Acute myeloid leukemia

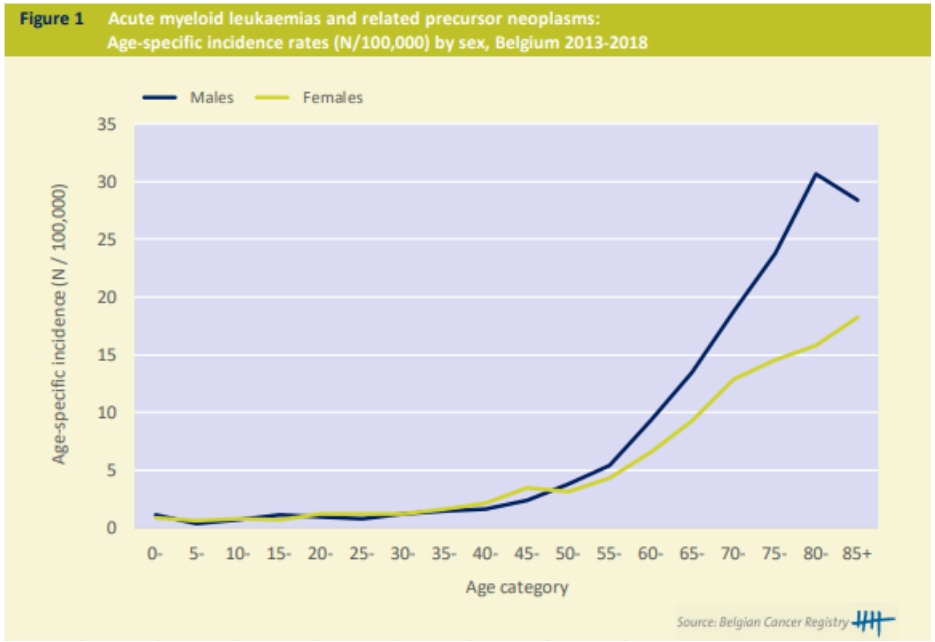
the molecular pathogenesis

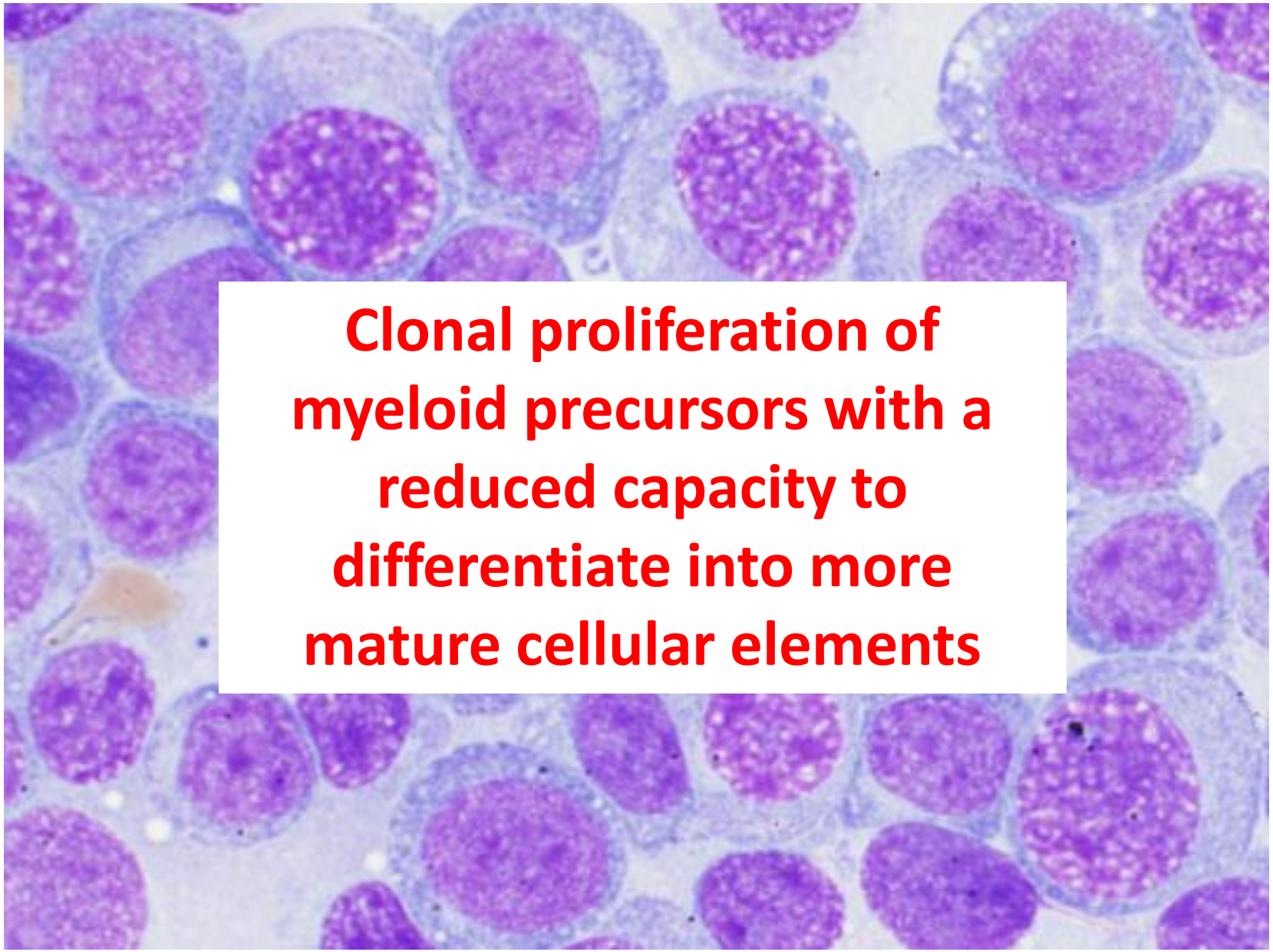
Pr Violaine Havelange, MD, PhD

Department of hematology

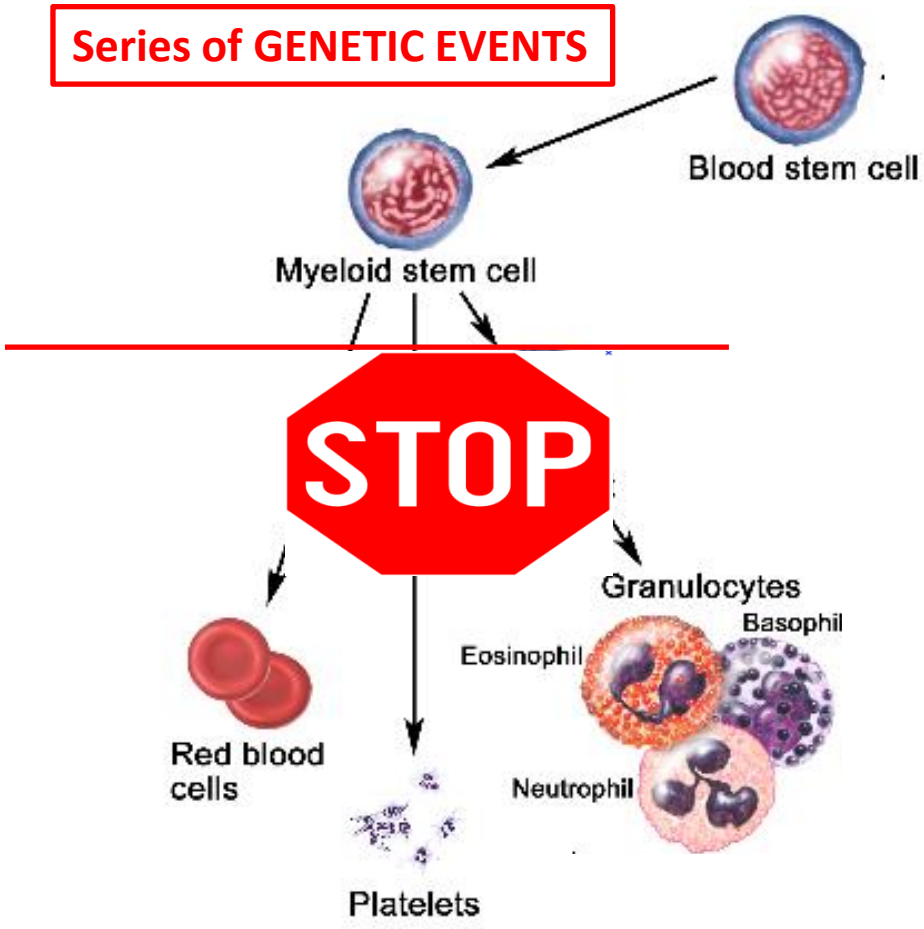
Acute myeloid leukemia (AML)

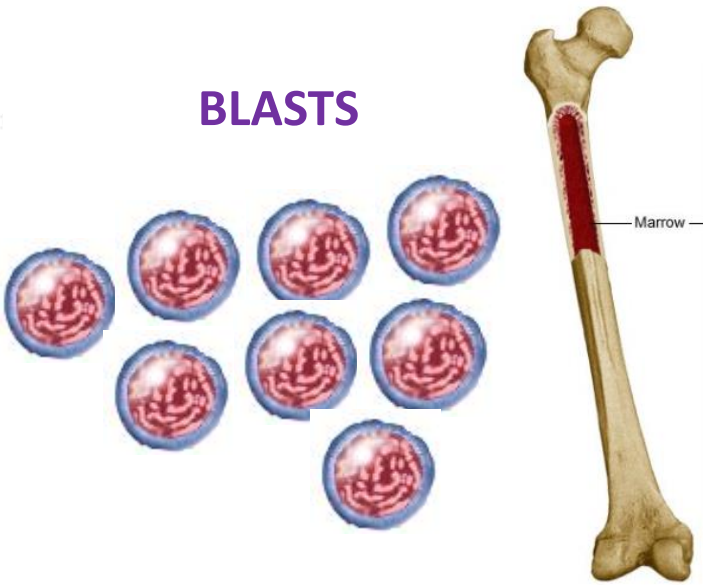
- **Incidence : 3-5 cases/100.000/year**
- **80% of acute leukemias in adults**
- **Median age : 65 years**




Clonal proliferation of myeloid precursors with a reduced capacity to differentiate into more mature cellular elements

PHYSIOPATHOLOGY





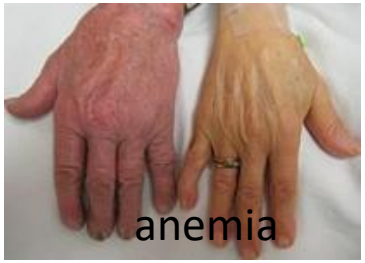
Accumulation of leukemic blasts or immature forms in BM, PB, other tissues

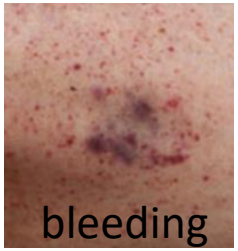
+

Reduction in the production of normal red blood cells, platelets, granulocytes

CLINICAL SYMPTOMS

- complications of pancytopenia

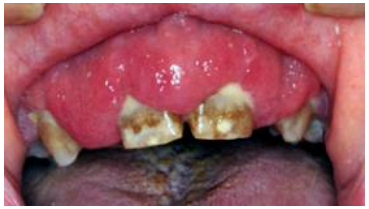

anemia

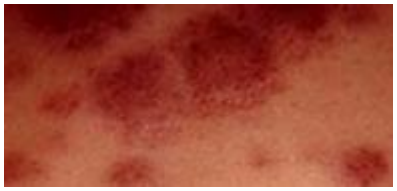

bleeding


increased risk of infections

- Extramedullary locations

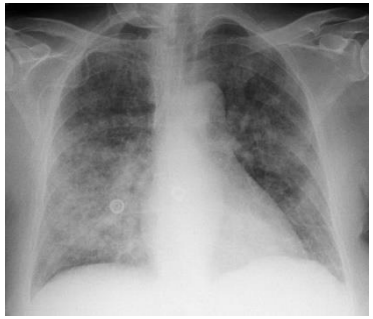
skin, CNS, oropharynx, organomegaly, joints, myeloid sarcomas





