Thursdays Webinars

EuroBloodNet

When is molecular analysis useful in MDS?

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European Reference Network
for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

Conflicts of interest

Advisory Boards : Novartis, Janssen, Celehen/BMS, Takeda , Pfizer, Menarini, Geron

Speaker at symposia: Novartis , Celgene/BMS, Geron

Practical issues before starting

- ✓ **30-35min presentation (30 slides max) + 15 min Q&A session**
- ✓ **Microphones will be muted by host to avoid back noise**
- ✓ **Please, stop your video to improve internet conexion**
- ✓ **Send your questions during the presentation through the chat, they will be gathered and answered after the presentations.**

Learning objectives of the webinar

1. Understand the frequence and recurrence of somatic mutations In MDS
2. Understand the prognostic role of number and type of somatic mutations In MDS
3. Identify the cases in which the molecular study in MDS is absolutely necessary

Most important molecular techniques applied to MDS

Technique	Application
PCR	Increasing the amount of specific DNA in the sample
Array Comparative genomic hybridisation (aCGH)	Checking the whole genome for large deletions or duplications (copy number variation)
Real Time polymerase chain (Q-PCR) reaction	Controlled PCR that allows the amplification and quantification of the number of copies of a specific genetic region.
DNA sequencing (Sanger)	Checking for alterations in the order of bases in the genetic code
Next generation sequencing (NGS)	Large-scale DNA sequencing producing vast amounts of data in a single test.

What is the role of molecular analysis in MDS in 2020?

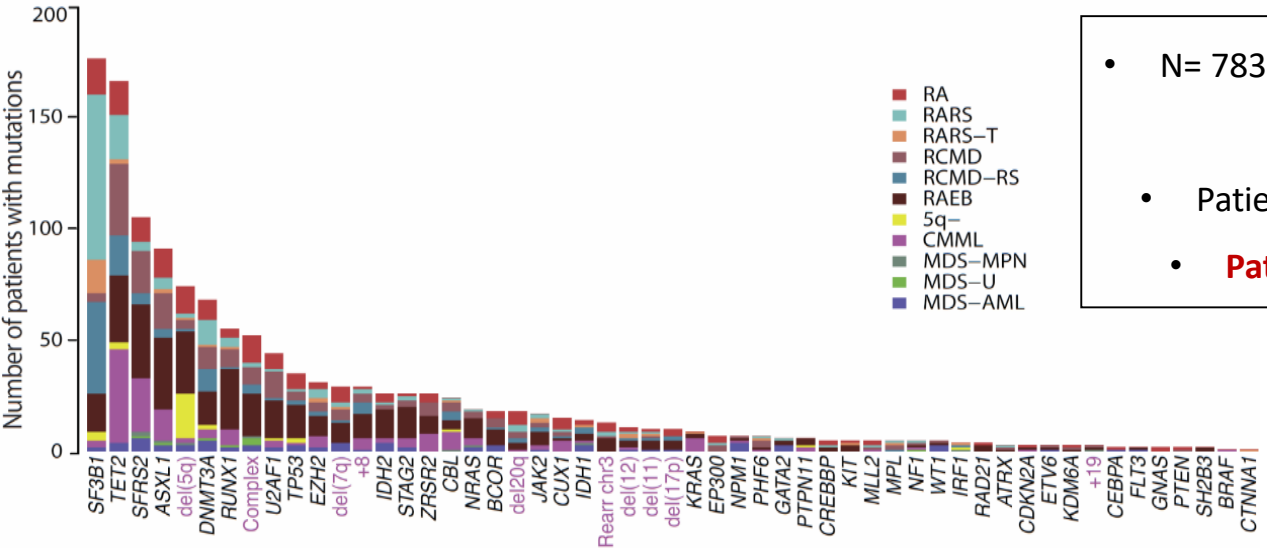
Prognostic importance of somatic mutations

Genetic predisposition

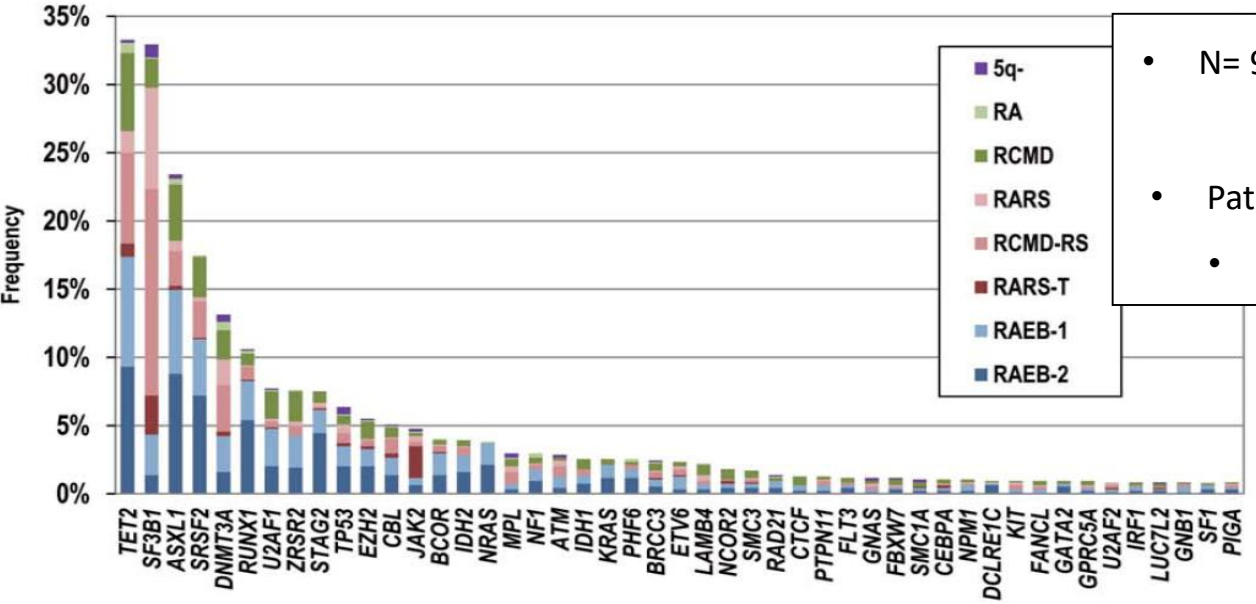
New therapeutic approaches

Novel Insights in pathophysiology (methylome, histone modifications)

Somatic Mutations in MDS are present in > 80% of cases



- N= 783 patients, 111 genes, 43 mutated genes
- Mean age; 68
- Patientes with cytogenetic aberrations: 33%
- **Patients with molecular aberrations: 78%**

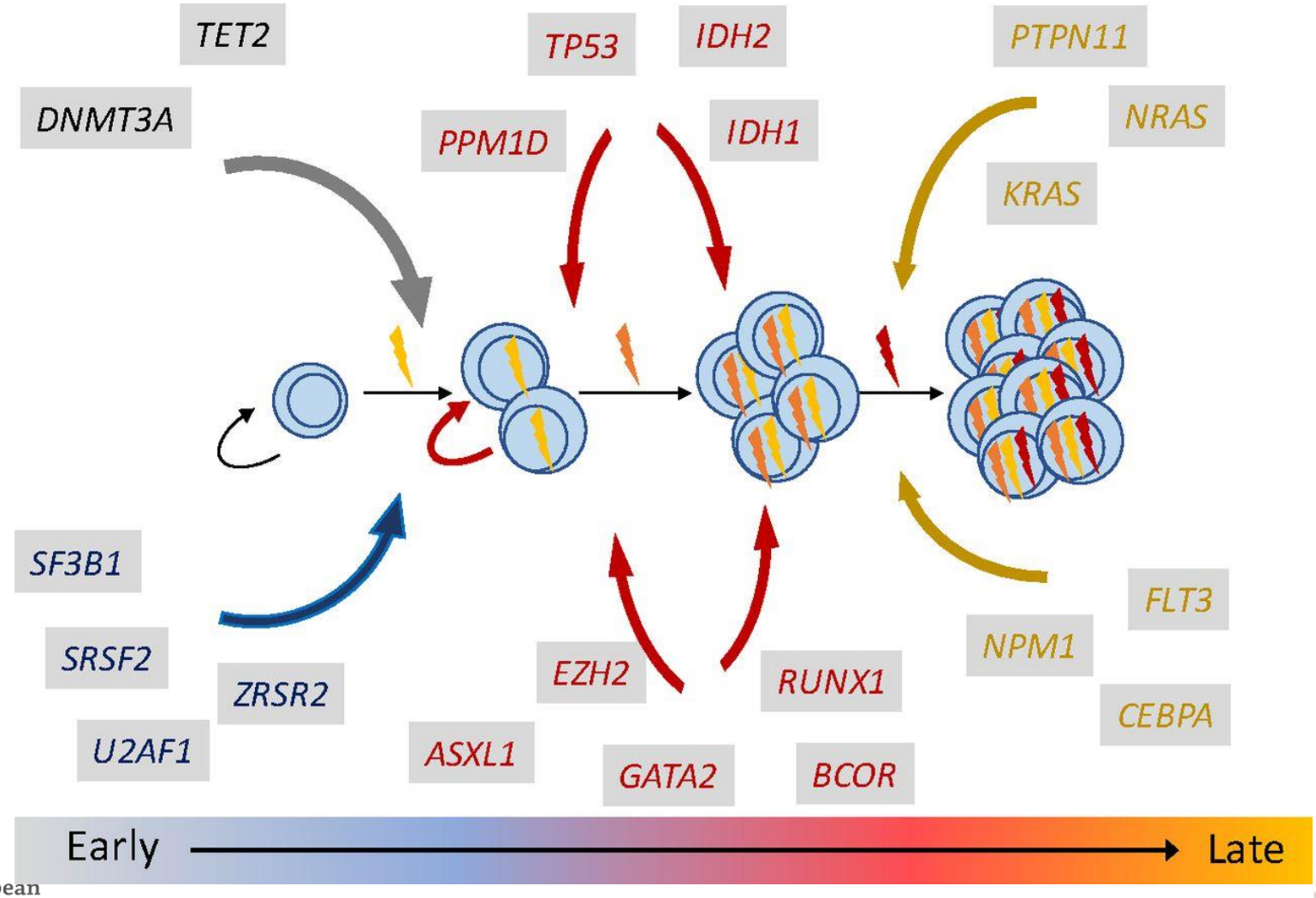


- N= 944 patients, 104 genes, 47 mutated genes
- Mean age; 72.8
- Patientes with cytogenetic aberrations: 31.4%
- **Patients with molecular aberrations: 89%**

Papaemmanuil et al. *Blood*. 2013.

Haferlach et al. *Leukemia*. 2014.

Gene mutations have stereotyped positions



R. Coleman Lindsley Hematology 2017;2017:447-452

Somatic mutation evaluation in MDS

1. Help refining diagnosis (according to WHO for MDS with RS).
2. Prompt to earlier intervention in presence of multiple (or prognostically negative) mutations
3. Prognostic established value in MDS with del5q
4. Prognostic value in HSCT
5. Identify inherited predisposition
6. Clonal hemopoiesis -Diagnosis of uncertain cases/Prediction of AML progression
7. Predictive of HMA response (?)
8. Indicate possibility of targeted therapy

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]

Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood

Table 15. PB and BM findings and cytogenetics of MDS

Name	Dysplastic lineages	Cytopenias*	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1			r	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3			r	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3			r rods	del(5q) alone or with 1 additional abnormality except −7 or del (7q)
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

- MDS with ring sideroblasts
  - RS > 15%,
  - SF3B1 and RS >5%
  - Unilineage/multilineage dysplasia

*Cytopenias defined as: hemoglobin, <10 g/dL; platelet count, <100 × 10^9/L; and absolute neutrophil count, <1.8 × 10^9/L. Rarely, MDS may present with mild anemia or thrombocytopenia above these levels. PB monocytes must be <1 × 10^9/L

†If *SF3B1* mutation is present.

‡One percent PB blasts must be recorded on at least 2 separate occasions.

§Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

Splicesome Mutations are very frequent in MDS

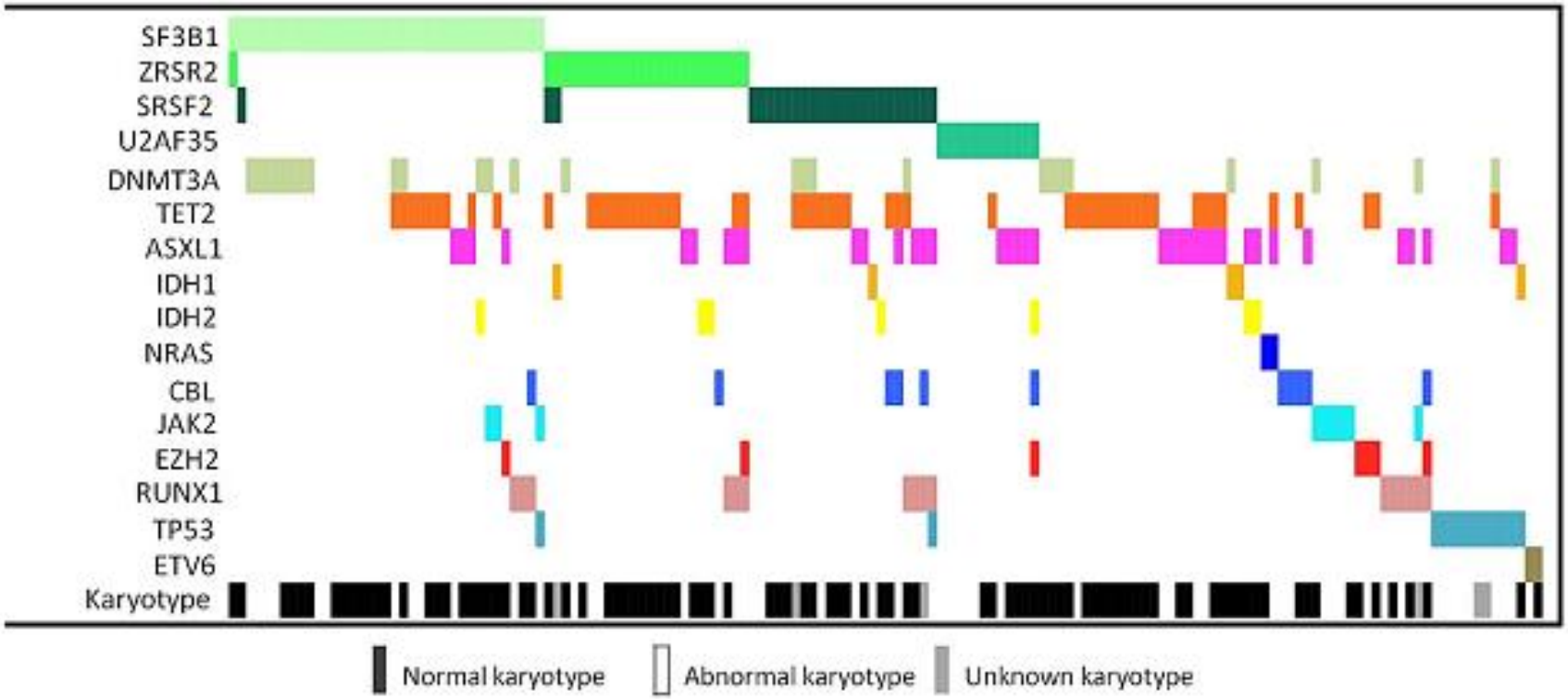
Genes	Mutations
SF3B1	Multiple
U2AF35 / U2AF1	Multiple
SRSF2	Multiple
ZRSR2	Multiple
LUC7LA	Single
PRPF8	Single
U2AF2	Multiple
SF1	Multiple
HCFC1	Single
SAP130	Single
SFRS6	Single
SON	Single
U2AF26	Single

85% myeloid neoplasias

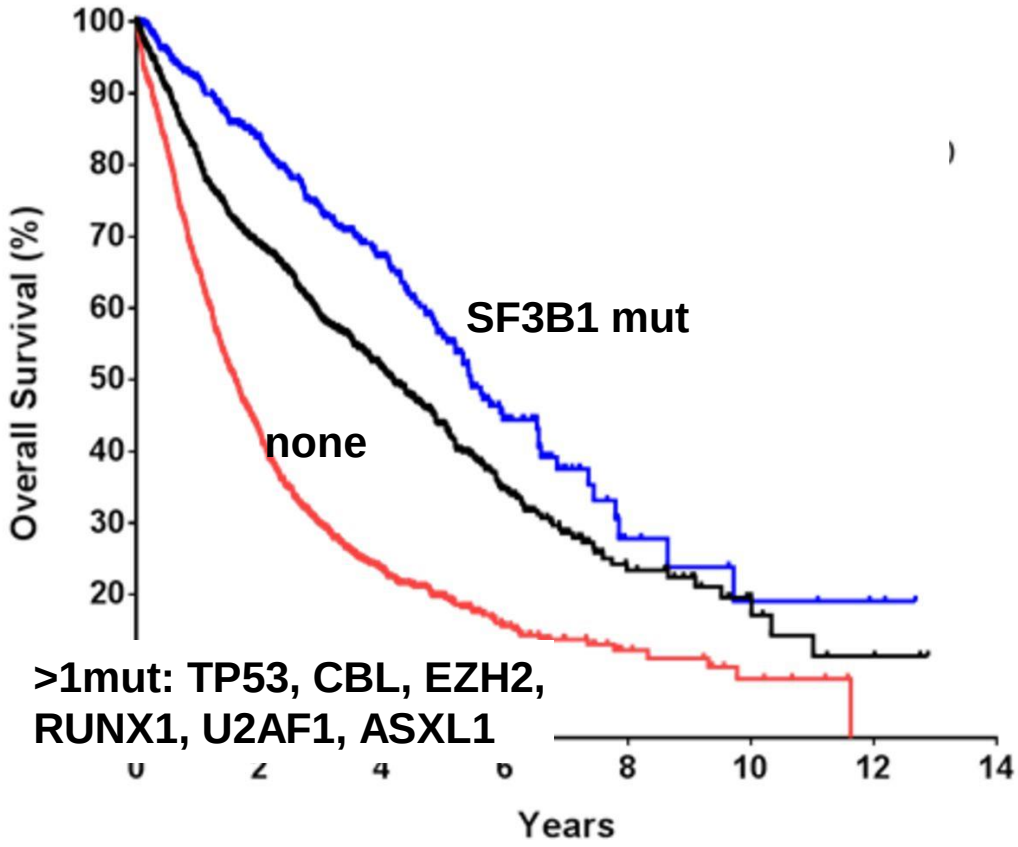
60% in MDS

Splicesome Mutations

- Heterozygous missense mutations at defined **hotspots**, leading to highly recurrent amino acid substitutions
- Mutations in the earlier phase
- **Mutually exclusive** of one another (?)



SF3B1 mutations in LR-MDS are independent good prognostic indicators



Rafael Bejar et al. Blood 2015;126:907

Somatic mutations in suspect of MDS:
a help in diagnosis?

