Acute myeloid leukemia

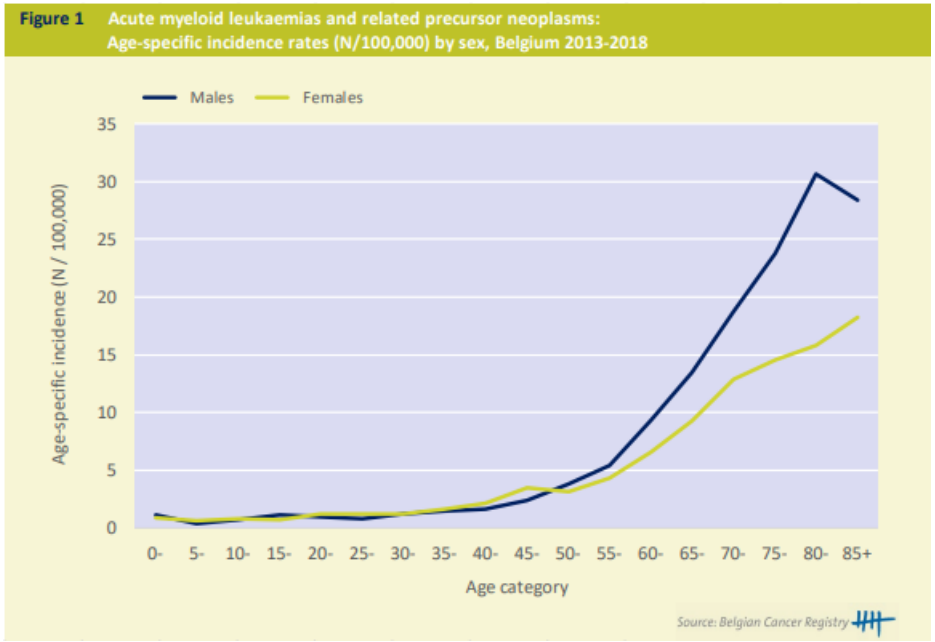
the molecular pathogenesis

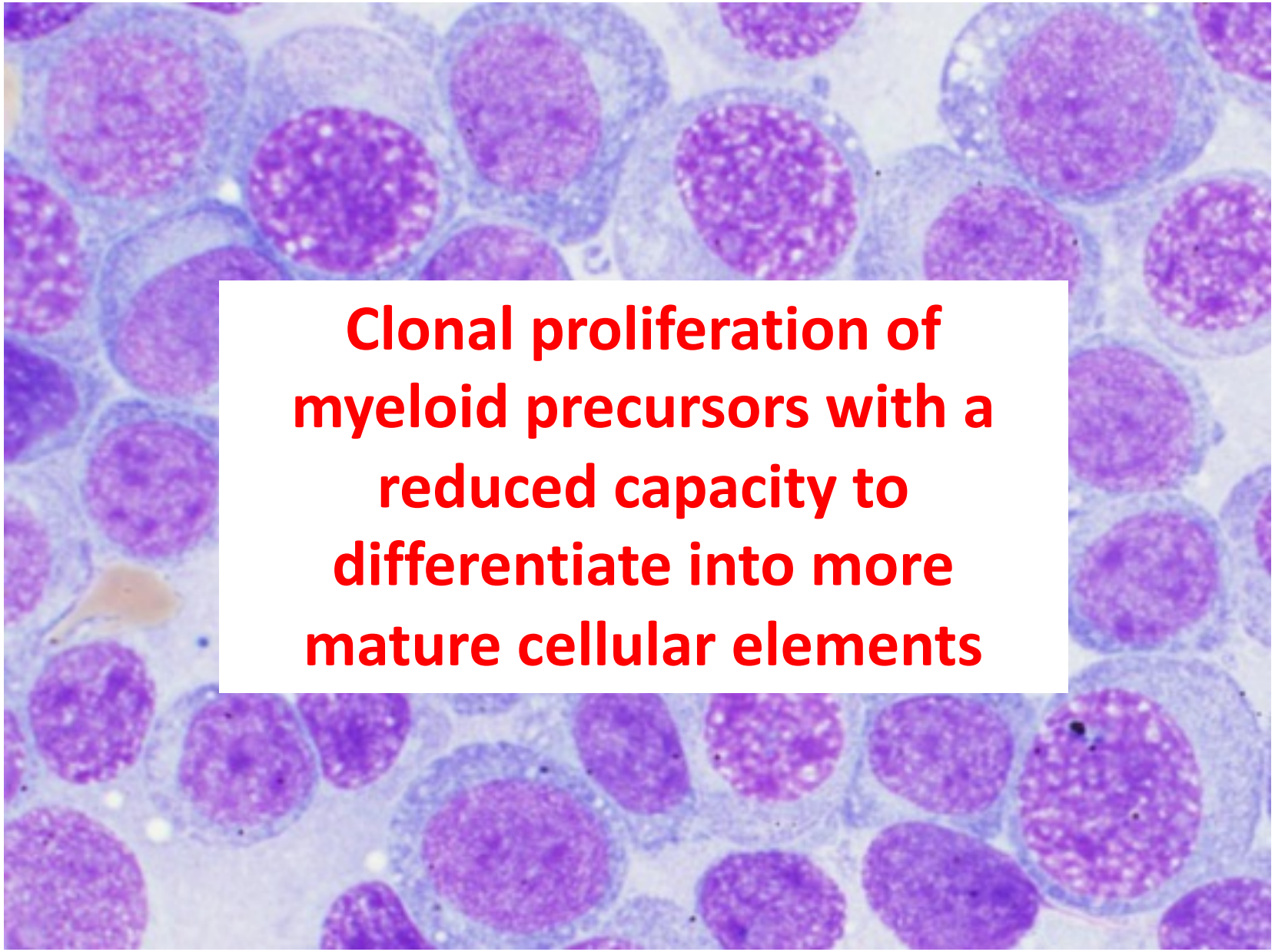
Pr Violaine Havelange, MD, PhD

Department of hematology

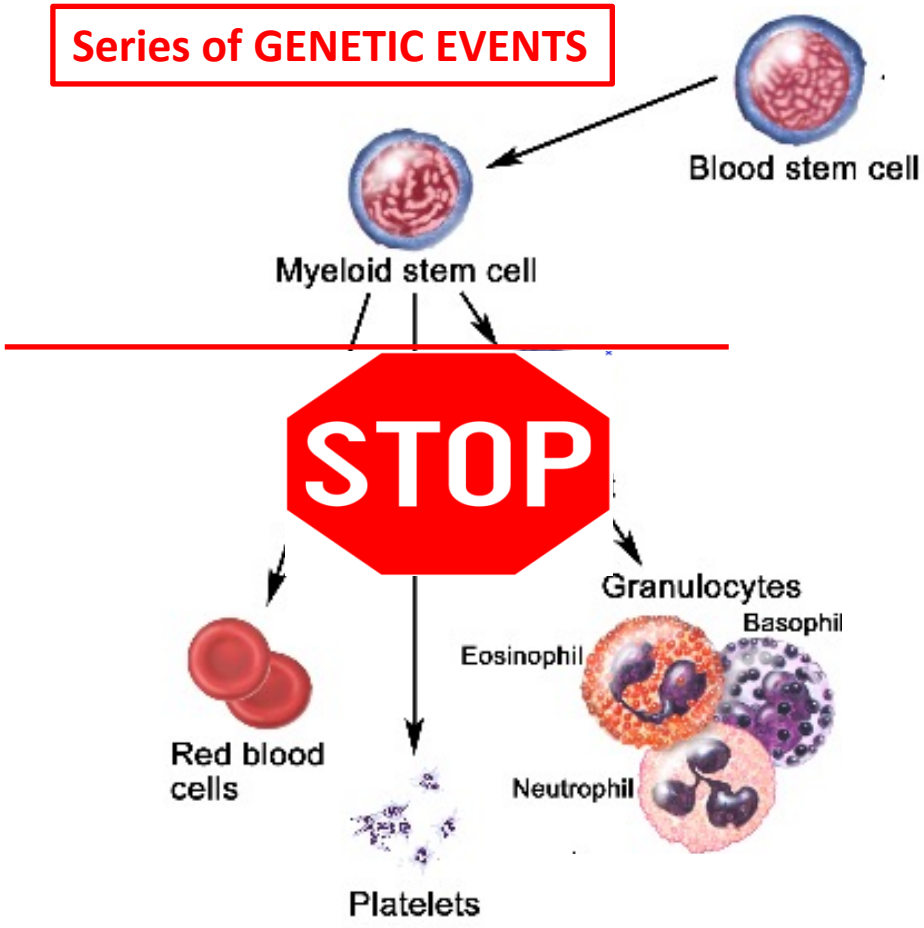
Acute myeloid leukemia (AML)

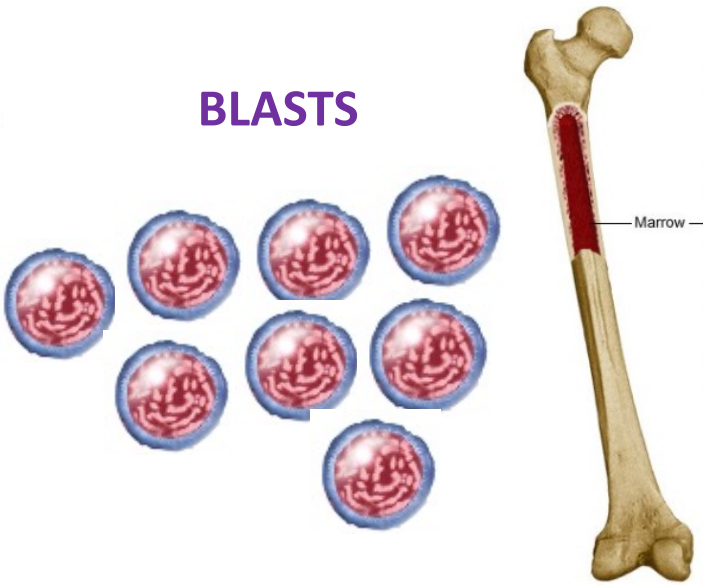
- **Incidence : 3-5 cases/100.000/year**
- **80% of acute leukemias in adults**
- **Median age : 65 years**




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

PHYSIOPATHOLOGY





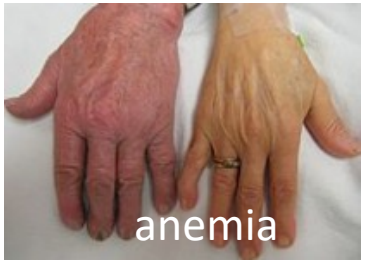
Accumulation of leukemic blasts or immature forms in BM, PB, other tissues

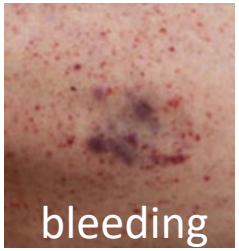
+

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

CLINICAL SYMPTOMS

- complications of pancytopenia

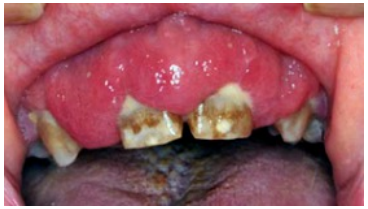

anemia

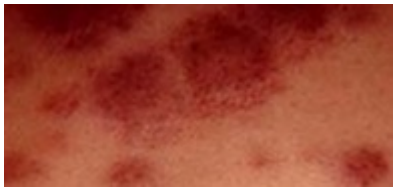

bleeding


increased risk of infections

- Extramedullary locations

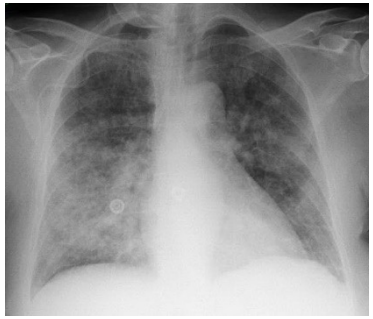
skin, CNS, oropharynx, organomegaly, joints, myeloid sarcomas