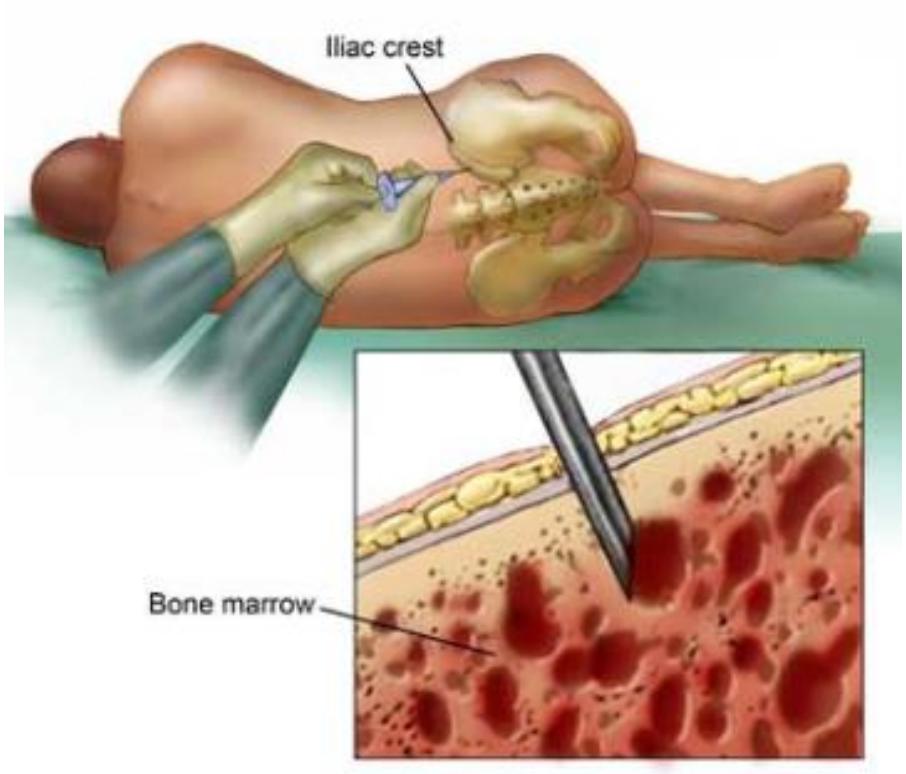
- Symptoms of leukostasis if extremely high white blood cell counts

fever, lung, CNS, heart



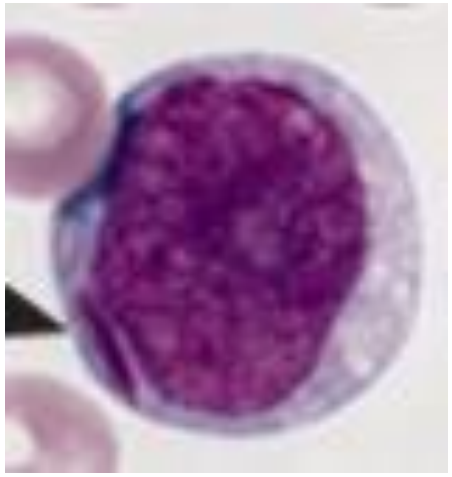
DIAGNOSIS

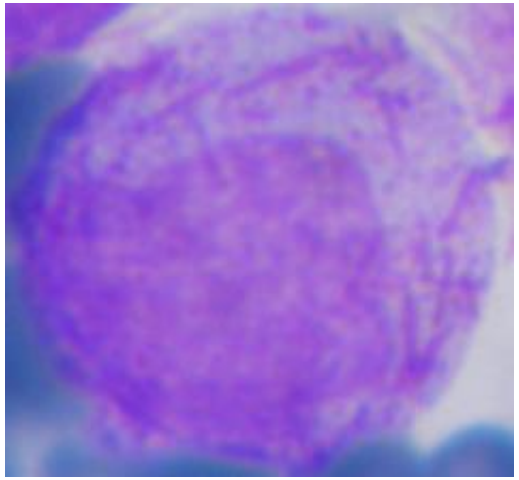
Bone marrow aspirate/biopsy



Mayo fondation for medical and research

MORPHOLOGY





staining with Wright Giemsa

blasts

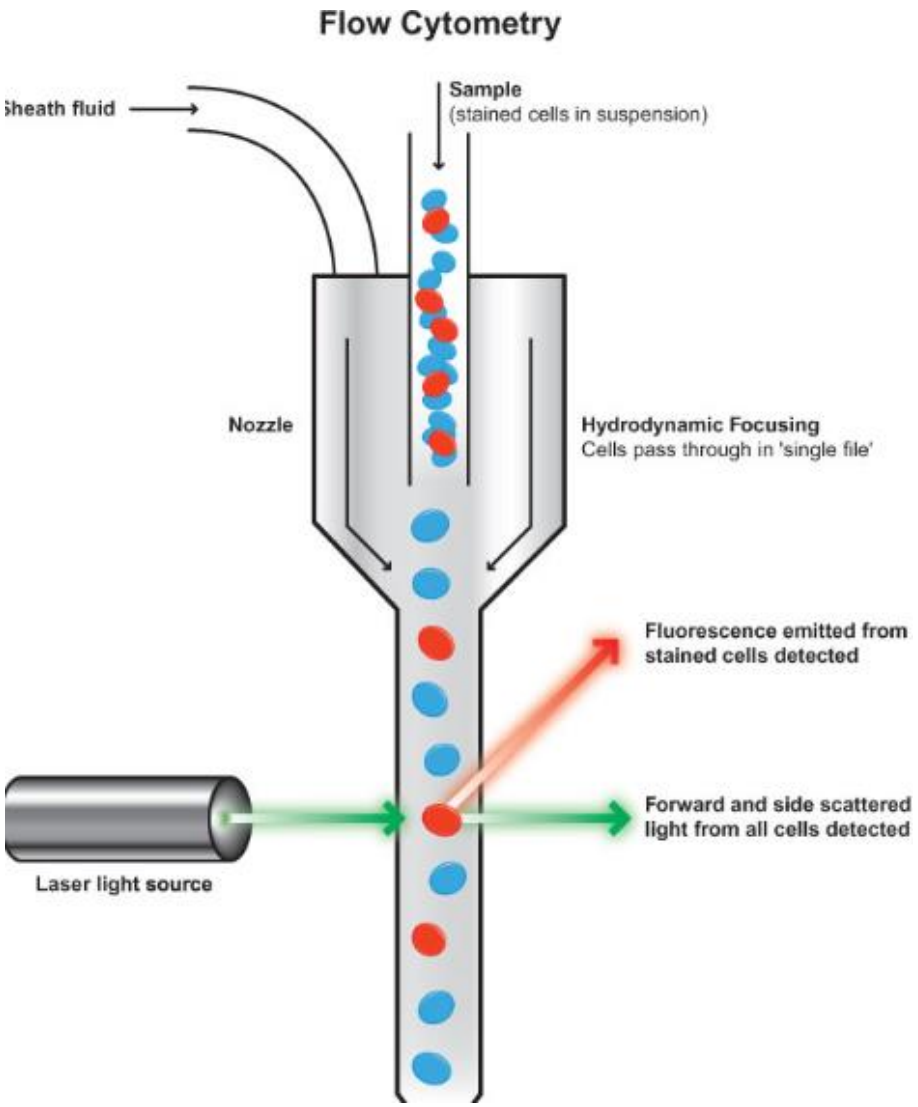
> 20%

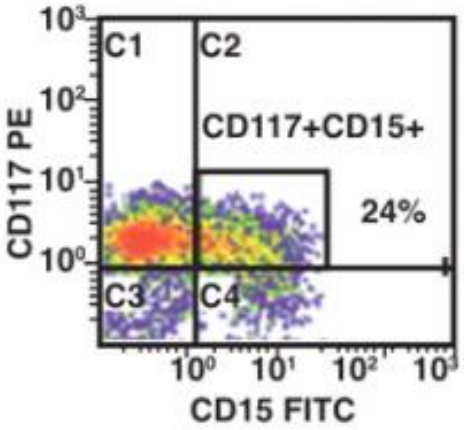
immature cells with large nuclei, with prominent nucleoli
pale blue cytoplasm

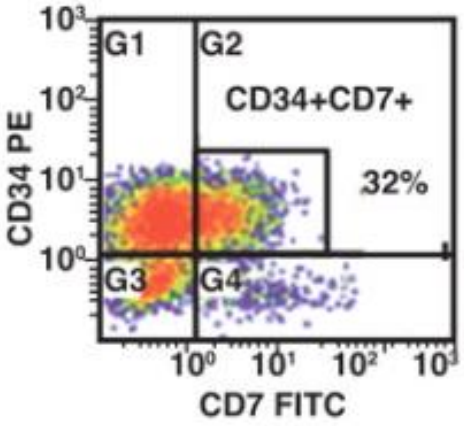
'auer rod' = pathognomonic of myeloblasts

Cytochemical studies : + sudan black B,
myeloperoxidase or esterase

IMMUNOPHENOTYPING







CD34+

HLA-DR +

CD117 +

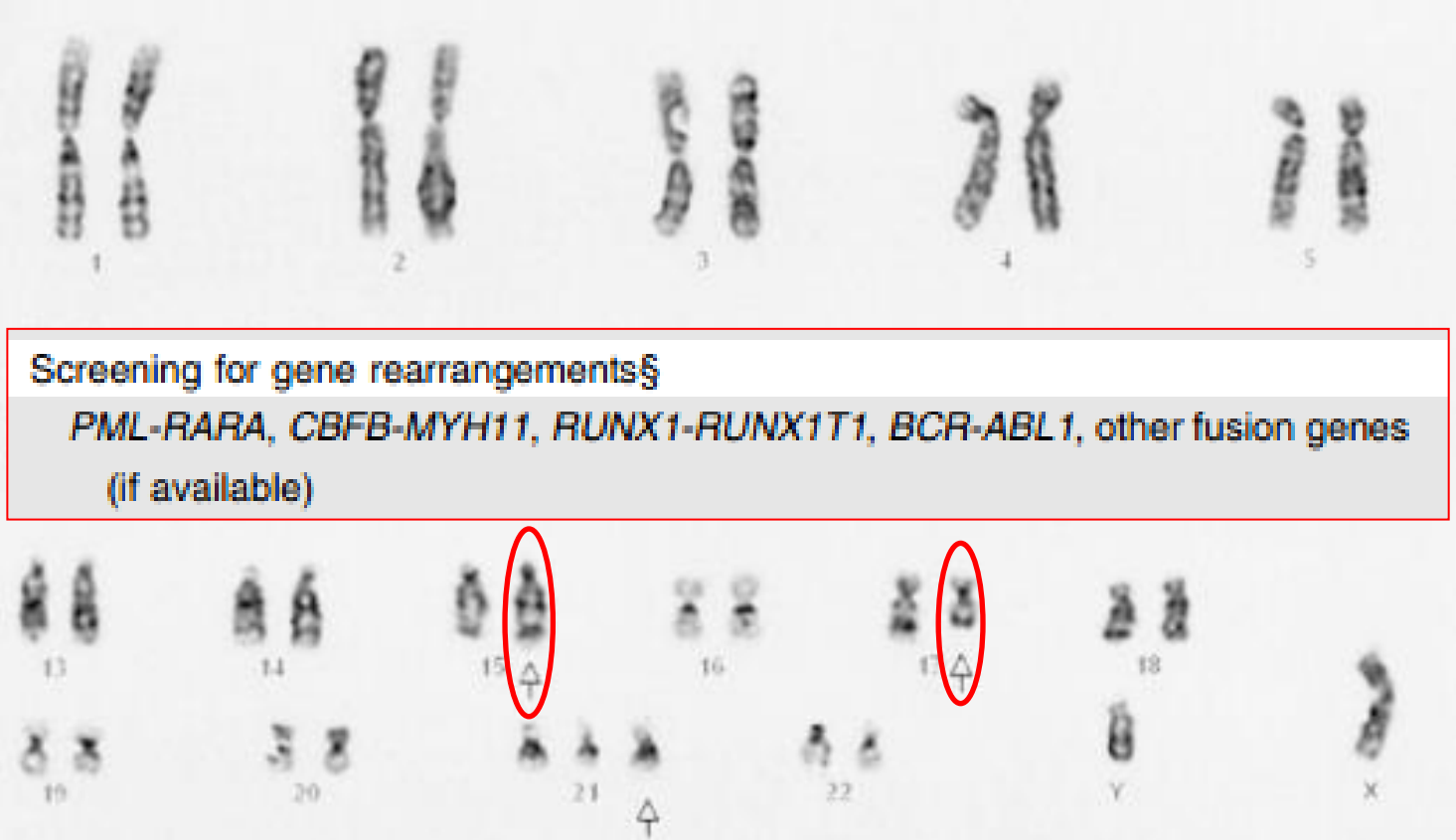
CD13 +

CD33 +

Mawali et al, Am J Clin Path 2009

CYTOGENETICS (karyotype and FISH)

recurrent cytogenetic abnormality in 55% of AML patients



t(15;17) in AML M3

(Acute Promyelocytic Leukemia)

MOLECULAR TESTINGS

Fusion transcripts : AML1/ETO; t(8;21)

PML/RARA; t(15;17)

CBFB/MYH11; inv(16)

Tandem internal duplication of *FLT3*

Internal duplication of *KMT2A*

Mutation of *CEBPalpha*

MOLECULAR TESTINGS

NGS Next Generation Sequencing

ASXL1 (exon 13)	*pronostic*
CEBPA (exon 1)	*diagnostic/pronostic*
DNMT3A (exon 8-23)	*diagnostic/pronostic*
FLT3 (exon 14, exon 15, exon 20-codon 835)	*pronostic/treatement*
IDH1 (exon 4-hotspot)	*pronostic/treatement*
IDH2 (exon 4-hotspot)	*pronostic/ treatement*
KIT (exon 8, exon 10, exon 17)	*pronostic/ treatement*
NPM1 (exon 11-codon 288)	*diagnostic/pronostic*
RUNX1 (exon 2-9)	*diagnostic/pronostic*
TET2 (exon 3, exon 9-11)	*diagnostic/pronostic*
TP53 (exon 2-11)	*pronostic/ treatement*
WT1 (exon 7, exon 9)	*pronostic*