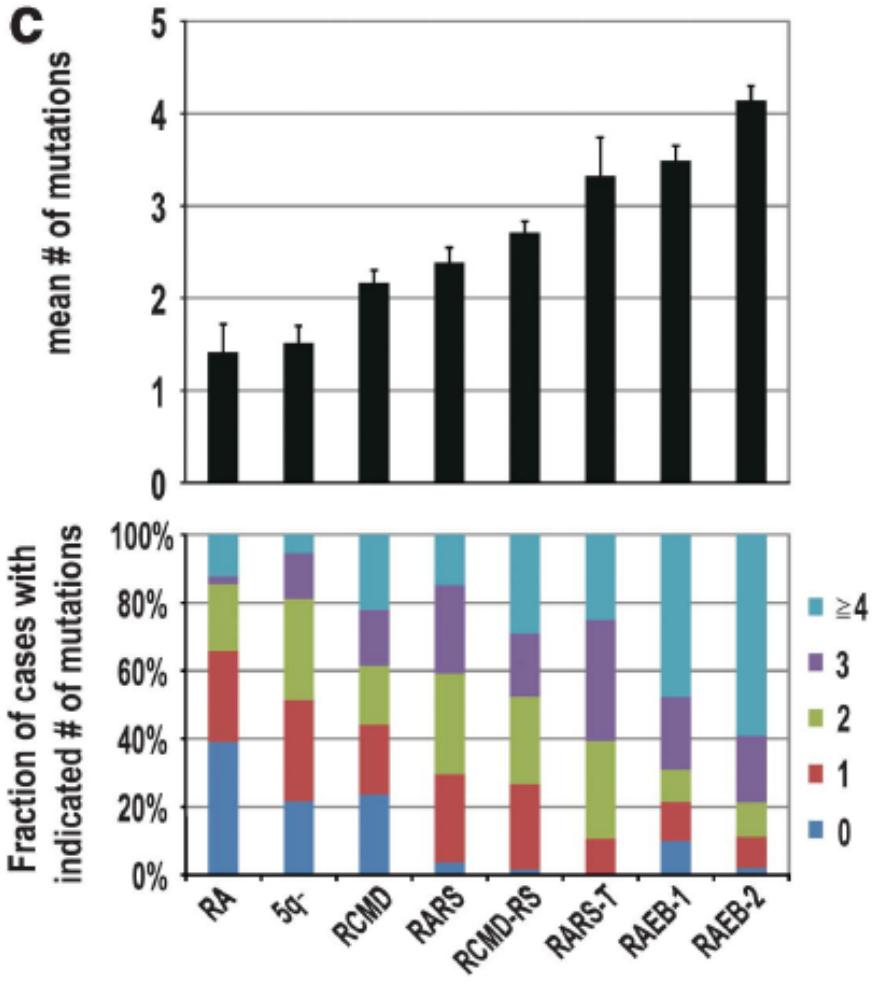
Dysplasia can be induced by other causes than MDS

Cytopenias without dysplasia may be tricking

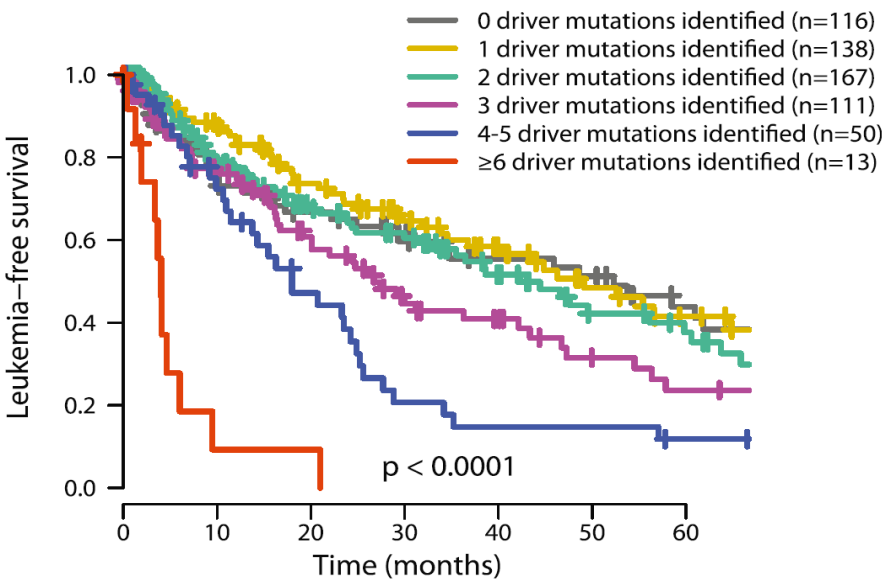
…

and definite diagnosis is often a challenge

Prognostic value of the number of somatic mutations



Mutations are higher in HR-MDS and number of mutations correlates with OS



Haferlach T et al. Leukemia 2014: 28:241-247

Papaemmanuil *et al.*, Blood (2013)

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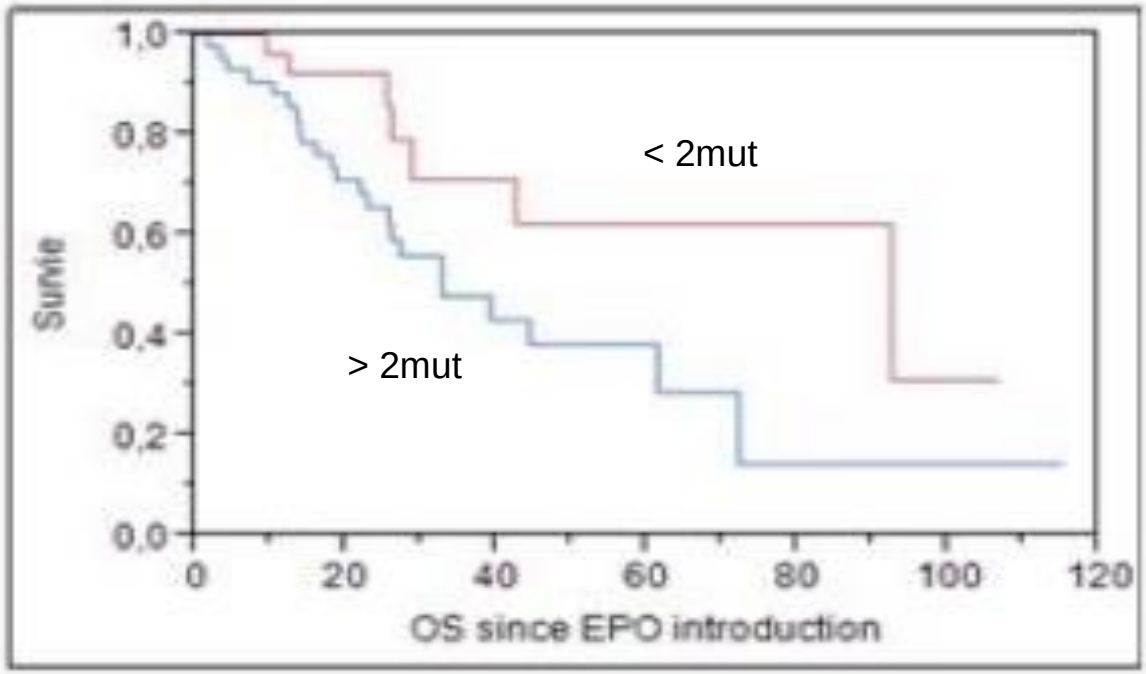
Number of mutations predicts OS after ESAs (79 LR-MDS anemic pts)

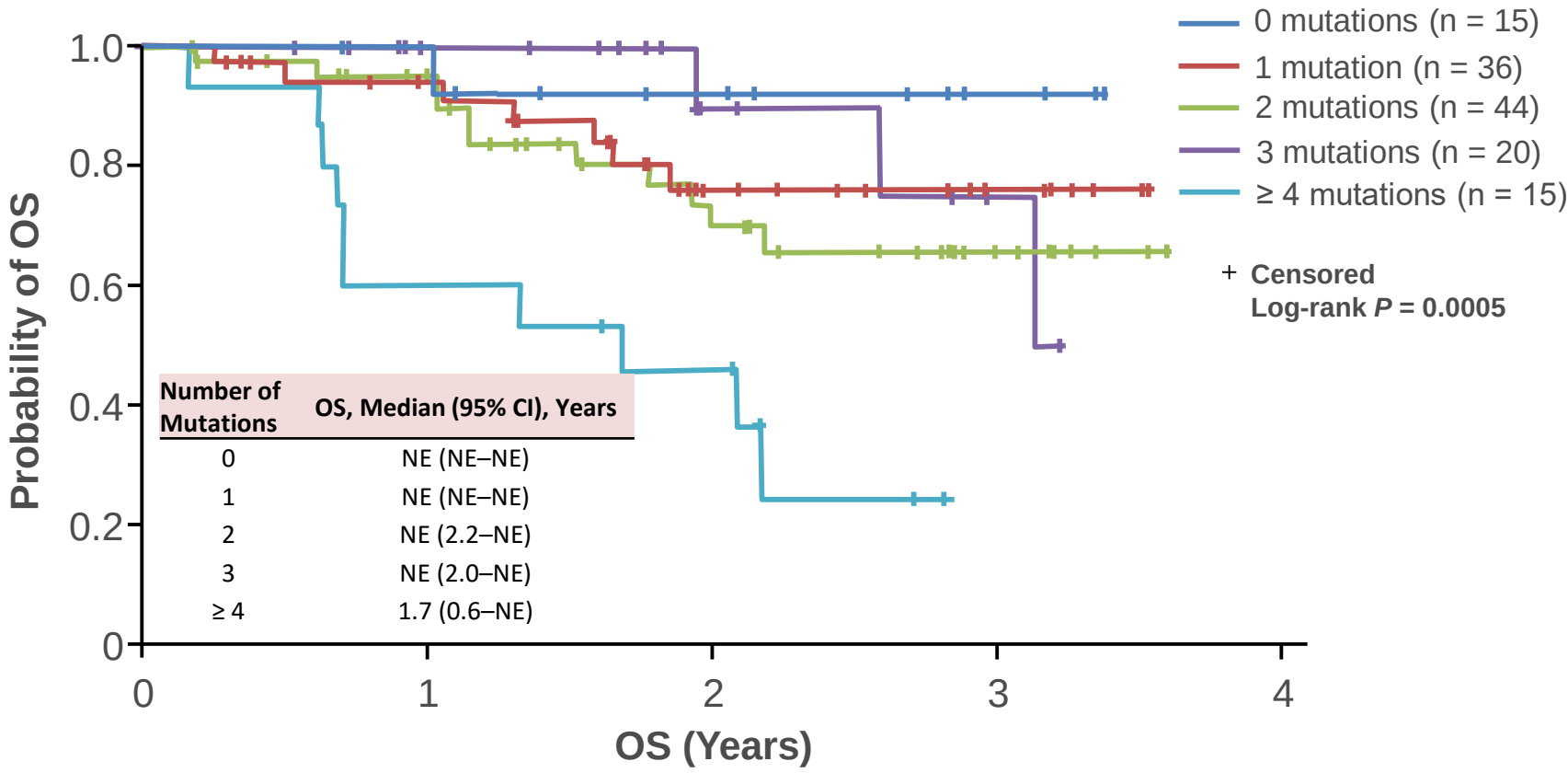


P=0.04

Kosmider O. et al Haematologica 2016; 101: e280-3.

OS According to Number of Mutations in nondel5q MDS Patients (#130)Treated with lenalidomide

- Higher number of mutations was significantly associated with shorter median OS (*P* = 0.0005)



Santini V et al Leukemia. 2020 Jul 13. doi: 10.1038/s41375-020-0961-3

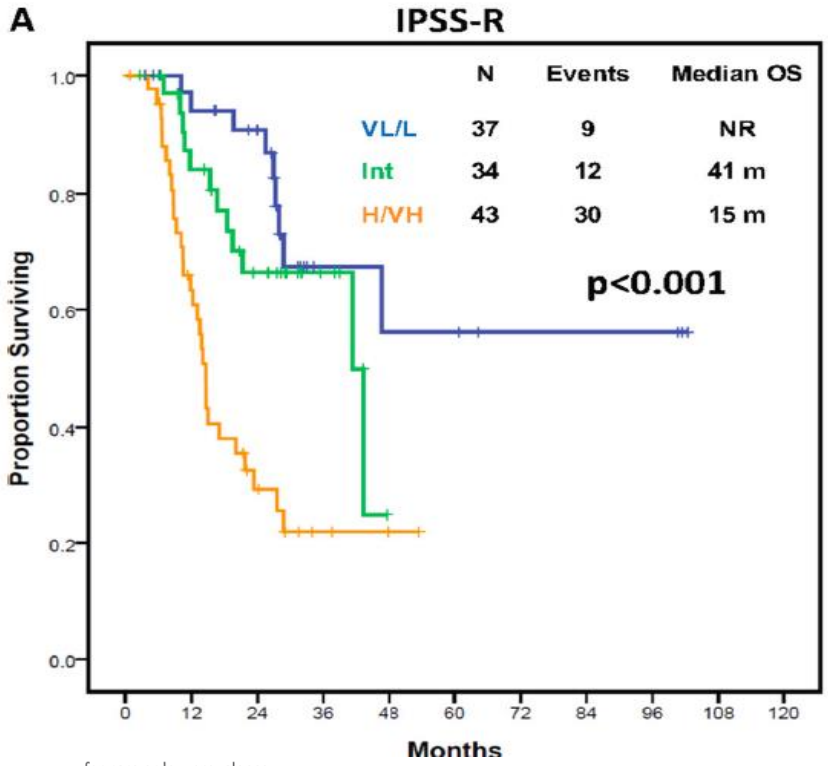
Gene mutations in LR-MDS are independent prognostic indicators

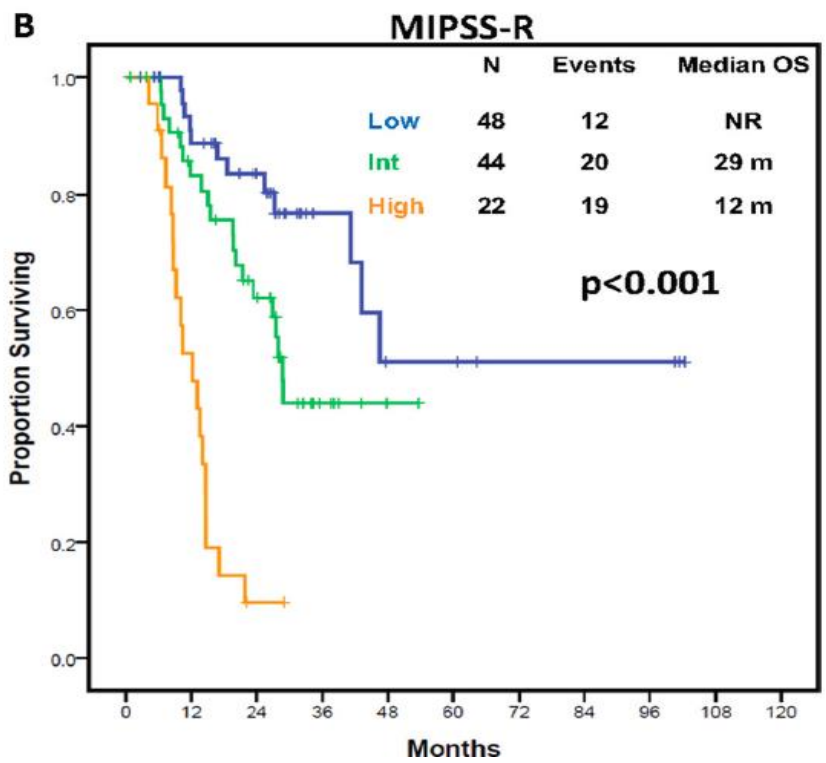
Gene	HR (95% CI)	P value
TP53	2.48 (1.60-3.84)	<0.001
EZH2	2.13 (1.36-3.33)	<0.001
ETV6	2.04 (1.08-3,86)	0.029
RUNX1	1.47 (1.01-2.25	0.047
ASXL1	1.36 (1.0-1.89)	0.049

Bejar R et al. N Engl J Med. 2011;364(26):2496-506

IPSS-R integrated model with molecular variables (83 pts)

Variable	Hazard Ratio	95% CI	*p* value	Score
IPSS-R				
Intermediate	1.45	0.56–3.77	0.45	0.5
High/Very high	4.66	2.01–10.84	< 0.001	1.5
TP53	3.12	1.3–7.49	0.011	1
Mutations 3 or more	2.51	1.32–4.76	0.005	1





for rare or low prevalence complex diseases

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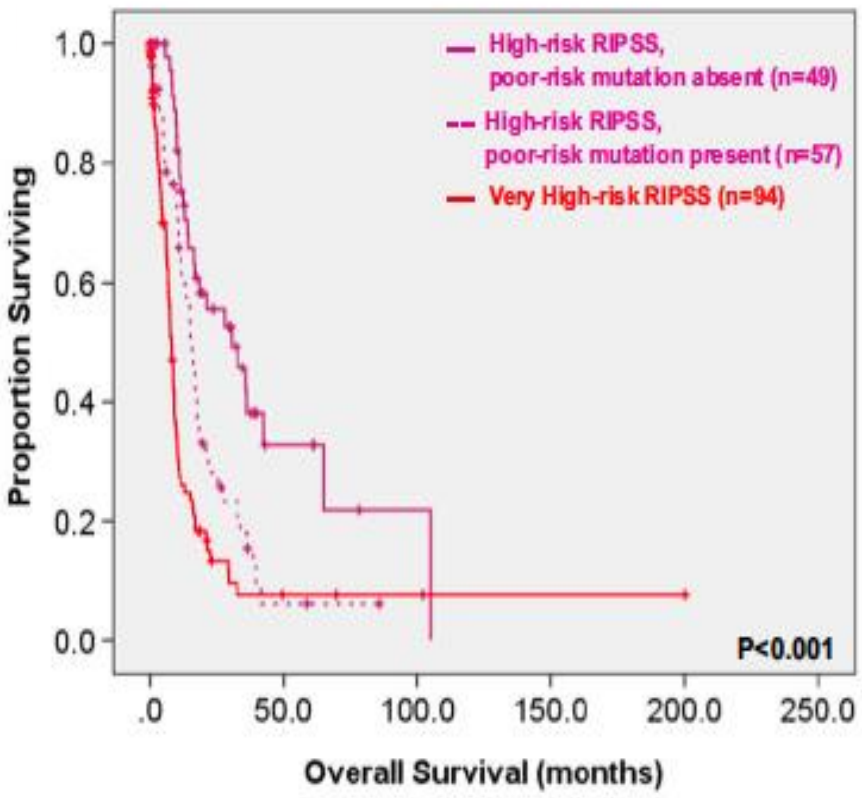
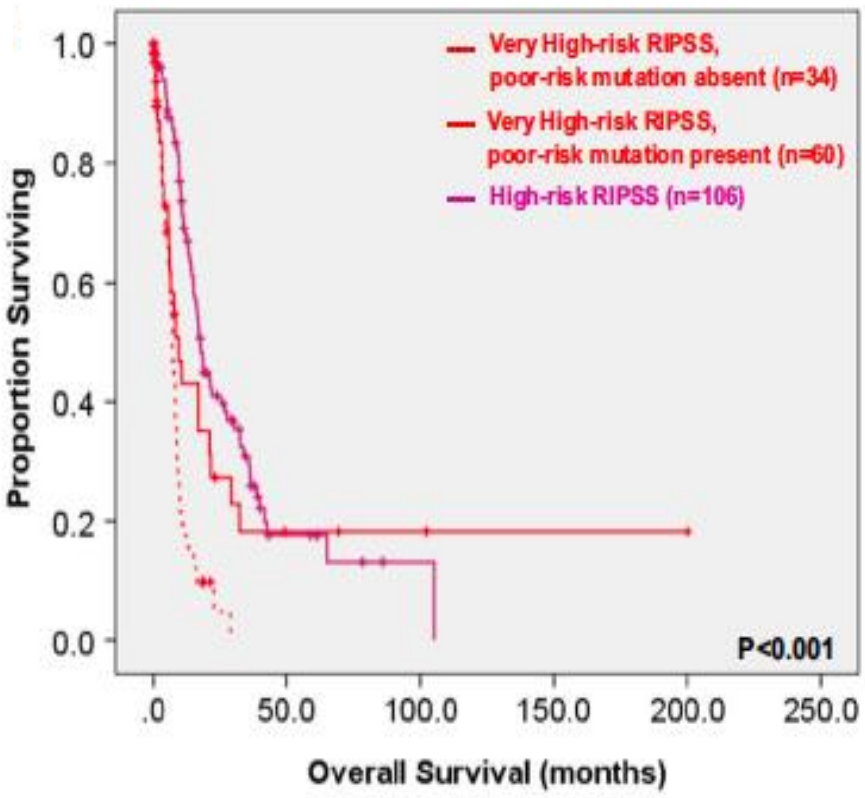
Montalban-Bravo G et Al,, Oncotarget 2018, Vol. 9, (No. 11), pp: 9714-9727

IPSS-R integrated model with molecular variables (426 pts)

Mutations of CBL, IDH2, ASXL1, DNMT3A, and TP53





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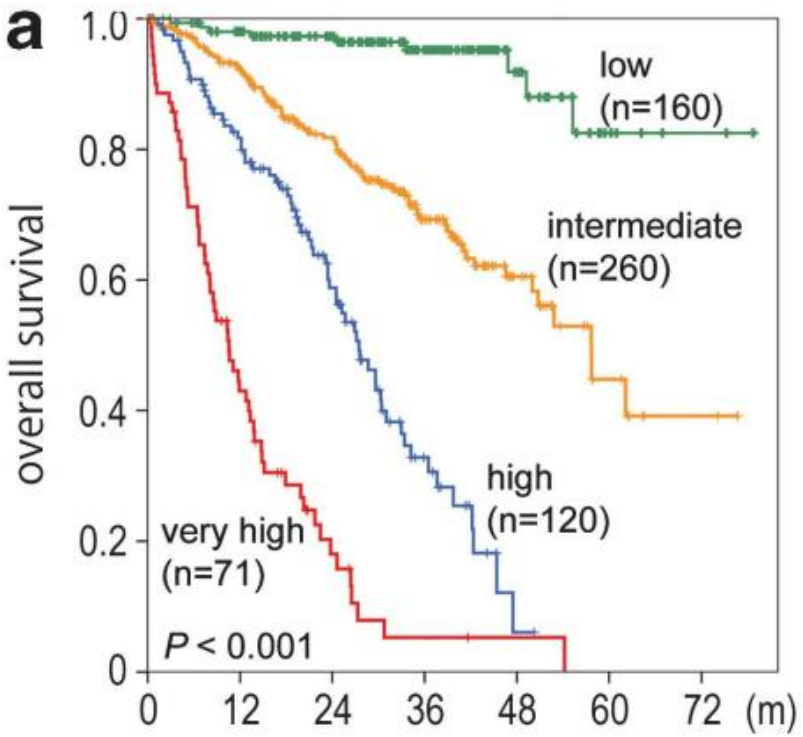
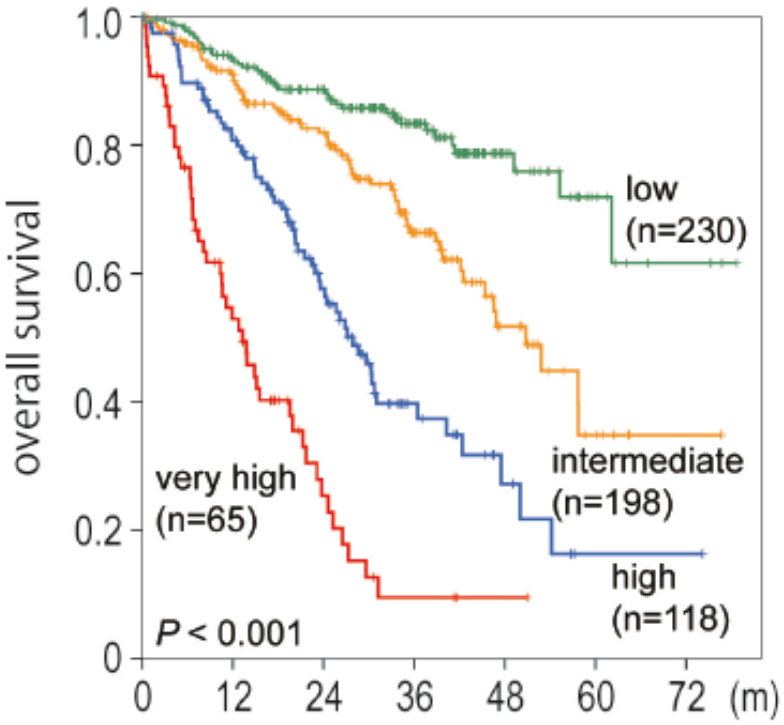
Network Hematological Diseases (ERN EuroBloodNet)

Hou HA et al; Blood Cancer J. 2018 Apr 4;8(4):39.

