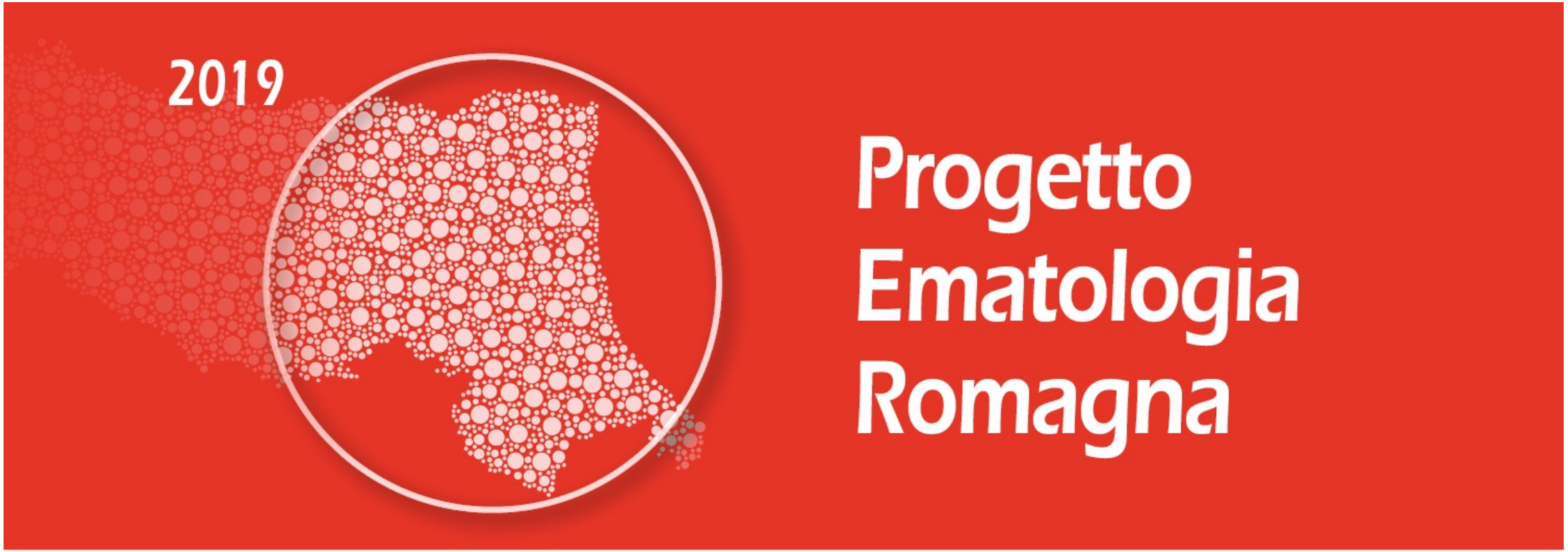


Da MDS a leucemia acuta

Emanuela Ottaviani

MDS

- Heterogeneous clonal bone marrow diseases
- Ineffective hematopoiesis
- Peripheral cytopenias
- Morphologic dysplasia
- Increased risk of progression to AML.

Diagnostic approach to MDS

Peripheral blood smear/ bone marrow aspirate:

- dysplasia in one or more cell lines
- blasts
- ring sideroblasts

Bone marrow biopsy:

- cellularity; CD34+; fibrosis

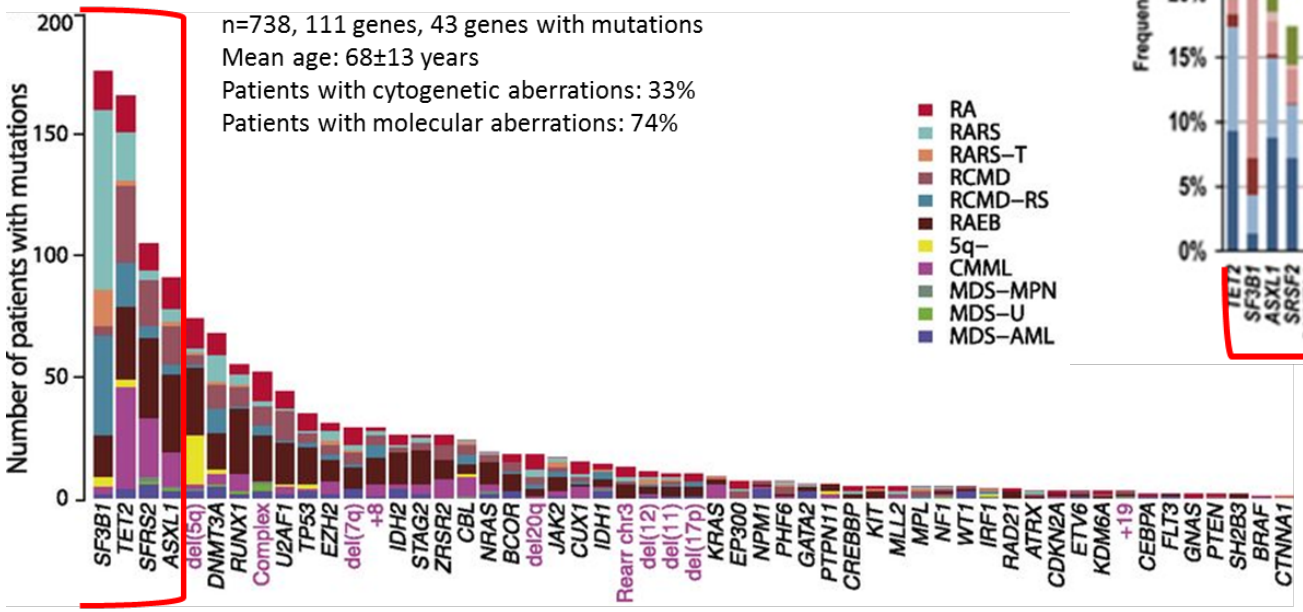
Cytogenetic analysis: +8, -Y, del(20q)

Mutation analysis

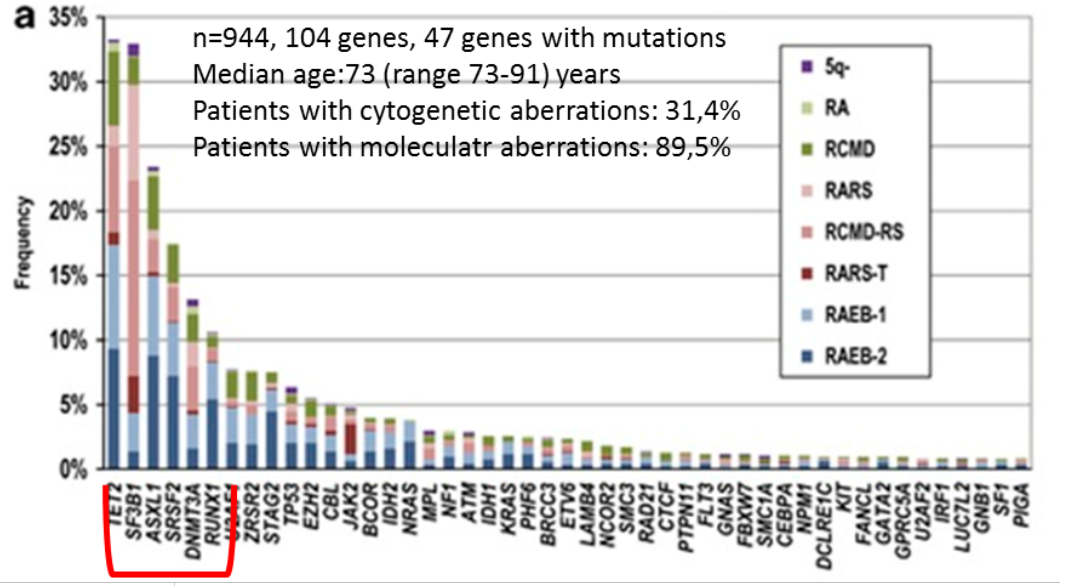
Abnormality	Frequency (%)*
−5 or del(5q)	10-15
−7 or del(7q)	10
i(17q) or t(17p)	2-3
del(12p) or t(12p)	1-2
del(11q)	1-2
−13 or del(13q)	1-2
del(9q)	1
idic(X)(q13)	1
inv(3)(q21q26.2)	1
t(6;9)(p23;q34)	1
t(3;21)(q26.2;q22.1)	<1
t(1;3)(p36.3;q21.2)	<1
t(11;16)(q23;p13.3)	<1
t(2;11)(p21;q23)	<1

*Frequencies reported in the table were extrapolated from Sole et al,[55] Haase et al,[56] and Schanz et al.[58]

