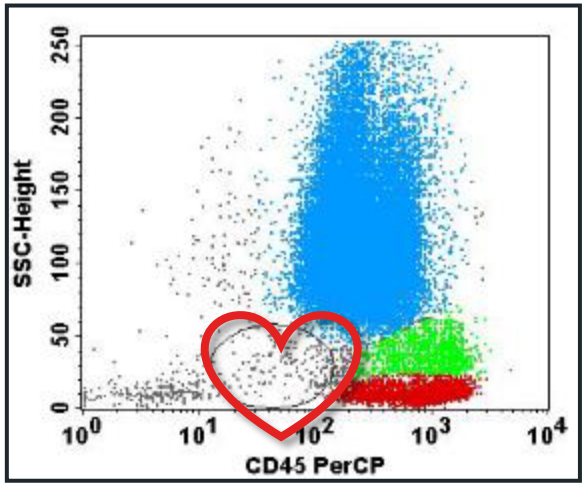
Immuno phenotyping



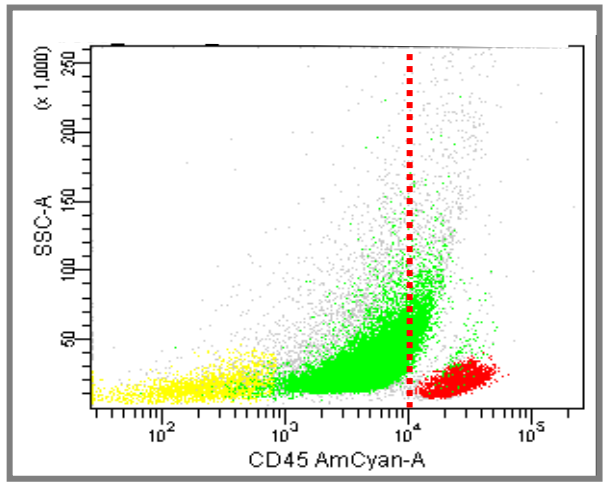
Immunophenotyping



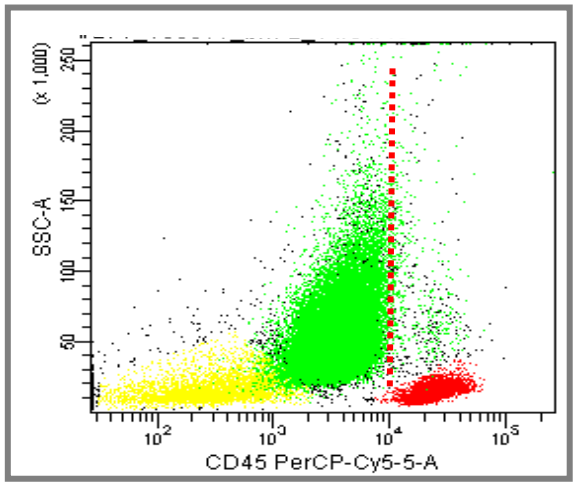
Identification of blast cells



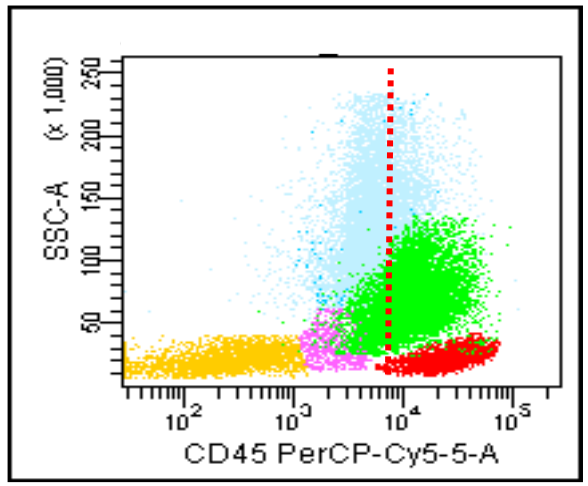
Control



AML without maturation

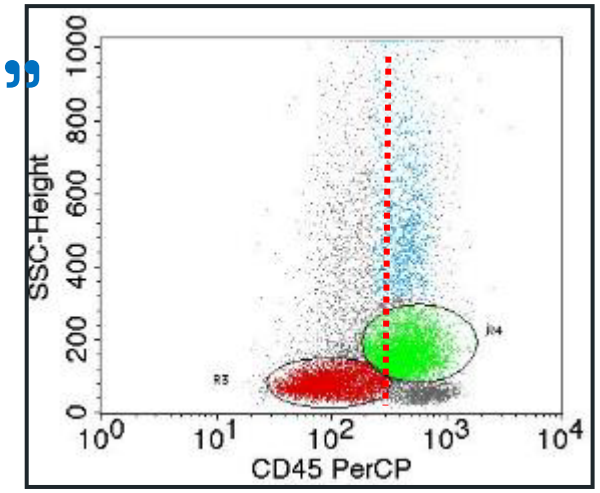


Acute promyelocytic



AML monocytic

“CD45 / SSC gating”



AML myelomonocytic

eha

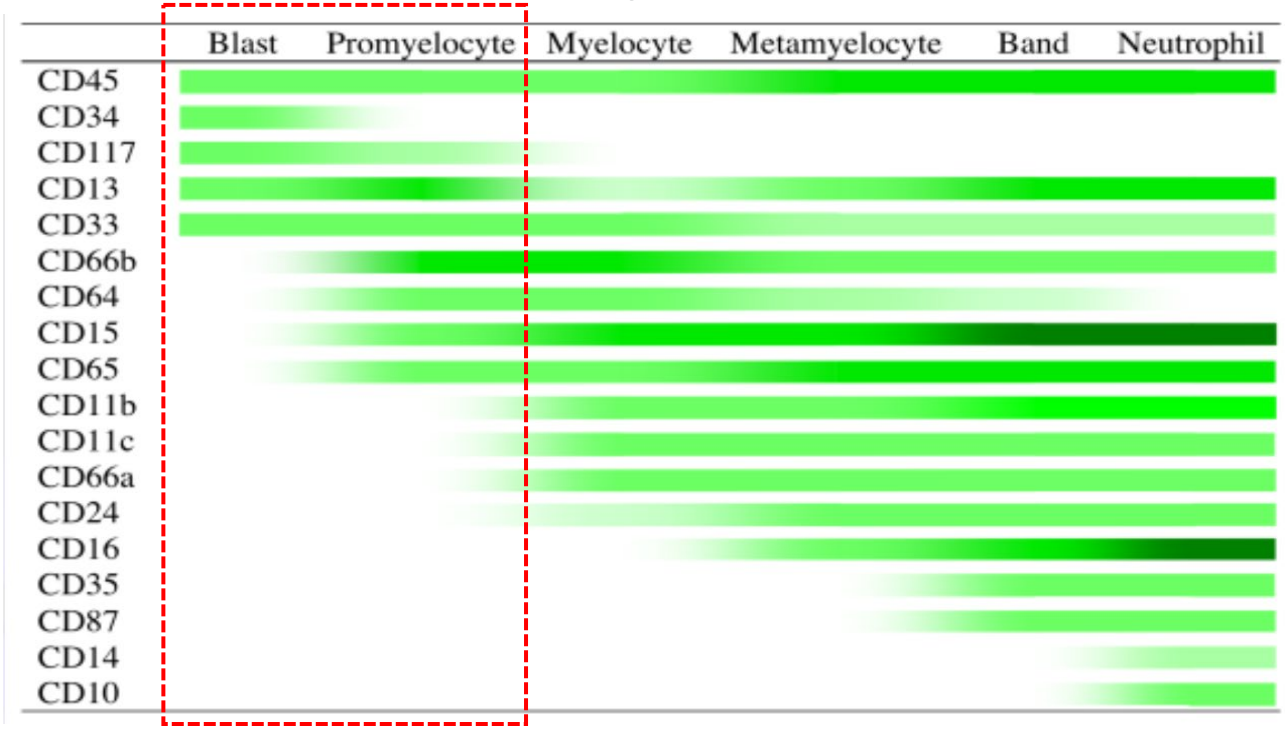
Lineage and maturation

AML diagnosis	Immunophenotypic markers
Precursor markers	CD34, CD117, HLA DR
Myeloid markers	Myeloperoxidase, or CD13, CD33 [≥2]
Myeloid maturation	CD11b, CD15, CD64, CD65
Monocytic markers	CD14, CD64, CD11c, lysozyme [≥2], CD36, CD4, CD38,
Megakaryocytic markers	CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD42b (glycoprotein Ib)
Erythroid markers	CD235a (glycophorin A), CD71, CD36
Core MRD markers	CD34, CD117, CD45, CD33, CD13, CD56, CD7, HLA-DR If monocytic: CD64, CD11b, CD4 (in addition)

