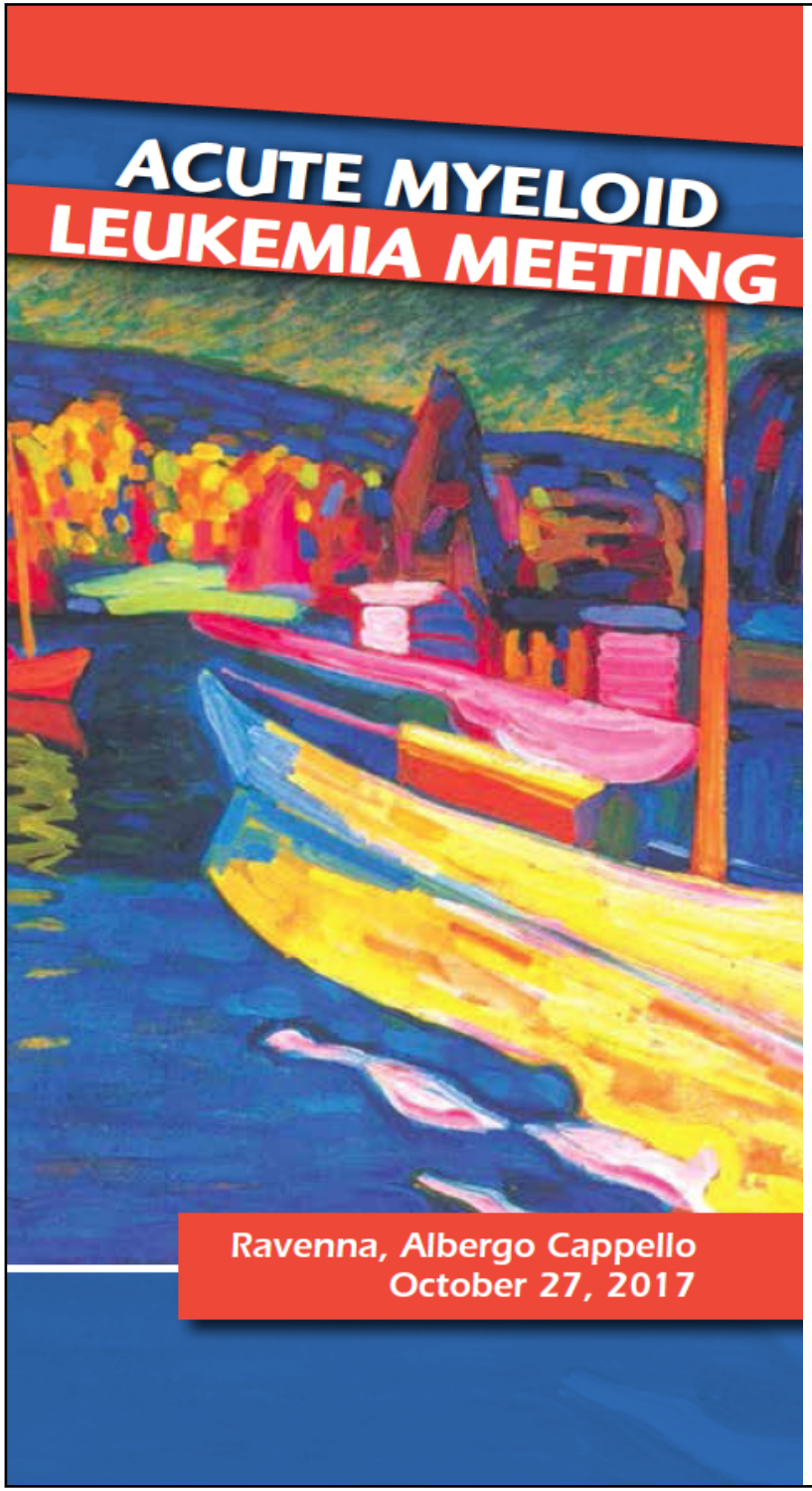


Updates of the WHO classification 2017: impact on the diagnosis of acute myeloid leukemia

Dr. Claudio Agostinelli
Haematopathology Unit, Sant'Orsola University Hospital,
Bologna

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

Blastic plasmacytoid dendritic cell neoplasm

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

WHO classification AML and related neoplasms (4th edition, 2017)

AML with recurrent cytogenetic abnormalities

WHO classification AML and related neoplasms (3th edition, 2008)

- AML with recurrent genetic abnormalities

AML with t(8;21) (q22;q22) (*RUNX1-RUNX1T1)*
AML with inv(16)(p13.1q22) or t(16,16) (p13.1;q22) (*CBFB-MYH11)*
Acute promyelocytic leukemia with t(15;17)(q24.1;q21.1) (*PML-RARA)*
AML with t(9;11)(p22;q23) (*MLLT3-MLL)*
AML with t(6;9)(p23;q34) (*DEK-NUP214)*
AML with inv(3)(q21q26.2) or t(3;3) (q21;q26.2) (*RPN1-EVI1)*
AML (megakaryoblastic) with t(1;22) (p13;q13) (*RBM15-MKL1)*
Provisional entity: AML with mutated *NPM1*
Provisional entity: AML with mutated *CEBPA*

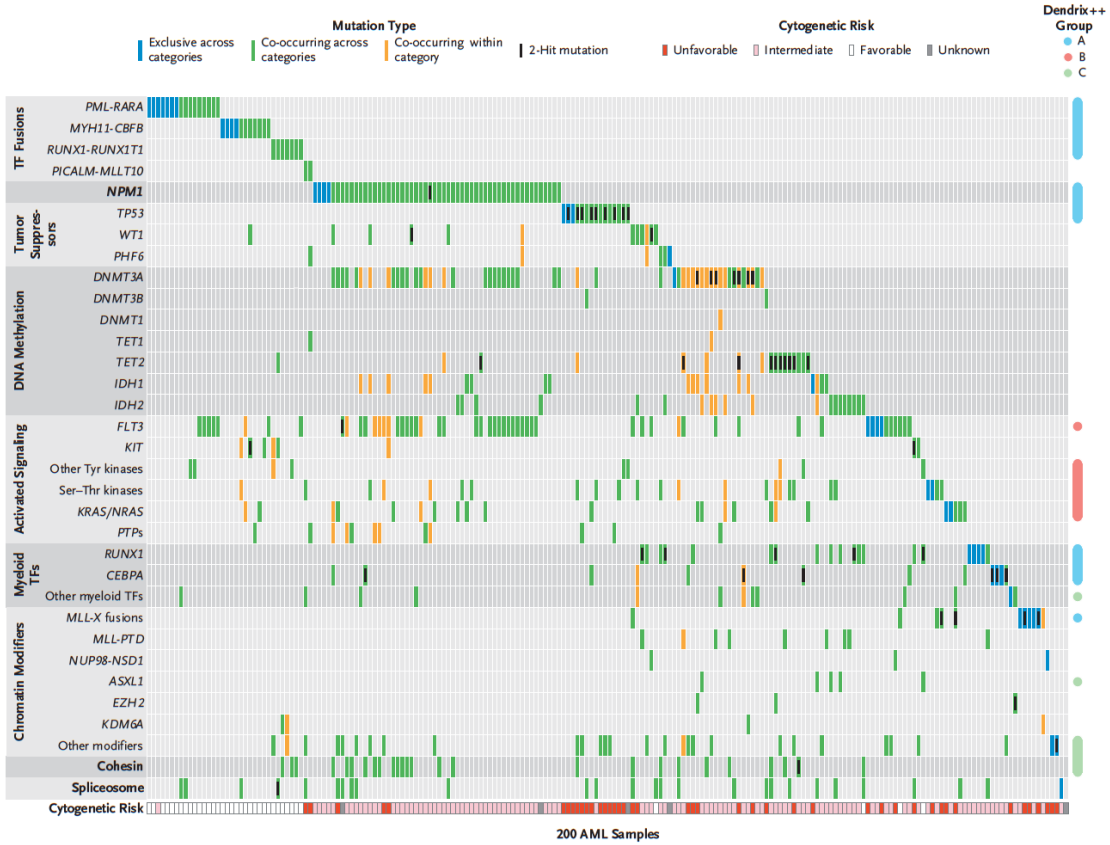
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, not otherwise specified

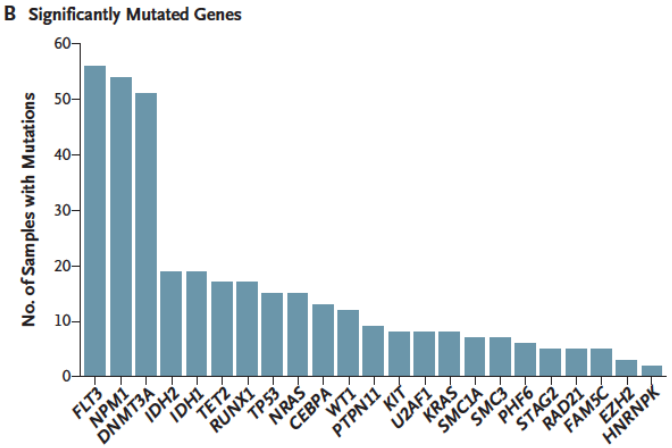
AML minimally differentiated
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic and monocytic leukemia
Acute erythroid leukemia
Acute megakaryocytic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis

- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network





- AML genomes have fewer mutations than others adult cancers (average of 13 mut./AML case)
- at least 23 recurrent mutations identified

NEJM 2013

From Swerdlow et al WHO 2017. At least 8 distinct categories of mutations in AML

	Before 2008: Cytogenetic and molecular genetic analysis	2008-12: NGS approaches	From2013: The Cancer Genome Atlas project
Functional groups	Class I Activated signalling e.g. FLT3, KIT, RAS mutations	Class I Activated signalling e.g. FLT3, KIT, RAS mutations	Class 1: transcription factor fusions e.g(t8;212, and t(15;17)
			Class 2: Nucleophosmin1 *NPM1* mutations
			Class 3: tumour suppressor genes e.g *TP53, PHF6* mutations
	Class II Transcrpition and differentiation e.g. e.g(t8;212, and t(15;17) *CEBPA* mutations	Class II Transcrpition and differentiation e.g. e.g(t8;212, and t(15;17) *CEBPA* mutations	Class 4:DNA methylation related genes *e.g. TET2, IDH2, IDH1, DNMT3A*
			Class 5:Activating signalling genes e.G *FLT3, KIT, RAS* mutations
			Class 6: Chromatin modifying genes e.g. *ASXL1, EZH2, KMT2A* fusions, *KMT2A-PTD*
		Epigenetic modifiers (so called Class III) *e.g. TET2, DNMT3A and AsXL1*	Class 7: Myeloid transcription factor genes e.g *CEBPA, RUNX* mutations
			Class 8: Cohesin complex genes e.g. *STAG2, RAD21, SMC1, SMC2* mutations
			Class 9: Spliceosome-complex genes e.g. *SRSF2, U2AF1, ZRSR2* mutations

