FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

Convegno della Fondazione Italiana Sindromi Mielodisplastiche

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Interpretazione di dati molecolari NGS nel contesto clinico: come cambia la diagnosi e la stratificazione dei pazienti

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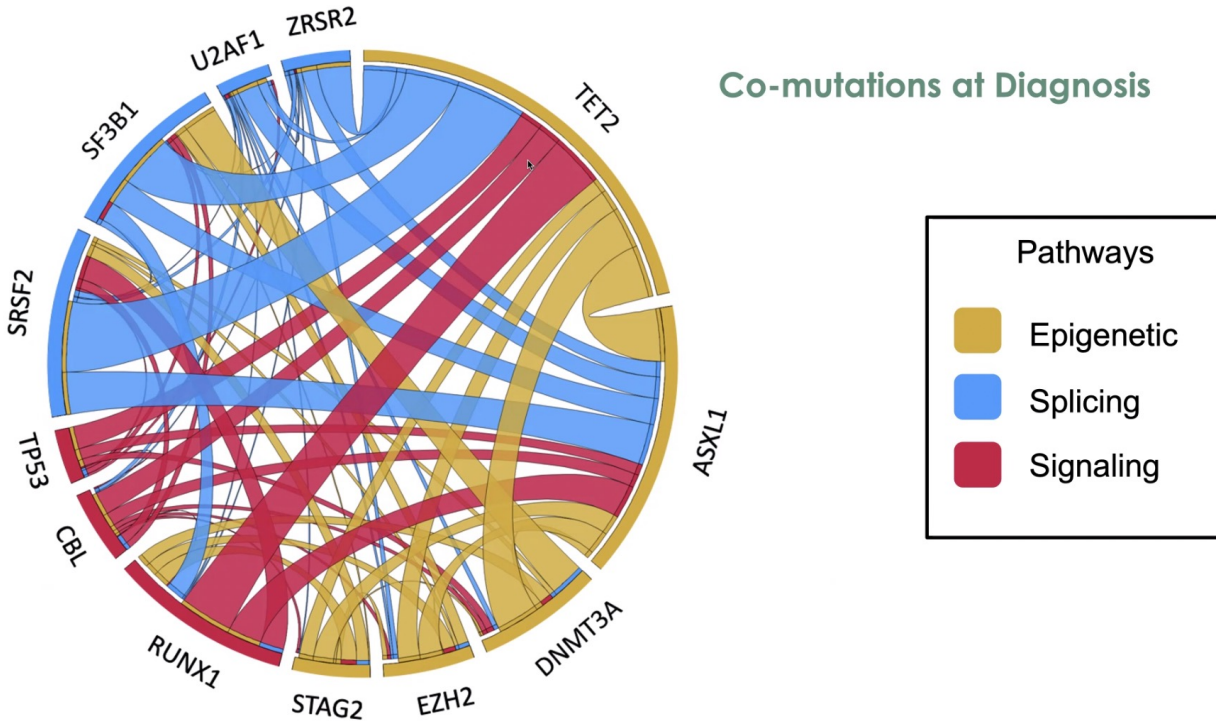
Disclosures of Daniela Cilloni

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Novartis					X		
Abbvie					X	X	
GSK					X		
Gentili					X		
Blueprint					X	X	
BMS					X	X	
Astellas	X				X		

Mutational landscape at diagnosis



Della Porta MG

2022 WHO CLASSIFICATION MDS (Myelodysplastic neoplasms)

	Blasts	Cytogenetics	Mutations
MDS with defining genetic abnormalities			
MDS with low blasts and isolated 5q deletion (MDS-5q)	<5% BM and <2% PB	5q deletion alone, or with 1 other abnormality other than monosomy 7 or 7q deletion	
MDS with low blasts and *SF3B1* mutation[a] (MDS-*SF3B1*)		Absence of 5q deletion, monosomy 7, or complex karyotype	*SF3B1*
MDS with biallelic *TP53* inactivation (MDS-bi*TP53*)	<20% BM and PB	Usually complex	Two or more *TP53* mutations, or 1 mutation with evidence of *TP53* copy number loss or cnLOH
MDS, morphologically defined			
MDS with low blasts (MDS-LB)	<5% BM and <2% PB		
MDS, hypoplastic[b] (MDS-h)			
MDS with increased blasts (MDS-IB)			
MDS-IB1	5–9% BM or 2–4% PB		
MDS-IB2	10-19% BM or 5–19% PB or Auer rods		
MDS with fibrosis (MDS-f)	5–19% BM; 2–19% PB		

Mutazione biallelica di TP53 prevale su MDS-5q and MDS-SF3B1

Khoury JD, et al. Leukemia (2022) 36:1703–1719

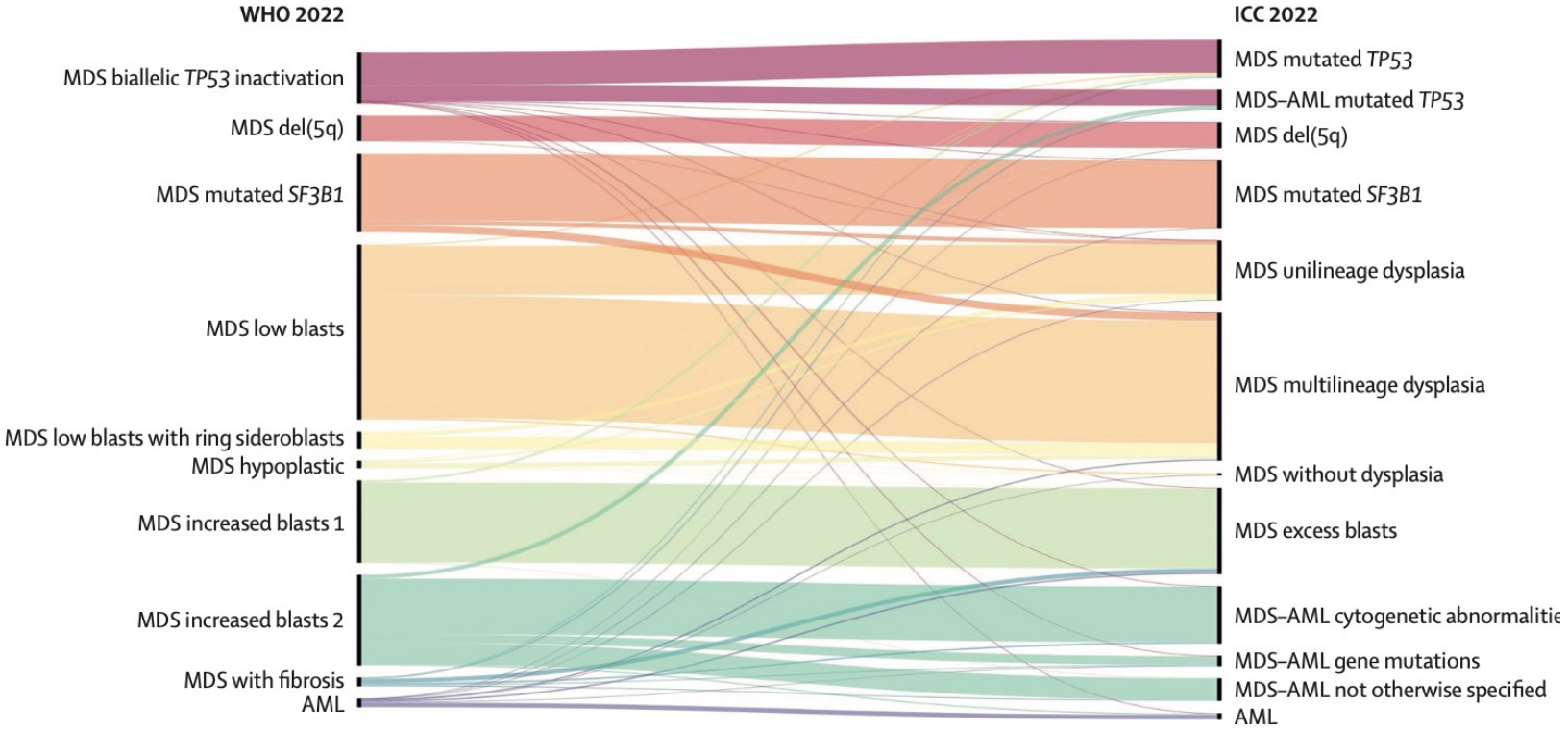
International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data

Premalignant clonal cytopenias and myelodysplastic syndromes

- Clonal cytopenia of undetermined significance
- Myelodysplastic syndrome with mutated *SF3B1*
- Myelodysplastic syndrome with del(5q)
- Myelodysplastic syndrome with mutated *TP53*
- Myelodysplastic syndrome, not otherwise specified (MDS, NOS)
  - MDS, NOS without dysplasia
  - MDS, NOS with single lineage dysplasia
  - MDS, NOS with multilineage dysplasia
- Myelodysplastic syndrome with excess blasts
- Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML)
  - MDS/AML with mutated *TP53*
  - MDS/AML with myelodysplasia-related gene mutations
  - MDS/AML with myelodysplasia-related cytogenetic abnormalities
  - MDS/AML, not otherwise specified

Type	Cytopenia	Blasts	Genetics
MDS with mutated *TP53*	Any	0-9% bone marrow and blood blasts	Multi-hit TP53 mutation* or *TP53* mutation (VAF > 10%) and complex karyotype often with loss of 17p†
MDS/AML with mutated *TP53*	Any	10-19% bone marrow or blood blasts	Any somatic *TP53* mutation (VAF > 10%)
AML with mutated *TP53*	Not required	≥20% bone marrow or blood blasts or meets criteria for pure erythroid leukemia	Any somatic *TP53* mutation (VAF > 10%)

Arber D.A, et al Blood, 2022



Komrokji R. et al. Lancet Haematol 2024

Data-driven, harmonised classification system for myelodysplastic syndromes: a consensus paper from the International Consortium for Myelodysplastic Syndromes

Panel: **Harmonised definition of genetically defined myelodysplastic syndrome subgroups**