WHO 2016

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber,[1] Attilio Orazi,[2] Robert Hasserjian,[3] Jürgen Thiele,[4] Michael J. Borowitz,[5] Michelle M. Le Beau,[6] Clara D. Bloomfield,[7] Mario Cazzola,[8] and James W. Vardiman[9]

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

DIAGNOSTIC

Arber et al, Blood 2016

Diagnosis and Management of AML in Adults: 2017 ELN Recommendations from an International Expert Panel

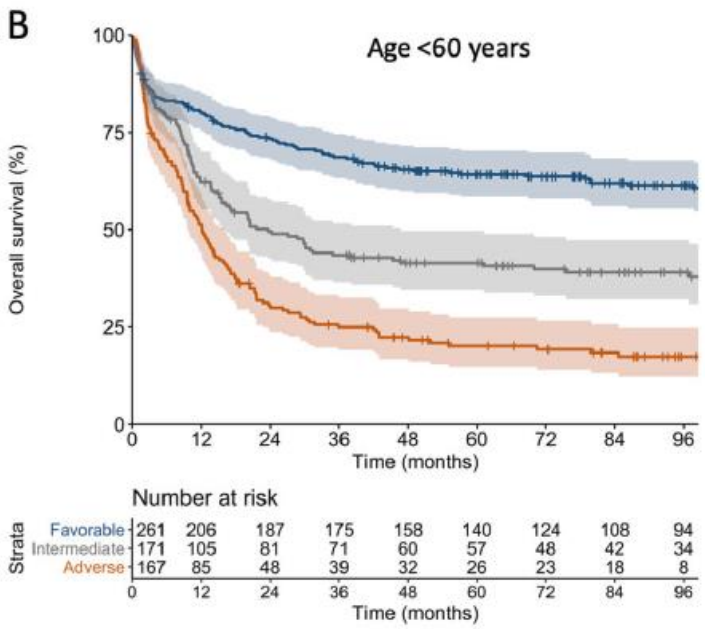
PROGNOSIS - TREATMENT

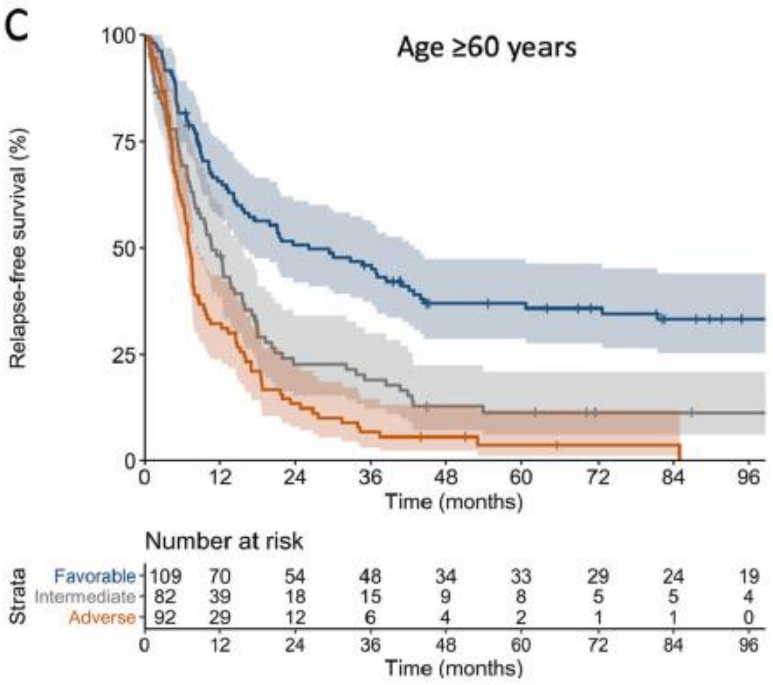
Table 5. 2017 European LeukemiaNet risk stratification by genetics[a]

Risk Category[b]	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 or with *FLT3*-ITD$^{low(c)}$ Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD$^{high(c)}$ Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ (w/o adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[d] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,[e] monosomal karyotype[f] Wild type *NPM1* and *FLT3*-ITD$^{high(c)}$ Mutated *RUNX1*[g] Mutated *ASXL1*[g] Mutated *TP53*[h]

Dohner et al, Blood 2017

PROGNOSIS





Herold et al, Leukemia 2020

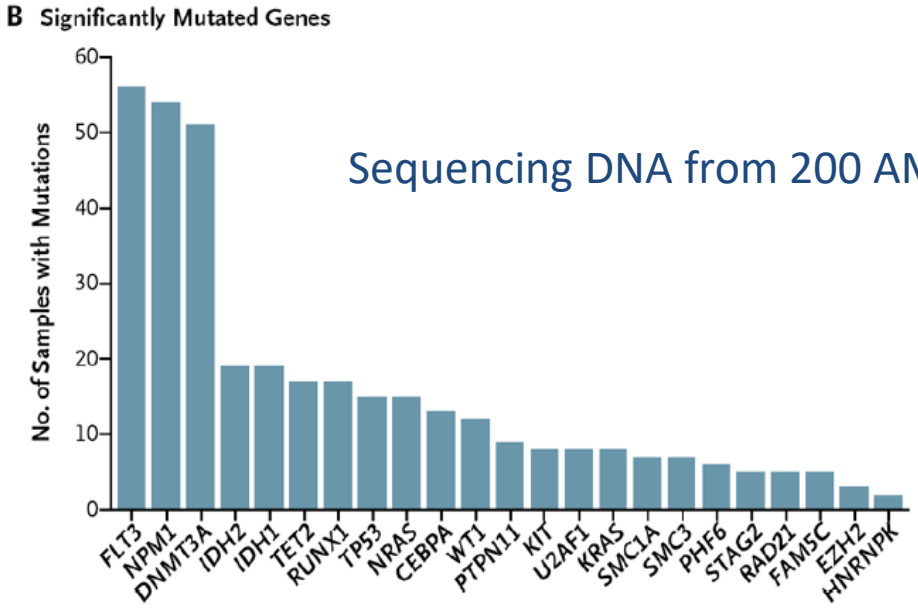
To understand the molecular pathogeneis of AML

Larges sequencing studies -> genetic heterogeneity
molecular pathogenesis of AML

Fewer mutations in AML genome …

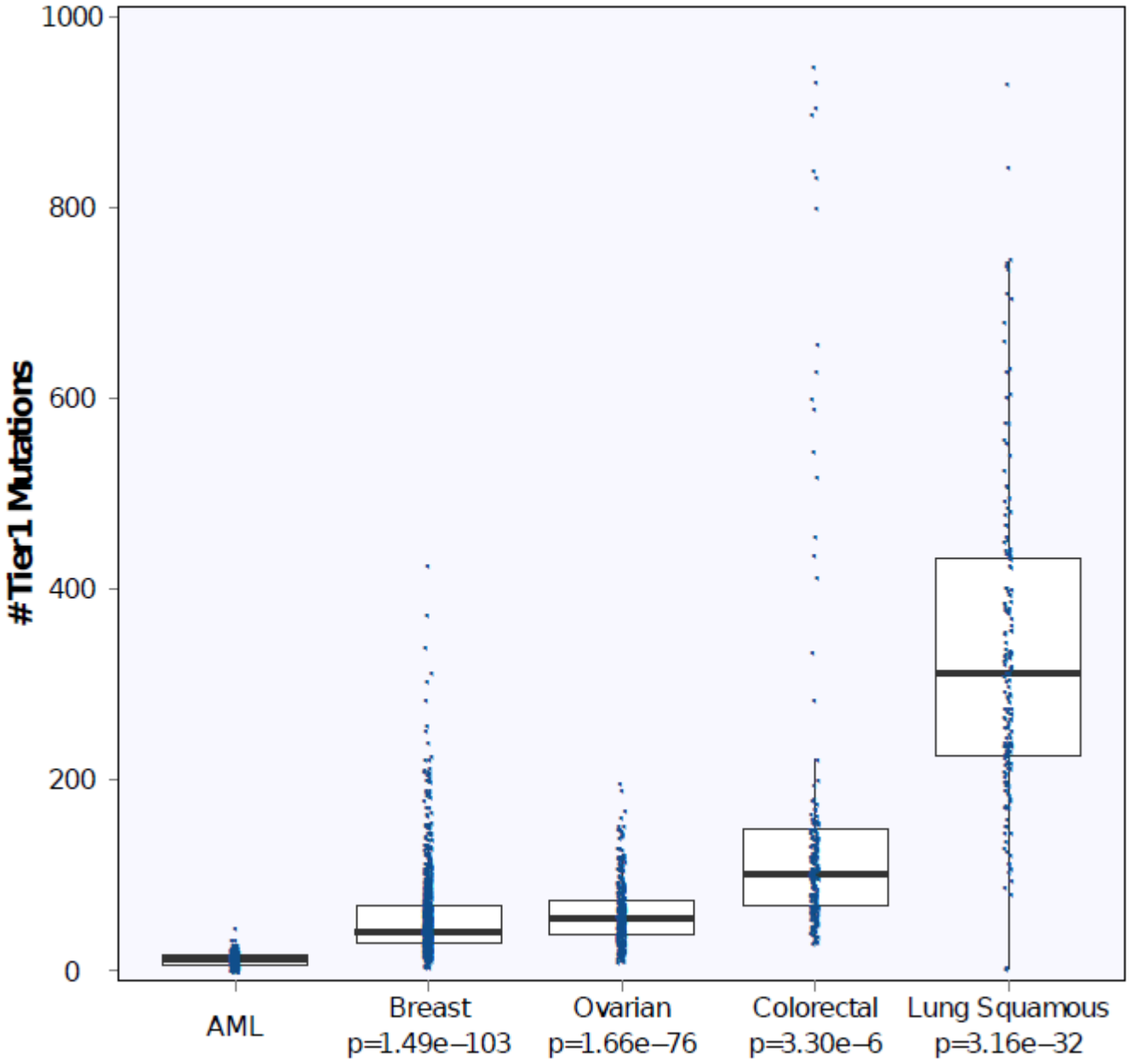
+- 13 mutations per patient – 5 in genes recurrently mutated in AML

