Genomics of high risk AML

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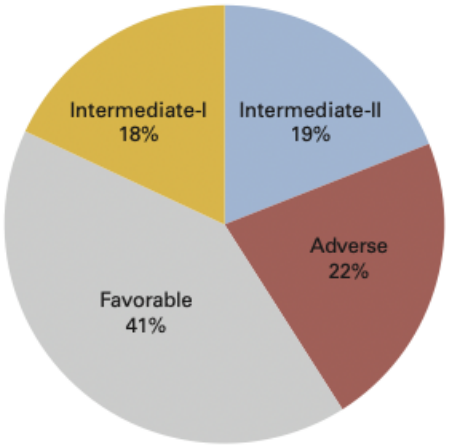
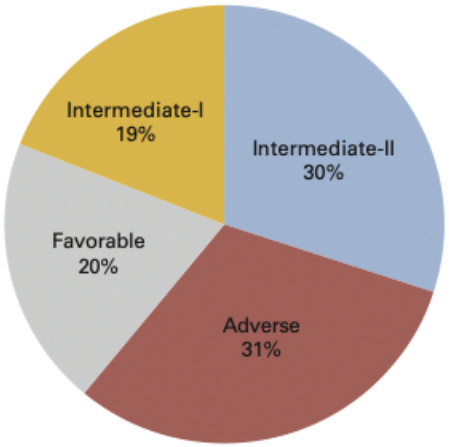
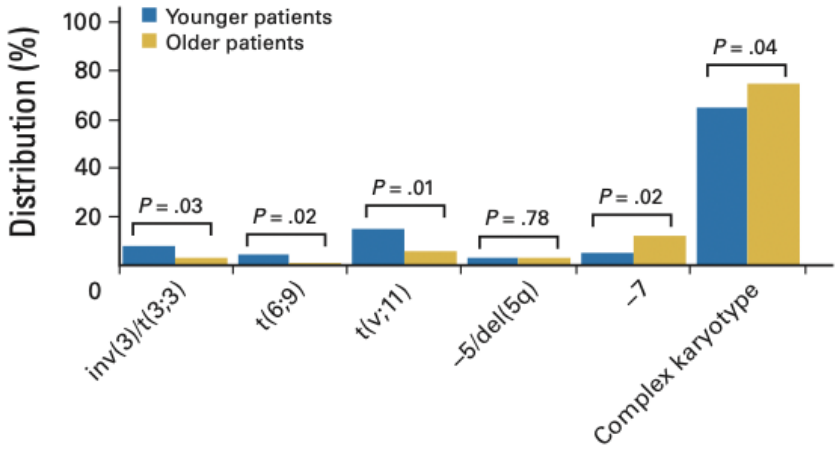
Adverse-risk AML



Younger adults



Older adults



Mrozek K, *J Clin Onc.* 2012

Adverse-risk AML molecular cytogenetic subsets (*ELN* 2017°)

- inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
- t(6;9)(p23;q34); *DEK-NUP214*
- t(v;11)(v;q23); *MLL* rearranged
- Monosomy 5 (-5) or del(5q)
- Monosomy 7 (-7)
- Complex karyotype/monosomal karyotype
- abnl(17p)

°Dohner H, *Blood*. 2017

Two major classes of therapy-related myeloid neoplasms

	Alkylating agent class	Topoisomerase II inhibitor class
Cytogenetics	del(5q), –7/del(7q)*	t(11q23.3), t(21q22.1)
Frequency	~70% of t-MN patients	~30% of t-MN patients
Latency	5–7 years	2–3 years
Presentation	MDS	AML
Implicated drugs	• Alkylating agents: bendamustine, busulfan, carmustine, chlorambucil, cyclophosphamide, dacarbazine, lomustine, melphalan, mitomycin C, nitrogen mustard, procarbazine, thiotepa • Platinum-based agents: cisplatin, carboplatin • Antimetabolite agents: azathioprine, fludarabine	• Anthracyclines: daunorubicin, epirubicin, doxorubicin • Other topoisomerase II inhibitors: etoposide, teniposide, amsacrine, mitoxantrone

*Loss of the short arm of chromosome 17 containing the *TP53* gene due to del(17p), unbalanced rearrangement or –17 is observed in association with del(5q) in 40% of cases.
AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; t-MN, therapy-related myeloid neoplasm.

AML with monosomy 7 (-7)

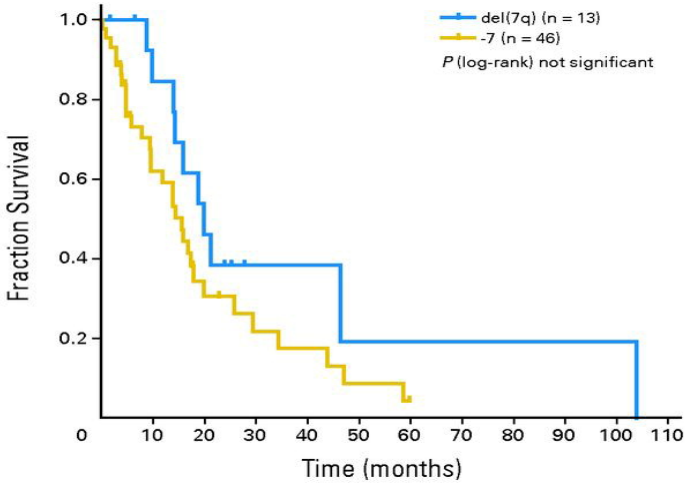
- **Monosomy 7 (-7) is the most frequent autosomal monosomy in AML. -7/del(7q) abnl is frequent in therapy-related/ AML*.**

- **-7 found in congenital evolving into myeloid neoplasms, e.g. germ-line *GATA2* mutations, neurofibromatosis, severe congenital neutropenia°.**

- **Tumor-suppressor genes located in chr. 7 are believed to act**
- **in a *haploinsufficient* manner:**
  - ***SAMD9/SAMD9L*** → endosomal proteins
  - ***EZH2*** **Histone modifying enzymes → frequently**
  - ***MLL3*** **associated with *Ras*-pathway mutations and *TP53* inactivation°.**

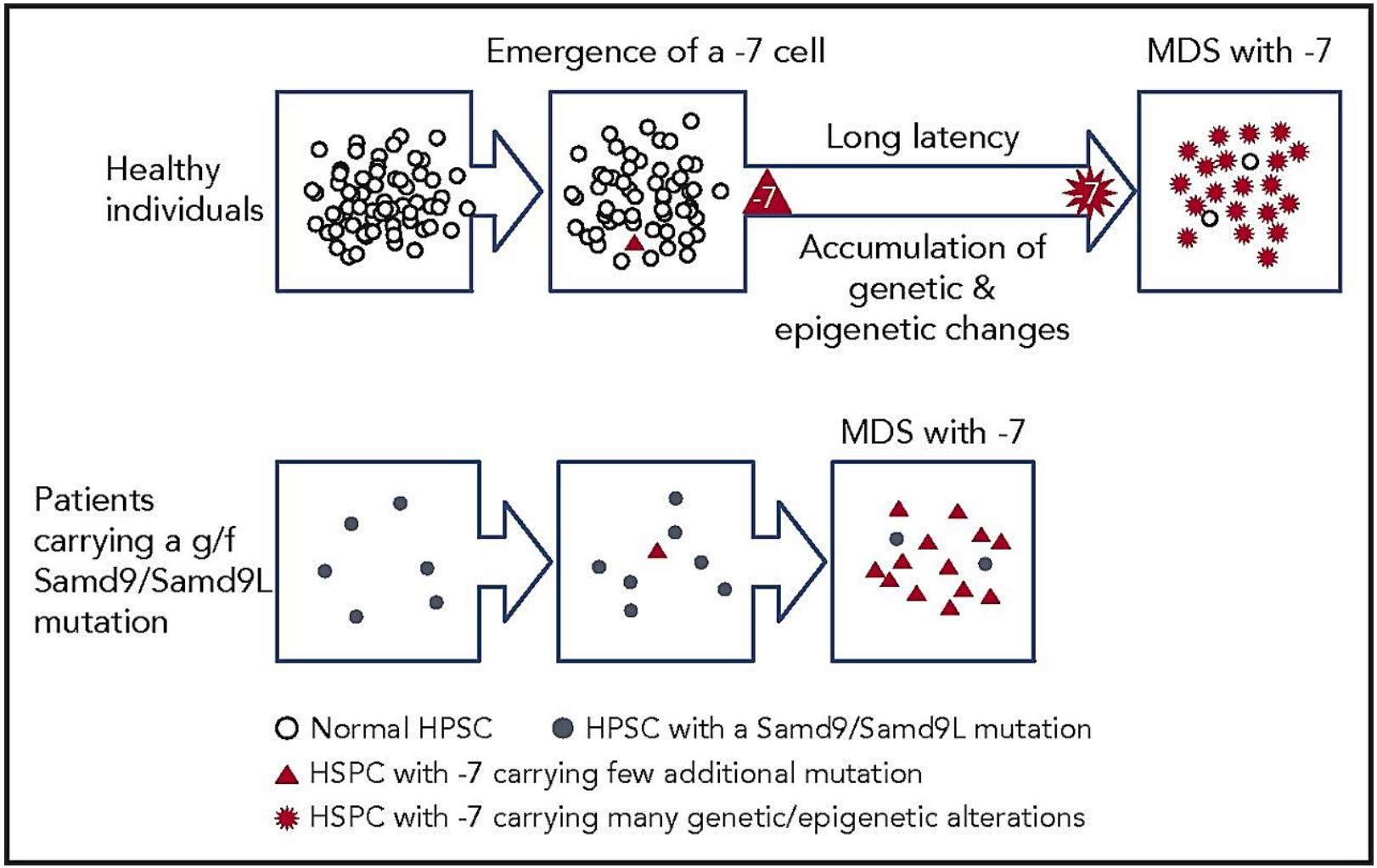
- **Prognosis of AML/MDS with -7 is worse than del(7q). Possibly for the more frequent association with unfavorable abnl (i.e. *EVI1*-r), del(7q)q) more commonly associated with AML with favorable karyotypes (i.e. inv(16), t(8;21)^)°,§.**

Survival of AML patients with -7/del(7q) abnl§



*^Grimwade D, Blood. 2010; *McNerney ME, Nat Rev Cancer.2017; °Inaba T, Blood. 2017; §Schanz J, J Clin Onc. 2010.*

The enigma of monosomy 7



Inaba T et al. The enigma of monosomy 7, Blood, 2018.