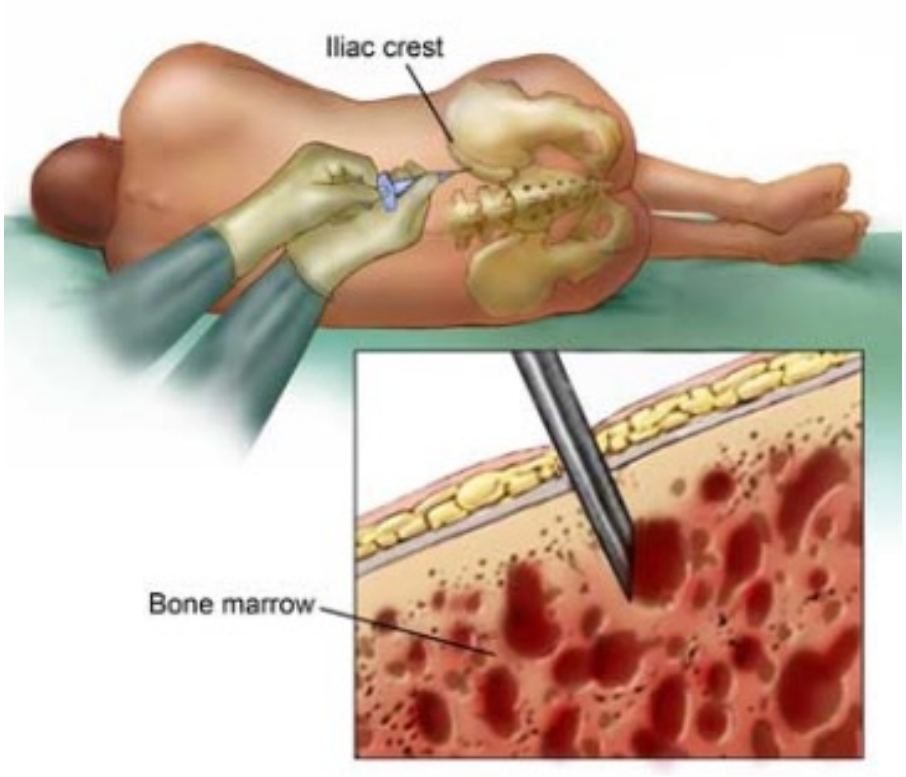
- Symptoms of leukostasis if extremely high white blood cell counts

fever, lung, CNS, heart



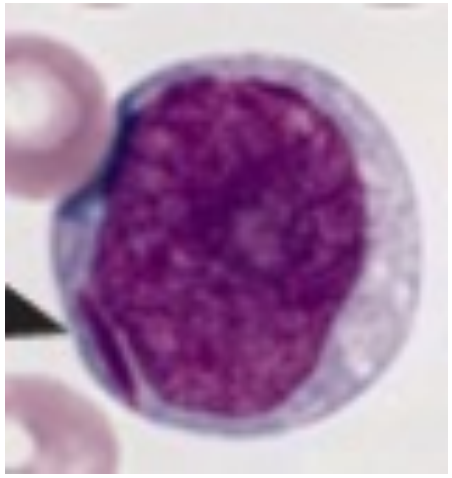
DIAGNOSIS

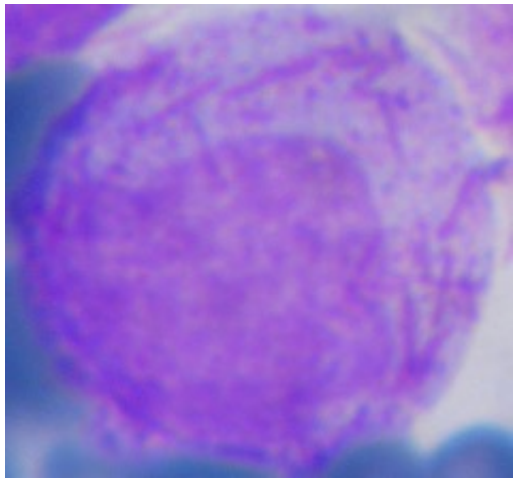
Bone marrow aspirate/biopsy



Mayo fondation for medical and research

MORPHOLOGY





staining with Wright Giemsa

blasts

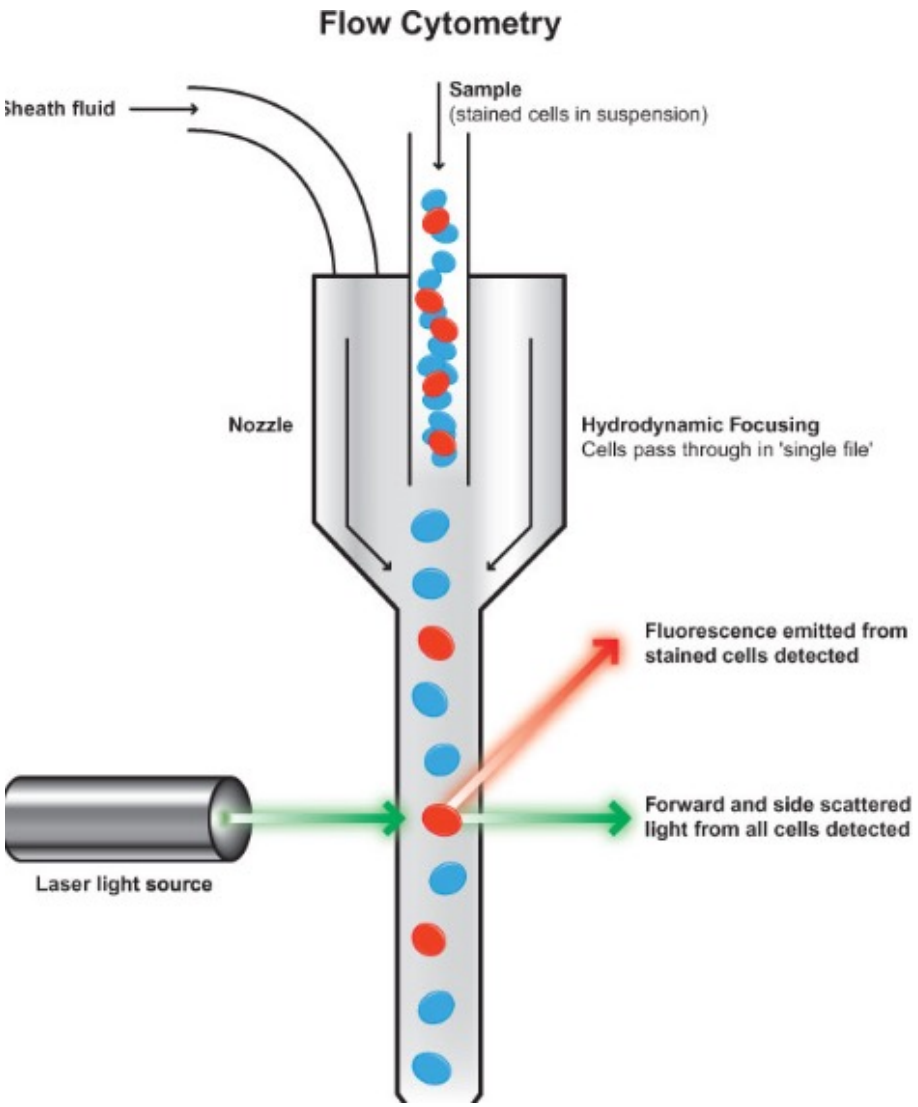
> 20%

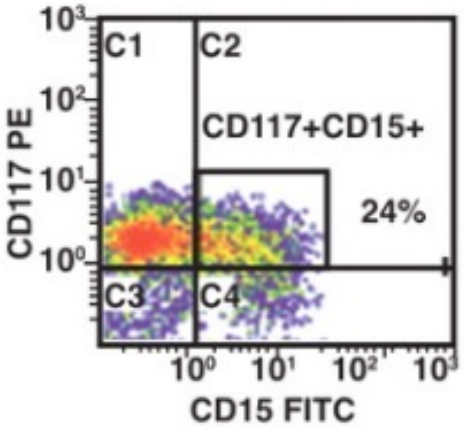
immature cells with large nuclei, with prominent nucleoli
pale blue cytoplasm

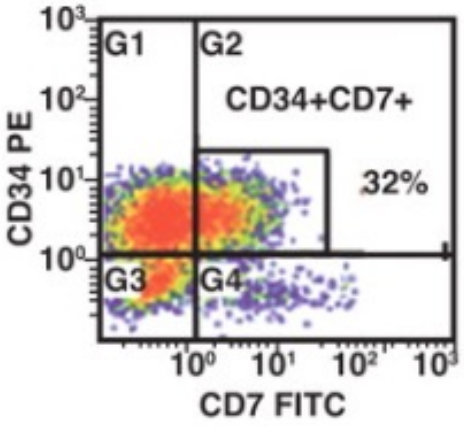
'auer rod' = pathognomonic of myeloblasts

Cytochemical studies : + sudan black B,
myeloperoxidase or esterase

IMMUNOPHENOTYPING







CD34+

HLA-DR +

CD117 +

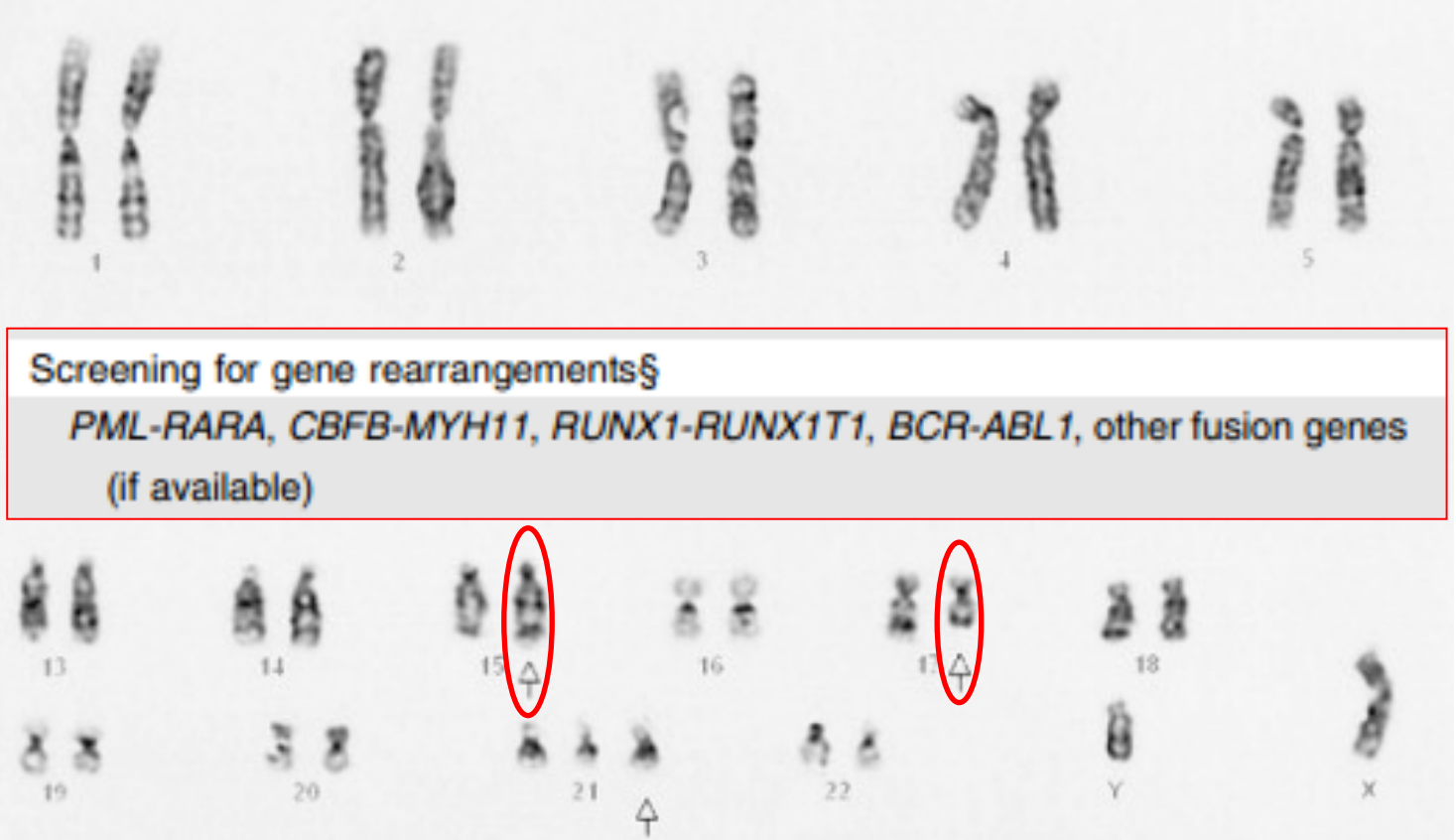
CD13 +

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 Acute Promyelocytic Leukemia (APL)

MOLECULAR TESTINGS

Fusion transcripts : PML/RARA; t(15;17)

AML1/ETO; t(8;21)

CBFB/MYH11; inv(16)