23 genes recurrently mutated - and 237 genes mutated in ≥ 2 patients

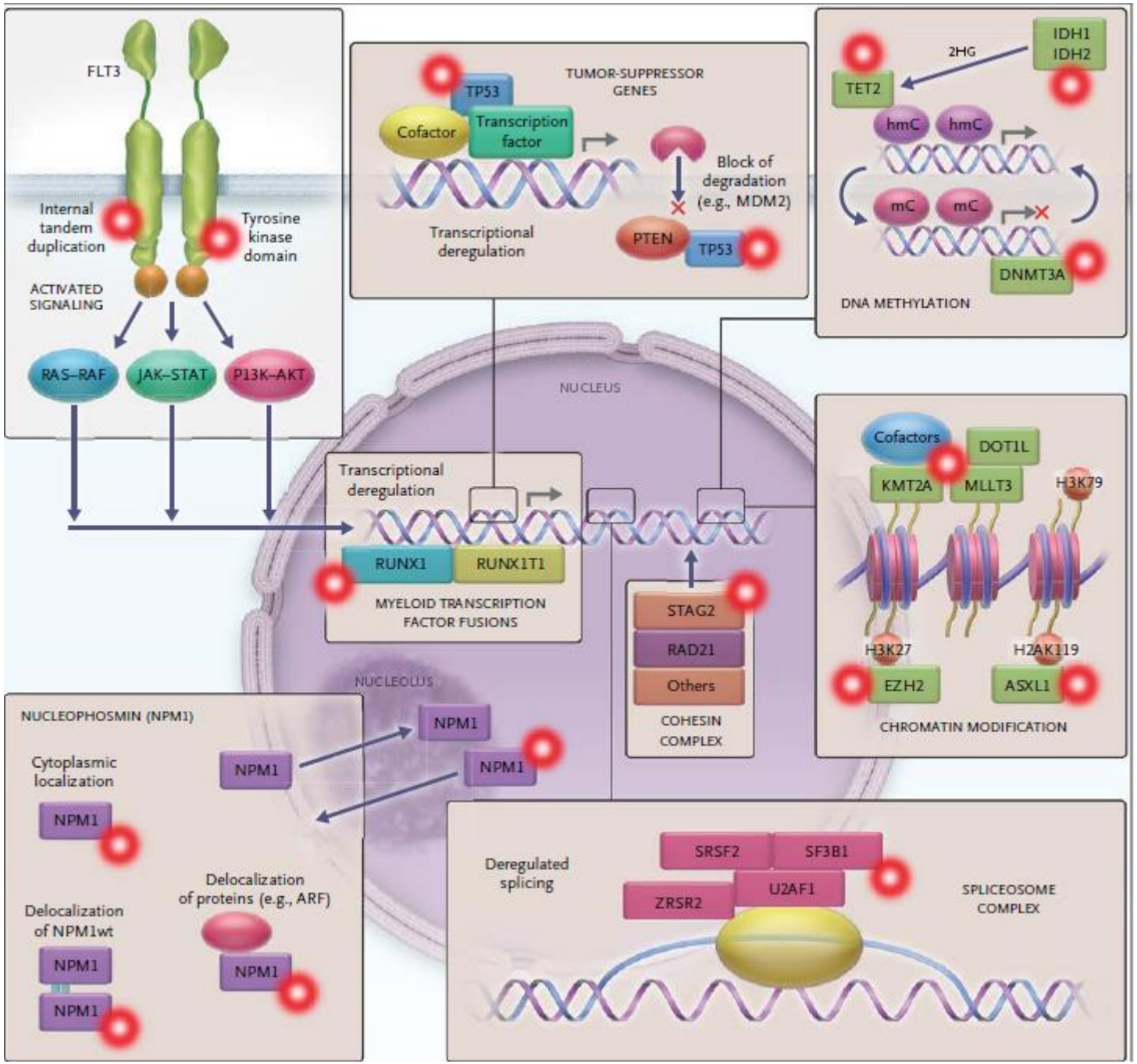


Sequencing DNA from 200 AML – BM and skin

Cancer Genome Atlas Research Network, NEJM 2013

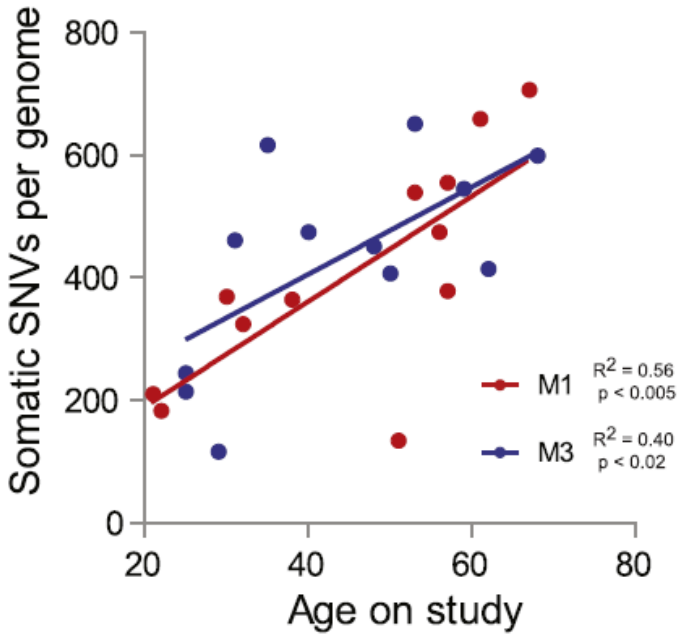


N Engl J Med 2013; 368:2059-2074



Dohner et al, NEJM 2015

Sequencing DNA of BM/skin from 12 AML M3 - t(15;17) /12 AML M1 normal KT



- 100s of MUTATIONS/ patient
- total number increases with AGE
- +- in all AML cells
- widely distributed in the genome
- similar numbers in M3 and M1
- very few are RECURRENT

Most of the mutations in founding clone

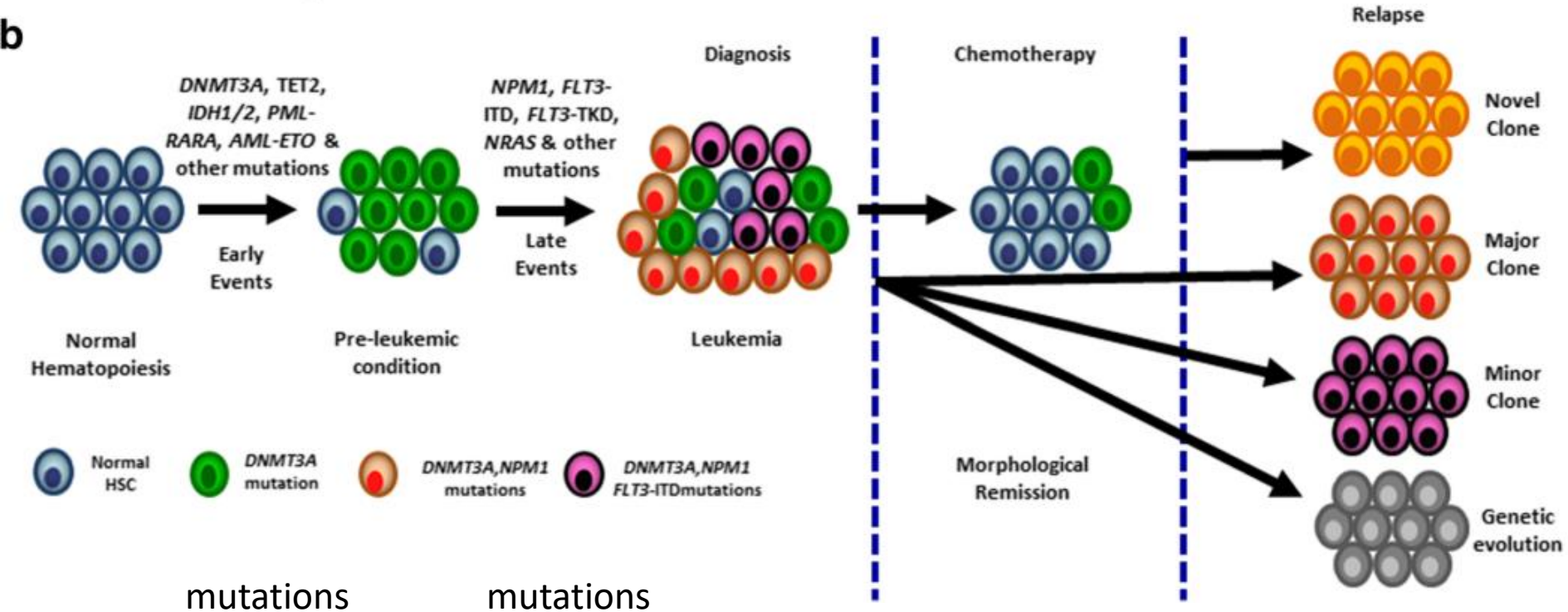
PASSENGER mutations : random events preexisted in HSC – lacking functional consequences

Acquisition of the **DRIVER mutation** (cancer initiating mutation) **-> growth advantage**

Welch et al, Cell 2012

AML = multistep clonal evolutionary process

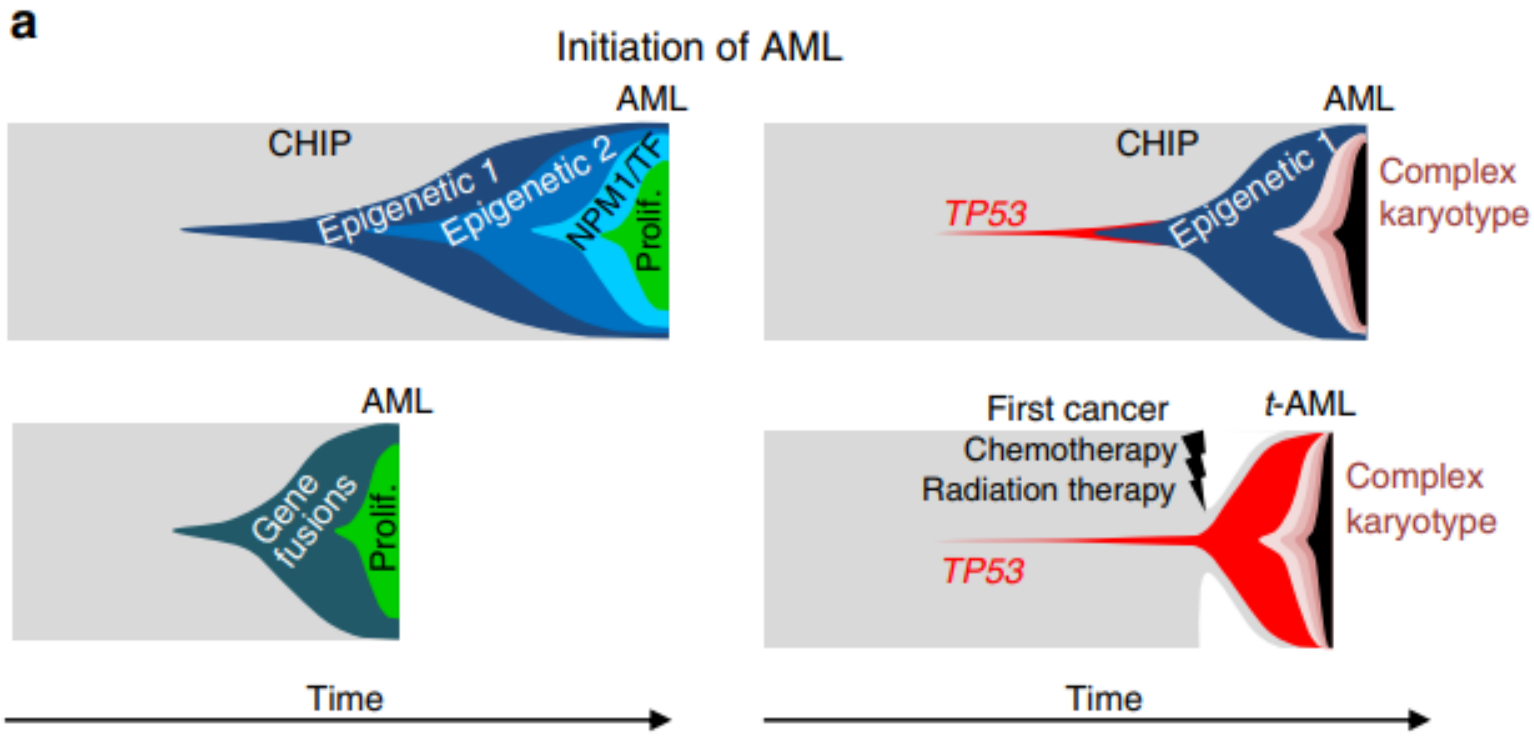
RELAPSE ?



mutations ‘landscape’

mutations ‘proliferation’