EVI1-rearranged AML

- ***EVI1*** **(ecotropic viral integration site 1) gene, also termed *MECOM* is located on chromosomal band 3q26.2.**

- **The inv(3)q21q26.2)/t(3;3)q21;q26.2)^ repositions a distal GATA2 enhancer to activate MECOM. Deregulated MECOM and GATA2 expression.**

- **High levels of *EVI1* in normal CD34+ cells*** and **5-10% of human AML°.**

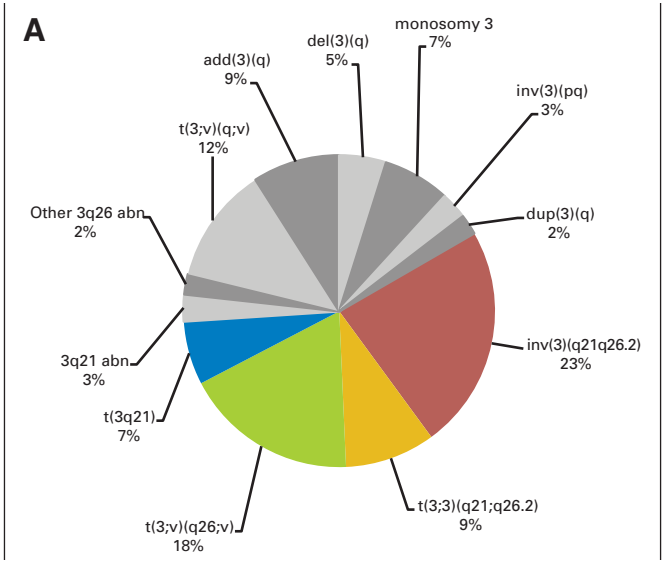
- **AML requires > 20% blasts. Usually CD34+. CD7+.**

- ***EVI1*-rearranged (*EVI1*-r) AML is frequently associated with monolobated megakaryocytes, multilineage dysplasia and normal/elevated blood platelet counts^. Poor prognosis.**

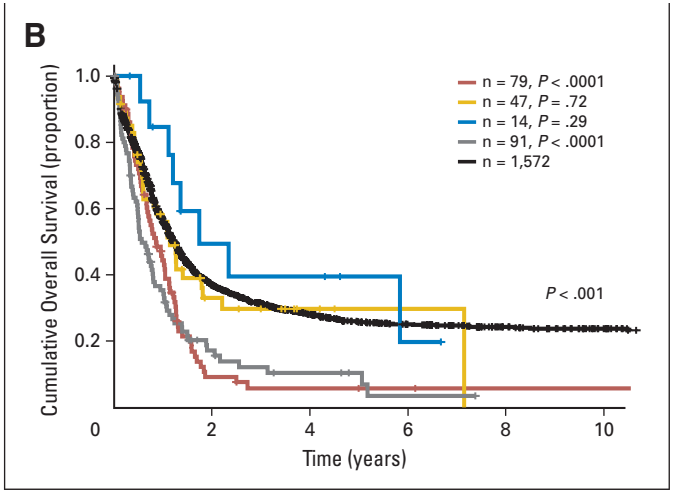
- **Monosomy 7 (-7) is the most frequent associated cytogenetic anomaly in *EVI1*-r AML (33-66% of cases of inv(3)/t(3;3)^.**

- **Mutational oncoprint featuring *EVI1-r* AML category§:**
  - ***RAS*-pathway** (*NRAS*, *KRAS*, *PTPTN11*, *NF1*) (~60-70%)
  - **Splicing factors** (*SF£B1*, *U2AF1*) (~60%)
  - *IKZF1* (~25%)
  - *TP53* (~25%), *ASXL1* (~15%)

Distribution of 3q abnl^



Survival of AML patients with 3q abnl^



*^Lughthart S, J Clin Oncol. 2010; *Steinleitner K, Anticancer Res. 2012; °Groschel S, J Clin Oncol. 2013; §Lavallée V-P, Blood. 2015.*

AML with complex karyotype

- **<u>Definition</u>: ≥3 chromosomal abn. in the absence of 1 of the WHO-designated recurring translocations or inversions**, including t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3)^.

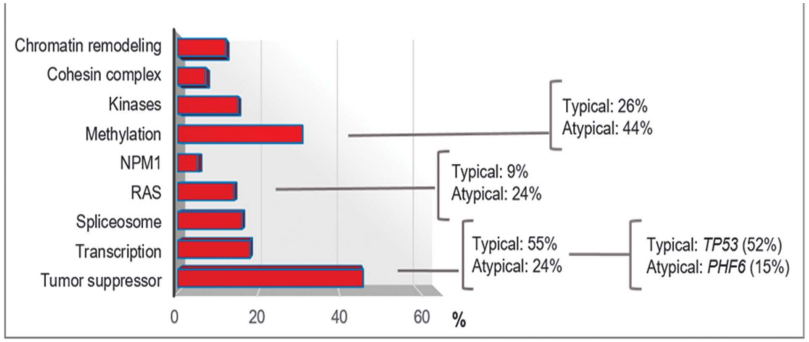
- **∽10-12% of adult AML cases, most frequent losses involve 5q (80% of cases), 7q and 17p chromosomes*.**

- **CK-AML was recently proposed to be further sub-clustered* into:**

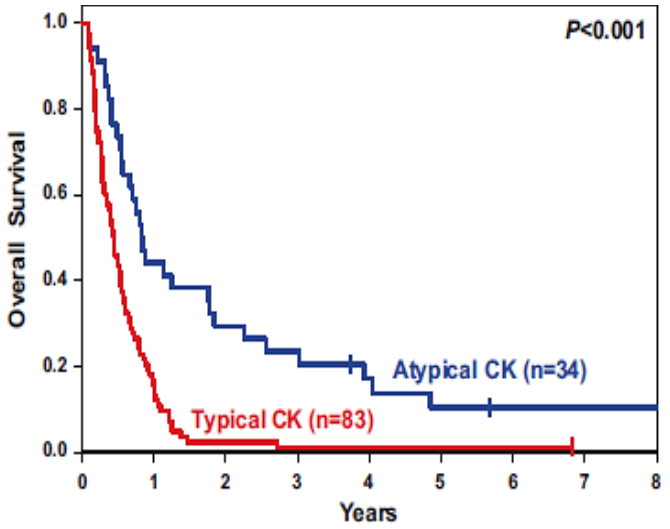
  - **<u>Typical CK</u>: presence of 5q, 7q abn. and/or 17p loss → association with *TP53* mutations (∽80% of cases) . Very poor prognosis.**

  - **<u>Atypical CK</u>: absence of above chromosomal abnl → less often *TP53* mutations . Better Outcome.**

Deregulated pathways in CK AML°



Survival of AML patients with CK*

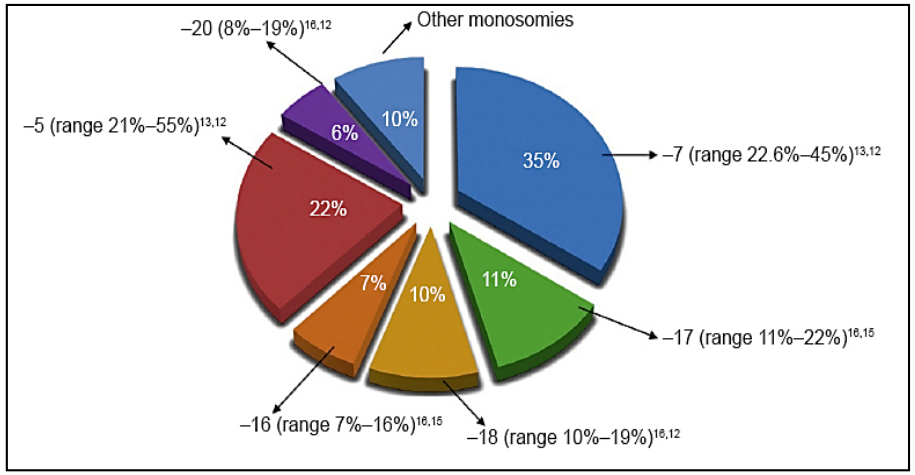


*^Dohner H, Blood. 2017; *Mrozek K, Leukemia. 2019; °Eisfeld AK, Leukemia. 2017.*