Tandem internal duplication of *FLT3*

Internal duplication of *KMT2A*

MOLECULAR TESTINGS

NGS Next Generation Sequencing

ASXL1 *(exon 13 = dernier exon)*	*pronostic*
BCOR *(tous les exons codants et les régions de sites de splicing)*	*diagnostic*
CEBPA *(exon 1 = entièrement)*	*diagnostic/pronostic*
DDX41 *(Tous les exons codants et les régions de sites de splicing)*	*diagnostic*
DNMT3A *(exon 8-23)*	*diagnostic/pronostic*
EZH2 *(exon 2-20 = entièrement)*	*diagnostic*
FLT3 *(exon 14, exon 15, exon 20-codon 835)*	*pronostic/ thérapie*
IDH1 *(exon 4-hotspot)*	*pronostic/ thérapie*
IDH2 *(exon 4-hotspot)*	*pronostic/ thérapie*
KIT *(exon 8, exon 10, exon 17)*	*pronostic/ thérapie*
NPM1 *(exon 11-codon 288)*	*diagnostic/pronostic*
RUNX1 *(exon 2-9 = entièrement)*	*diagnostic/pronostic*
SF3B1 *(exon 14, exon 15)*	*diagnostic*
SRSF2 *(exon 1-codon 95)*	*diagnostic*
STAG2 *(Tous les exons codants et les régions de sites de splicing)*	*diagnostic*
TET2 *(exon 3, exon 9-11)*	*diagnostic/pronostic*
TP53 *(exon 2-11)*	*pronostic/ thérapie*
U2AF1 *(exon 2-codon 34, exon 6-codon 157)*	*diagnostic*
WT1 *(exon 7, exon 9)*	*pronostic*
ZRSR2 *(Tous les exons codants et les régions de sites de splicing)*	*diagnostic*

Classification ICC 2022 based on cytogenetic and mutational profiles

AML and related neoplams

AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)*

- APL with t(15;17)(q24.1;q21.2)/*PML*::*RARA*†
- 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*
- AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*‡
- AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*§
- AML with other rare recurring translocations‖
- AML with mutated *NPM1*
- AML with in-frame bZIP mutated *CEBPA*¶
- AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1**

Myeloid sarcoma

Acute leukemia of ambiguous lineage

- Acute undifferentiated leukemia
- MPAL with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*
- MPAL with t(v;11q23.3)/*KMT2A*-rearranged
- MPAL, B/myeloid, not otherwise specified
- MPAL, T/myeloid, not otherwise specified

impact on disease phenotype and outcome

Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts in BM or PB)

- AML with mutated *TP53*#
- AML with myelodysplasia-related gene mutations
  Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*
- AML with myelodysplasia-related cytogenetic abnormalities**
- AML not otherwise specified

Myeloid proliferations related to Down syndrome

- Transient abnormal myelopoiesis associated with Down syndrome
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Diagnostic qualifiers††

Therapy-related‡‡
- Prior chemotherapy, radiotherapy, immune interventions

Progressed from MDS
- MDS should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis

Progressed from MDS/MPN (specify type)
- MDS/MPN should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis

Germline predisposition (specify type)

Dohner et al, Blood 2022

PROGNOSIS

Table 6. 2022 ELN risk classification by genetics at initial diagnosis*

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 *CEBPA*‖
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 • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*‡‡ • Mutated *TP53*[a]