Myelodysplastic syndromes with mutated *TP53*

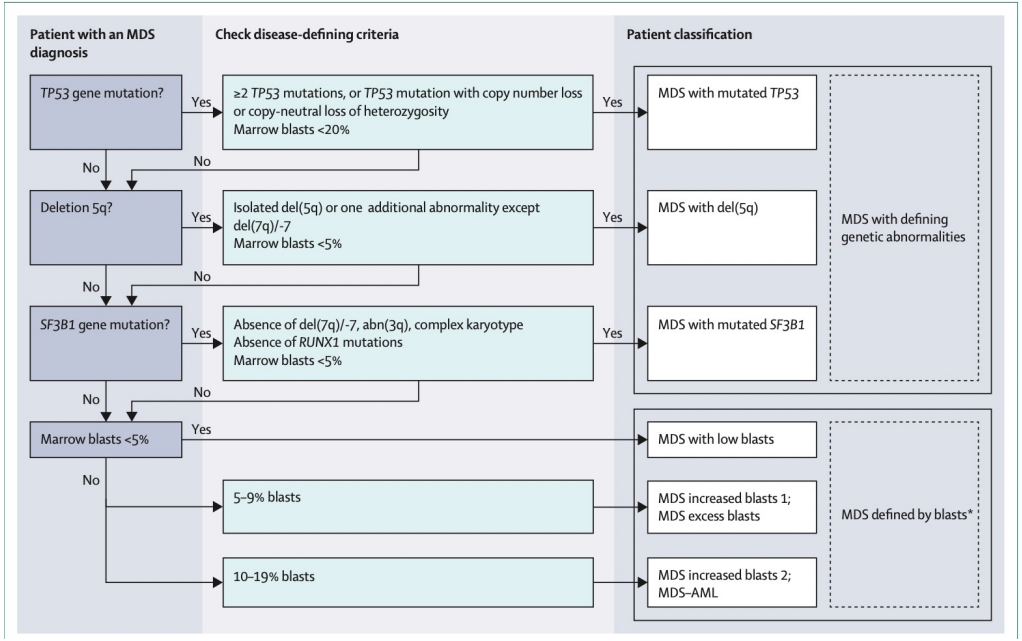
- Two or more *TP53* mutations, or one mutation with *TP53* locus copy number loss or copy-neutral loss of heterozygosity
- Bone marrow blasts less than 20%
- *TP53* mutation supersedes presence of del(5q) or *SF3B1* mutation
- Complex karyotype frequently detected
- *TP53* variant allele frequency of more than 10% frequently detected

Myelodysplastic syndromes with del(5q)

- Presence of del(5q)
- Bone marrow blasts less than 5%
- Absence of del(7q)/-7 or complex karyotype
- Absence of biallelic *TP53* inactivation
- Del(5q) supersedes *SF3B1* mutations

Myelodysplastic syndromes with mutated *SF3B1*

- Presence of *SF3B1* mutation
- Bone marrow blasts less than 5%
- Absence of isolated del(5q), del(7q)/-7, abn(3q26.2), or complex karyotype
- Absence of biallelic *TP53* inactivation
- Absence of *RUNX1* mutations
- *SF3B1* variant allele frequency of more than 10% in 96% of individuals, and more than 5% in 100% of individuals



Komrokji R. et al. Lancet Haematol 2024

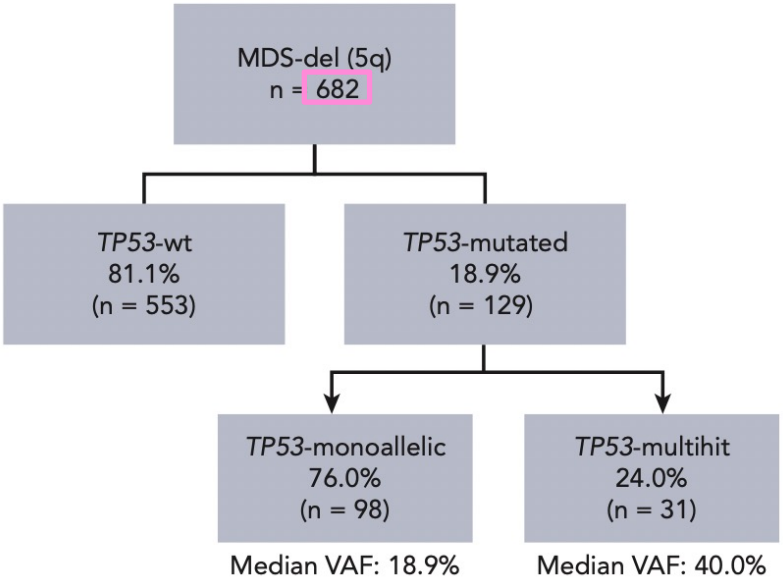
- **Una classificazione armonizzata, basata su dati reali e modelli predittivi permette di stabilire:**

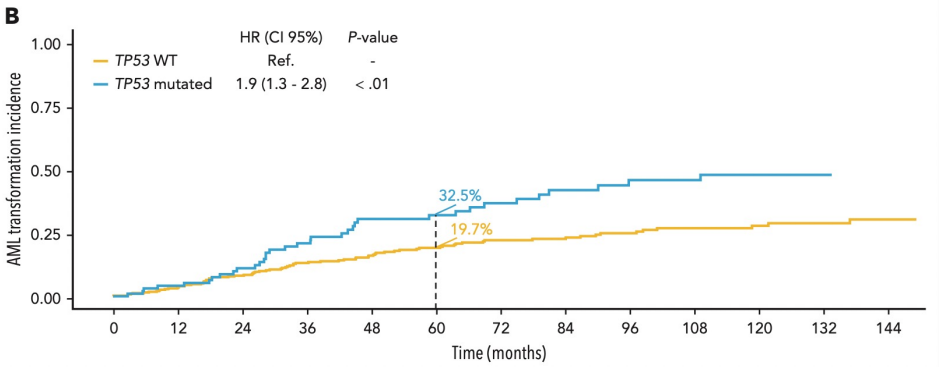
- 1) Una gerarchia tra le mutazioni, utile nella pratica clinica:
  - **Mutazioni TP53** prevalgono su del(5q) e SF3B1.
  - **del(5q)** prevale su SF3B1.

- 3) **Mutazioni di geni dello splicing** oltre a SF3B1: **SRSF2** e **U2AF1** definiscono MDS
  - Queste mutazioni si sviluppano precocemente nella malattia, ne guidano l'evoluzione.
  - Spesso queste mutazioni si associano a mutazioni come **RUNX1** e **ASXL1**, che peggiorano la prognosi.

  4) Ruolo delle caratteristiche morfologiche
  - **Sideroblasti ad anello**:se manca la mutazione SF3B1, la presenza di sideroblasti non migliora la prognosi.
  - **Il numero di linee cellulari displastiche** nel midollo mantiene valore prognostico.
  - **Le MDS ipoplastiche** (con ridotta cellularità del midollo) restano una categoria riconosciuta, associata a buona risposta agli immunosoppressori, ma con grande variabilità genetica.
  - **MDS con aumento dei blasti:** La percentuale di blasti è ancora un indicatore importante per la prognosi

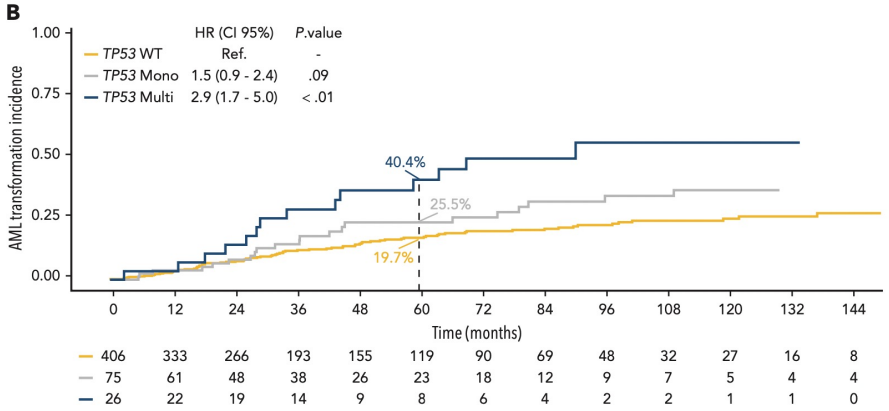
TP53 mutations have an impact on leukemic transformation in MDS 5q-

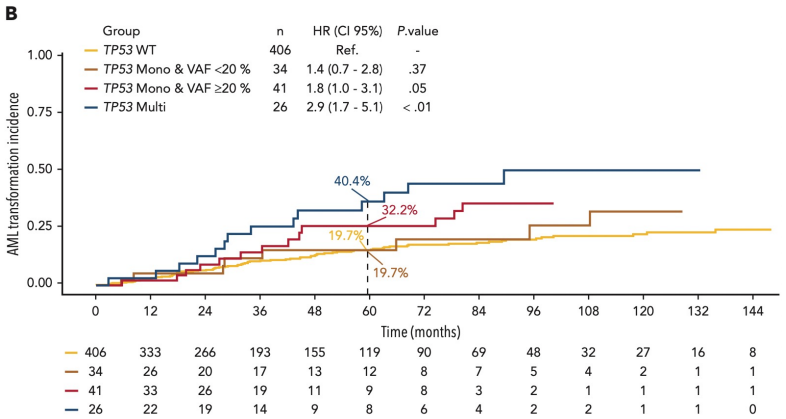




Montoro M.J. et al. Blood 2024

TP53 mutations have an impact on leukemic transformation in MDS 5q-





Patients with TP53 monoallelic mutations and VAF <20% exhibited behavior similar to TP53 wild type

TP53-monoallelic mutations and VAF ≥20% presented outcomes equivalent to TP53-multihit patients

Montoro M.J. et al. Blood oct 2024

IPSS-M score

Blast
Hb
PLTs
Neutrophil
Age

Cytogenetic

*Presence of

del(5q)	No	Yes
-7/del(7q)	No	Yes
-17/del(17p)	No	Yes
Complex Karyotype	No	Yes

Molecular abnormalities

N° of TP53 mutations: 0-1-2
TP53 Loss of heterozygosity Y/N
MLL PTD
FLT3 ITD or TKD

mutations

ASXL1
CBL
DNMT3A
ETV6
EZH2
IDH2
KRAS
NPM1
NRAS
RUNX1
SF3B1
SRSF2
U2AF1
BCOR
BCORL1
CEBPA
ETNK1
GATA2
GNB1
IDH1
NF1
PHF6
PPM1D
PRPF8
PTPN11
SETBP1
STAG2
WT1

Risk category

Very Good	-Y, del(11q).
Good	Normal, del(5q), del(12p), del(20q), double including del(5q).
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones.
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities.
Very Poor	Complex: > 3 abnormalities.

