Hot topics in myeloid neoplasms

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Outline

- *Premalignant clonal myeloid proliferations*
- *Myeloid neoplasms with germline predisposition*
- *Mutations and MDS diagnosis*
- *What is new in the genetic testing workflow for MPN, eosinophilia, AML*
- *MRD for AML*

Premalignant clonal myeloid proliferations

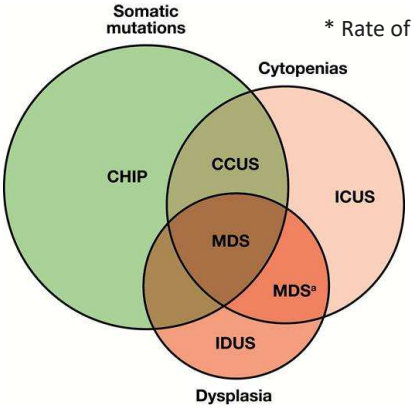
ARCH / CH/ CHIP = somatic mutations of genes frequently mutated in hematologic malignancies (*DNMT3A> ASXL1> TET2> JAK2> PPM1D> SF3B1> SRSF2>TP53..)* with a VAF ≥ 2%, with no clinical/morphologic evidence of a hematologic malignancy

* Accompanies aging (10% over age 70, 20% over age 90)

* Rate of progression to hematologic neoplasm ~0.5%-1% per year

*Rate of progression is proportional to the size (VAF) of the somatic clone (the higher the VAF, the higher the risk)

**TP53* and *PPM1D* mutations are associated with increased risk of therapy-related myeloid neoplasm, and lymphoma post ASCT



	Cytopenia	Dysplasia	CH	VAF	No. of Variants	Genes	Risk
Normal	–	–	–	–	–		–
CHIP	–	–	+	≥2% but <20%	Rarely >2	*TET2, DNMT3A, ASXL1, SF3B1, PPM1D*	1%/y
ICUS	+	–	–	–	–		low
CCUS	+	–	+	>10%	Often >1	*SRSF2, IDH1, NRAS, RUNX1, U2AF1, BCOR, EZH2, STAG2*	10%/y
MDS	+	+	+/–	>10%	On average ≥2		–
IDUS	–	+	–	–	–		–

Clonal hematopoiesis of indeterminate potential [CHIP]
Idiopathic cytopenia of undetermined significance [ICUS]
Clonal cytopenias of undetermined significance [CCUS]
Idiopathic dysplasia of undetermined significance [IDUS]
Myelodysplastic syndrome [MDS]
Clonal hematopoiesis [CH]
Age-related clonal hematopoiesis [ARCH]

Nardi V et al, AJCP 2019

Jaiswal S., et al., N Engl J Med 2014; Genovese G. et al, NEJM 2014;

Premalignant clonal myeloid proliferations: CH vs AML MRD

Genetic abnormality	Type	Usually cleared after successful therapy	Persistence after therapy associated with adverse outcome
RUNX1-RUNX1T1, CBFB-MYH11, PML-RARA	AML-related	Yes	Yes
NPM1	AML-related	Yes	Yes
KMT2A rearrangement, *DEK-NUP214, BCR-ABL1*	AML-related	Unknown	Unknown
NRAS/KRAS	AML-related	Yes	Yes
FLT3-ITD/FLT3-TKD	AML-related	Yes (but may be lost at relapse or acquired at relapse of previously *FLT3* wild-type AML)	Unknown
KIT	AML-related	Yes	Yes
PTPN11	AML-related	Yes	Yes
IDH1/IDH2	CH (potentially AML-related)	Variable	Yes
DNMT3A	CH	Usually not	No
ASXL1	CH	Variable	No
TET2	CH	Usually not	No

CH may persist at AML Remission: ddx with MRD!