Prognostic Models beyond IPSS-R





Model 1:
Age, Sex
WBC, Hb, Plt, % blasts (IPSS-R)
Cytogenetics (IPSS-R)
14 genes

Model 2:
14 genes
(13/14 from Model 1)

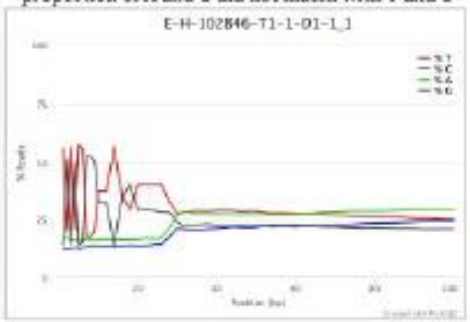
Haferlach et al., Leukemia, 2014

IWG molecular study

DNA samples sequenced
N=4,105

QC of fastq data and bam data
- FastQC
- Picard tools
- Excess of missense or synonymous calls

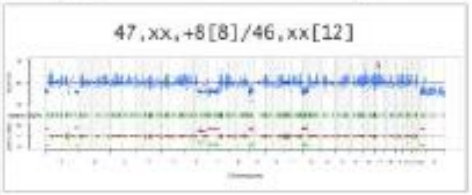
Typical example of a poor quality sample where the proportion of A and G did not match with T and C



Good DNA quality
N=3,968

Match between clinical and sequencing data
- Fingerprinting
- Sex and coverage on sex chromosomes
- Cytogenetic and NGS copy-number profiles

Typical example of a sample with discordant clinical and sequencing data, where both sex and cytogenetic did not match with NGS profile



Match with clinical data
N=3,458

Further validation
- Remove treated samples (N=131)
- Exclude pediatric MDS (N=3)

Included in the study
N=3,324

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Bernard et al, presented at ASH 2019

IWG-MDS cohort (N=3324)		
Characteristic	**No. of cases (%)**	**Median (1Q - 3Q)**
Gender		
Male	2005 (60%)	-
Female	1319 (40%)	-
Age at diagnosis	-	71 (63 - 78)
Missing data	85 (2.6%)	-
Type of MDS		
De-novo	2855 (86%)	-
Therapy-related	229 (7%)	-
Secondary	51 (1%)	-
Missing data	189 (6%)	-
WHO 2016 classification[4]		
MDS		
MDS-del5q	142 (4.3%)	-
MDS-SLD/MLD	914 (27.5%)	-
MDS-RS-SLD/MLD	460 (13.8%)	-
MDS-EB1	451 (13.6%)	-
MDS-EB2	429 (12.9%)	-
MDS-U	92 (2.8%)	-
AML		
AML-MRC	103 (3%)	-
AML*	64 (2%)	-
MDS/MPN		
CMML	425 (12.8%)	-
aCML	46 (1.4%)	-
MDS/MPN-U	50 (1.5%)	-
MDS/MPN-RS-T	42 (1.3%)	-
Other	11 (0.3%)	-
Missing data	95 (2.9%)	-
Cytogenetics IPSS-R		
Very-good	125 (3.8%)	-
Good	1992 (59.9%)	-
Int	421 (12.7%)	-
Poor	149 (4.5%)	-
Very-poor	254 (7.6%)	-
Missing data	383 (11.5%)	-
IPSS-R risk group		
Very-good	372 (14.6%)	-
Good	1106 (33.3%)	-
Int	630 (19%)	-
Poor	448 (13.5%)	-
Very-poor	372 (11.2%)	-
Missing data	282 (8.5%)	-
Blood counts		
Hemoglobin (g/dL)	-	9.7 (8.6 - 11.2)
Platelets (10^9/L)	-	123 (65 - 229)
ANC (10^9/L)	-	2 (1 - 3.7)
Bone Marrow Blasts %	-	3 (1 - 8)
Missing data	108 (3.2%)	-
Outcome		
Median follow-up (years)[5]	-	3.44
Missing OS data	152 (4.5%)	-
Missing AML data	163 (4.9%)	-

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Bernard et al, 2020

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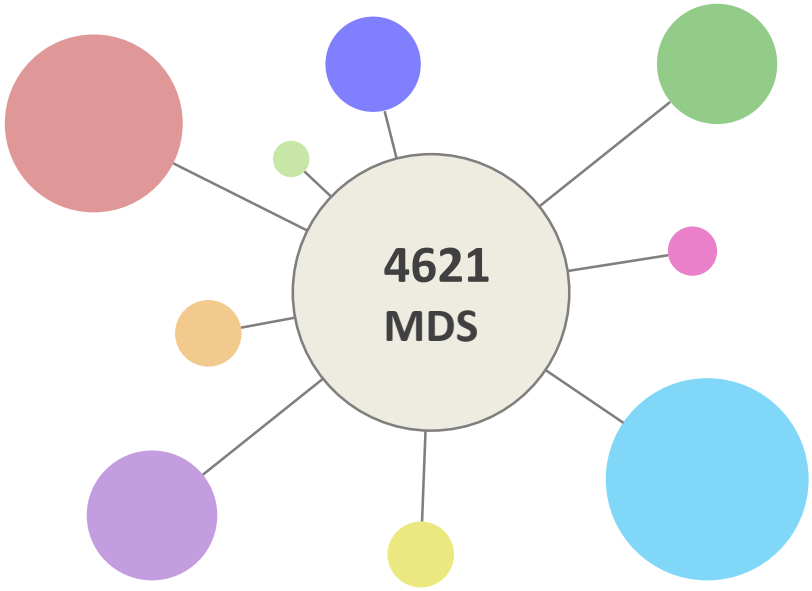
IWG-PM Molecular Status Study Project

2019 update

MDS sample data collected from 25 centers
in 13 countries in Europe, the United States, and Asia



Analysis : **Kristen Stevenson** **Donna Neuberg** **Heinz Tuechler**

Coordinating Committee:
Elli Papaemmanuil ; Ben Ebert ; Rafael Bejar; Peter Greenberg

European

Complete sequencing June 2018
Complete variant calling and sequencing QC August | September 2018
Complete variant annotation by November 2018

Hematological
Diseases (ERN EuroBloodNet)

Data Summary

- <u>Overall Survival Data</u>:
  - available for 3359
  - 3.6 years follow-up
  - 1780 deaths
  - median OS 2.65 years

<u>Treatment Status</u>

803 HMA, 302 chemo, 327 transplant

Prognostic Impact

Table 1. Summary of driver mutations in myelodysplastic syndromes (MDS)

Mutated genes	Associated phenotypes	MDS types	Other disease	Frequency in MDS (%)	Effect on outcome	Application to treatment
RNA splicing (mutually exclusive)				60–70		None
SF3B1	Ring sideroblasts	RARS, RCMD-RS	RARS-T	15–30	Good	
SRSF2		RCMD, RAEB	CMML	10–20	Poor	
U2AF1		RCMD, RAEB	CMML	5–10	Poor	
ZRSF2		RCMD, RAEB	CMML	5–10	None	
DNA methylation (*TET2* and *IDH1/2* are exclusive)				40–50		DNA methyltransferase inhibitors
TET2	Myeloid dominancy	All MDS, normal karyotype	CMML	20–30	None	IDH1/2 inhibitors
IDH1/2		RCMD, RAEB	CMML	5	Poor (*IDH2*)	
DNMT3A		All MDS	AML	10	None	
Chromatin modification				20–30		Deacetylase inhibitors
ASXL1		RCMD, RAEB	CMML	15–20	Poor	
EZH2	-7/7q-	RCMD, RAEB	CMML	5	Poor	
BCOR		RCMD, RAEB		5	Poor	
Transcriptional factor				20–30		None
RUNX1	Thrombocytopenia	RCMD, RAEB	CMML, AML	10	Very poor	
CEBPA		RCMD, RAEB	AML	<5	None–poor	
ETV6		RCMD, RAEB		<5	Poor	
Signal transduction (mutually exclusive)				20–30		Kinase inhibitors
NRAS/KRAS		All MDS	JMML, CMML	10	Poor	
CBL		All MDS	JMML, CMML	5	Poor	
JAK2	Megakaryocytosis	All MDS	RARS-T, MPN	5	None	JAK inhibitors
NF1		All MDS	JMML	<5	Poor	
FLT3		All MDS	AML	<5	Poor	FLT3 inhibitors
Cohesin complex (mutually exclusive)				10		None
STAG2		RCMD, RAEB	AML, CMML	5–10	None–poor	
TP53	Complex karyotype	RAEB, isolated del(5q)		10	Very poor	None

Bejar R et al. N Engl J Med 2011

Prognostic Impact

Table 1
Somatic mutations in MDS.

Gene function	Gene symbol	Gene name	location	Mutation; effect	Prognostic implication	Protein function	Frequency %	Common attributes
Signaling	*NRAS*	Neuroblastoma RAS viral (v-ras) oncogene homolog	1p13.2	Missense; activation	Poor (? Increased risk of AML evolution)	GTPase signal transducer controlling cell growth	~ 10	Frequent in CMML, increased risk of leukemic transformation
	FLT3-ITD	Fms-related tyrosine kinase 3	13q12		Unclear	FMS-like receptor tyrosine kinase, class III	~ 2	Uncommon in MDS, increased frequency in MDS progressing to AML
	CBL	CBL proto-oncogene, E3 ubiquitin pritein ligase	11q23.3	Missense; inactivation possibly dominant negative	Unclear	Tyrosone kinase-associated ubiquitin ligase	~ 1	Associated with UPD 11q; frequent in CMML and MD/MPN overlap
	JAK2	Janus kinase 2 V617F	9p24	Somatic mutation	Negative	Tyrosine kinases that are associated with cytokine receptors	~5MDS, ~50% MDS/MPN	Increased platelet count in MDS, megakaryocytic proliferation, RARS-T, and AML; associated with a subgroup of del(5q31)
	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	4q11-12	Missense; activation	unclear	Receptor tyrosine kinase	~ 1	High risk, involved in leukemic transformation
Transcription factors	*RUNX1*	Runt-related transcription factor 1	21q22.3	Missense in the runt domain; dominant negative. Nonsense/indel/splice site distal nonfunctional	Negative	Member of transcription protein complex	~ 15	Thrombocytopenia, high risk MDS, common in t-MDS, increased risk of AML
	TP53	Tumor protein p53	17p13.1	Missense/indel; nonfunctional	Negative	Multiple: DNA repair, apoptosis	~ 10	Asspciated with complex cytogenetics and isolated 5q-, poor prognosis
	ETV6	Ets variant 6	12p13	Missense/indel; nonfunctional	Negative	ETS family transcription factor	~ 2	Heterozygous mutations alter protein that is incapable of repressing transcription and shows dominant negative effects
	CEBPA	CCAAT/enhancer-binding protein (C/EBP), alpha	19q13.1	Indel/nonsense; nonfunctional	Unclear; favorable in AML when 2 mutations are present	Basic leucine zipper (bZIP) transcription factor; cell cycle regulation	~1–4	Familial predisposition to AML and MDS
	NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	5q35.1	Indel; cytoplasmic localization, p53 inactivation	Unclear	Phosphoprotein, nuclear and cytoplasmic	~2	Ribosome biogenesis, centrosome duplication, protein chaperoning, histone assembly, cell proliferation
	BCOR	BCL6 Corepressor, a POZ/zinc finger transcription repressor	Xp11.4	Subclonal driver mutation	Negative	A transcription repressor; may influence apoptosis	< 5	Associated with RCMD or RAEB
	GATA2	GATA binding protein 2	3q21.3	Somatic mutation	Unclear;? risk of progression to AML	Zinc-finger transcription factors; development and proliferation of hematopoietic cells	< 5	Germline mutations causes familial predisposition to AML and MDS; monocytopenia
Epigenetic modifiers	*TET2*	Tet methylcytosine deoxygenase 2	4q24	Nonsense/indel throughout; non-functional, missense in catalytic domain; nonfunctional	Unclear; possibly positive	Alpha ketoglutarate-dependent dioxygenase	~ 20	UPD or microdeletion in 4q associated with advanced age and normal karyotype
	IDH1	Isicitrate dehydrogenase 1 (NADP+), soluble	2q33.3	Missense; altered function	Negative	NADP-dependent isocitrate dehydrogenase	~ 2	Mutually exclusive to TET2 mutations, associated with normal karyotype, IDH 1 mutations impact adverse prognosis
	IDH2	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	15q26.1	Missense; altered function	None	NADP-dependent isocitrate dehydrogenase	~ 2	
	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	2p23	Missense; dominant negative	Negative	DNA methyltransferase	~ 8	Not associated with normal karyotype, increased risk of AML

Mutations of myelodysplastic syndromes (MDS): An update, Ganguly et al, Mutations Research, 2016

Prognostic Impact

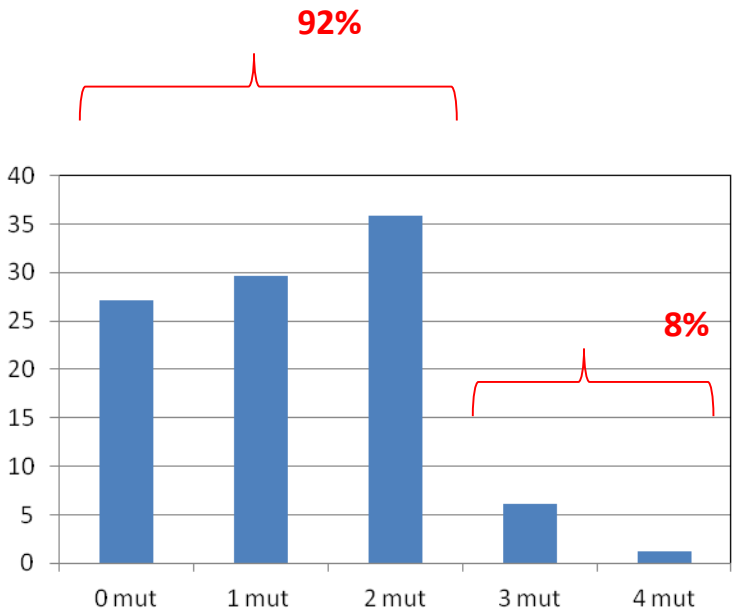
Table 1 (*Continued*)

Gene function	Gene symbol	Gene name	location	Mutation; effect	Prognostic implication	Protein function	Frequency %	Common attributes
Epigenetic modifiers	*TET2*	Tet methylcytosine deoxygenase 2	4q24	Nonsense/indel throughout; non-functional, missense in catalytic domain; nonfunctional	Unclear; possibly positive	Alpha ketoglutarate-dependent dioxygenase	~ 20	UPD or microdeletion in 4q associated with advanced age and normal karyotype
	IDH1	Isicitrate dehydrogenase 1 (NADP+), soluble	2q33.3	Missense; altered function	Negative	NADP-dependent isocitrate dehydrogenase	~ 2	Mutually exclusive to TET2 mutations, associated with normal karyotype, IDH 1
	IDH2	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	15q26.1	Missense; altered function	None	NADP-dependent isocitrate dehydrogenase	~ 2	mutations impact adverse prognosis
	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	2p23	Missense; dominant negative	Negative	DNA methyltransferase	~ 8	Not associated with normal karyotype, increased risk of AML
Histone modification	*ASXL1*	Additional sex combs like 1 (Drosophila)	20q11	Nonsense/indel; dominant negative or activation	Adverse	Chromatin-binding protein	~ 10–20	Excess of blasts, intermediate risk IPSS, shorter OS, increased risk of AML
	EZH2	Enhancer of zeste homolog 2 (Drosophila)	7q35-36	Missense in the SET domain; non-functional nonsense/indel; nonfunctional	Negative	Histone-methylating protein	~ 7	UPD or microdeletion in 7q, worse outcome in low-risk MDS
	SF3B1	Splicing factor 3b, subunit 1, 155 kDa	2q33.1	Missense; possible dominant negative or gain of function	Favorable	RNA-splicing factor 3b subunit 1, part of U2	~ 20	Common in RARS-RARS-T, coexistence with DNMT3A
RNA splicing	*U2AF1*	U2small nuclear RNA auxillary factor 1	21q22.3	Missense; possibly dominant negative or gain of function	Unclear	U2 small nuclear RNA splicing factor	~ 7	High risk, enriched in patients with del20q11 and ASXL1 mutations
	SRSF2	Serine/arginine-rich splicing factor 2	17q25.1	Missense; possible dominant negative or gain of function	Negative	Serine/arginine-rich pre RNA splicing factor	~ 12	Male gender, advanced age and coexistence of RUNX1, IDH1, ASXL1 mutations
	ZRSR2	Zinc finger (CCCG type), RNA-binding motif and serine/argentine rich 2	Xp22.1	Nonsense/indel/splice sites; non-functional	Unclear	Zinc finger RNA-binding associated with U2	~ 3	Male predilection, isolated neutropenia and clustering with TET2 mutations
	PRPF8	Pre-mRNA processing factor 8	17p13.3	Missense/Deletions; defects in proof-reading functions	Unclear (poor in AML)	Catalytic step II in pre-mRNA splicing; sister chromatid cohesion	~1–4	Associated with ring sideroblast phenotype in common with SRF3B1; retinitis pigmentosa
Cohesin complex	*STAG2*	Cohesin complex factor	Xq25	Indel; loss of function; subclonal mutation	Negative	Regulates separation of sister chromatids during cell division	1–10%	Associated with RCMD or RAEB
Other mutations	*SETBP1*	SET binding protein 1	18q21.1	Missense in SKI-homologous domain, Impairs degrdation	Negative; high risk of leukemic evolution	Binds SET, unclear function	~2–5	Co-occur with –ASXL1 and BL mutations, 7/del(7q); mutual exclusiveness to TP53

Mutations of myelodysplastic syndromes (MDS): An update, Ganguly et al, Mutations Research, 2016

Evaluation of Gene Mutations

In MDS Sequential Cases 2018 in MDS UNIT Florence

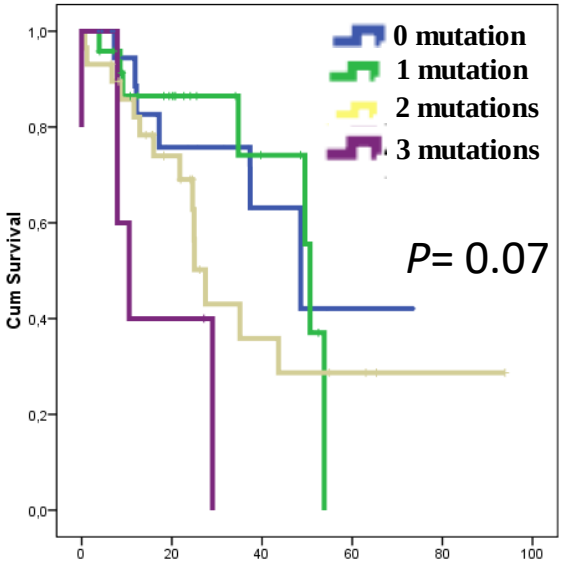


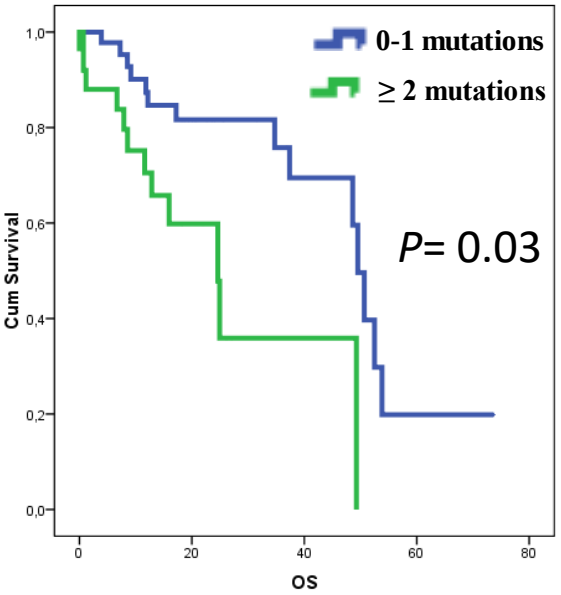
- 75% of MDS patients carried at least 1 mutation
- Only 8% of MDS patients showed ≥ 3 mutations, consistent with the majority of cases belonging to IPSS-R higher risk and with recent diagnosis

Brogi et al, unpublished

Number of Mutations: Prognostic Impact on OS

Characteristics	n	Median (months)	P
TET2			
Wild type	62	48	0.655
Mutated	19	49	
SRSF2			
Wild type	64	49	0.212
Mutated	17	35	
ASXL1			
Wild type	65	49	0.389
Mutated	16	43	
SF3B1			
Wild type	68	49	0.892
Mutated	13	35	
DNMT3A			
Wild type	68	49	0.592
Mutated	13	35	
RUNX1			
Wild type	73	49	**0.001**
Mutated	8	12	
TP53			
Wild type	75	48	0.844
Mutated	6	49	
Number of mutations			
0 -1	51	49	**0.03**
≥ 2	30	25	