Bernard E, et al. NEJM Evidence Aug 2022

LFS, OS and AML transformation according to IPSS-R and IPSS-M

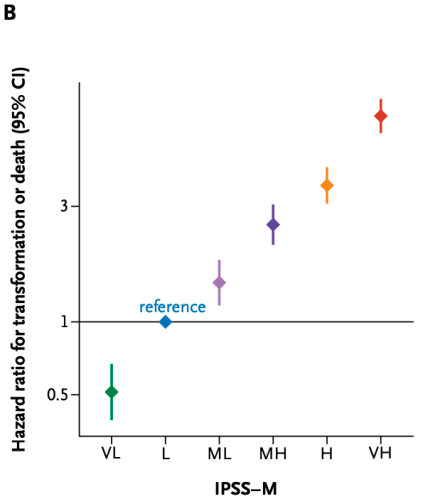
2957 pts

94% at least one molecular abnormality

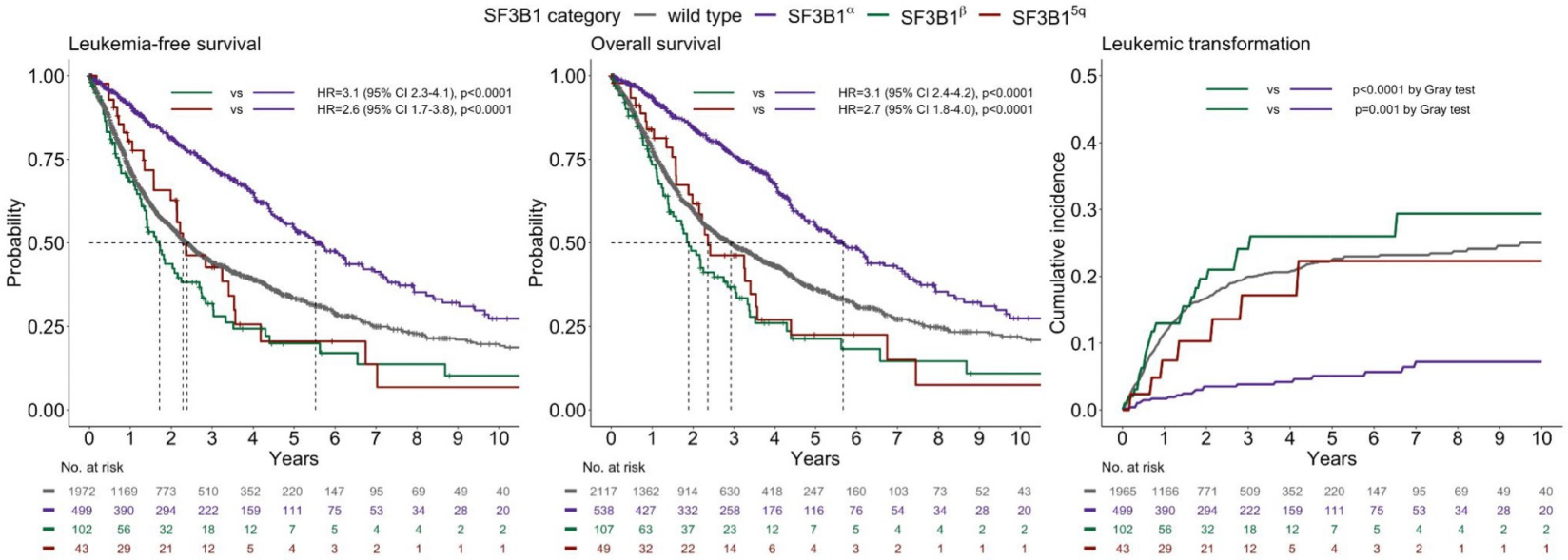
90% mutations

- 53% mutation only
- 37% mutations + cytogenetic alteration

4% cytogenetic alterations only



Bernard E, et al. NEJM Evidence Aug 2022



SF3B1^{5q} = *SF3B1* mutation with concomitant presence of isolated del(5q) only or with one additional cytogenetic alteration excluding -7/del(7q));
SF3B1$^{\beta}$ plus *BCOR*, *BCORL1*, *NRAS*, *RUNX1*, *SRSF2* or *STAG2*
SF3B1α as any other mutant *SF3B1*

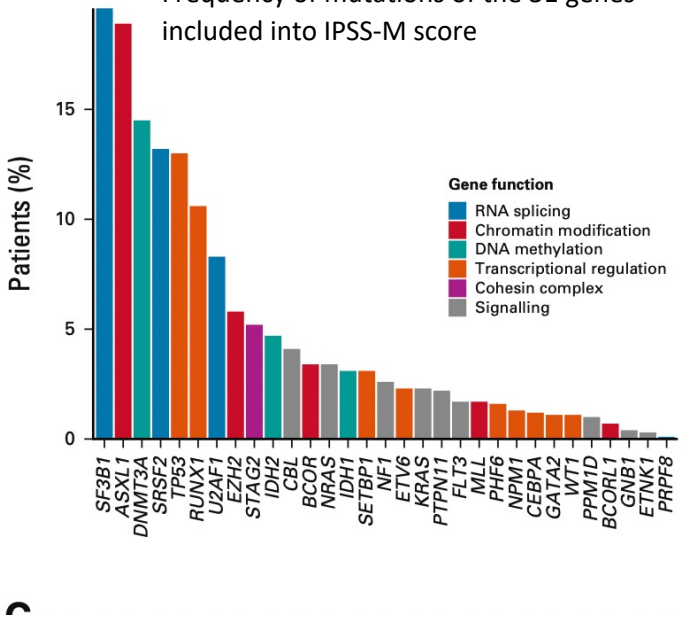
Bernard E, et al. NEJM Evidence Aug 2022

Real-World Validation of Molecular International Prognostic Scoring System for Myelodysplastic Syndromes

2876 pts in the GenoMed4all cohort

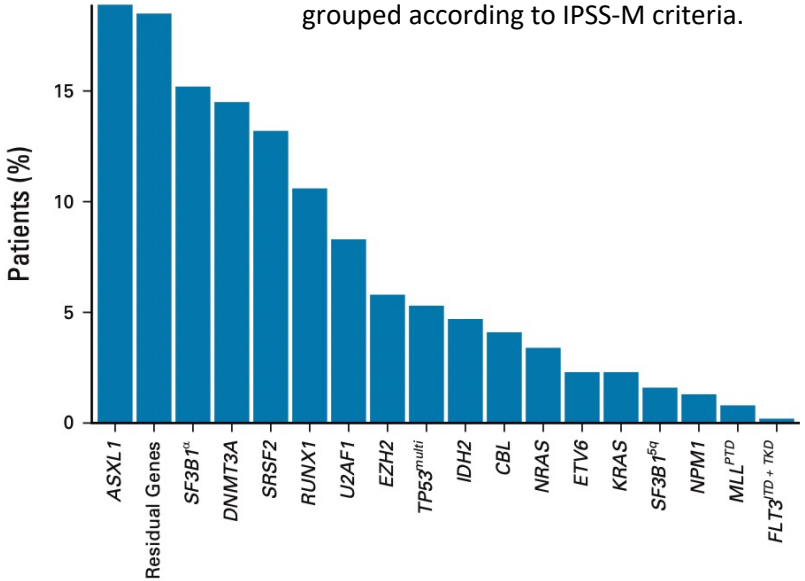
A

Frequency of mutations of the 31 genes included into IPSS-M score

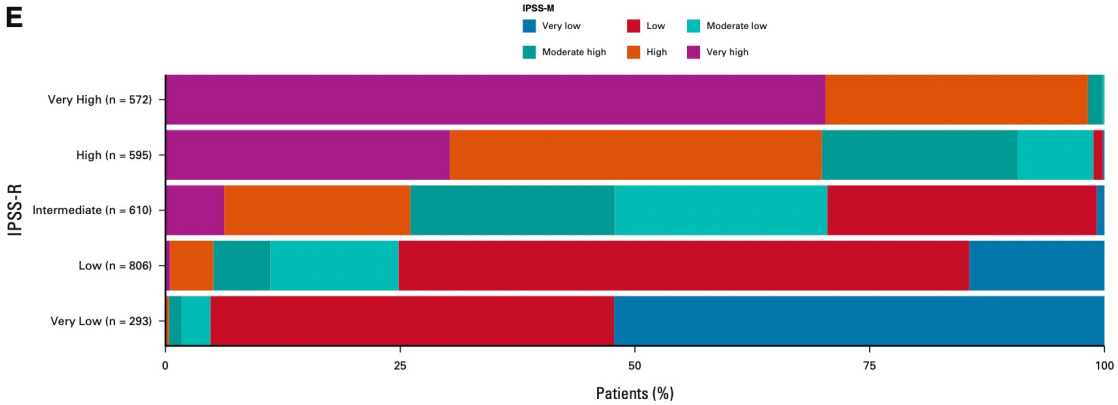


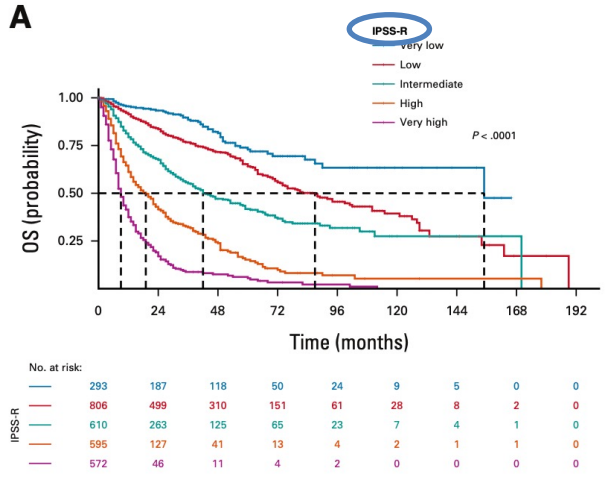
B

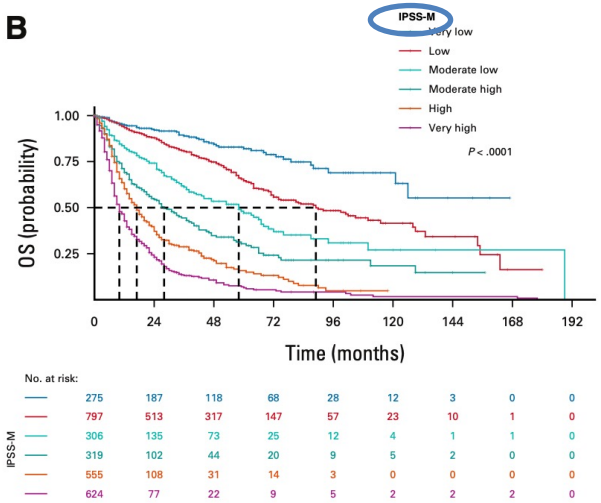
Frequency of mutations on genomic features grouped according to IPSS-M criteria.