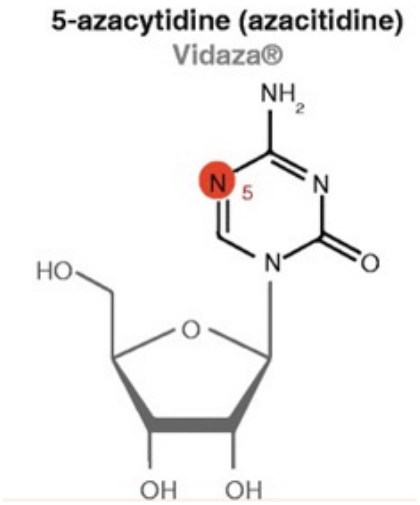
Dohner et al, Blood 2022

TREATMENT

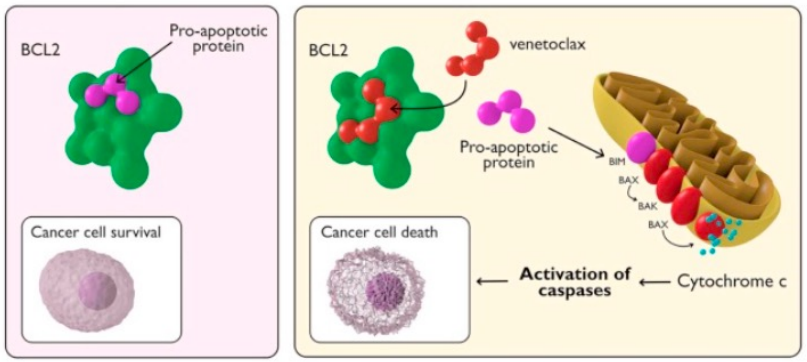
Young patients < 65 y

7 + 3

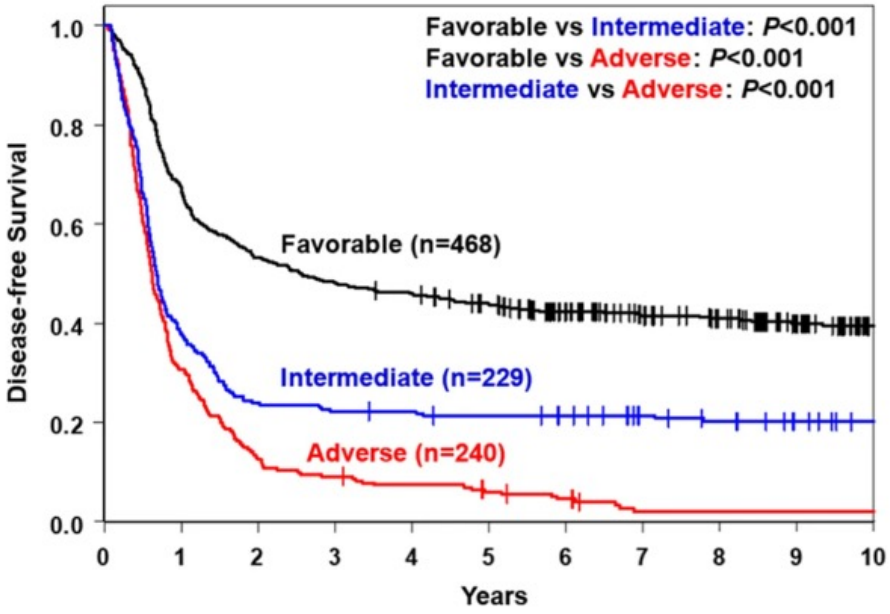
Older patients > 65 y

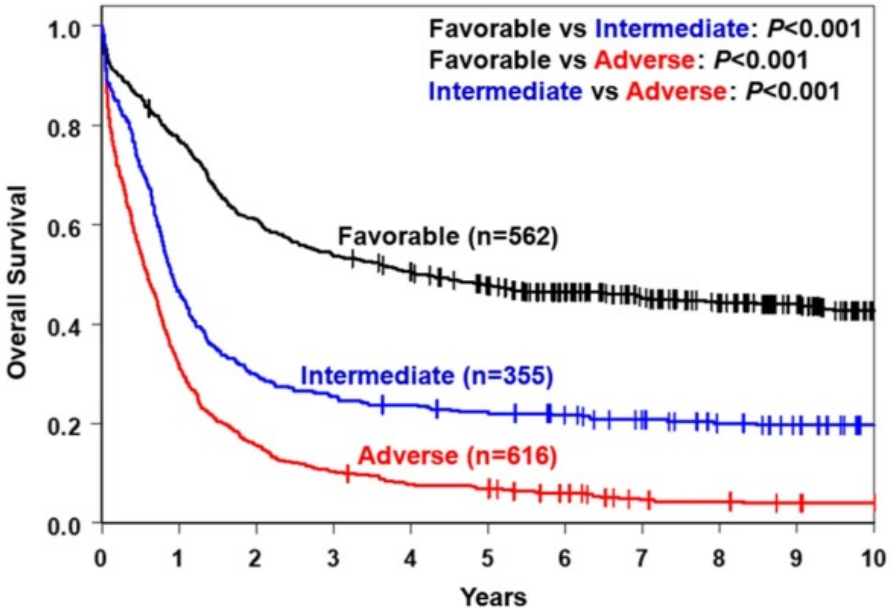


venetoclax



SURVIVAL





to better understand the underlying mechanisms of leukemogenesis

-> to develop more targeted therapies

-> to understand and treat relapses

Mrozek et al, Leukemia 2023

To understand the molecular pathogenesis of AML

1/ cytogenetic heterogeneity of leukemia cells

high-throughput NGS seqencing

2/ molecular heterogeneity of leukemia cells

some genomic alterations are shared by the entire tumor,

BUT not all cancer cells show identical genomic and molecular profiles

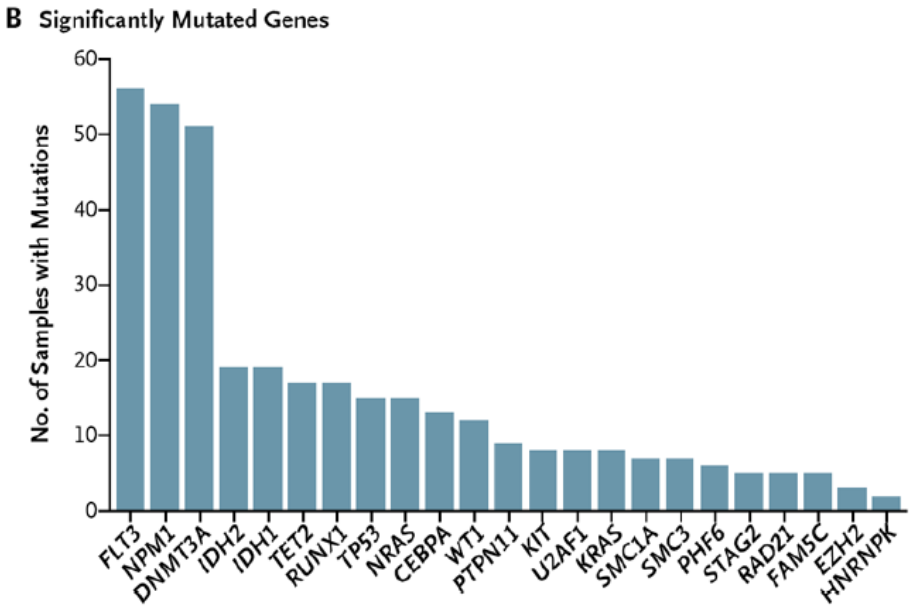
single-cell sequencing

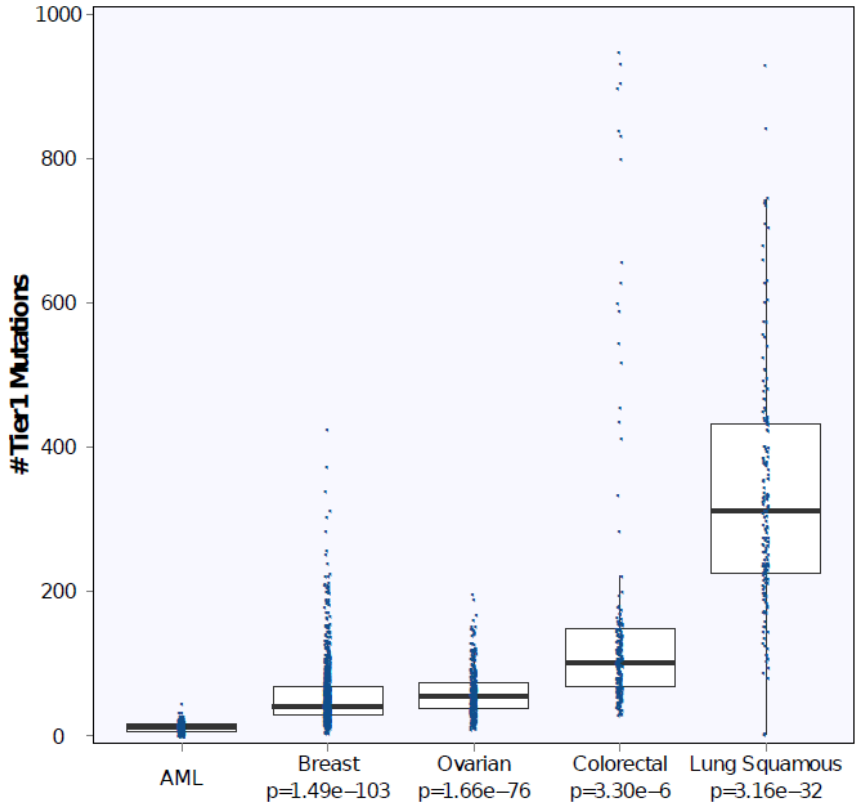
high-throughput NGS sequencing

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

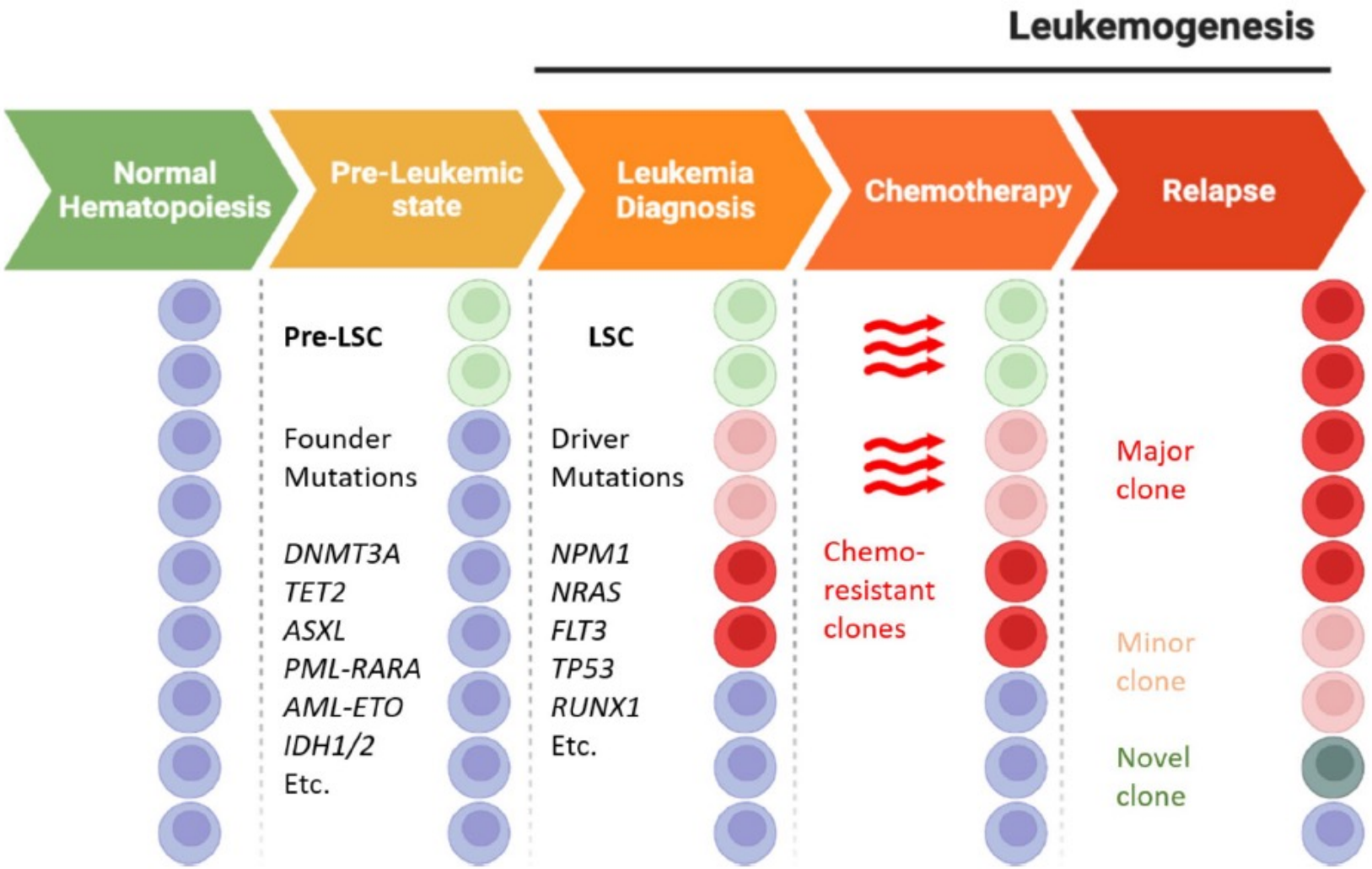




Sequencing DNA from 200 AML – BM and skin

Cancer Genome Atlas Research Network, NEJM 2013

N Engl J Med 2013; 368:2059-2074



Peroni, J of exp and clin cancer res 2023

Founder mutation

genes involved in epigenetic regulation

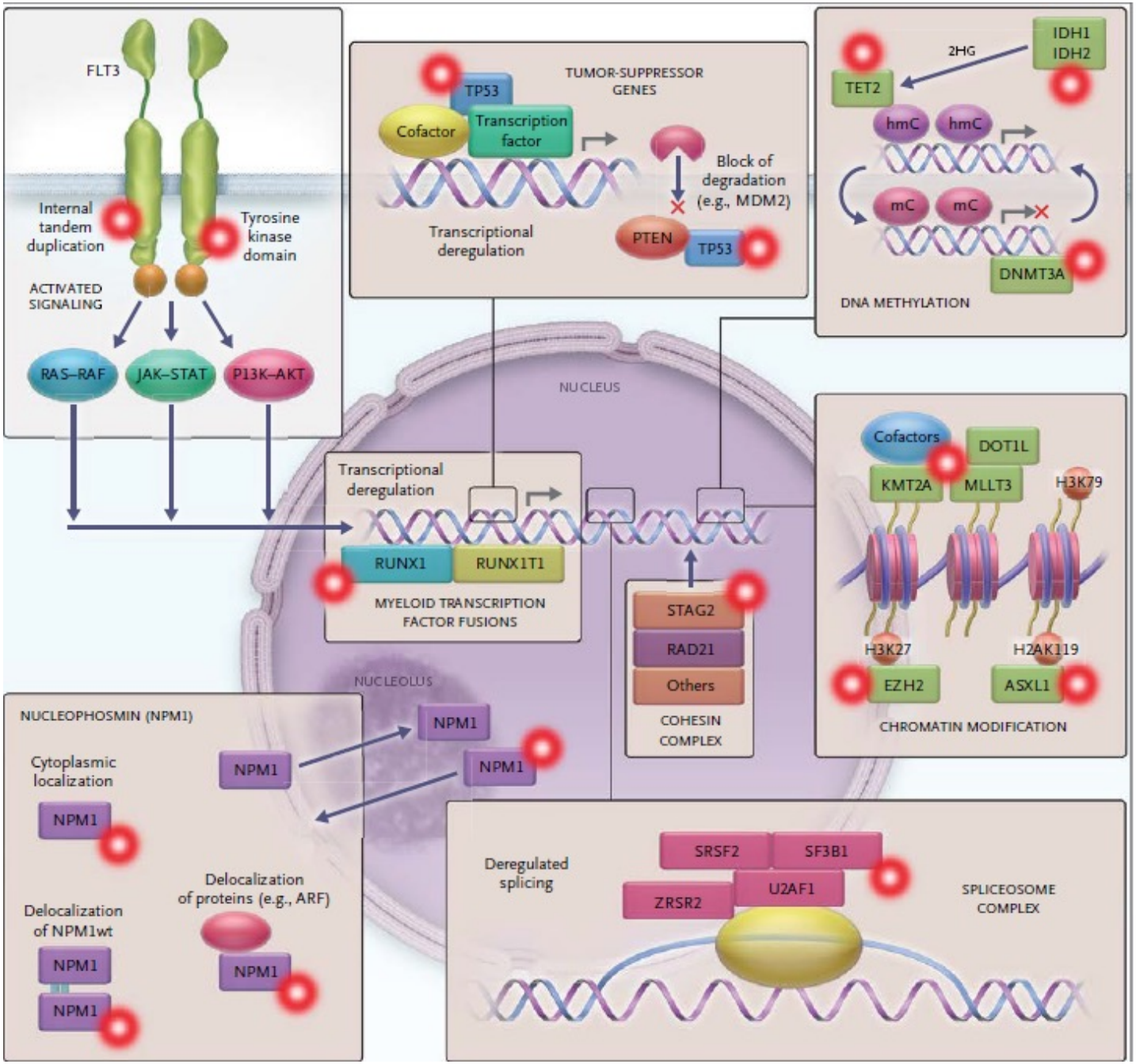
compromise maturation of blasts + promote self-renewal and clonal expansion