Brogi et al, unpublished

Prognostic Impact of single mutations on OS

Characteristics	n	Median (months)	P
TET2			
Wild type	62	48	0.655
Mutated	19	49	
SRSF2			
Wild type	64	49	0.212
Mutated	17	35	
ASXL1			
Wild type	65	49	0.389
Mutated	16	43	
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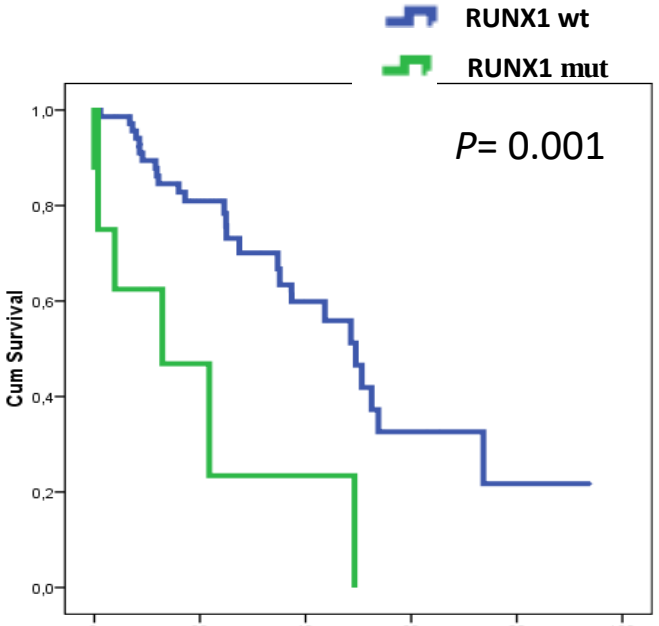

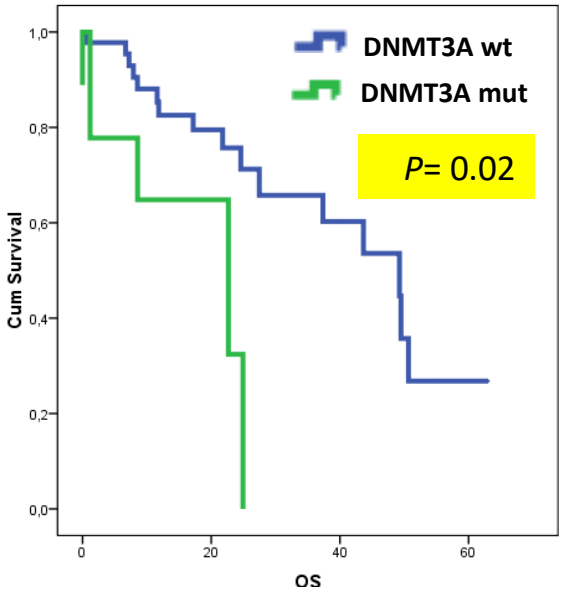

- *DNMT3* mutations in MDS patients with **normal karyotype** were associated with a significantly worst OS

Normal karyotype, n= 54

Brogi et al, unpublished

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TP53 Mutations in MDS

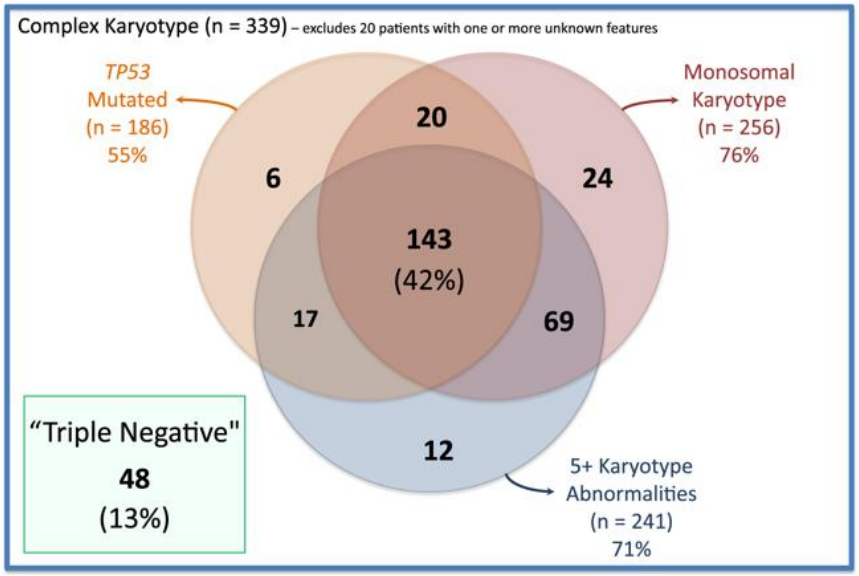
Leukemia. 2019 Jan 11. doi: 10.1038/s41375-018-0351-2. [Epub ahead of print]

TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups.

Haase D[1], Stevenson KE[2], Neuberg D[2], Maciejewski JP[3], Nazha A[3], Sekeres MA[3], Ebert BL[2], Garcia-Manero G[4], Haferlach C[5], Haferlach T[5], Kern W[5], Ogawa S[6], Nagata Y[3], Yoshida K[6], Graubert TA[7], Walter MJ[8], List AF[9], Komrokji RS[9], Padron E[9], Sallman D[9], Papaemmanuil E[10], Campbell PJ[11], Savona MR[12], Seegmiller A[12], Adès L[13], Fenaux P[13], Shih LY[14], Bowen D[15], Groves MJ[16], Tauro S[16], Fontenay M[17], Kosmider O[17], Bar-Natan M[18], Steensma D[2], Stone R[2], Heuser M[19], Thol F[19], Cazzola M[20], Malcovati L[20], Karsan A[21], Ganster C[1], Hellström-Lindberg E[22], Boultwood J[23], Pellagatti A[23], Santini V[24], Quek L[25,26], Vyas P[25,26], Tüchler H[27], Greenberg PL[28], Bejar R[29], International Working Group for MDS Molecular Prognostic Committee.

A

B



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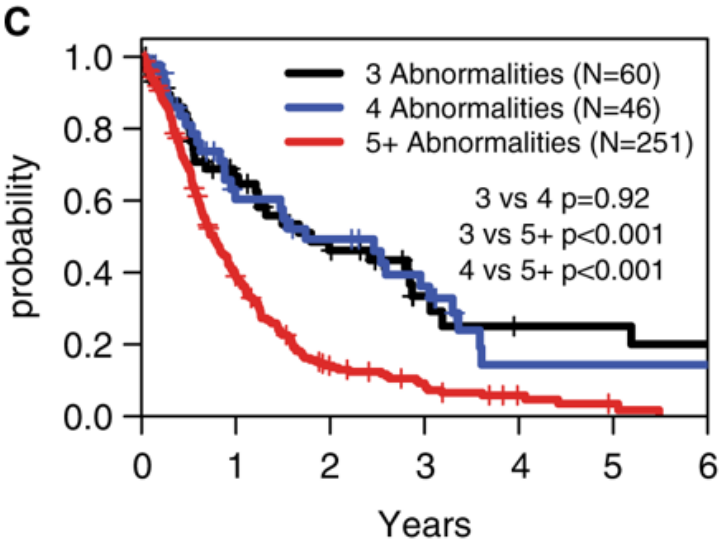
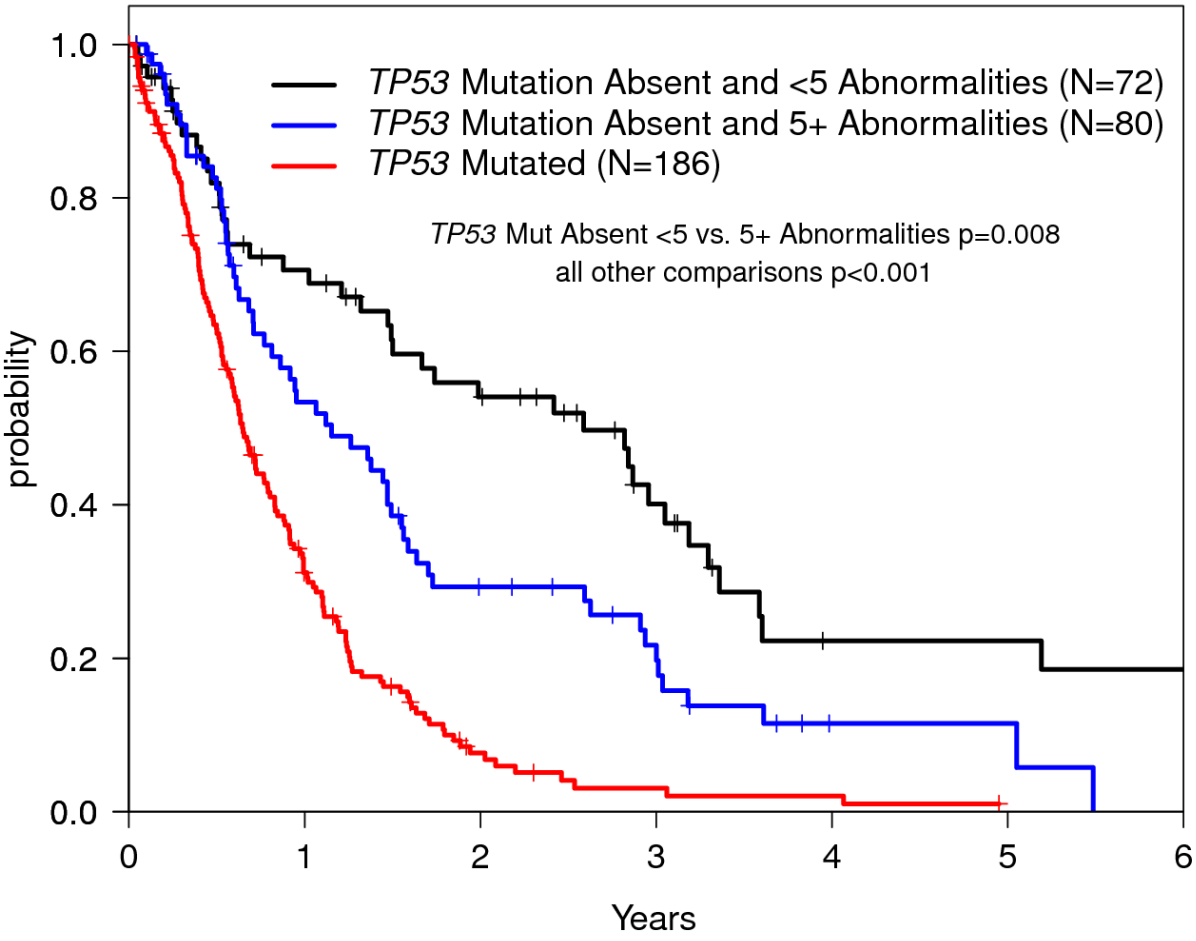


MDS Overall Survival Stratified by *TP53* Mutation and High Complexity Status.

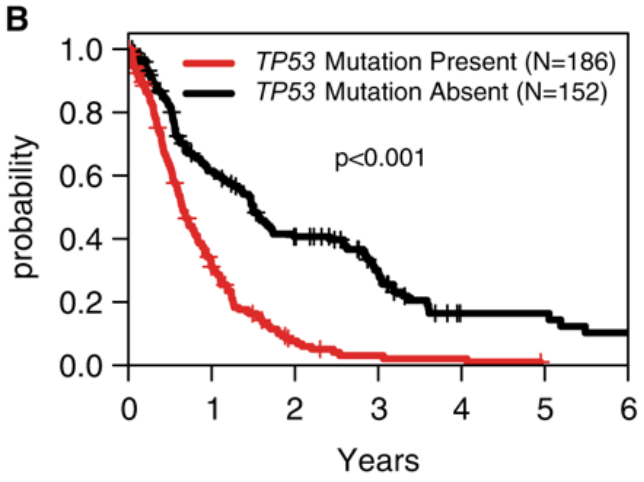
N : 359

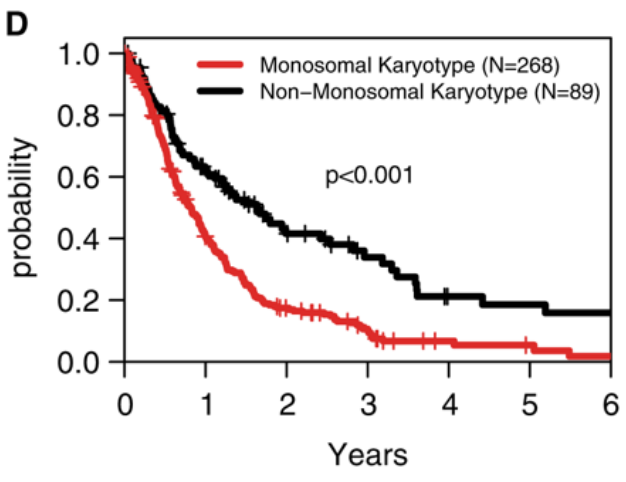


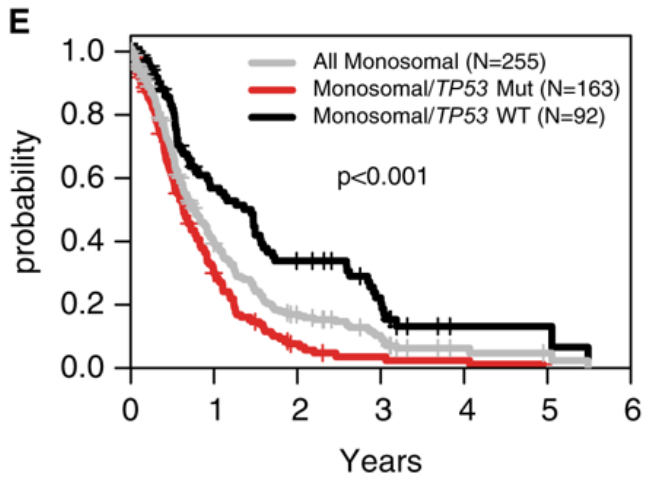
Haase et al , Leukemia 2019

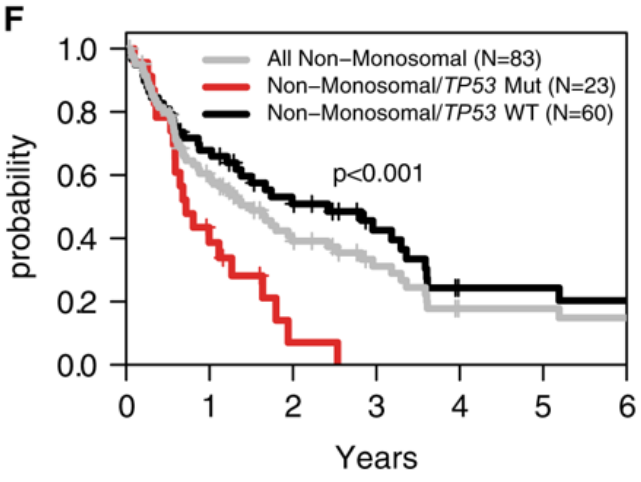
TP53 Mutations and overall survival

***TP3 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups*,**
Haase et al, Leukemia 2019



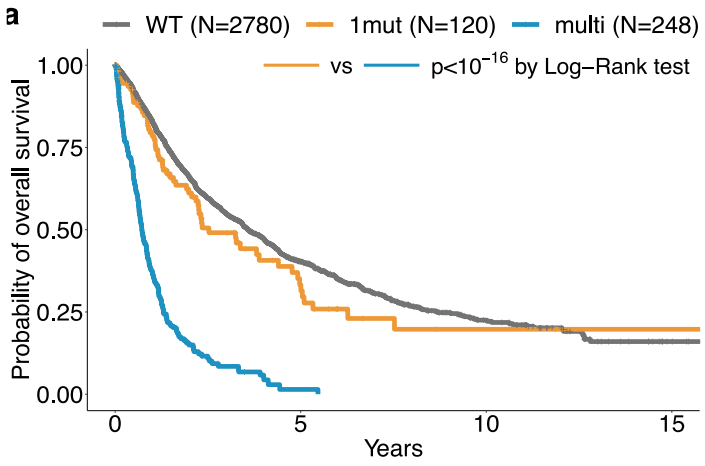




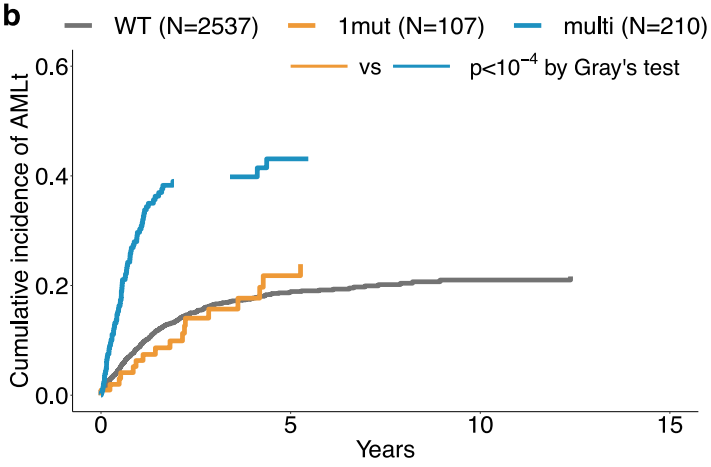


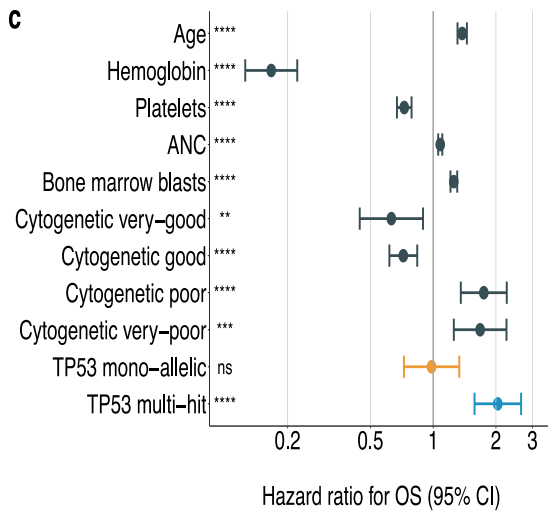
TP53 allelic state shapes clinical outcomes

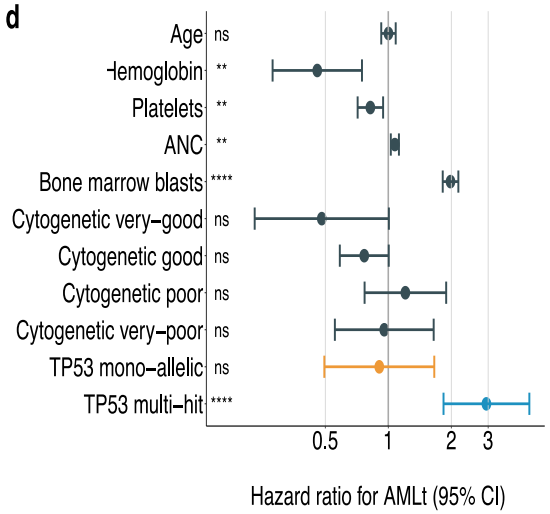
Overall Survival



AML Transformation







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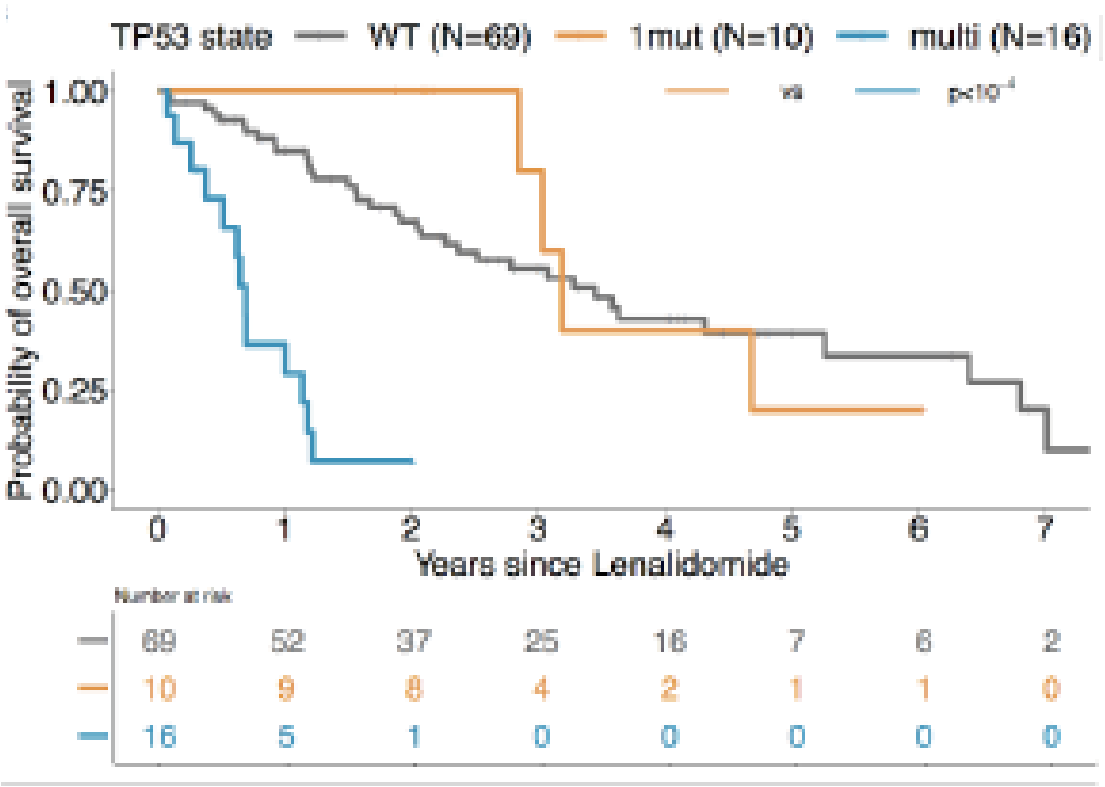
Bernard E, et al. Nat Med. 2020 Oct;26(10):1549-1556

