WHO 2016 classification of AML

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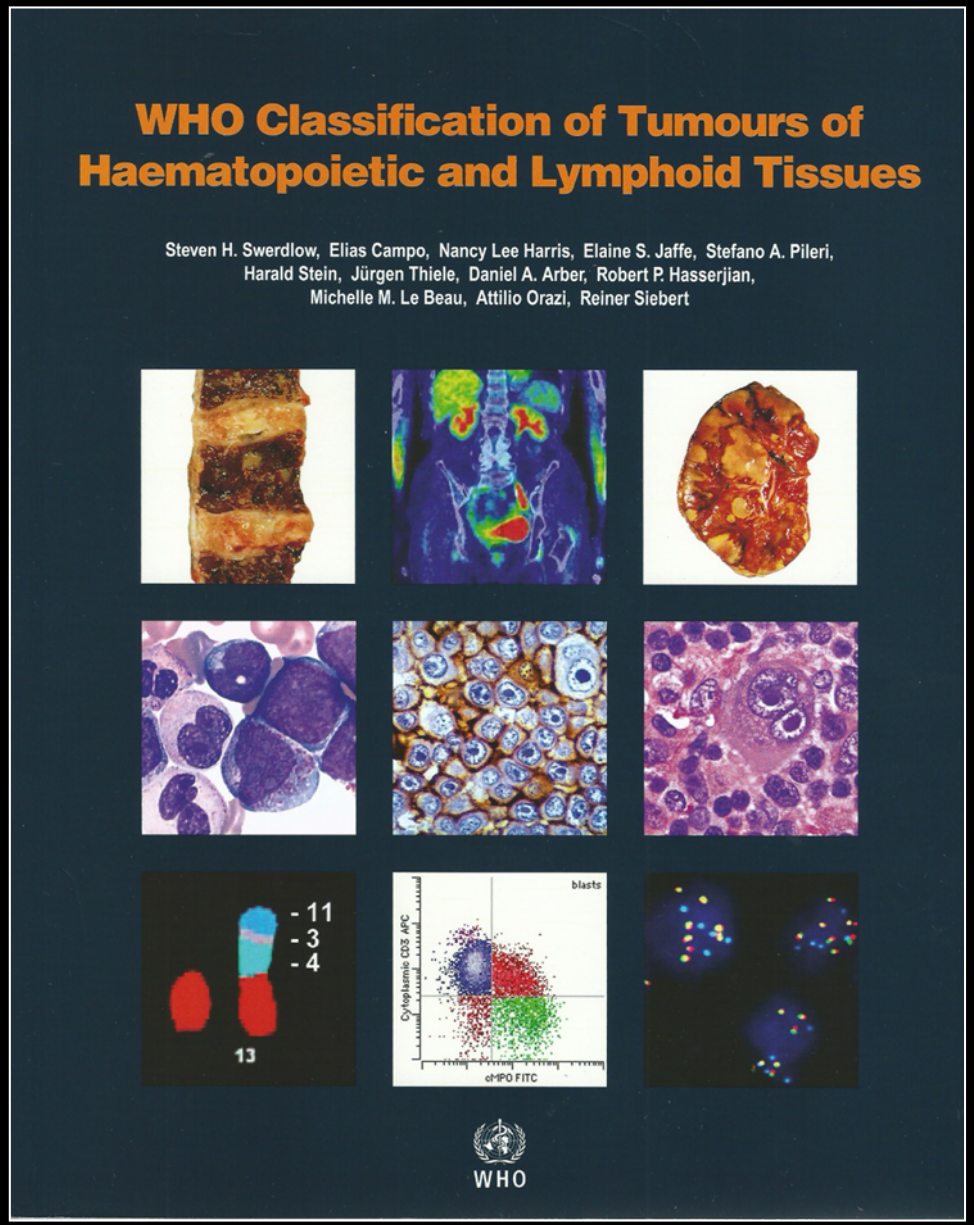
Disclosure: Patent on the clinical use of NPM1 mutants

Myeloid CAC Meeting Participants Chicago 3/31/14

WHO Classification (2016)



Printed in 2017

AML categories (2016)

- AML with recurrent genetic abnormalities
- AML with myelodysplasia related changes (MRC)
- -Therapy-related AML/MDS
- AML not otherwise specified (NOS)
- Myeloid sarcoma
- Myeloid proliferations related to Down sindrome

AML with recurrent genetic abnormalities (WHO-2008)

a) AML with t(8;21)(q22;q22); *RUNX1.RUNX1T1*	25%*
b) AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	
c) Acute promyelocytic leukaemia (AML with t(15;17)(q22;q12); *PML-RARA*	
d) AML with t(9;11)(p22;q23); *MLLT3-MLL*	
e) AML with t(6;9) (p23;q34); *DEK-NUP214* AML	
f) AML with inv(3) (q21q26.2) or t(3;3) (q21;q62); *RPN1-EVI1*	
g) AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MLK1*	
h) AML with mutated *NPM1* (provisional entity)	30%*
i) AML with mutated *CEBPA* (provisional entity)	5%*

* Of all AML.

New AML provisional entity defined by translocations: AML with *BCR-ABL1*

- **Difficult to distinguish from myeloid blast crisis of chronic myelogenous leukemia**
- **Deletion of antigen receptors genes (*IGH*, *TCR*), *IKZF1* and/or *CDKN2A* may support a diagnosis of de novo disease**
- **Subset of cases have mutated *NPM1***
- **Important to recognize due to presence of targeted (TKI) therapy**

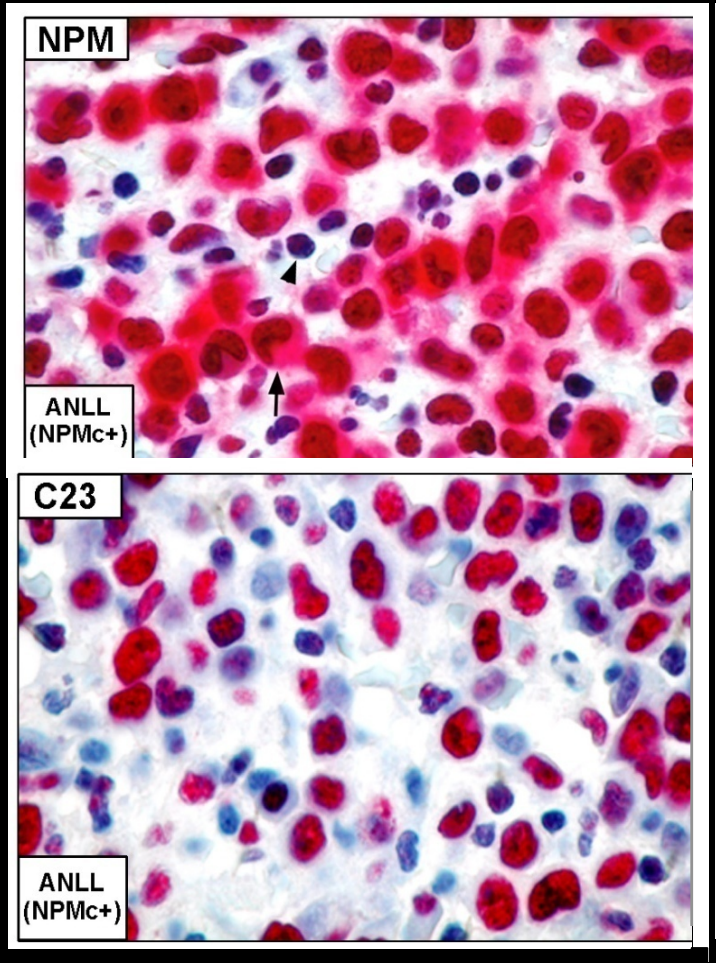
Soupir CP, et al. Am J Clin Pathol 127:642, 2007
Konoplev S, et al. Leuk Lymphoma 54:138, 2013
Nacheva EP, et al. Br J Haematol 161:541, 2013

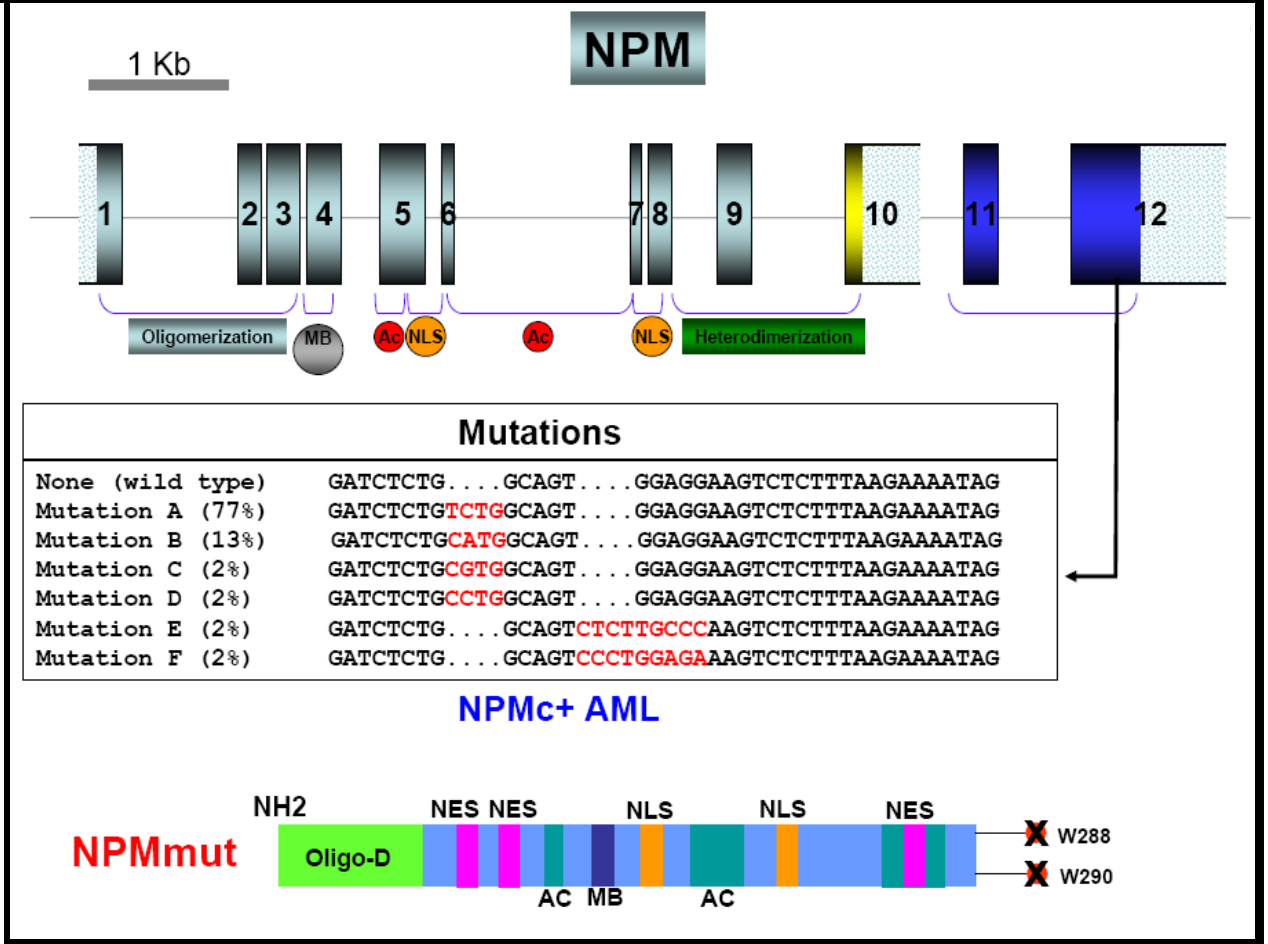
WHO 2016: major changes in the category of "AML with recurrent genetic abnormalities"