Driver mutation

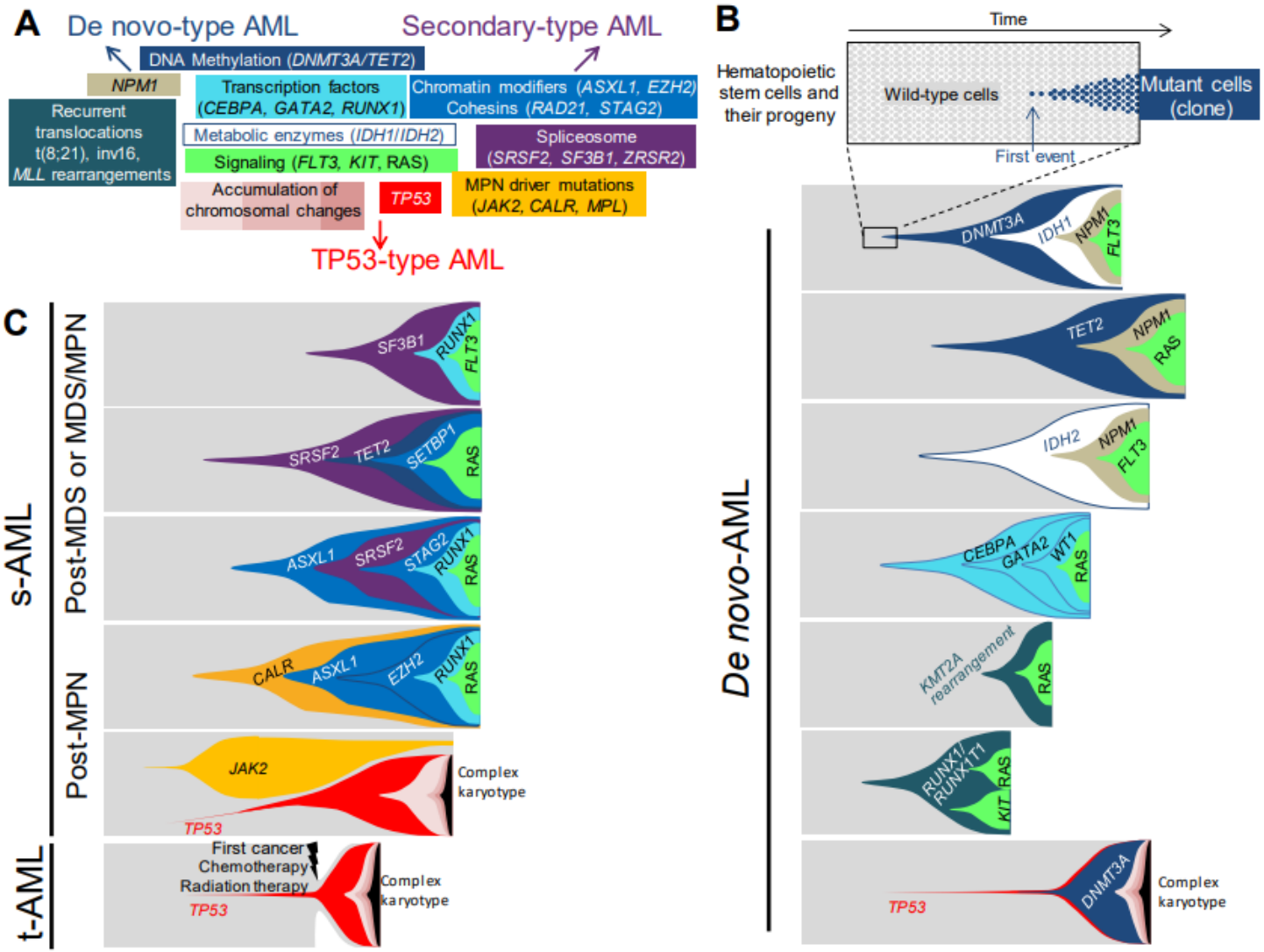
genes involved in proliferation

proliferative advantages to tumor cells by impairing normal apoptotic activity

DEREGULATION OF SEVERAL PATHWAYS



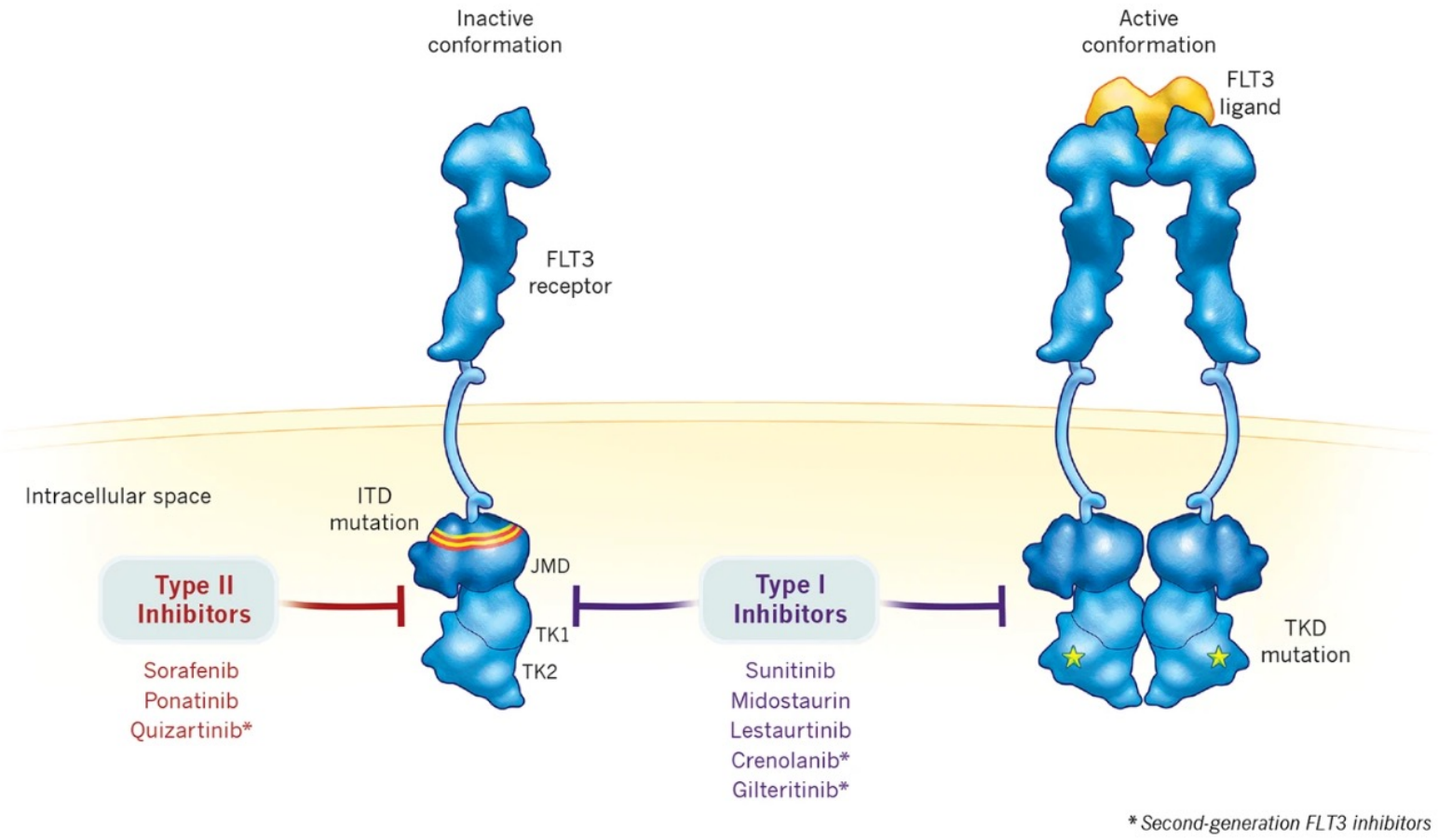
Dohner et al, NEJM 2015



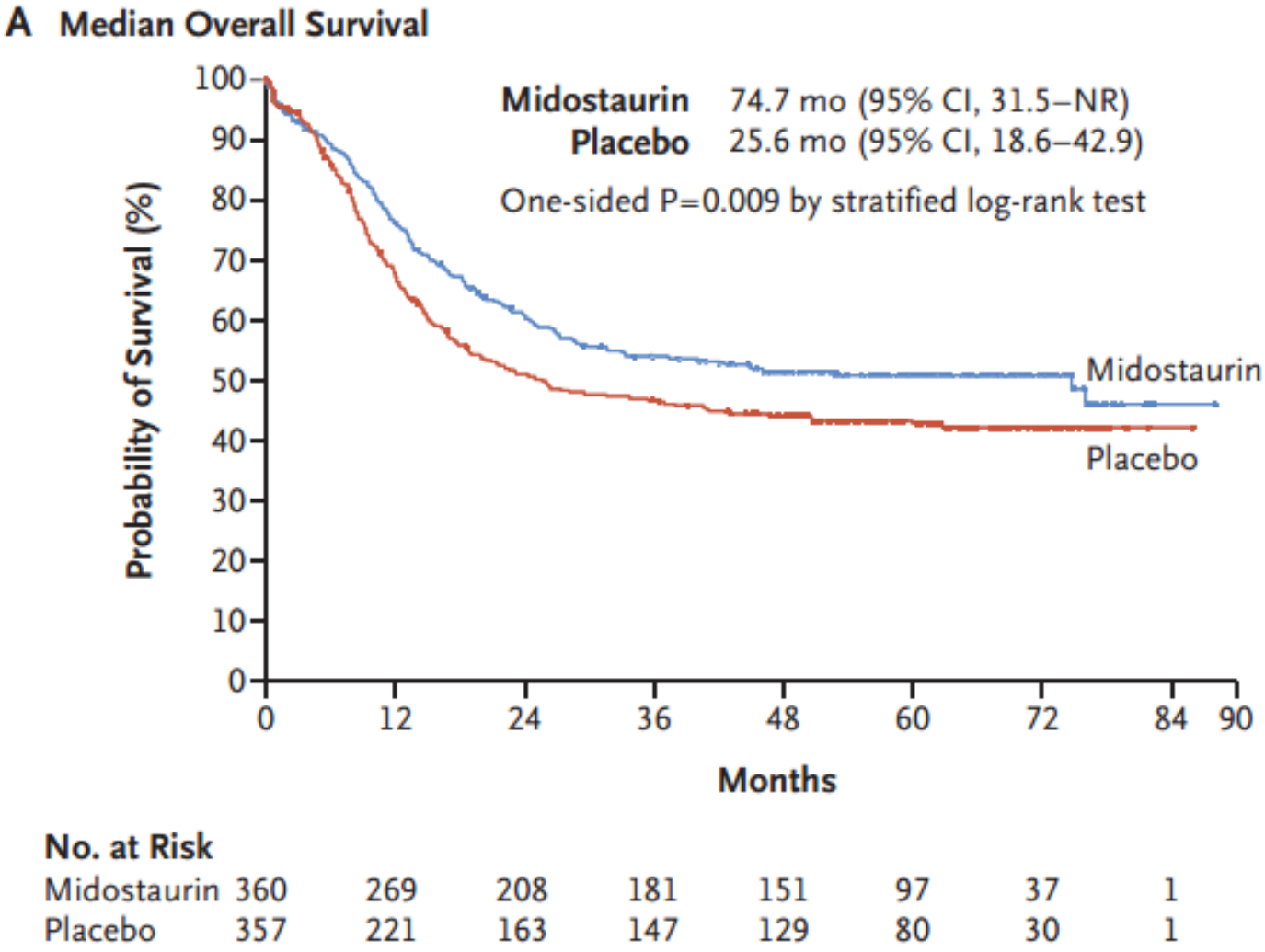
Martignoles, IJMS 2018

-> targeted therapies

FLT3 inhibitors

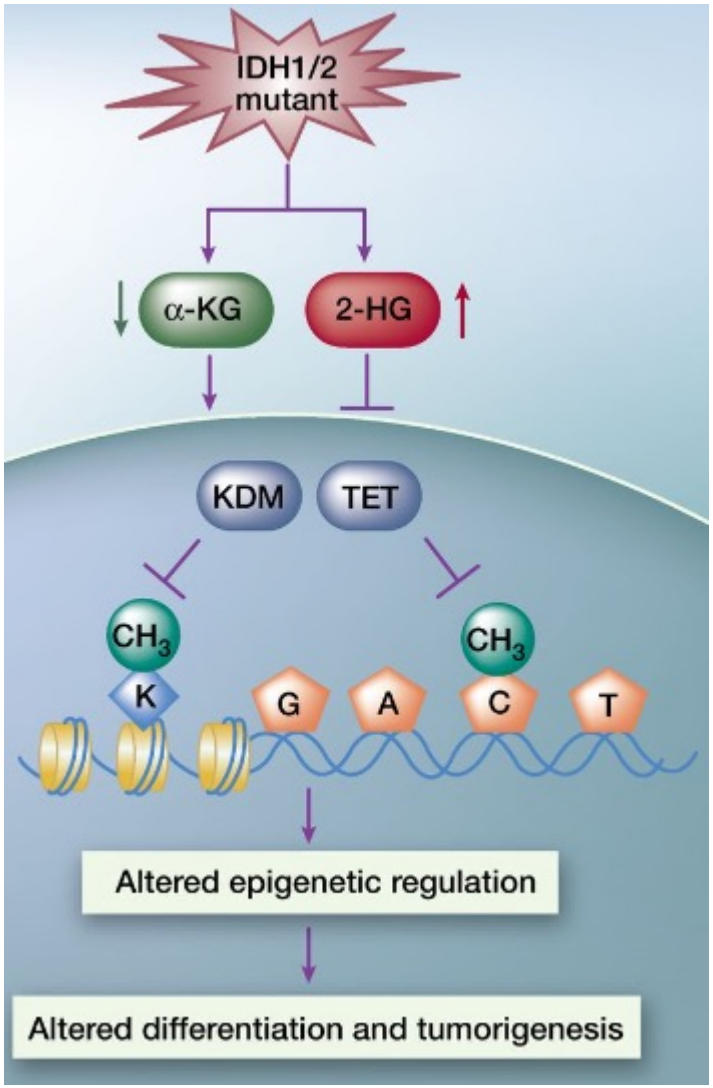


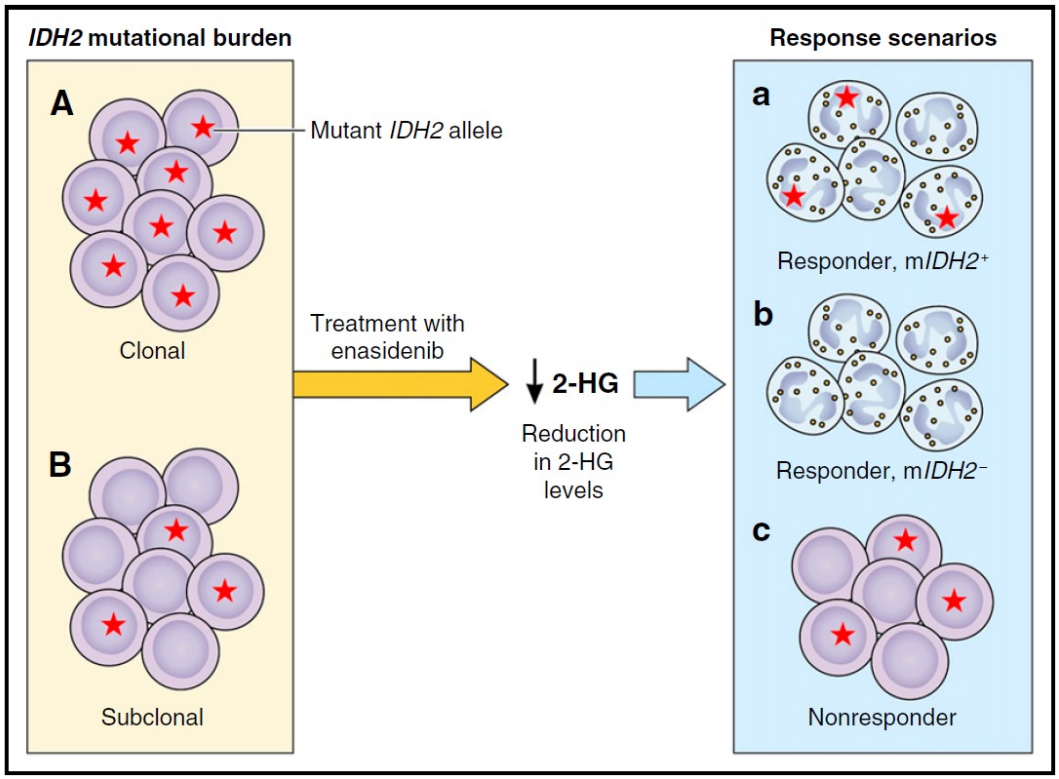
Daver et al, Leukemia 2017



Stone et al, NEJM 2017

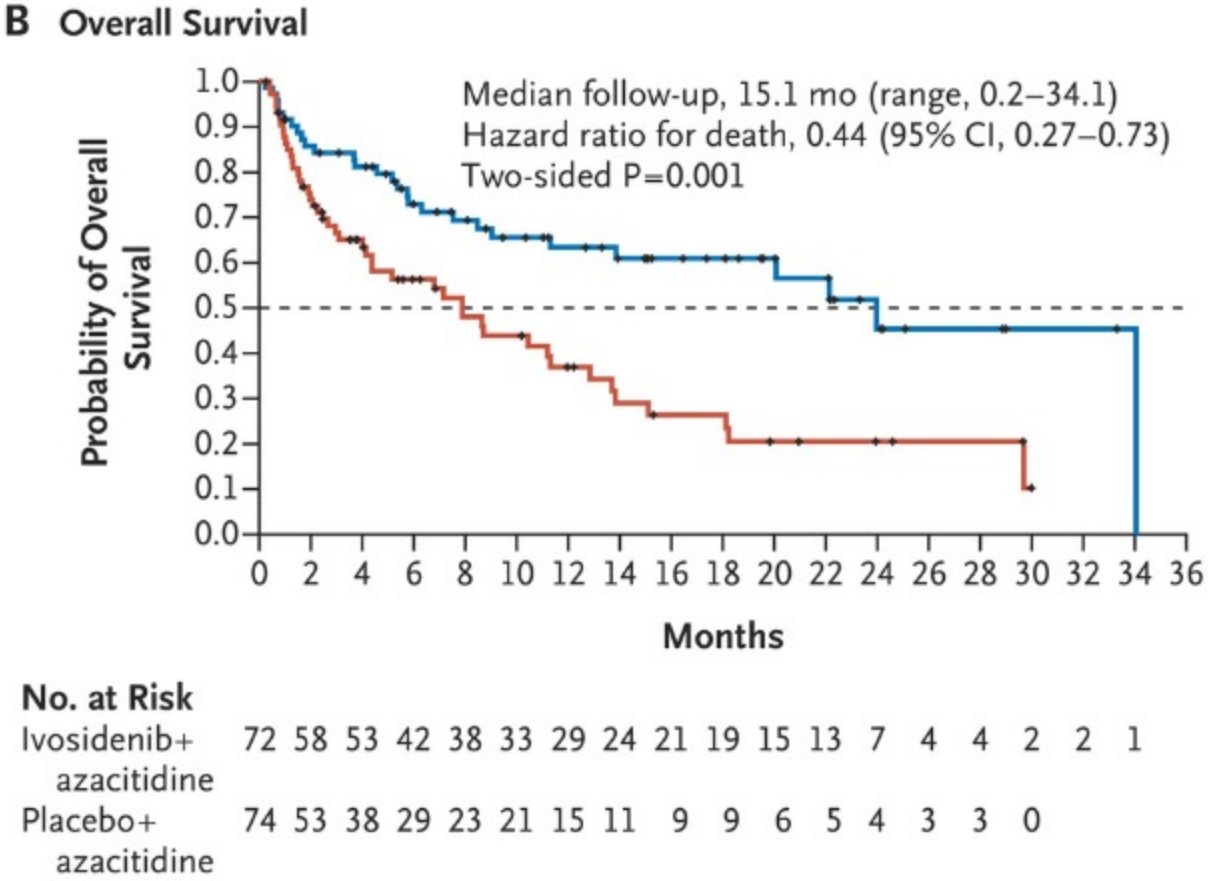