Somatic mutations in MDS

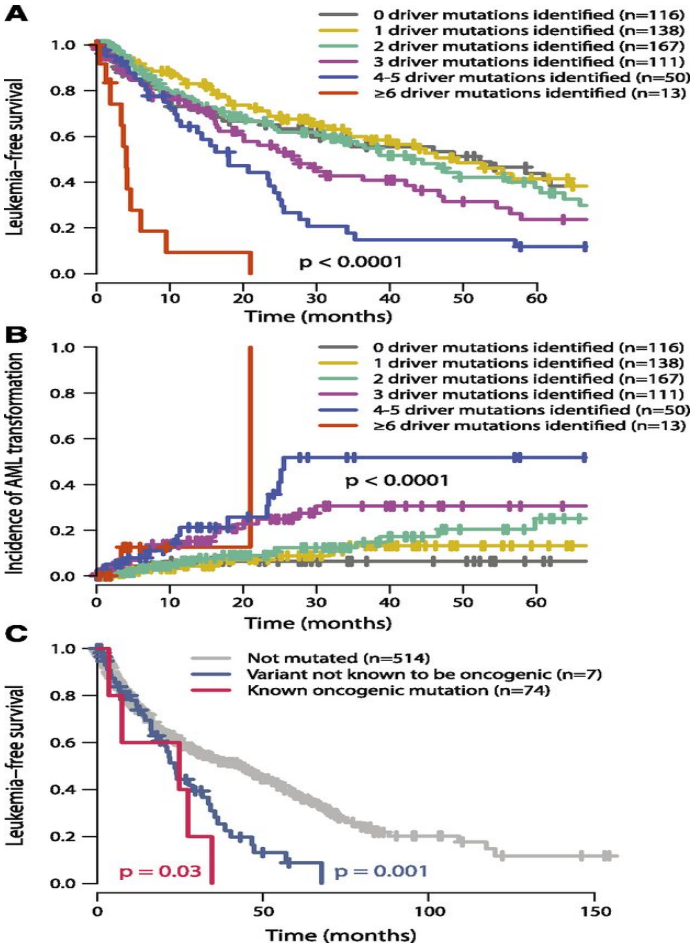


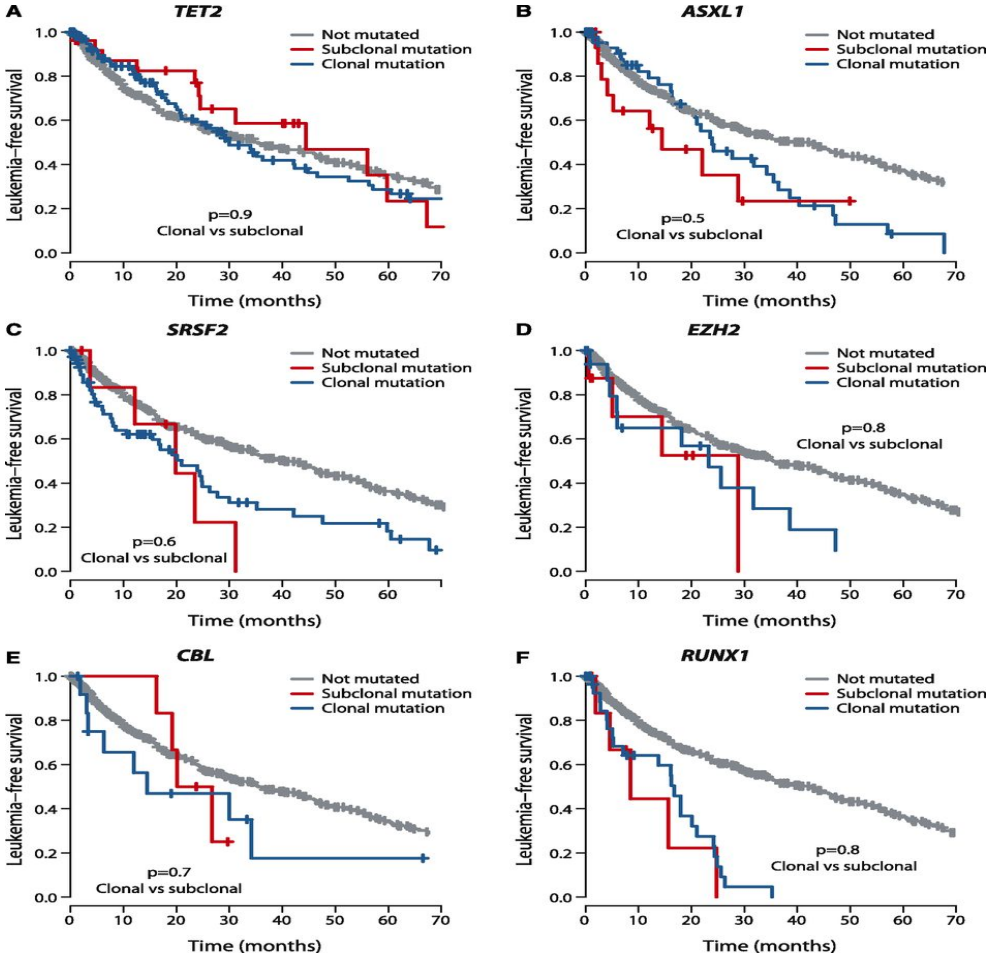
Papaemmanuil E. et al, Blood 2013



Haferlach, Leukemia 2014

1Numerous recurrently mutated genes;
2.Mostly infrequently mutated genes <5%;
3.Most patients have more than one mutation;
4.Clonal heterogeneity;
5.Systematic –pairwise interactions;
6.Increasing complexity in variables to be considered in clinical practice.


A
0 driver mutations identified (n=116)
1 driver mutations identified (n=138)
2 driver mutations identified (n=167)
3 driver mutations identified (n=111)
4-5 driver mutations identified (n=50)
≥6 driver mutations identified (n=13)
Leukemia-free survival
p < 0.0001
Time (months)
B
Incidence of AML transformation
p < 0.0001
Time (months)
C
Not mutated (n=514)
Variant not known to be oncogenic (n=7)
Known oncogenic mutation (n=74)
Leukemia-free survival
p = 0.03
p = 0.001
Time (months)


A
TET2
Not mutated
Subclonal mutation
Clonal mutation
Leukemia-free survival
p=0.9
Clonal vs subclonal
Time (months)
B
ASXL1
p=0.5
C
SRSF2
p=0.6
D
EZH2
p=0.8
E
CBL
p=0.7
F
RUNX1
p=0.8

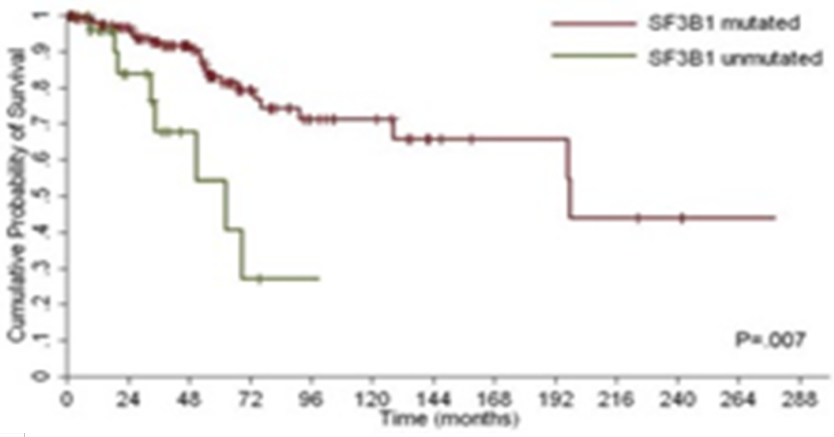
2016 WHO CLASSIFICATION

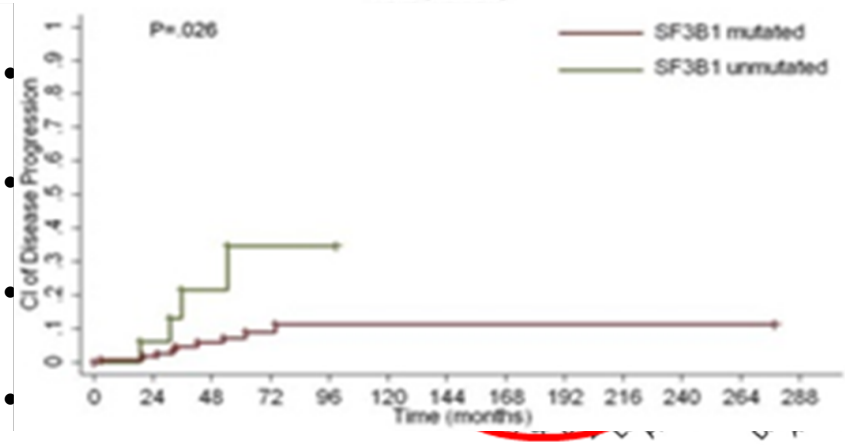
Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory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