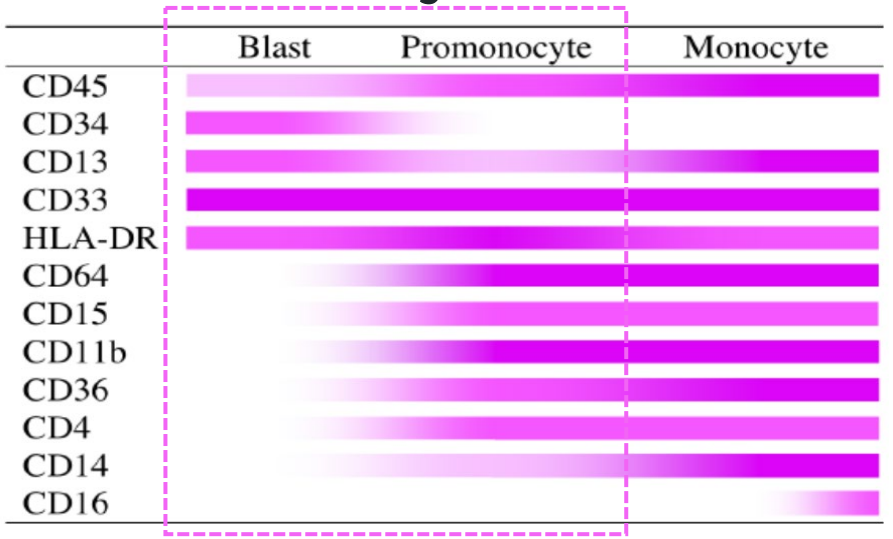
Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.; Khoury et al. The 5th edition of the WHO of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719.

Normal immunophenotypic patterns

Granulocytic



Monocytic



Wood, Cherian & Borowitz. Henry's Laboratory Methods, 2017

Required for diagnosis

WHO – HAEM5
Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with PML::RARA fusion
Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion
Acute myeloid leukaemia with CBFB::MYH11 fusion
Acute myeloid leukaemia with KMT2A rearrangement
Acute myeloid leukaemia with DEK::NUP214 fusion
Acute myeloid leukaemia with MECOM rearrangement
Acute myeloid leukaemia with BCR::ABL1 fusion
Acute myeloid leukaemia with NPM1 mutation
Acute myeloid leukaemia with CEBPA mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with RBM15::MRTFA fusion
Acute myeloid leukaemia with NUP98 rearrangement
AML with other defined genetic alterations
Acute myeloid leukaemia, defined by differentiation
Acute myeloid leukaemia with minimal differentiation
Acute myeloid leukaemia without maturation
Acute myeloid leukaemia with maturation
Acute basophilic leukaemia
Acute myelomonocytic leukaemia

Some entities require phenotype for diagnosis

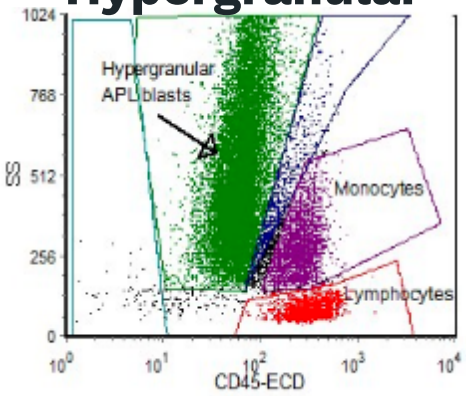
- **AML with minimal differentiation:**
  ≥ 2 CD13, CD33, CD117
- **Acute erythroid leukemia:**
  CD235, CD71, CD36
- **Acute megakaryoblastic leukemia:**
  CD41, CD42, CD61
- **Differential diagnosis:**
  MPAL – My/T; My/B

WHO Classification of Tumours Editorial Board. Haematolymphoid tumours. Lyon (France): IARC; 2024.

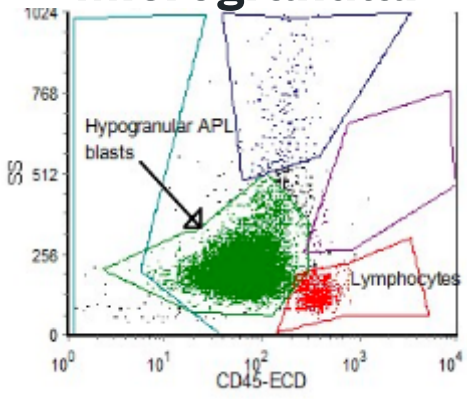
eha

Or quick guide to diagnosis:
APL PML::RARA

Hypergranular



Microgranular



SSC^{high} 100% - CD117, MPO, CD33

	Hypergranular	Microgranular
CD2	23%	90%
CD34	2%	75%

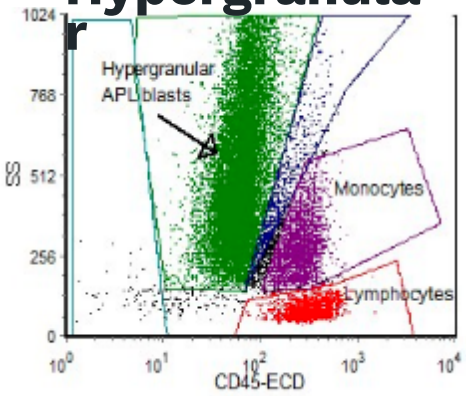
SSC^{low} 0% - HLA DR

Fang et al. Acute promyelocytic leukemia: Immunophenotype and differential diagnosis by flow cytometry. Cytometry B Clin Cytom. 2022;102(4):283-291.