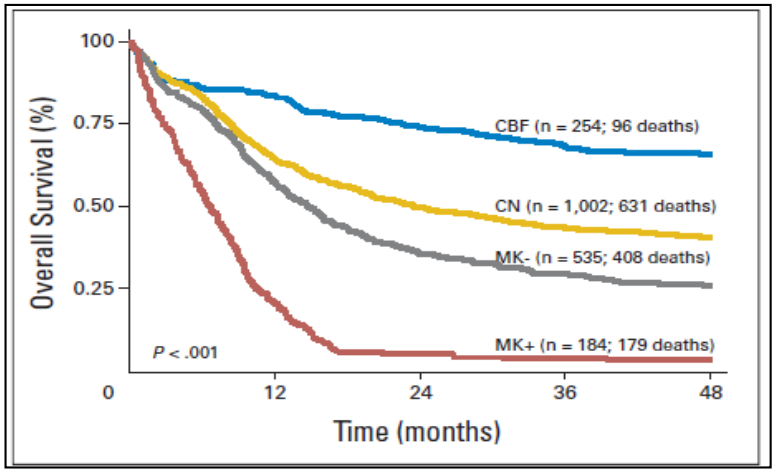
AML with monosomal karyotype

- **Definition**: ≥2 distinct autosomal monosomies or one single autosomal monosomy in the presence of structural abnormalities^. Deletions of - X and –Y are non considered monosomies.
- More frequent in therapy-related than *de novo* AML
- *TP53* gene alterations occur in ∽70% of MK AML patients, leading to a markedly increased chromosomal instability* and poor response to conventional chemotherapy^,*.

Frequency of autosomal abnl observed in AML with monosomal karyotype°



Prognosis of AML patients with monosomal karyotype^



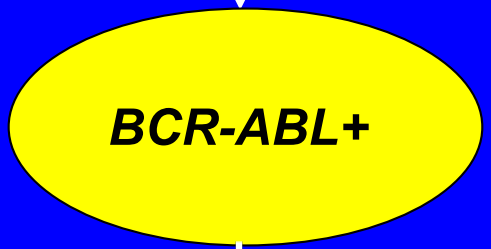
*^Breems DA, J Clin Onc. 2008; *Leung GM, Am J Hematol. 2019; °Anelli L, Oncotargets & Therapy, 2015.*

AML with *BCR-ABL1* (Provisional WHO entity)

- **Difficult to distinguish from myeloid blast crisis of CML. No previous CML history, < 2% basophils, rarely splenomegaly**
- **Deletion of antigen receptors genes (*IGH*, *TCR*), *IKZF1* and/or *CDKN2A* supports a diagnosis of de novo disease**
- **Aberrant expression of CD19, CD7 and TdT is common**
- **Presence of t(9;22) or BCR/ABL fusion (p210). A subset of cases carry *NPM1* mutations**
- **Important to recognize due to availability 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

Distinction between *BCR-ABL* AML(provisional WHO category) and *NPM1*- mutated AML



- CD34 negative
- Good response if FLT3/ITD absent in the few cases reported) *

* *Arber, 2017 WHO Book; Koplonev, Leuk & Lymphoma, 54:138, 2013*

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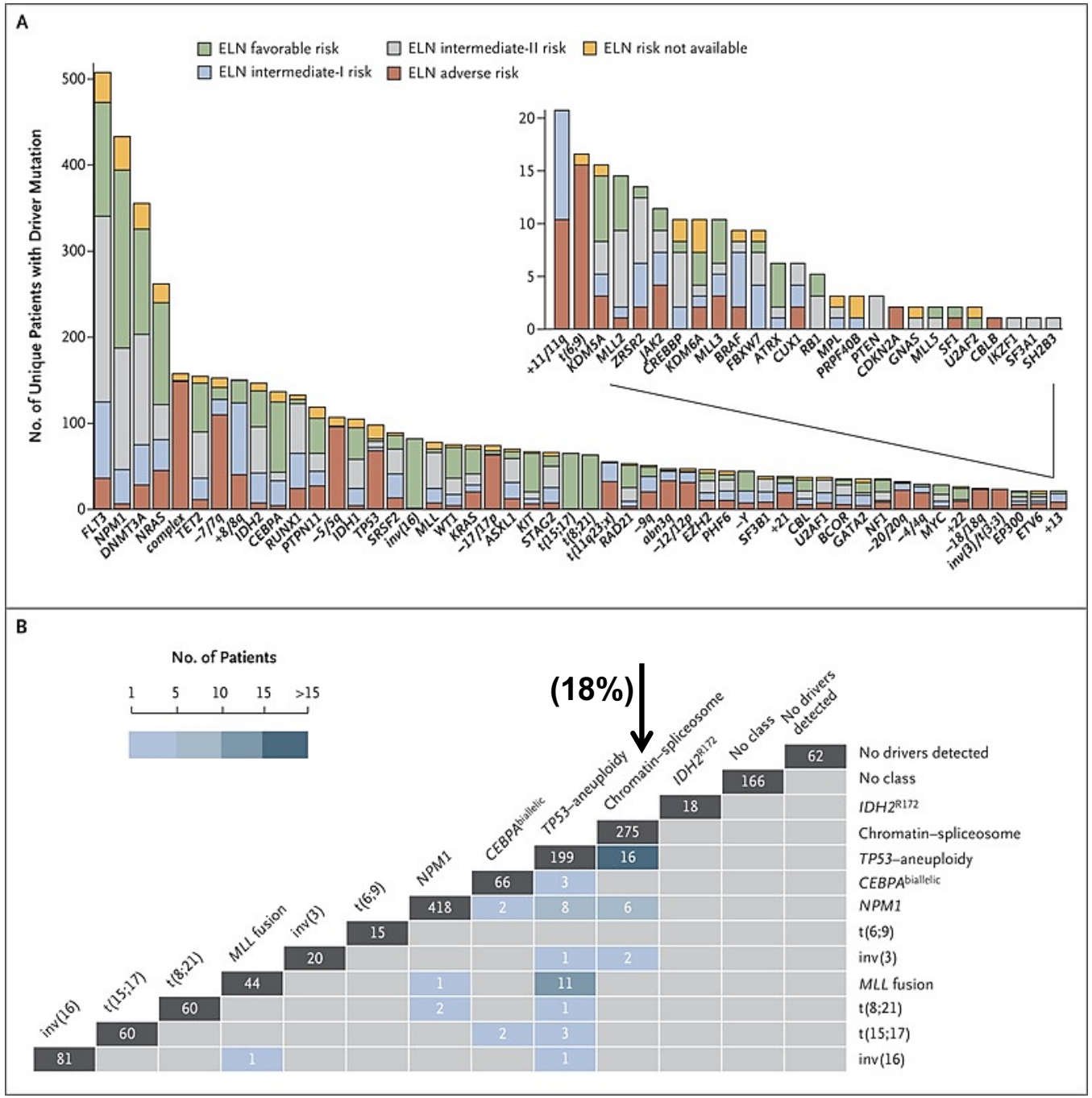
2017 ELN risk stratification by genetics

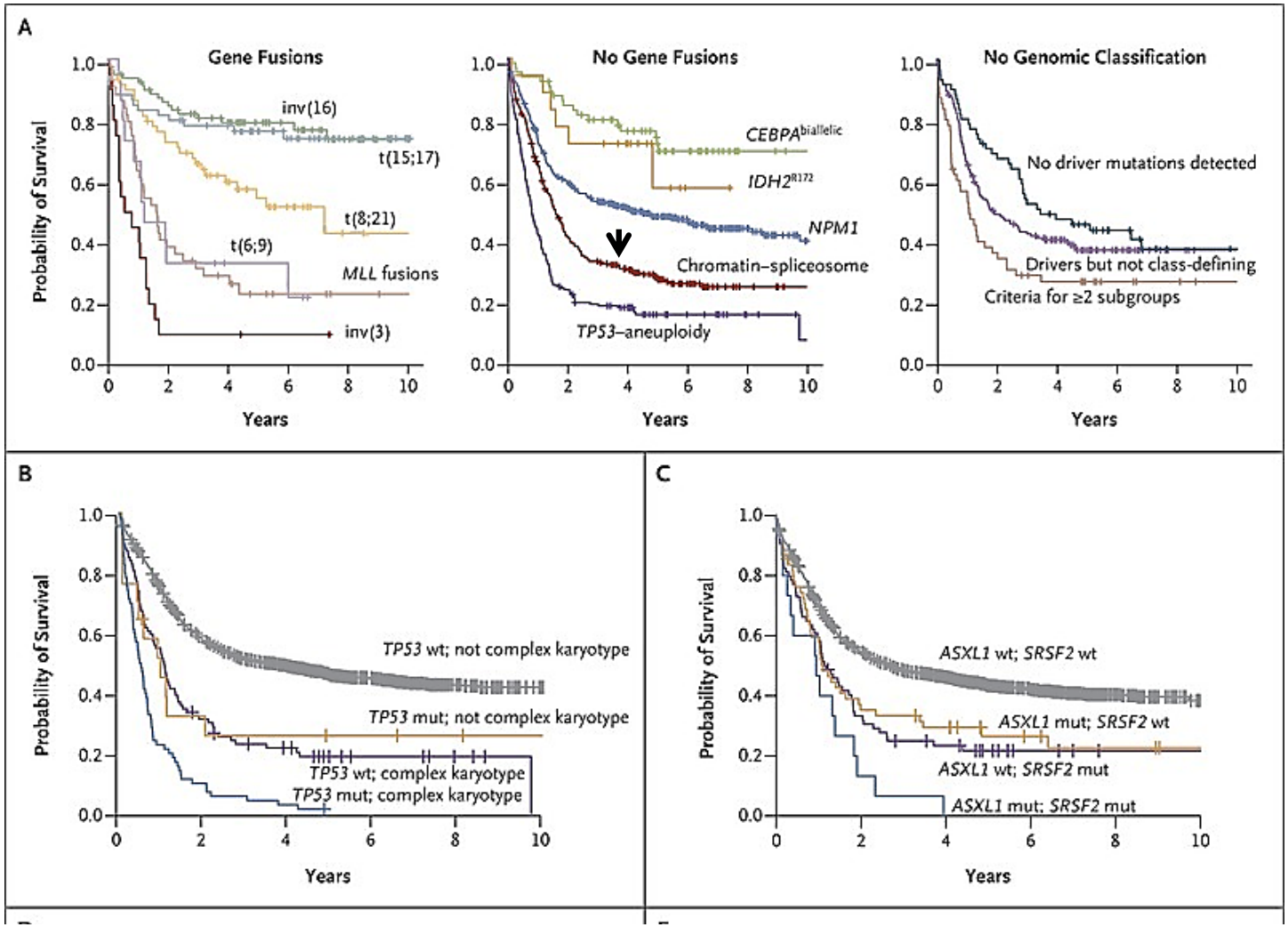
Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
→	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	→ Mutated *NPM1* and *FLT3*-ITD^{high}†
	→ Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	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,§ monosomal karyotype‖
→	Wild-type *NPM1* and *FLT3*-ITD^{high}†
→	Mutated *RUNX1*¶
→	Mutated *ASXL1*¶
→	Mutated *TP53*#