- AML entities defined by mutations now include:

AML with mutated NPM1 (distinct entity)
AML with double mutated CEBPA (distinct entity)
AML with mutated RUNX1 (provisional entity)

NPM1-mutated AML (NPMc+ AML)





Mutations

None (wild type)	GATCTCTG....GCAGT....GGAGGAAGTCTCTTTAAGAAAATAG
Mutation A (77%)	GATCTCTGTCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG
Mutation B (13%)	GATCTCTGCATGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG
Mutation C (2%)	GATCTCTGCGTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG
Mutation D (2%)	GATCTCTGCCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG
Mutation E (2%)	GATCTCTG....GCAGTCTCTTGCCCAAGTCTCTTTAAGAAAATAG
Mutation F (2%)	GATCTCTG....GCAGTCCCTGGAGAAAGTCTCTTTAAGAAAATAG

NPMc+ AML

Falini B et al., NEJM 352:254-266, 2005

Properties of most common mutations in AML-NK

Features	*NPM1**	*FLT3-ITD*	*DNMT3A*	*IDH1/2*
Frequency	55-60%	25-30%	35-40%	10-15%
Specificity	AML	AML, MDS ETP-ALL	AML, MDS, MPN, PTCL T-ALL	AML, MDS, MPN, gliomas
GEP	Distinct	No	No	No
Micro-RNA	Distinct	No	No	No**
Appearance time	Later	Later	Earlier	Earlier (*IDH2*)

ETP: Early T-cell precursors ALL; * Falini B. et al (NEJM, 2005). Most of these features also apply to double-*CEBPA* mut. AML; ** Only AML with R172 IDH2 mut.

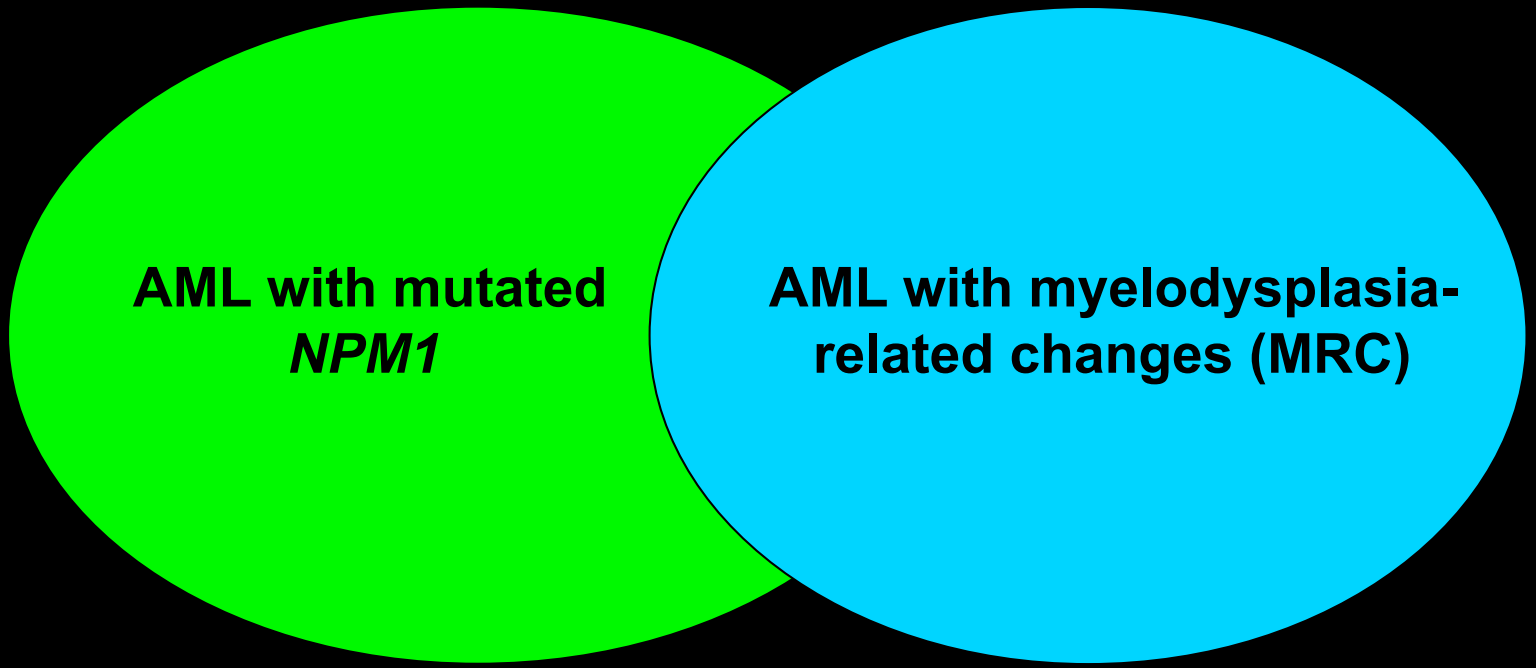
Main features of *NPM1*-mutated AML (NPMc+ AML)

- About 35% adult AML (age : 15-60)
- About 60% of AML with normal cytogenetics
- Exclusive with major cytogenetic abnormalities
- Wide morphological spectrum (often M4/M5)**
- Negativity for CD34 and CD133
- Up-regulation of HOX genes (GEP)
- Concomitant *FLT3*-ITD and *DNMT3A* mutations
- Good response to induction therapy
- Favourable prognosis (if FLT3-ITD absent)

* Based on 591 GIMEMA patients; ** 80-90% of M5b are NPMc+ ;

(Falini B et al., NEJM 352:254, 2005)

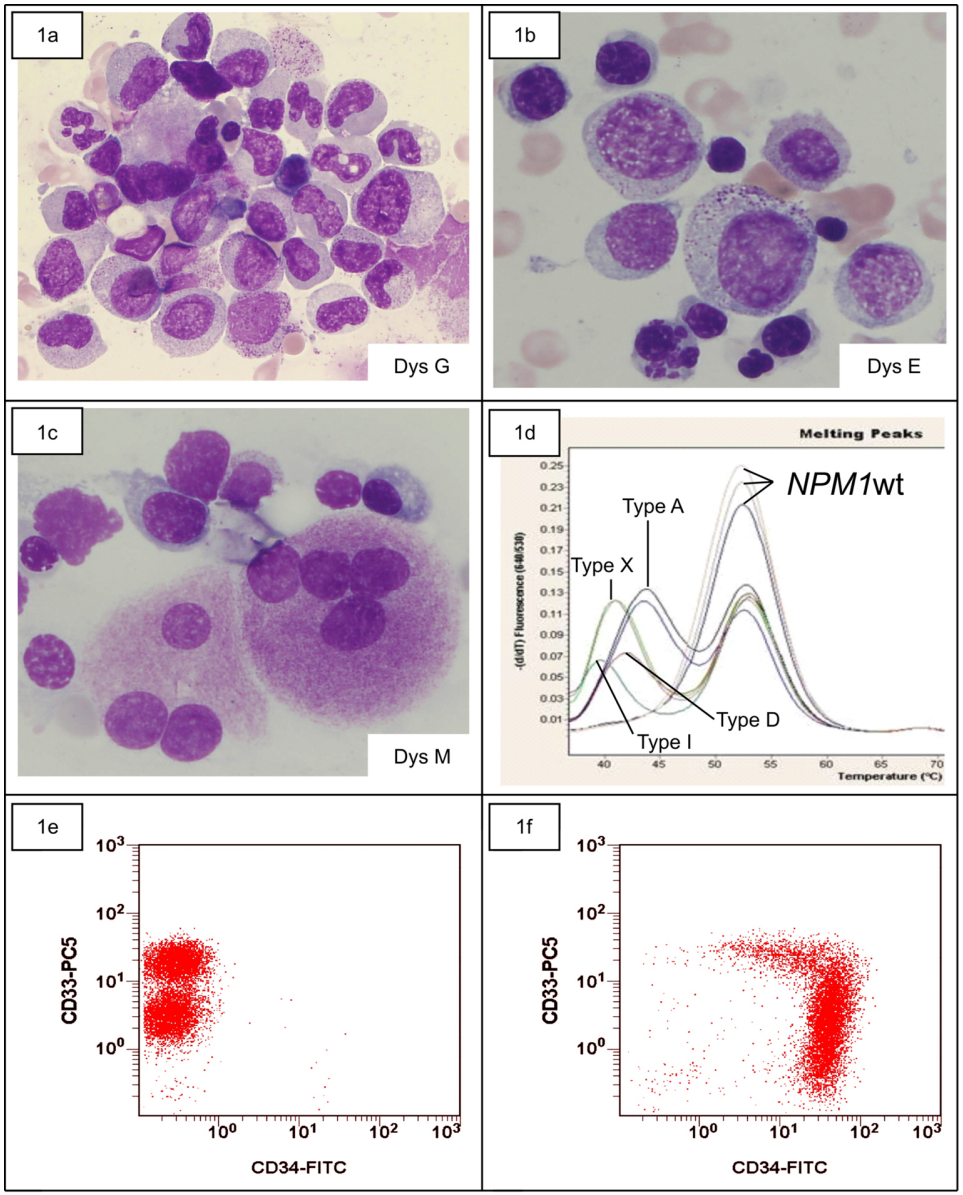
NPM1-mutated AML: provisional entity (WHO-2008)*. Unclear how it correlates with other WHO categories.