Sauta et al. JCO 2023



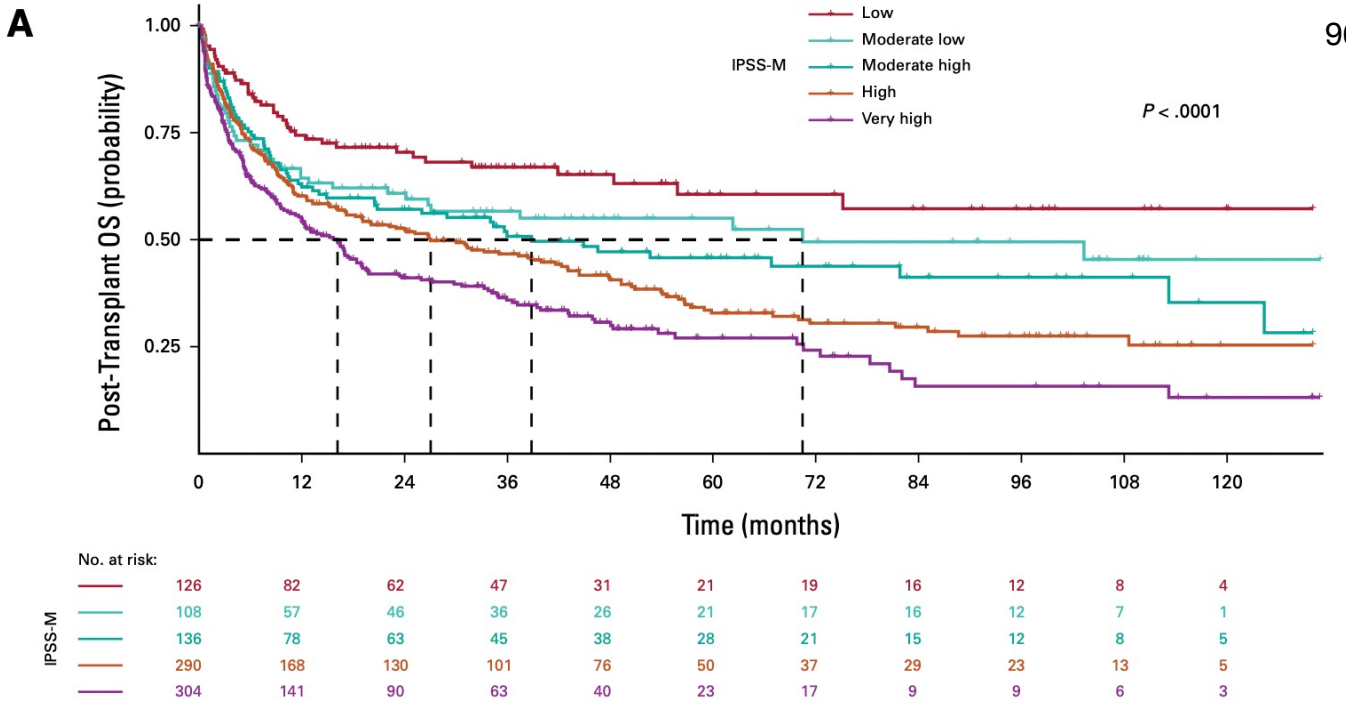




IPSS-M changed group in 46% of pts
23.6% up-staged
22.4 down-staged

Sauta et al. JCO 2023

IPSS-M improves the prediction of OS in MDS treated with HSCT

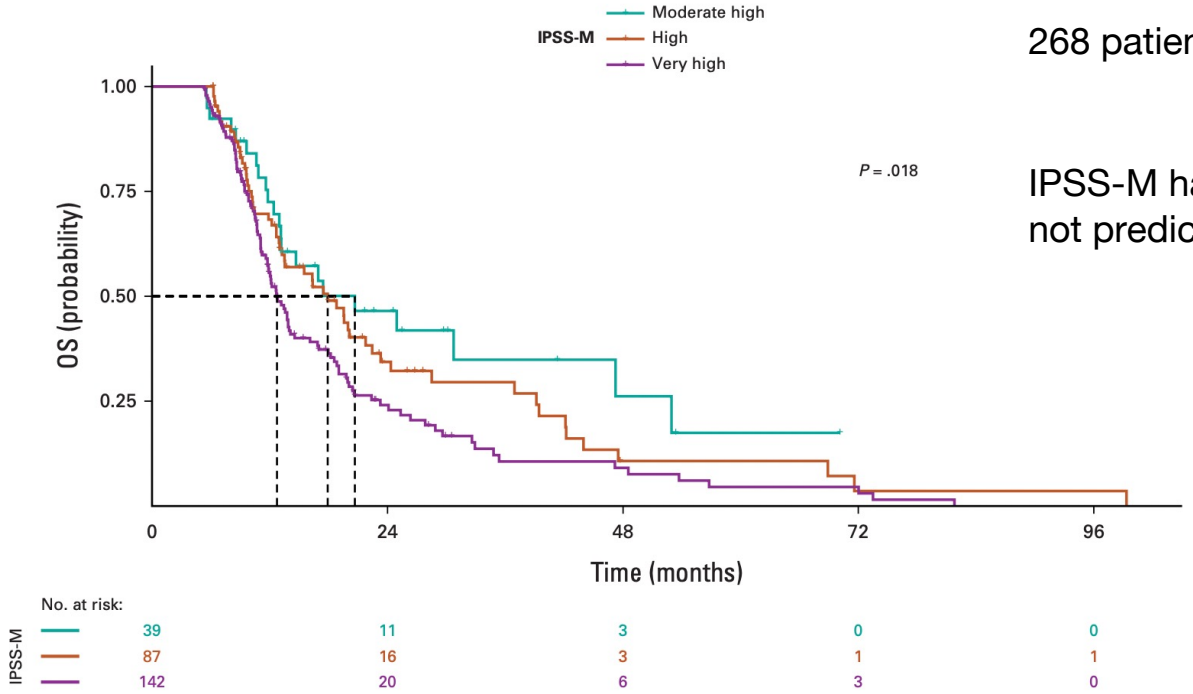


964 pts undergo HSCT

Sauta et al. JCO 2023

IPSS-M prediction of OS in MDS treated with HMA

A

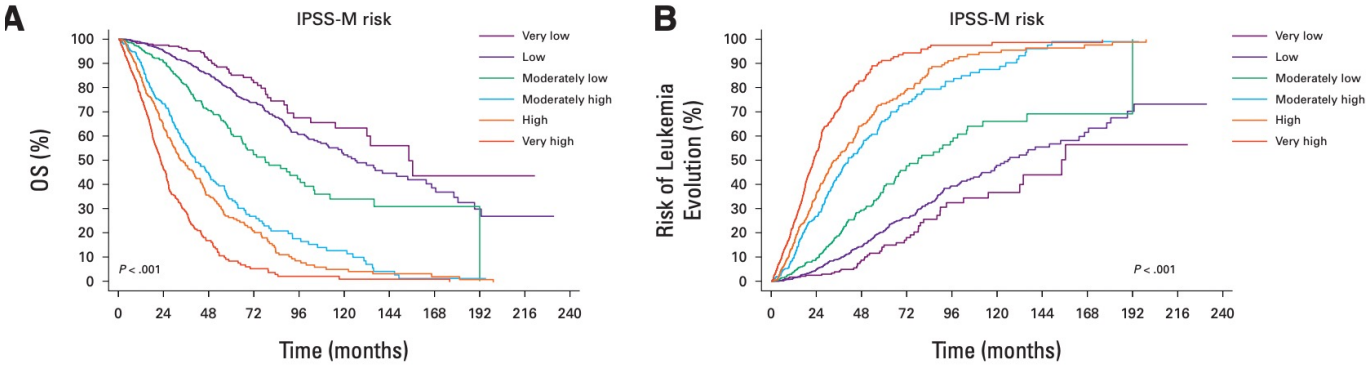


268 patients included

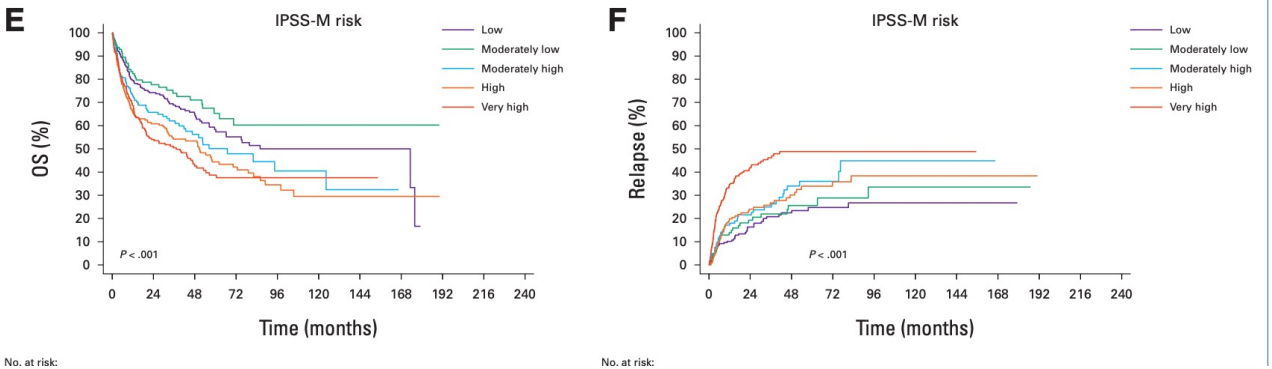
IPSS-M has prognostic value but not predictive