Chromatin-spliceosome modifiers

2017 ELN recommendations (Dohner et al. Blood 2017)



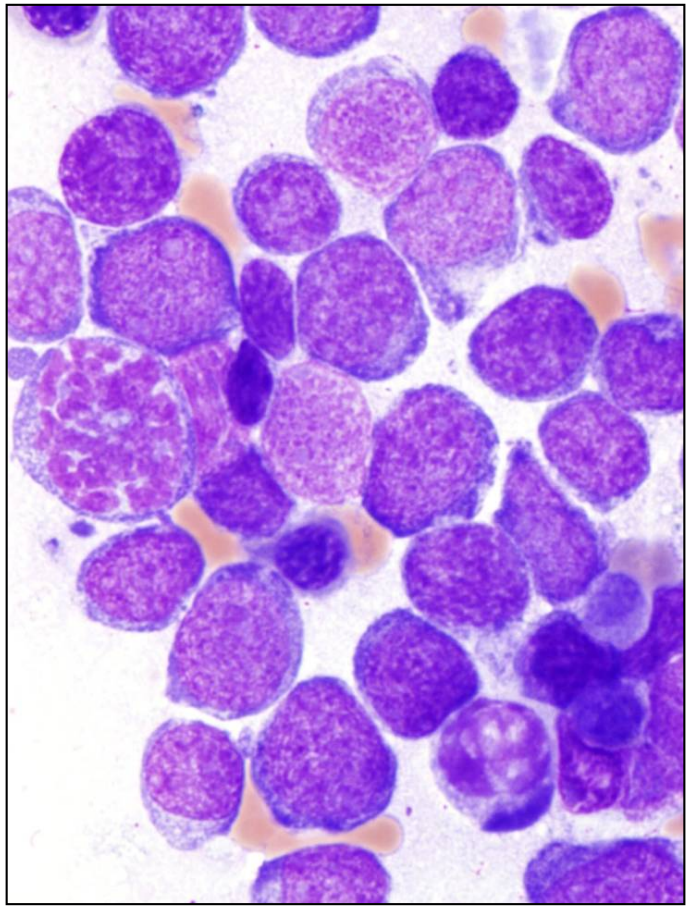
Papaemmanuil E, et al. NEJM 374:2209-2221, 2016



Papaemmanuil E, et al. NEJM 374:2209-2221, 2016

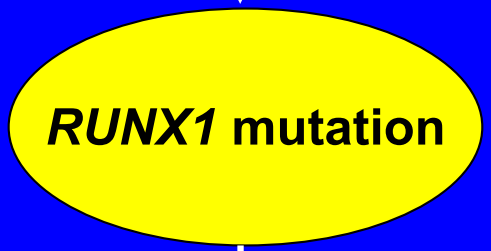
AML with mutated *RUNX1*
(Provisional WHO entity)

- Mutations usually involve the RHD or TAD domains of RUNX1 (located at 21q22) and encoding the alpha subunit of CBF
- Search for germline mutations in the family (exclude autosomal dominant thrombocytopenia). Predisposes to AML
- Mutations in 4-16.0% of AML
- More frequent in older male patients
- Frequent prior history of MDS, or prior exposure to radiation
- May be preceded by Fanconi anemia or
- Congenital neutropenia
- Immature morphology (60% M0) and phenotype. Usually CD34+
- Frequent associated *MLL*-PTD or *ASXL1* mutations
- Poor response to therapy and short survival



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

Distinction between *RUNX1*-mutated AML (provisional WHO category) and *NPM1*-mutated AML



- *RUNX1* mutations different from other AMLs
- In-frame, outside RD region
- Often germline
- Unclear functions

** *Mendler et al. Haematologica, 98:e92, 2013*

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Table 1. Proposed Genomic Classification of Acute Myeloid Leukemia (AML).

Genomic Subgroup	Frequency in the Study Cohort (N=1540)	Most Frequently Mutated Genes*
	no. of patients (%)	*gene (%)*
AML with *NPM1* mutation	418 (27)	***NPM1*** (100), *DNMT3A* (54), *FLT3*^{ITD} (39), *NRAS* (19), *TET2* (16), *PTPN11* (15)
AML with mutated chromatin, RNA-splicing genes, or both†	275 (18)	***RUNX1*** (39), *MLL*^{PTD} (25), ***SRSF2*** (22), *DNMT3A* (20), ***ASXL1*** (17), ***STAG2*** (16), *NRAS* (16), *TET2* (15), *FLT3*^{ITD} (15)
AML with *TP53* mutations, chromosomal aneuploidy, or both‡	199 (13)	**Complex karyotype** (68), **–5/5q** (47), **–7/7q** (44), ***TP53*** (44), **–17/17p** (31), **–12/12p** (17), +8/8q (16)
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB–MYH11*	81 (5)	**inv(16)** (100), *NRAS* (53), +8/8q (16), +22 (16), *KIT* (15), *FLT3*^{TKD} (15)
AML with biallelic *CEBPA* mutations	66 (4)	***CEBPA***^{biallelic} (100), *NRAS* (30), *WT1* (21), *GATA2* (20)
AML with t(15;17)(q22;q12); *PML–RARA*	60 (4)	**t(15;17)** (100), *FLT3*^{ITD} (35), *WT1* (17)
AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1*	60 (4)	**t(8;21)** (100), *KIT* (38), –Y (33), –9q (18)
AML with *MLL* fusion genes; t(x;11)(x;q23)§	44 (3)	**t(x;11q23)** (100), *NRAS* (23)
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM(EVI1)*	20 (1)	**inv(3)** (100), –7 (85), *KRAS* (30), *NRAS* (30), *PTPN11* (30), *ETV6* (15), *PHF6* (15), *SF3B1* (15)
AML with *IDH2*^{R172} mutations and no other class-defining lesions	18 (1)	***IDH2***^{R172} (100), *DNMT3A* (67), +8/8q (17)
AML with t(6;9)(p23;q34); *DEK–NUP214*	15 (1)	**t(6;9)** (100), *FLT3*^{ITD} (80), *KRAS* (20)
AML with driver mutations but no detected class-defining lesions	166 (11)	*FLT3*^{ITD} (39), *DNMT3A* (16)
AML with no detected driver mutations	62 (4)	
AML meeting criteria for ≥2 genomic subgroups	56 (4)	

* Genes with a frequency of 15% or higher are shown in descending order of frequency. Key contributing genes in each class are shown in boldface type.