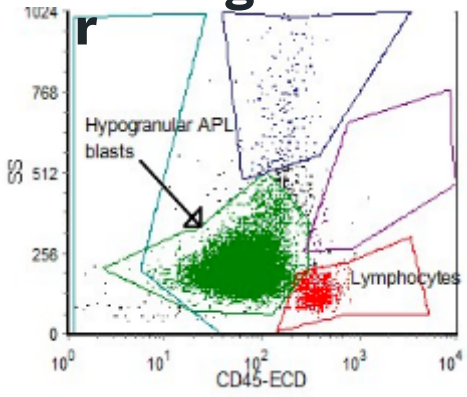
Or quick guide to diagnosis:

APL PML::RARA

Hypergranular



Microgranular



SSC^{high} **100% -** CD117, MPO, CD33

SSC^{low} **0% -** HLA DR

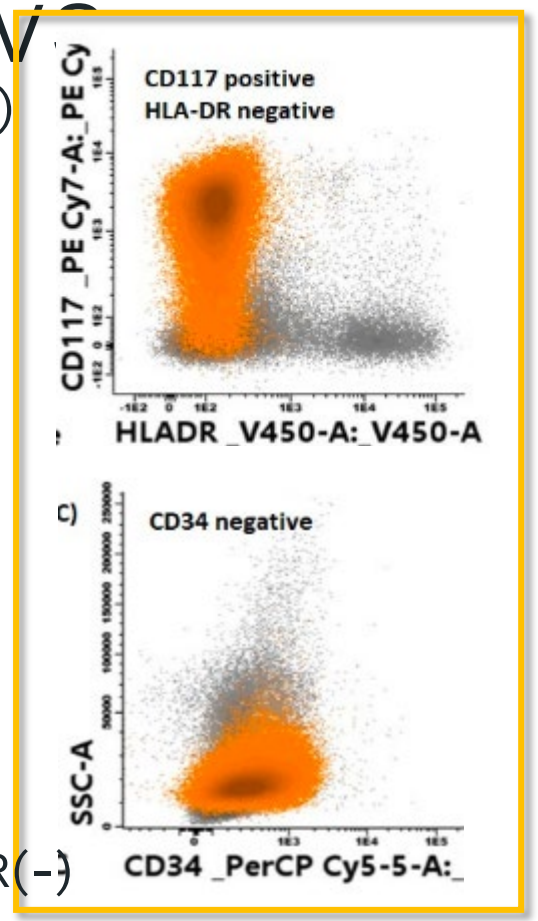
	Hypergranular	Microgranular
CD2	23%	90%
CD34	2%	75%

VS

CD117, MPO(+)

APL-like NPM1^{mut} AML

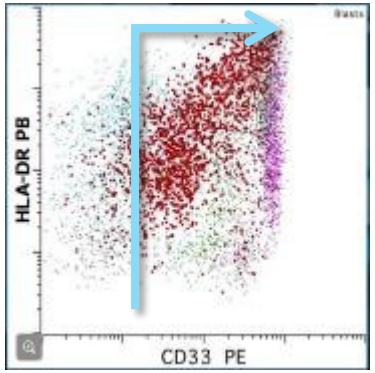
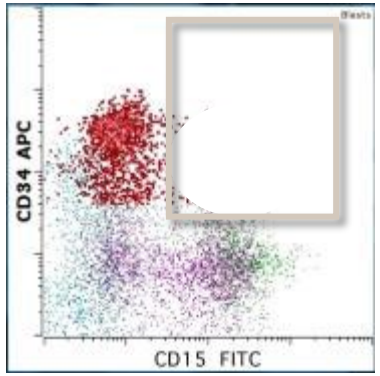
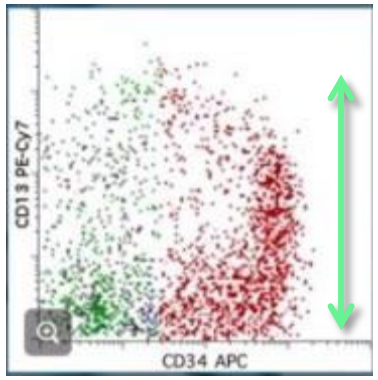
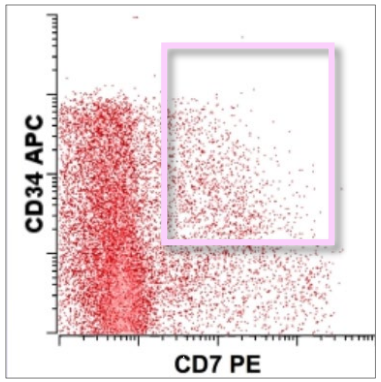
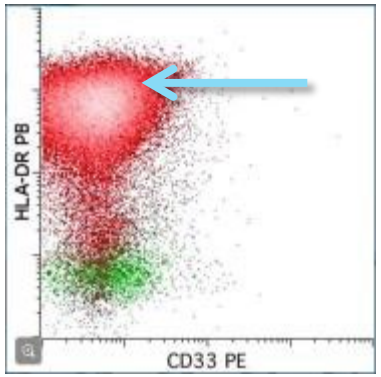
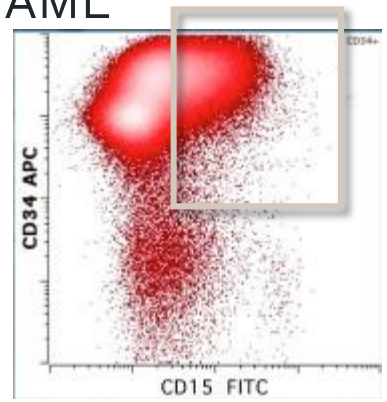
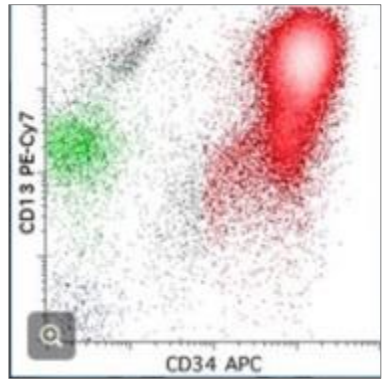
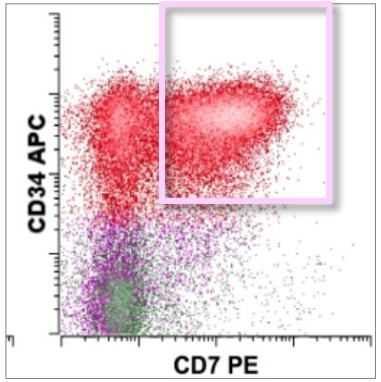
CD34, HLA DR(-)



Fang et al. Acute promyelocytic leukemia: Immunophenotype and differential diagnosis by flow cytometry. Cytometry B Clin Cytom. 2022;102(4):283-291.