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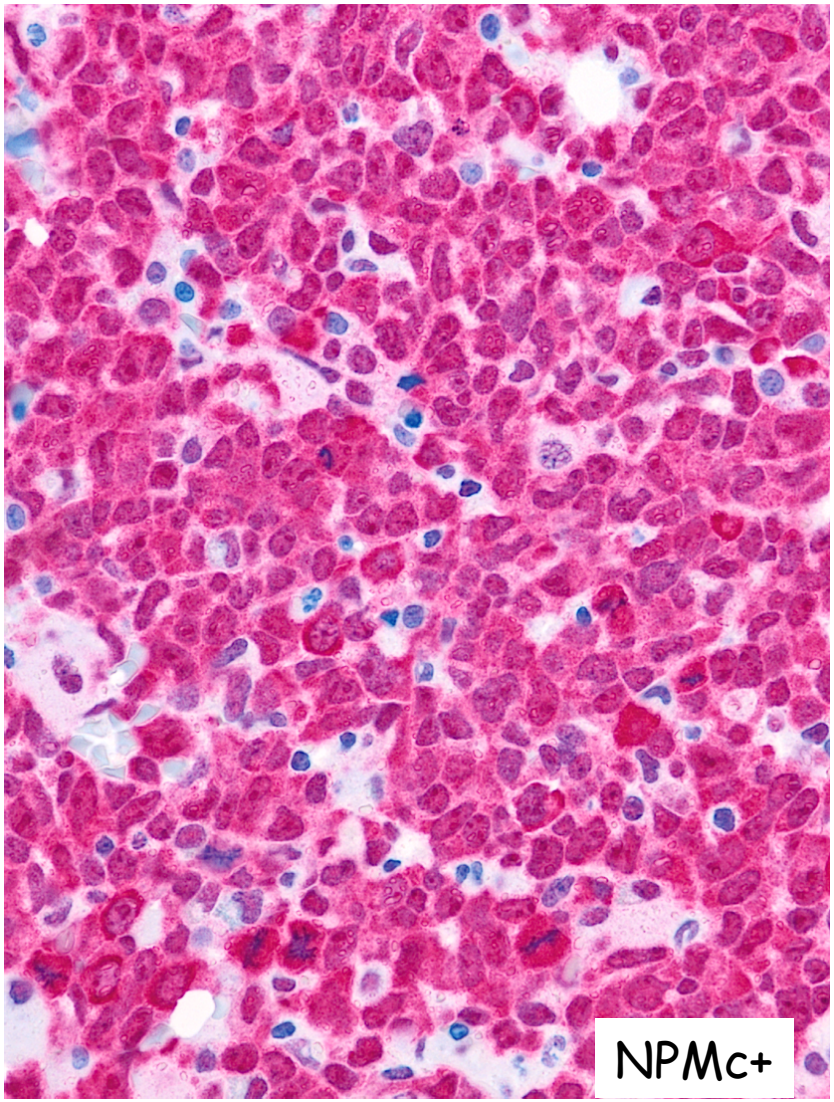
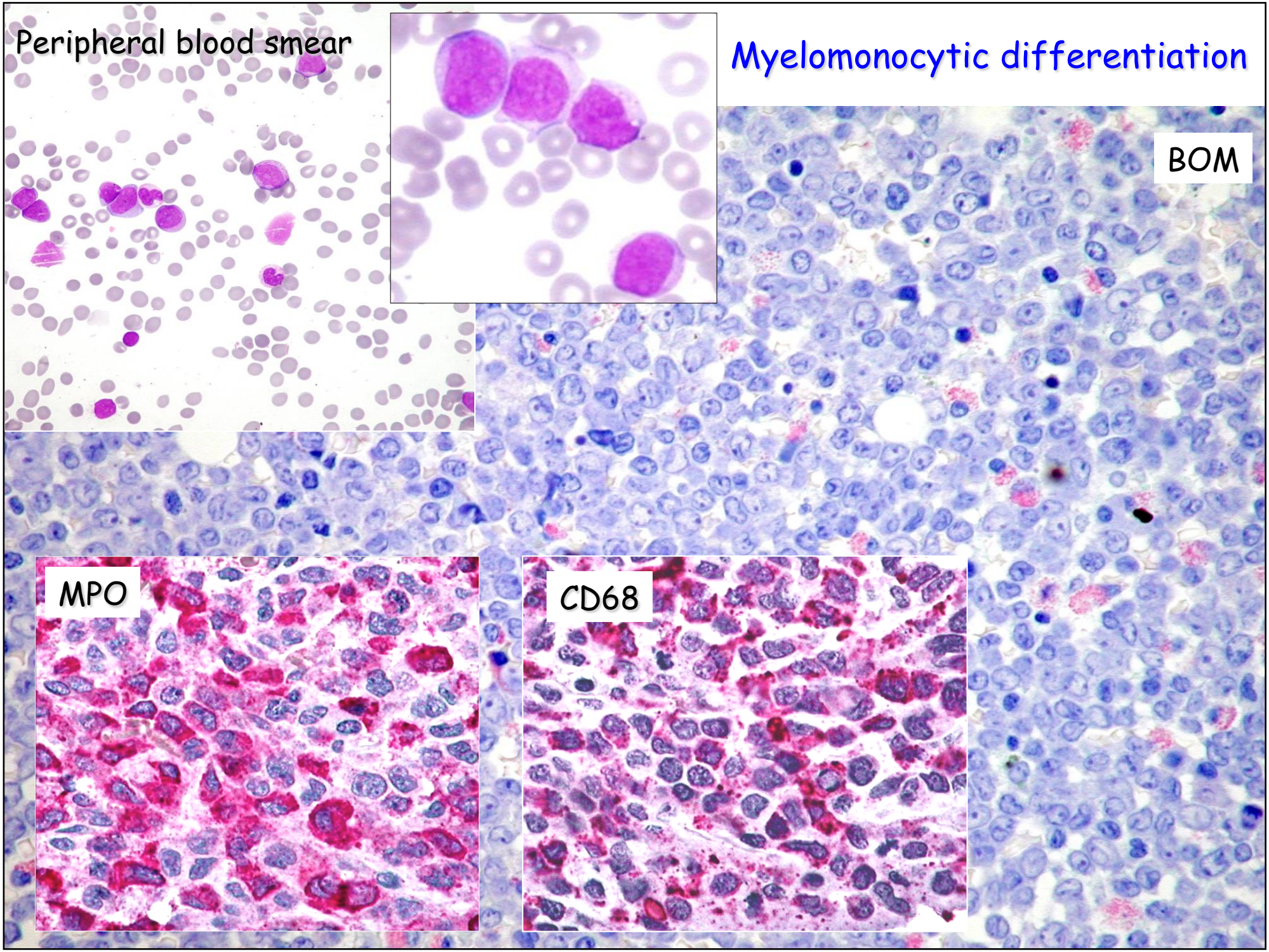
WHO classification AML and related neoplasms (4^{th} edition, 2017)

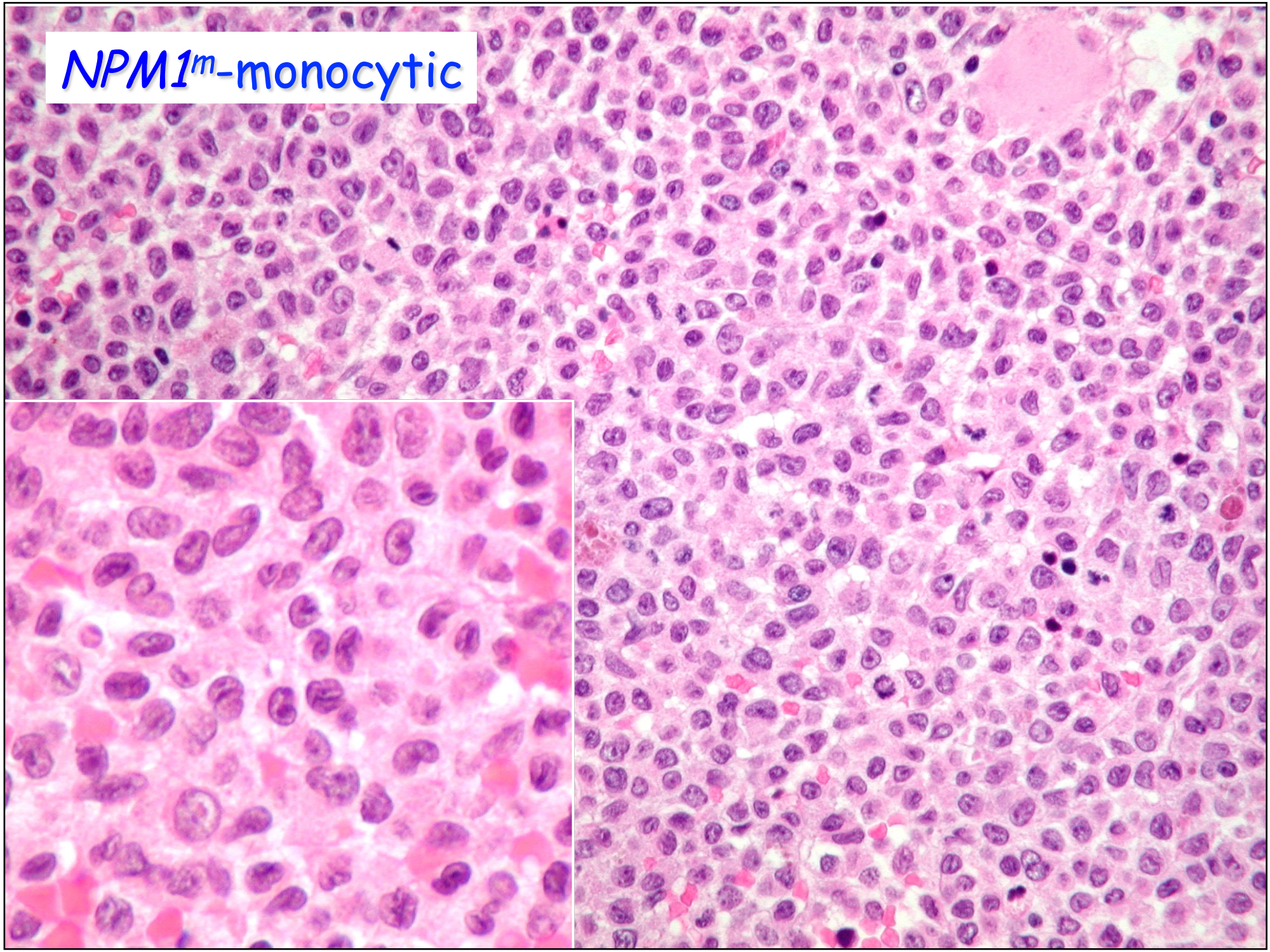
AML with mutated NPM1

- NPM1^{m} usually in exon 12
- One of most common recurrent genetic lesion in AML:
  27-35% of adult AML,
  2-8% of childhood AML
  45-64% of AML with normal karyotype
- Abnormal karyotype identified in 5-15% of NPM1 mutated AML cases: +8, +4, -Y, del(9q) and +21 most frequent
- Distinct GEP with up *HOX* genes
- Association with myelomonocytitc and monocytic leukaemia
  (80% of monocytic AML *NPM1*^{m})