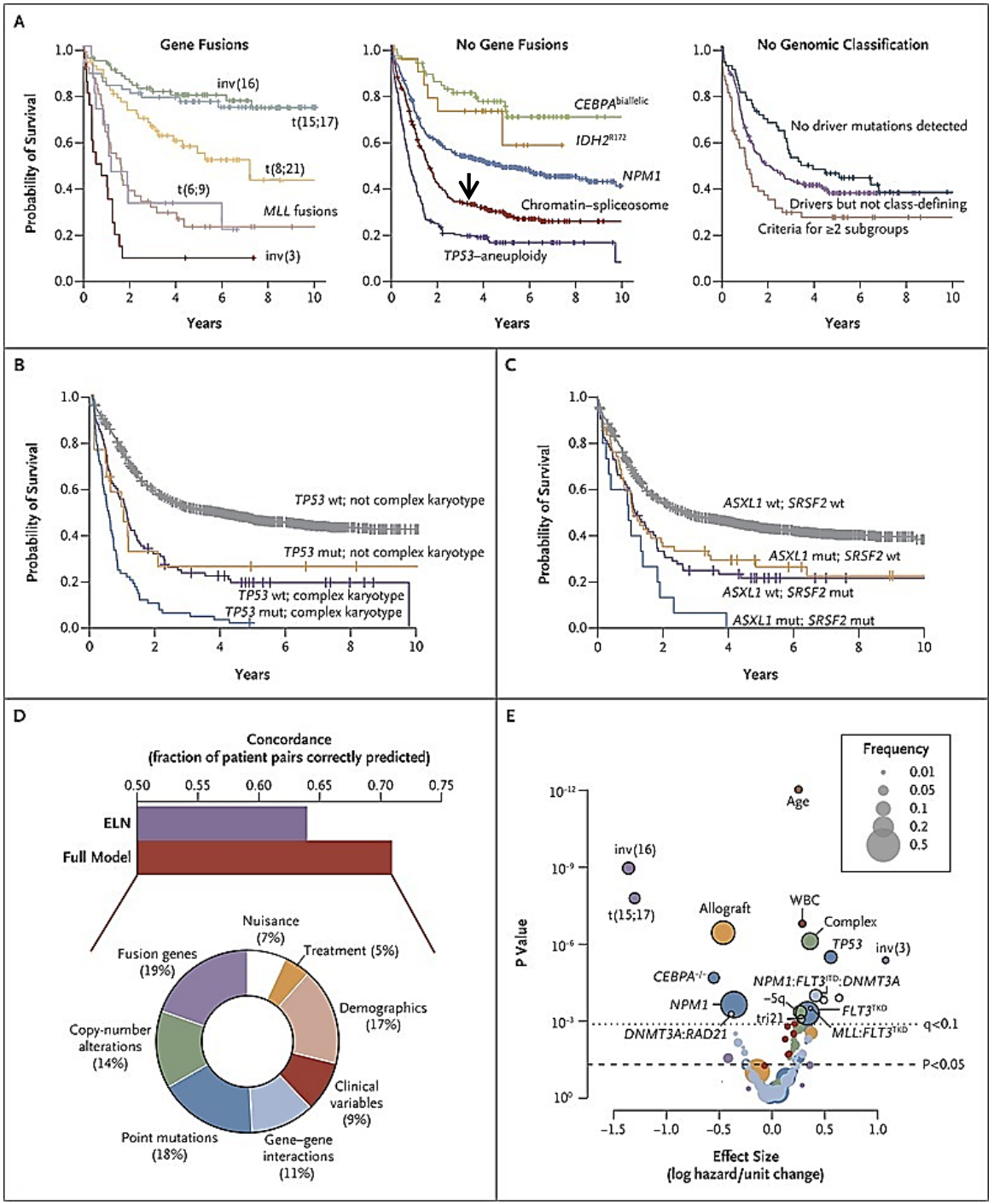
† Classification in this subgroup requires one or more driver mutations in *RUNX1*, *ASXL1*, *BCOR*, *STAG2*, *EZH2*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, or *MLL*^{PTD}. In the presence of other class-defining lesions — namely, inv(16), t(15;17), t(8;21), t(6;9), *MLL* fusion genes, or complex karyotype or driver mutations in *TP53*, *NPM1*, or *CEBPA*^{biallelic} — two or more chromatin–spliceosome mutations are required.

‡ Classification in this subgroup requires *TP53* mutation, complex karyotype, or in the absence of other class-defining lesions, one or more of the following: –7/7q, –5/5q, –4/4q, –9q, –12/12p, –17/–17p, –18/18q, –20/20q, +11/11q, +13, +21, or +22.

§ Multiple fusion partners for *MLL* were found, with the clinical implications depending on the specific fusion partner.

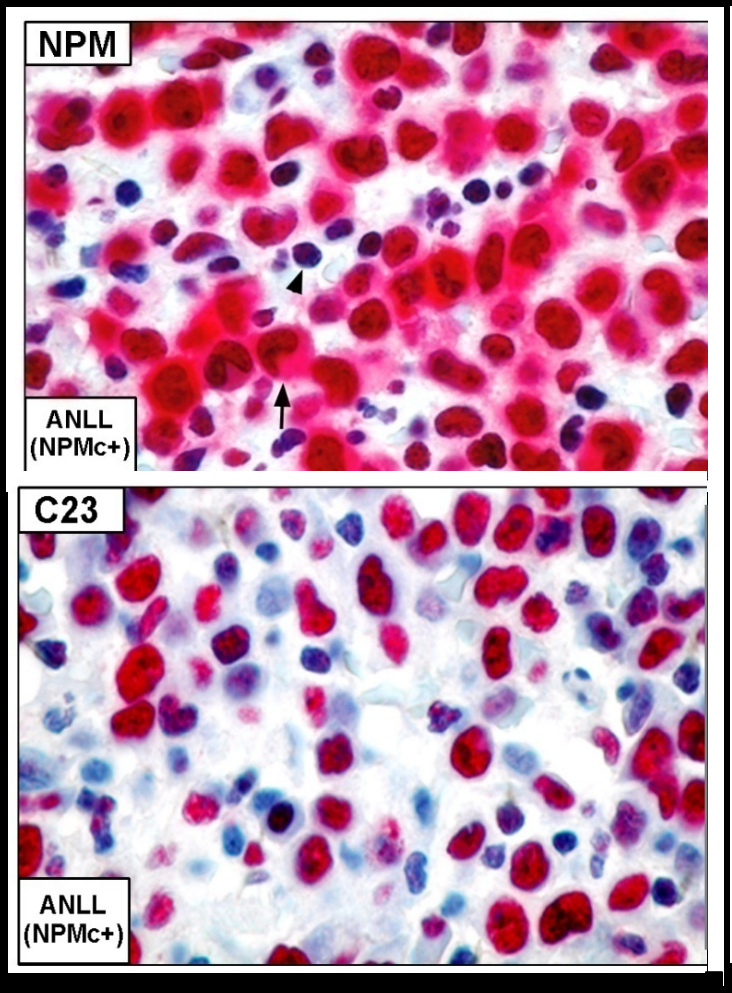
Papaemmanuil E, et al. NEJM 374:2209-2221, 2016.

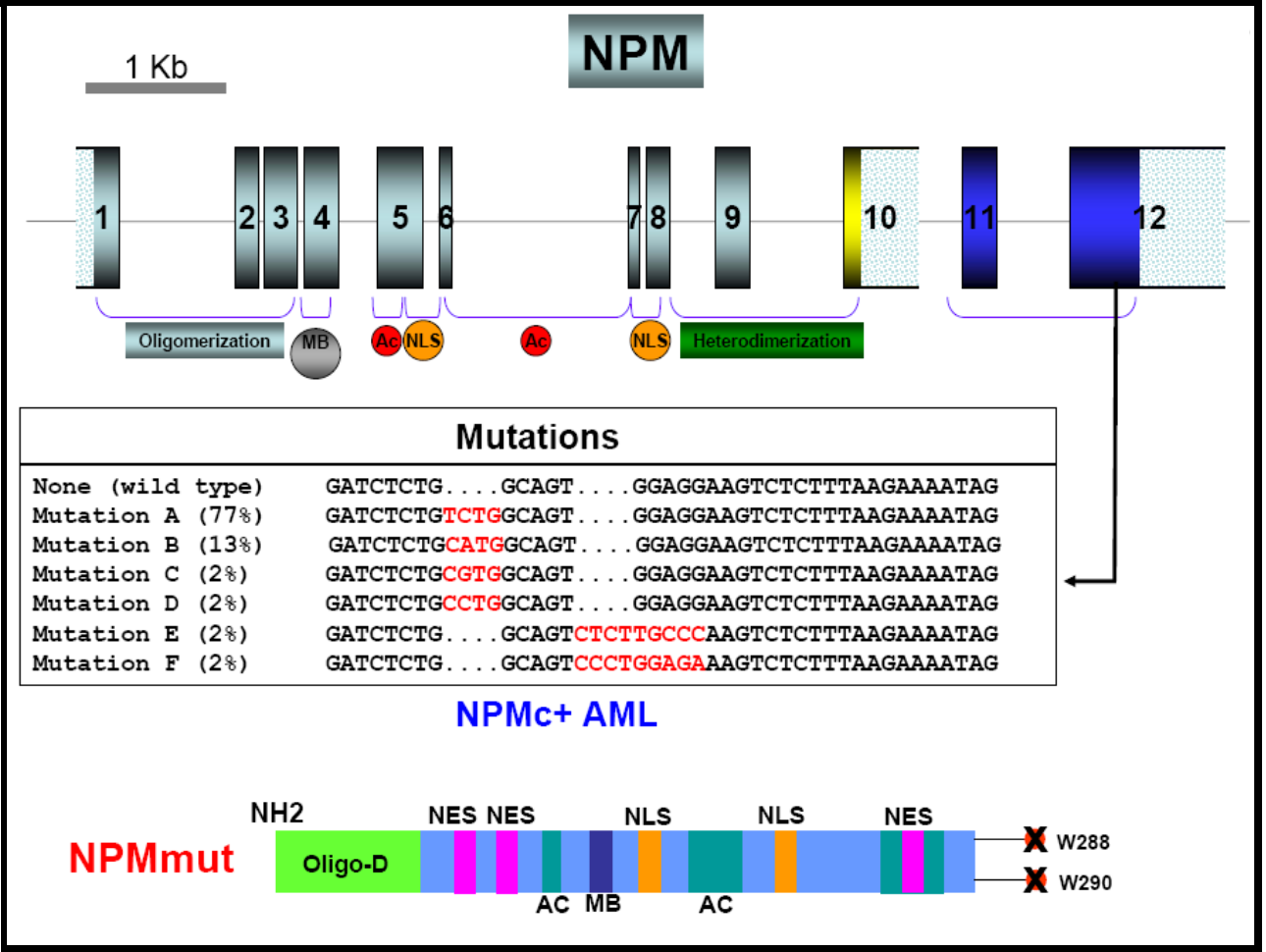
TP53



Papaemmanuil E, et al. NEJM 374:2209-2221, 2016

NPM1-mutated AML (NPMc+ AML)