Adapted from Hasserjian RP, et al., Blood 2020

Myeloid neoplasms with germline predisposition

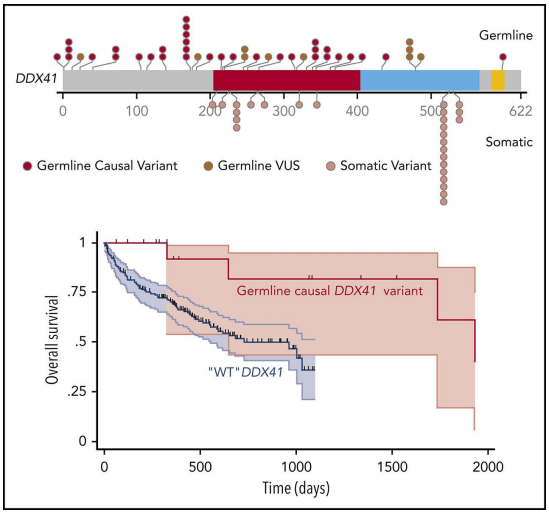
- Increased recognition; relevance for bone marrow donor selection
- Many of the genes mutated in the germline can also be mutated as acquired events in MDS/AML; importance of family and personal history and awareness
- Skin fibroblasts, nails, hair for germline testing

Mutated gene	Region	Inheritance	1st report	Median age at diagnosis (range), years	Low platelets	Other organ dysfunction	Type of neoplasm	Risk of HM
CEBPA	19q13.1	AD	2004	25 (2–46)	no	no	AML	100%
DDX41	5q35.3	AD	2015	62 (40–85)	no	no	AML, MDS, rarely CML, CMML, lymphoma, myeloma	?%
RUNX1	21q22.12	AD	1999	39 (7–53)	yes	no	AML, MDS, rarely CMML, T-ALL, hairy-cell leukemia	40%
ANKRD26	10p12.1	AD	2011	38 (1–84)	yes	no	AML, MDS, rarely CML, CMML, CLL	8%
ETV6	12p13.2	AD	2015	uncertain	yes	no	B-ALL, AML, MDS, CMML, myeloma, PV, solid tumors	8%
GATA2	3q21.3	AD	2010	20 (<1 to 78)	no	yes	AML, MDS, CMML, aCML	80%
SAMD9/SAMD9L	7q21.2	AD	2016	uncertain	yes	yes	MDS, AML	?%

-Bone marrow failure syndrome
-Telomeropathies
-JMML

Adapted from Geyer J. T. , Myeloid Neoplasms with Germline Predisposition. Pathobiology. 2019;

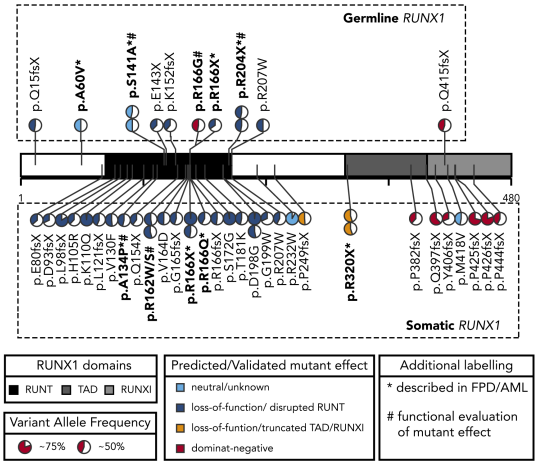
Myeloid neoplasms with germline DDX41 mutations



Sebert M., et al, Blood 2019

- DEAD-box helicase 41 (*DDX41*), essential for cell growth and viability of hematopoietic stem and progenitor cells
- ~1-4% of myeloid neoplasms
- Antecedent cytopenias, particularly leukopenia
- Male gender
- Average age of MDS/AML onset in mutation carriers is notably older at 65 years
- Most germline mutations are truncating (or M1 or codon R525)
- Most common somatic mutation is a second *DDX41* mutation, usually missense
- HSCT from *DDX41* mutation carriers may promote donor cell leukemias
- Lenalidomide has been suggested as an effective treatment strategy for myeloid malignancies with *DDX41* mutations [and without del(5q)]