Sauta et al. JCO 2023

Probability of OS and risk of leukemia evolution in the nontransplant study population

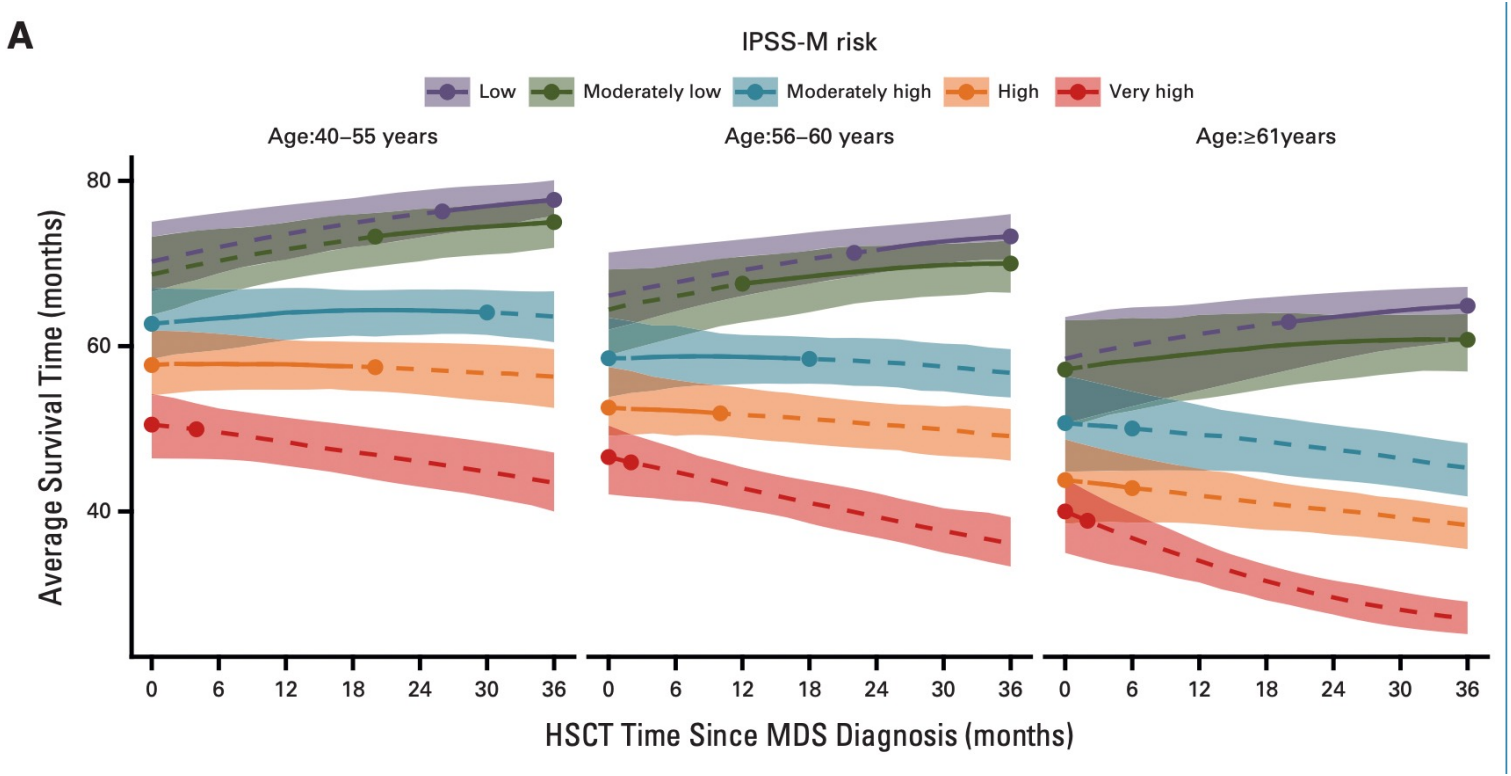


Probability of post-transplantation survival and risk of disease relapse after transplant

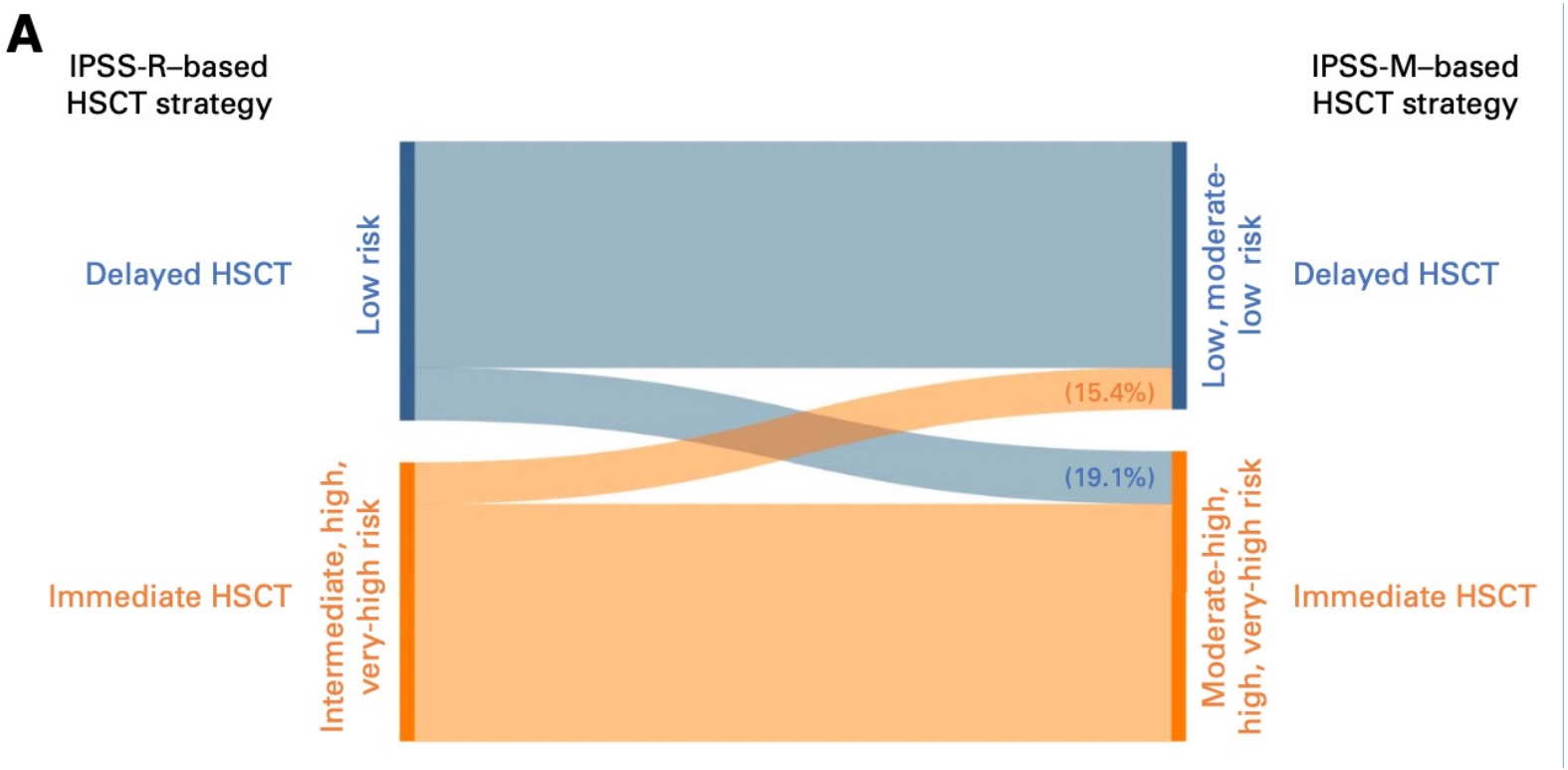


Tentori CA et al JCO 2024

Identification of the time when the transplant can provide the greatest benefit



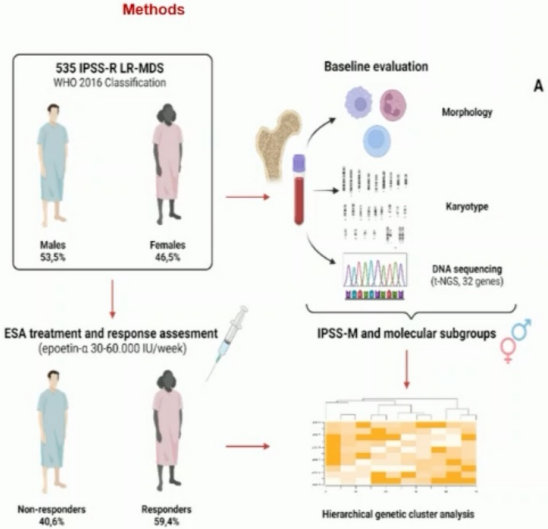
Tentori CA et al JCO 2024

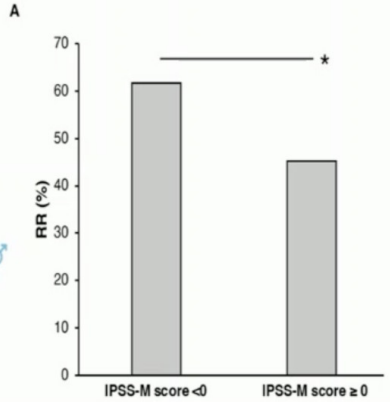


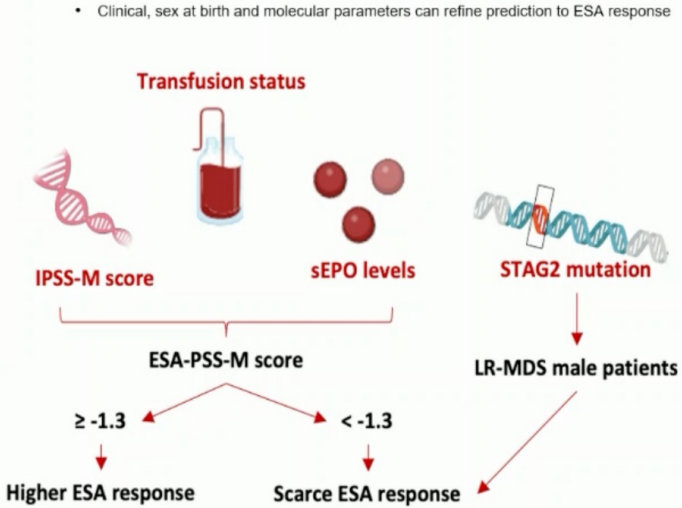
Tentori CA et al JCO 2024

IPSS-M risk and sex-associated somatic mutations improve prediction of response to ESAs in LR-MDS: building a new score