IDH1,2 inhibitors





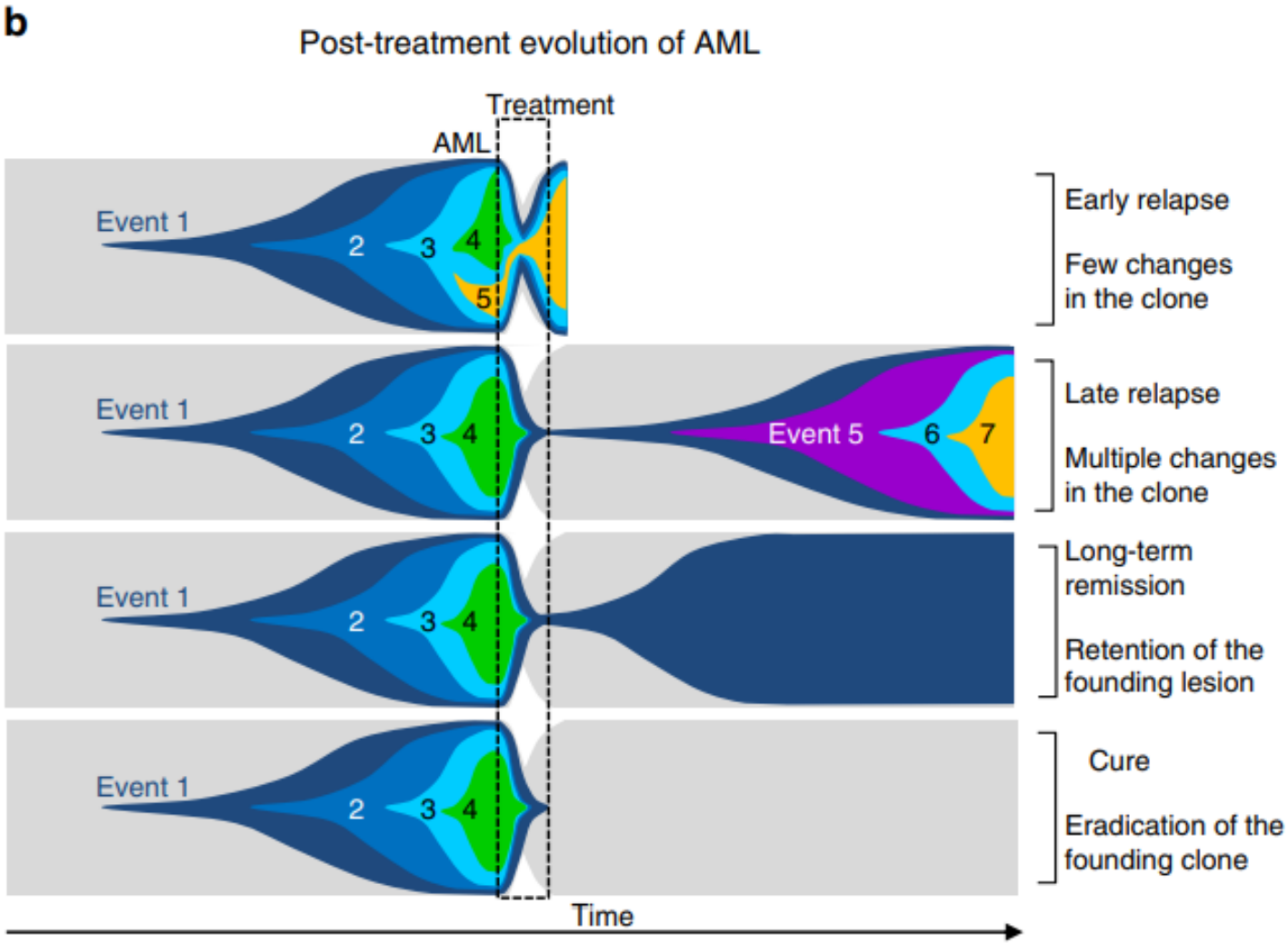
Yang et al, CCR 2012

Wouters et al, Blood 2017



Montesinos, NEJM 2022

-> relapses



Hirsch, nature com 2016

Single-cell approaches

extreme heterogeneity of leukemic blasts

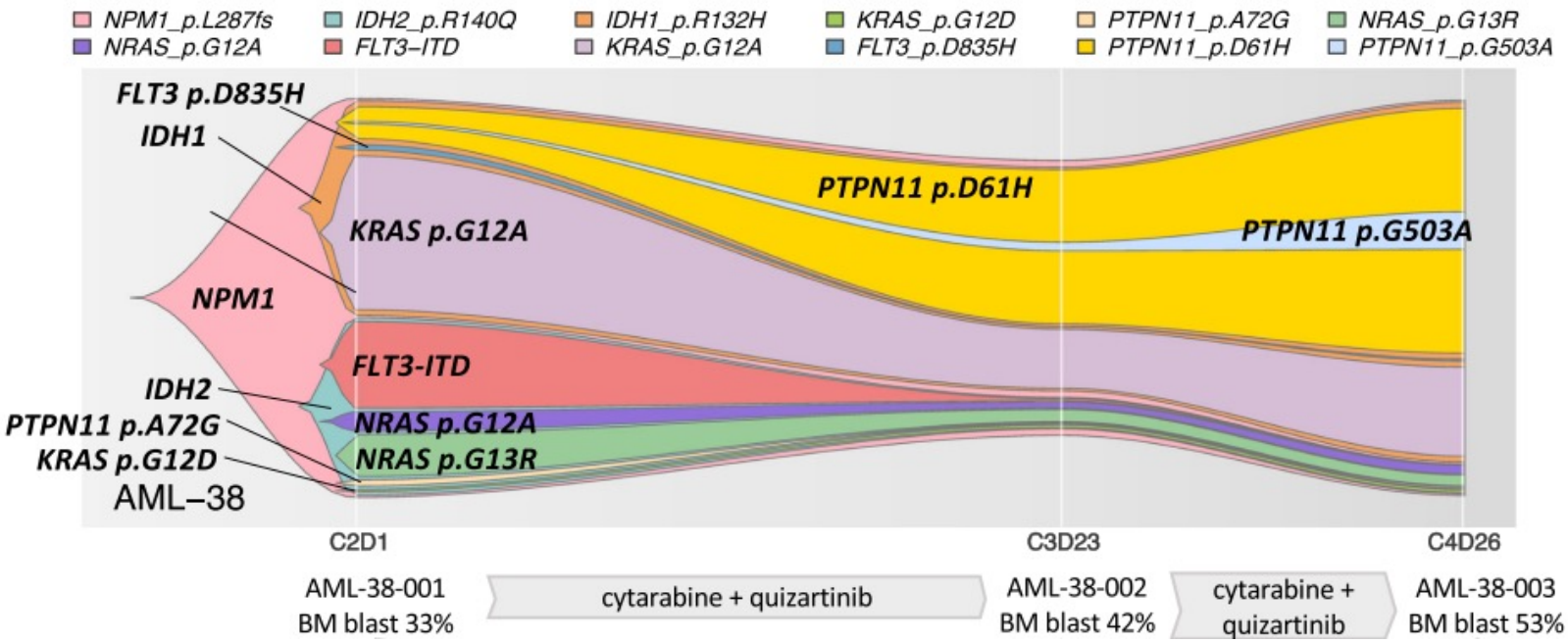
To profile the leukemia at diagnosis and at relapse

-> identify underrepresented cellular subclones

-> identify resistant clones to therapeutic approaches

-> personalize the therapy to the genetic and transcriptional profile of leukemia