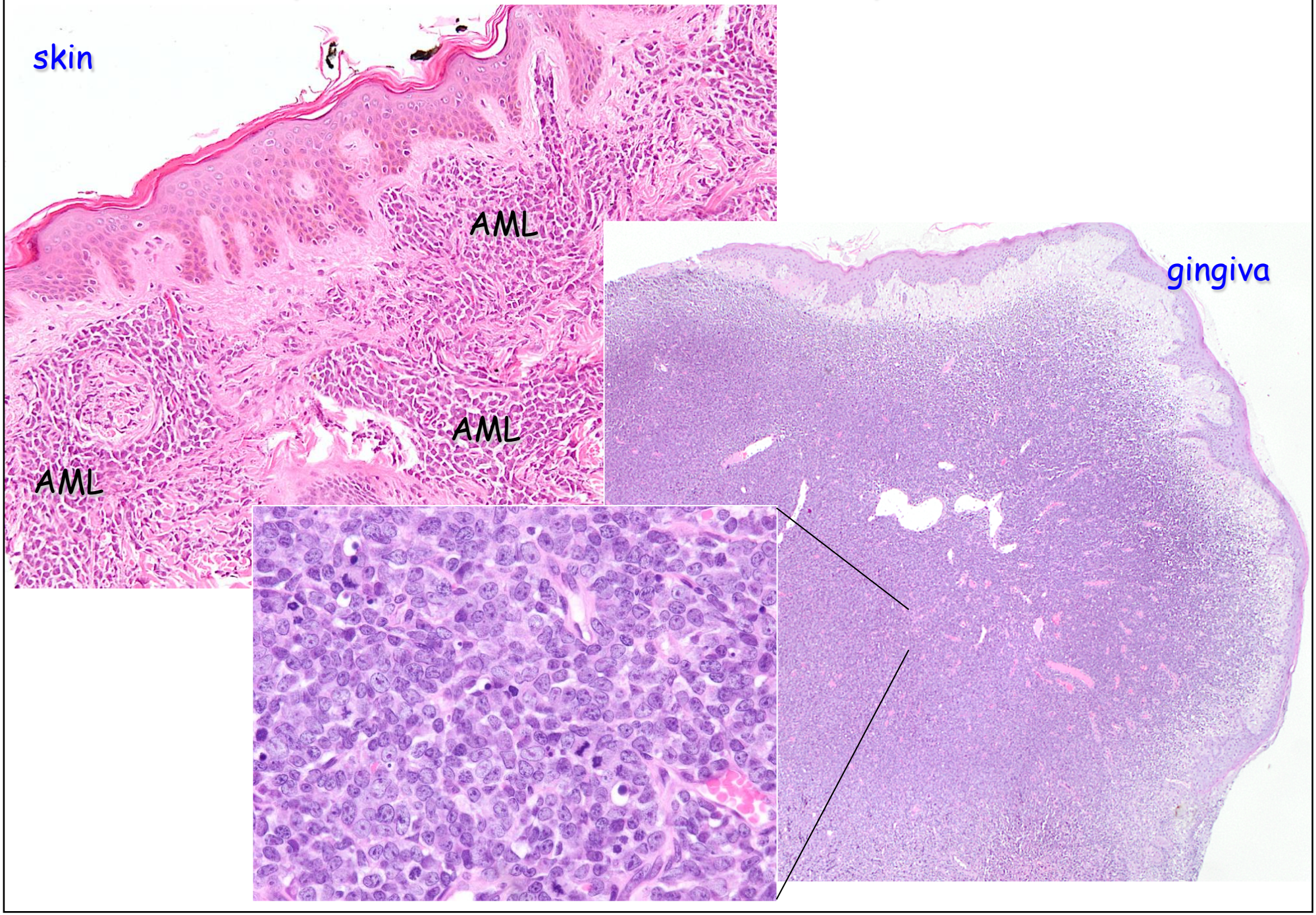


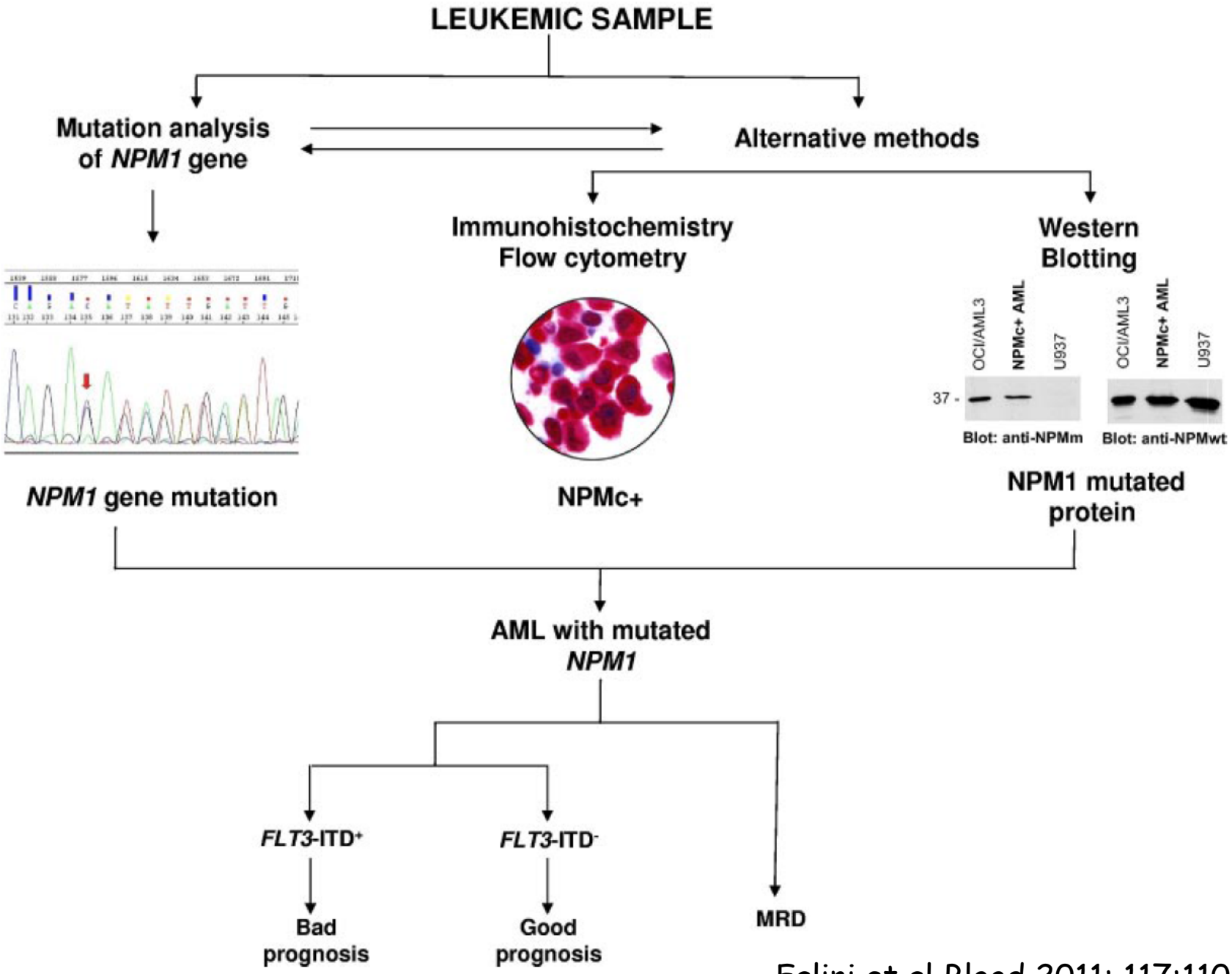
Myelomonocytic differentiation




NPM1m-monocytic

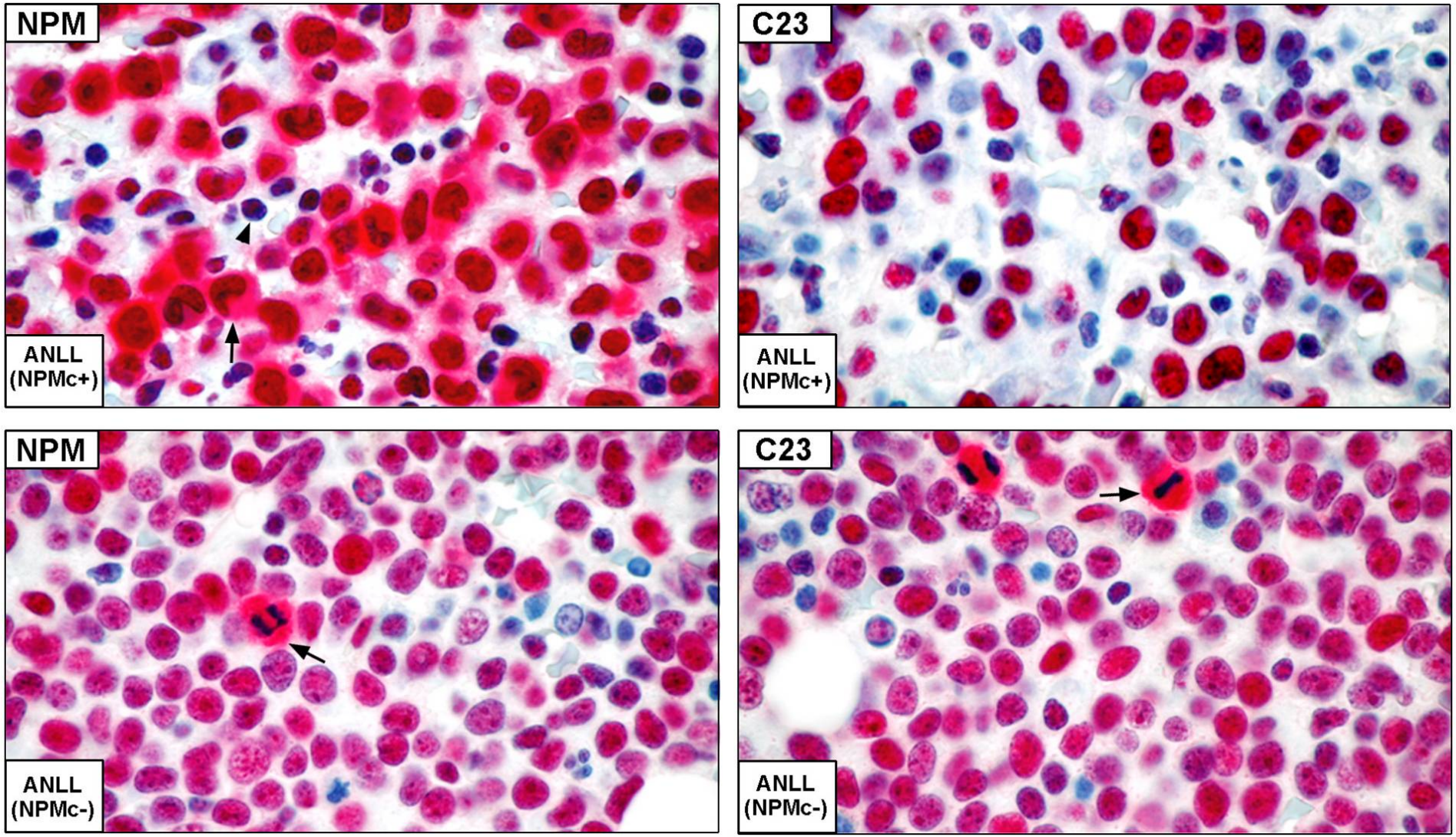
Many cases shows extramedullary involvement





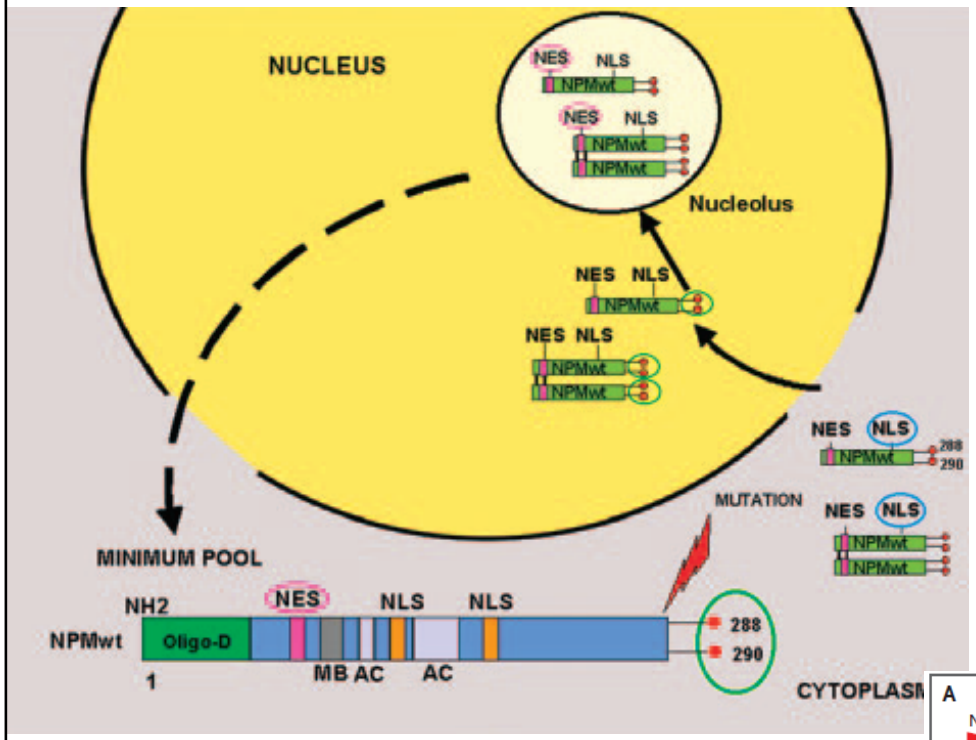
Falini et al Blood 2011; 117:1109-20

ABERRANT CYTOPLASMIC EXPRESSION OF NPM IN AML (NPMc+ AML)



Falini et al NEJM 2005 Jan 20;352(3):254-66.

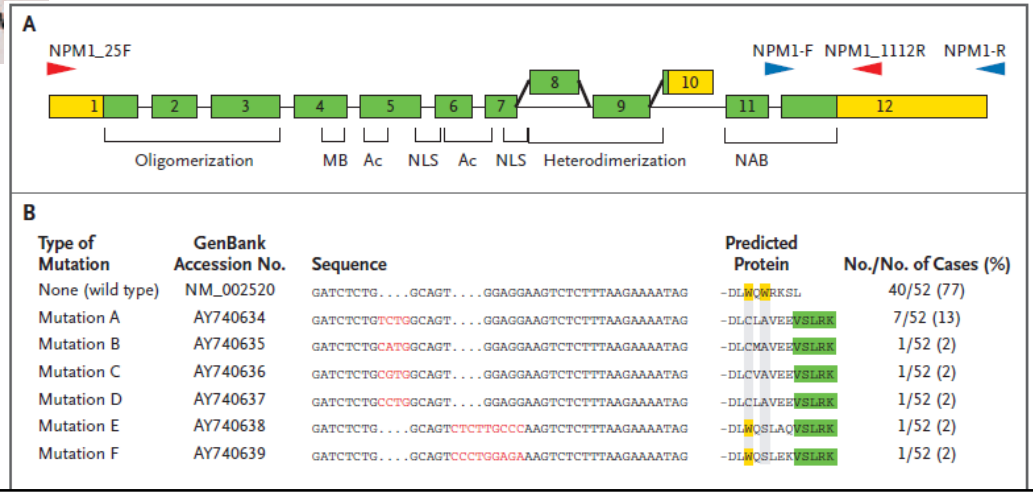
Mutations results in common changes in C-terminus of the protein that consists in the creation of a new nuclear export signal