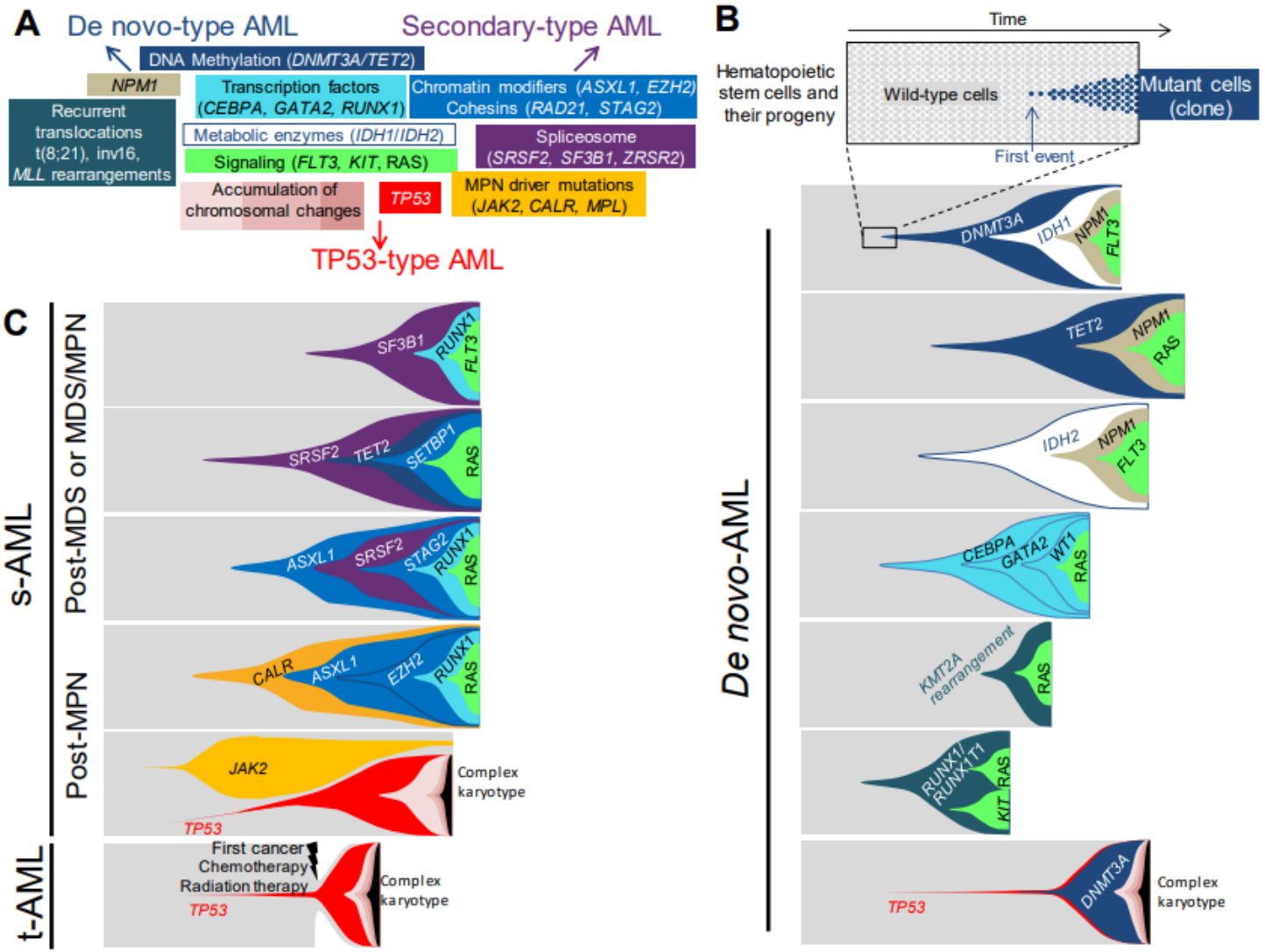
Kirtonia, JCMM 2020



initiating pre-leukaemic lesions

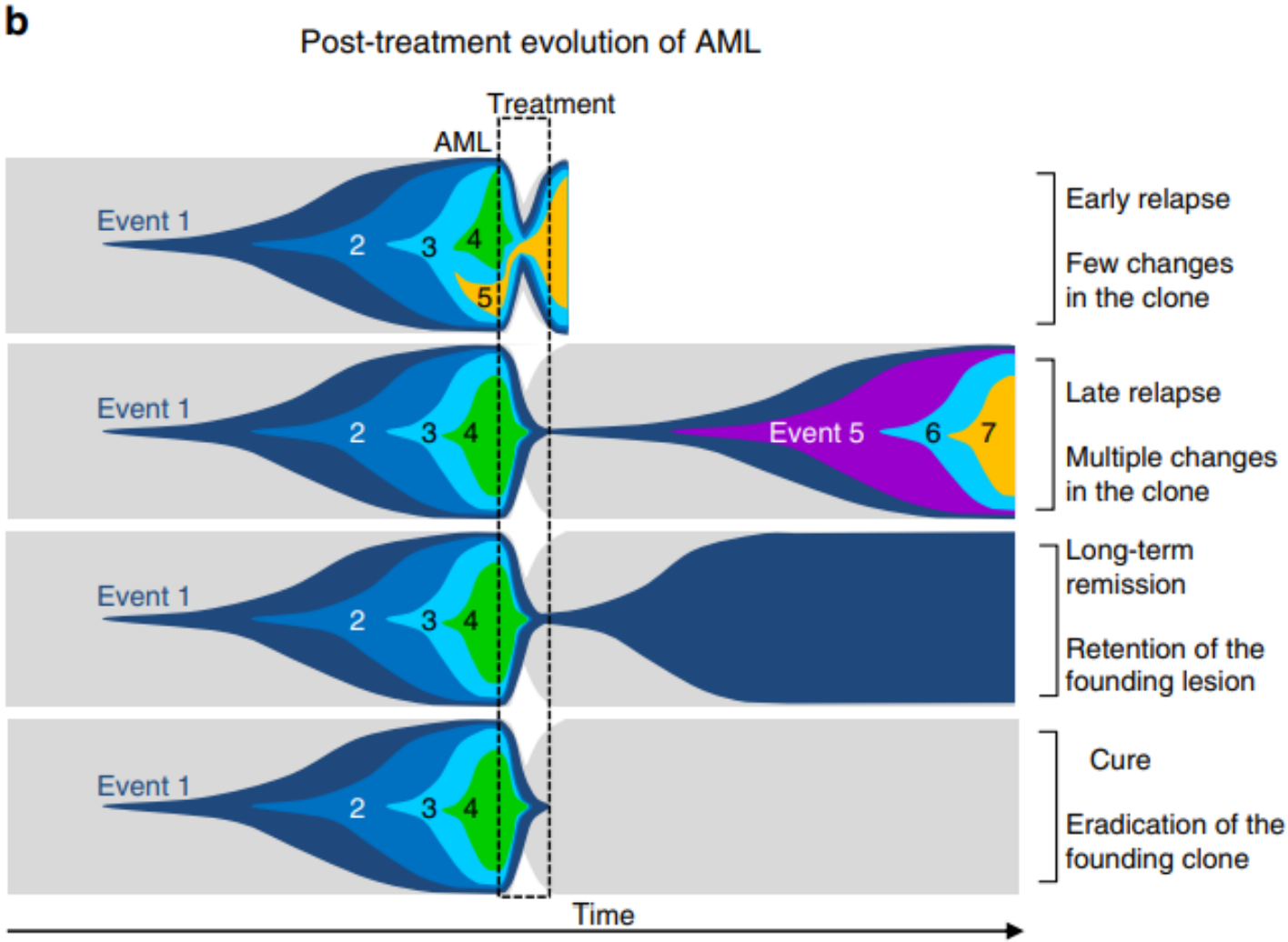
- early occurrence in the clone
- persistence at relapse
- ability to initiate multilineage haematopoietic repopulation and leukaemia *in vivo*