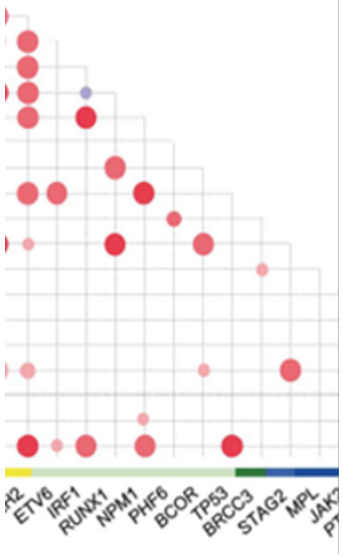
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*$^-$
Juvenile myelomonocytic leukemia (JMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
MDS/MPN, unclassifiable

MDS and SF3B1

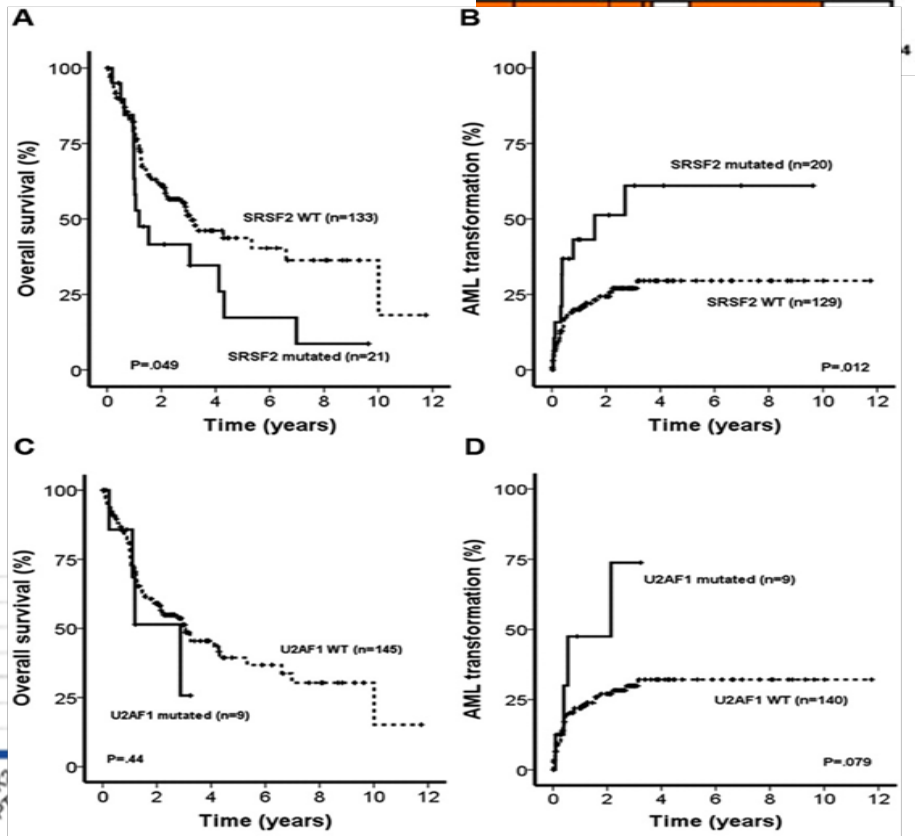
- Distint molecular and clinical subgroup





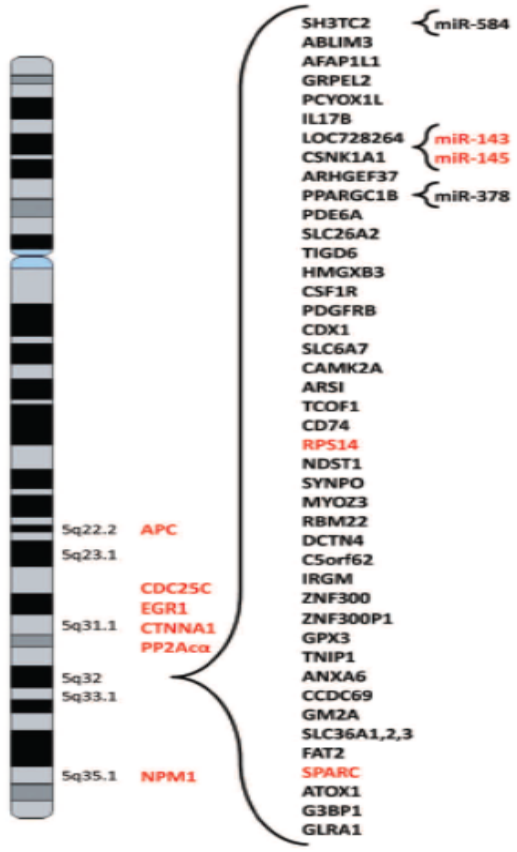


SRSF2: significantly shorter OS and shorter time to AML progression

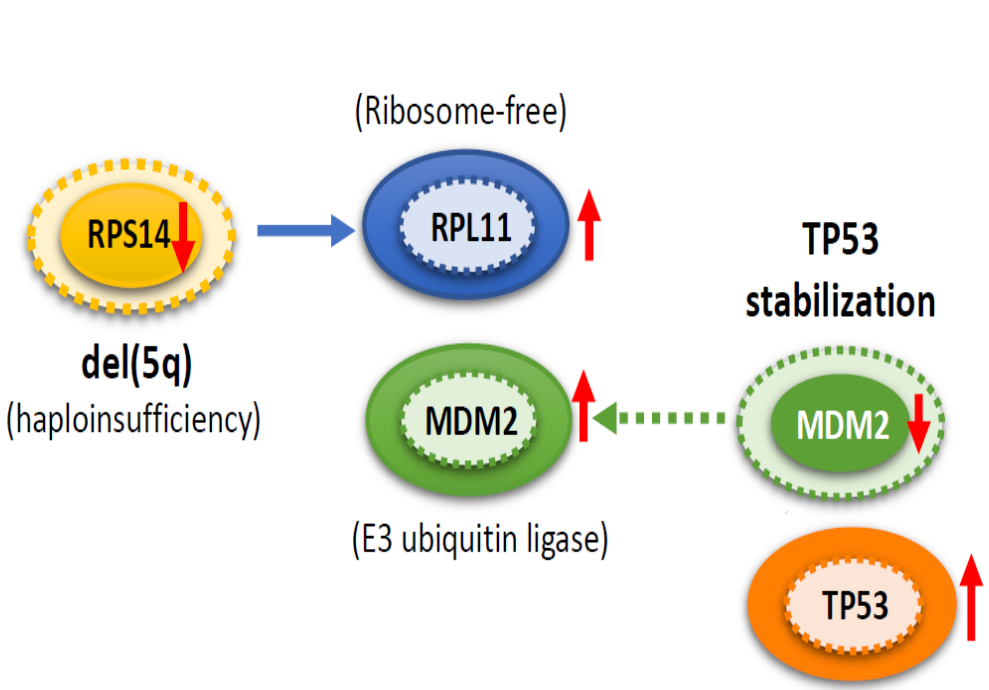


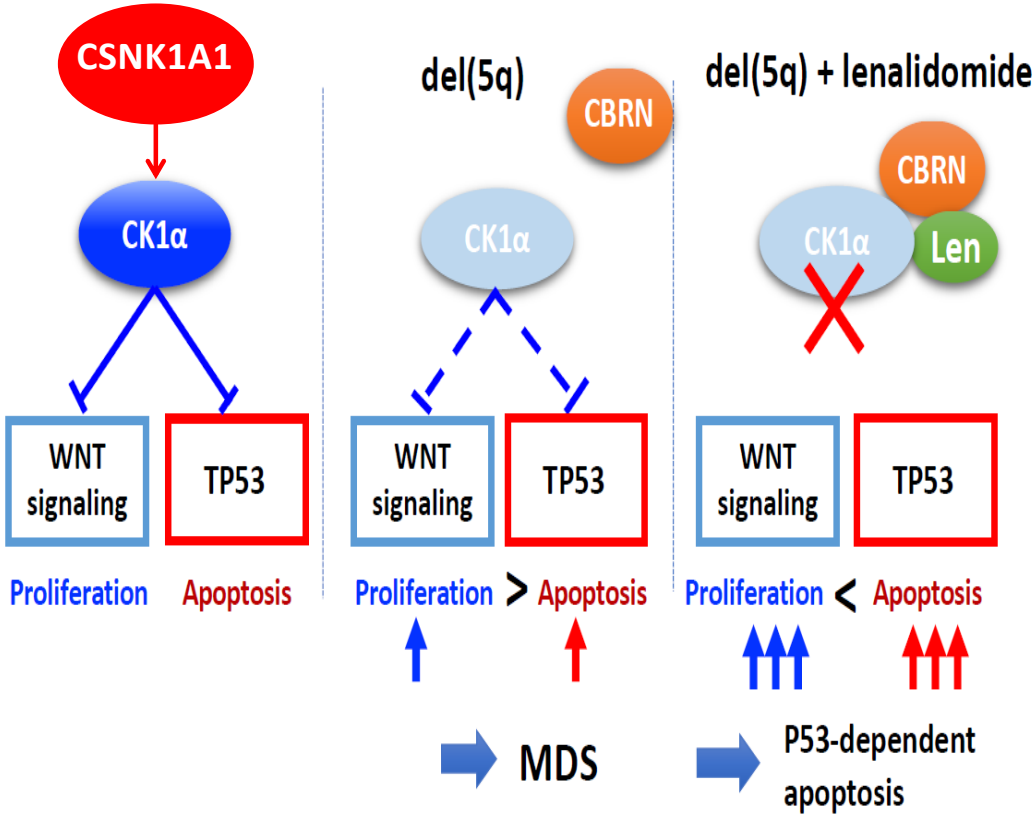
MDS with del(5q)