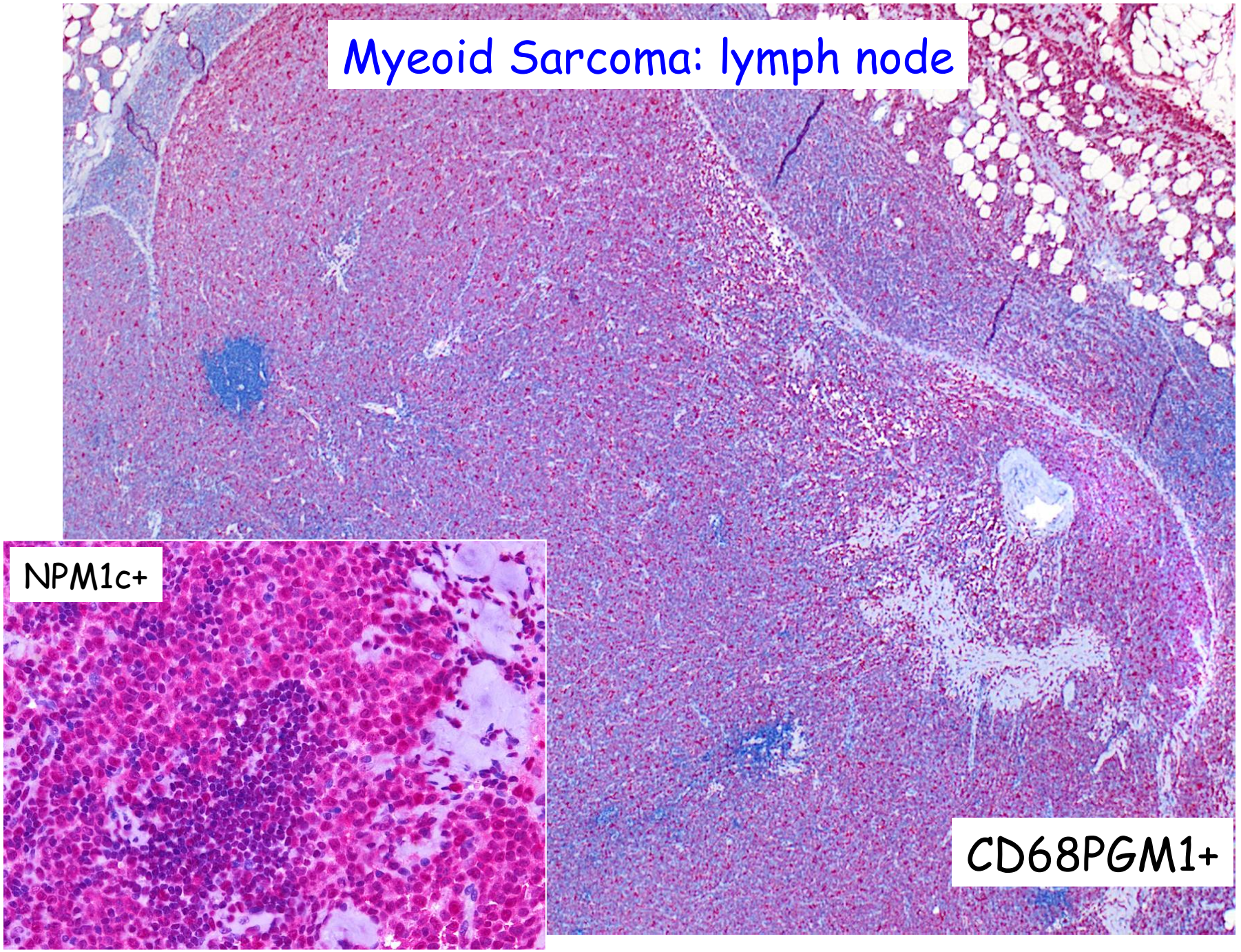


NPMc+ usefull tool:

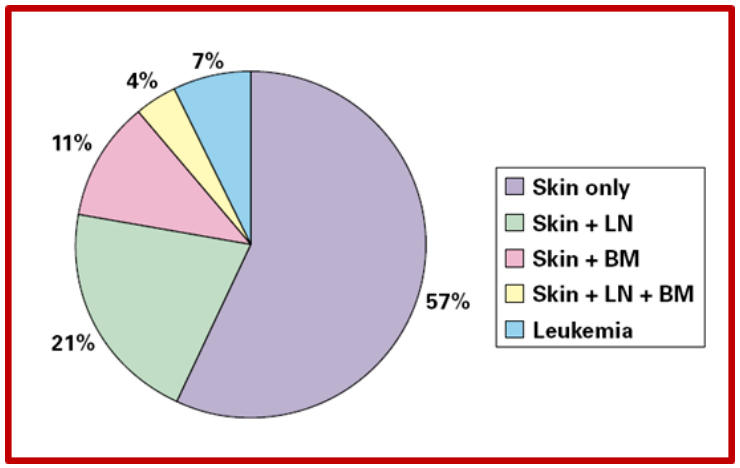
- *diagnosis in BOM*
- *differential diagnosis:*
  MDS, MDS/MPN (CMML)
- *detection of MRD in tissues*
- *NPM1^{m} in Myeloid sarcoma*

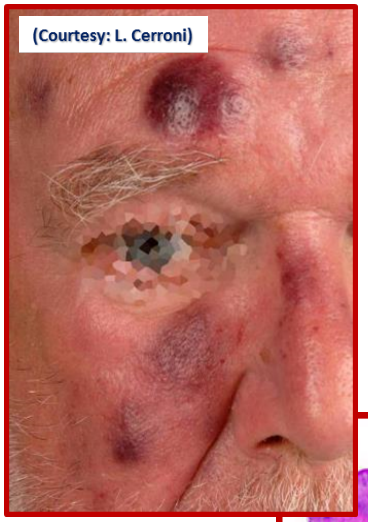
Falini et al Blood 2011
Falini et al NEJM 2005
Falini et al Blood 2006

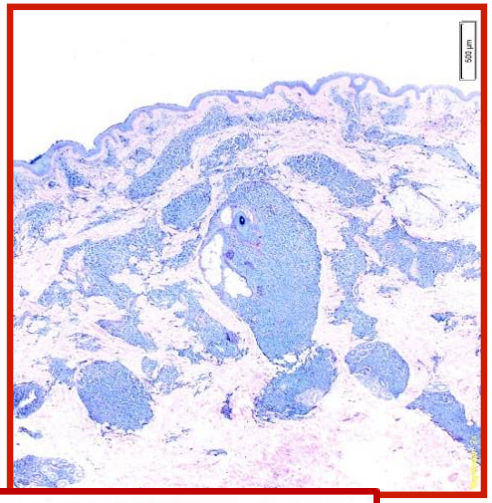


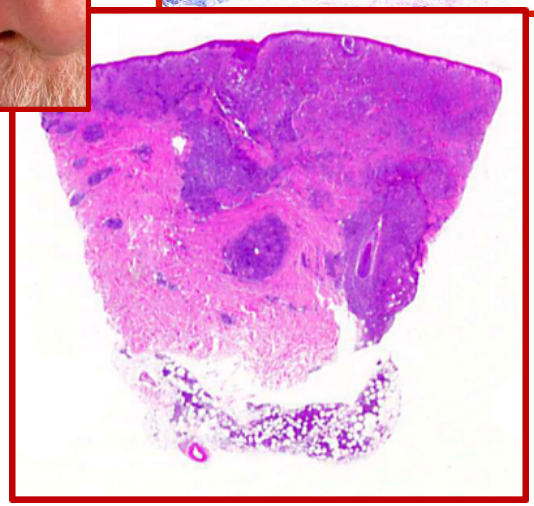

Myeoid Sarcoma: lymph node
NPM1c+
CD68PGM1+

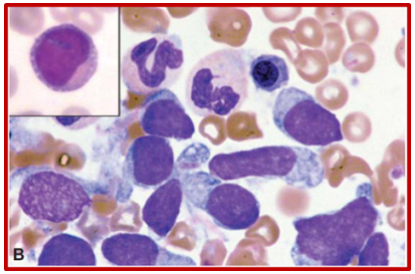
Blastic plasmacytoid dendritic cell neoplasm

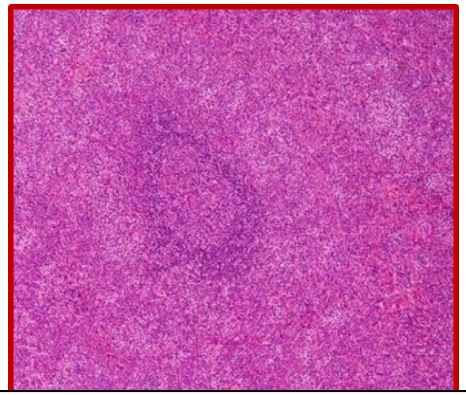


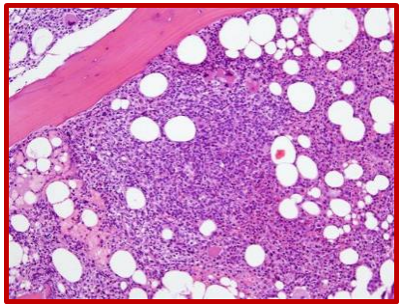








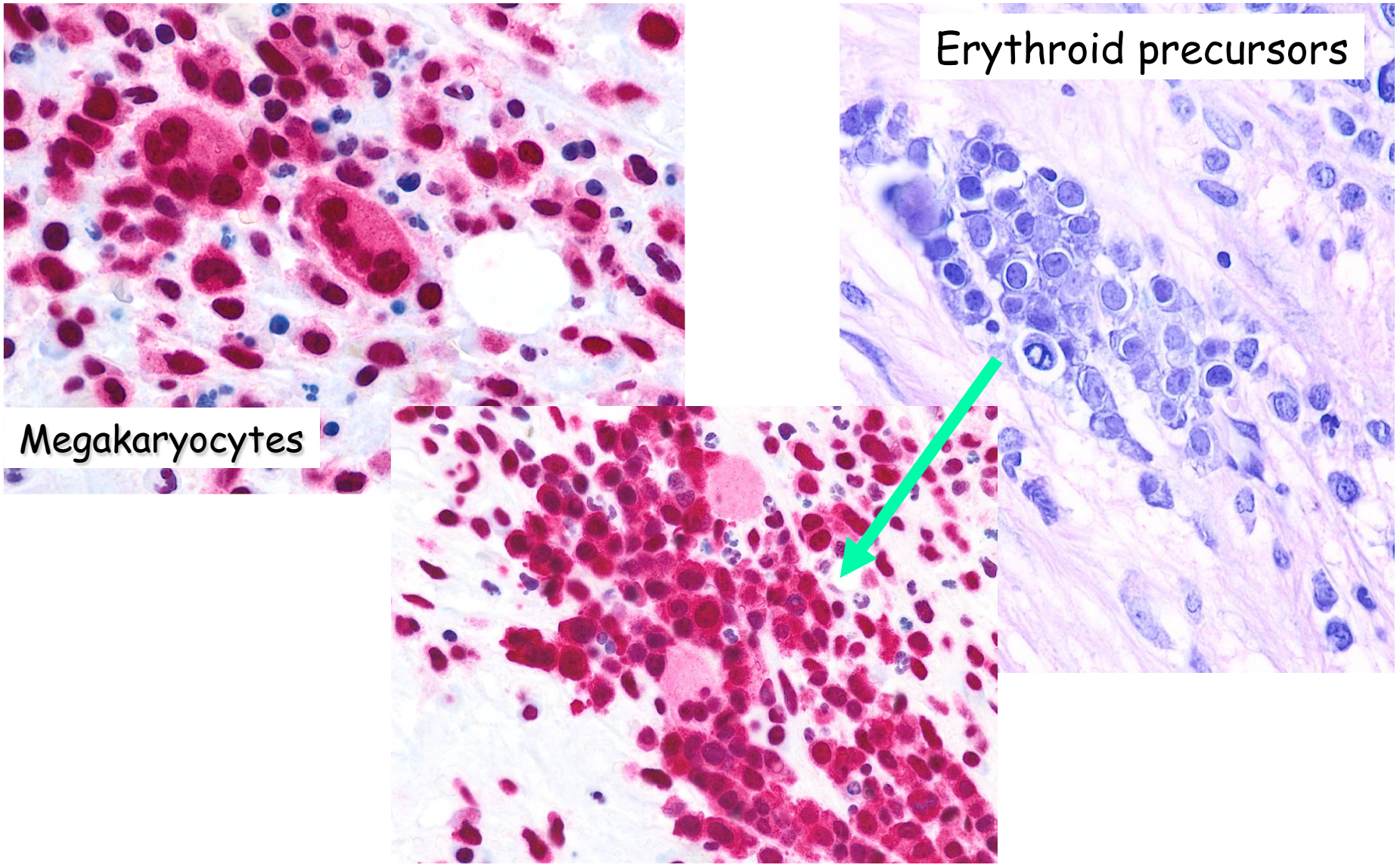




Cytoplasmic nucleophosmin is not detected in blastic plasmacytoid dendritic cell neoplasm

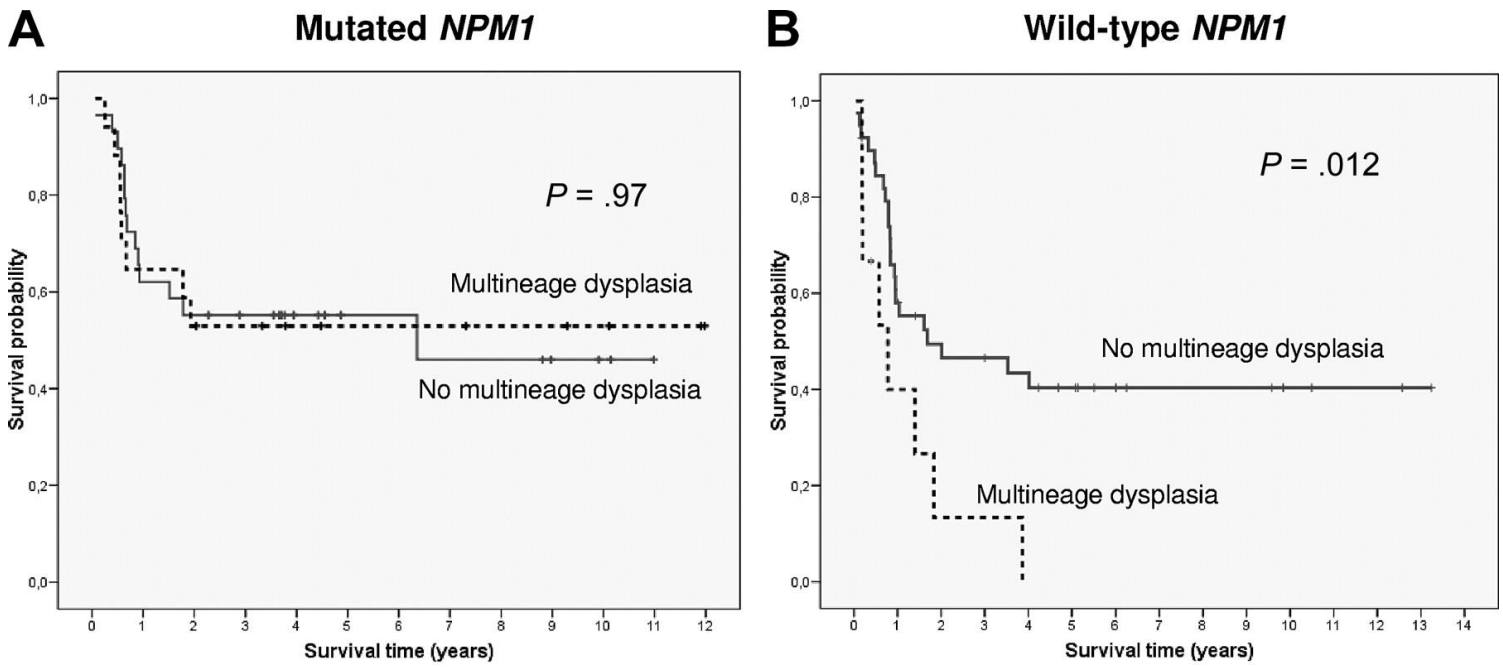
Fabio Facchetti,[1] Stefano A. Pileri,[2] Claudio Agostinelli,[2] Maria Paola Martelli,[3] Marco Paulli,[4] Adriano Venditti,[5] Massimo F Martelli,[3] and Brunangelo Falini[3]