- **The ESA-PSS-M score**: 0.05*[sEPO U/L] -4.5*[IPSS-M score] -5*[TD, yes =1, no =0]







Raddi et al, Blood 2025

IPSS-M correlates with luspatercept response

	Response	No response	*p* Value
IPSS-M, *n* (%)			
Very low	1 (1.2)	0 (0.0)	0.031
Low	56 (70.0)	37 (50.0)	
Moderate low	12 (15.0)	21 (28.4)	
Moderate high	10 (12.5)	10 (13.5)	
High	1 (1.2)	5 (6.8)	
Very high	0 (0.0)	1 (1.4)	
IPSS-R, *n* (%)			
Very low	6 (4.2)	4 (2.7)	0.247
Low	123 (85.4)	116 (78.9)	
Intermediate	13 (9.0)	24 (16.3)	
High	2 (1.4)	3 (2.0)	
SF3B1 status, *n* (%)			
*SF381*mut	91 (91.0)	78 (85.7)	0.267
SF381 WT	9 (9.0)	13 (14.3)	
SF3B1 hotspot, *n* (%)			
K700E	45 (57.7)	31 (48.4)	0.312
Others	33 (42.3)	33 (51.6)	
SF3B1 VAF, *n* (%)			
≥38%	37 (52.1)	42 (66.7)	0.113
<38%	34 (47.9)	21 (33.3)	
SF3B1 co-mutations, *n* (%)			
SF3B1$^{\alpha}$	72 (90.0)	57 (82.6)	0.046
SF3B1$^{\beta}$	8 (10.0)	7 (10.1)	
SF3B1^{5q}	0 (0.0)	5 (7.2)	

IPSS-M but not IPSS-R correlates with the response

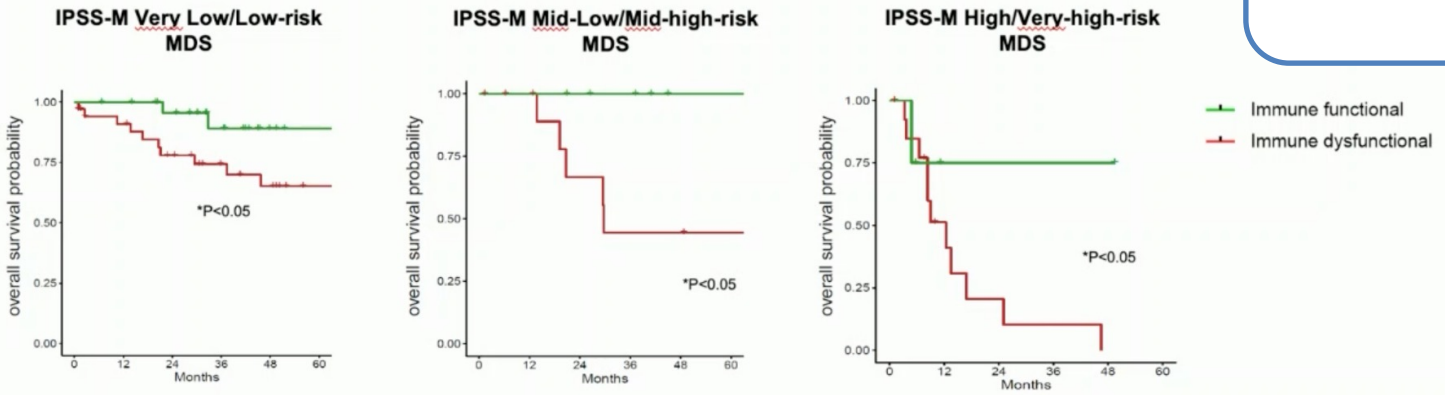
Responses in SF3B1$^{\alpha}$ and SF3B1$^{\beta}$ are higher than in SF3B1^{5q}

SF3B1α, as any other mutant
SF3B1$^{\beta}$, SF3B1 and any gene from BCOR, BCORL1, NRAS, RUNX1, SRSF2, or STAG2;
SF3B1^{5q}, concomitant presence with isolated del5q;

Consagra A. et al HemaSphere. 2025;9:e70086.

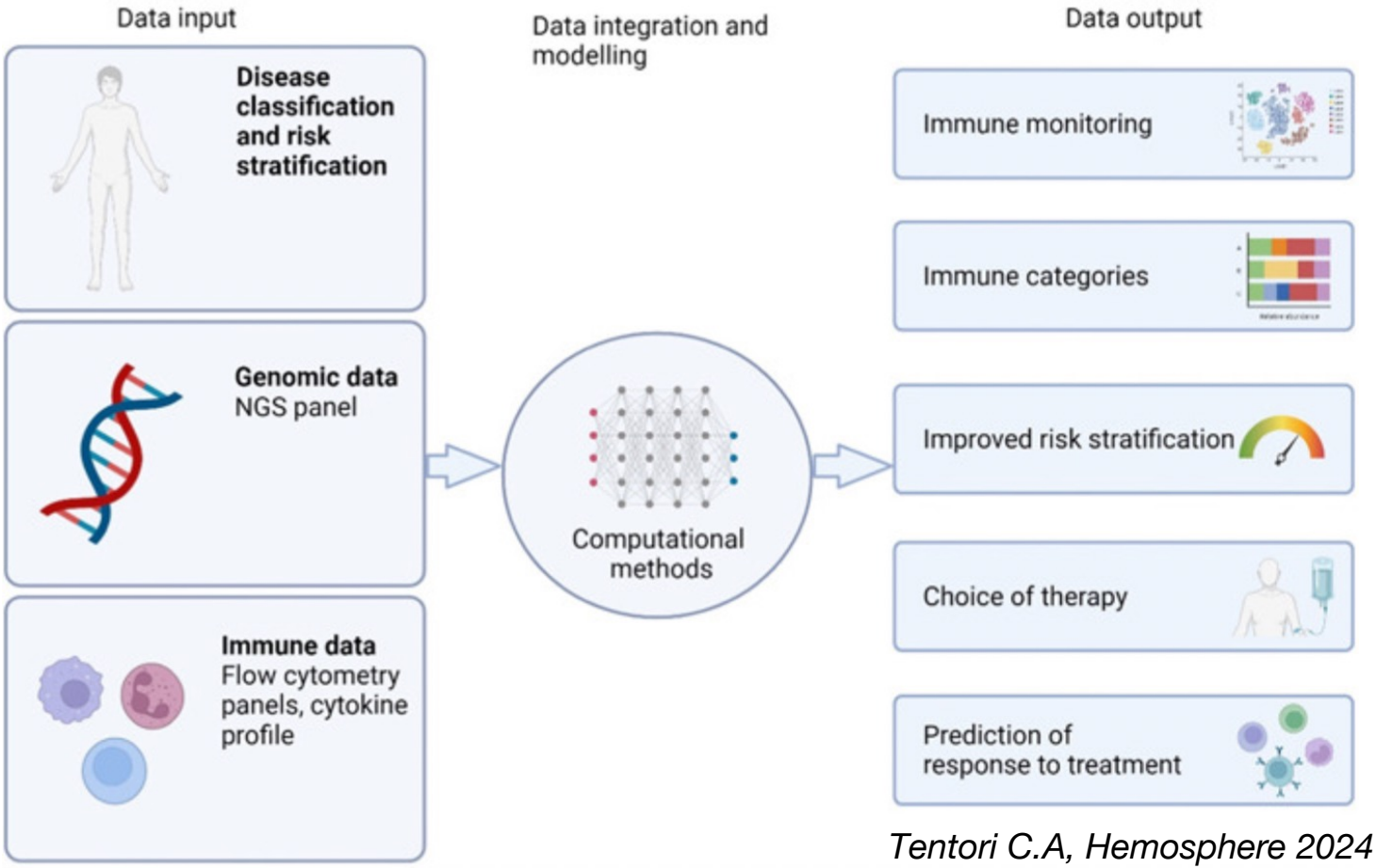
Have we accounted for everything?

Immune ecosystem in 286 MDS patients



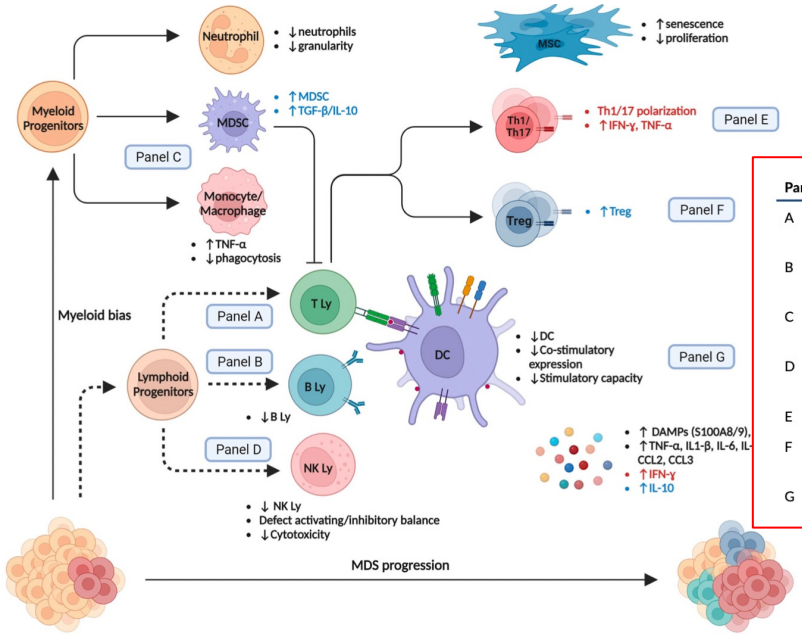
- Immune score was able to further refine the prognosis of patients stratified according to IPSS-M risk groups.
- Integrating immune cell signatures with molecular profiles improved the accuracy of predicting patient outcomes
- Is it the only way to go?