- del(5q): most common aberration (10-20% of MDS)
- Often single abnormality; sometime in a complex karyotype
- Middle age to older
- Female
- Blasts: <1% in PB and <5% in BM
- Clinical presentation:
  - refractory macrocytic anemia, often severe
  - thrombocitosis (may be present)
  - neutropenia
  - hypolobulated megakariocytes
- Associated with no or one mutation (SF3B1, DNMT3A, TP53, TET2, CSNK1A1, ASXL1, JAK2)
- Good prognosis; lower risk for progression to LAM
- Good response to treatment

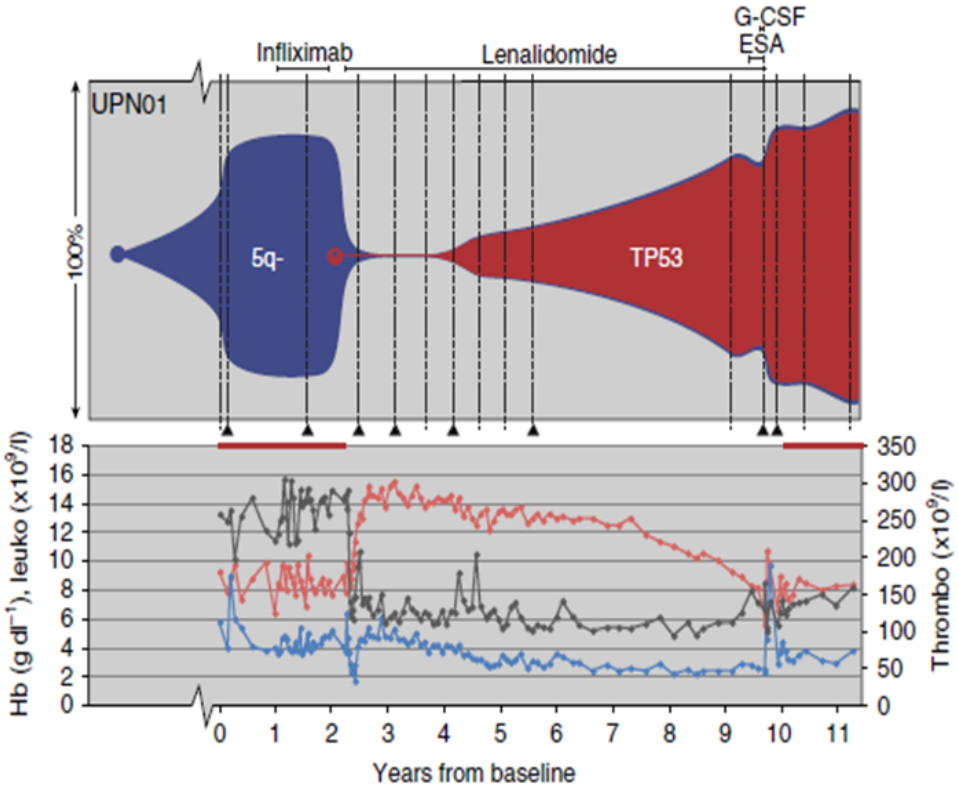


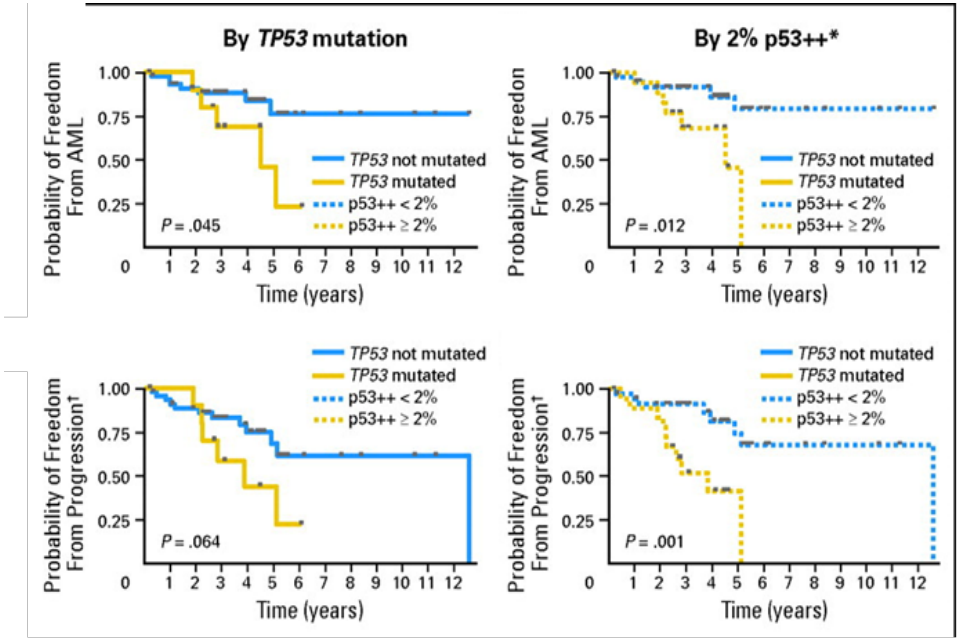
RPS14 and CSNK1A1 haploinsufficience





TP53 and MDS with del(5q)



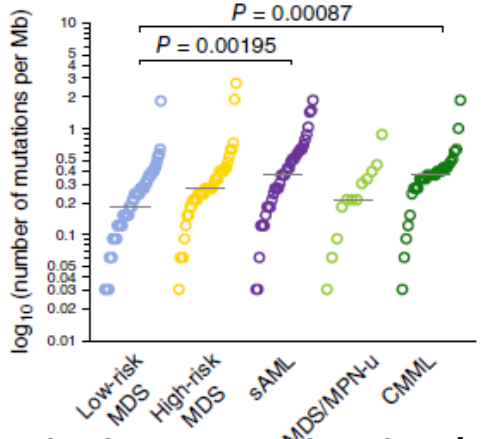


TP53 mutations predict outcome in del (5q) myelodysplastic syndrome and are related to p53 status. (*) p53++, proportion of marrow cells with strong p53 staining by immunohistochemistry. (†) Progression is defined as marrow blast increase from 10% to 19% or acquisition of complex karyotype.

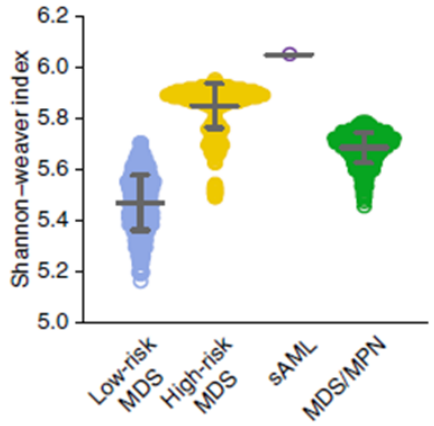
Martin Jädersten; Journal of Clinical Oncology 2011

Clonal evolution in MDS

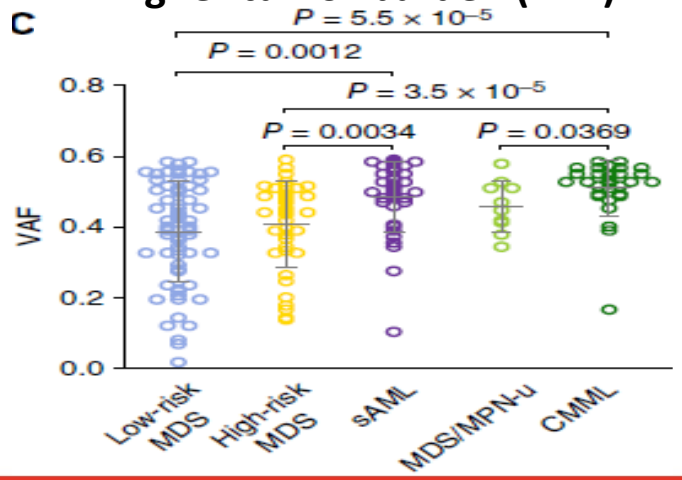
sAML: significantly higher mutation rate