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

Transgenic

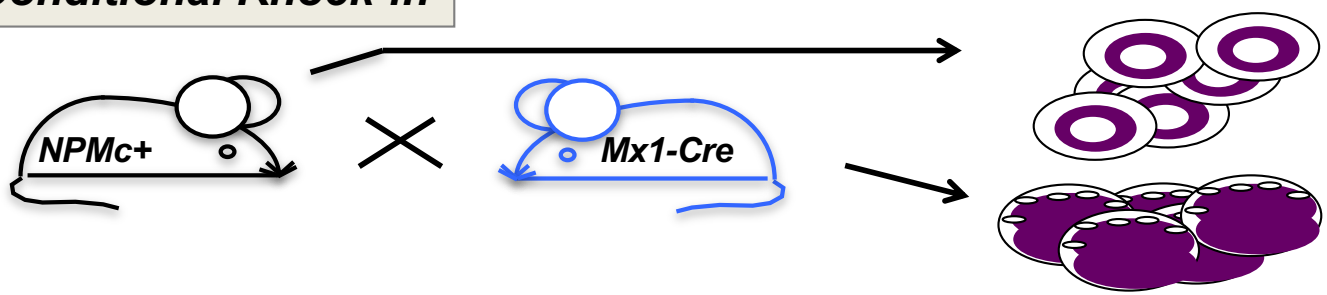
Myeloproliferation
Some mice
Cheng et al. 2010

"Non conventional" conditional Knock-in

Block of Mk differentiation
Sportoletti et al. 2013

AML late-onset
Some mice
Mallardo et al. 2013

Conditional Knock-in



MPD
Some mice
Chou et al. 2012

AML late-onset
Some mice
Vassiliou et al. 2011

Compound Mutants

AML rapid-onset
All mice
Mupo et al. 2013
Mallardo et al. 2013
Rau et al. 2013

Sportoletti P et al. Leukemia 2015

AML triple mutated *NPM1*/FLT3-ITD/DNMT3A

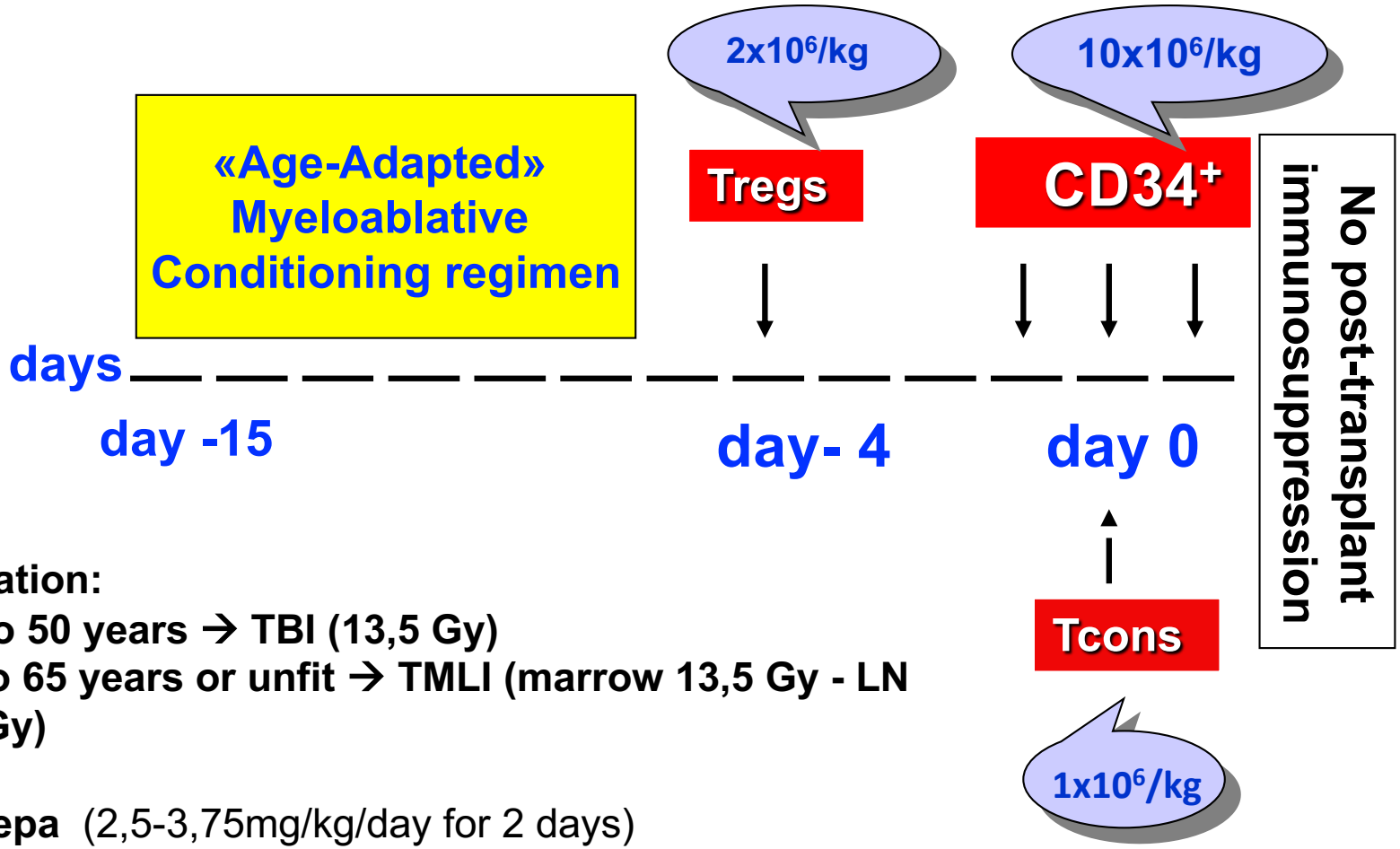
- Median age 56 years; mostly women
- High blood cell count and percentage of BM blasts
- Normal cytogenetics
- Short event-free survival (EFS)

Post-remission therapy in high-risk AML

- Eligible patients with high risk AML should receive allo-HSCT

- Allo-HSCT should point to reduce at maximum post-transplant relapse

Clinical Trial: Age-adapted myeloablative T cell depleted haploidentical transplantation with Treg/Tcon immunotherapy in AML

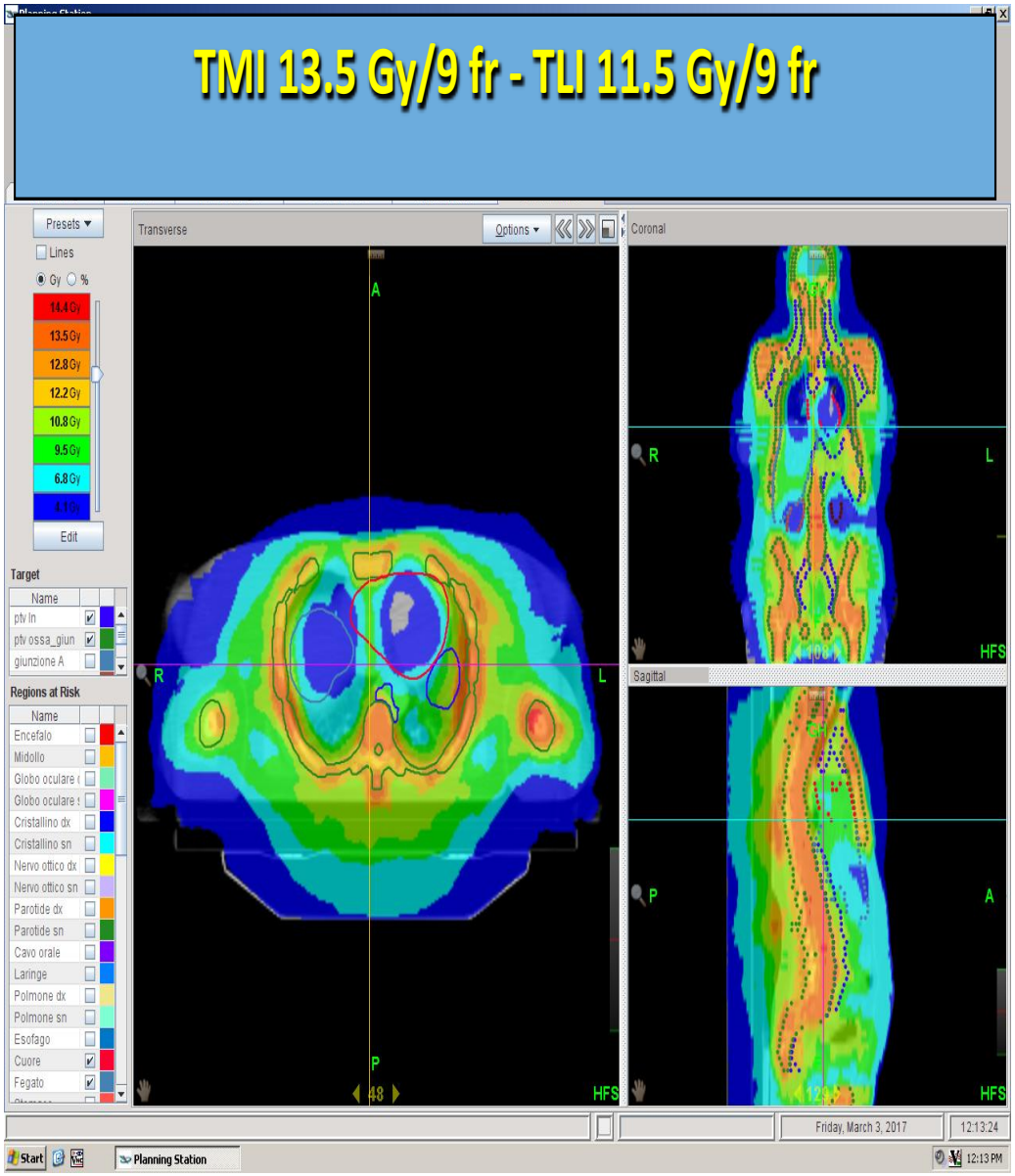


Irradiation:
- up to 50 years → TBI (13,5 Gy)
- 50 to 65 years or unfit → TMLI (marrow 13,5 Gy - LN 11,5 Gy)
+
Thiotepa (2,5-3,75mg/kg/day for 2 days)
Fludarabine (30 mg/m2/day for 5 days)
Cyclophosphamide (15 mg/kg/day for 2 days)