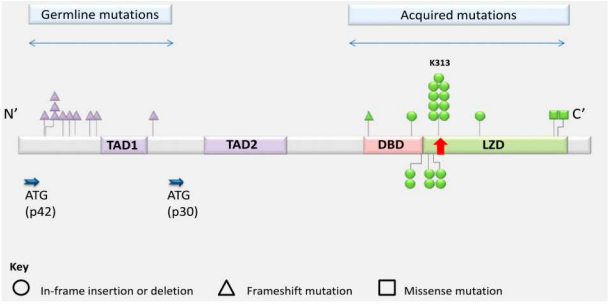
Myeloid neoplasms with germline RUNX1 mutations: familial platelet disorder (FPD) with associated myeloid malignancy (FPDMM, also referred to as FPD/AML)

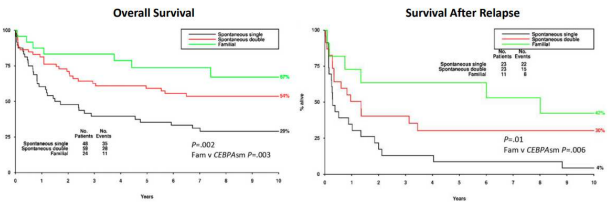


- *RUNX1* encodes a TF that is a master regulator of hematopoiesis
- Germline mutations in *RUNX1* occur in ~15--30% of patients with AML and *RUNX*1 mutations
- Variable clinical presentation, with mostly mild to moderate bleeding tendency since childhood
- Platelet count are often normal but there platelet dysfunction
- Germline *RUNX1* mutations encompass partial and whole gene deletions and frameshift, stop-gain, and missense mutations
- Median age of onset of MDS/AML is 33 ys
- Somatic mutations in *RUNX1* are frequently observed in leukemic progression; *NRAS* mutations are also common

Simon et al, *Blood*. 2020

Myeloid neoplasms with germline CEBPA mutations





- CEBPA is a transcription factor involved in the control of myeloid progenitor differentiation and proliferation
- Germline mutations occur typically in the N-terminal
- AML develops with acquisition of a somatic CEBPA mutation in the C-terminal region
- Patients usually develop AML as children and young adults (a median age of 25 years)
- Approximately 10% of AML cases with biallelic mutations represent a germline and a somatic mutation
- Somatic *CEBPA* mutations appear unstable throughout the disease course, with novel independent clones frequently identified at recurrence
- Favorable prognosis

Tawana K, et al, Blood 2015

Mutations and diagnosis of MDS

*1. **MDS-defining** cytogenetic aberrations in the absence of significant morphologic dysplasia*

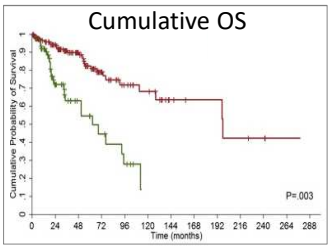
Chromosomal abnormality	Frequency	
	MDS overall	Therapy-related MDS
Balanced		
t(11;16)(q23.3;p13.3)		3%
t(3;21)(q26.2;q22.1)		2%
t(1;3)(p36.3;q21.2)	1%	
t(2;11)(p21;q23.3)	1%	
inv(3)(q21.3q26.2) / t(3;3)(q21.3;q26.2)	1%	
t(6;9)(p23;q34.1)	1%	

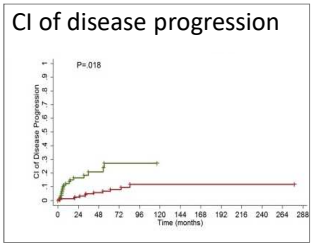
Chromosomal abnormality	Frequency	
	MDS overall	Therapy-related MDS
Unbalanced		
~~Gain of chromosome 8[a]~~	10%	
Loss of chromosome 7 or del(7q)	10%	50%
del(5q)	10%	40%
~~del(20q)[a]~~	5–8%	
~~Loss of Y chromosome[a]~~	5%	
Isochromosome 17q or t(17p)	3–5%	25–30%
Loss of chromosome 13 or del(13q)	3%	
del(11q)	3%	
del(12p) or t(12p)	3%	
del(9q)	1–2%	
idic(X)(q13)	1–2%	

Adapted from Swerdlow SH, et al.: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition).