Hirsch, nature com 2016



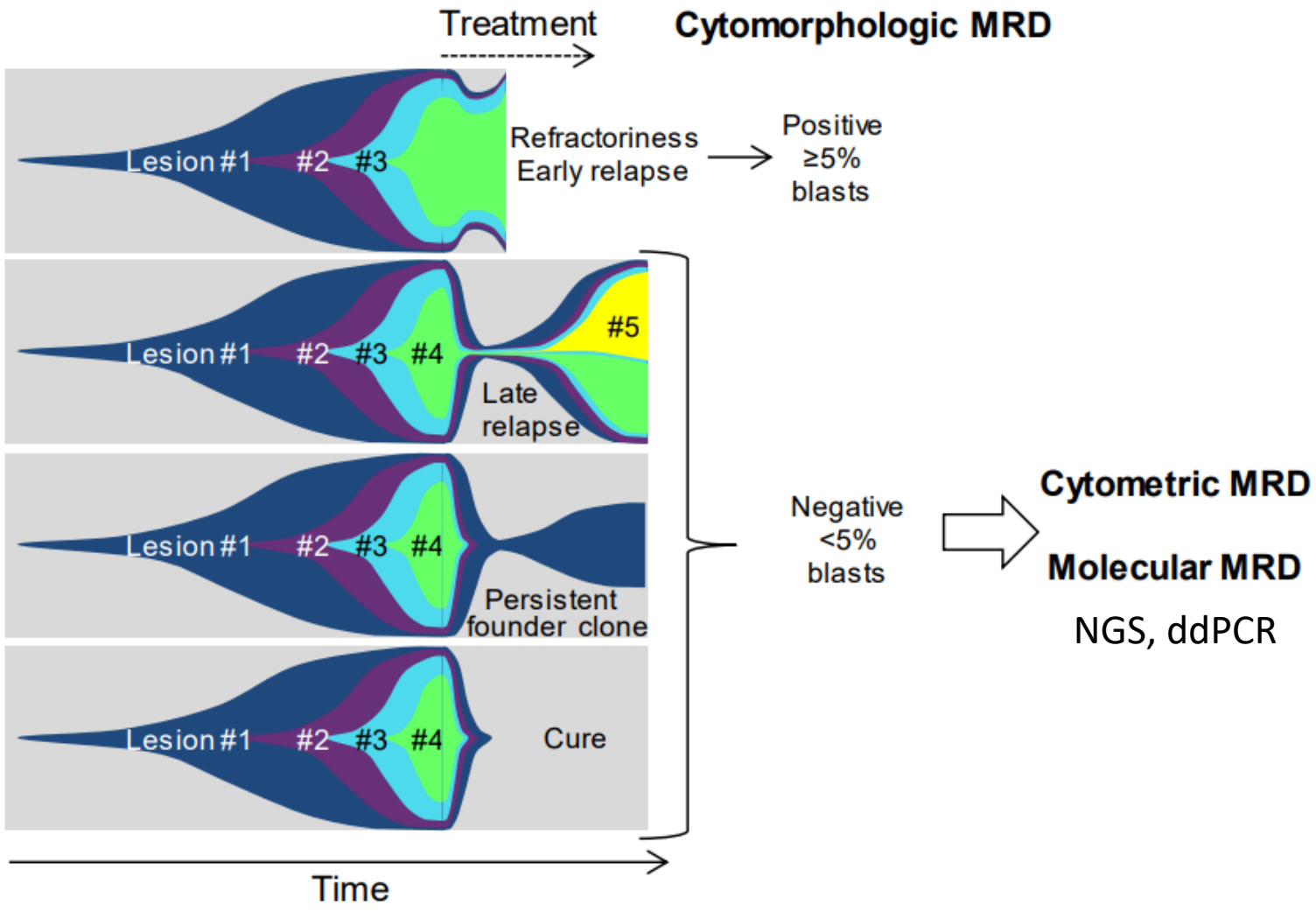
Martignoles, IJMS 2018

-> therapy : **relapses**/MRD/ targeted therapies

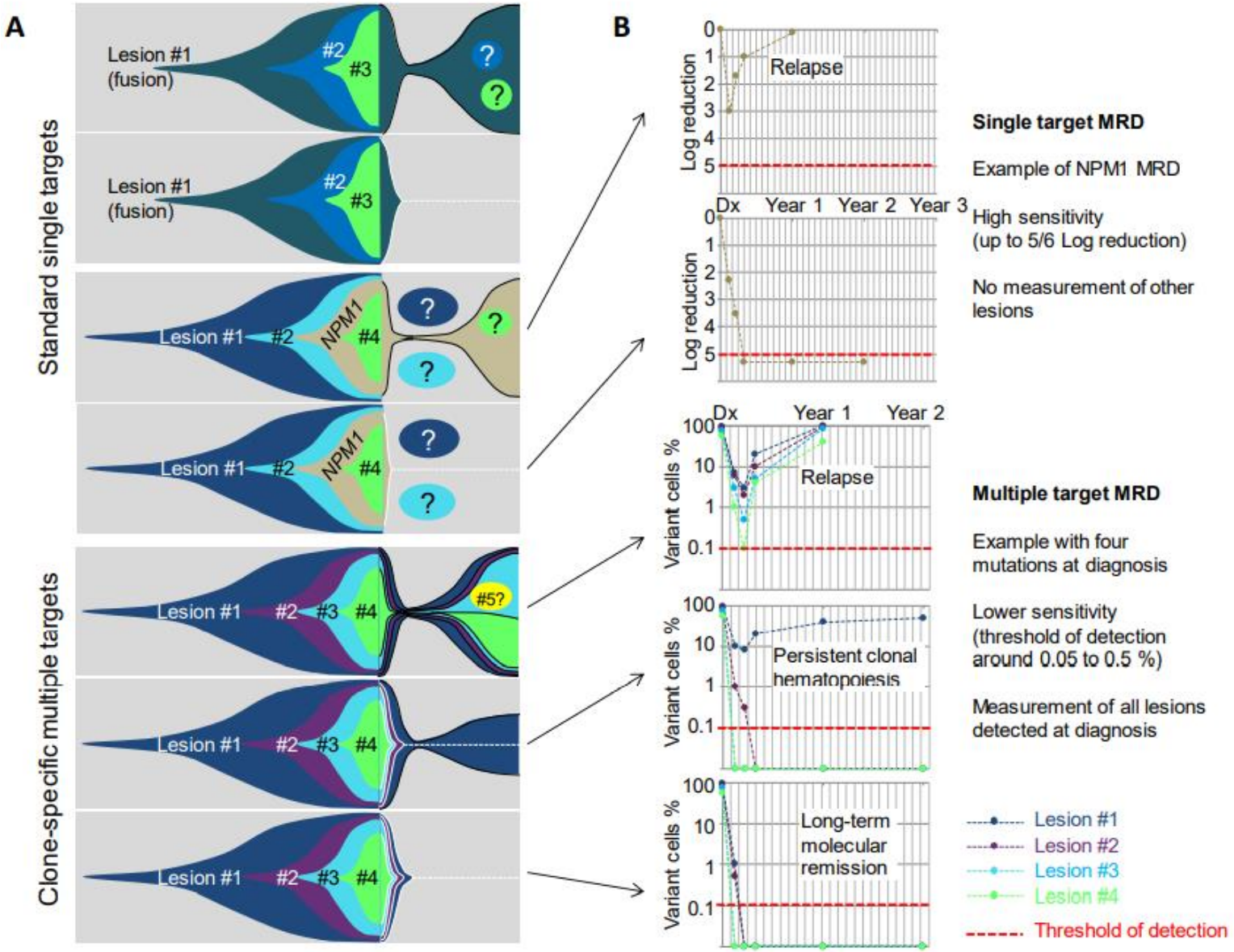


Hirsch, nature com 2016

-> therapy : relapses/**MRD**/ targeted therapies

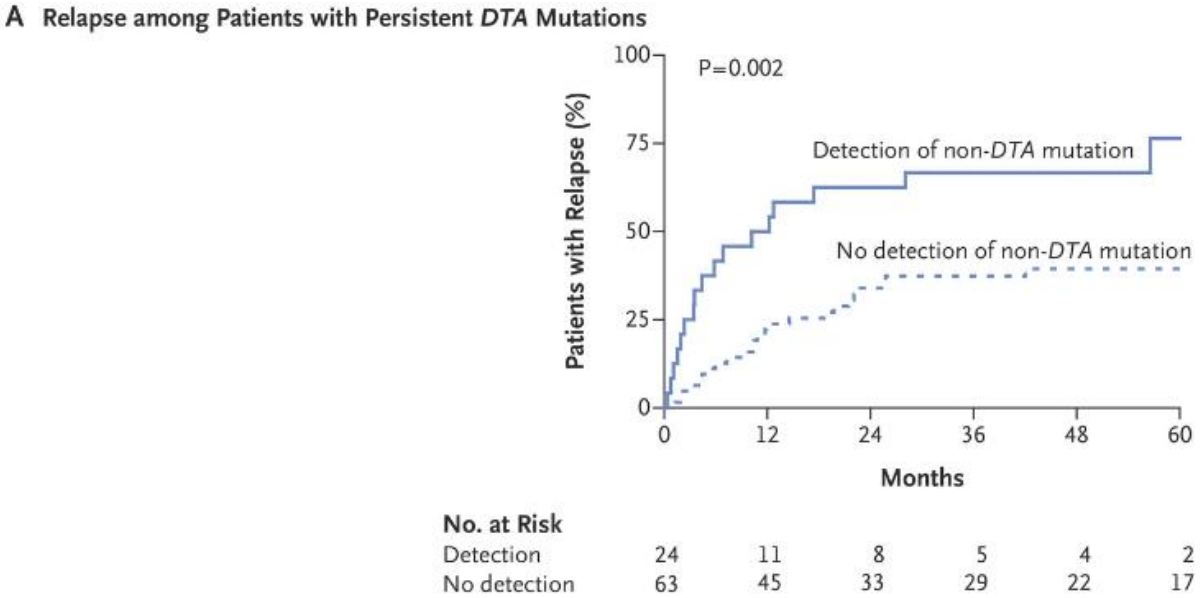


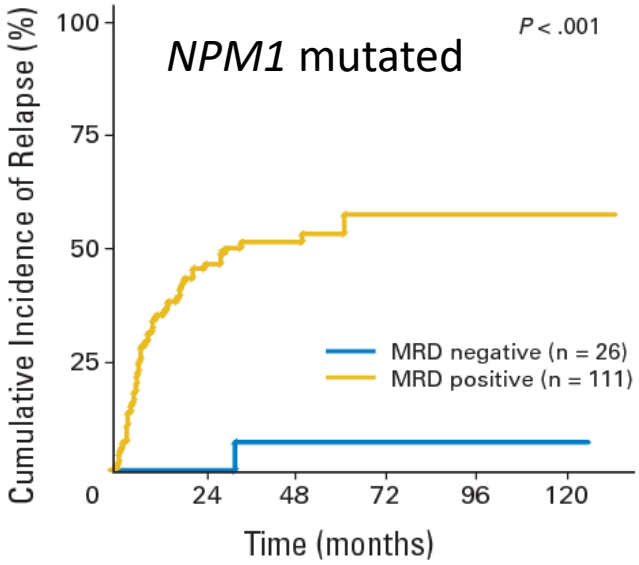
Martignoles, IJMS 2018



Martignoles, IJMS 2018

Molecular MRD associated with relapse



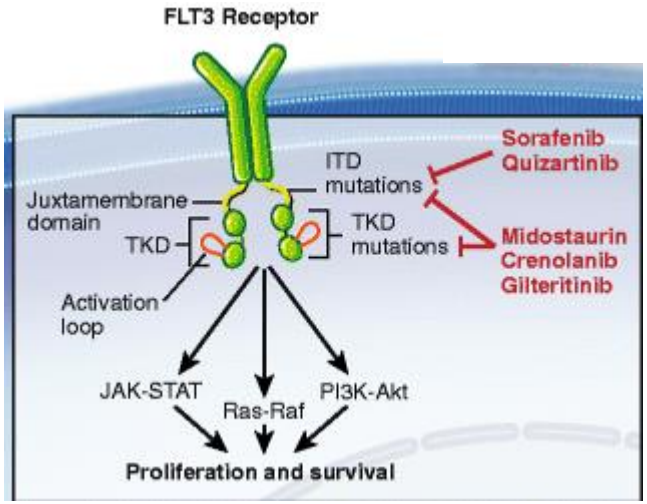


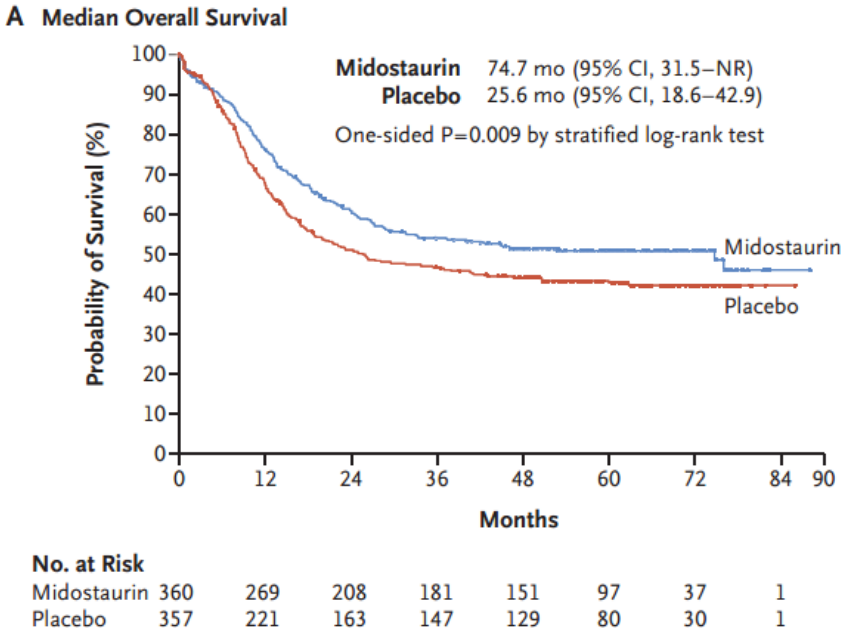
Jongen-Lavrencic et al, NEJM 2018

Kronke et al, JCO 2011

-> therapy : relapses/MRD/ **targeted therapies**

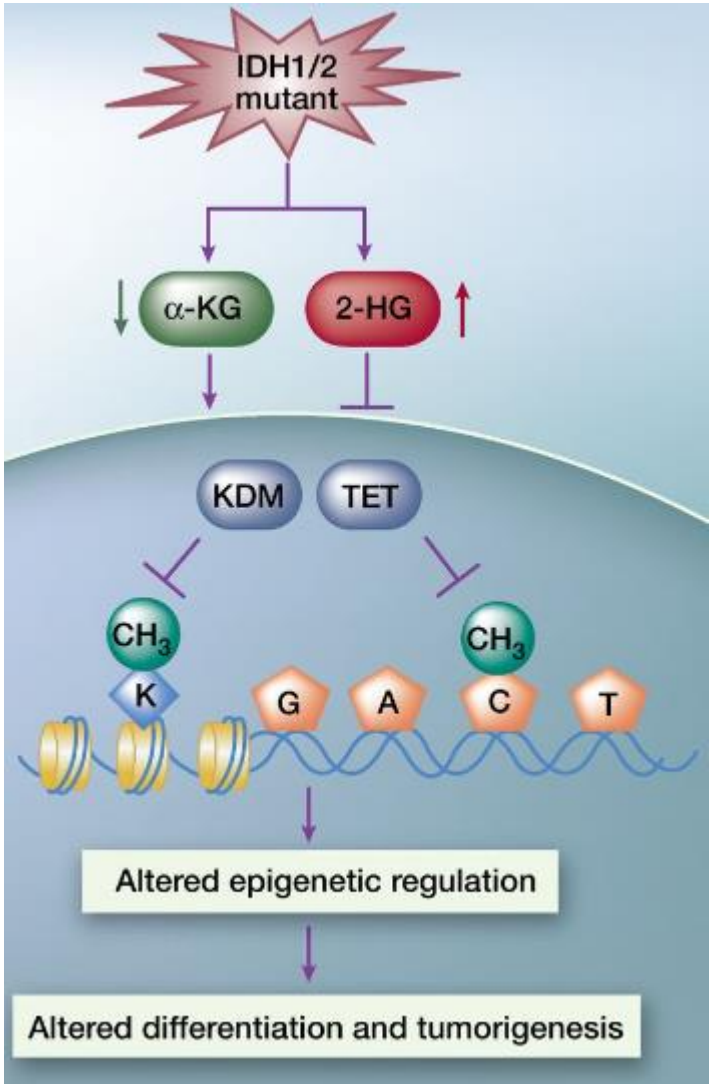
FLT3 inhibitors



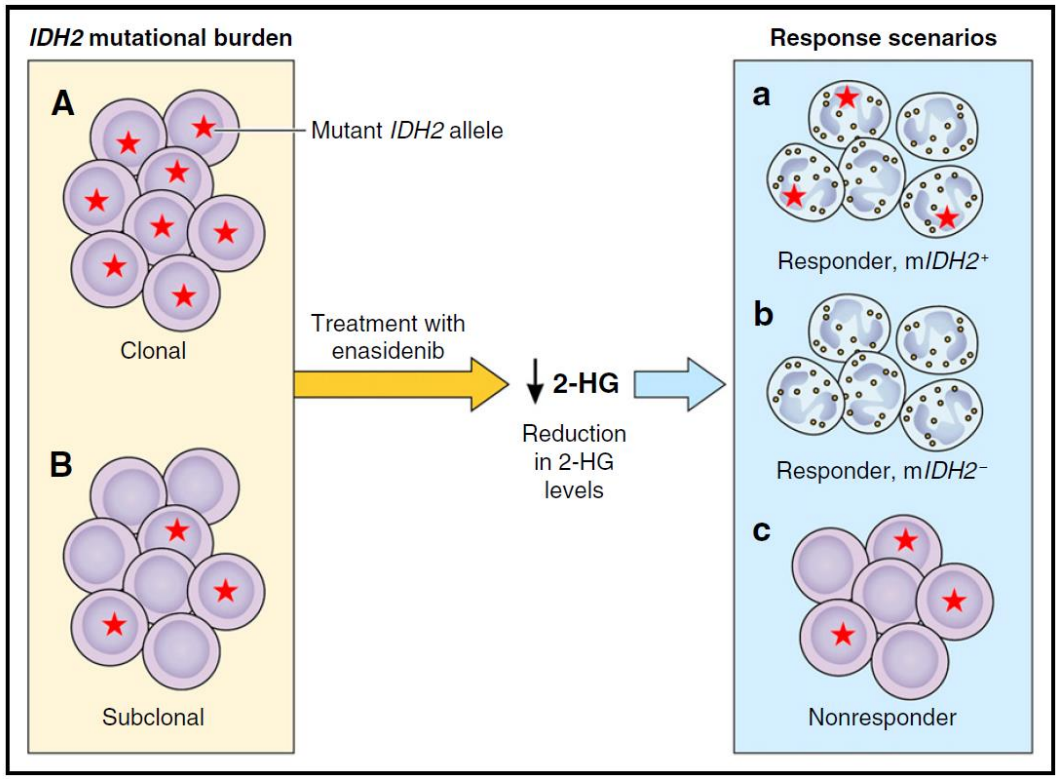


Stone et al, NEJM 2017

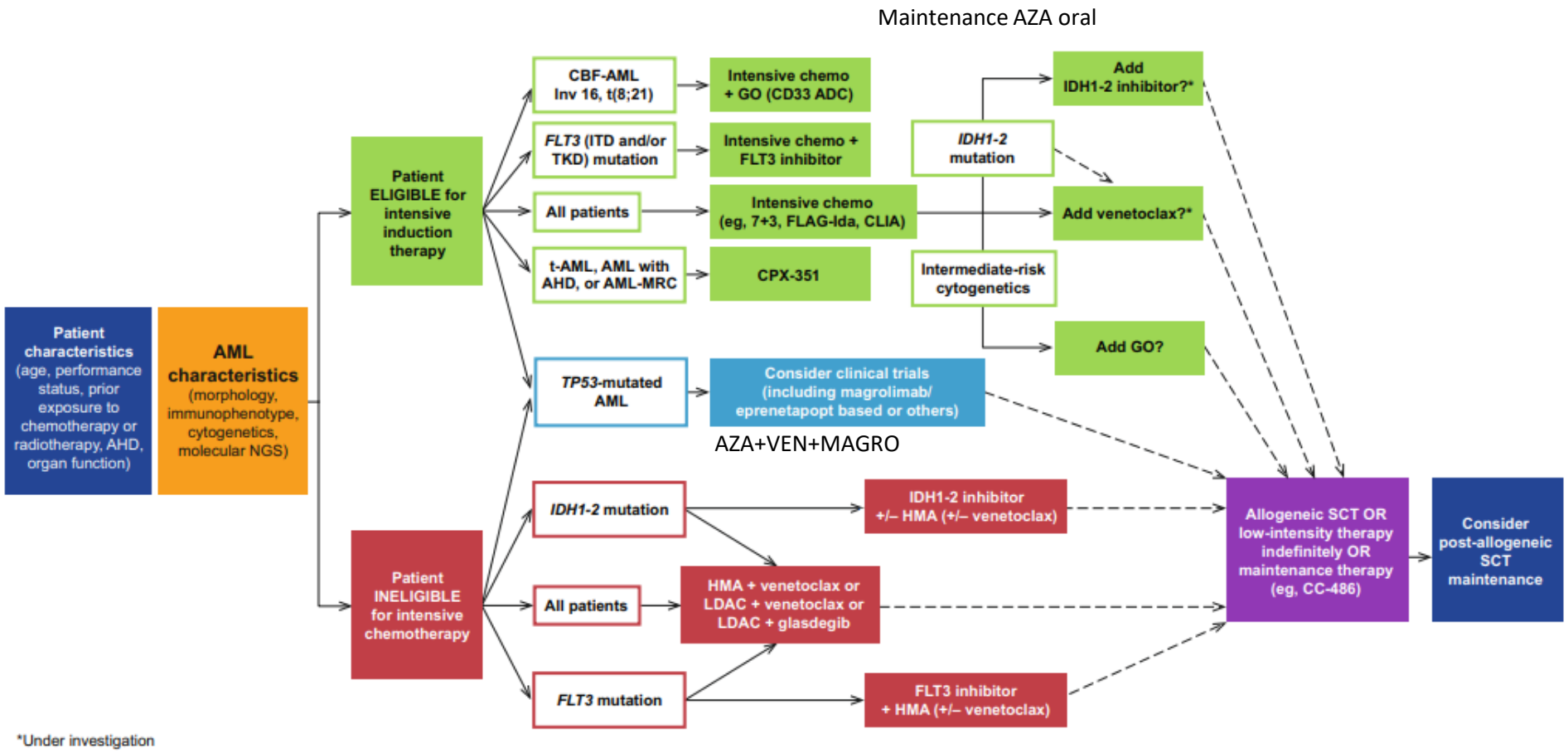
IDH1,2 inhibitors



Yang et al, CCR 2012



Wouters et al, Blood 2017



Daver et al. Blood Cancer Journal (2020)

To understand the 'preleukemic states'

* CHIP/CHOP

Clonal Hematopoiesis : a population of blood/bone marrow cells that share an acquired mutation

CHIP : Clonal Hematopoiesis of Indeterminate Potential

Somatic mutations associated with AML/MDS in healthy subjects

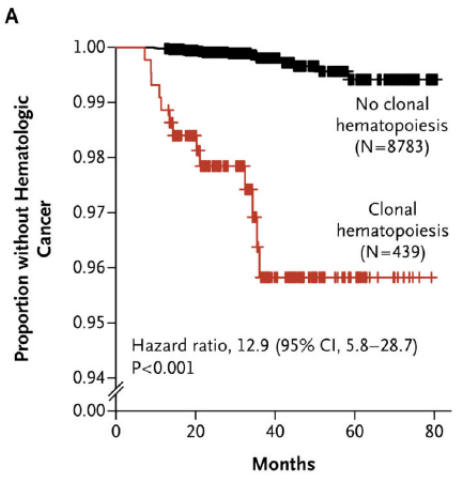