Riva E.… Della Porta ASH 2024 abstr



Tentori C.A, Hemosphere 2024

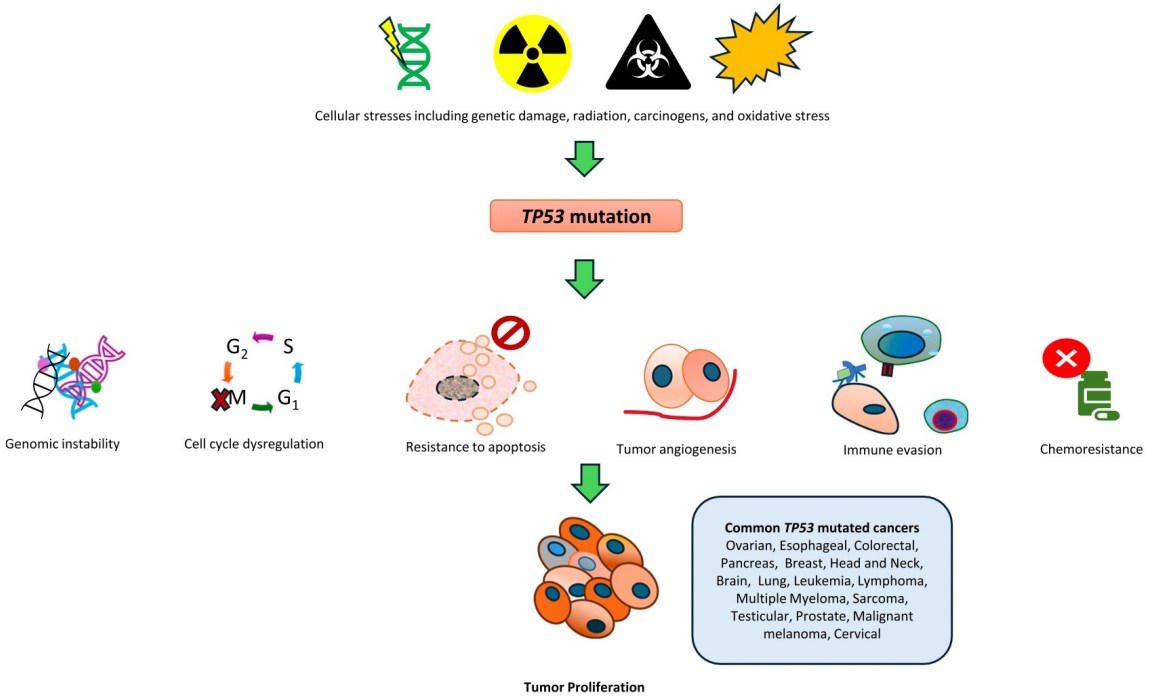
Immune-monitoring of myelodysplastic neoplasms: Recommendations from the i4MDS consortium



Panel	Immune subsets	Recommended markers	Optional (immune)	Optional (malignant)
A	T cell	CD45, CD45RA, CD3, CD4, CD8, CD5, CD7, CD16/56, CD27, TCRγδ	HLADR, CD38, CD95, CD62L, CD45RO, PD1/CTLA4/TIM3	TRBC1, CD26
B	B cell	CD45, CD20, CD19, CD10, CD27, CD38, IgD, IgM, CD25, CD22	CD305, CD185	kappa, lambda, CD5, CD23
C	Monocyte/MDSC	CD45, CD14, CD16, CD64, CD300e, CD56, HLADR, CD11b, CD33, CD15, Lin	SLAN, CD141, CD45RO	CD34, CD13
D	NK cell	CD7, CD56, CD8, CD94, CD57, CD161, CD16, CD3, KIR3DL1/DL2, CD45RO	DNAM1, NKG2D, NKp30, NKp46, KIR2DL2	
E	T cell subset – 1	CD3, CD4, CD8, CD19, CD25, CD127, CXCR5, CXCR3, CCR4, CCR6	CD95, CD28, CCR7	
F	T cell subset – 2	CD45RA, CD3, CD4, CD25, CD127, CD194 (CCR4), CD95, CD28, CCR7, CD8	FOXP3, CXCR5, CXCR3, CCR6, CD45RO	
G	Dendritic cells	CD45, Lin, CD123, CD88, HLADR, CD5, CD11C, CD141, CD163, CD11B, CD14	CD1c, CD303, CD11b. CD45RO	

Tentori C.A, Hemosphere 2024

TP53 mutations



Shahzad M. et al Blood Cancer J 2024

77% missense mutations
8% non sense mutations

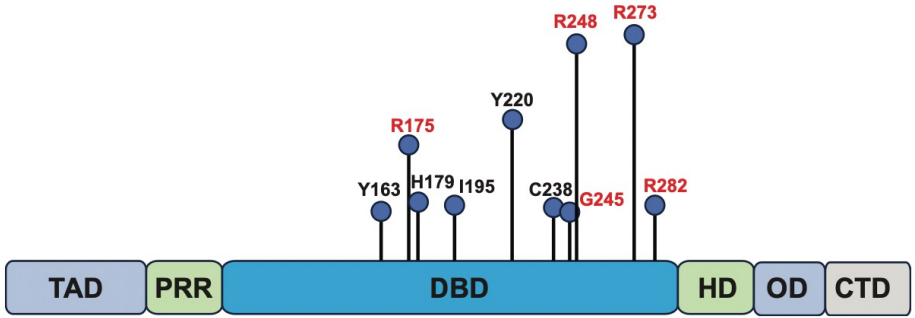


Diagram representing the 10 most common *TP53* mutations identified in AML/MDS.3 Mutations highlighted in red are also among the 5 most commonly mutated residues in overall malignancies. CTD, C-terminal domain; DBD, DNA-binding domain; HD, hinge domain; OD, oligomerization domain; PRR, proline-rich region; TAD, transactivation domain.

Multiple hit

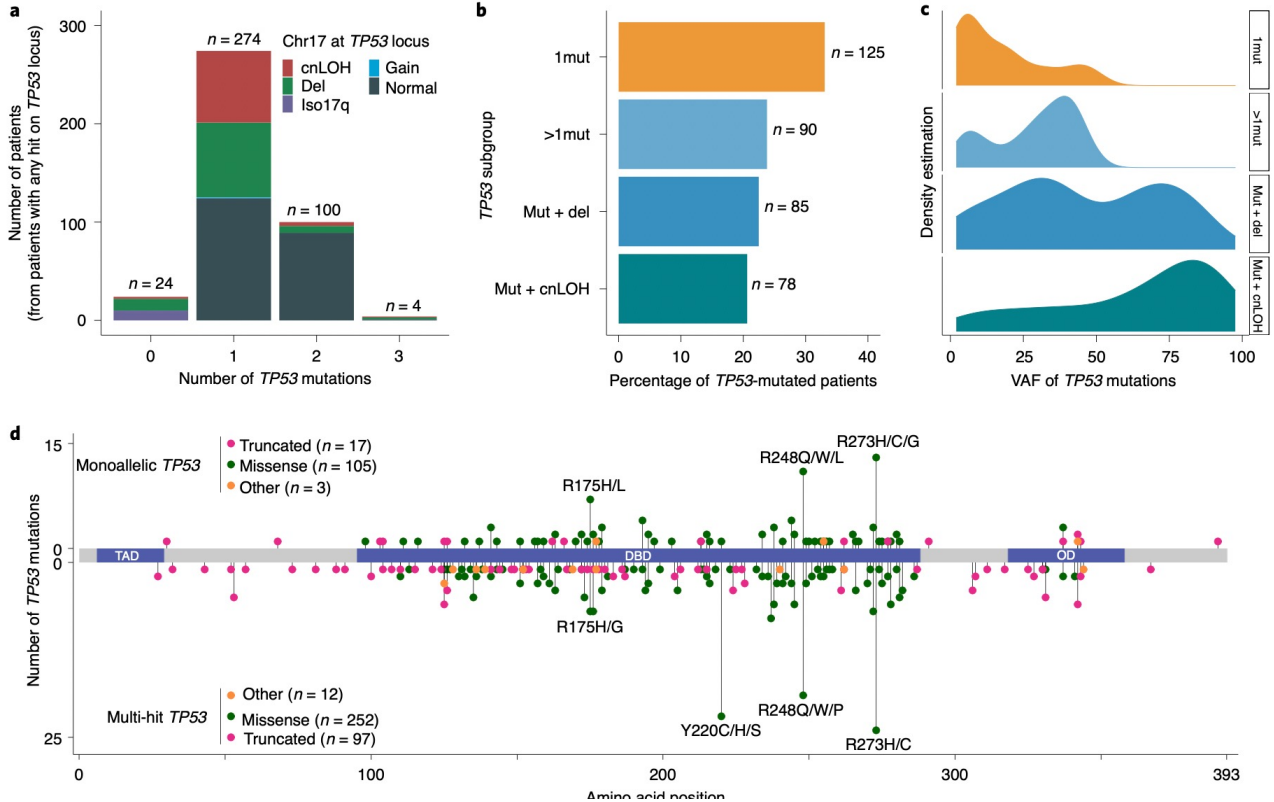
2 different mutations with VAF>10%
1 mutation with VAF>50%
1 mutation with VAF>10% + LOH (del17p)

25% of patients with VAF<50% are misclassified as single hit because they have LOH