NPM1c+ in two or more lineages



Multilineage dysplasia in the presence of *NPM1* mutation

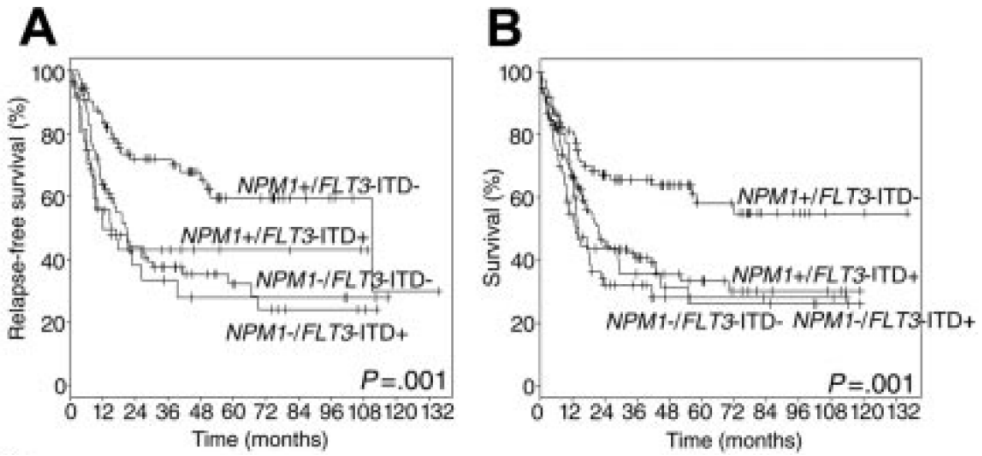
- 25% de novo AML *NPM1*^{m}
- Usually cases with normal karyotype
- blasts usually CD34-
- do not result in a worse prognosis



Díaz-Beyá M et al. Blood 2010;116:6147-6148

Mutant nucleophosmin *(NPM1)* predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations

Konstanze Döhner, Richard F. Schlenk, Marianne Habdank, Claudia Scholl, Frank G. Rücker, Andrea Corbacioglu, Lars Bullinger, Stefan Fröhling, and Hartmut Döhner, for the AML Study Group (AMLSG)



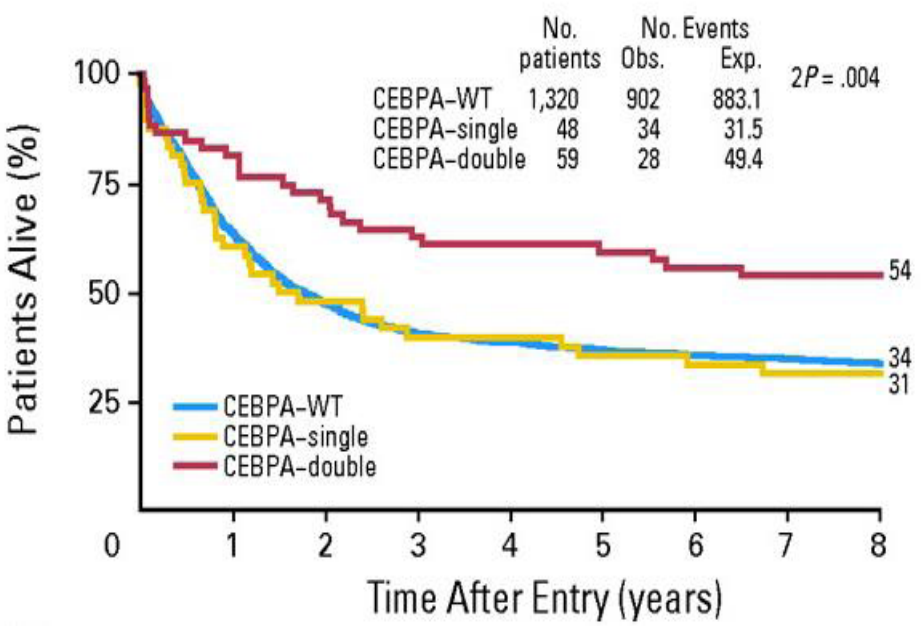
Coexistence of FLT3-ITD is associated to a poorer prognosis at least in yunger patients

Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden *FLT3*-ITD mutation and concomitant *NPM1* mutation: relevance to post-remission therapy

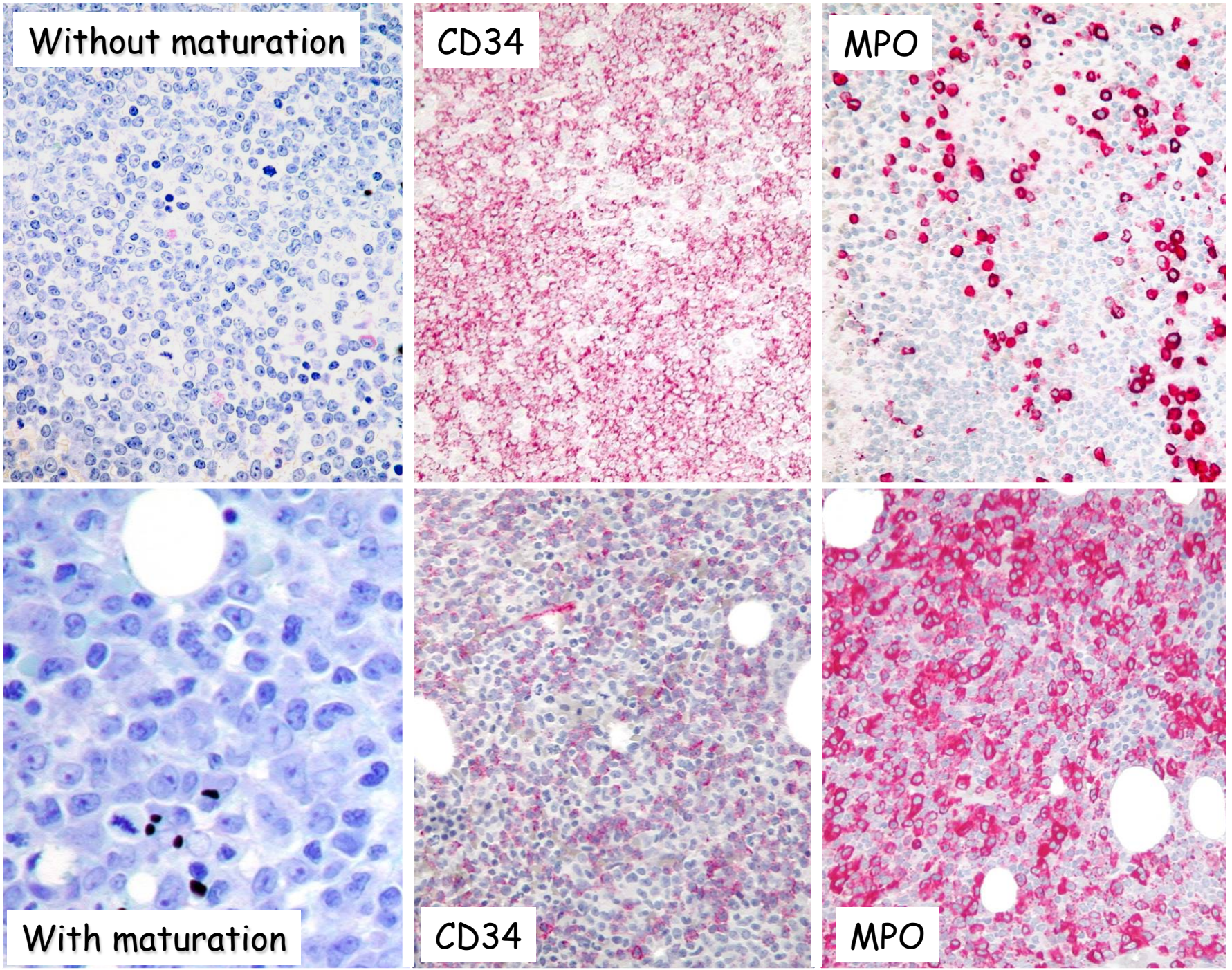
Marta Pratcorona,[1] Salut Brunet,[2] Josep Nomdedéu,[2] Josep Maria Ribera,[3] Mar Tormo,[4] Rafael Duarte,[5] Lourdes Escoda,[6] Ramon Guàrdia,[7] M. Paz Queipo de Llano,[8] Olga Salamero,[9] Joan Bargay,[10] Carmen Pedro,[11] Josep Maria Martí,[12] Montserrat Torrebadell,[1] Marina Díaz-Beyá,[1] Mireia Camós,[13] Dolors Colomer,[1] Montserrat Hoyos,[2] Jorge Sierra,[2] and Jordi Esteve,[1] on behalf of the Grupo Cooperativo Para el Estudio y Tratamiento de las Leucemias Agudas Mieloblásticas

AML with biallelic mutation of *CEBPA*

- Biallelic mutation confer good prognosis
- GEP $CBPA^{bm} \neq CBPA^{sm}$
- 4-9% of AML
- Usually de novo AML
- AML with or without maturation
- Subset abnormal karyotype: del(9q) do not influences prognosis
- 25% multylineage dysplasia no prognostic impact
- 70% normal karyotype



Green CL, et al. J Clin Oncol 28:2739, 2010


Without maturation
CD34
MPO
With maturation
CD34
MPO

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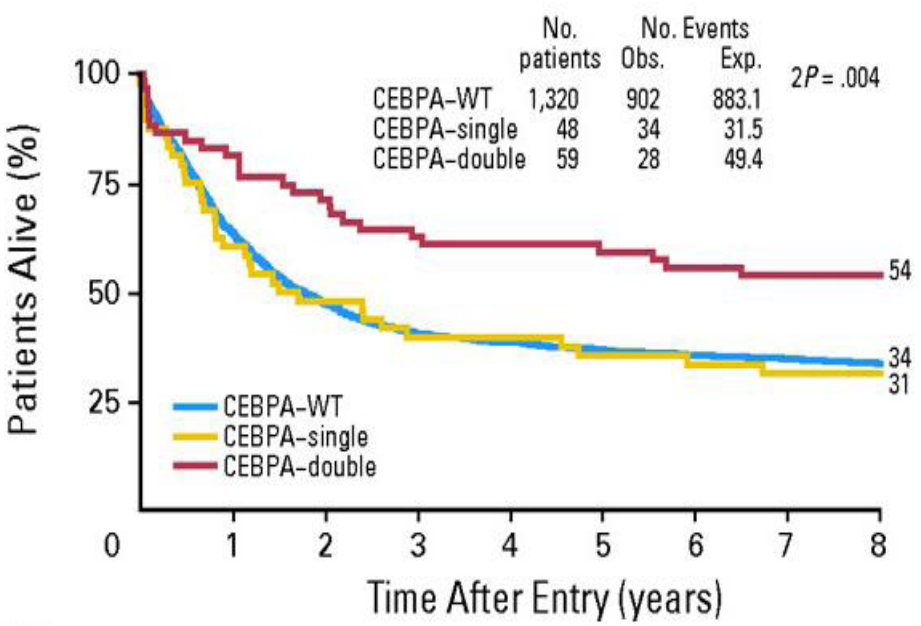

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AML with mutated *RUNX1* (provisional entity)

- Gene located at 21q22
- Encodes the alpha subunit of the core binding factor
- Mutation in 4-16% of AML
- More frequent in older male patients
- $RUNX1^{m}$ frequent in prior history of MDS, MDS/MPN; or prior exposure to RT or alkylating agents: excluded diagnosis
- cases associatted with $CEBPA^{bm}$ or *NPM1* mutations: AML with $CEBPA^{bm}$ or AML with NPM^{1m}
- variable morphology and phenotype
- Frequent associated *KMT2A-PTD*, or *ASXL1* mutations
- Poor response to therapy with shortened survival