Thursdays Webinars

TP53 allelic state influences response to therapy: LEN in lower risk MDS



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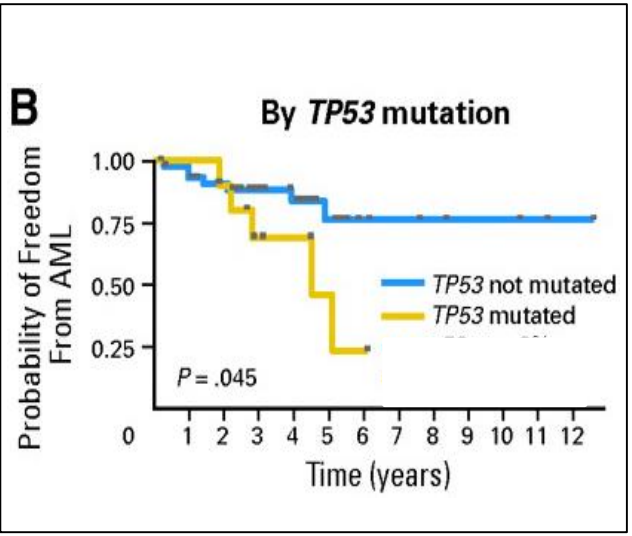
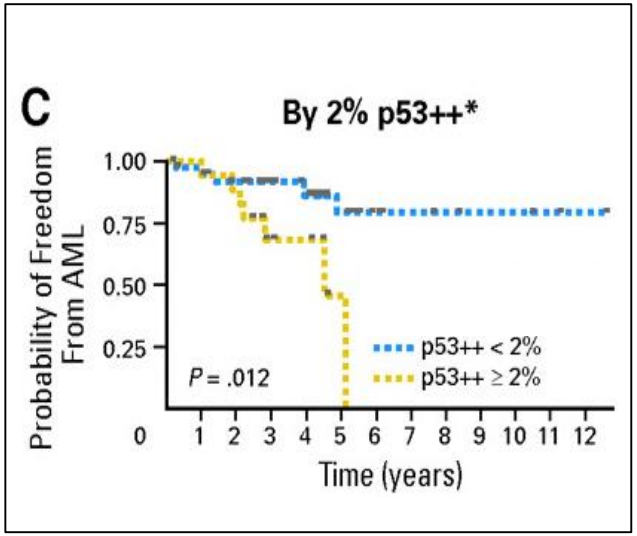
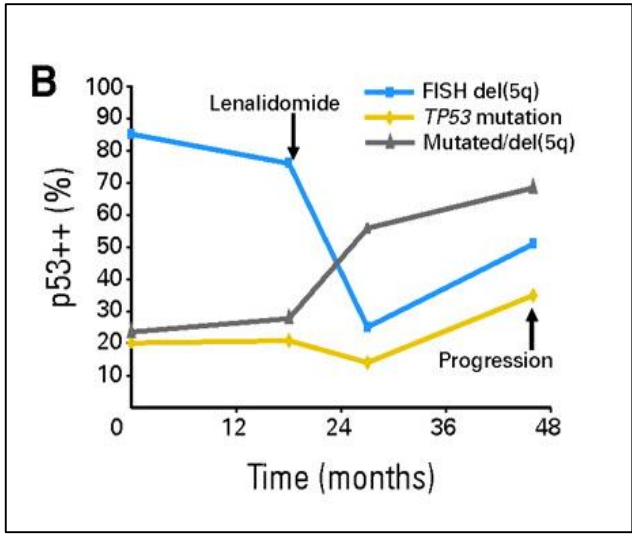
Lenalidomide does not improve OS in del(5q) MDS with TP53mut

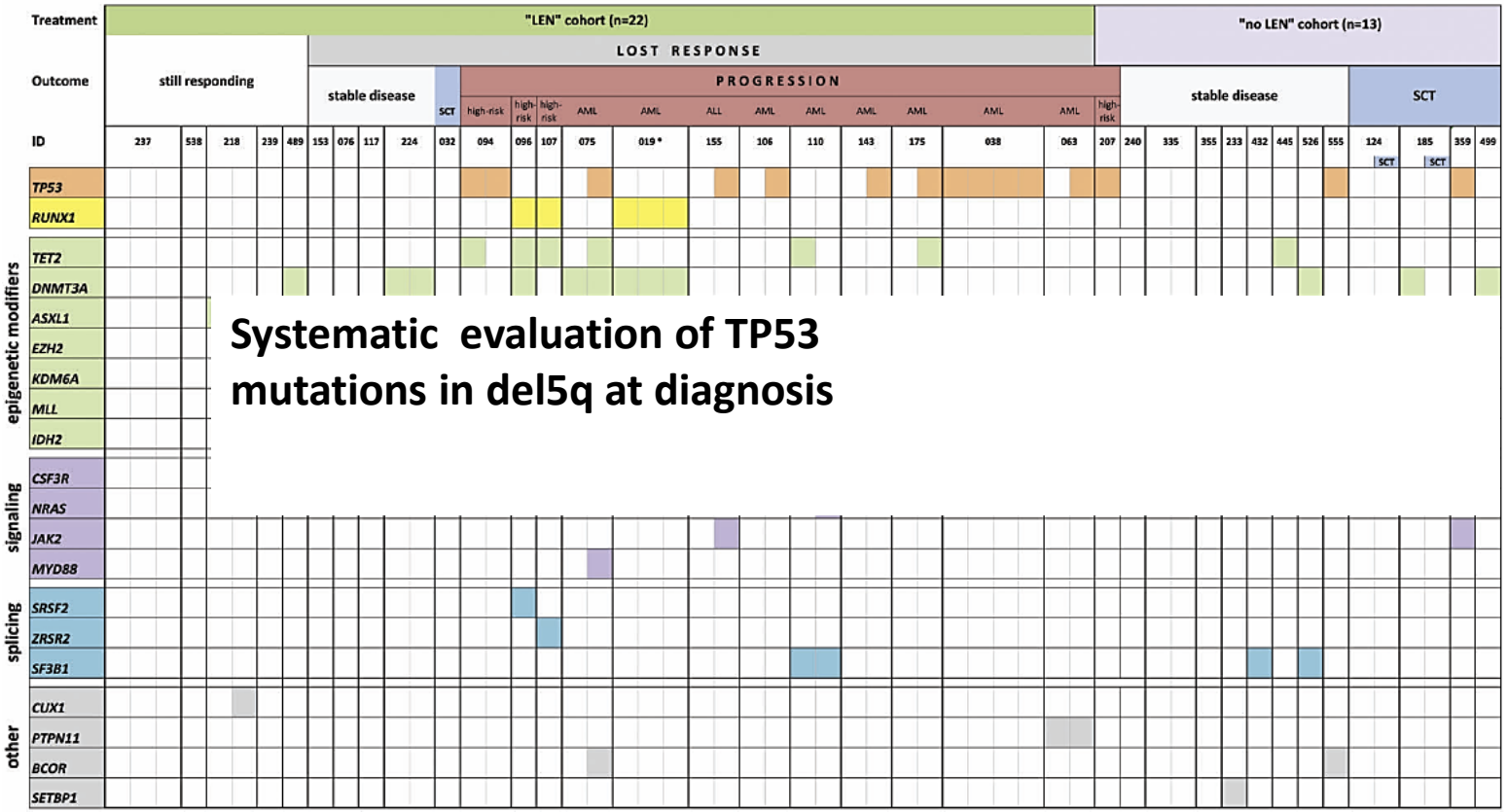






Martin Jädersten et al. JCO 2011;29:1971-1979

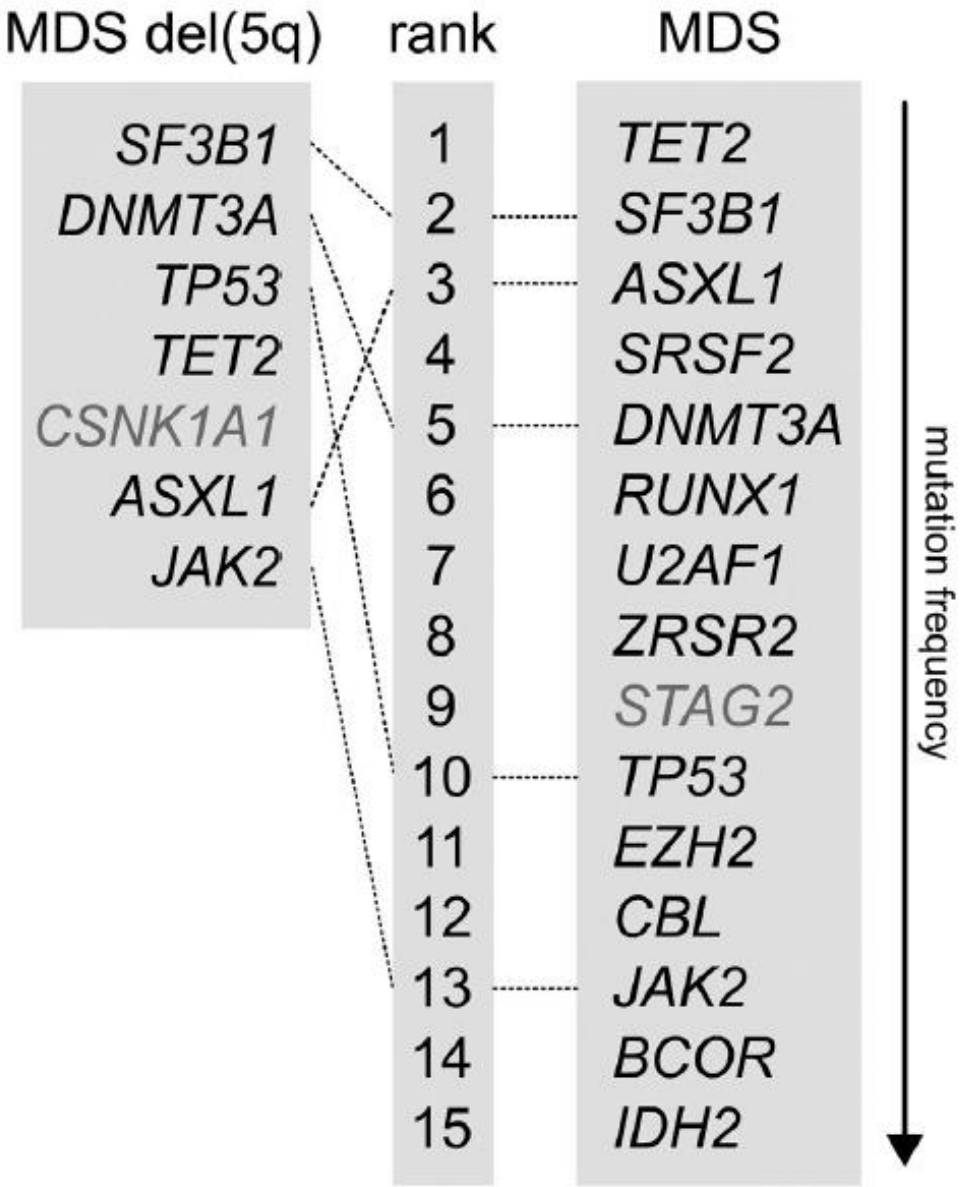
Lenalidomide does not improve OS in del(5q) MDS with TP53mut



Systematic evaluation of TP53 mutations in del5q at diagnosis

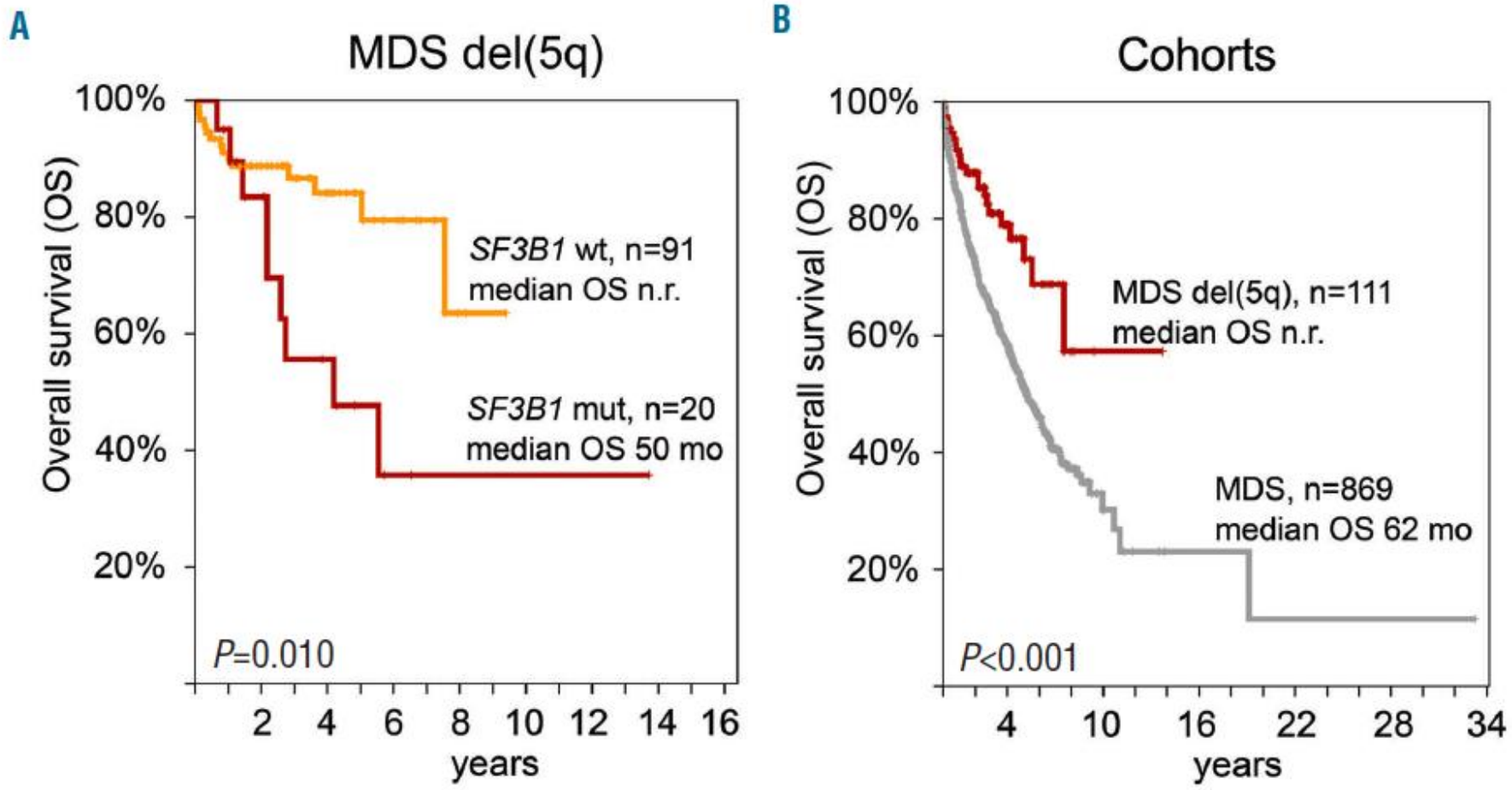
Christian Scharenberg et al. *Haematologica* 2017;102:498-508.

Most frequently mutated genes in MDS with isolated del5q and comparison with nondel5q MDS



Meggendorfer et al .
Haematologica 2017 ; 102(9):1502

SF3B1 mutations are not a good prognostic factor In MDS with isolated del5q



Meggendorfer et al . Haematologica 2017 ; 102(9):1502-1510

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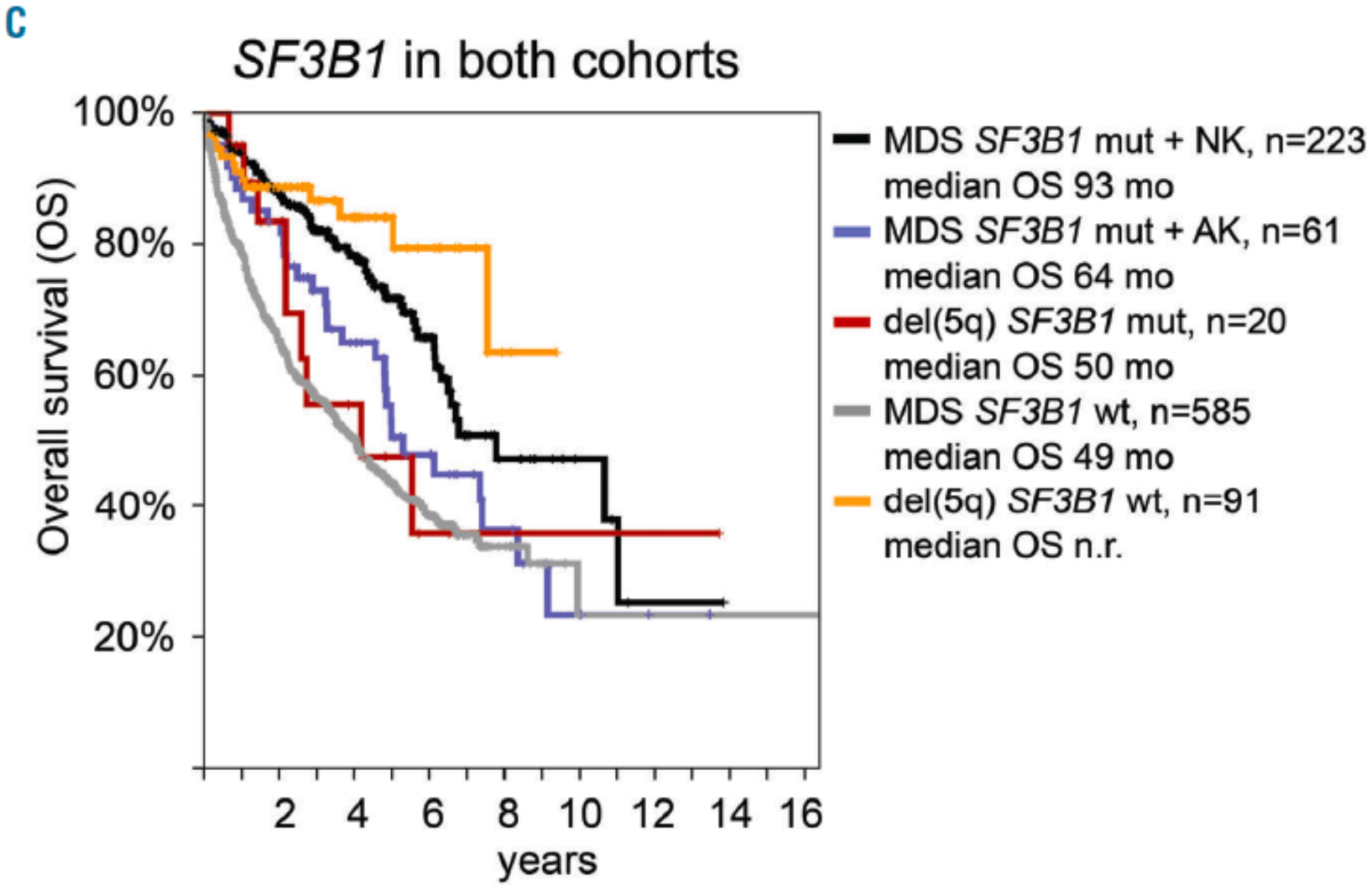
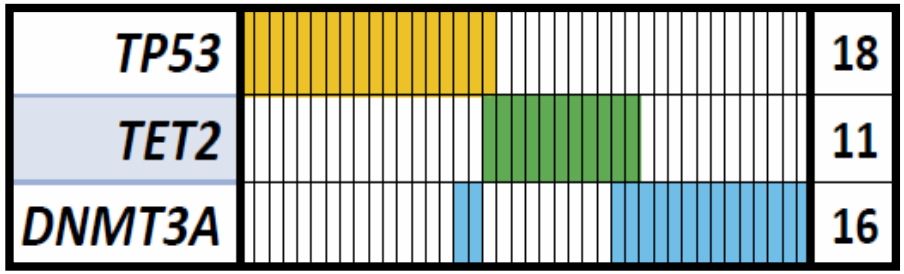
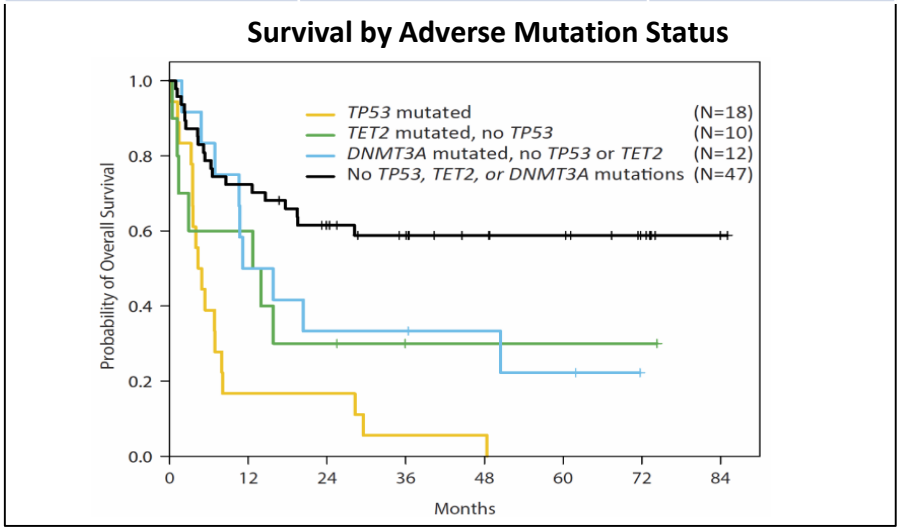