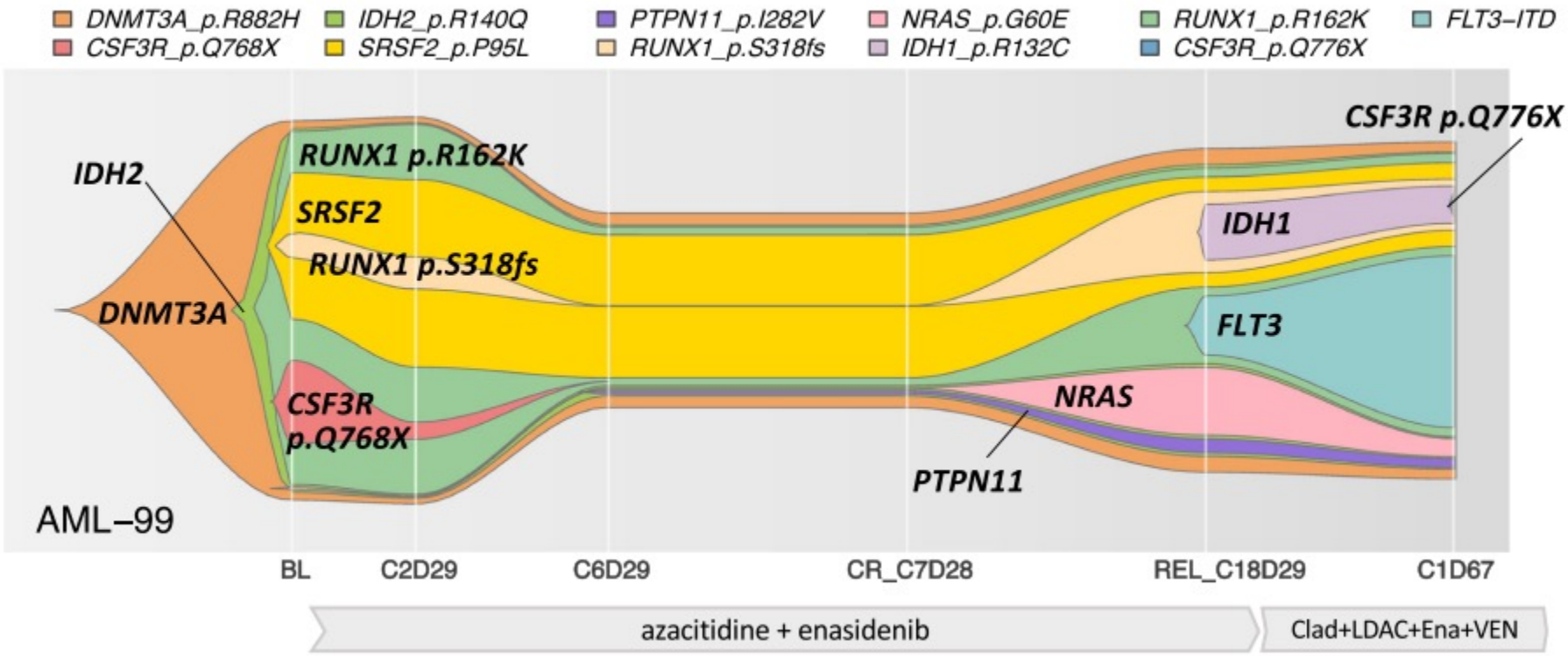
After targeted therapies …



single-cell DNA sequencing

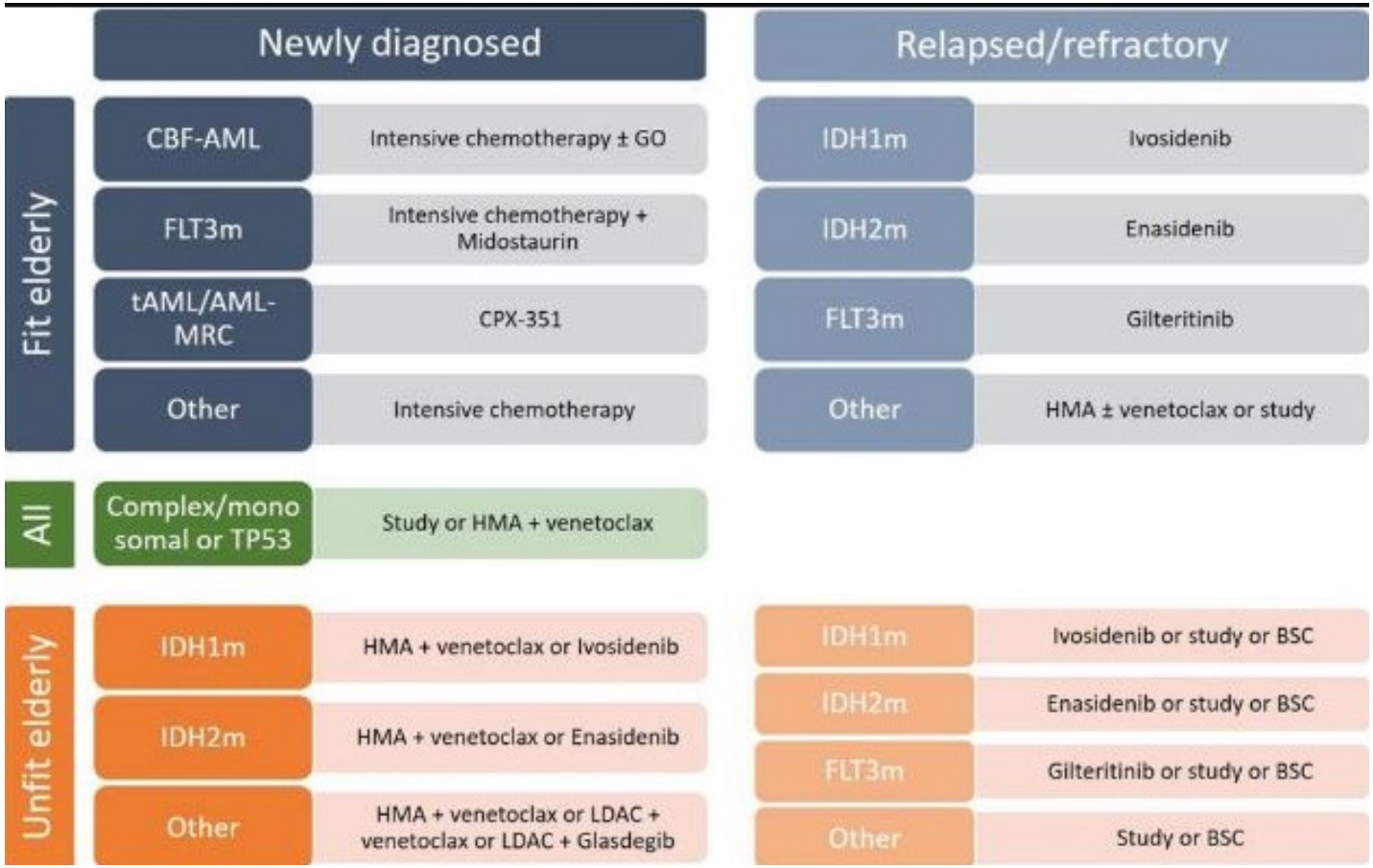
Morita et al, Nature comm 2020

After targeted therapies …



single-cell DNA sequencing

Morita et al, Nature comm 2020


Newly diagnosed
Relapsed/refractory
Fit elderly
CBF-AML
Intensive chemotherapy ± GO
FLT3m
Intensive chemotherapy + Midostaurin
tAML/AML-MRC
CPX-351
Other
Intensive chemotherapy
IDH1m
Ivosidenib
IDH2m
Enasidenib
FLT3m
Gilteritinib
Other
HMA ± venetoclax or study
All
Complex/monosomal or TP53
Study or HMA + venetoclax
Unfit elderly
IDH1m
HMA + venetoclax or Ivosidenib
IDH2m
HMA + venetoclax or Enasidenib
Other
HMA + venetoclax or LDAC + venetoclax or LDAC + Glasdegib
IDH1m
Ivosidenib or study or BSC
IDH2m
Enasidenib or study or BSC
FLT3m
Gilteritinib or study or BSC
Other
Study or BSC

-> preleukemic states ?

1. Germline predisposition

Critical for proper diagnosis

Impact on management

-> Allogeneic stem cell transplantation

exclude a donor with the same mutation

->health surveillance strategies patient/family

Genetic counseling

Table 24. ICC of hematologic neoplasms with germline predisposition

Hematologic neoplasms with germline predisposition without a constitutional disorder affecting multiple organ systems
Myeloid neoplasms with germline *CEBPA* mutation
Myeloid or lymphoid neoplasms with germline *DDX41* mutation
Myeloid or lymphoid neoplasms with germline *TP53* mutation
Hematologic neoplasms with germline predisposition associated with a constitutional platelet disorder
Myeloid or lymphoid neoplasms with germline *RUNX1* mutation
Myeloid neoplasms with germline *ANKRD26* mutation
Myeloid or lymphoid neoplasms with germline *ETV6* mutation
Hematologic neoplasms with germline predisposition associated with a constitutional disorder affecting multiple organ systems
Myeloid neoplasms with germline *GATA2* mutation
Myeloid neoplasms with germline *SAMD9* mutation
Myeloid neoplasms with germline *SAMD9L* mutation
Myeloid neoplasms associated with bone marrow failure syndromes
Fanconi anemia
Shwachman-Diamond syndrome
Telomere biology disorders including dyskeratosis congenita
Severe congenital neutropenia
Diamond-Blackfan anemia
JMML associated with neurofibromatosis
JMML associated with Noonan-syndrome-like disorder (CBL-syndrome)
Myeloid or lymphoid neoplasms associated with Down syndrome
Acute lymphoblastic leukemia with germline predisposition*
Acute lymphoblastic leukemia with germline *PAX5* mutation
Acute lymphoblastic leukemia with germline *IKZF1* mutation

Arber et al, Blood 2022

Table 3. Clinical features prompting consideration of clinical testing for a germline predisposition allele(s)

Clinical features
Personal history of ≥2 cancers, 1 of which is a hematopoietic malignancy (order does not matter)
Personal history of a hematopoietic malignancy plus: • Another relative within two generations with another hematopoietic malignancy, or • Another relative within two generations with a solid tumor diagnosed at age 50 or younger, or • Another relative within two generations with other hematopoietic abnormalities
Presence of a deleterious gene variant in tumor profiling that could be a germline allele, especially if that variant is present during remission*
Age of diagnosis of hematopoietic malignancy at an earlier age than average (eg, MDS diagnosed ≤ 40 y)
Germline status of a variant is confirmed by: Its presence in DNA derived from a tissue source not likely to undergo somatic mutation frequently (eg, cultured skin fibroblasts or hair follicles) AND at a variant allele frequency consistent with the germline (generally considered between 30-60%), or Its presence in at least two relatives at a variant allele frequency consistent with the germline

*Certain gene alleles (eg, *CHEK2* I200T and truncating *DDX41* variants) are overwhelmingly likely to be germline and should prompt consideration of germline testing when identified even once.

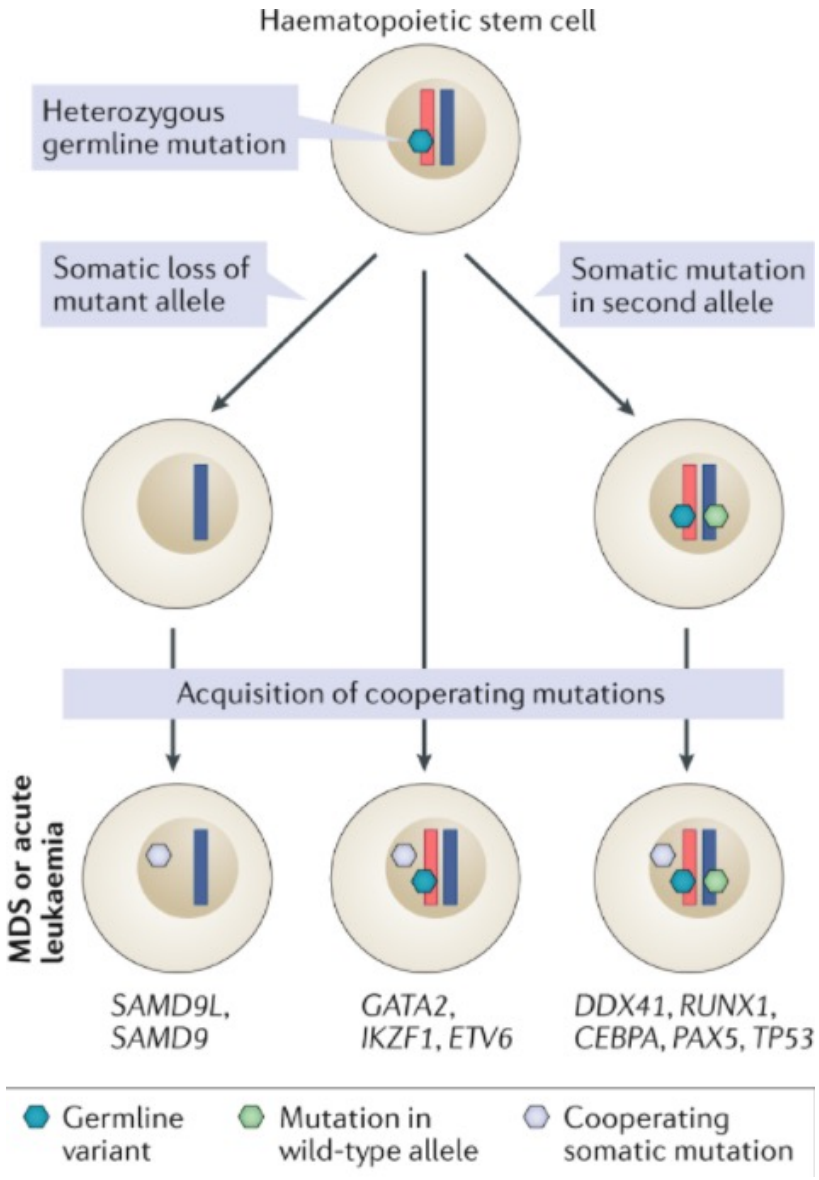
*pathogenic or likely pathogenic germline variants

* Regardless of age (*DDX41* 44-88y)