AML with recurrent cytogenetic abnormalities (translocations)

- AML with recurrent cytogenetic abnormalities
- many balanced transl. and inv. recognized but not represent new disease category (uncommon, >paediatric pts)

- Refine APL with PML-RARA fusion

- AML with *BCR-ABL1 (provisional entity)*

Chromosomal Translocations with Higher Prevalence in Pediatric AML than in Adult AML (Swerdlow et Blood 2016)

Translocation	Gene Fusions	Frequency in Children /Frequency in Adults	Age Group Predilection	Comments/ Prognosis
t(1;22)(p13.3;q13.1)	*RBM15(OTT)-MKL1(MAL)*	0.8%/0%	Infants	AMKL – FAB M7/ Intermediate
t(7;12)(q36.3;p13.2)	*MNX1-ETV6*	0.8%/<0.5%	Infants	+19 seen as secondary abnormality Adverse
t(8;16)(p11.2;p13.3)	*KAT6A-CREBBP*	0.5%/<0.5%	Infants and children	Can spontaneously remit in infancy; intermediate prognosis in later childhood
t(6;9)(p23;q34.1)	*DEK-NUP214*	1.7%/1%	Older children; rare in infants	Adverse; 65% with *FLT3*-ITD
11q23.3	*KMT2A* (*MLL*) translocated	25%/5-10%	Infant 50%	Prognosis dependent on the partner gene
t(9;11)(p21.3;q23.3)	*KMT2A-MLLT3*	9.5%/2%	Children	Intermediate
t(10;11)(p12;q23.3)	*KMT2A-MLLT10*	3.5%/1%	Children	Include subtle and cryptic *KMT2A* rearrangements/ Adverse
t(6;11)(q27;q23.3)	*KMT2A-MLLT4*	2%/<0.5%	Children	Adverse
t(1;11)(q21;q23.3)	*KMT2A-MLLT11*	1%/<0.5%	Children	Favorable
Cryptic Chromosomal Translocations				
t(5;11)(q35.3;p15.5)	*NUP98-NSD1*	7%/3% 16% of *FLT3*-ITD patients	Older children and young adults	Adverse; 80% with *FLT3*-ITD. In combination associated with induction failure
inv(16)(p13.3q24.3)	*CBFA2T3-GLIS2*	3%/0%	10% of Infants, 20% of FAB M7	Adverse
t(11;12)(p15.5;p13.5)	*NUP98-KDM5A*	3%/0%	Children <5 years 10% of FAB M7	Intermediate

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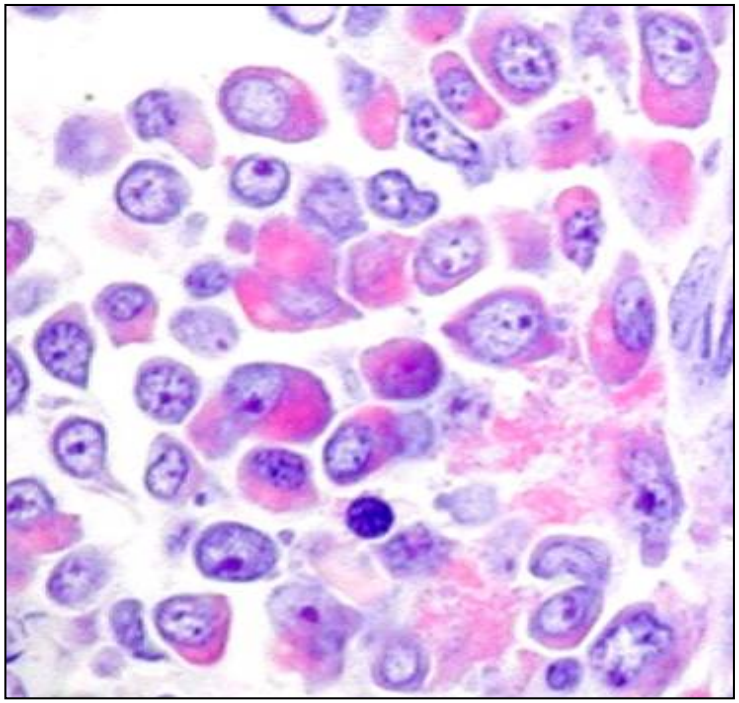
APL PML-RARA

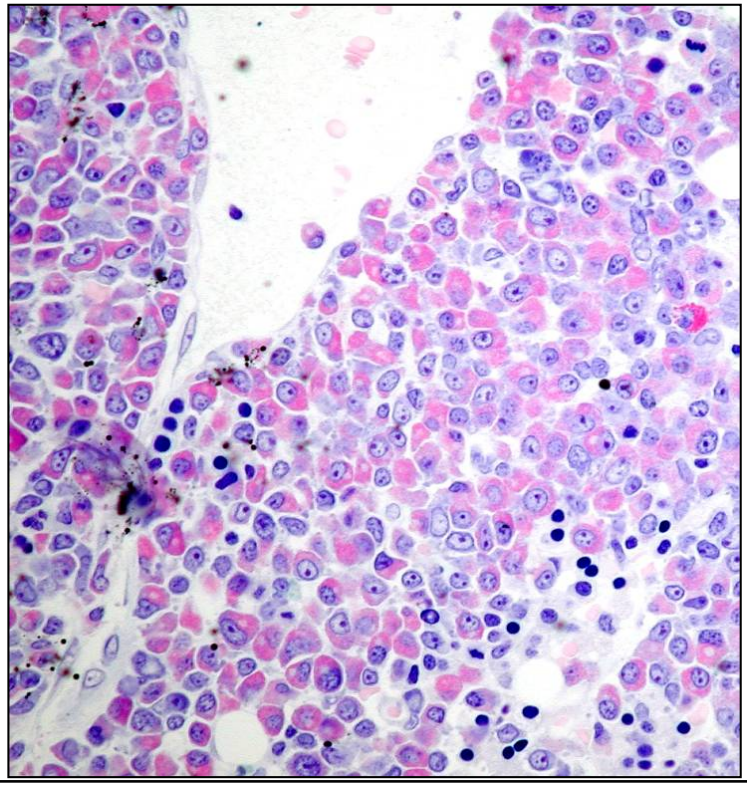
- Rare cases lacking

classic t(15;17)(q24.1;q21.2)
with *PML-RARA* transcripts
- complex cytogenetic rearrangements involving chr 15,17 and additional chr
- submicroscopic insertion of RARA in PML

included in APL *PML-RARA* as cryptic or masked t(15;17)(q24.1;q21.2)

No morphological differences →





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- Refine APL with PML-RARA fusion

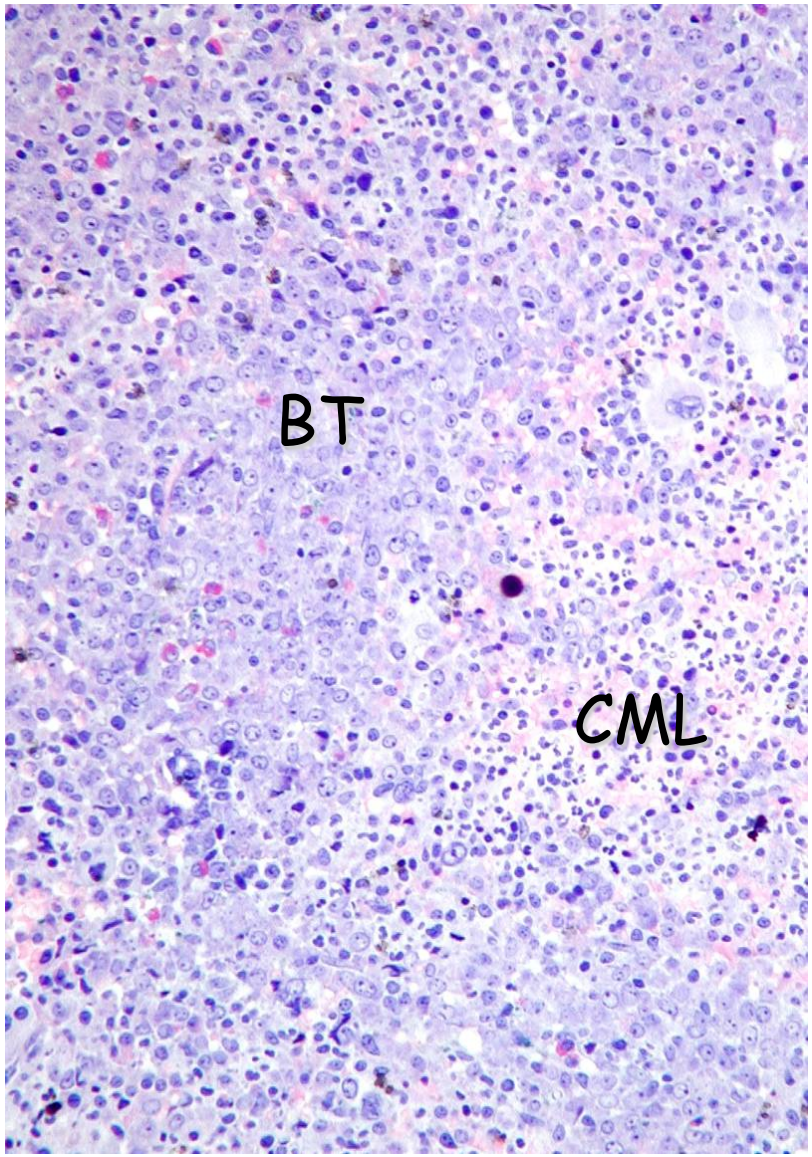
- AML with *BCR-ABL1 (provisional entity)*

AML with *BCR-ABL1*

- de novo AML <1% of AML
- genetic profile: t(9;22)(q34.1;q11.2) or BCR-ABL1 fusion
- no evidence before or after therapy of CML
- excluded cases of: mixed phenotype AL, therapy related myeloid neoplasms, AML with other recurrent abnormalities, late acquisition of BCR-ABL1 fusion in pre-existing AML
- Clinics: adults, M>F
  leukocytosis with blast predominance
  anaemia and thrombocytopenia
  < splenomegaly and PB basophilia compared to basts crisis of CML

AML with *BCR-ABL1*

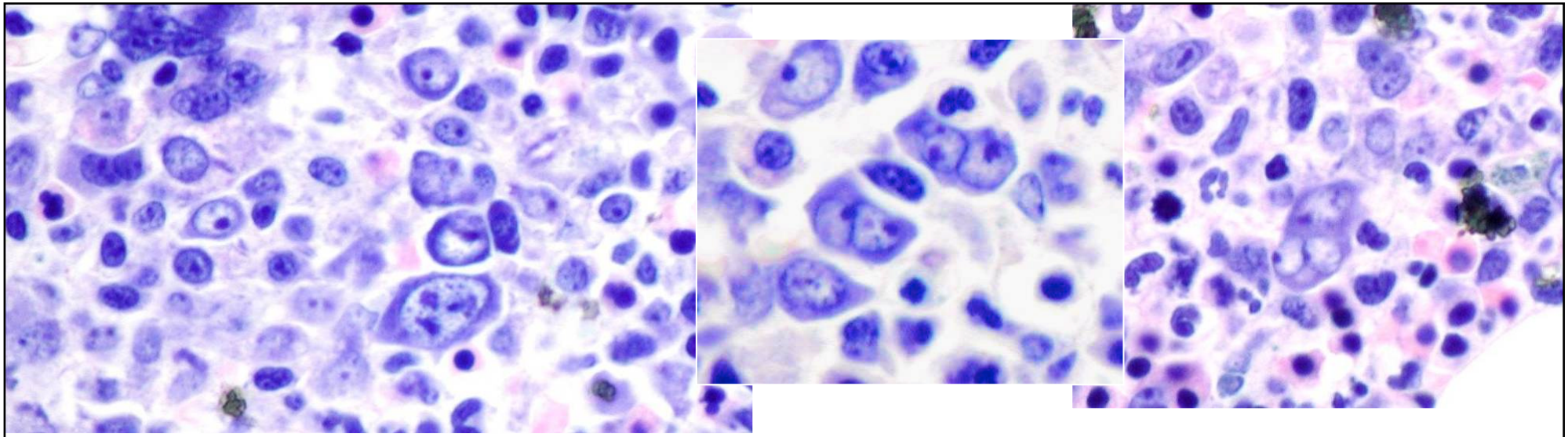
- Difficult to distinguish from myeloid blast crisis of chronic myelogenous leukaemia
- Frequent loss of CDKN2A and/or IKZF1 (ICAROS) (absent in CML blast crisis)
- Deletion of antigen receptors, particularly IGH and TRG, specific for de novo disease (reported in B-ALL but not in CML blast crisis)
- Important to recognize due to presence of targeted (TKI) therapy