Minimal VAF of 2%

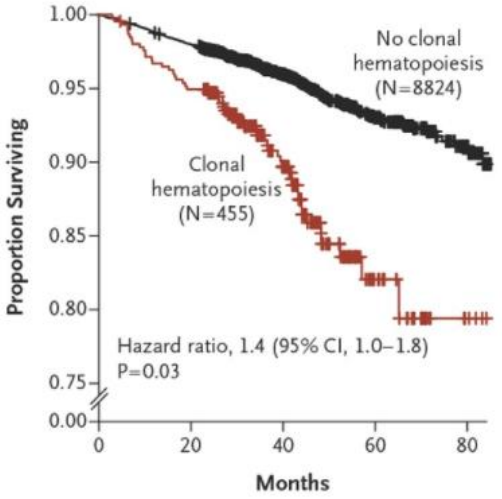
Table 1. Mutations observed in blood of healthy older persons, separated by frequency of detection of variants

Common (>50) variants, n		Less common (10-49) variants, n		Uncommon (5-9) variants, n*	
DNMT3A	403	*TP53*	33	*GNAS*	8
TET2	72†	*JAK2*	31	*BRCC3*	6
ASXL1	62†	*SF3B1*	27	*CREBBP*	6
		GNB1	22	*NRAS*	6
		CBL	12	*RAD21*	6
		SRSF2	11	*SETDB1*	6
		PPM1D	†	*U2AF1*	5
				SETD2	5

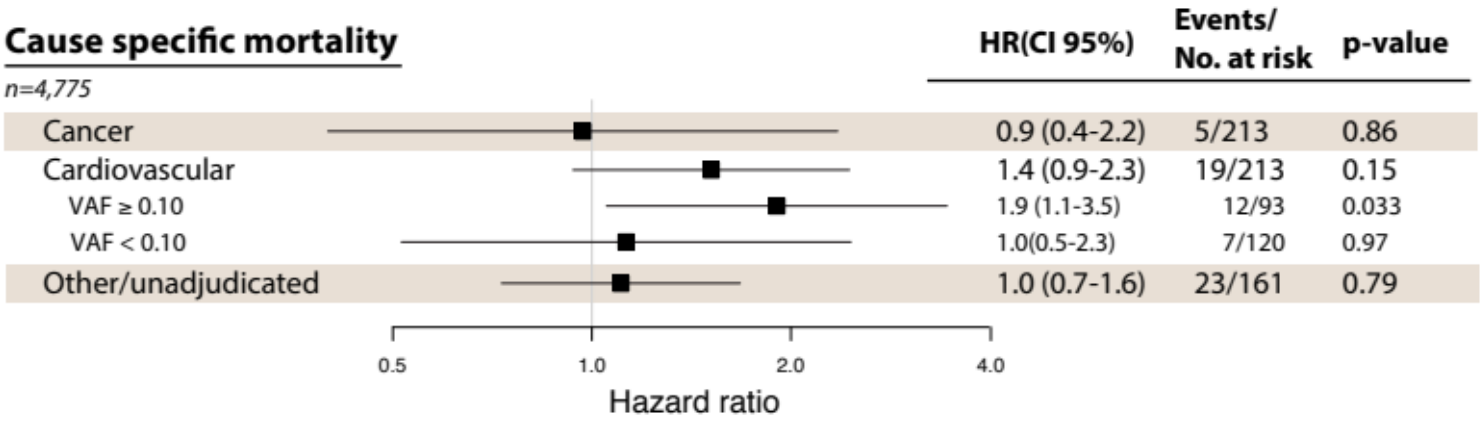
Steensma, Educational ASH 2018

Low risk of transformation to myeloid malignancies





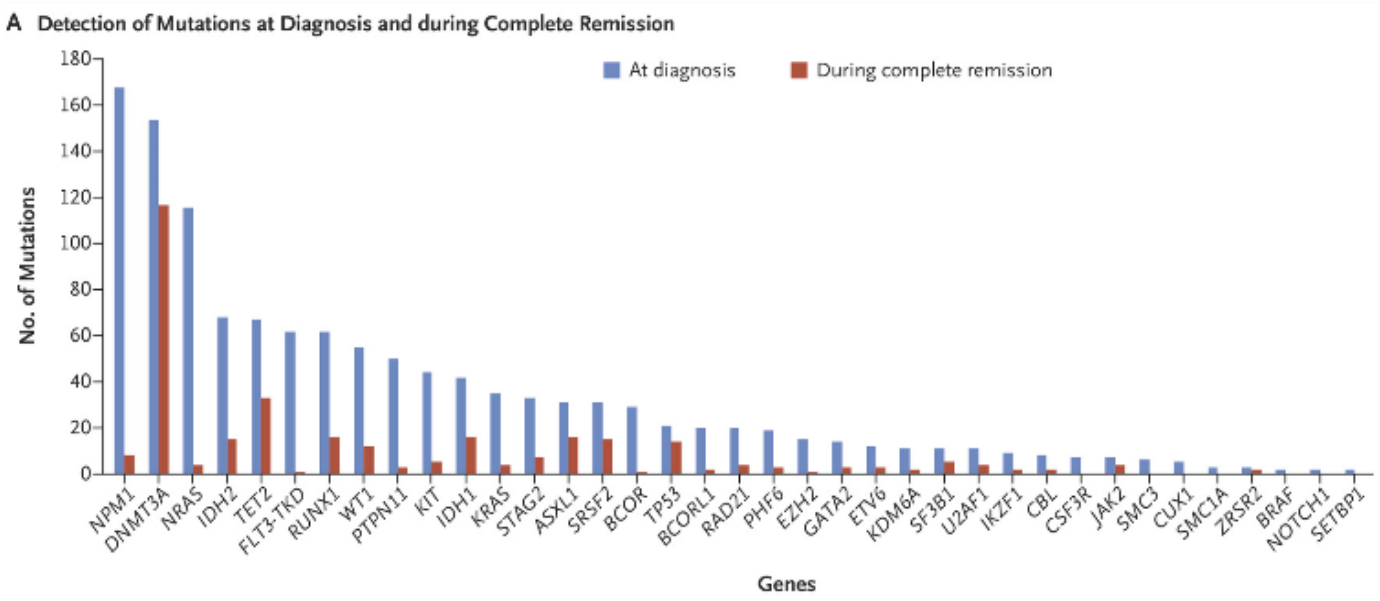
0,5-1% per year

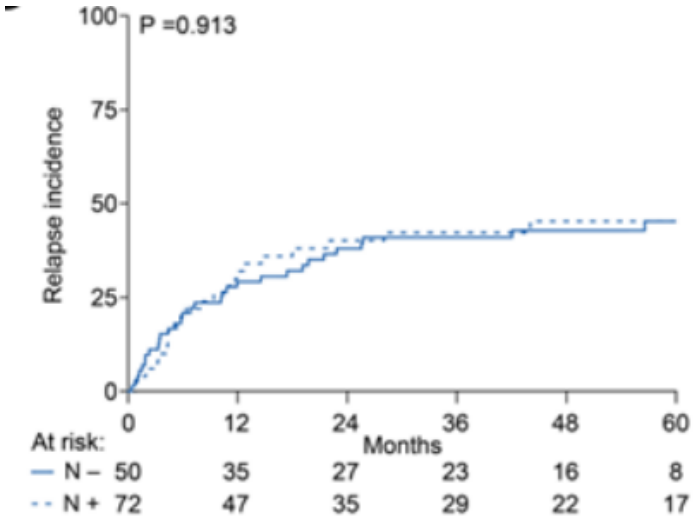


coronary heart disaes, early onset myocardial infarction, heart failure, ischemic stroke

Genovèse et al, NEJM 2014 Jaiswal et al, NEJM 2014

Can persist in AML in remission (DNMT3A, TET2…)





persistence of DTA mutation was not associated with relapse

Jongen-Lavrencic et al, NEJM 2018

CHOP : Clonal Hematopoiesis with Oncogenic Potential

disease related/specific lesion that trigger differentiation and/or proliferation

high risk of transformation to myeloid malignancies

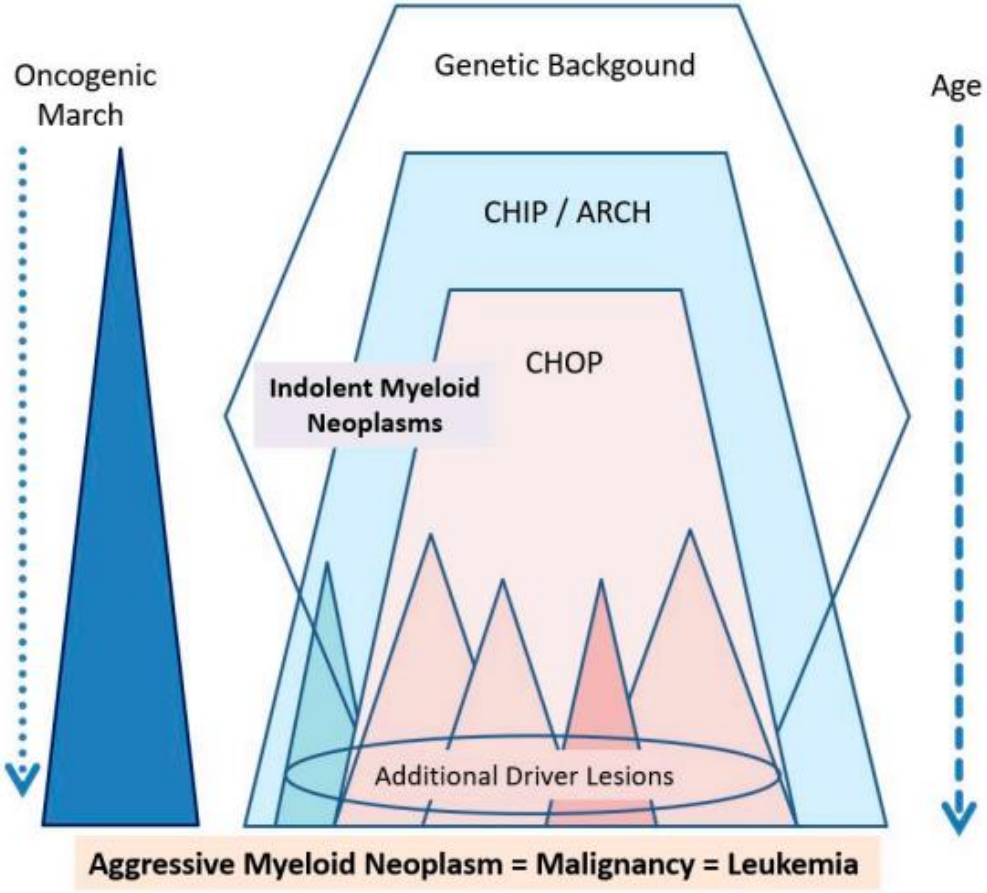
indicate adverse risk if persistent in CR ?