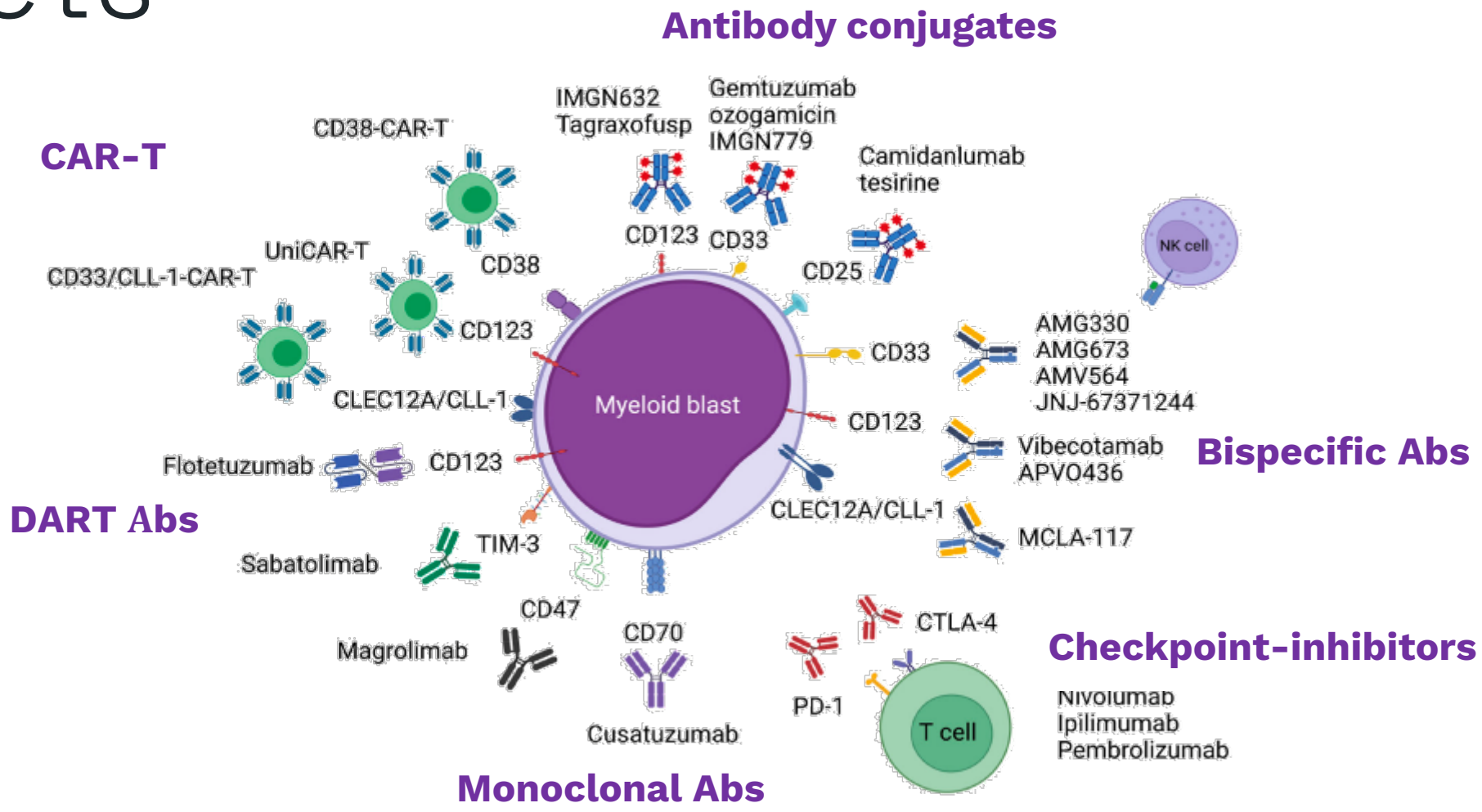
eha

Aberrant phenotypes (LAP)

Abnormal expression intensity ↑↓ ∅	Asynchronous "mature" + "immature"	Homogeneous instead of variable expression	Mixed lineage expression
normal	normal	normal	normal
			
AML	AML	AML	AML
			
CD33(-)	CD34(+)CD15(+)	CD34(+)bright	CD7(+) My blasts

Wood, Cherian & Borowitz. Henry's Laboratory Methods, 2017

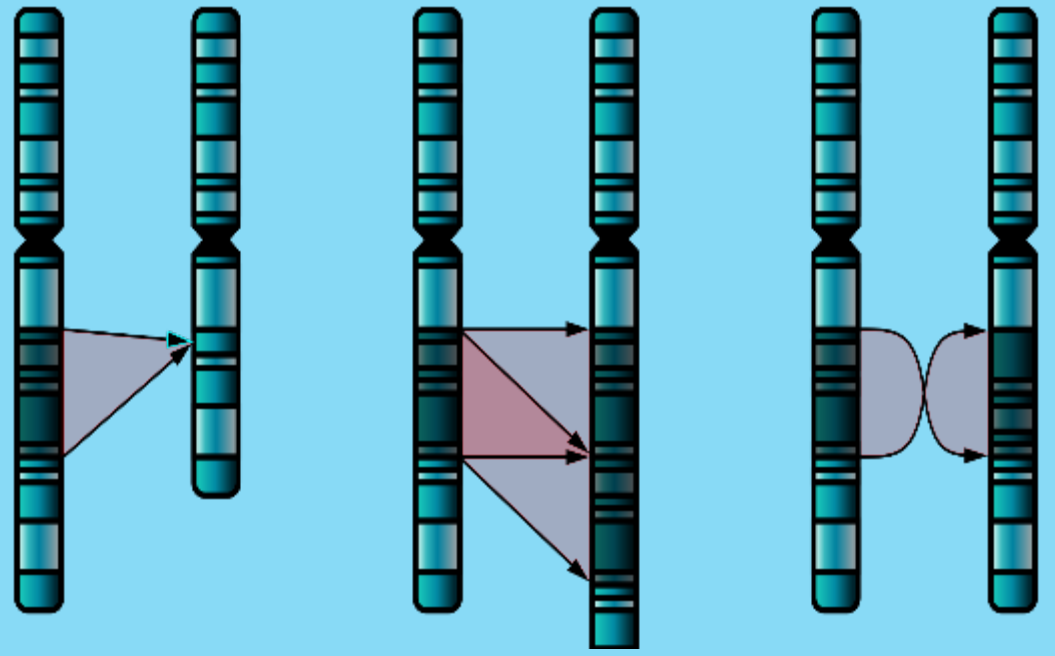
Identification of therapeutic targets



Schürch CM. Front Oncol. 2018;8:152. ; Böhme et al, Cancers (Basel). 2021;14:105.; Perna et al. Cancer Treat Res. 2022;183:225.

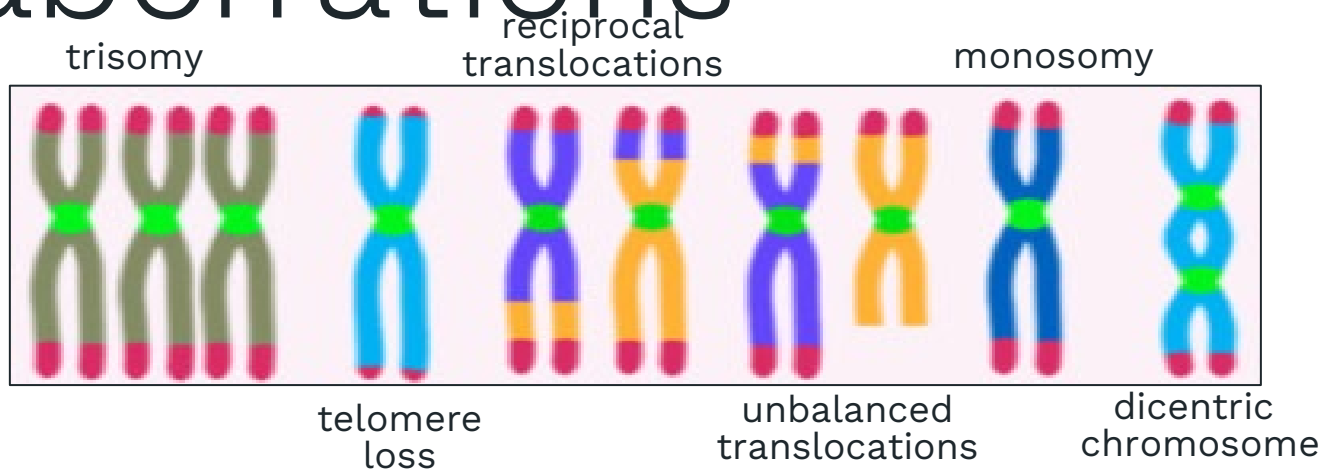
04

Cytogenetics



- Detection of chromosomal aberrations
- Classification of AML entities
- Risk stratification

Detection of chromosomal aberrations



Complex karyotype:

- presence of ≥3 different chromosomal aberrations in the same clone.