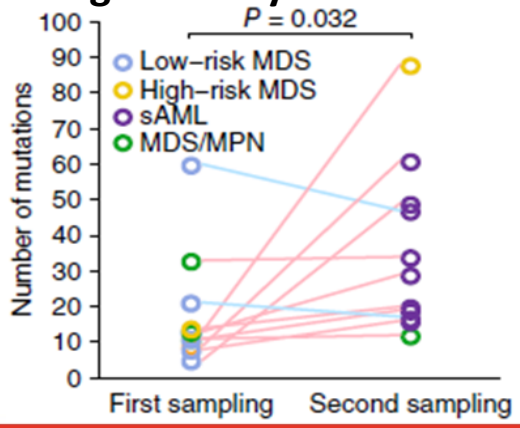
sAML: more frequent intratumor heterogeneity



sAML: higher tumor burden (VAF)

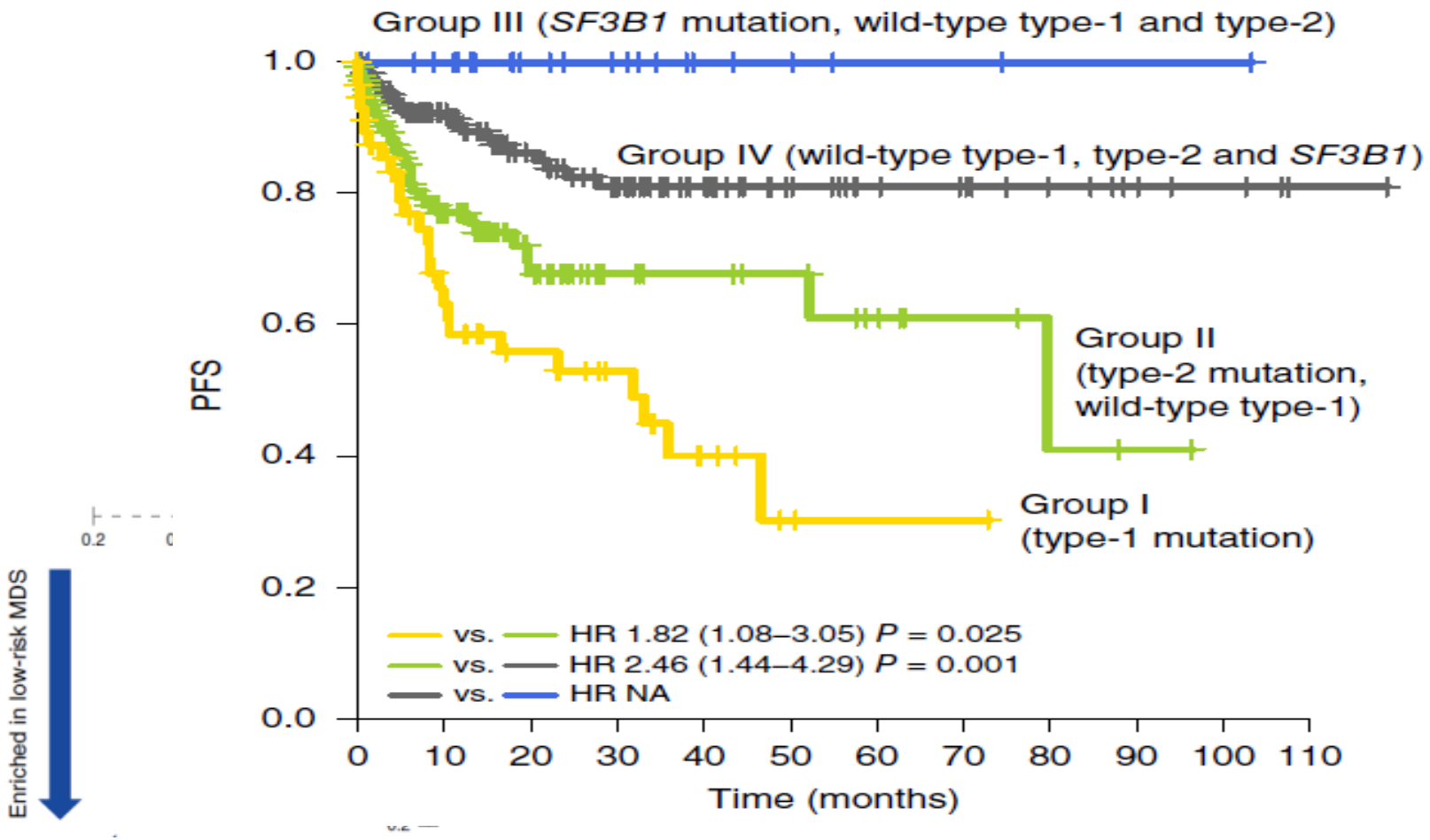


sAML: significantly increase of mutations number



Makishima H., Nature Genetics 2017

MDS: progression to sAML



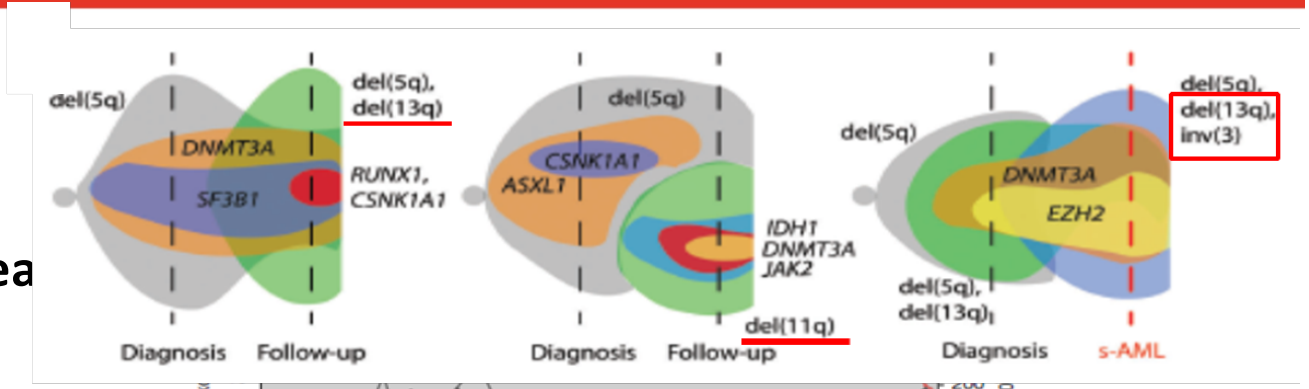
ıt disease subtype:
vs high risk MDS
2, NRAS, KRAS, KIT,

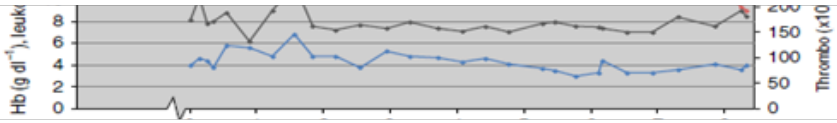
isk vs low risk MDS
ASXL1, ZRSR2, TET2)

MDS follow-up

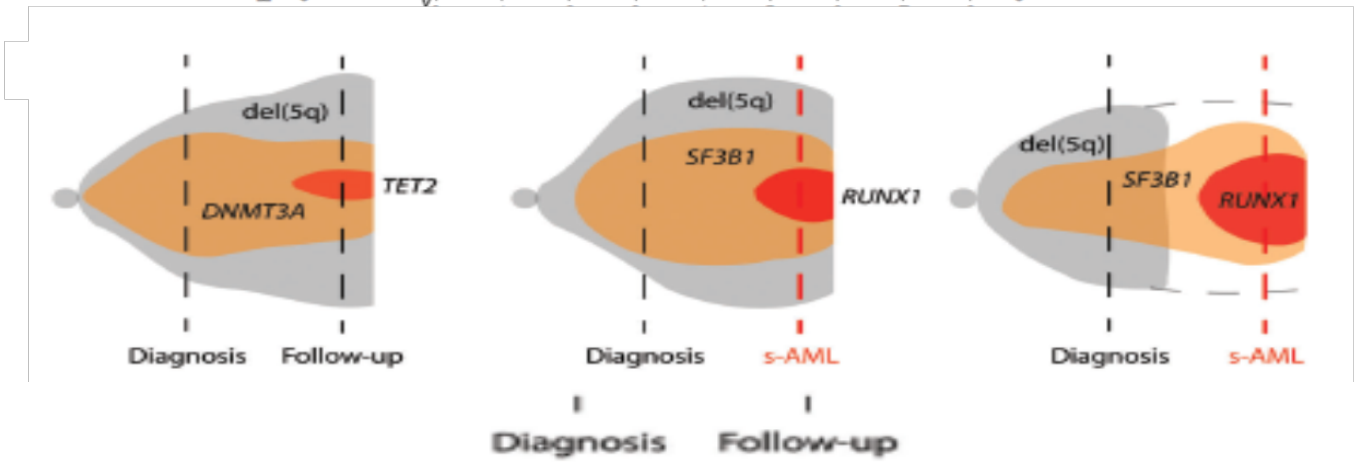
During follow-up:

- Gain of cytogenetic alterations
- Stable mutations → stable disea



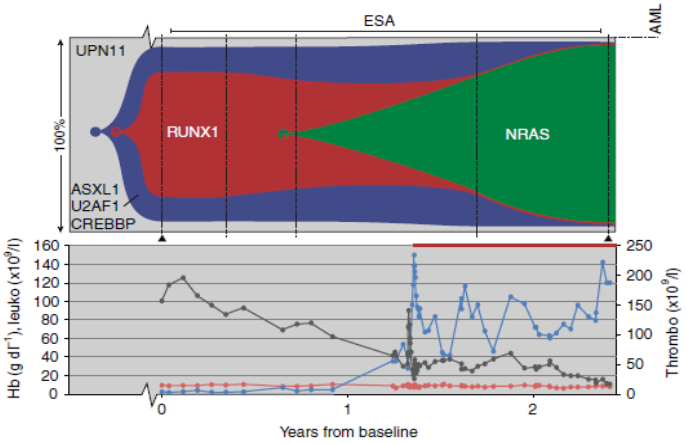


- Gain of gene mutations
- Loss of gene mutations

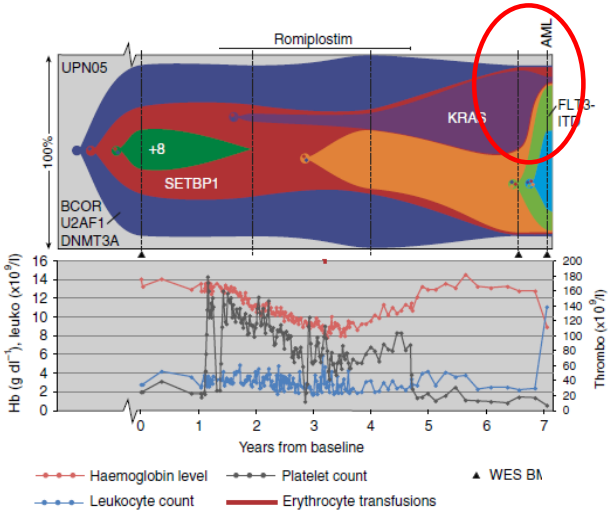


Meggendorfer M., Haematologica 2017
Jansen J. H., nature communications 2017

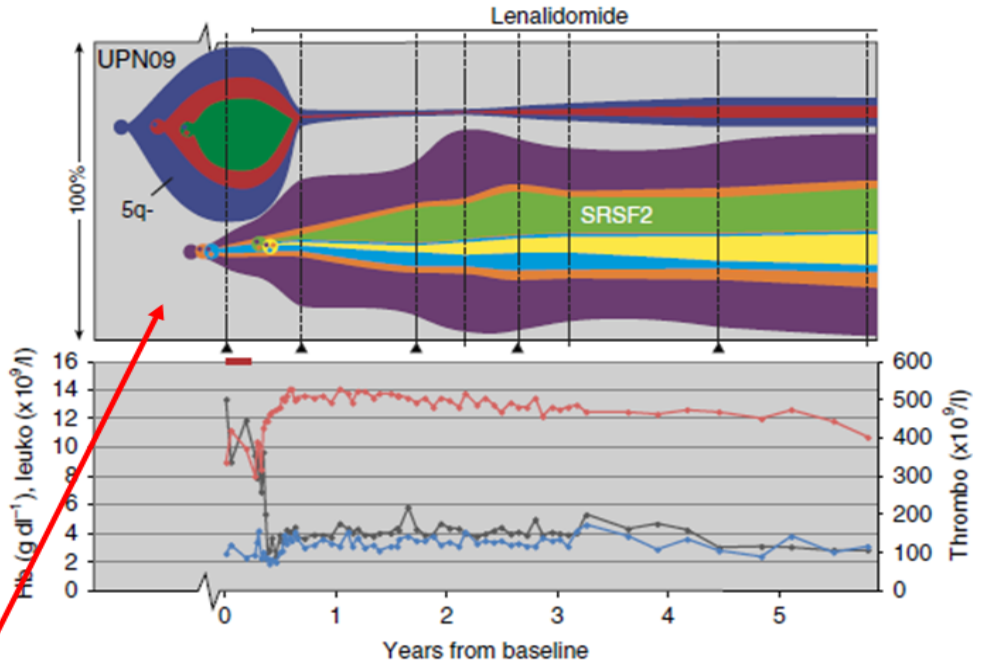
Linear evolution



Branching clonal evolution



Clonal evolution of treated patients



Conclusions

- MDS: a very heterogeneous group of haematopoietic neoplasms
- Important role for mutational data in clinical management
- Various clonal evolution patterns can be observed
- Important molecular monitoring during disease

Which is the role of molecular diagnostic lab????

NGS IN DIAGNOSTIC WORK FLOW

Sophia Genetics, Myeloid Solution Panel

ThermoFisher, AmpliSeq Myeloid Panel

Myeloid Solution

ABL1	ASXL1	BRAF	CALR	CBL	CEBPa
CSF3R	DNMT3A	ETV6	EZH2	FLT3	HRAS
IDH1	IDH2	JAK2	KIT	KRAS	MPL
NPM1	NRAS	PTPN11	RUNX1	SETBP1	SF3B1
SRSF2	TET2	TP53	U2AF1	WT1	ZRSR2

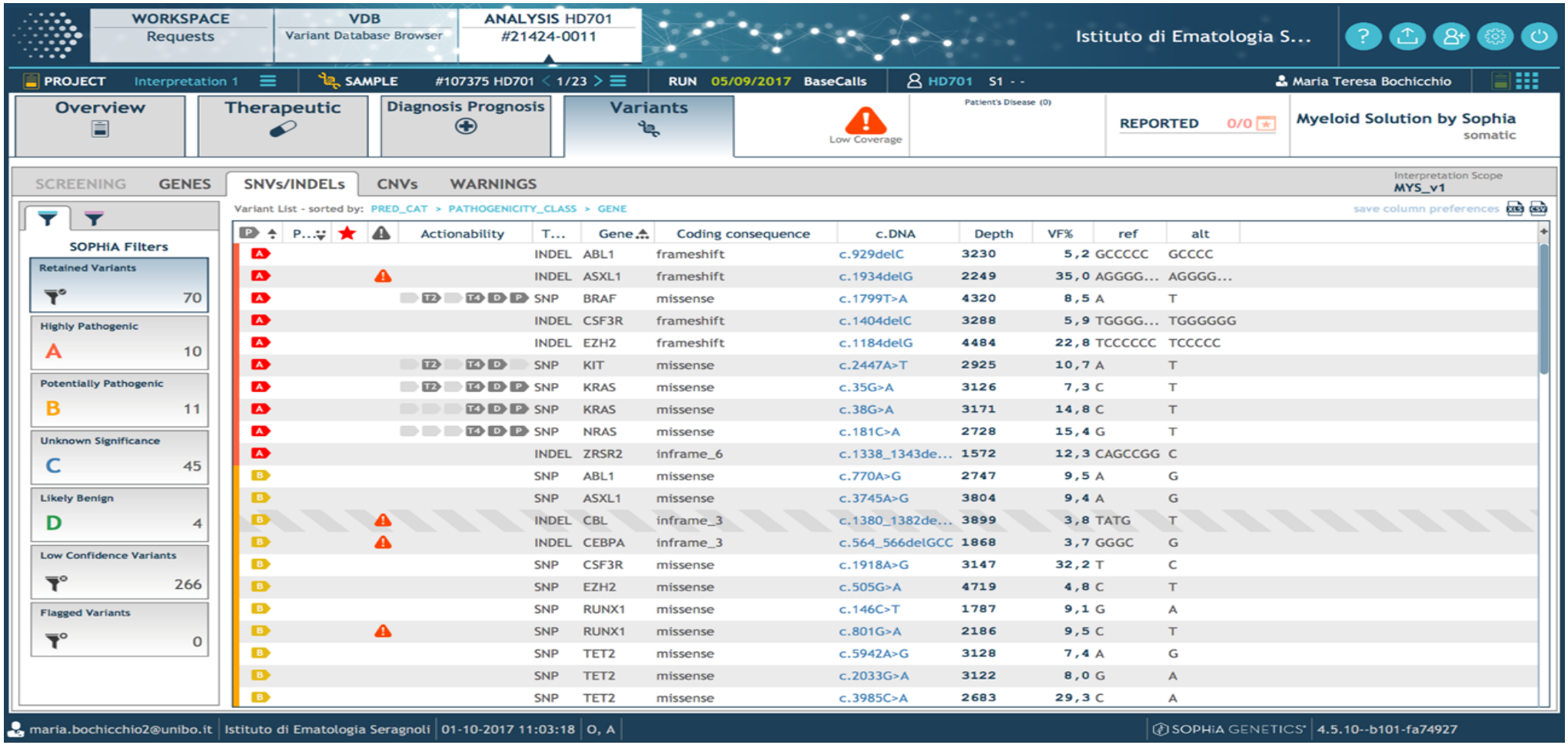
30 geni: 10 full,
20 regioni “hotspots”
coverage minimo 1000X (98%)