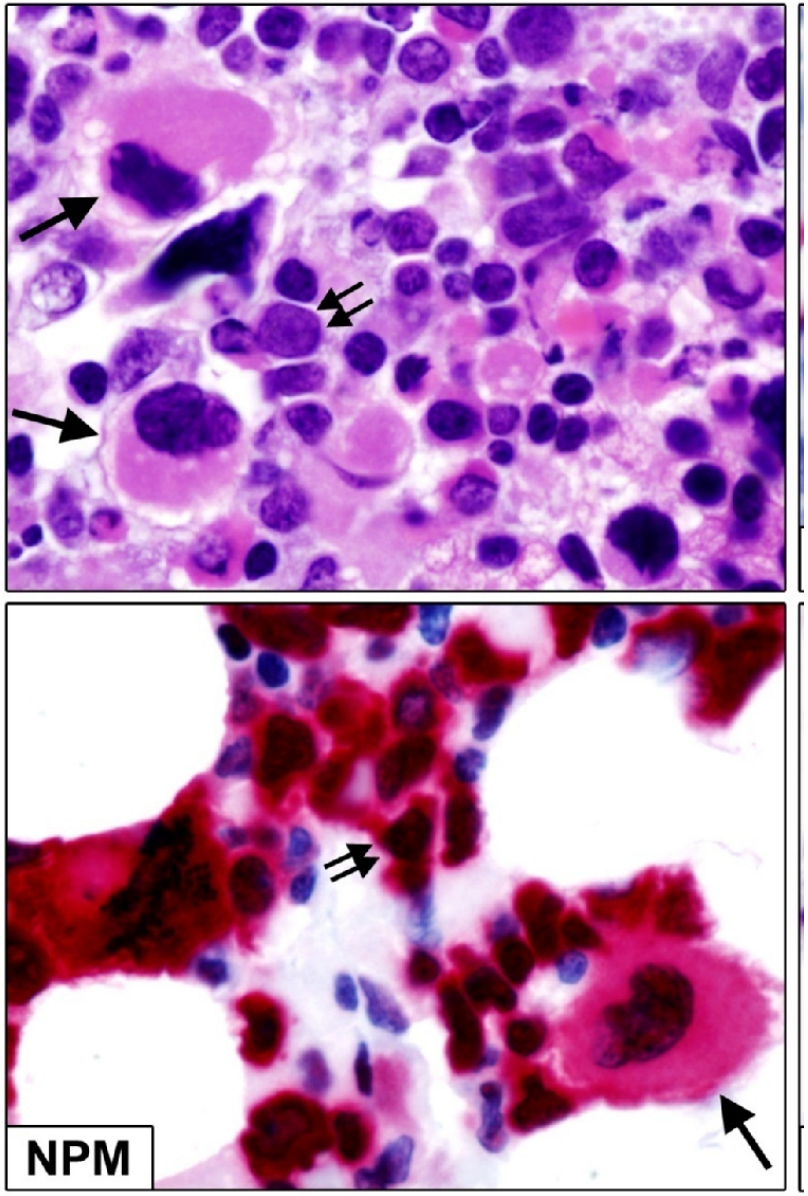


AML with MRC (myelodysplasia-related changes) is defined by one or more of the followings: 1) Multilineage dysplasia; 2) Previous history of MDS; 3) MDS-related karyotype.

* The same problems apply to *CEBPA*-mutated AML.

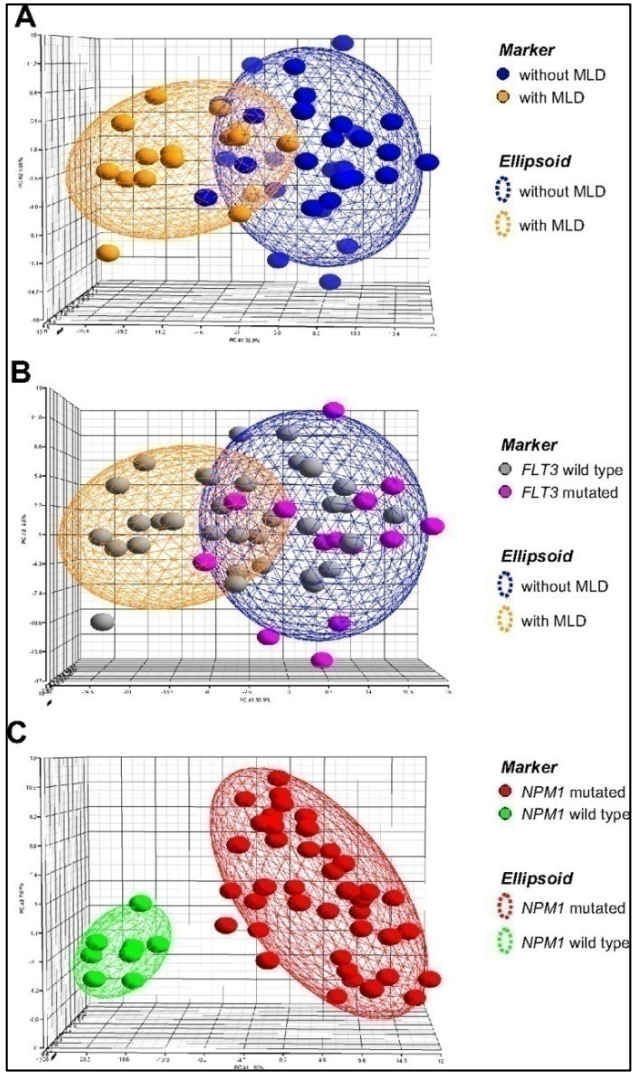
Multilineage dysplasia in *NPM1*-mutated AML (n=318) (Frequency: 23.3%)



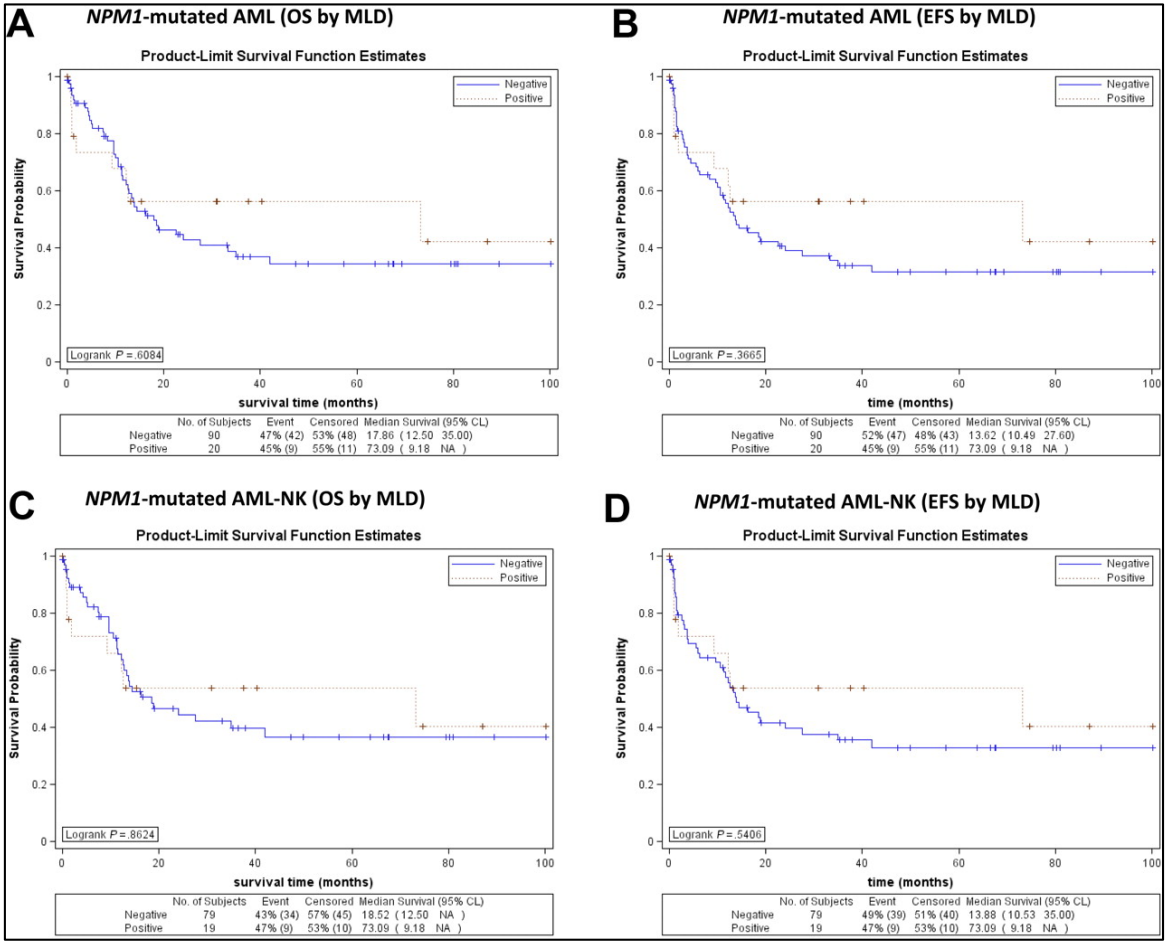


(Falini B et al., Blood 115:3776, 2010)

No difference in GEP and survival in *NPM1*-mutated AML with and without multilineage dysplasia



Principal component analysis



Survival curves from GIMEMA

(Falini B. et al. Blood 2010;115:3776-3786)

AML with mutated *NPM1* (WHO 2016)*

- Change from “provisional” to a “distinct entity”

- Cases showing multilineage dysplasia as the only defining criterion for AML with MDS-related changes are classified as AML with mutated *NPM1*

- The entity includes only *de novo* cases that lack an MDS-related karyotype except del(9q) and previous history of MDS or MDS/MPN, and are not therapy-related **