Monosomal karyotype:

- presence of ≥ 2 autosomal monosomies

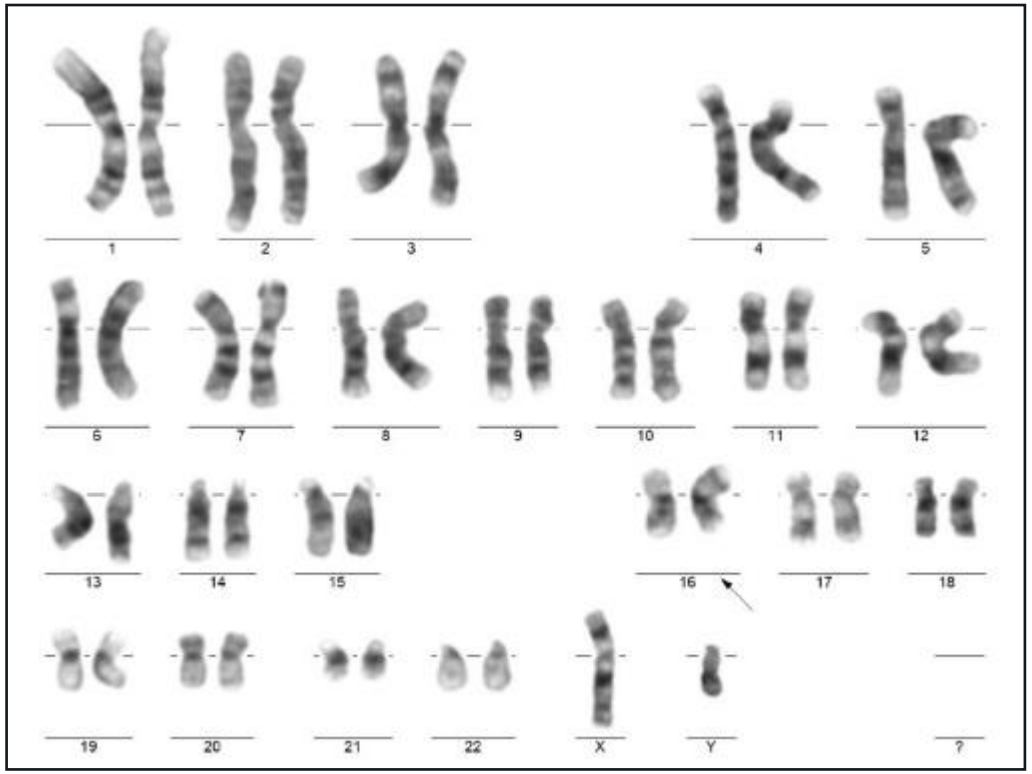
  or

- presence of 1 autosomal monosomy in combination with at least 1 structural aberration

de Oliveira Lisboaet al. "Chromosomal instability in acute myeloid leukemia." Cancers 13.11 (2021): 2655.

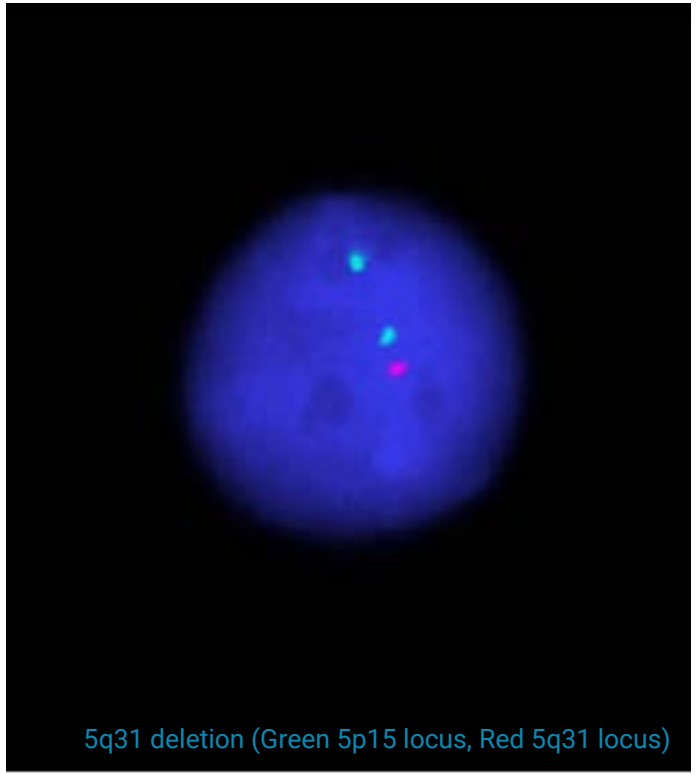
Technical aspects

Conventional cytogenetics



Courtesy prof. G.Balatzenko

Fluorescence in situ hybridization



Within 5-7 days[1]

1) Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377

Genetic abnormalities define entities

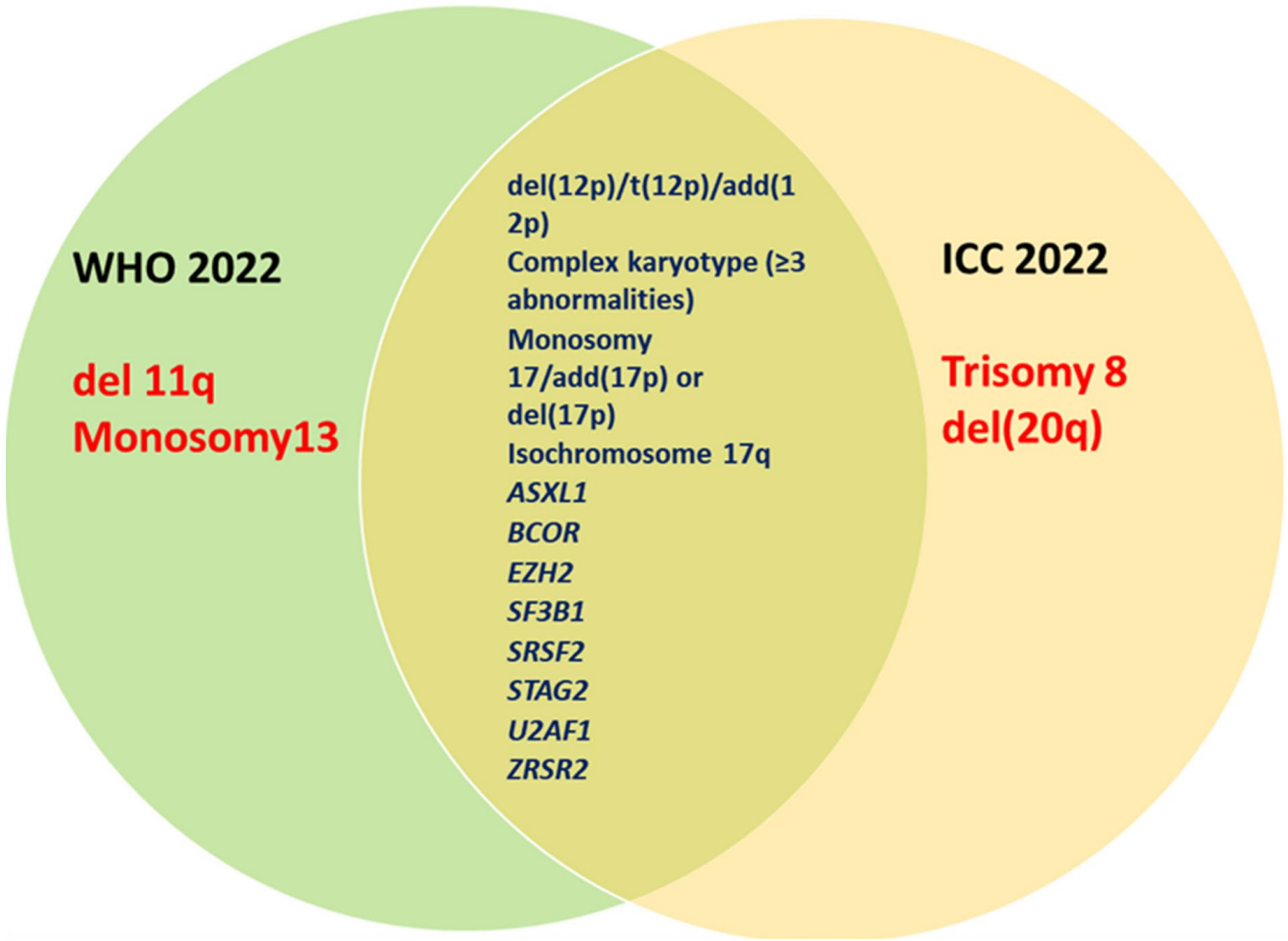
WHO – HAEM5	ICC
Acute myeloid leukaemia with defining genetic abnormalities	
Acute promyelocytic leukaemia with **PML::RARA** fusion	Acute promyelocytic leukemia with **t(15;17)(q24.1;q21.2)/PML::RARA**
	APL with other RARA rearrangements*
Acute myeloid leukaemia with **RUNX1::RUNX1T1** fusion	AML with **t(8;21)(q22;q22.1)/RUNX1::RUNX1T1**
Acute myeloid leukaemia with **CBFB::MYH11** fusion	AML with **inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11**
Acute myeloid leukaemia with **KMT2A** rearrangement	AML with **t(9;11)(p21.3;q23.3)/MLLT3::KMT2A**
	AML with other KMT2A rearrangements
Acute myeloid leukaemia with **DEK::NUP214** fusion	AML with **t(6;9)(p22.3;q34.1)/DEK::NUP214**
Acute myeloid leukaemia with **MECOM** rearrangement	AML with **inv(3)or t(3;3)(q21.3;q26.2)/GATA2; MECOM**
	AML with other MECOM rearrangements
Acute myeloid leukaemia with **BCR::ABL1** fusion	AML with **t(9;22)(q34.1;q11.2)/BCR::ABL1**
Acute myeloid leukaemia with **NPM1 mutation**	AML with mutated **NPM1**
Acute myeloid leukaemia with **CEBPA mutation**	AML with in-frame **bZIP CEBPA** mutations ≥ 10%
Acute myeloid leukaemia, myelodysplasia-related	AML with **myelodysplasia-related cytogenetic abnormalities**
	MDS/AML (10-19%) / AML (≥ 20%)
	AML (≥ 20%) and MDS/AML (10-19%) with **myelodysplasia-related gene mutations**
	AML (≥ 20%) and MDS/AML (10-19%) with **mutated TP53**
Acute myeloid leukaemia with **RBM15::MRTFA** fusion	
Acute myeloid leukaemia with NUP98 rearrangement	
AML with other defined genetic alterations	AML with other rare recurring translocations