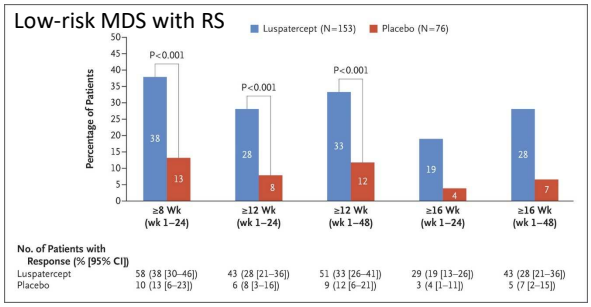
Mutations and diagnosis of MDS

*2. **SF3B1 mutations** and 5-15% ring sideroblasts*





SF3B1 mutated
SF3B1 wild type

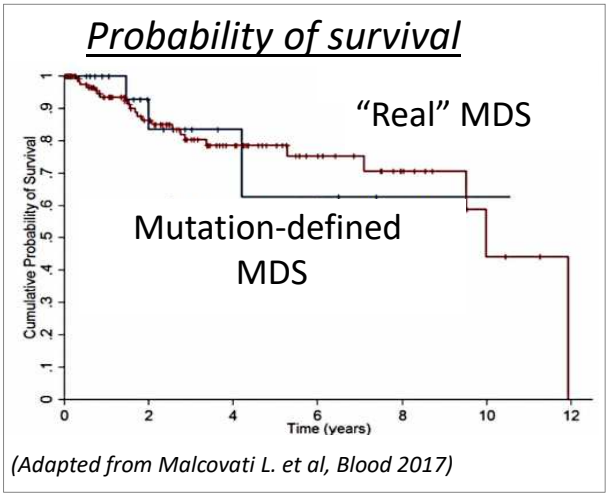


Adapted from Swerdlow SH, et al.: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition) and Malcovati L. et al, Blood 2015; Fenaux P. et al., NEJM 2020

Mutations and diagnosis of MDS

3. Presence of specific ***mutation patterns, "high" VAF (>10% of)*** *and unexplained* ***cytopenias*** *~/= MDS*

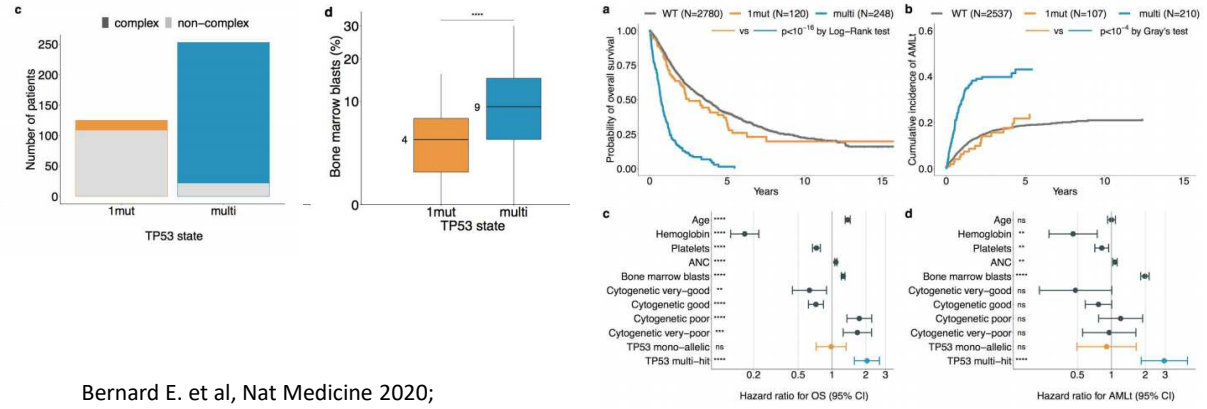
- ***Spliceosome*** *mutations (SF3B1, SRSF2, U2AF1, ZRSR2)*
- ***DNMT3A/TET2/ASXL1*** *("DTA" mutation)* ***+*** *another mutation*