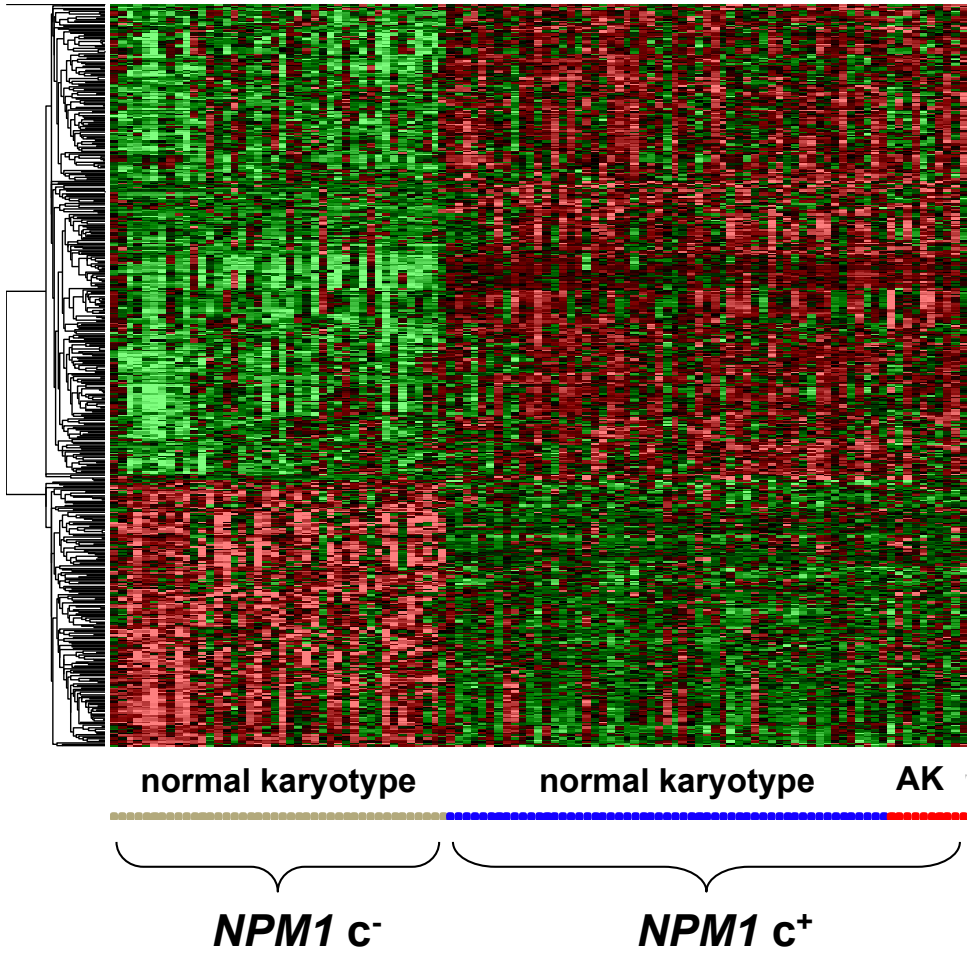
** Almost all cases of *NPM1*-mutated AML, including the 15% with abnormal karyotype (AK), will be classified as a distinct entity (no previous history of MDS, AK different from that of AML with MDS-related changes).

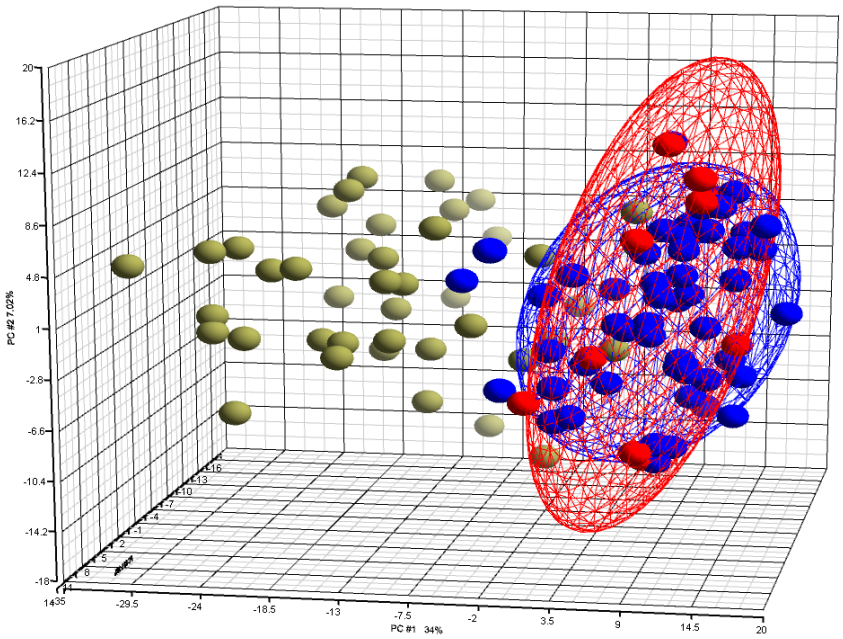
CHROMOSOME ABNORMALITIES FOUND IN 15% of *NPM1*-MUTATED AML ARE RARELY (<1%) THOSE DEFINING AML-MRC

Karyotype	AML				
	NPM1-mut* (N=632)	t(8;21) (N=63)	Inv16 (N=37)	t(15;17) (N=83)	11q23/MLL (N=83)
Additional Abnormalities	93/632 (14.7%)	44/63 (69.8%)	13/37 (35.1%)	39/83 (47%)	28/83 (33.7%)
-X/-Y	11	32	1	3	
+4	11	2			2
-7	3				
+8	33	2	5	12	8
+13	2				2
+19					4
+21	5				4
+22	1		6		2
del(7q)			2		
del(9q)	9	10		2	
del(11q)		2			
Ider(17)(q10)t(15;17)			7		
Other	67	11	8	20	30
Total	142	59	22	44	52

(Blood 114:3024, 2009)

NPM1-mutated AML with and without abnormal karyotype show the same GEP and outcome





- 42 AML normal karyotype, *NPM1* c⁻
- 55 AML normal karyotype, *NPM1* c⁺
- 10 AML other aberrations, *NPM1* c⁺

Upregulation of HOX genes and downregulation of CD34

(Blood 114:3024, 2009)

WHO 2016: major changes in the category of "AML with recurrent genetic abnormalities"

- AML entities defined by mutations will most likely include:

  AML with mutated NPM1 (distinct entity)

  AML with double mutated CEBPA (distinct entity)*

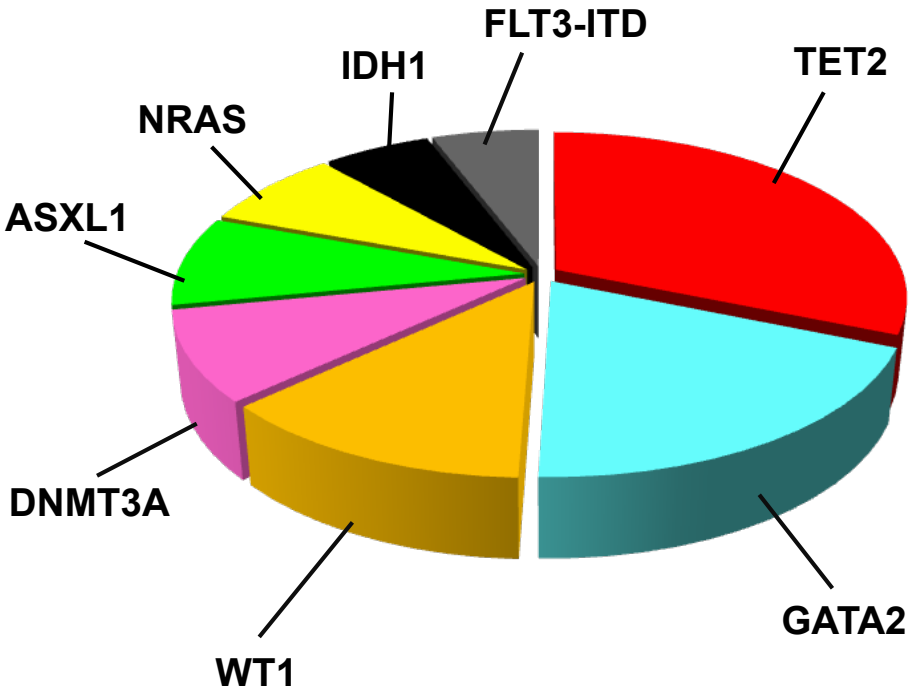
  AML with mutated RUNX1 (provisional entity)**

* Same recommenations as for *NPM1*-mutated AML concerning distinction from AML-MRC.