AmpliSeq Myeloid Panel

Hotspot (23)	Full-gene (17)	Fusion	Expression
ABL1	ASXL1	ABL1	BAALC
BRAF	BCOR	ALK	MECOM
CBL	CALR	BCL2	MYC
CSF3R	CEBPA	BRAF	SMC1A
DNMT3A	ETV6	CCND1	WT1
FLT3	EZH2	CREBBP	
GATA2	IKZF1	EGFR	*Controls:*
HRAS	NF1	ETV6	HMBS
IDH1	PHF6	FGFR1	ITGB7
IDH2	PRPF8	FGFR2	LRP1
JAK2	RB1	FUS	MYC
KIT	RUNX1	HMGA2	TBP
KRAS	SH2B3	JAK2	
MPL	STAG2	KMT2A (MLL)	
MYH88	TET2	MECOM	
NPM1	TP53	MET	
NRAS	ZRSR2	MLLT10	
PTPN11		MLLT3	
SETBP1		MYH11	
SF3B1			
SRSF2			
U2AF1			
WT1			

- 2 pools DNA, 1 pool RNA
- Coverage of mutations & fusions
- Compatible sample types:
  - Blood
  - Bone marrow samples
- Compatible systems:
  - Ion Chef™ with Ion S5™ Sequencing System
  - Ion Chef™ with Ion S5™ XL Sequencing System
  - Ion OneTouch™ 2 with Ion PGM™
  - Ion Chef™ with Ion PGM™
- Compatible software:
  - Torrent Suite™ software v5.2.1
  - Ion Reporter™ Server system 5.2 or later -Required for fusion calling
- Target 5% MAF for SNV

Myeloid Solution by SG: results

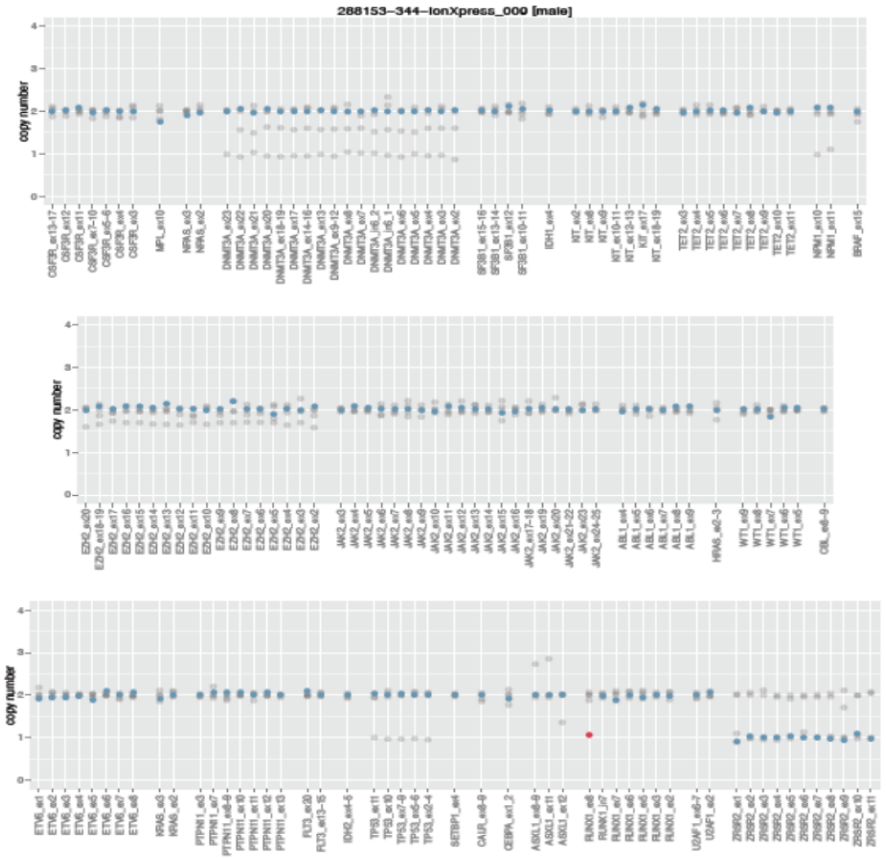


2019

Case report

1. CNV DETECTION

1.5.1 Plot for sample 288153-344-IonXpress_009



-PRESENZA DI ALTERAZIONI IN:

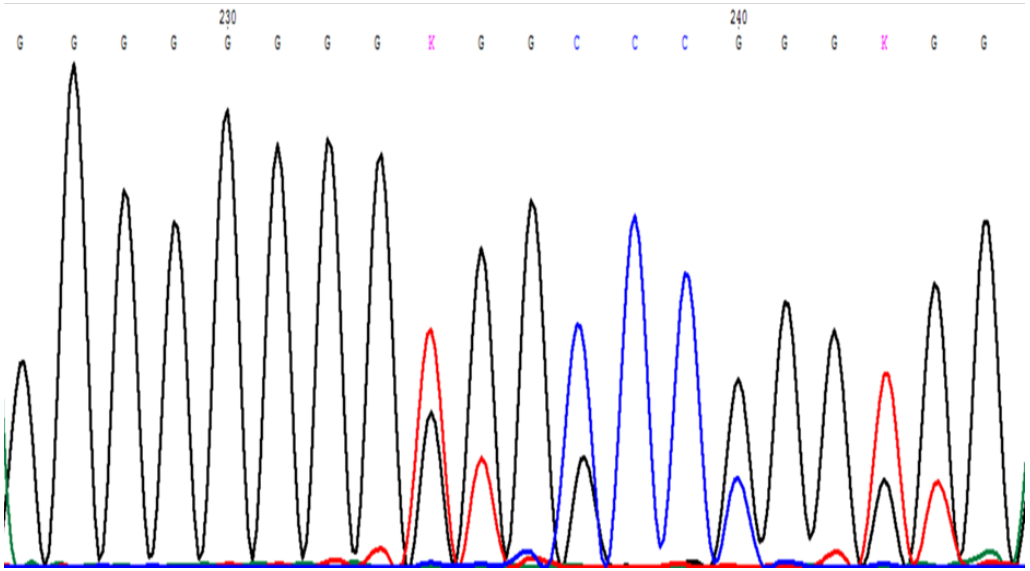
FLT3 mutazione puntiforme in esone 20: p.Asn841His (c.2521A>C) al 26%;

ASXL1 frameshift in esone 13: p.Gly646Trpfs*12 (c.1934dupG) al 19%.

Entrambe le alterazioni sono state evidenziate anche mediante sequenziamento Sanger.

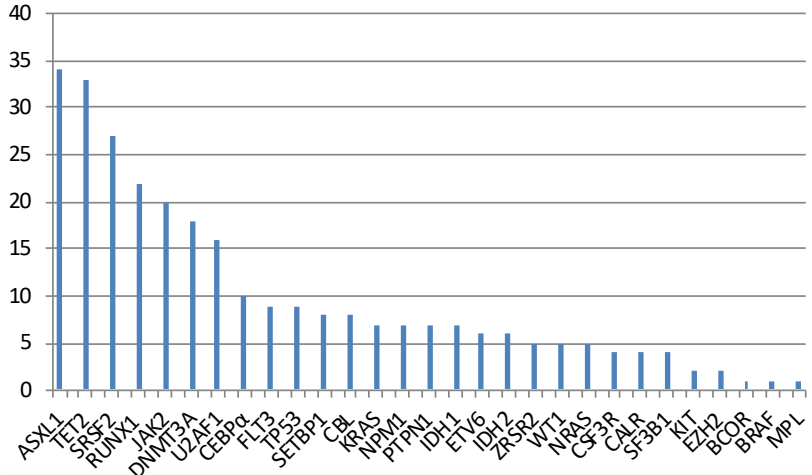
Presente una CNV di delezione a livello dell'esone 8 di RUNX1 (cromosoma 21). Allego plot delle CNV.