Minimal panel

PML::RARA, CBFB::MYH11, RUNX1::RUNX1T1, KMT2A rearrangements, BCR::ABL1

Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377
Arber et al. ICC of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022; 140(11)
WHO Classification of Tumours Editorial Board. Haematolymphoid tumours. Lyon (France): IARC; 2024.

AML, myelodysplasia-related



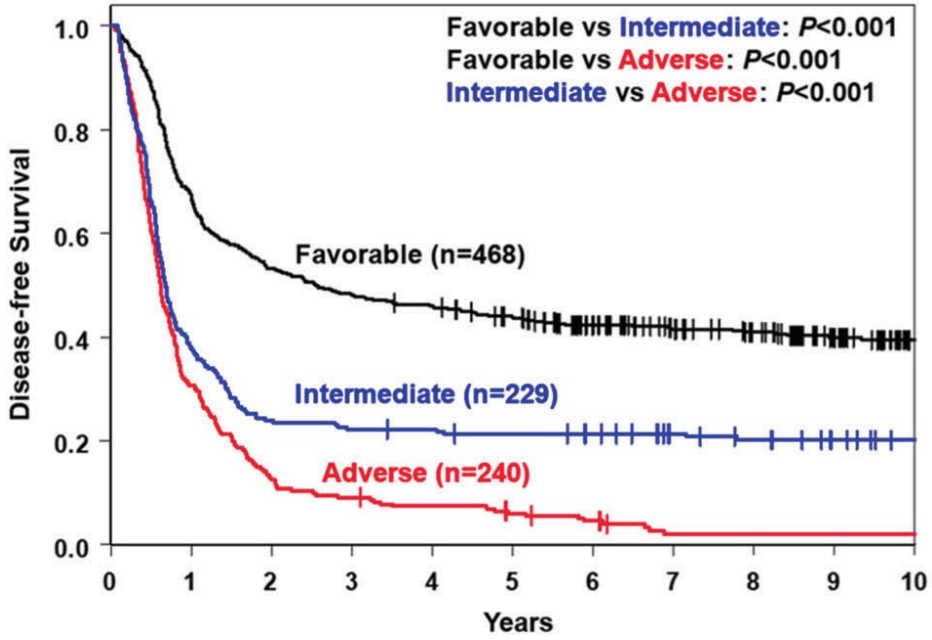
Salman H. Comparative Analysis of AML Classification Systems: Evaluating the WHO, ICC, and ELN Frameworks and Their Distinctions. Cancers (Basel). 2024 Aug 22;16(16):2915.

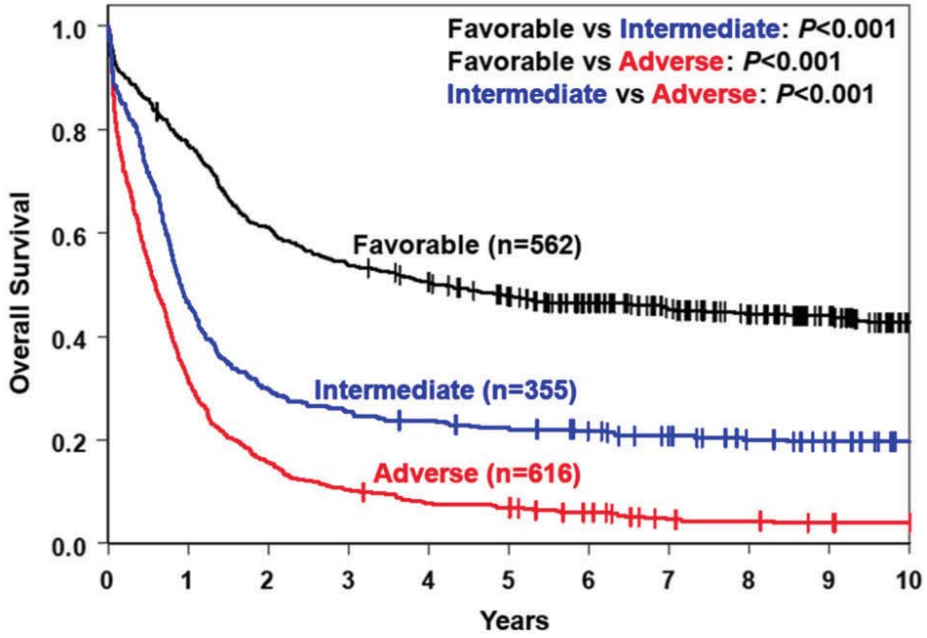
Risk classification by genetics ELN2022

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 CEBPAk
Intermediate	• Mutated NPM1†,§ with FLT3-ITD • Wild-type NPM1 with FLT3-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/MLLT3::KMT2A • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/DEK::NUP214 • t(v;11q23.3)/KMT2A-rearranged • t(9;22)(q34.1;q11.2)/BCR::ABL1 • t(8;16)(p11.2;p13.3)/KAT6A::CREBBP • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1) • t(3q26.2;v)/MECOM(EVI1)-rearranged • 25 or del(5q); 27; 217/abn(17p) • Complex karyotype, monosomal karyotype • Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2 • Mutated TP53a