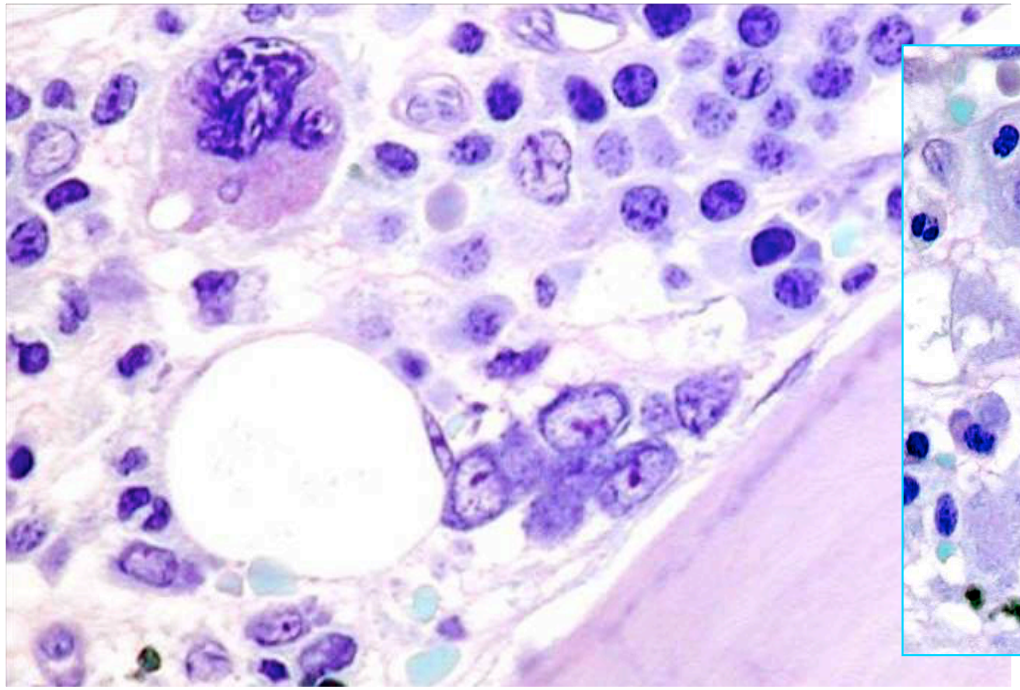
AML with myelodisplasia-related changes

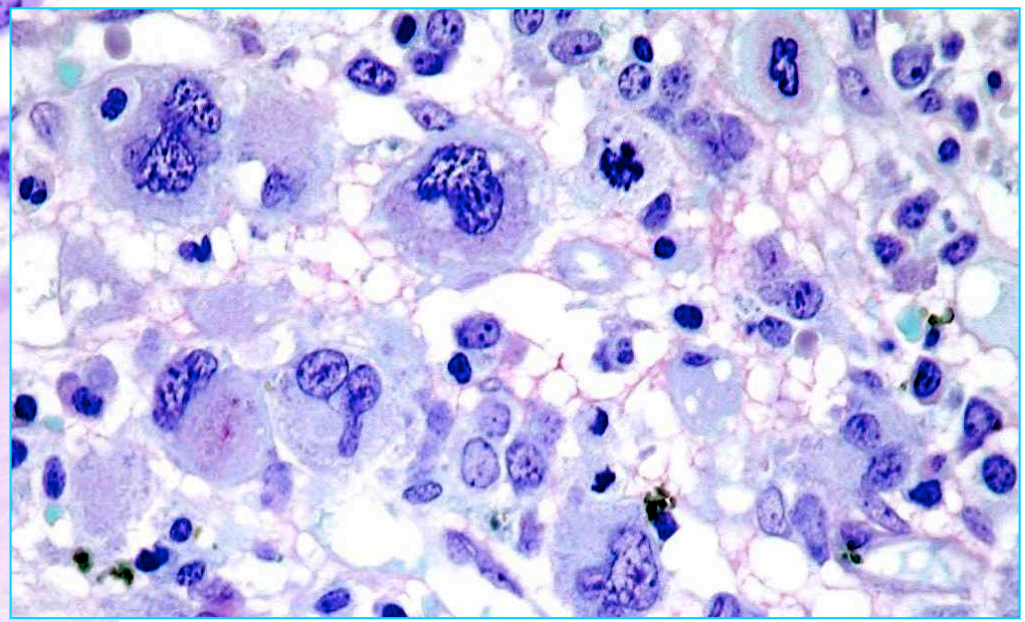
Diagnostic criteria:

1) ≥20% blood or BM blasts
2) one of the following:
   - *history of MDS, MDS/MPN,*
   - *MDS-related cytogenetic changes*
   - *multilineage dysplasia (≥50% dysplastic cells in 2 or more lineages)*
3) absence of both the following:
   - *prior cytotoxic or RT therapy for unrelated disease*
   - *recurrent cytogenetic abnormalities described in AML*



multilineage dysplasia
≥50% dysplastic cells in 2 or more lineages





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AML with myelodisplasia related changes

Revised criteria for AML with myelodysplasia related changes:

- Excluded cases with *NPM1* mutated or *CEBPA* biallelic mutation in absence of MDS-related cytogenetic abnormalities (no prognostic impact of multilineage dysplasia)

- MDS-related cytogenetic abnormalities: del(9q) alone in cases with *NPM1*^{m} and *CEBPA*^{bm}, in absence of MDS-related cytogenetic abnormalities, has no prognostic impact

MDS-related cytogenetic abnormalities

- Complex caryotype (≥3 abnormalities)
- Unbalanced abnormalities
  loss of chr 7 or del(7q)
  del(5q) or t(5q)
  isochr. (17q) or t(17p)
  loss of chr. 13 or del(13q)
  del(11q)
  del(12p)/t(12p)
  idic(X)(q13)

- Balanced abnormalities

t(11;16)(q23.3;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2)
t(2;11)(p21;q23.3)
t(5;12)(q32;p13.2)
t(5;7)(q32;q11.2)
t(5;17)(q32;p13.2)
t(5;10)(q32;q21.2)
t(3;5)(q25.3;q35.1)

AML not otherwise specified

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

- Only a single change:

  move erythroid/myeloid type of acute erythroid leukemia to the MDS sectio or other AML types

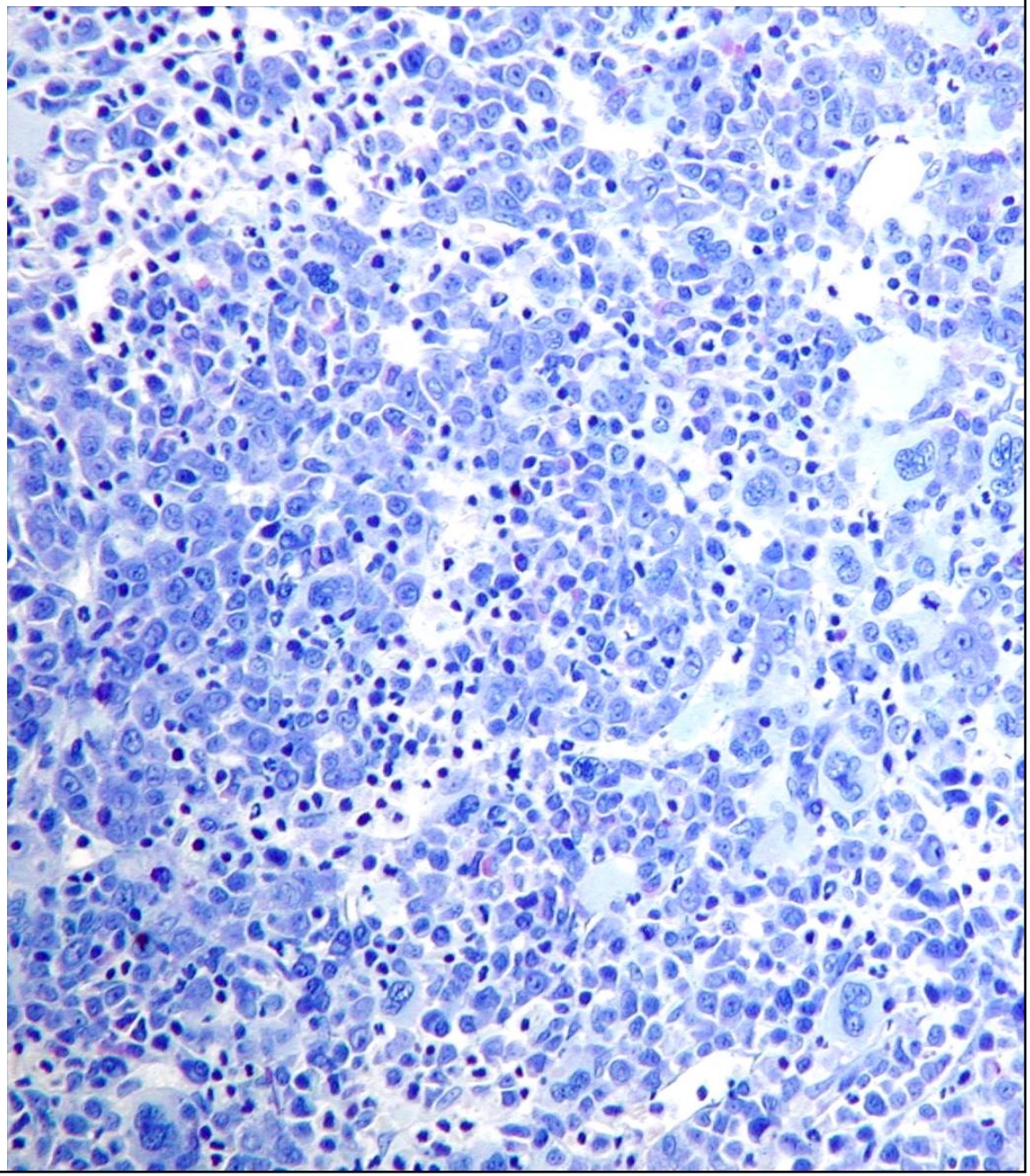
Acute erythroid leukaemia (WHO 2008)

predominant erythroid population.

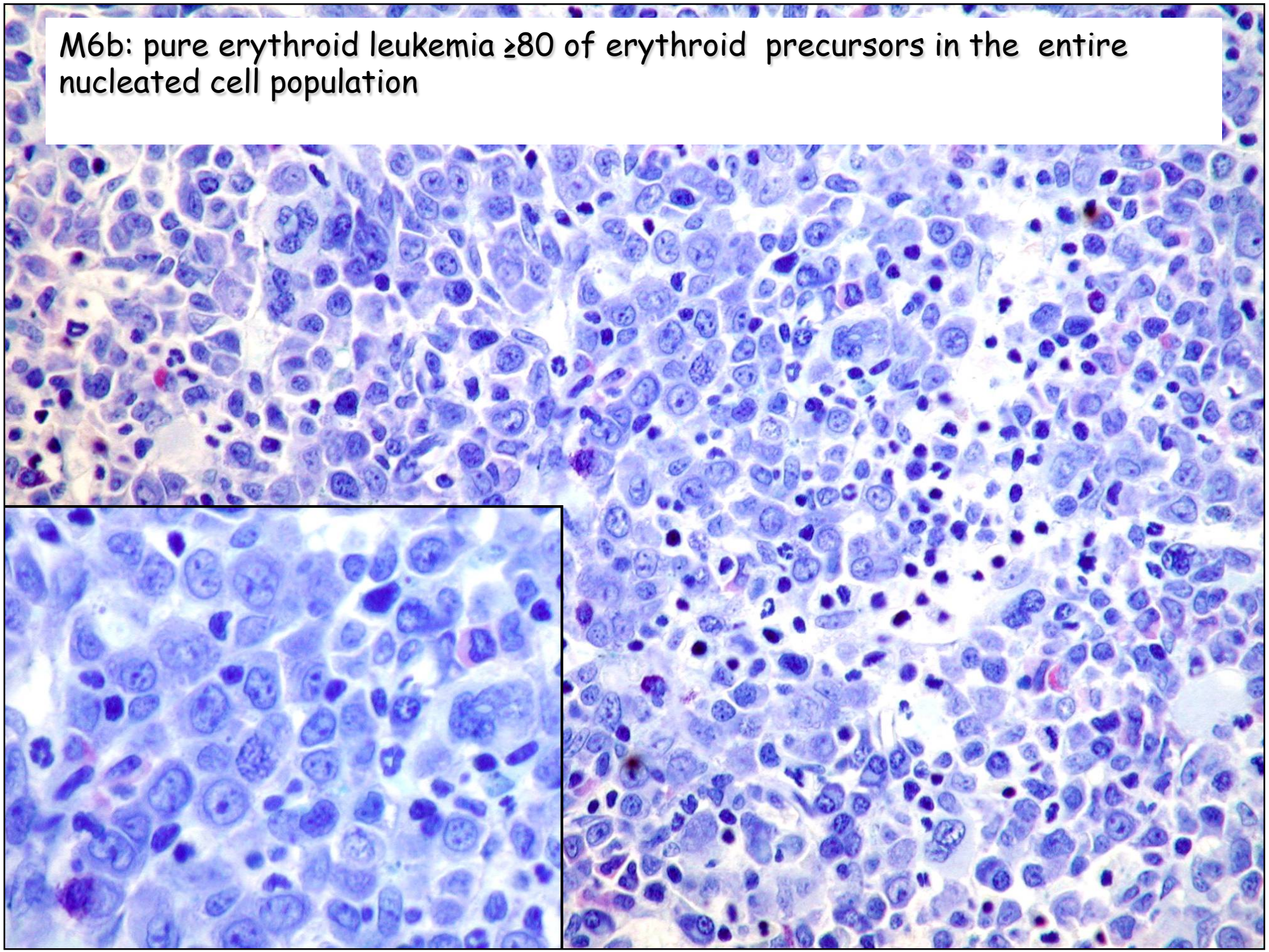
In WHO 2008 two subtypes based on the presence or absence of a myeloid component:

M6a: erythroleukaemia (erythroid/myeloid type)

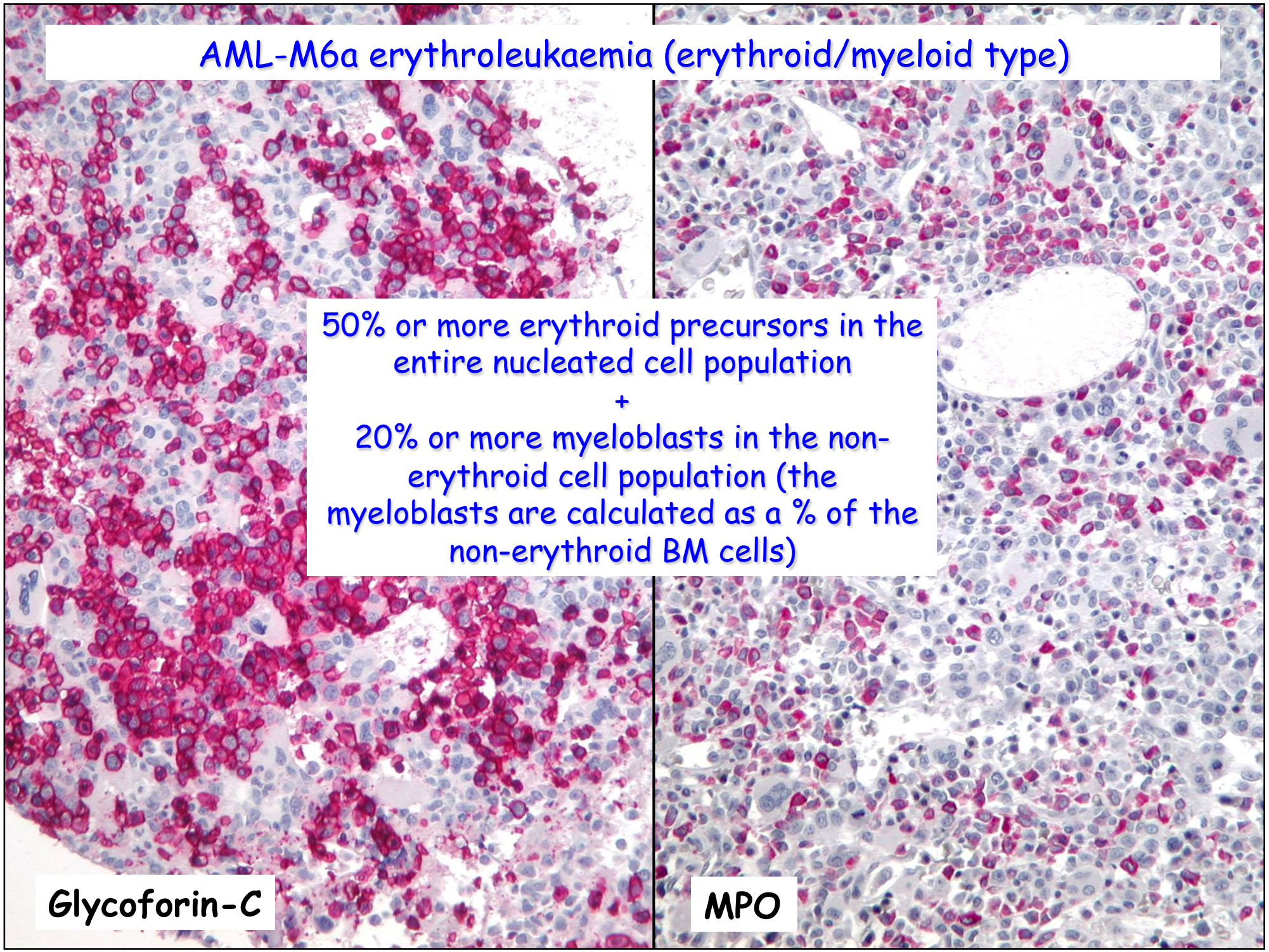
M6b: pure erythroid leukemia



M6b: pure erythroid leukemia ≥80 of erythroid precursors in the entire nucleated cell population



AML-M6a erythroleukaemia (erythroid/myeloid type)



50% or more erythroid precursors in the entire nucleated cell population

+

20% or more myeloblasts in the non-erythroid cell population (the myeloblasts are calculated as a % of the non-erythroid BM cells)

Glycoforin-C

MPO

Pure erythroid leukemia (Who 2017)

- "AML committed exclusively to the erythroid lineage (>80% of the bone marrow cells are erythroid with > 30% proerythroblasts) without significant myeloblastic component"

- Myeloblasts are counted as % total cells and M6a were moved to:

  -other AML categories , if ≥20% (often AML-MRC)

  -MDS, if blasts ≤20%

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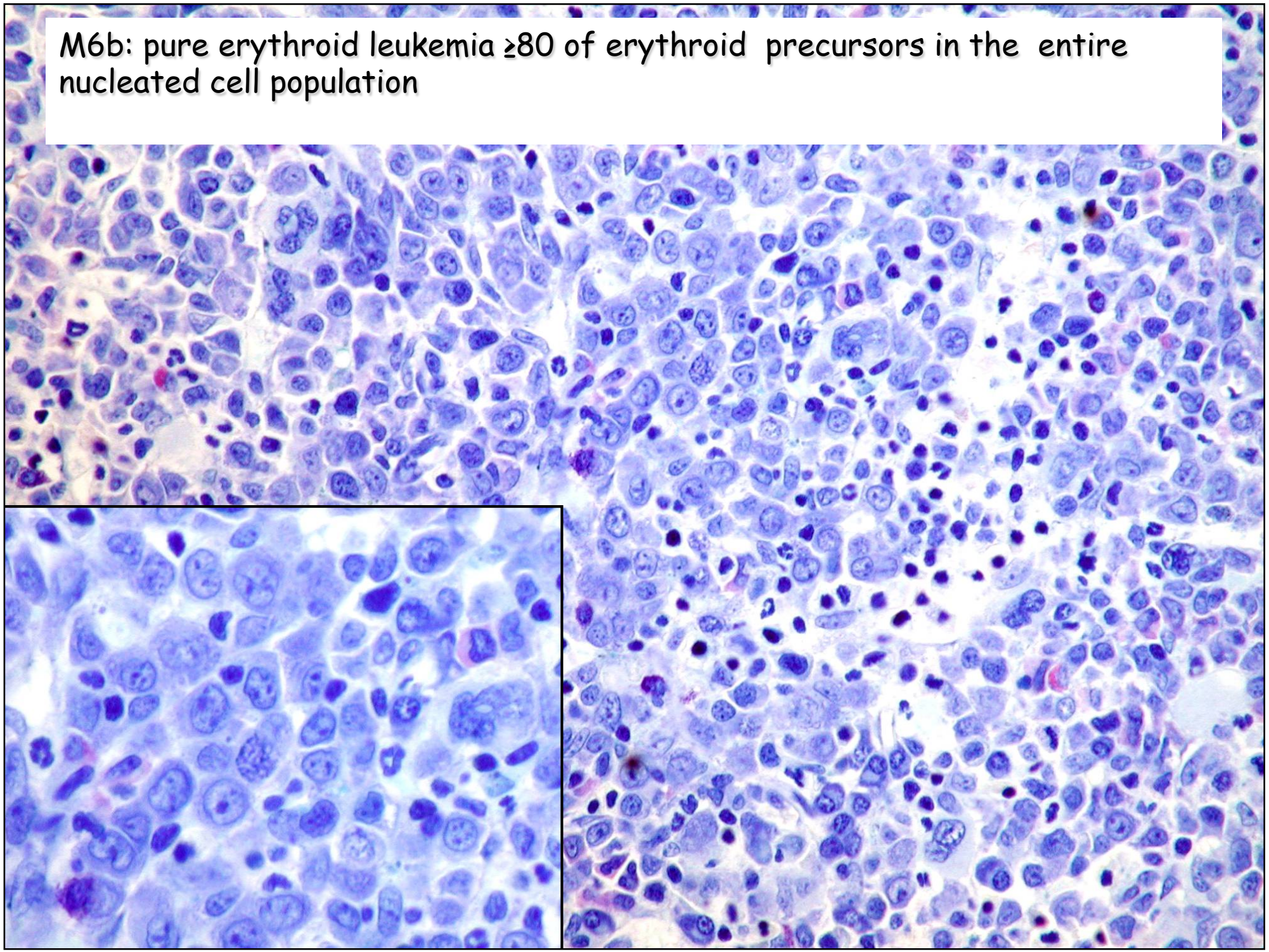

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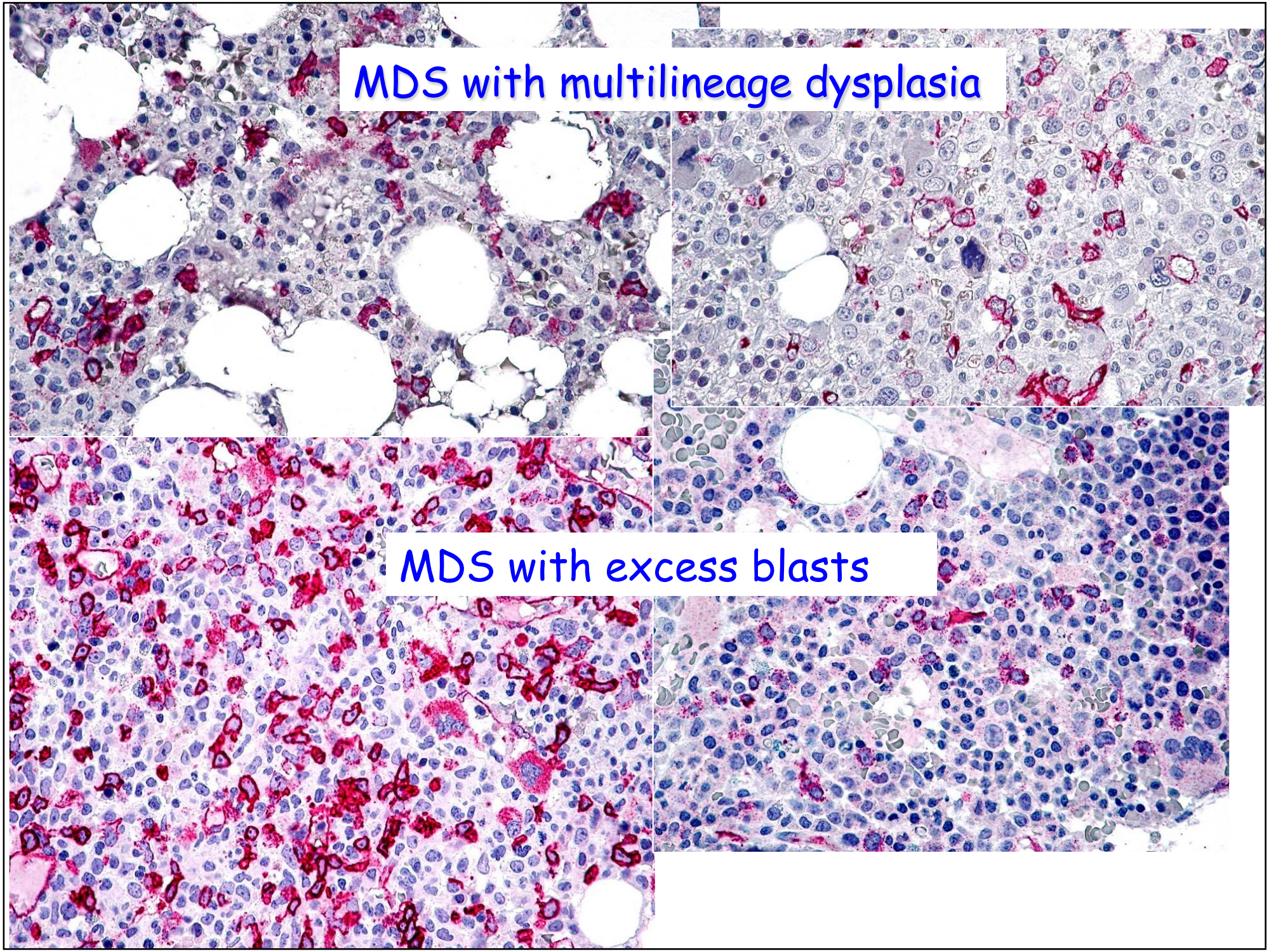
MDS with multilineage dysplasia
MDS with excess blasts

Summary

- New cytogenetic subgroups

– many rare balanced transl. and inv. recognized

– AML with *BCR-ABL1*

– Refine APL with *PML-RARA fusion*

- New and revised mutation subgroups

- Revised criteria AML-MRC

- MLD in the setting of specific mutation

- MDS-related cytogenetic abnormalities revised

- AML-NOS: erythroid/myeloid type removed