*validation by culture and sequencing of skin fibroblasts

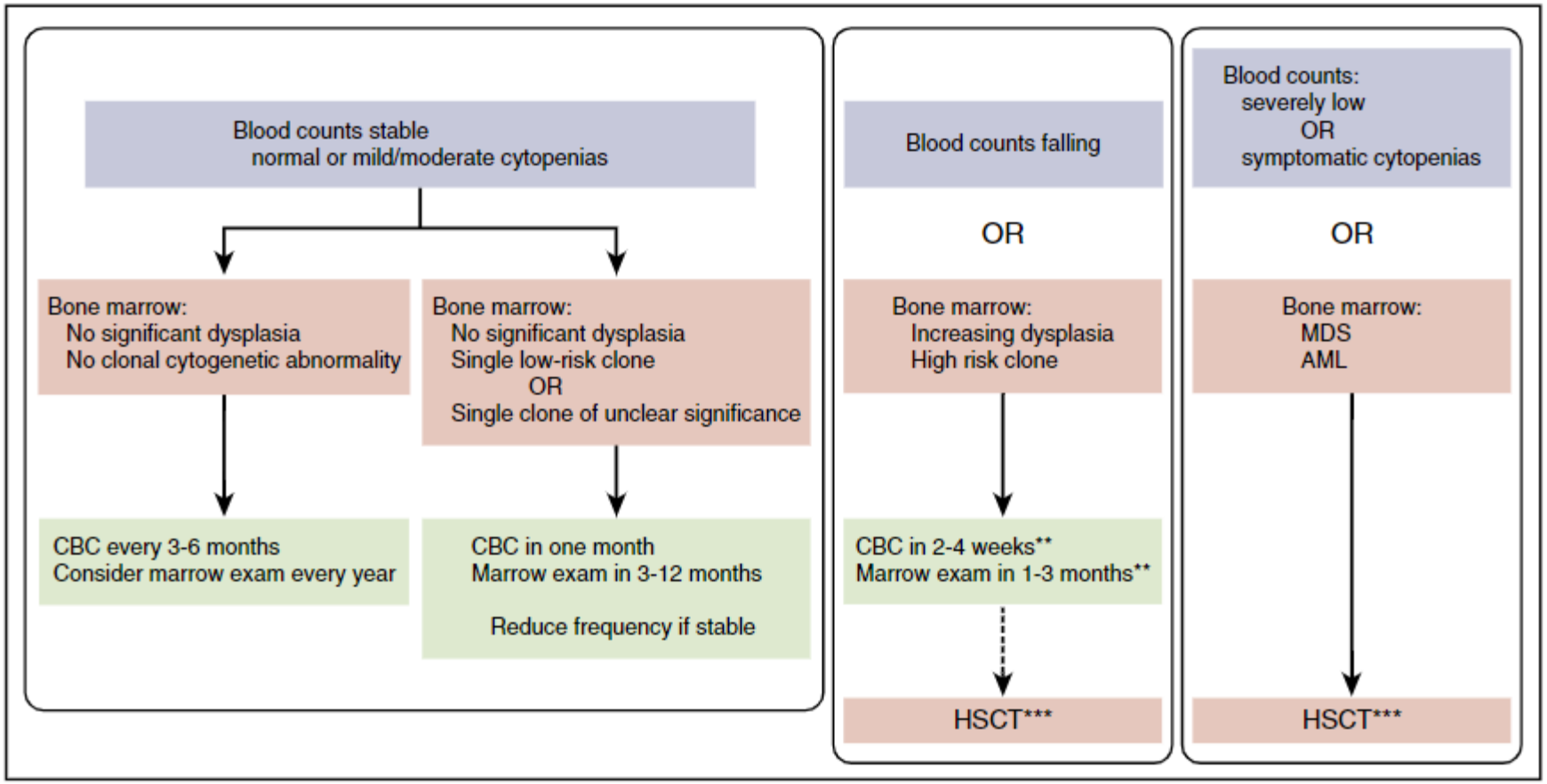
Dohner et al, Blood 2022

Model of disease progression



Klco, Nature review cancer 2021

How to manage germline predisposition to AML ?



Godley et al, Blood 2017

Table 2

Follow-up of individuals with a germline predisposition to MDS/AML

	Baseline	Follow-up
Complete blood count (CBC)	YES	Every 6 months
Bone marrow aspirate/biopsy	YES	Only in case of change in CBC
NGS-myeloid gene panel	YES (bone marrow)	Once a year[a] (blood)
Control of other relevant organs	As indicated depending on the underlying condition	As indicated depending on the underlying condition

CBC = complete blood count; NGS = Next-generation sequencing.

[a] The emergence of a clone should not solely be an indication for action. The gene, the variant allele frequency (VAF), the number of pathogenic variants as well as the dynamics over time should be taken into account.

Baliakas et al, Hemasphere 2022

2. Clonal hematopoiesis

CH: the outsized contribution of expanded clones of HSC and progenitor cells to blood cell production

prevalence of CH increase with age

CH > somatic mutations in individual genes or

> gains /losses of larger chromosomal segments

CH = premalignant state

somatic mutations in CH = initiating mutations for hematological malignancies

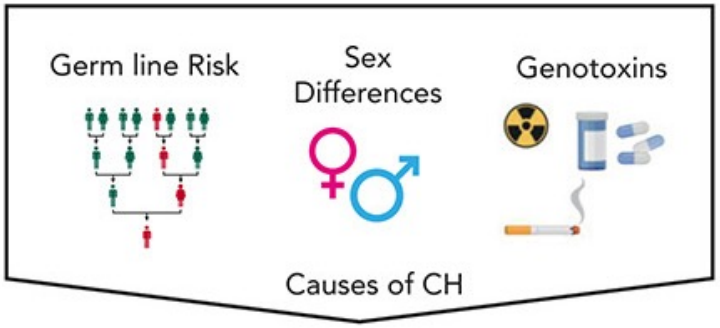
CH = strong predictor of development of blood cancers

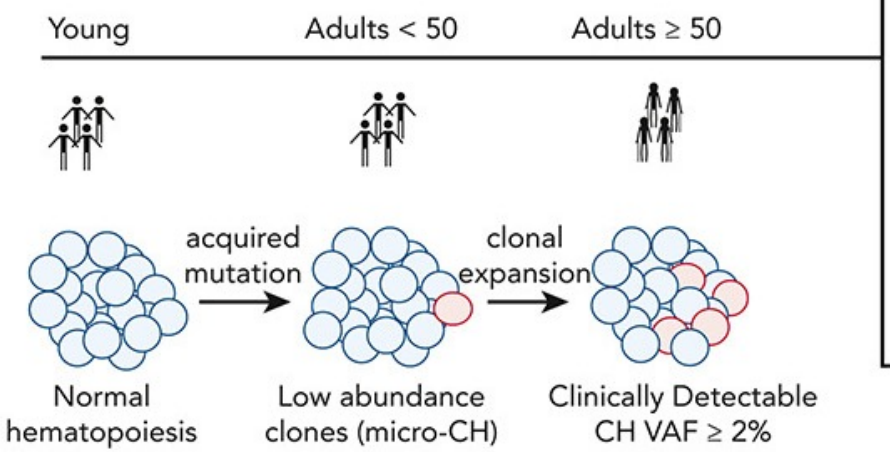
mutations alter the function of terminally differentiated blood cells

including release of elevated levels of inflammatory cytokines -> inflammatory disorders

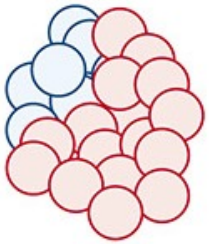
Weeks et al, Blood 2023

Causes and Consequences of Clonal Hematopoiesis (CH)



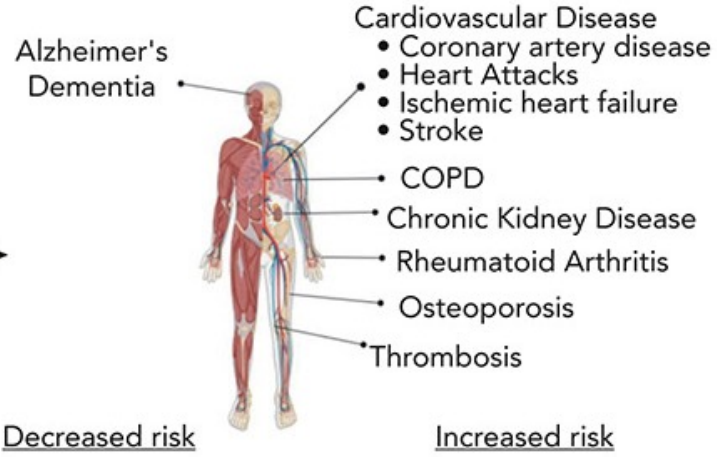


Malignant Outcomes



Myeloid CH CHIP/CCUS, M-mCA	→ MDS, CMML, AML, ET, PV, MF, Ph- CML
Lymphoid CH L-CHIP, L-mCA	→ CLL, SLL, Lymphomas

Nonmalignant Outcomes



Weeks et al, Blood 2023

CHIP : **CH of indeterminate potential**

CH possessing somatic mutations in leukemia driver genes

at a variant allele fraction (VAF) of ≥2% in the absence of cytopenia

CCUS : **Clonal cytopenia of undetermined significance** (CCUS)

CHIP in the presence of persistent, unexplained cytopenia in which

dysplastic features of myelodysplastic syndrome (MDS) are absent

10% to 20% of individuals aged >70 years

DNMT3A, ASXL1, and TET2 : 87%

JAK2, TP53, SF3B1, and SRSF2 remaining cases

Weeks et al, Blood 2023

- How does clonal hematopoiesis progress to AML ? 10 -15 years pre-AML

4 patterns

Linear evolution : successive mutations in a single dominant clone

Clonal competition : multiples clones with clear evidence of clonal interference

Static evolution : expanded clones have stopped growing ?

Late evolution : mutations only detected close to AML diagnosis or not at all

when ?

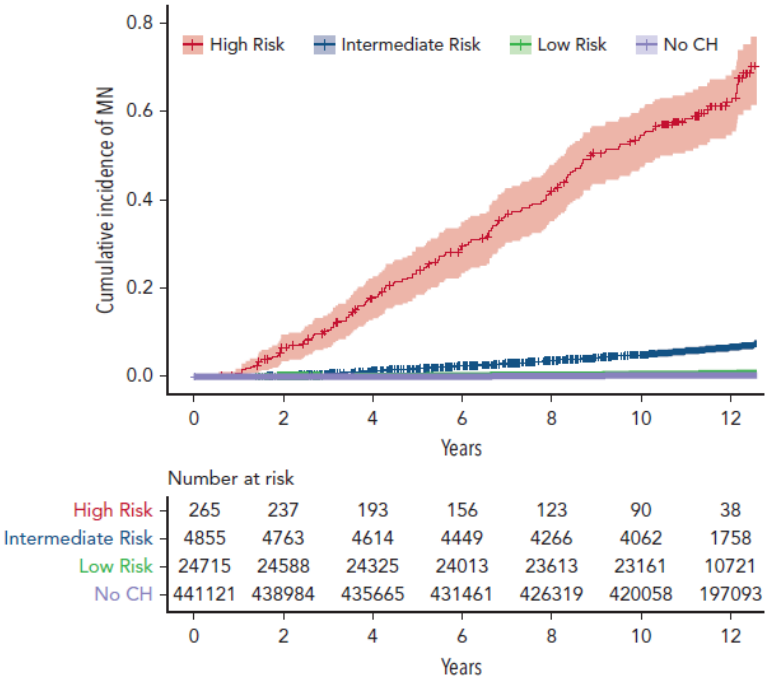
1st driver mutation : > 50 years pre-AML (linear) - 4 years pre-AML (late)

Watson C, ESH 2023

CHIP : risk of malignant transformation 0,5 – 1% per year

depend on specific mutations and hematological features

CHRS Prognostic Variable Scores					
Prognostic Variable	0.5	1	1.5	2	2.5
Single *DNMT3A*	present	absent	–	–	–
High Risk Mutation	–	absent	–	–	present
Mutation Number	–	1	–	≥ 2	–
Variant Allele Fraction	–	< 0.2	–	> 0.2	–
Red Cell Distribution Width	–	< 15	–	–	≥ 15
Mean Corpuscular Volume	–	< 100	–	–	> 100
Cytopenia	–	CHIP	CCUS	–	–
Age	–	< 65y	≥ 65y	–	–



Weeks et al, Blood 2023

10-year Risk of Myeloid Malignancy

> 50%

7-8%

< 1%

Malignancy Risk Mitigation Strategy

High Risk:
- Q3-6 month CBC+D
- Repeat NGS annually
- Repeat bone marrow with clinical change
- Consider risks/benefits of clinical trial participation

Intermediate Risk:
- Annual CBC+D
- Repeat NGS with clinical change
- Repeat bone marrow with clinical change

Low Risk:
- Annual CBC+D
- Repeat NGS with clinical change
- Repeat bone marrow with clinical change

Management of Non-Malignant Disease Risk

All Patients

- Assessment for risk factors or symptoms of ischemic cardiovascular disease
- Referral to primary care provider or preventative cardiologist for guidelines-based cardiovascular disease prevention strategy
- Other sub-specialists (rheumatology, endocrinology, hepatology) may be engaged based on patient symptoms, physical exam findings, and personal risk

Conclusions

To understand the molecular mechanisms of AML

extreme heterogeneity of leukemic blasts

-> targeted therapies/relapse

To understand the 'preleukemic states'

* Germlines mutations

* Clonal hematopoiesis - CHIP


Thank you for your attention