Pierini A et al. Blood Adv, 2021

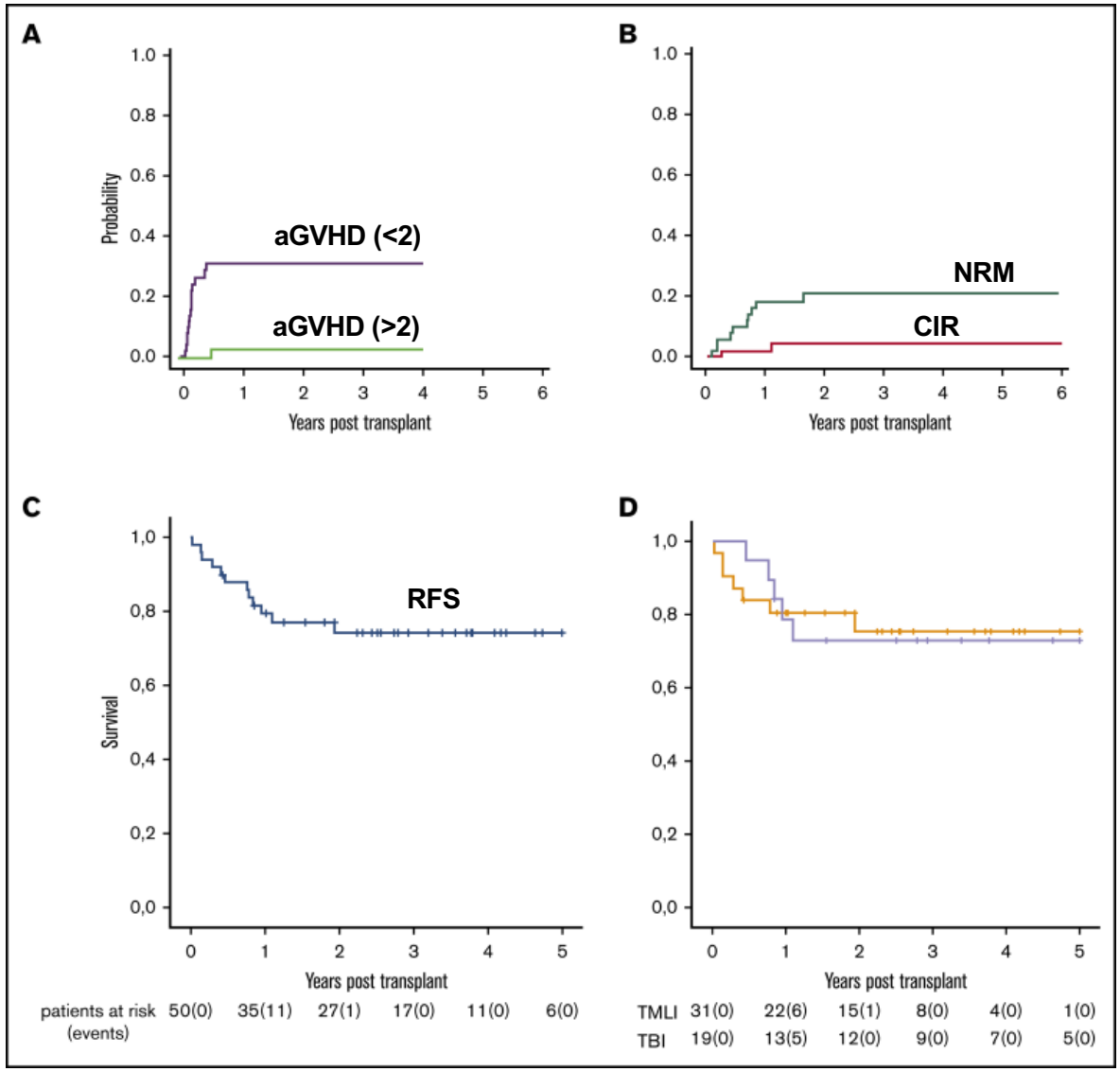
Total Marrow/Lymphoid Irradiation (TMLI) technology -

Boosting irradiation in marrow and lymph nodes while sparing vital organs



Courtesy of Prof. C. Aristei

Haploidentical age-adapted myeloablative transplant and regulatory and effector T cells for AML



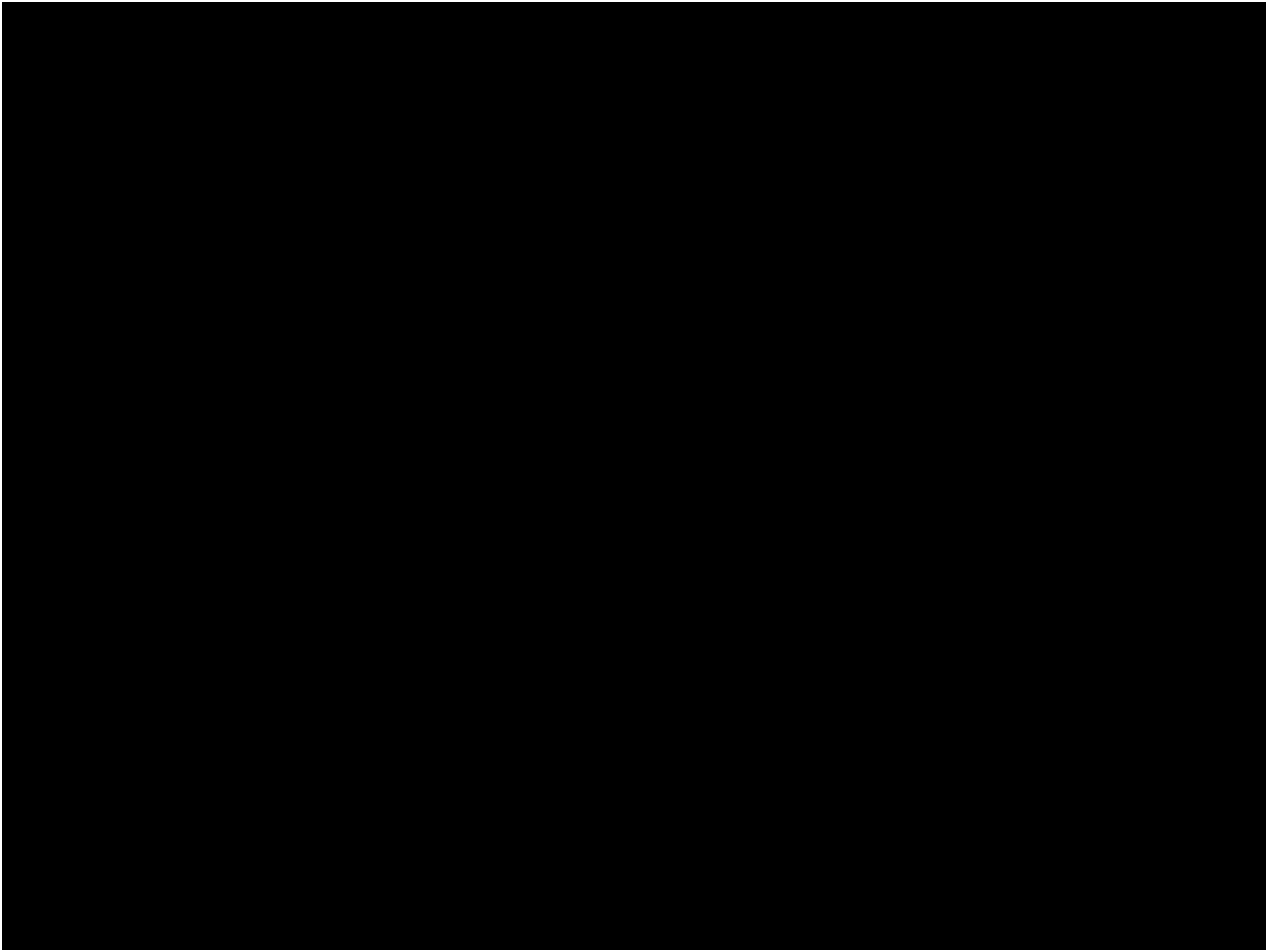
Pierini A. et al Blood Adv, 2021

Conclusions

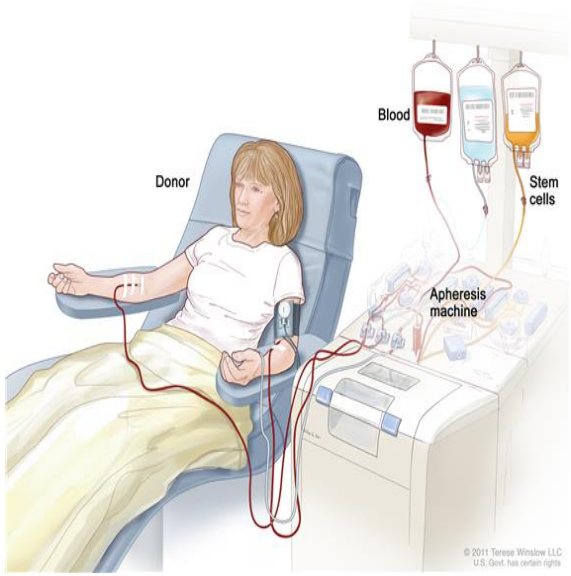
- Both cytogenetic/FISH and mutation analysis are critical for identifying high-risk AML