2):1345-1377

Outcome prediction by ELN^{2022} genetic-risk classification

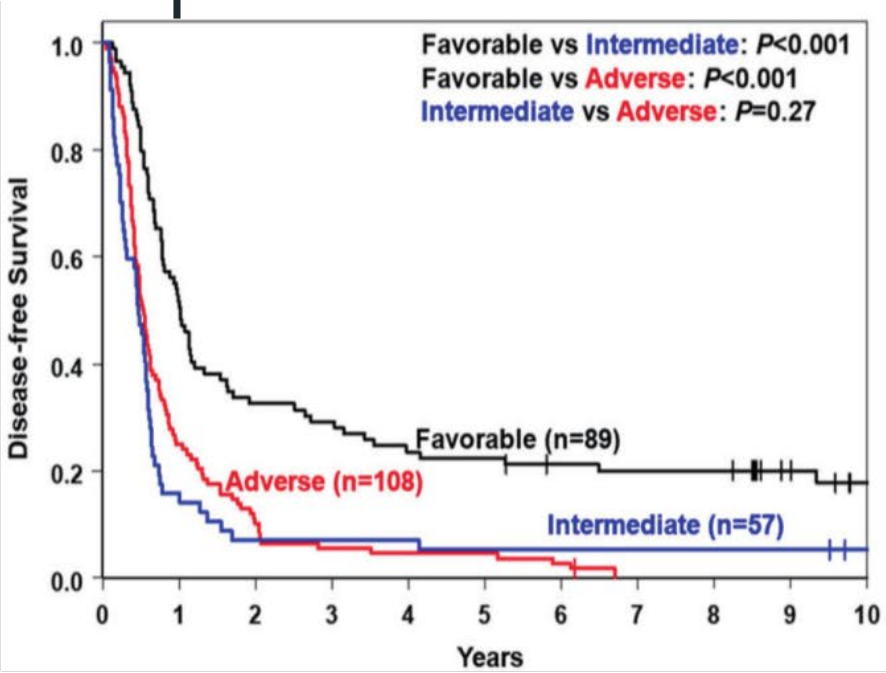


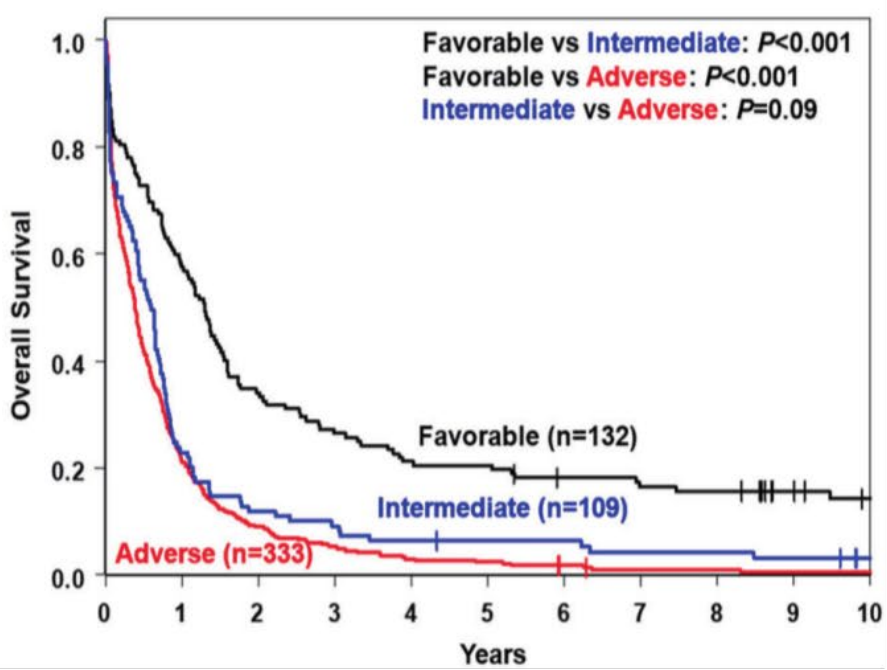


n=1637 adults with AML, treated with cytarabine/anthracycline regimens

Mrózek K, et al. Outcome prediction by the 2022 European LeukemiaNet genetic-risk classification for adults with acute myeloid leukemia: an Alliance study. Leukemia. 2023 Apr;37(4):788-798.

Suboptimal for elderly and for adults receiving less-intensive therapies





de novo AML patients,
aged ≥60 years

Mrózek K, et al. Outcome prediction by the 2022 European LeukemiaNet genetic-risk classification for adults with acute myeloid leukemia: an Alliance study. Leukemia. 2023 Apr;37(4):788-798.

Genetic risk classification ELN^{2024} for adults receiving less-intensive therapies

Risk category	Genetic abnormality
Favorable	• Mutated *NPM1* (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt}) • Mutated *IDH2* (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt}) • Mutated *IDH1*[b] (*TP53*^{wt}) • Mutated *DDX41*[c] • Other cytogenetic and/or molecular abnormalities[d] (*FLT3*-ITD^{neg}, *NRAS*^{wt}, *KRAS*^{wt}, *TP53*^{wt})
Intermediate	• Other cytogenetic and molecular abnormalities[d] (*FLT3*-ITD^{pos} and/or *NRAS*^{mut} and/or *KRAS*^{mut}; *TP53*^{wt})
Adverse	• Mutated *TP53*

Döhner H, et al. Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations. Blood. 2024 Aug 12:blood.2024025409.

05

Molecular testing

should screen for all the genetic abnormalities that:

- define disease
- define risk categories
- that are needed for targeted treatment modalities

Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377

Molecular testing at AML diagnosis

AML-associated gene fusions

- t(15;17)(q24.1;q21.2)/*PML::RARA*
- t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
- inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
- t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*
- t(6;9)(p22.3;q34.1)/*DEK::NUP214*
- inv(3)or t(3;3)(q21.3;q26.2)/*GATA2; MECOM(EVI1)*
- t(9;22)(q34.1;q11.2)/*BCR::ABL1*

Detectable by RNA based PCR

AML-associated gene mutations

- *FLT3-ITD/ FLT3-TKD, IDH1, IDH2*
- *NPM1*
- *CEBPA, DDX41, TP53; ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*

- Additional genes recommended to test at Dg: *ANKRD26, BCORL1, BRAF, CBL, CSF3R, DNMT3A, ETV6, GATA2, JAK2, KIT, KRAS, NRAS, NF1, PHF6, PPM1D, PTPN11, RAD21, SETBP1, TET2, WT1*