(Adapted from Malcovati L. et al, Blood 2017)

TP53 Mutations in MDS

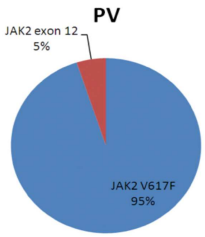
- **Biallelic** not mono-allelic **TP53 mutations** are associated with complex karyotype, high risk presentation, poor outcome, resistance to conventional therapy.
- Biallelic/two hits: mutation, deletion, or loss of heterozygosity

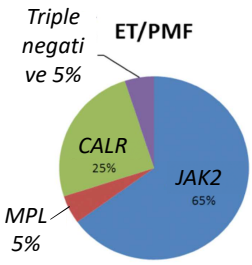


Bernard E. et al, Nat Medicine 2020;

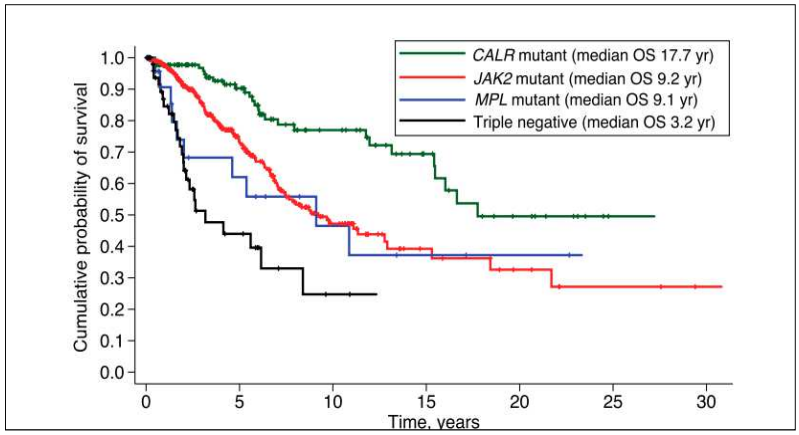
Genetic testing for diagnosis and classification of MPN (except eosinophilias)

- ***Karyotype*** *(clonality, progression..)*
- ***BCR-ABL1; ABL1 mutations***
- ***JAK2*** *V617F (and exon 12 mutations),* ***MPL*** *and* ***CALR****. Broader myeloid mutation panel if triple-negative.*
- ***CSF3R*** *in suspected chronic neutrophilic leukemia*





Risk stratification of MPN according to driver mutations

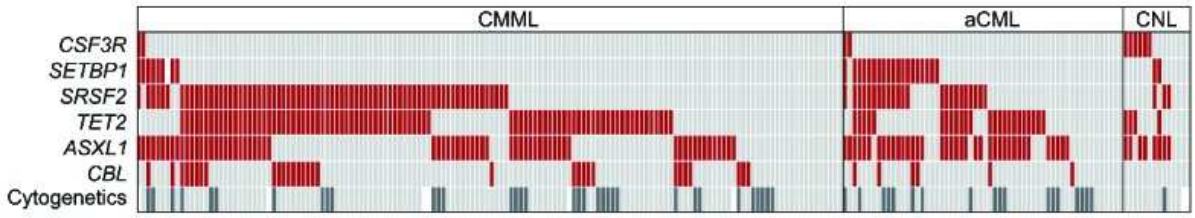


N=617
PMF

Elisa Rumi et al, Blood 2016

CALR mutations: ***favorable prognosis, indolent clinical course***
JAK2/MPL mutations: ***intermediate prognosis. Increased risk of thrombosis***
Triple neg: unfavorable prognosis; high risk of transformation to AML