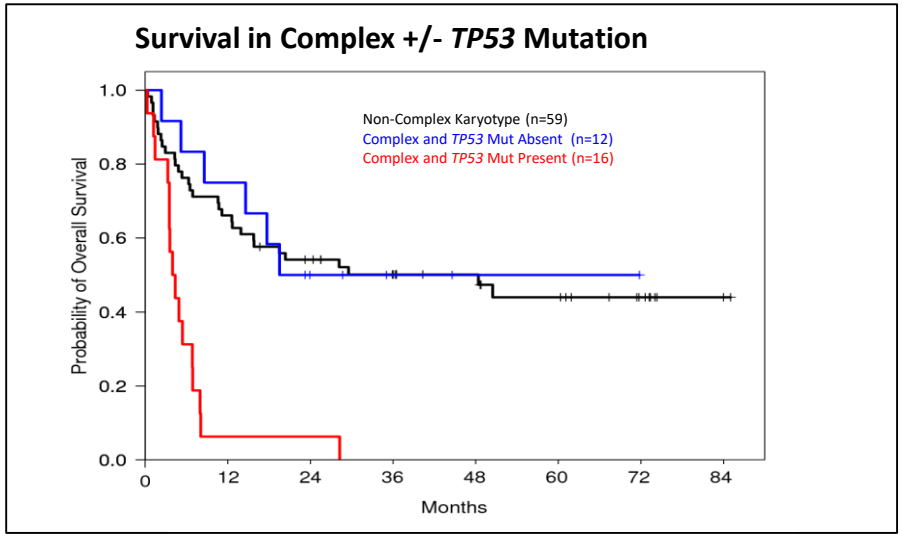
Meggendorfer et al . Haematologica 2017 ; 102(9):1502-1510

TP53 mutation and HSCT

Gene	Adjusted HR (95% CI)	p-value
TP53 (n=18)	**2.30** (1.10, 4.81)	0.027
TET2 (n=11)	**2.40** (1.07, 5.38)	0.033
DNMT3A (n=16)	**2.08** (1.00, 3.26)	0.049

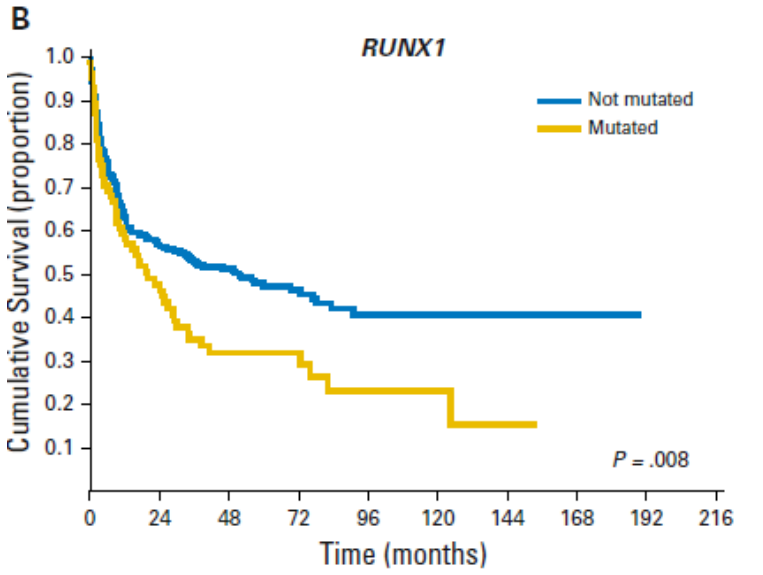


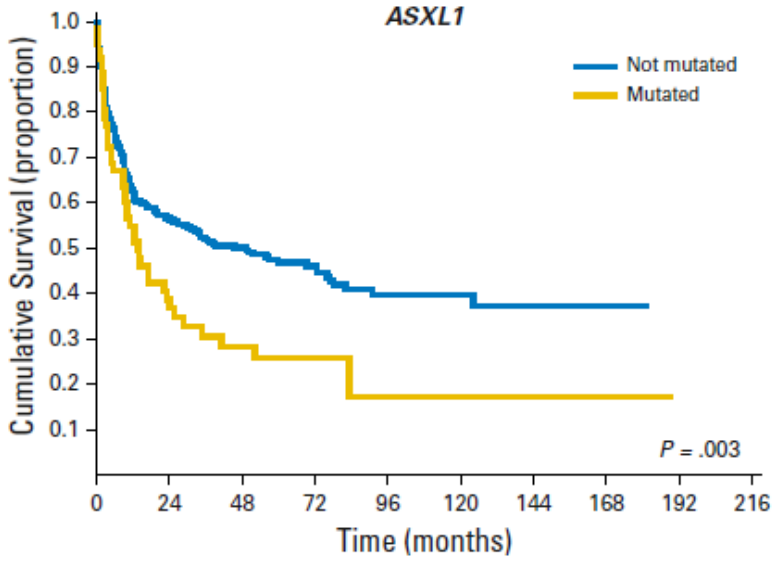


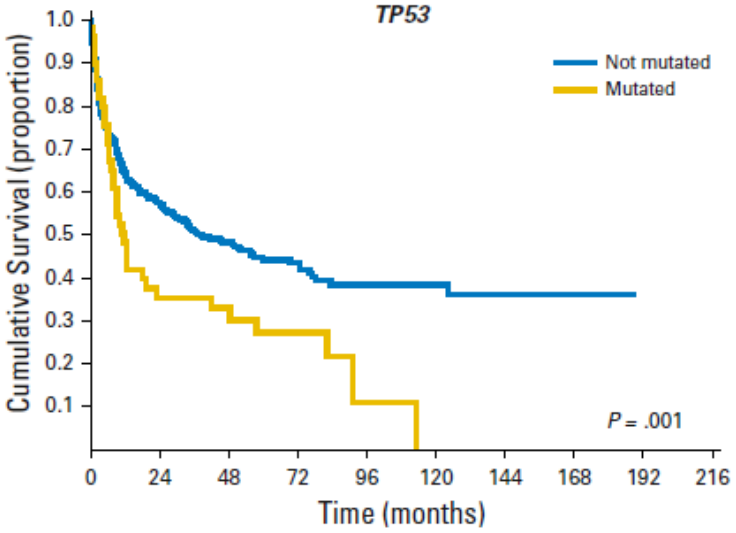


Bejar et al., *JCO*. 2014

Relationship between type of oncogenic mutations and overall survival of MDS receiving allo-HSCT



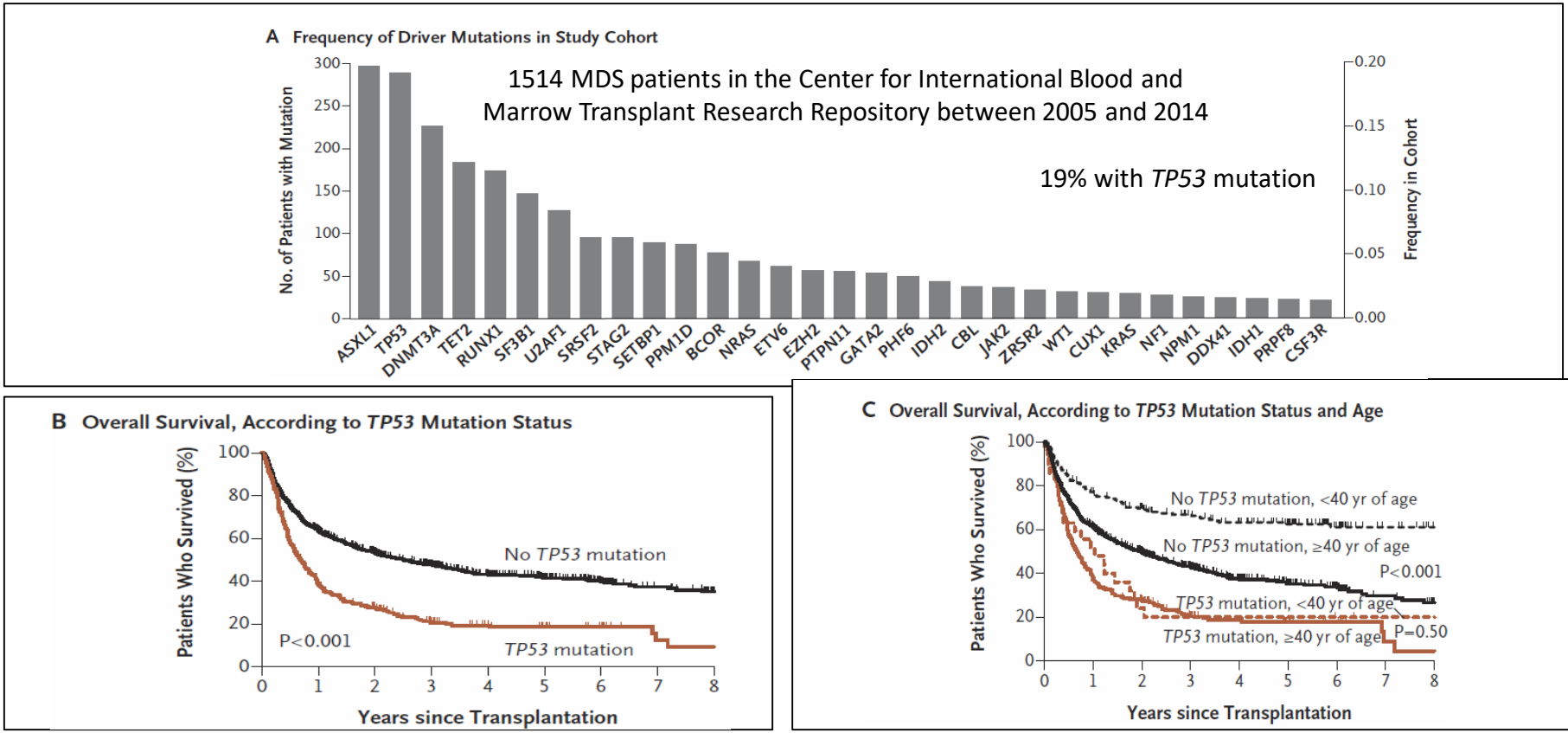




Multivariable analysis				
MDS patients	**Probability of relapse**		**Overall Survival**	
Variable	HR	P	HR	P
ASXL1	1.89	.003	1.72	.008
RUNX1	1.67	.02	1.59	.035
TP53	1.90	.019	1.82	.022

Matteo G. Della Porta et al. JCO doi:10.1200/JCO.2016.67.3616

TP53 mutation and HSCT



Lindsley et al. *NEJM* 2017;376(6):536-47.

WHO 2016 classification of myeloid neoplasms with germ line predisposition

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 dysfunctior
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.

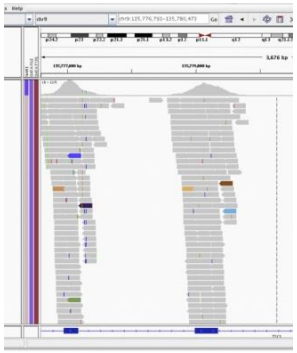
Arber et al Blood 2016 : 127:2391

- **Patient with de novo MDS *at a younger age* (< 50yrs)**
- **Patient with MDS and familial history of AML**
- **Patient with MDS and peculiar extra hematological symptoms:**

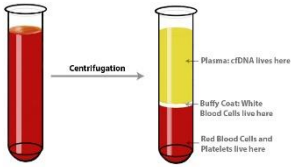
1. **Perform an accurate family and personal history**
2. **Search for signs and symptoms of congenital syndromes**
3. **Perform mutational analysis for genes involved in inherited predisposition**
4. **Select accurately HSCT donor (completely avoid related matched donor?) Slow engraftment, donor derived leukemia**
5. **Familial genetic counseling (anticipation of onset through generations)**

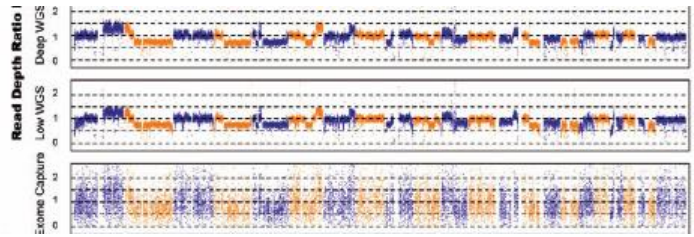
We decided to analyze a group of patients with "juvenile MDS" (between 40 and 50 years) with the following protocol:

- WES on bone marrow (with variant filtering and analysis of CNVs)



- lc-WGS on liquid biopsy (to identify rearrangements not visible with karyotype)





- WES on saliva to try to detect "congenital driver variants"

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Giglio et al 2019, unpublished

Thursdays Webinars

20% of young MDS patients tested carry germline and predisposing mutations

Somatic mutations in suspect of MDS: a help in diagnosis?

Dysplasia can be induced by other causes than MDS

Cytopenias without dysplasia may be tricking

…

and definite diagnosis is often a challenge

ICUS idiopathic cytopenia of unknown significance

IDUS

idiopathic dysplasia of unknown significance

CHIP/ARCH

clonal hemopoiesis of indeterminate potential/ age related clonal hemopoiesis

CCUS

clonal cytopenia of unknown significance

Clonal hemopoieis of indeterminate potential (CHIP)

- Clonality defined by presence of MDS-associated genes:

DNMT3A, ASXL1, TET2, (JAK2) with loss of function

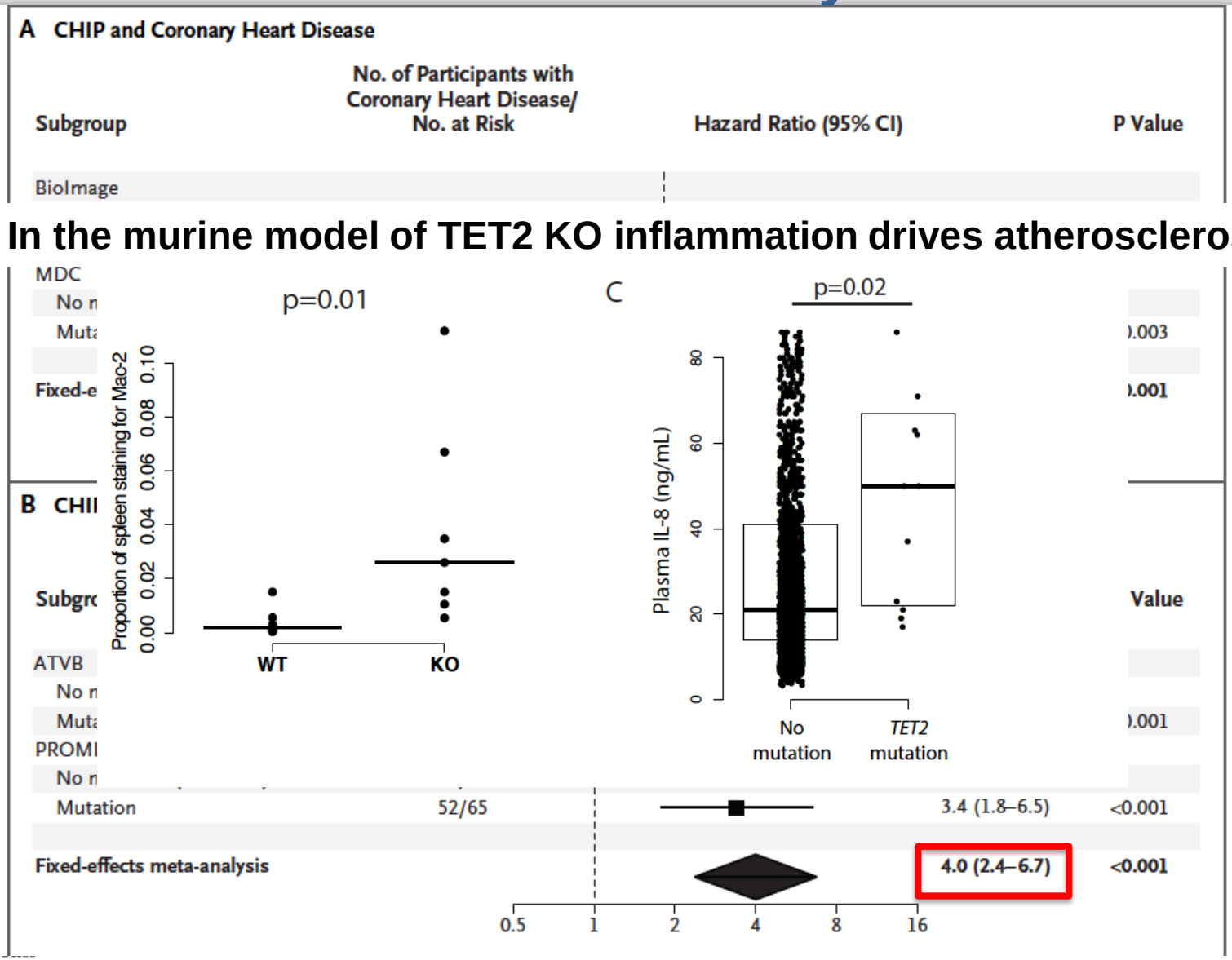
- Little propensity to develop MDS (0,5-1% /year)

- Present in 15% of persons aged > 70yrs

Triggered by (?) :

Stochastic event
Environment (smoke, radiation, chemotherapy, inflammation)
Hereditary/predisposition conditions

CHIP correlates with coronary heart disease



In the murine model of TET2 KO inflammation drives atherosclerosis

Jaiswal S et al NEJM June 21, 2017

	ICUS	ARCH	CHIP	CCUS	Lower Risk MDS	Higher Risk MDS
Clonality	-	+	+	+	+	+
Dysplasia	-	-	-	-	+	+
Cytopenia	+	-	-	+	+	+
BM Blast %	<5%	<5%	<5%	<5%	<5%	<19%
AML Overall Risk	Very low	Very low	Very low but cardiopathy	Low /intermediate	Low	High
Median Num of mutations	0	1	1	>1<2	>2	>2
Typical VAF	-	1-10%	9-12% (>10)	30-40% (40)	>50%	>50%
Types of mutations		*DNMT3A, TET2,ASXL1, JAK2, TP53*	*DNMT3A, TET2,ASXL1, JAK2, TP53*	*TET2, DNMT3A,ASXL1, SRSF2, TP53* ***Later*** *TET2, SRSF2, ASXL1, U2AF1, DNMT3A*	***SF3B1, TET2, ASXL1, SRSF2, DNMT3A*** *and all the less frequent*	

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Modified from Steensma et al, *Blood* 2015 and Bejar R Leukemia, 2017 online

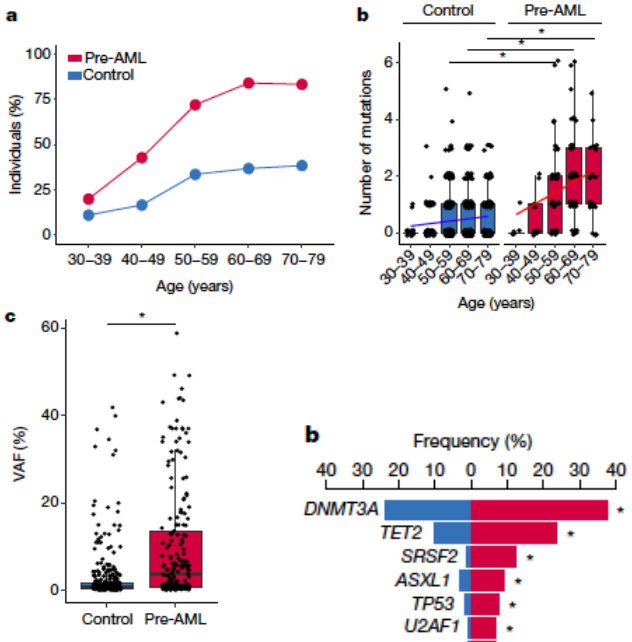
Somatic mutations can confirm a diagnosis of MDS
IF :

Present in a young patient with isolated cytopenia

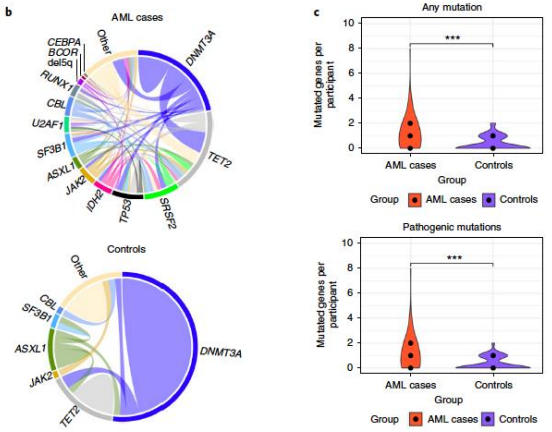
Present with elevated VAF in an elderly patient, mostly if different than those found in CHIP

Predicting progression to AML

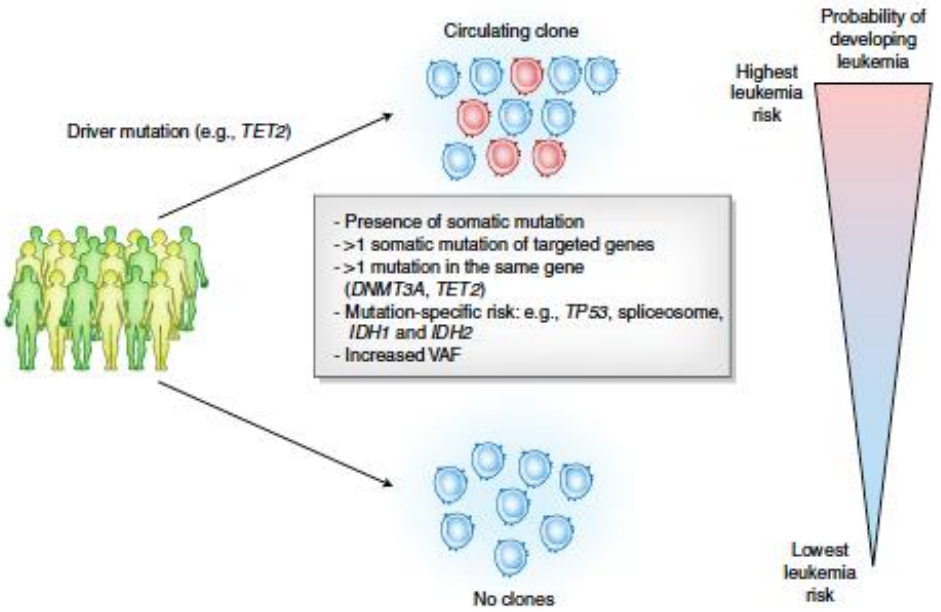
Abelson S et al, Nature. 2018 Jul;559(7714):400-404.