** Cases with associated multilineage dysplasia will be still called AML-MRC.

AML with mutated *CEBPA*

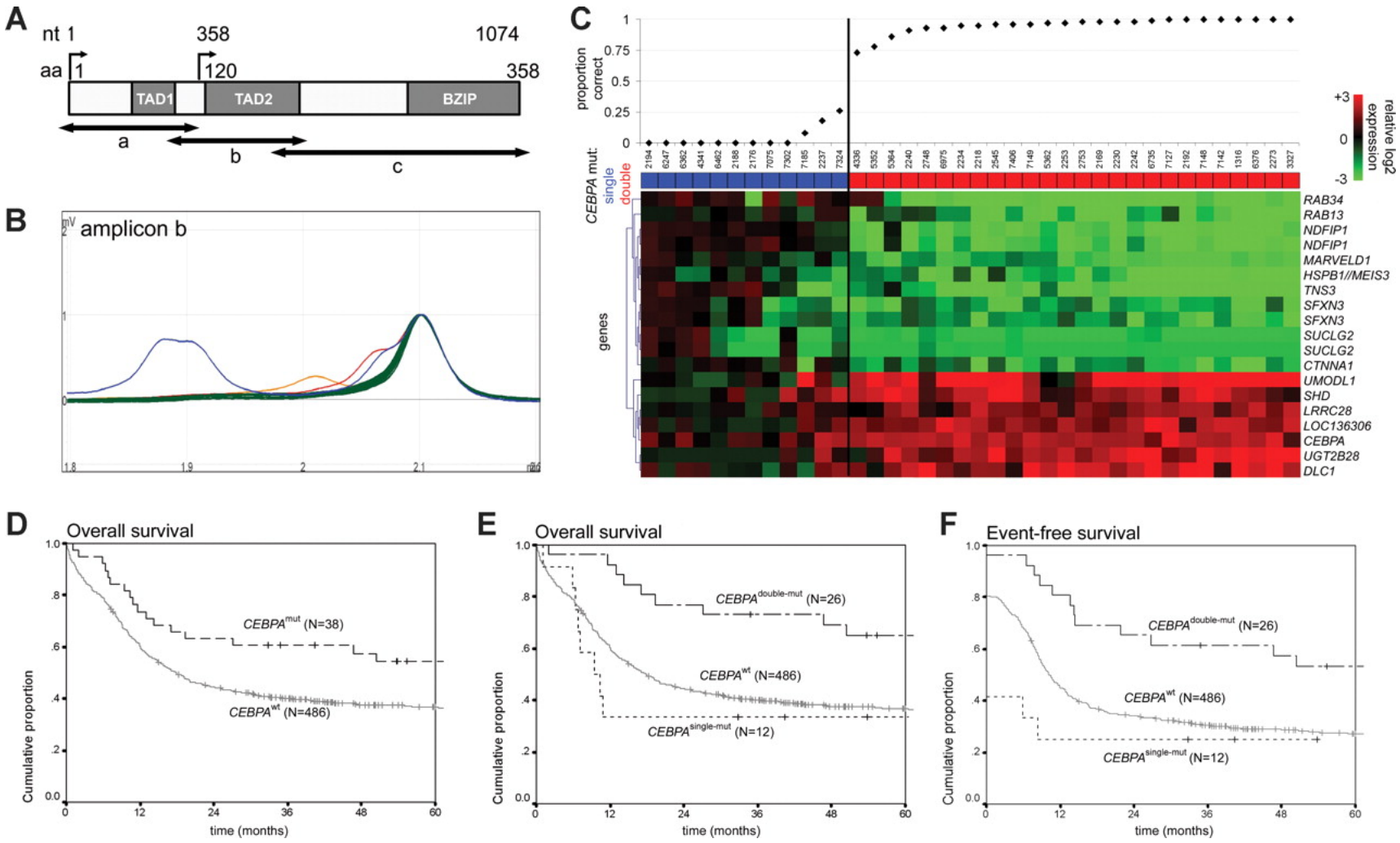
- About 10% of AMLs have mutations of *CEBPA*
  - *More frequent with normal or intermediate karyotype*
- About 50% are single/monoallelic
- Double mutant/biallelic (*CEBPA*^{dm}) predict a favorable prognosis
- Possible association with other mutations (especially *GATA2, TET2 and WT1)*



Associated mutations

Only double *CEBPA* mutations define a subgroup of AML with a distinctive GEP and favorable outcome*

(Wouters BJ, et al. *Blood.* 2009;113:3088-91)



Subsequent studies (Bacher, Blood 119:4719; 2012; Schlenk RF Blood 122: 1576, 2013): no impact of MLD or accompanying chromosomal aberrations

blood
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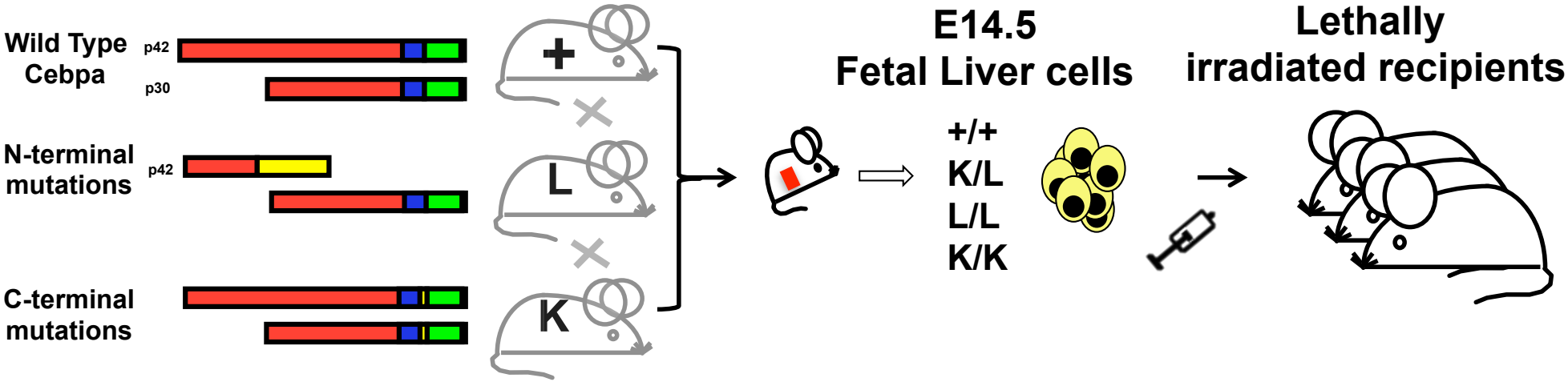
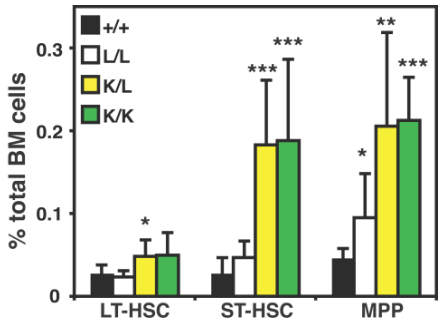
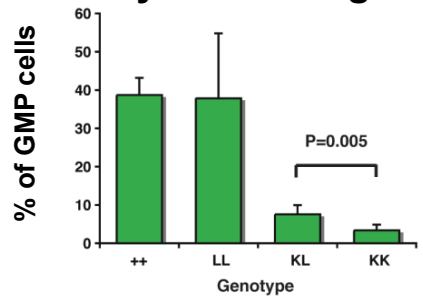
Combining N- and C-terminal *Cebpa* mutations causes accelerated AML in the mouse