Overlap in mutational landscape of CMML, aCML and CNL



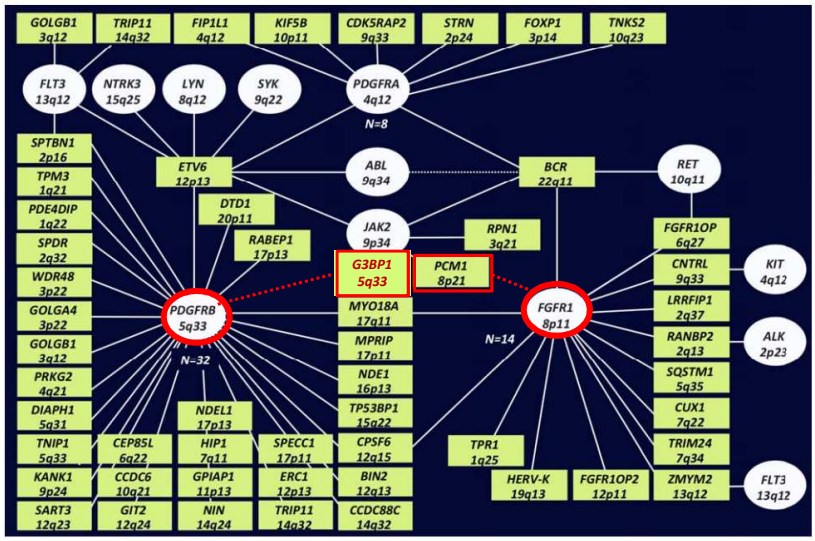
CSF3R mutations in 90% of patients with **CNL** (truncation mutations → constitutive overexpression of the receptor and ligand hypersensitivity; membrane proximal mutation →constitutive activation of the receptor)

Meggendorfer et al, Haematologica 2014

Genetic testing for diagnosis and classification of eosinophilias

- ***Karyotype*** *(clonality → Chronic eosinophilic leukemia NOS)*
- ***BCR-ABL1*** *(FISH, RT-PCR, karyotype)*
- ***FIP1L1-PDGFRA*** *by FISH (cryptic)*
  **Consider PDGFRB FISH, FGFR1 FISH, Fusion panel*
- ***Broad myeloid-mutation*** *panel (prove clonality) including* ***KIT*** *(exclude mastocytosis)*

Fusion genes associated with myeloid/lymphoid neoplasms with eosinophilia



Reiter A, Gotlib J, Blood 2018

Jan M. et al, A cryptic imatinib-sensitive G3BP1-PDGFRB rearrangement in a myeloid neoplasm with eosinophilia. Blood Advances 2020
Kasbekar M et al. Targeted FGFR inhibition results in a durable remission in an FGFR1-driven myeloid neoplasm with eosinophilia. Blood Adv 2020

Genetic testing for diagnosis and classification of AML (CAP-ASH guidelines 2017)

- ***Karyotype***
- ***FLT3 ITD and TKD*, NPM1, CEBPA , RUNX1, IDH1/2****
- *May also perform **additional mutational analysis** (WT1, **TP53**, ASXL1..)*
- ***KIT** in core binding factor AML*
- ***NUP98 rearranged AML** (pediatric AML>>)*