Progression to AML in ARCH/CHIP 6.7 yrs in advance



Desai P et al, Nat Med. 2018 Jul;24(7):1015-1023.



Sellar et al, Nat Med. 2018 Jul;24(7):904-906.

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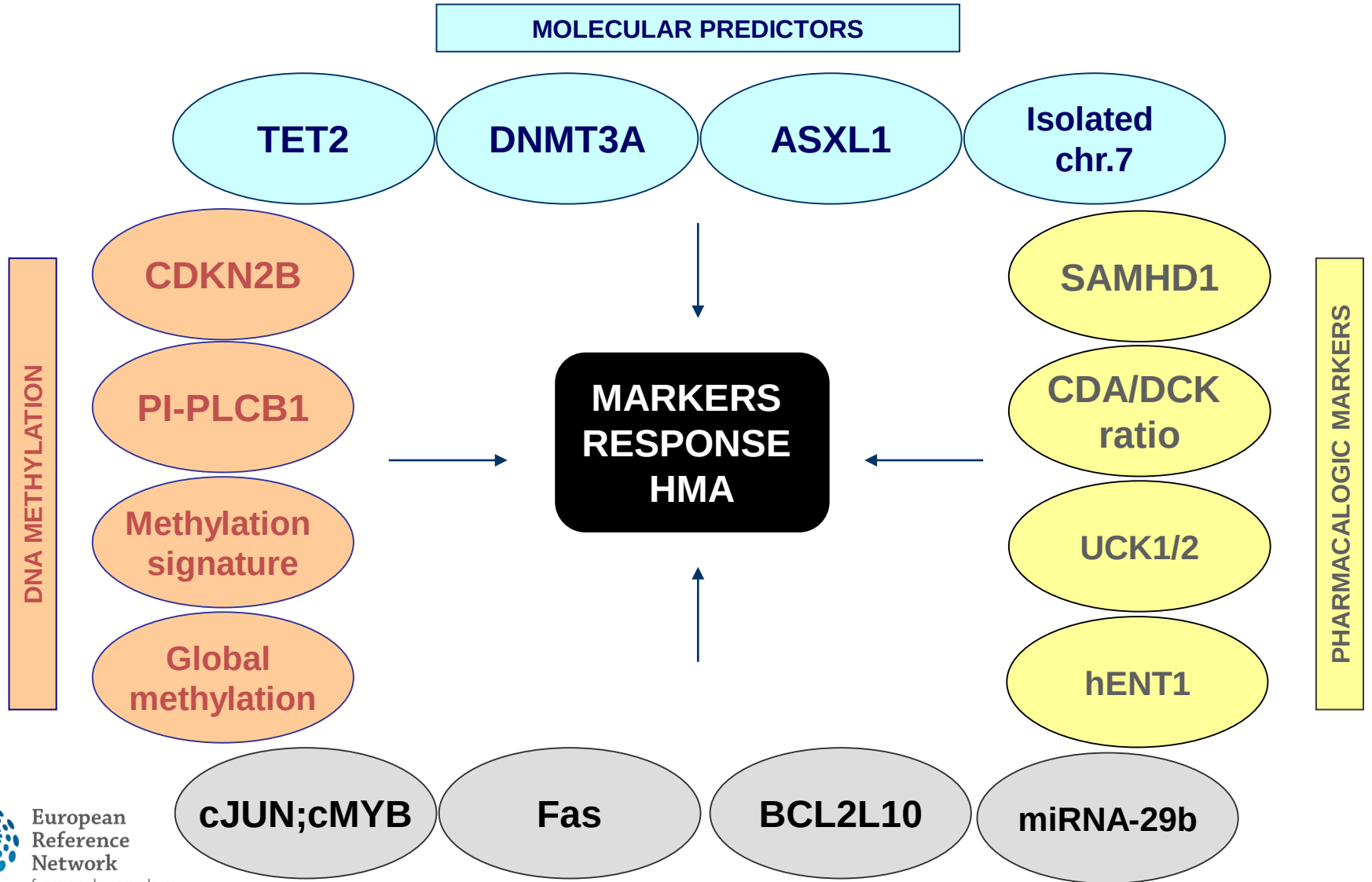
Thursdays Webinars

Somatic mutations can predict response to therapy?

Proposed Biomarkers for HMA response



Molecular predictors

Responses rates are higher in the subset of TET2^{mut} and DNMT3A^{mut} patients

Table 4 Multivariate analysis (logistic regression) of overall response according to *TET2* status, cytogenetics and previous therapy

	Including SD with HI		*Excluding SD with HI*	
	OR (95% CI)	P	*OR (95% CI)*	P
Mutated *TET2*	5.92 (1.05–33.33)	0.044	5.92 (1.43–24.39)	0.014
Cytogenetic risk[a]				
Intermediate	0.24 (0.06–0.98)	0.048	2.41 (0.60–9.71)	0.22
Poor	0.33 (0.11–0.95)	0.040	2.11 (0.68–6.45)	0.19
Previous therapy	1.56 (0.47–5.15)	0.47	0.47 (0.13–1.65)	0.24

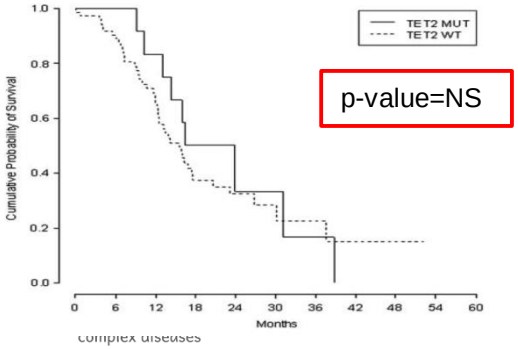
Abbreviations: CI, confidence interval; HI, hematological improvement; OR, odds ratio; SD, stable disease; TET2, ten-eleven-translocation 2.
[a]Compared with good risk.

Mutated gene*	Unadjusted OR (95% CI)	*P* value	Adjusted† OR (95% CI)	*P* value
Mutations with VAF ≥10%				
TET2-mut vs *TET2*-WT	1.99 (1.05, 3.80)	.036	1.98 (1.02, 3.85)	.044
ASXL-mut vs *ASXL1*-WT	0.69 (0.40, 1.20)	.19	0.68 (0.38, 1.19)	.17
TET2-mut + *ASXL1*-WT vs other	3.65 (1.38, 9.67)	.009	3.64 (1.35, 9.79)	.011
TET2-mut + *ASXL1*-WT vs both WT	3.40 (1.24, 9.35)	.011	3.36 (1.20, 9.38)	.013
TET2-WT + *ASXL1*-mut vs both WT	0.77 (0.41, 1.46)	.35	0.80 (0.39, 1.46)	.39
TET2-mut + *ASXL1*-mut vs both WT	1.11 (0.48, 2.61)	.62	1.07 (0.44, 2.61)	.59
CBL-mut vs *CBL*-WT	0.27 (0.06, 1.29)	.10	0.28 (0.06, 1.40)	.12

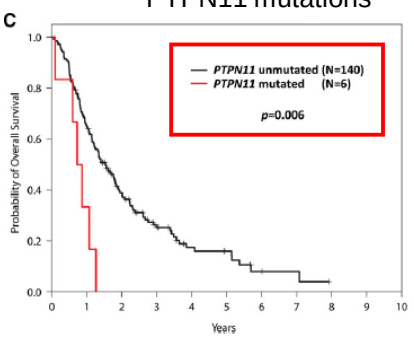
Feature	Category
Platelets, x10^9/L	≥100 < 100
WBC , x10^9/L	<3.0 ≥3.0
TET2/*DNMT3A* mutation	One or both genes mutated Both genes wild type

Mutations of TP53, PTPN11, and ASXL1 affected OS but not TET2^{mut}

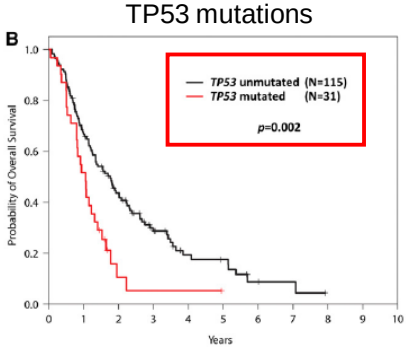
TET2 mutations



PTPN11 mutations



TP53 mutations



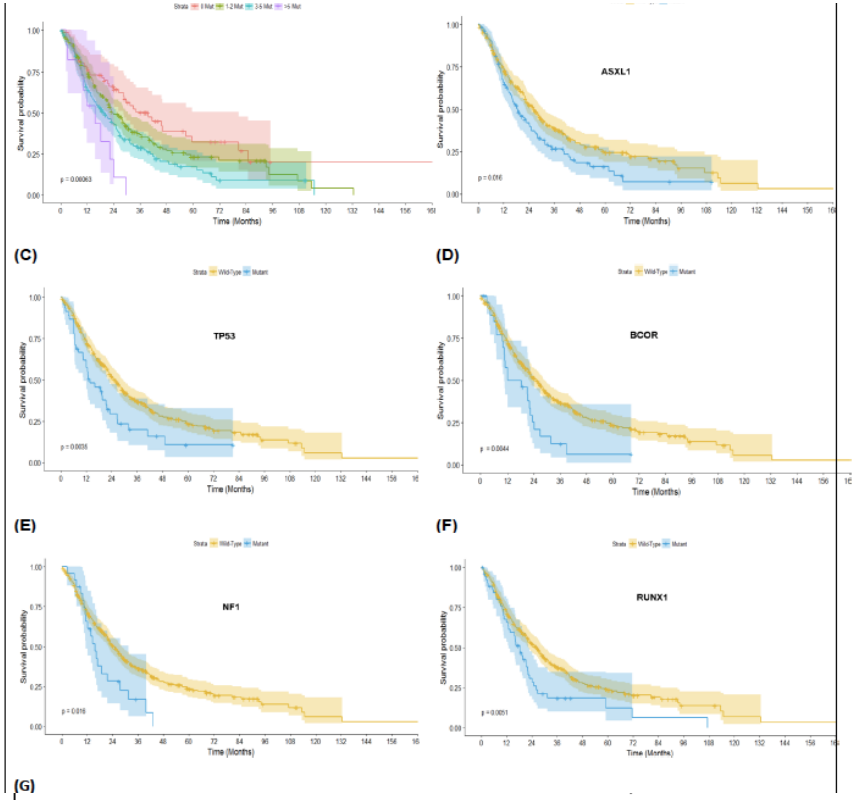
ASXL1 mutations

Feature	Category
Cytogenetic Risk	Good Intermediate or no growth Poor
ASXL1	Wild type Mutated
Hemoglobin, g/dL	≥10 <10
Age	< 60 ≥ 60
SF3B1	Mutated Wild type

Itzykson et al, *Leukemia* 2011; Bejar et al, *Blood* 2014; Traina F et al, *Leukemia* 2013

Molecular predictors of AZA response

(elaborated with artificial intelligence)



433 MDS patients

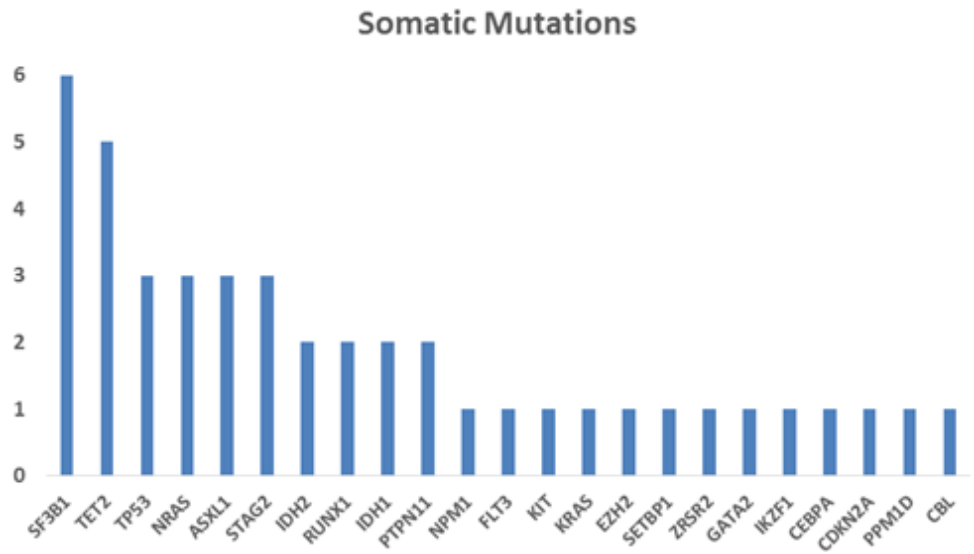
Possible in 30% of cases

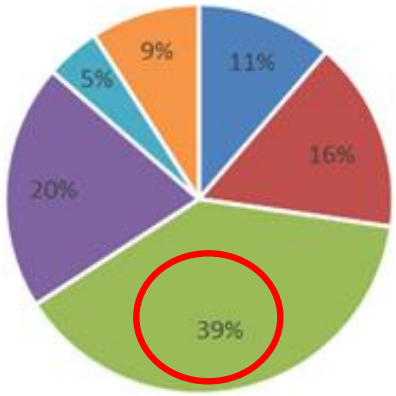
Association Rules for Resistance to HMA
ASXL1, NF1
ASXL1, EZH2, TET2
ASXL1, EZH2, RUNX1
EZH2, SRSF2, TET2
ASXL1, EZH2, SRSF2
ASXL1, RUNX1, SRSF2
ASXL1, TET2, SRSF2
ASXL1, BCOR, RUNX1

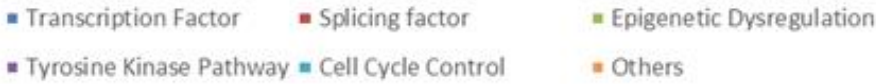
Nazha et al, JCO Prec Oncol 2019

None of the most frequently mutated genes in MDS correlates with response to AZA (77 cases)

- **39% of mutated genes involved in Epigenetic regulation**





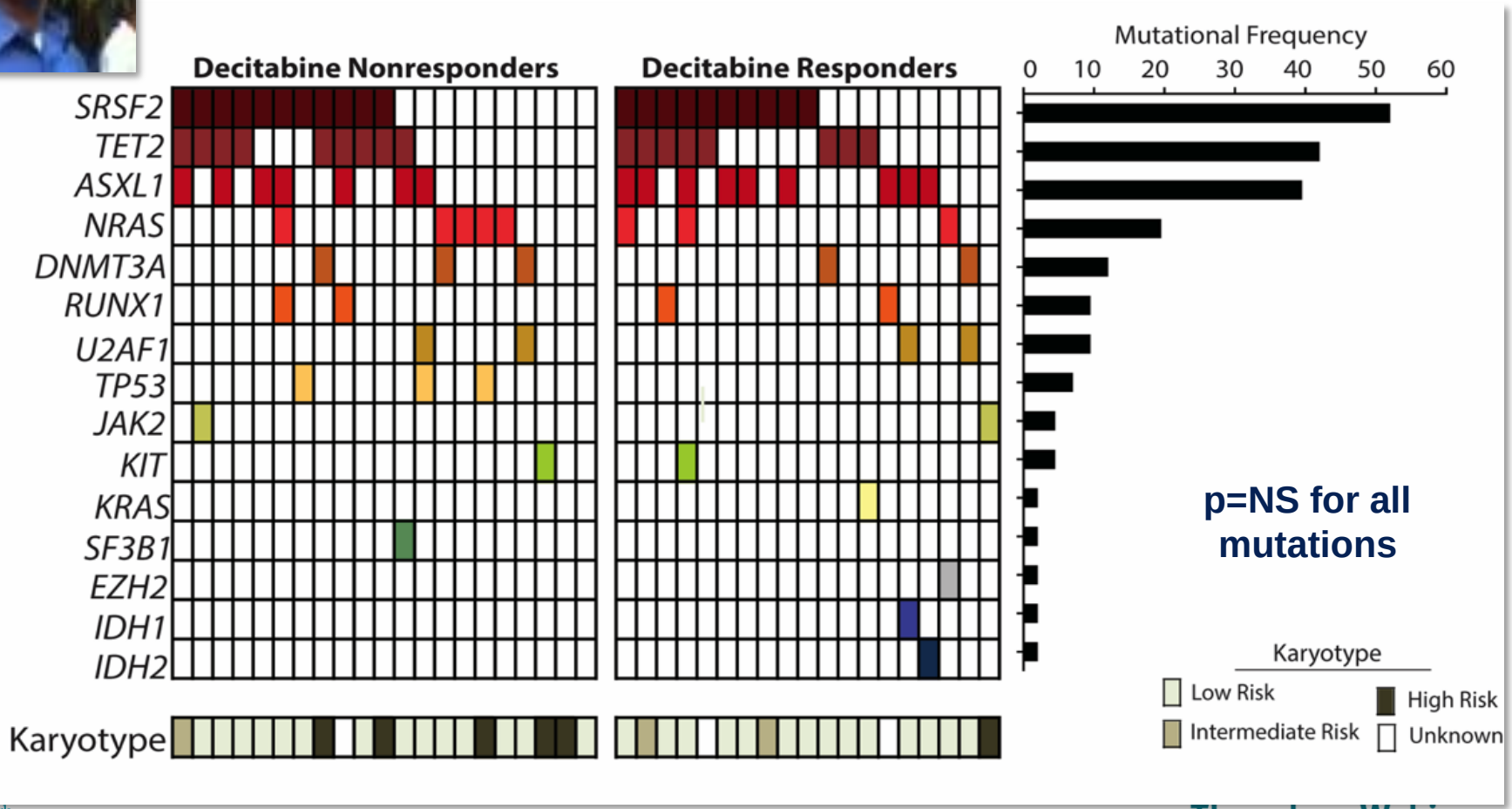


Masala e et al, submitted 2020

In CMML Mutational profiles do not correlate with response to decitabine

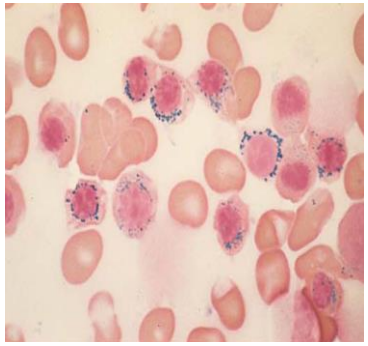
Responders

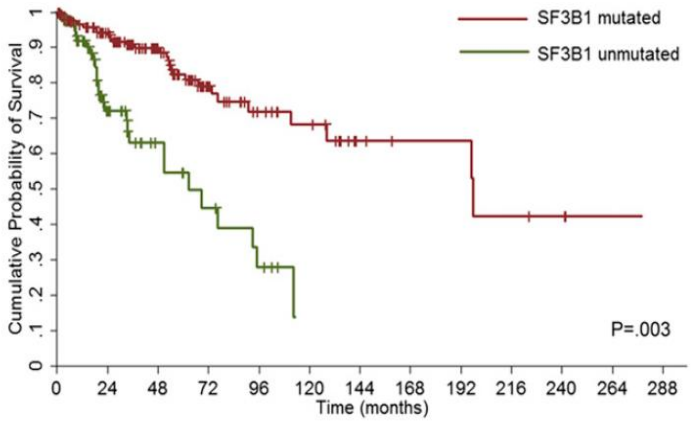
Non-Responders



Meldi et al 2015; Santini et al 2017

Myelodysplastic syndromes with ring sideroblasts (MDS-RS)





Malcovati L et al Blood. 2011 Dec 8;118(24):6239-46.

Ringed sideroblasts (RS) are erythroblasts in which iron accumulates in the mitochondria in the form of mitochondrial ferritin .

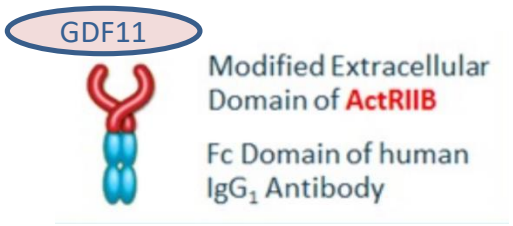
MDS-RS are characterized by ineffective erythropoiesis, severe anemia, transfusion dependency but relatively low risk of leukemic transformation.