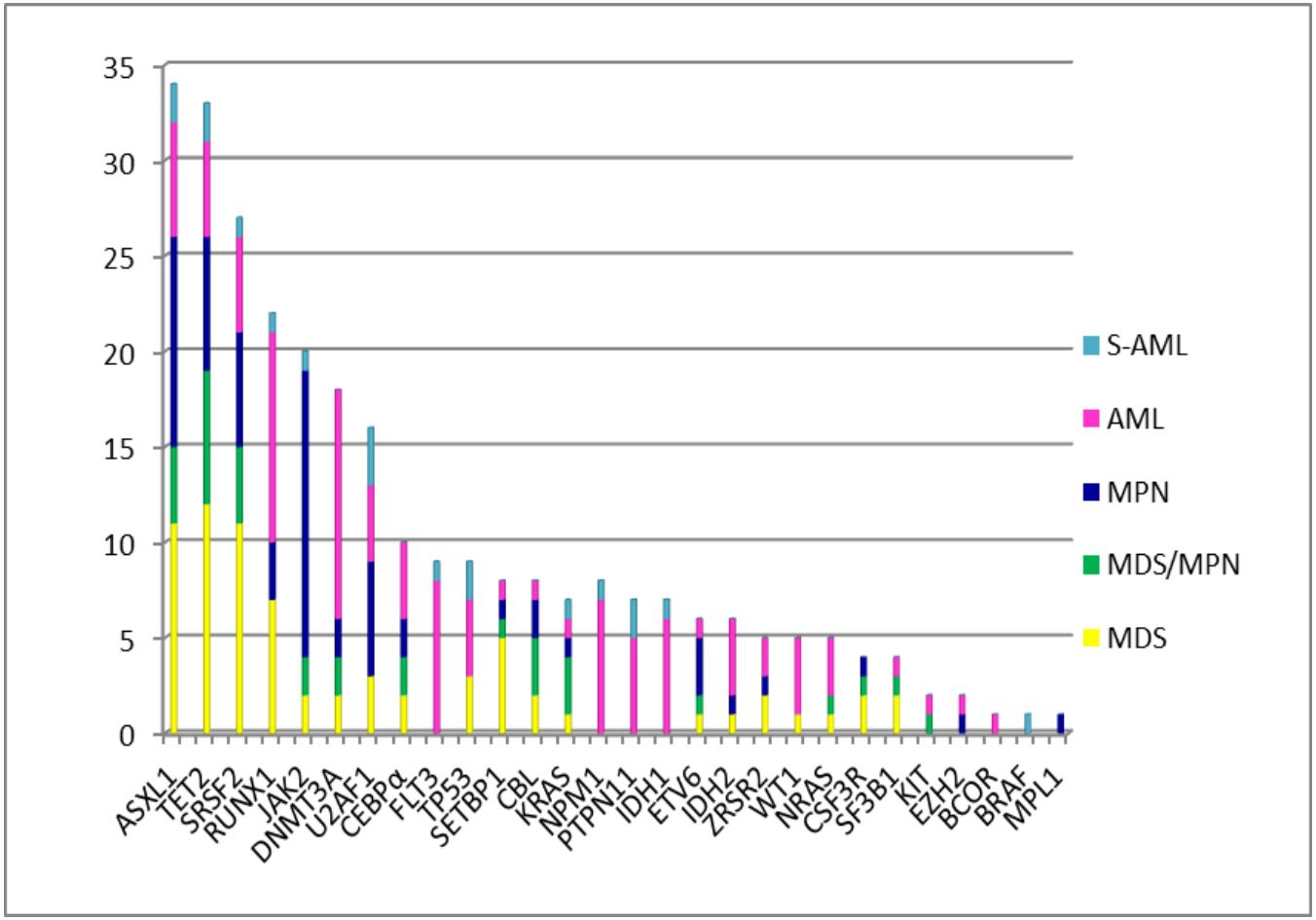
Inoltre sono presenti alcune alterazioni riportate come polimorfismi e/o con significato clinico incerto in: ASXL1 (p.Leu815Pro al 100%), TET2 (p.Leu1721Trp al 48%) e TP53 (Pro72Arg al 100%).

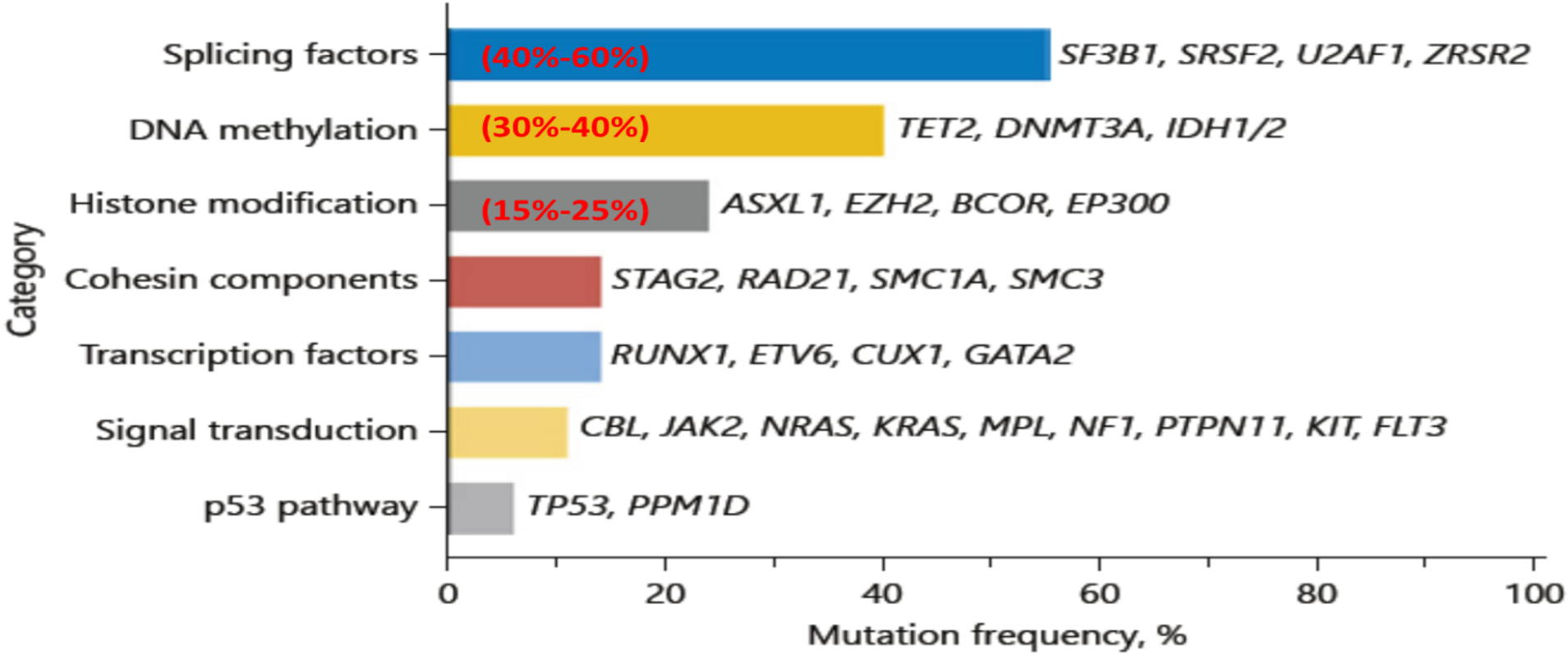


Result patients: our casistic

Disease		N° alterations (median)
MDS	32	2 (0-7)
MDS/MPN	8	3,5 (2-6)
MPN	20	3,5 (1-6)
AML	36	3 (0-7)
sAML	5	3 (3-5)
	101	






Splicing factors
(40%-60%)
SF3B1, SRSF2, U2AF1, ZRSR2
DNA methylation
(30%-40%)
TET2, DNMT3A, IDH1/2
Histone modification
(15%-25%)
ASXL1, EZH2, BCOR, EP300
Cohesin components
STAG2, RAD21, SMC1A, SMC3
Transcription factors
RUNX1, ETV6, CUX1, GATA2
Signal transduction
CBL, JAK2, NRAS, KRAS, MPL, NF1, PTPN11, KIT, FLT3
p53 pathway
TP53, PPM1D
Category
0
20
40
60
80
100
Mutation frequency, %

Conclusions

Molecular characterization by NGS at diagnosis and during follow-up

Several gene panels compatible with different instruments

Essential bioinformatics analysis

Possibility to identify even small altered clones

……**BUT**

- Sharing panels and analytic pipelines
- Dedicated technicians/biologists and facilities
- Recognized diagnostic activity in NGS

Thanks!!!!