** Need rapid Turnaround time*

Role of the molecular laboratory in the era of FDA approved drugs

2000- 2010

FDA approval of ***Gemtuzumab (Mylotarg)***

Gemtuzumab *removed from market in 2010*

2017

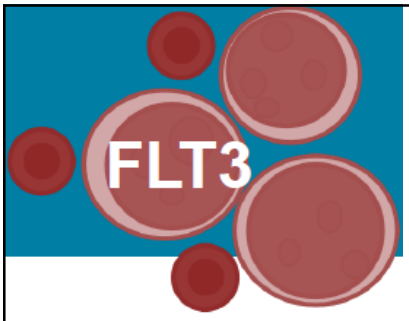
- April 28th: PKC-412 (***Midostaurin***) For ***FLT3***m AML (+"7+3")
- August 1st:***Enasidenib*** for R/R ***IDH2***m AML
- August 3rd:***Vyxeos*** for t-AML or AML MRC
- September 1st: ***Gemtuzumab (Mylotarg)*** for CD33+ AML (CBF AML)

2018

- July 20st:***Ivosidenib*** for R/R ***IDH1***m AML*
- Nov 21th:***Glasegib & Venetoclax*** for frail > 75ys with AML
- Nov 28th: *Gilteritinib* for ***FLT3***m R/R AML

** 2019: also upfront in >75ys/frail patients*

R/R: relapsed/refractory
MRC: myelodysplasia related changes

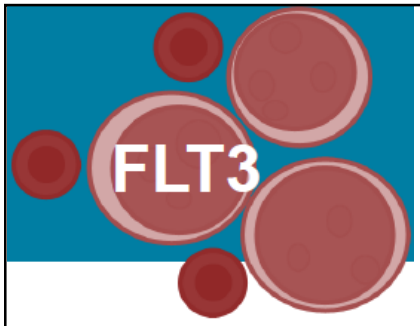


Role of molecular laboratory

Test at AML diagnosis (and relapse):

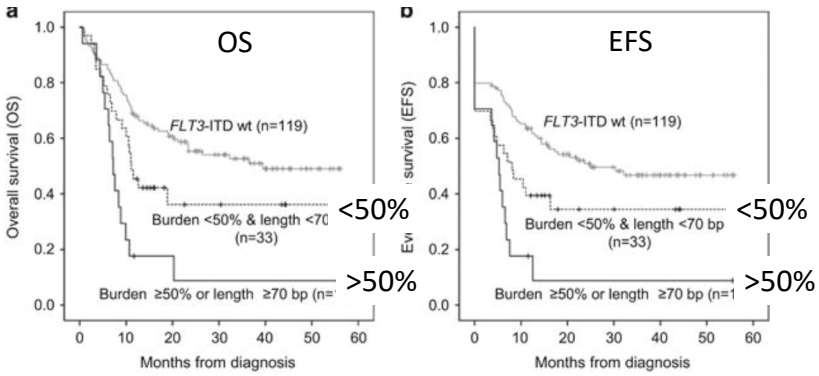
-FLT3 ITD
-- fragment analysis
-- NGS

-FLT3 TKD
-- allele specific PCR
-- Sanger sequencing
-- NGS

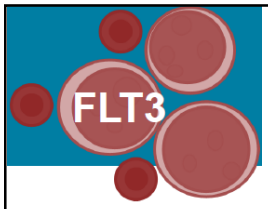


FLT3 ITD burden matters (allelic ratio (AR))

Part of ELN risk Stratification:
ITDlow: AR<0.5
ITDhigh: AR>0.5



Kim Y, et al. Blood Cancer Journal 2015

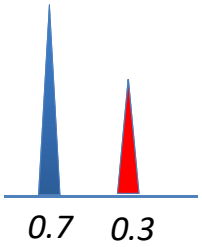


FLT3 ITD AR controversies

1. How is it calculated?

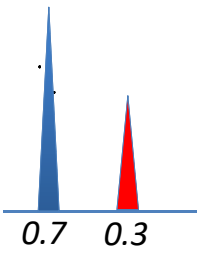
VAF: ratio AF ITD/ AF (ITD+WT) (NGS)