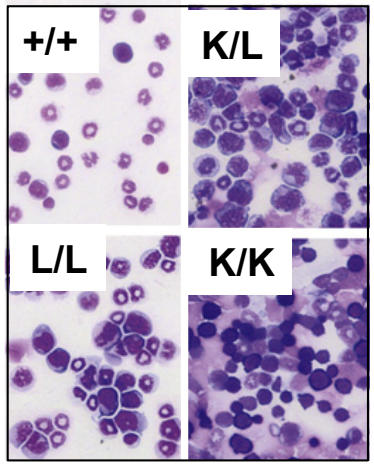
Hematopoietic stem cells expansion



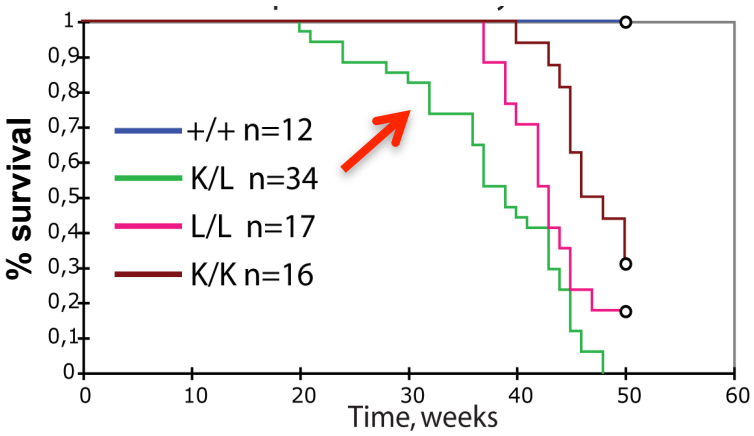
Altered myeloid lineage commitment



Myeloid Leukemia



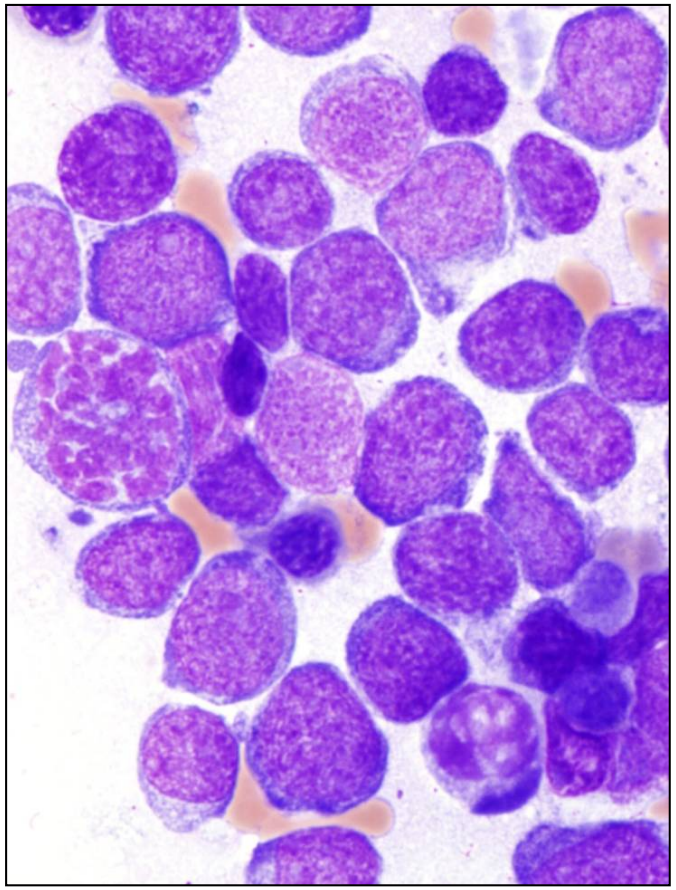
Reduced K/L mice survival



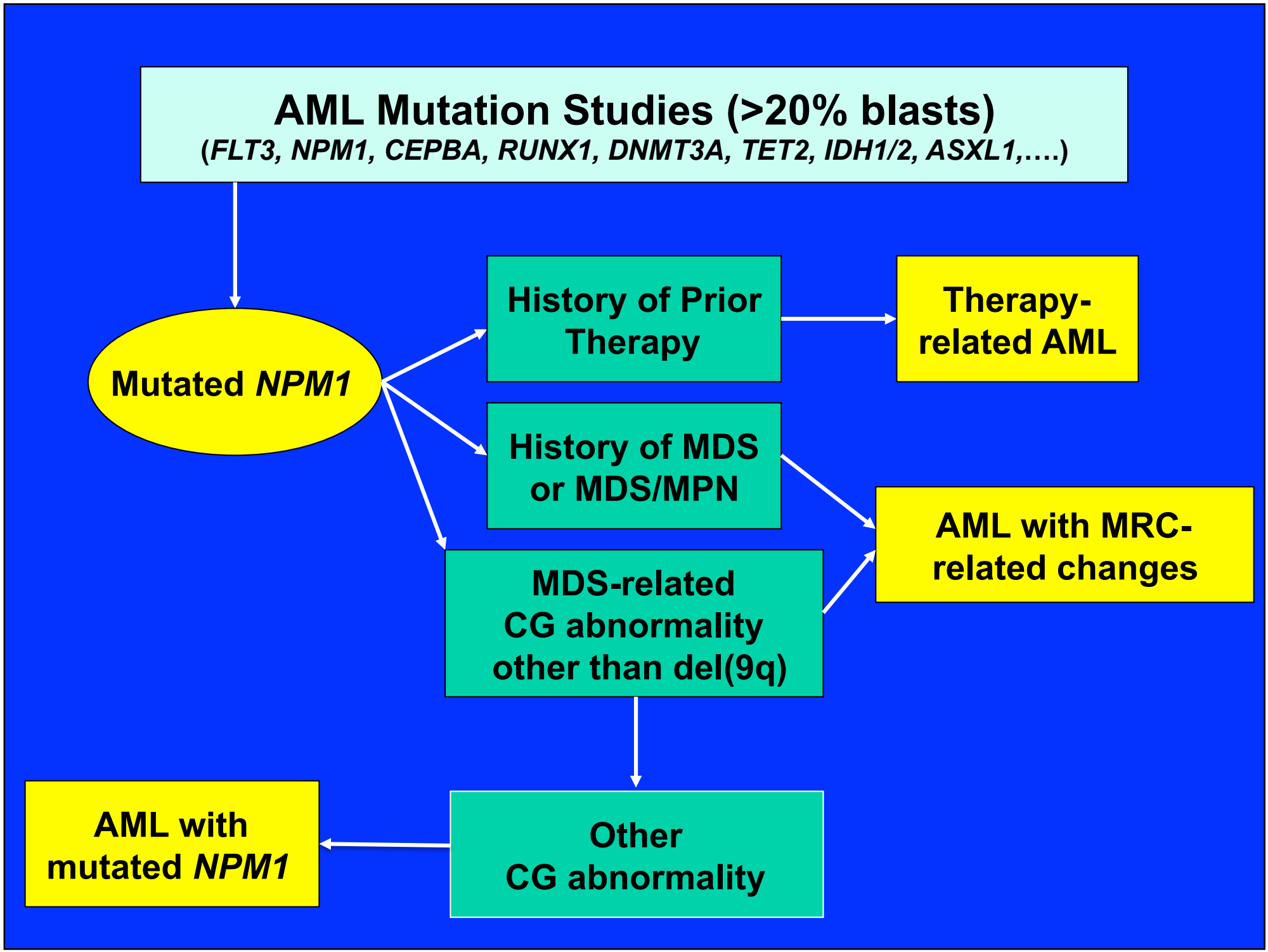
(Bereshchenko et al., Cancer Cell 2009)

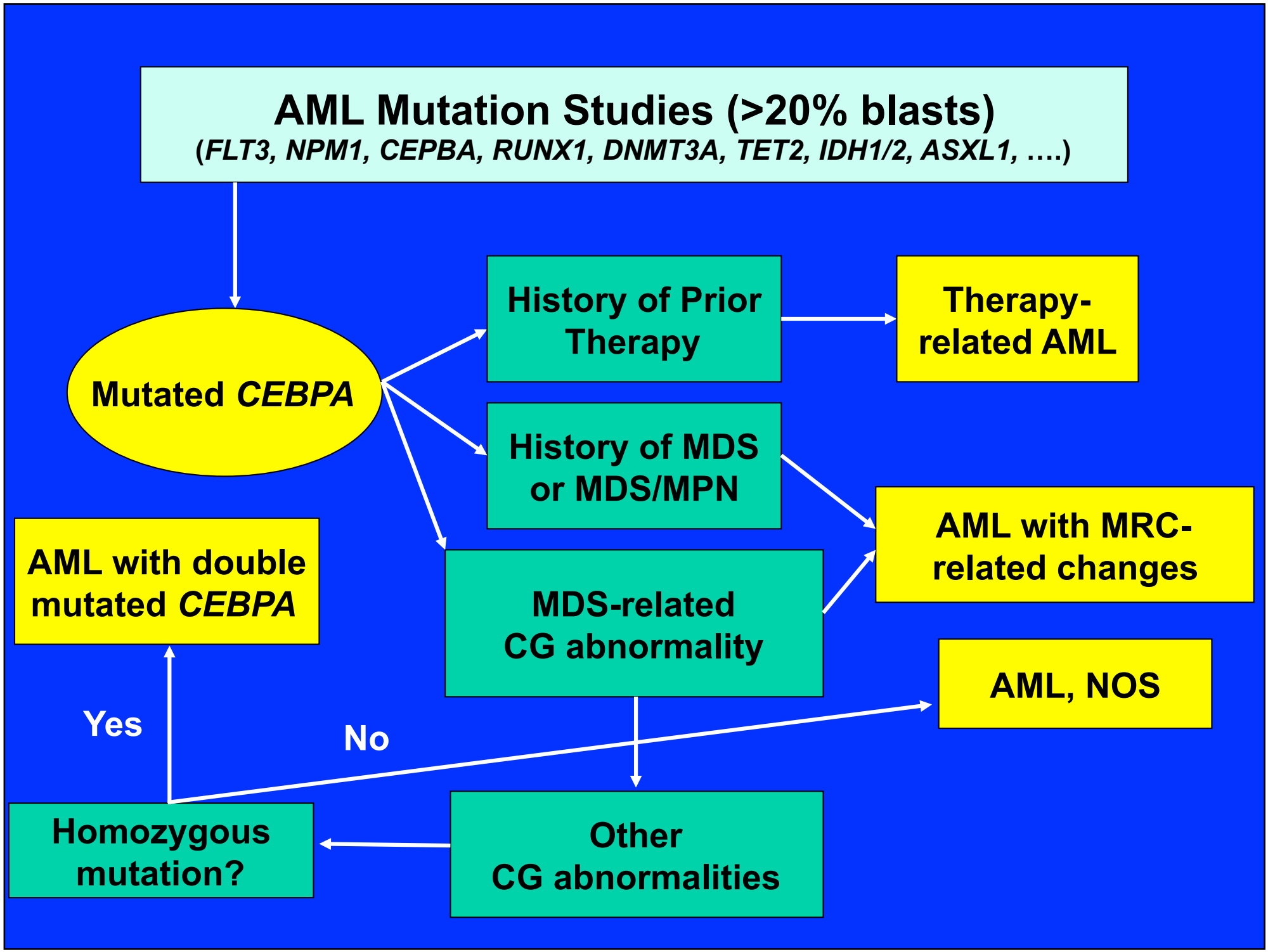
AML with mutated *RUNX1*

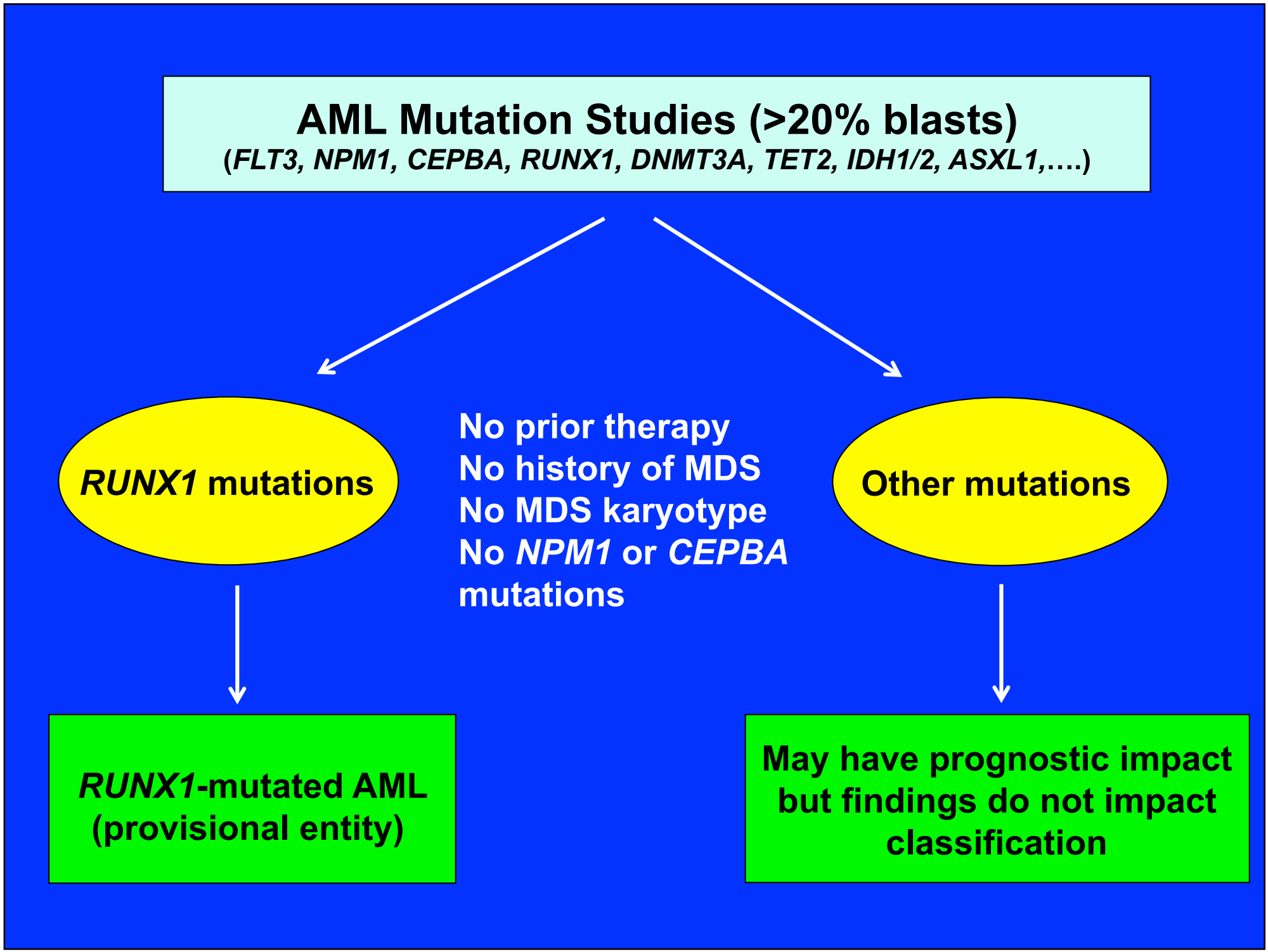
- Gene located at 21q22
- Encodes the alpha subunit of the core binding factor
- Mutation in 12.5-13.2% of AML
- More frequent in older male patients
- Frequent prior history of MDS, or prior exposure to radiation
- Immature morphology (60% M0) and phenotype
- Frequent associated *MLL*-PTD or *ASXL1* mutations
- Poor response to therapy with shortened survival