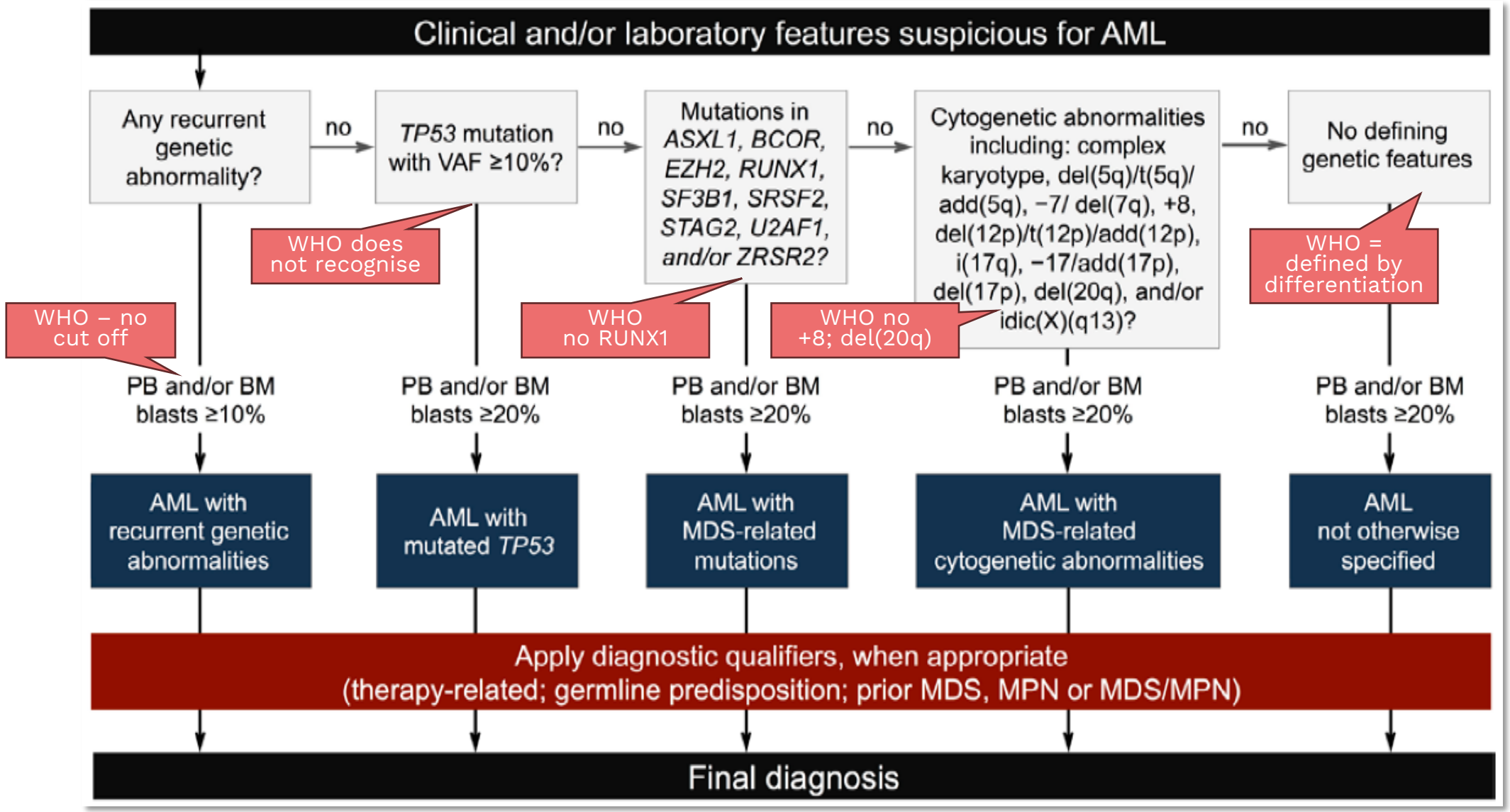
Detectable by DNA based PCR or NGS

Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377

Molecular markers guiding AML therapy

	Target	Frequency	Selected agent
AML with defining genetic abnormalities	*PML::RARA*	1-25%	All-Trans Retinoic Acid(ATRA), Arsenic Trioxide (ATO)
	RUNX1::RUNX1T1	1-5%	Anti-CD33 ADC (Gemtuzumab ozogamicin)
	CBFB::MYH11	1-5%	Anti-CD33 ADC (Gemtuzumab ozogamicin)
	KMT2A rearranged	1-3%	Menin inhibitors (not approved)
	BCR::ABL1	1%	Imatinib
	NPM1 mutation	15-30%	Anti-CD33 ADC (Gemtuzumab ozogamicin)
	Myelodysplasia relat	10-50%	CPX-351, Venetoclax
Signaling molecules	*FLT3 mutation*	15-30%	Midostaurin, Gilteritinib, Quizartinib
Epigenetic modifiers	*IDH1 mutation*	6-8%	Ivosidenib, Olutasidenib
	IDH2 mutation	10-15%	Enasidenib
Tumor suppressor	*TP53*	5-25%	Nothing approved so far!

Modified after: Döhner H., Progress in myeloid neoplasms: prognostic and therapeutic consequences of hierarchical molecular genetic classification of acute myeloid leukemia. 22nd Meeting of the EA4HP, Dubrovnik, 21 to 26 Sept 2024.



Pizzi et al. What's New in the Classification, Diagnosis and Therapy of Myeloid Leukemias. Hemato 4.2 (2023): 112-134.; Döhner et al, Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022; 140(12):1345-1377; WHO Classification of Tumours Editorial Board. Haematolymphoid tumours. Lyon (France): IARC; 2024.

In conclusion

- AML is a complex and diverse disease characterized by a wide range of clinical manifestations, morphological and immunophenotypic characteristics, as well as chromosomal and genomic abnormalities.
- The assessment of morphology and immunophenotyping can provide valuable insights into the potential cytogenetic aberrancies present in certain AML cases. Utilizing both modalities together allows for a highly accurate diagnosis and can highlight the necessity for prompt, targeted treatment even before cytogenetic or molecular confirmation is available (e.g., in cases of acute promyelocytic leukemia, APL).

In conclusion

- Genetic evaluation forms the foundation of contemporary classifications and risk stratification in AML. Karyotyping, FISH for cases where karyotyping is not feasible, and molecular testing should be conducted for every AML patient, regardless of their age.
- The rapid growth in our understanding of AML genomics has driven a paradigm shift in classification systems, moving from morphology-based to integrated genomic diagnosis. This has enabled better risk stratification and paved the way for personalized therapy in AML.
- The importance of multidisciplinary diagnostics in a holistic approach to treating patients with AML is crucial for achieving enduring therapeutic success.

National Specialised Hematology Hospital

Lab Haematopathology and Immunology

Rositza Vladimirova
Milan Jagurinoski
Johan Dimitrov
Georgi Vasilev
Maria Muhtarova
Valentina Madjarova
Tihomir Dikov
Lidia Gartcheva

Lab Medical Genetics

Gueorgui Balatzenko
Svetlana Angelova
Inna Ivanova
Siana Ivanova

Hematology Clinic

Martin Donchev
Emil Alexov
Penka Ganeva
Janitza Davidkova

Reading list:

1. Arber DA, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022 Sep 15;140(11):1200-1228.
2. Bain BJ, Béné MC. Morphological and Immunophenotypic Clues to the WHO Categories of Acute Myeloid Leukaemia. Acta Haematol. 2019;141(4):232-244.
3. Bullinger L, Döhner K, Döhner H. Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. J Clin Oncol. 2017 Mar 20;35(9):934-946.
4. Döhner H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
5. Falini B, Martelli MP. Comparison of the International Consensus and 5th WHO edition classifications of adult myelodysplastic syndromes and acute myeloid leukemia. Am J Hematol. 2023 Mar;98(3):481-492.
6. Kayser S, Levis MJ. The clinical impact of the molecular landscape of acute myeloid leukemia. Haematologica. 2023 Feb 1;108(2):308-320.
7. Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719.
8. Lucas F, Hergott CB. Advances in Acute Myeloid Leukemia Classification, Prognostication and Monitoring by Flow Cytometry. Clin Lab Med. 2023 Sep;43(3):377-398.
9. Narayanan D, Weinberg OK. How I investigate acute myeloid leukemia. Int J Lab Hematol. 2020 Feb;42(1):3-15.
10. Saft L. The role of flow cytometry in the classification of myeloid disorders. Pathologie (Heidelb). 2023 Dec;44(Suppl 3):164-175.
11. WHO Classification of Tumours Editorial Board. Haematolymphoid tumours. Lyon (France): International Agency for Research on Cancer; 2024. (WHO classification of tumours series, 5th ed.; vol. 11). https://publications.iarc.who.int/637.