- Some on the mutations with prognostic value (e.g. *NPM1*, *RUNX1)* define also new entities in the WHO-2016 classification of AML

- Post-remission allo-HSCT is fundamental to achieve cure in eligible high risk AML



Easy clinical scale selection of CD4+/CD25+ regulatory T Cells



Fully automated immunomagnetic selection by commercially available kits and device

1st step: Depletion of CD8+/CD19+cells

2nd step: Selection of CD25+ cells

«Treg»

Final product

- Cells ($x10^9$) = 280 (202- 390)
- CD4/CD25+ = 92% (90-97%)
- FOXP3+ cells up to 90%

Table 1. Proposed Genomic Classification of Acute Myeloid Leukemia (AML).

Genomic Subgroup	Frequency in the Study Cohort (N=1540)	Most Frequently Mutated Genes*
	no. of patients (%)	*gene (%)*
AML with *NPM1* mutation	418 (27)	***NPM1*** (100), *DNMT3A* (54), *FLT3*^{ITD} (39), *NRAS* (19), *TET2* (16), *PTPN11* (15)
AML with mutated chromatin, RNA-splicing genes, or both†	275 (18)	***RUNX1*** (39), *MLL*^{PTD} (25), ***SRSF2*** (22), *DNMT3A* (20), ***ASXL1*** (17), ***STAG2*** (16), *NRAS* (16), *TET2* (15), *FLT3*^{ITD} (15)
AML with *TP53* mutations, chromosomal aneuploidy, or both‡	199 (13)	**Complex karyotype** (68), **–5/5q** (47), **–7/7q** (44), ***TP53*** (44), **–17/17p** (31), **–12/12p** (17), +8/8q (16)
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB–MYH11*	81 (5)	**inv(16)** (100), *NRAS* (53), +8/8q (16), +22 (16), *KIT* (15), *FLT3*^{TKD} (15)
AML with biallelic *CEBPA* mutations	66 (4)	***CEBPA***^{biallelic} (100), *NRAS* (30), *WT1* (21), *GATA2* (20)
AML with t(15;17)(q22;q12); *PML–RARA*	60 (4)	**t(15;17)** (100), *FLT3*^{ITD} (35), *WT1* (17)
AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1*	60 (4)	**t(8;21)** (100), *KIT* (38), –Y (33), –9q (18)
AML with *MLL* fusion genes; t(x;11)(x;q23)§	44 (3)	**t(x;11q23)** (100), *NRAS* (23)
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM(EVI1)*	20 (1)	**inv(3)** (100), –7 (85), *KRAS* (30), *NRAS* (30), *PTPN11* (30), *ETV6* (15), *PHF6* (15), *SF3B1* (15)
AML with *IDH2*^{R172} mutations and no other class-defining lesions	18 (1)	***IDH2***^{R172} (100), *DNMT3A* (67), +8/8q (17)
AML with t(6;9)(p23;q34); *DEK–NUP214*	15 (1)	**t(6;9)** (100), *FLT3*^{ITD} (80), *KRAS* (20)
AML with driver mutations but no detected class-defining lesions	166 (11)	*FLT3*^{ITD} (39), *DNMT3A* (16)
AML with no detected driver mutations	62 (4)	
AML meeting criteria for ≥2 genomic subgroups	56 (4)	

* Genes with a frequency of 15% or higher are shown in descending order of frequency. Key contributing genes in each class are shown in boldface type.

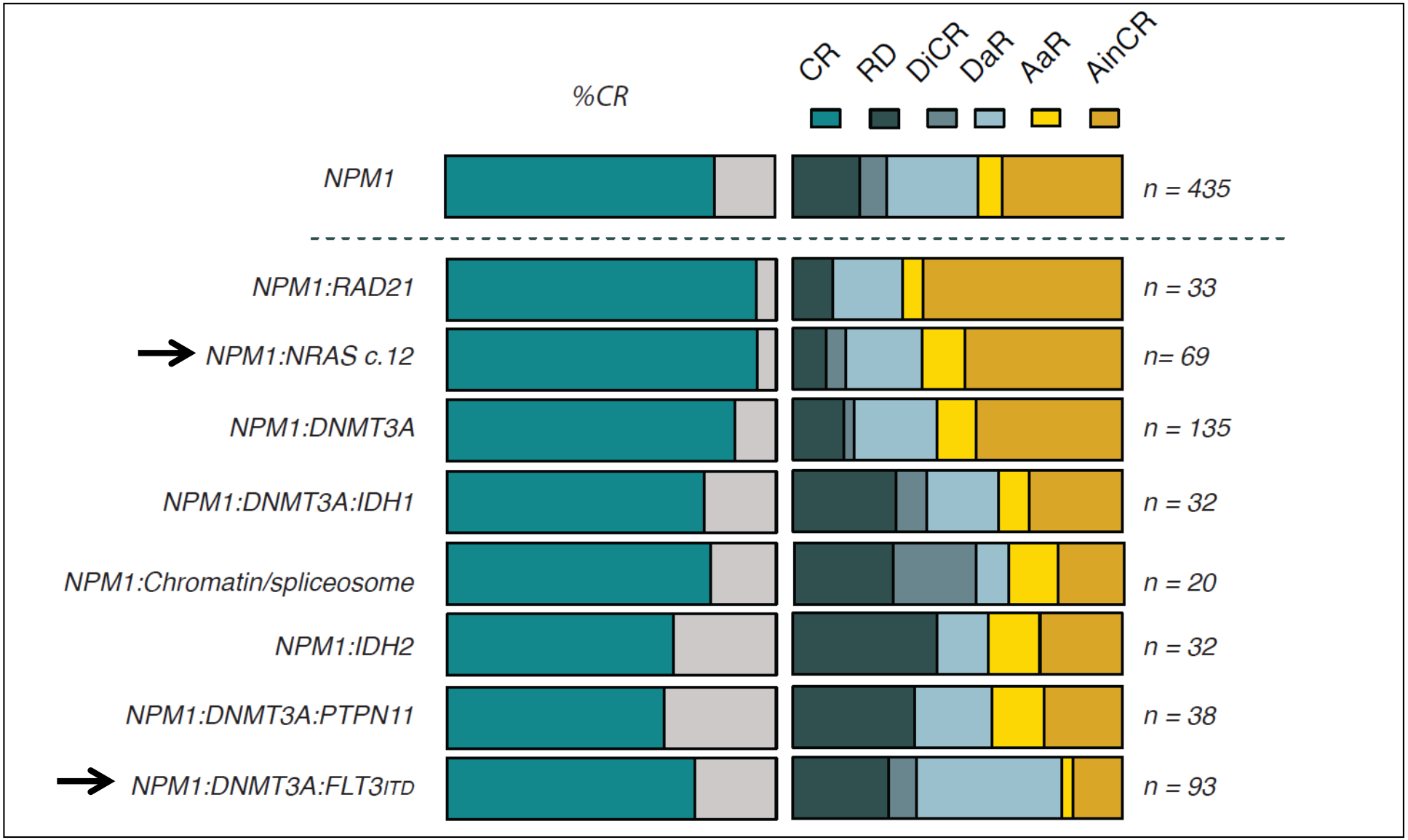
† Classification in this subgroup requires one or more driver mutations in *RUNX1*, *ASXL1*, *BCOR*, *STAG2*, *EZH2*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, or *MLL*^{PTD}. In the presence of other class-defining lesions — namely, inv(16), t(15;17), t(8;21), t(6;9), *MLL* fusion genes, or complex karyotype or driver mutations in *TP53*, *NPM1*, or *CEBPA*^{biallelic} — two or more chromatin–spliceosome mutations are required.

‡ Classification in this subgroup requires *TP53* mutation, complex karyotype, or in the absence of other class-defining lesions, one or more of the following: –7/7q, –5/5q, –4/4q, –9q, –12/12p, –17/–17p, –18/18q, –20/20q, +11/11q, +13, +21, or +22.

§ Multiple fusion partners for *MLL* were found, with the clinical implications depending on the specific fusion partner.

Papaemmanuil E, et al. NEJM 374:2209-2221, 2016

NPM1 mutations in the risk stratification of AML



Papaemmanuil E. et al., NEJM 374:2209-2221, 2016.