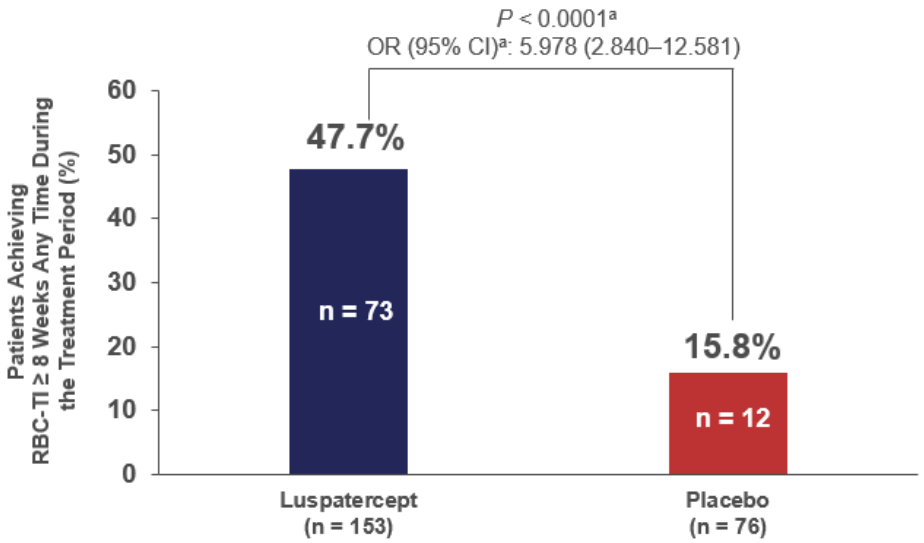
>90% of MDS-RS patients have somatic heterozygous mutations in *SF3B1*

Hepcidin is lower in MDS-RS vs other MDS subtypes

SF3B1 is associated with better overall survival

Luspatercept induces Transfusion independence in *RS(+)* LR-MDS pts



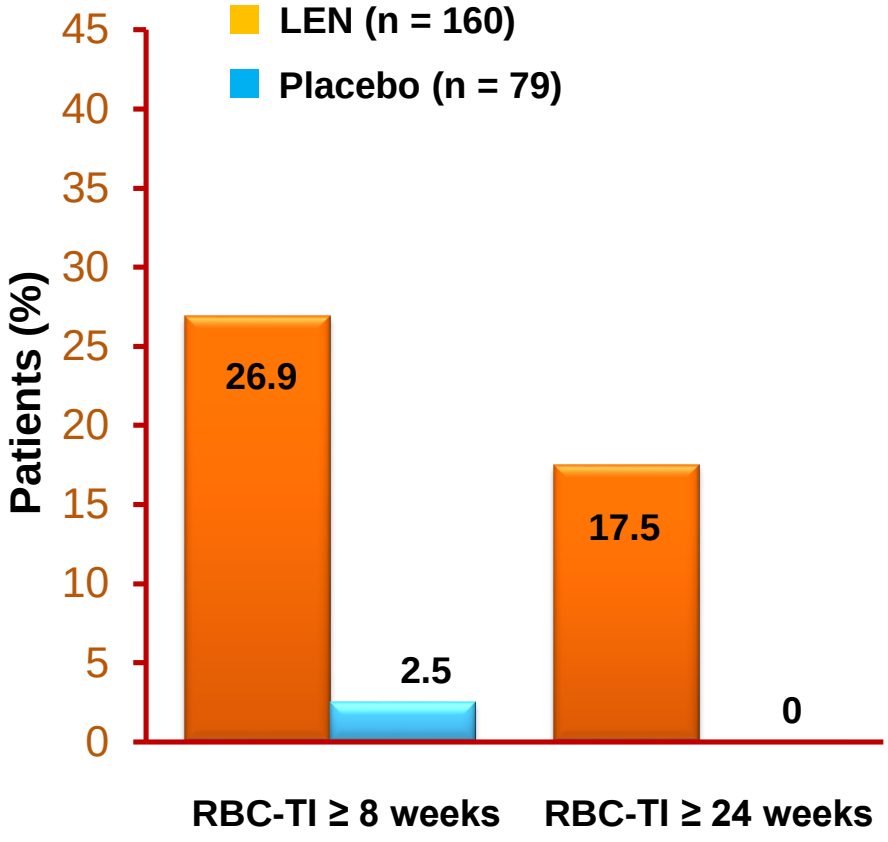


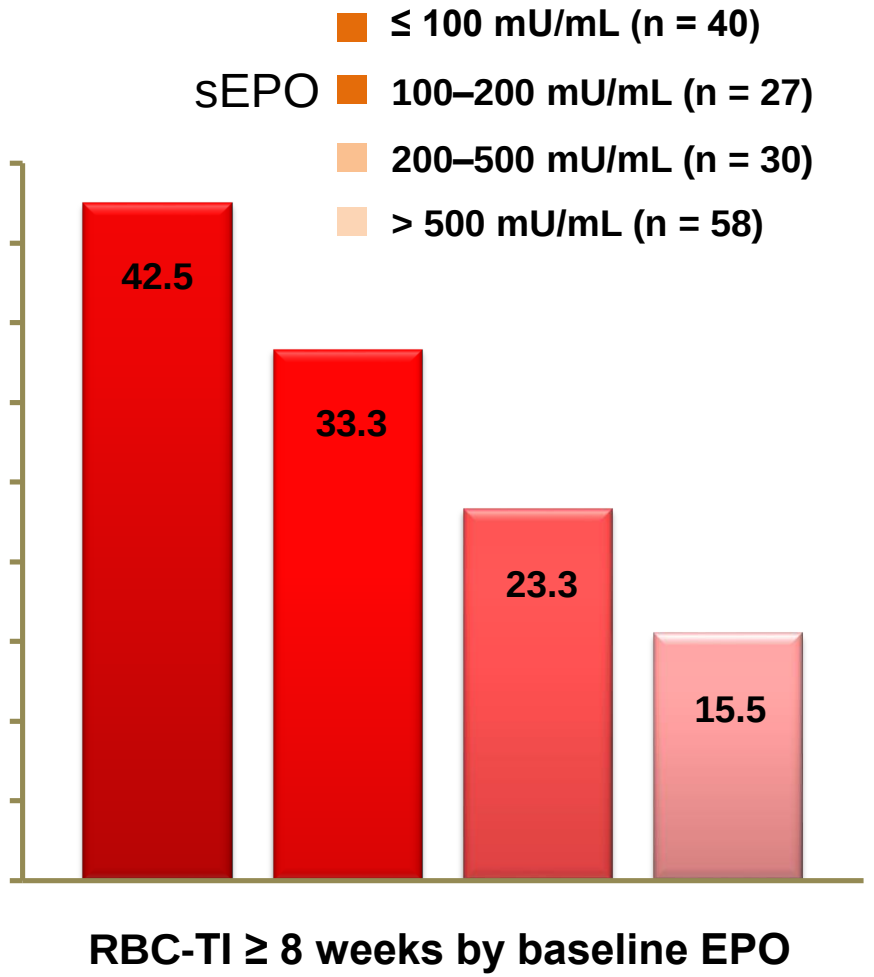
When assessed during the entire treatment period, a greater proportion of luspatercept-treated patients achieved RBC-TI ≥ 8 weeks compared with placebo than previously reported (37.9% of patients receiving luspatercept achieved RBC-TI ≥ 8 weeks during Weeks 1–24 of treatment vs 13.2% of placebo-treated patients; $P < 0.0001$)[1]

Luspatercept has been approved by FDA and EMA in 2020 for TD MDS-RS

Fenaux et al, N Engl J Med. 2020 Jan 9;382(2):140-151.

Lenalidomide in non-del5q MDS induces RBC-TI





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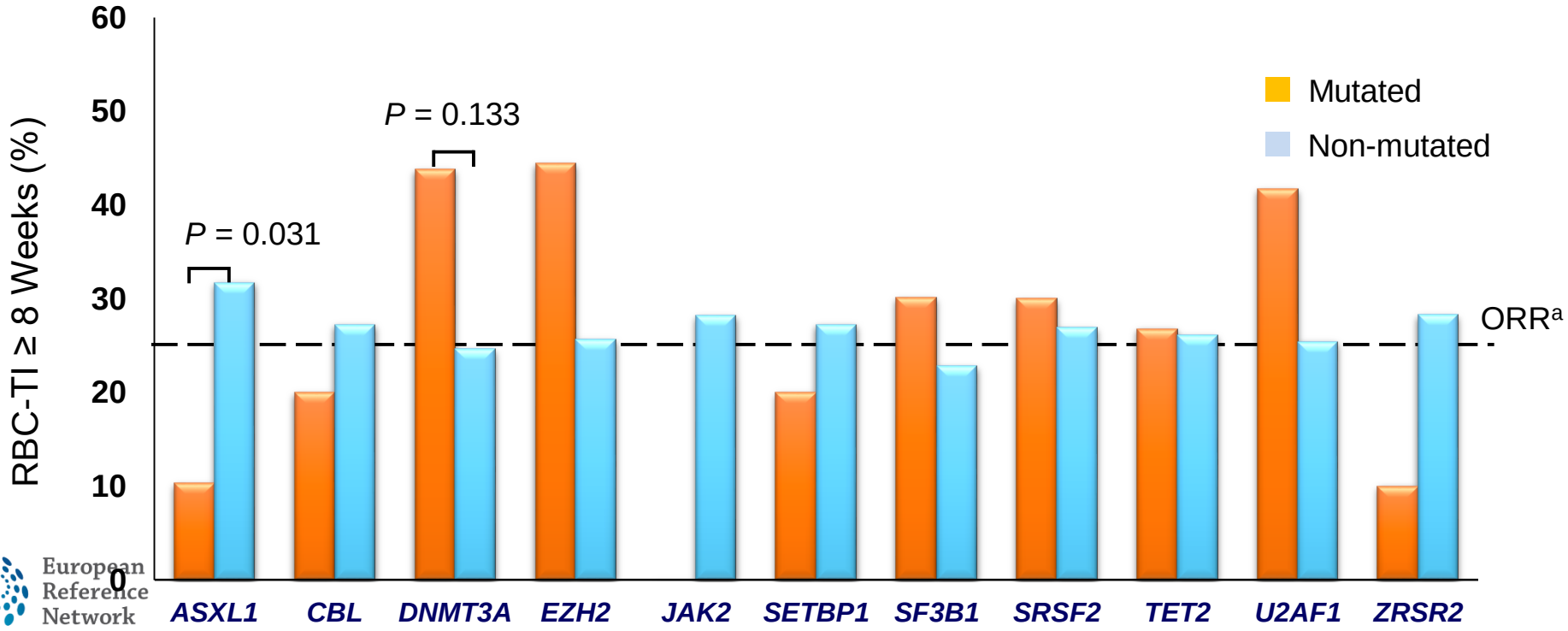
Santini V J Clin Oncol. 2016 Sep 1;34(25):2988-96.

ASXL1 mutation is associated with lower RBC-TI ≥ 8 Weeks Response in LEN-treated non-del 5q Patients
DNMT3A and EZH2 trend to better response

[a]Dotted line represents the RBC-TI ≥ 8 weeks response rate in the overall population (26.9%).

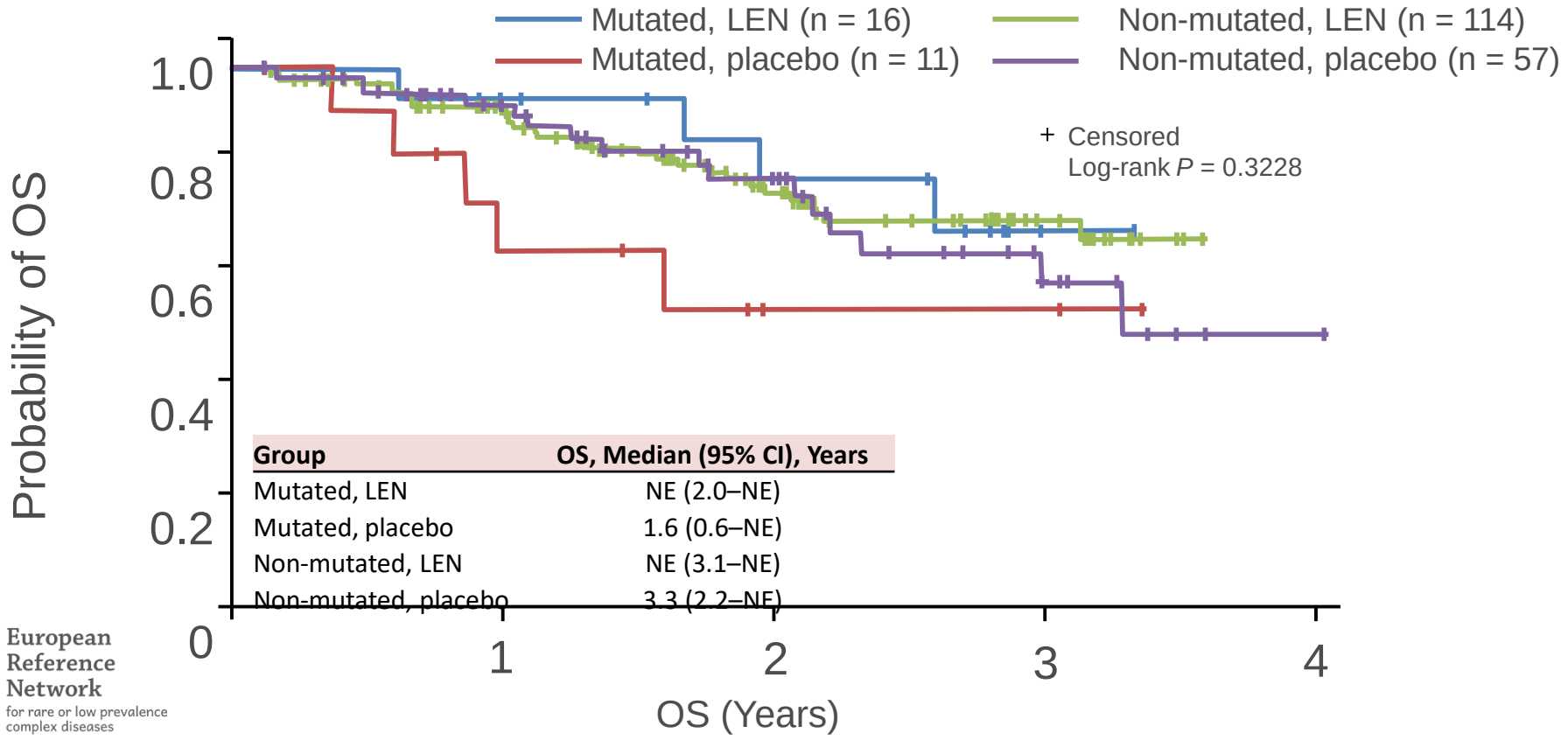


Santini V et al, manuscript in preparation

OS According to DNMT3A Mutation

Len vs PBO

- *DNMT3A* mutations were not significantly associated with different OS (*P* = 0.3228) in patients treated with placebo
- *DNMT3A* mutant patients had a trend for improved OS with LEN treatment compared with placebo (*P* = 0.123)



Group	OS, Median (95% CI), Years
Mutated, LEN	NE (2.0–NE)
Mutated, placebo	1.6 (0.6–NE)
Non-mutated, LEN	NE (3.1–NE)
Non-mutated, placebo	3.3 (2.2–NE)

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Santini V et al Leukemia. 2020 Jul 13. doi: 10.1038/s41375-020-0961-3

Isocitrate Dehydrogenase (IDH) Mutations in MDS

- Somatic *IDH1* and *IDH2* mutations result in accumulation of oncometabolite **2-HG**

  → epigenetic changes, impaired cellular differentiation

- m*IDH* identified in multiple solid and hematologic tumors, rare in MDS, more frequent in AML

	mIDH1	mIDH2
% of MDS patients	**~5%**	**~5–10%**

- **Enasidenib (AG-221):** inhibitor of m*IDH2*
- **Ivosidenib (AG-120):** inhibitor of m*IDH1*

Response to mIDH2 and mIDH1 inhibitors in R/R AML (……and few MDS)

	AG-221 (Enasedinib)	AG-120 (Ivosedinib)
Clinical trial	NCT01915498. (Stein et al-Blood. 2017;130(6):722-731)	NCT02074839.(DiNardo et al NEJM June 2, 2018 DOI: 10.1056/NEJMoa1716984), Pollyea ASCO 2018
Pts dosed	258	239
Overall Response Rate	40.3%	39.1%

ONGOING:
HMA-naïve high risk MDS enasidenib in combination with azacitidine (NCT03383575).

Median time to response	1.9 months	1.9 months
Overall survival	9.3 months	9.0 months
Duration of response if CR	8.8 months	10.1 months
Toxicity	-Indirect hyperbilirubinemia (inhibiting UGT1A1) -nausea -leukocytosis	-QT prolongation -diarrhea -nausea -leukocytosis

17 MDS pts 50% ORR
21% CR

A single-arm phase II multicenter study of IDH1 (AG 120) inhibitor in patients with IDH1 mutated myelodysplastic syndrome

EMSCO
MYELODYSPLASTIC SYNDROMES

Cohort A: Higher risk MDS (IPSS int-2, high) without response (CR, PR, marrow CR, stable disease with HI) after at least 6 cycles of azacitidine or relapse after a response.
but without overt progression (defined by at least doubling of marrow blasts, compared to pre azacitidine bone marrow, or by AML progression beyond 30% blasts)

- **Cohort B: Untreated higher risk MDS (IPSS int-2, high) without life threatening cytopenias (ie ANC < 500/mm3 or any recent severe infection and/ or platelets below 30,000/mm3 and any bleeding symptom). Azacitidine will be added after 3 cycles of AG 120 in the absence of response. Azacitidine will be given at the standard dose of 75mg/m2 over 7 days (7 consecutive days, 4-10; or 2+5 (i.e., days 4-5 and 8-12) as a subcutaneous injection or intravenous**
- **Cohort C: Lower risk MDS with anemia resistant to erythropoietic stimulating agents (primary or secondary resistance)**

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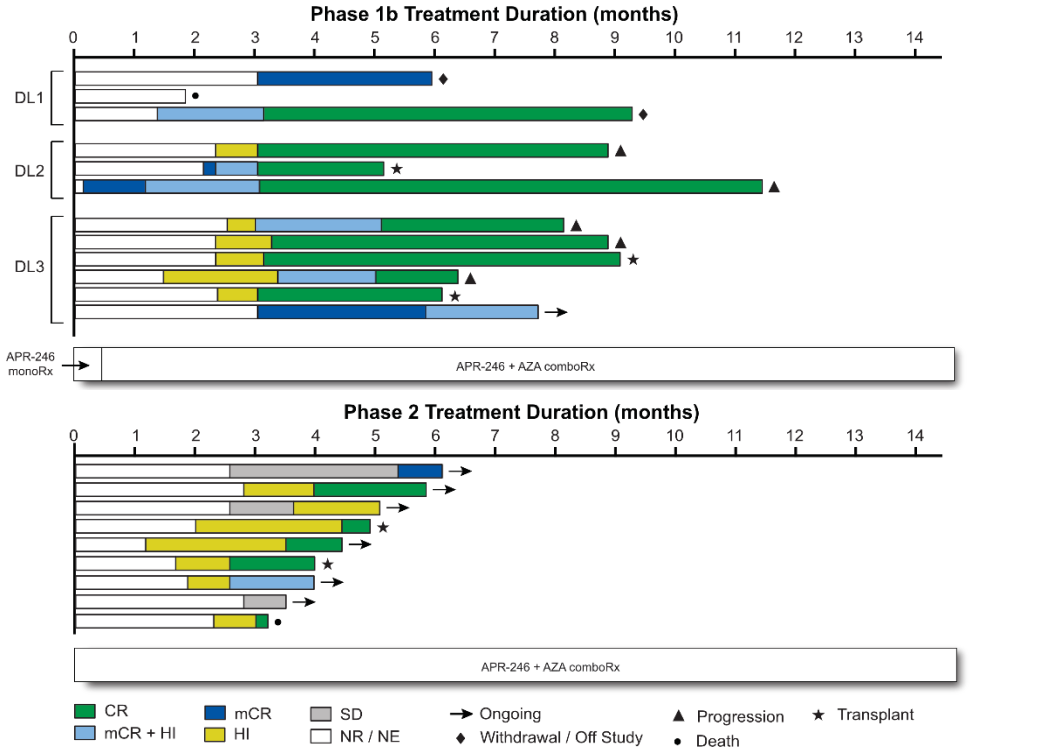
Phase 1b/2 Combination Study of APR-246 and Azacitidine (AZA) in Patients with *TP53* Mutant Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML)

Treatment Duration and Response



Best Response at Cutoff						
	Ph1b	Ph2	MDS	AML	All	AZA Historical
Evaluable Patients	11	9	15	5	20	
Response Rate	100%	89%	93%	100%	95%	30-50%
CR Rate	82%	56%	67%	80%	70%	20-30%

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Sallman D t al, ASH 2018

Thursdays Webinars

bjh guideline

Spanish Guidelines for the use of targeted deep sequencing in myelodysplastic syndromes and chronic myelomonocytic leukaemia

Laura Palomo,[1] Mariam Ibáñez,[2,3,4] María Abáigar,[5] Iria Vázquez,[6,7] Sara Álvarez,[8] Marta Cabezón,[9] Bárbara Tazón-Vega,[10,11] Inmaculada Rapado,[12,13,14] Francisco Fuster-Tormo,[1] José Cervera,[2,3,15] Rocío Benito,[5] María J. Larrayoz,[6,7] Juan C. Cigudosa,[8] Lurdes Zamora,[9] David Valcárcel,[10,11] María T. Cedena,[12,13,14] Pamela Acha,[1] Jesús M. Hernández-Sánchez,[5,16] Marta Fernández-Mercado,[6,17,18] Guillermo Sanz,[2,3] Jesús M. Hernández-Rivas,[5,16,19] María J. Calasanz,[6,7] Francesc Solé,[1] Esperanza Such,[2,3,4] On behalf of the Spanish Group of MDS (GESMD)

UNIVERSITÀ DEGLI STUDI FIRENZE

DIPARTIMENTO DI MEDICINA SPERIMENTALE E CLINICA

Azienda Ospedaliero Universitaria Careggi

FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

MDS UNIT

EMSCO MYELODYSPLASTIC SYNDROMES

AIRC

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Take home messages

1. Molecular analysis in MDS can refine diagnosis (in MDS-RS with < 15% RS)
2. Molecular analysis with NGS at diagnosis can improve the prognostic stratification (number of mutations and some specific mutations – biallelic TP53, ASXL1)
3. The presence of a actionable somatic mutations may help to select a therapy, expecially in second line treatment