Tang et al. Blood 114:5352, 2009
Mendler et al. JCO 30:3109, 2012


AML Mutation Studies (>20% blasts)
(FLT3, NPM1, CEPBA, RUNX1, DNMT3A, TET2, IDH1/2, ASXL1,….)
Mutated NPM1
History of Prior Therapy
Therapy-related AML
History of MDS or MDS/MPN
AML with MRC-related changes
MDS-related CG abnormality other than del(9q)
AML with mutated NPM1
Other CG abnormality


AML Mutation Studies (>20% blasts)
(FLT3, NPM1, CEPBA, RUNX1, DNMT3A, TET2, IDH1/2, ASXL1, ….)
Mutated CEBPA
History of Prior Therapy
Therapy-related AML
History of MDS or MDS/MPN
AML with MRC-related changes
MDS-related CG abnormality
AML with double mutated CEBPA
AML, NOS
Yes
No
Homozygous mutation?
Other CG abnormalities

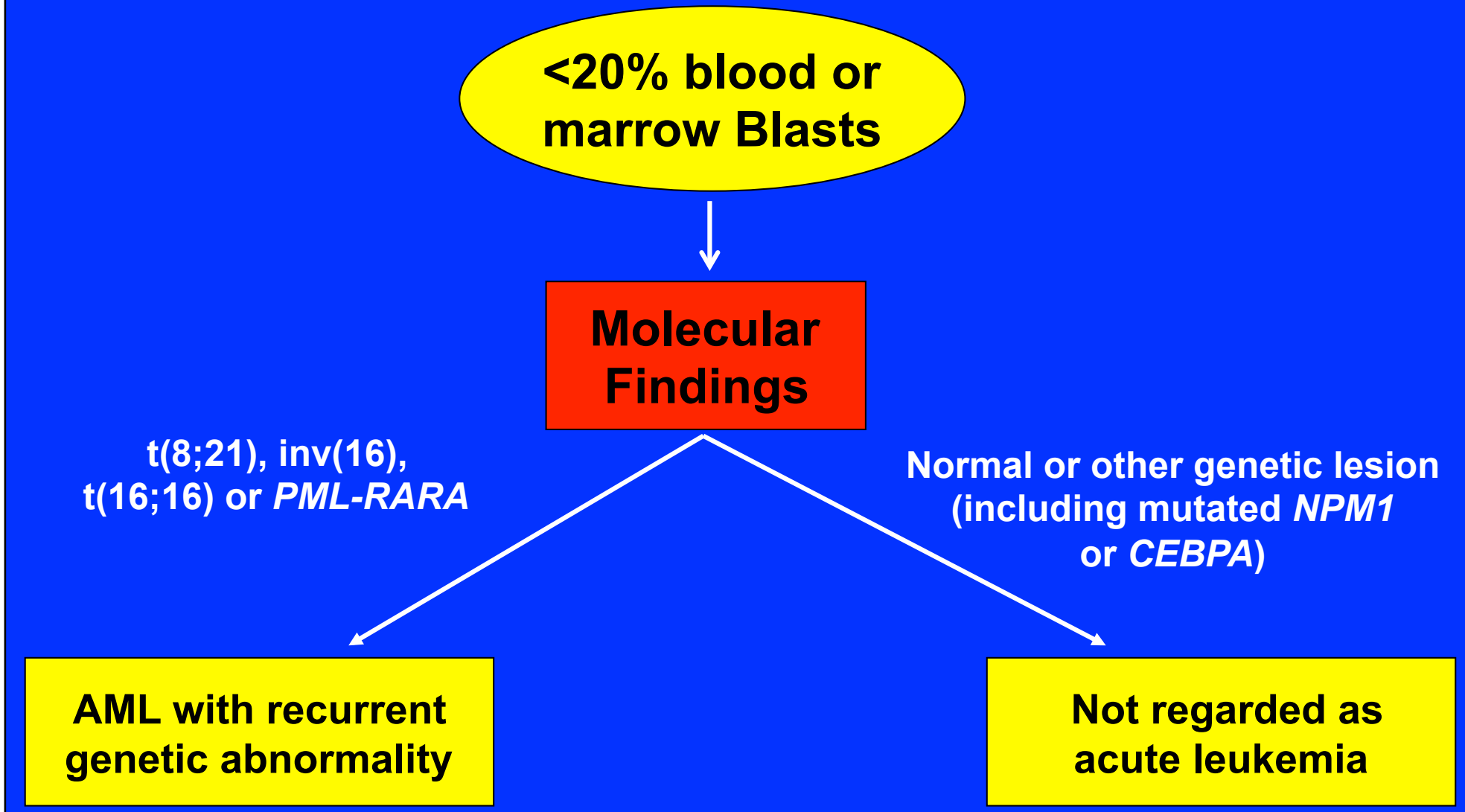

AML Mutation Studies (>20% blasts)
(FLT3, NPM1, CEPBA, RUNX1, DNMT3A, TET2, IDH1/2, ASXL1,….)
RUNX1 mutations
No prior therapy
No history of MDS
No MDS karyotype
No NPM1 or CEPBA mutations
Other mutations
RUNX1-mutated AML (provisional entity)
May have prognostic impact but findings do not impact classification

Prognostic value of mutations in CN-AML*

Mutated gene	Frequency	Prognostic value	
NPM1	~55%	Favourable (in absence of FLT3-ITD)	
CEBPA	5-10%	Favourable (only double-mutated)	
FLT3-ITD	~30%	Unfavourable (especially if high Mut/Wt ratio and/or insertions in TKD1 β1 sheet)	
RUNX1	~10%	Unfavourable (especially if ASLX1-mut)	
ASLX1	~5%	Unfavourable (especially if RUNX1-mut)	
DNMT3A	~35%	Controversial	*Patel NEJM, 2012* *Grossmann Blood 2012* *Marcucci JCO 2012* *Gaidzig Blood 2013*
IDH1/2	20-25%	Controversial	
TET2	10-20%	Controversial	

* Adapted from Dhöner K et al., *Hematology* 2014 (ASH Education Program)

Blasts percentage and AML: an unsolved issue



Conclusions

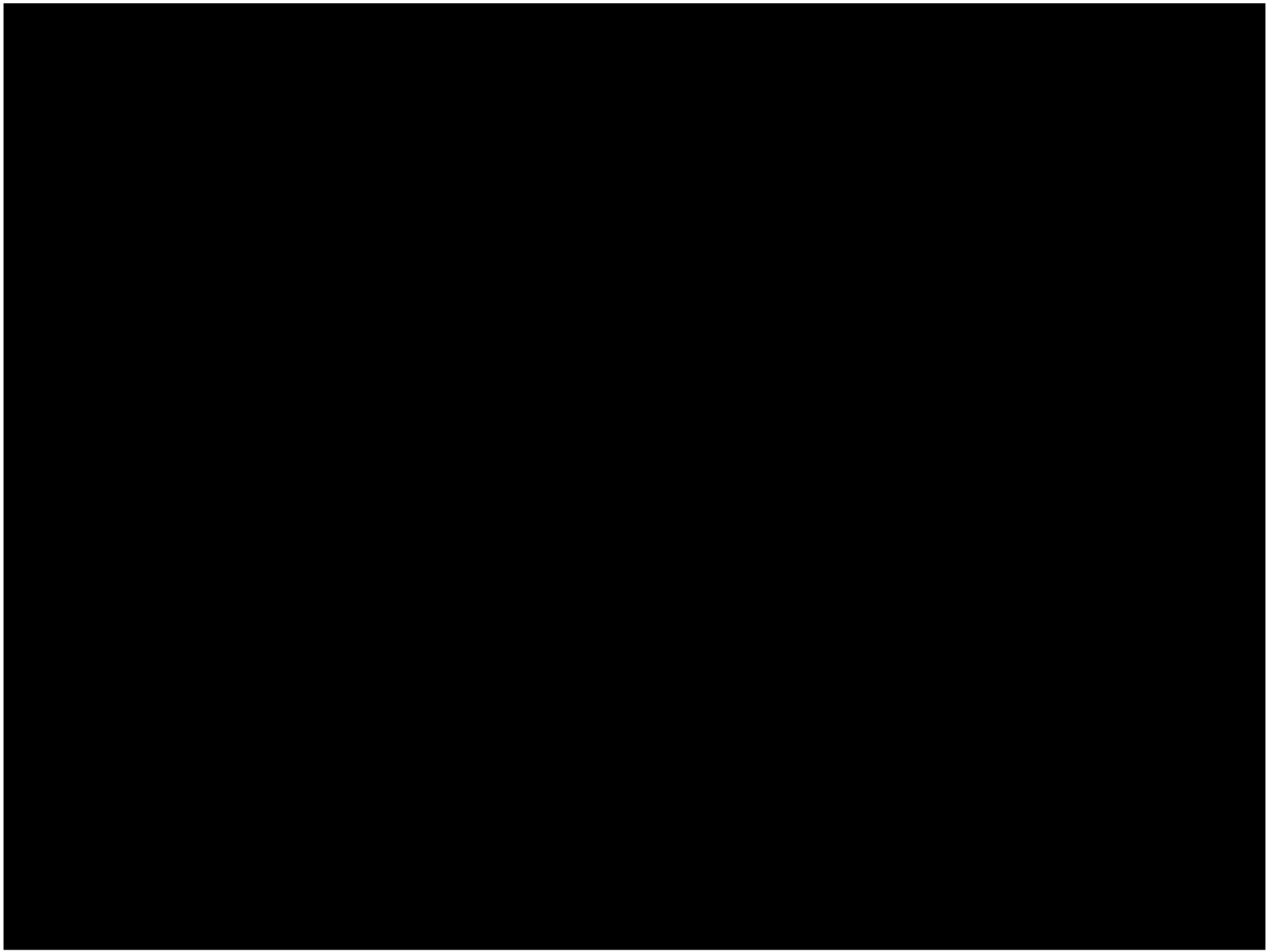
- Shift of *NPM1*-mutated and double-*CEBPA* mutated AML from provisional to distinct entities