FLT3 wt
FLT3 ITD

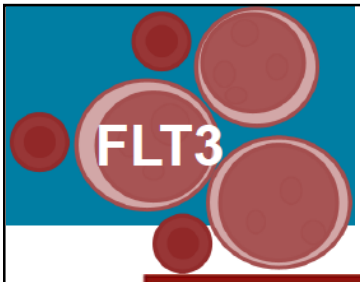


0.3/(0.7+ 0.3)=0.3 VAF

AR: ratio AUC ITD/ AUC WT (sizing assay)



0.3/0.7=0.42 AR



FLT3 ITD: allelic ratio controversies

2. What AR cutoff?

Reference	Allelic ratio			Patients, N	Population
	Low	Intermediate	High		
Thiede C, et al.	≤ 0.78	NA	> 0.78	979	Adult, > 18 y
Gale RE, et al.*	< 25%	25%-50%	> 50%	1425	Adult, 18-60 y
Meshinchi S, et al.	≤ 0.4	NA	> 0.4	630	Pediatric, 0-21 y
Linch DC, et al.*	< 25%	25%-50%	> 50%	1609	Adult
Schlenk RF, et al.	< 0.51	NA	≥ 0.51	323	Adult, 16-62 y

https://www.medscape.org/viewarticle/898477_transcript

Role of molecular laboratory

*Test at AML **diagnosis for elderly and frail patients**:*

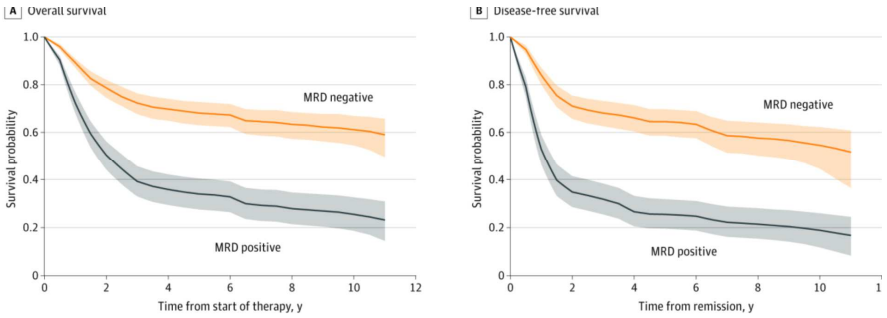
-IDH1 codon 132 mutations

*Test at AML **relapse** or in **refractory** disease:*

-IDH1 codon 132
-IDH2 codon 140 and 172

Minimal (Measurable) residual disease (MRD) in AML

- *MRD should be standard of care*
- *MRD positivity after CR in a patient with AML is associated with a higher risk of relapse and shorter survival*
- *MRD in AML can be assessed using **MFC** and **PCR** approaches (more in Dr. Annette Kim's presentation)*
  PCR <-> RUNX1-RUNX1T1, CBFBMYH11, PML-RARA and mutations in NPM1.
  *(While **NGS** may be applicable to another 40%-50% of AML patients, its use is still being standardized)*
- *The strongest evidence for MRD is in **core-binding AML**, but persistence of NPM1 mutation in remission by ultrasensitive techniques is also being used to make treatment decisions, though has not yet made its way into the guidelines.*



Short N., et al. Association of Measurable Residual Disease With Survival Outcomes in Patients With Acute Myeloid Leukemia. *JAMA Oncology, 2020;* Schuurhuis GJ, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2018;

Conclusions

- *Be aware of germline pathogenic mutations conferring increased risk of hematological malignancies*
- *Mutations in myeloid elements can represent clonal hematopoiesis, CCUS , MDS, AML MRD*
- *Need of rapid test results for patients with AML (FLT3, IDH1/2 at a minimum)*
- *Testing for cryptic gene fusions in patients with unexplained eosinophilia*
- *Minimal residual disease detection in AML is being adopted and used for treatment decisions*

Thank you!