Table 3. Somatic mutations producing clonal hematopoiesis of oncogenic potential (CHOP).

	Effects of the Mutant on Clonal Cells			Affected
Mutation	**Differentiation**	**Proliferation**	**Oncogenesis**	**Myeloid Neoplasm**
BCR-ABL1$_{p210}$	+	+	+*	Ph+ CML
JAK2 V617F	+	+/-	-	MPN
CALR mutations	+	+/-	-	MPN
MPL mutations	++	+/-	-	
KIT D816V	++	+/-	-	ISM and AdvSM
FIP1L1-PDGFRA	+	+/-	-	CEL, MPN-eo
RUNX1- RUNX1T1	+/-	++	+	AML
CBFβ-MYH11	+/-	++	+	AML
FLT3 ITD mutations	+/-	+	+/-	AML
NPM1 mutations	-	++	+/-	AML
KRAS, HRAS mutations	-	++	+	AML
TP53 mutations	-	+	+	MPN, CMML, AML

Valent et al, Int J Mol Sc 2019

Model of progression



Valent et al, Int J Mol Sc 2019

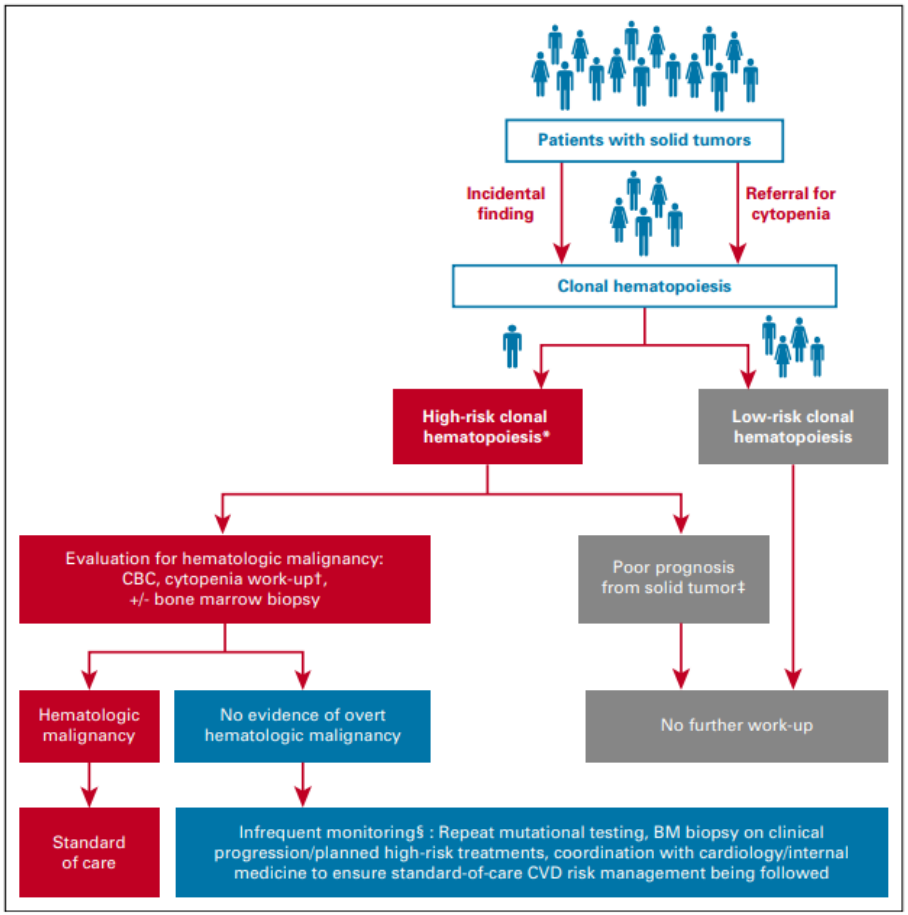
How to manage CHIP ? CHOP ?

Blood count 1x/6 months

Bone marrow exam if cytopenia

Repeat NGS to follow clonal burden evolution ?

Cardiovascular follow-up



Bolton et al, JCO 2018

* Germlines mutations

Table 17. Classification of myeloid neoplasms with germ line predisposition

Myeloid neoplasm classification
Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction
AML with germ line *CEBPA* mutation
Myeloid neoplasms with germ line *DDX41* mutation*
Myeloid neoplasms with germ line predisposition and preexisting platelet disorders
Myeloid neoplasms with germ line *RUNX1* mutation*
Myeloid neoplasms with germ line *ANKRD26* mutation*
Myeloid neoplasms with germ line *ETV6* mutation*
Myeloid neoplasms with germ line predisposition and other organ dysfunction
Myeloid neoplasms with germ line *GATA2* mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome or
Noonan syndrome-like disorders
Myeloid neoplasms associated with Down syndrome*

*Lymphoid neoplasms also reported.

(anémie de Fanconi, Shwachman-Diamond, …)

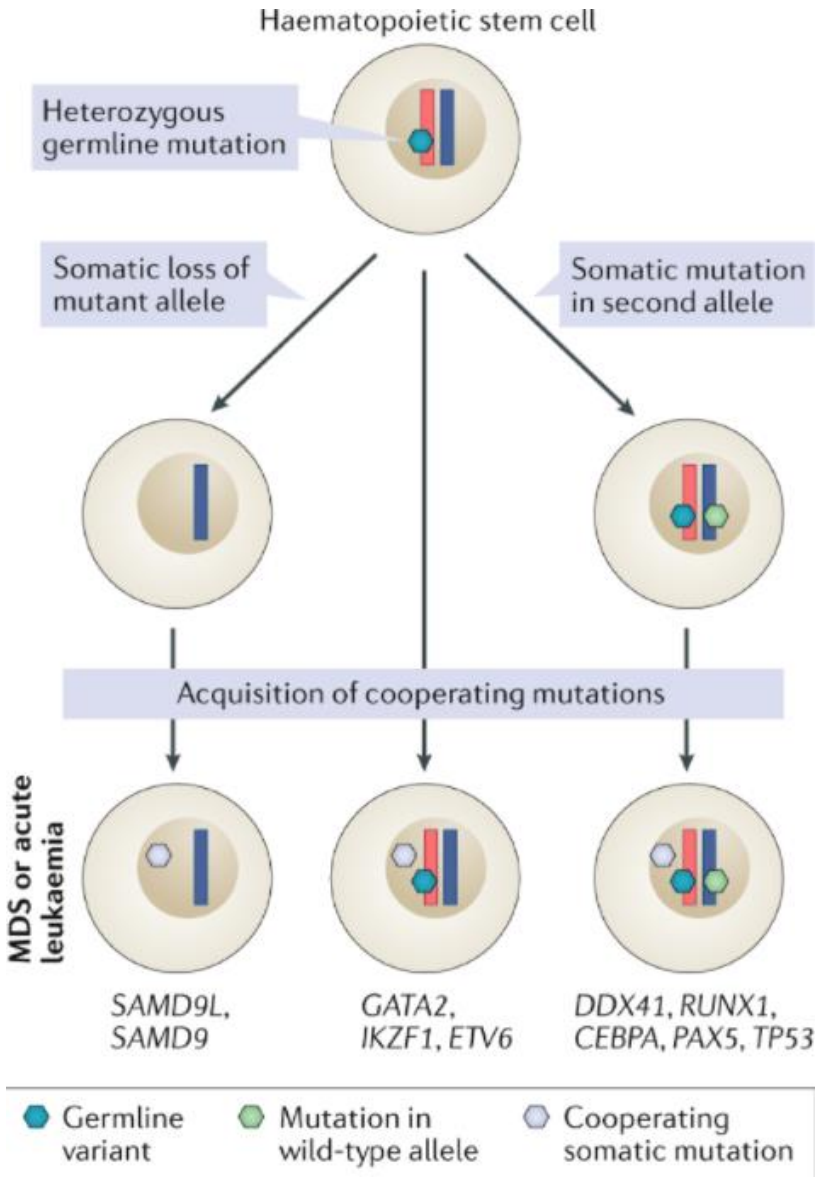
Polymorphismes (KMT5B, HLA, RAVER2, AK4)

! only a minority willl represent suspicious anamnesis

Arber et al, Blood 2016

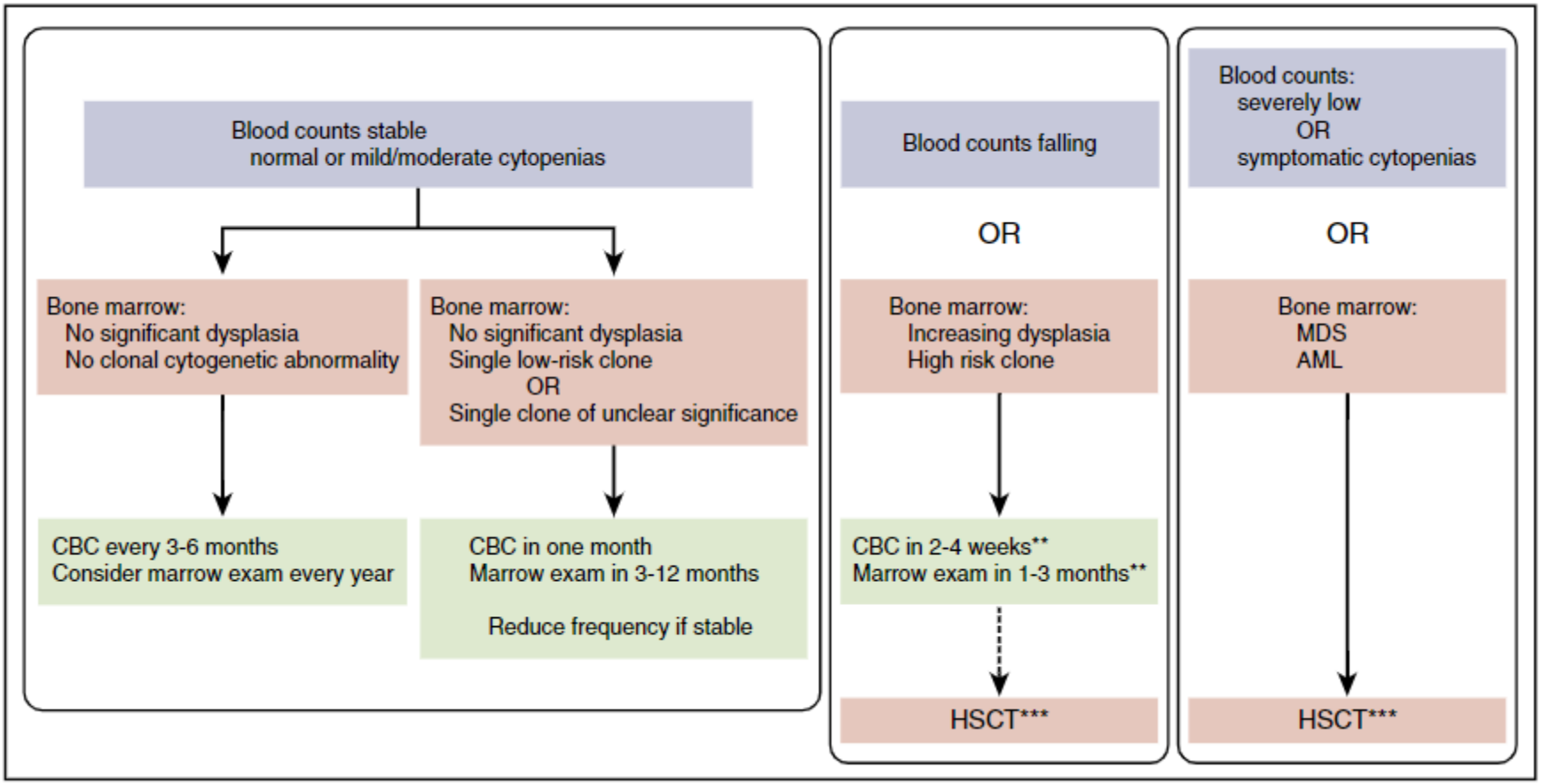
Lin et al, Nat Com 2021

Model of disease progression



Klco, Nature review cancer 2021

How to manage germline predisposition to AML ?



Godley et al, Blood 2017

Conclusions

To understand the molecular mechanisms of AML

-> therapy : relapses/MRD/ targeted therapies

To understand the 'preleukemic states'

* CHIP/CHOP

* Germlines mutations


Thank you for your attention