- Addition of *RUNX1*-mutated AML and Bcr/Abl as new provisional entities

- Most AML (65%-70%) are now genetically well defined and included in the category of AML with recurrent genetic abnormalities

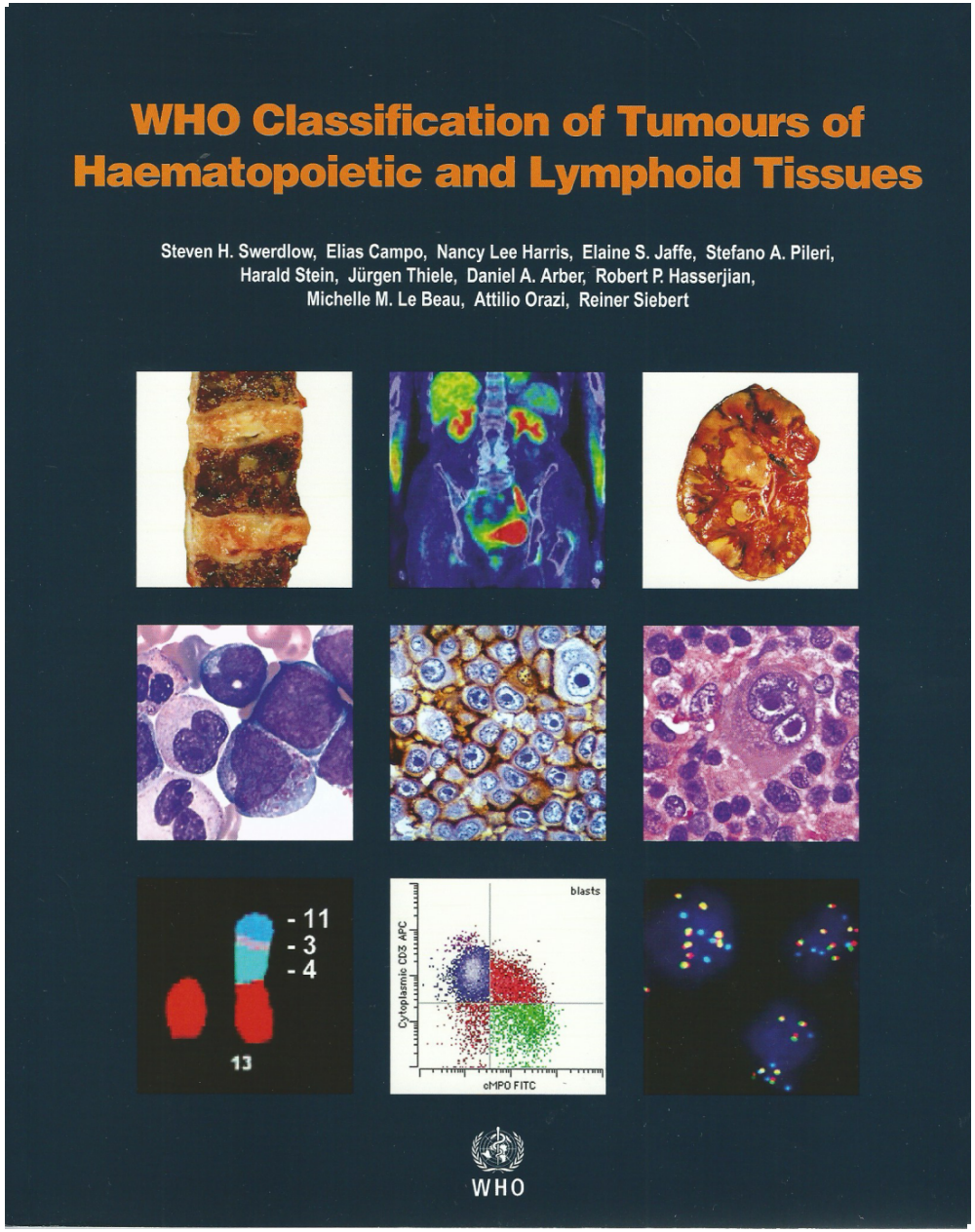
- Refinement of category of AML with myelodysplasia related changes: *NPM1* and double *CEBPA* mutations supersede morphologic criteria, deletion of del(9q)

- Other mutations: no impact in classification, potential prognostic markers and therapeutic targets (*FLT3*, *IDH1/2*)

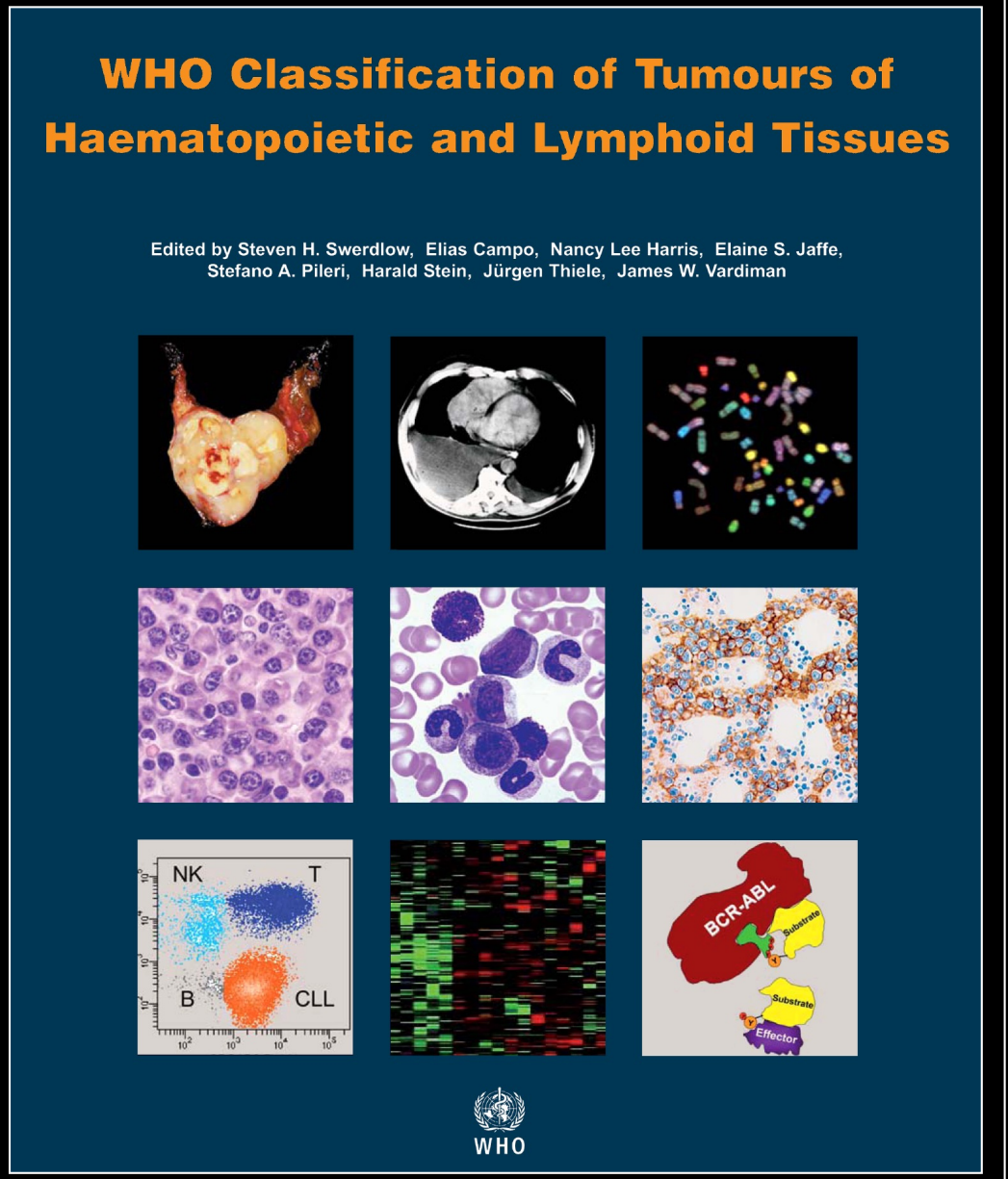


What about new translocations in AML?

WHO Classification (2017)



WHO Classification (2008)



AML categories (2008)

- **AML with recurrent genetic abnormalities**
- AML with MDS-related changes
- -Therapy-related AML/MDS
- AML not otherwise specified (NOS)
- Myeloid sarcoma
- Myeloid proliferations related to Down sindrome

Criteria for diagnosis of AML with myelodyslasia-related changes (WHO 2016)

1. Multilineage dysplasia (50% or more dysplastic cells in at least 2 cell lines (only in the absence of *NPM1* or biallelic *CEBPA* mutations)

2. MDS-related cytogenetic abnormality (with the exception of del(9q)

3. History of myelodysplasia

Cytogenetics abnormalities sufficient for diagnosis of AML with myelodysplasia-related changes

Complex karyotype (≥ 3)

Unbalanced abnormalities

- 7 or del(7q)
- 5 or del(5q)
i(17q) or t(17p)
-13 or del(13q)
del(11q)
del(12p) or t(12p)
~~del(9q)~~
idic(X)(q13)

Balanced abnormalities

t(11;16)(q23;p13.3)
t(3;21) (q26.2;q22.1)
t(1;3)(p36.3;q21.1)
t(2;11)(q21;q23)
t(5;12)(q33;p12)
t(5;7)(q33;q11.2)
t(5;17)(q33;p13)
t(5;10)(q33;q21)
t(3;5)(q25;q34)