⇩

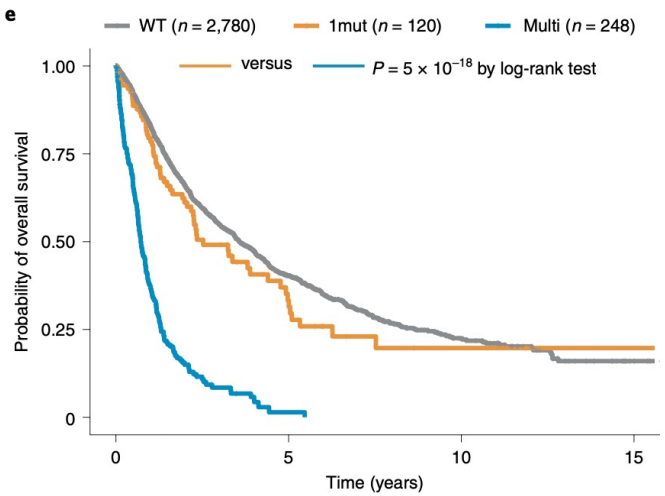
Whole genome sequencing

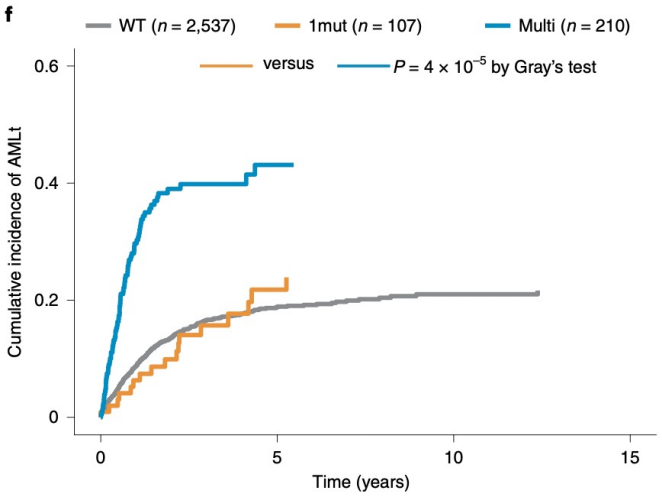
Implications of *TP53* allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes



Bernard E, et al. Nat Med , 2020 26, 1549–1556

Implications of *TP53* allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes





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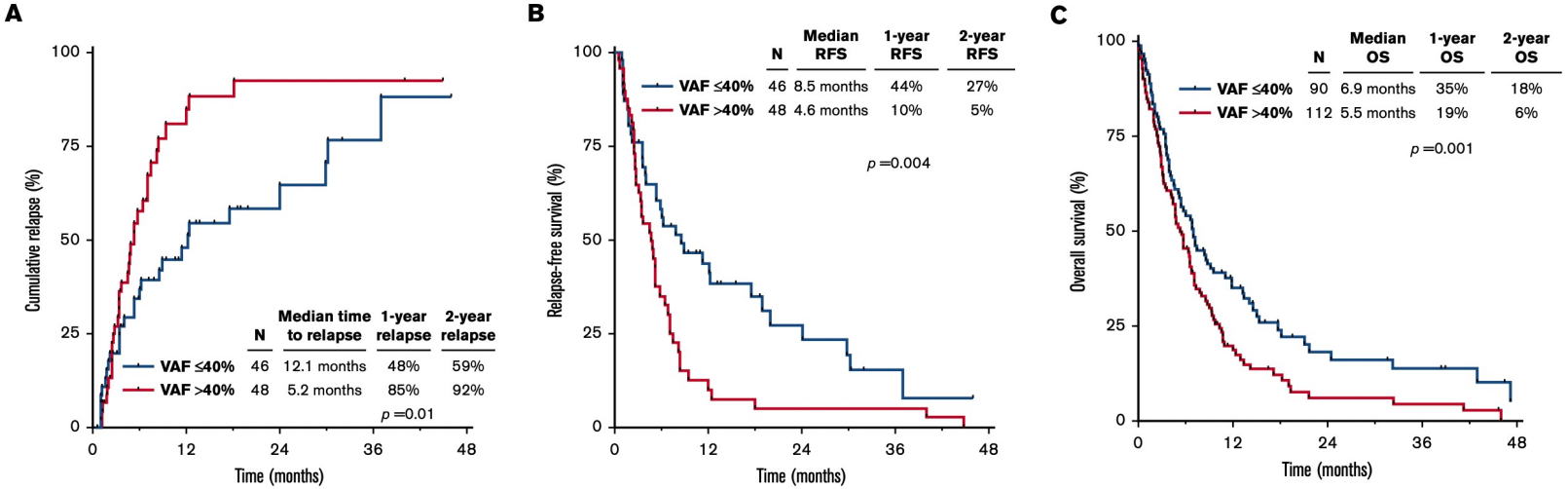
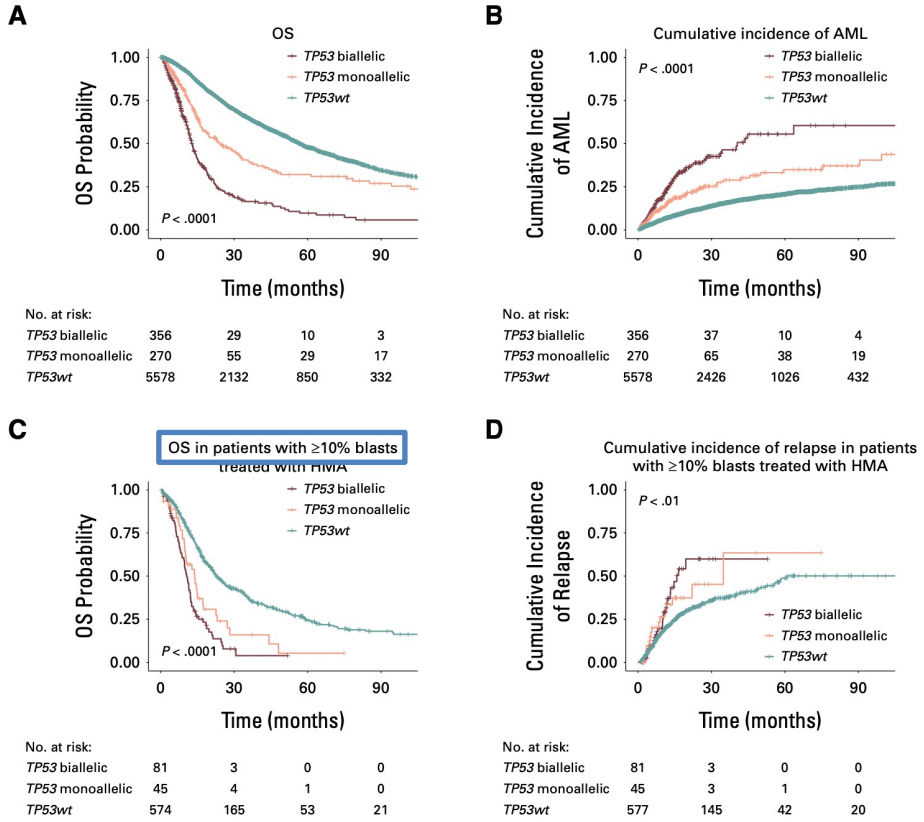
Prognostic and therapeutic impacts of mutant *TP53* variant allelic frequency in newly diagnosed acute myeloid leukemia

Nicholas J. Short,[1] Guillermo Montalban-Bravo,[1] Hyunsoo Hwang,[2] Jing Ning,[2] Miguel J. Franquiz,[1] Rashmi Kanagal-Shamanna,[3] Keyur P. Patel,[3] Courtney D. DiNardo,[1] Farhad Ravandi,[1] Guillermo Garcia-Manero,[1] Koichi Takahashi,[1] Marina Konopleva,[1] Naval Daver,[1] Ghayas C. Issa,[1] Michael Andreeff,[1] Hagop Kantarjian,[1] and Tapan M. Kadia[1]



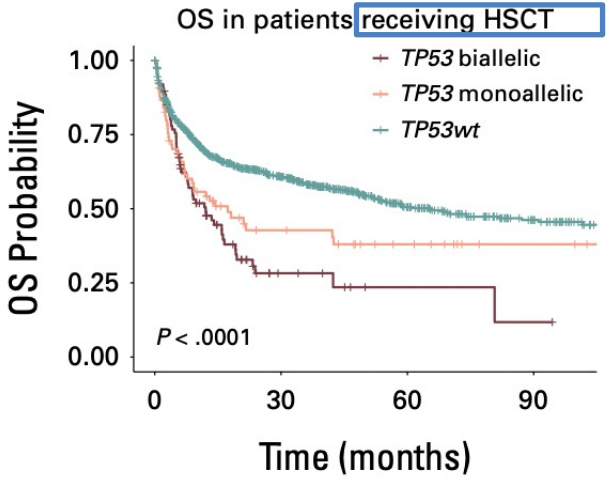
Blood Adv 2020; 22: 5681

Characterization and Clinical Implications of p53 Dysfunction in Patients With Myelodysplastic Syndromes



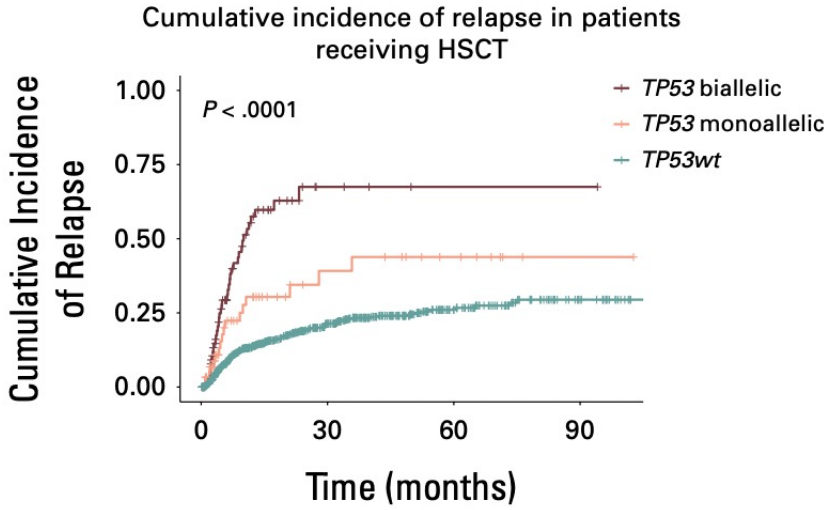
Zampini et al. J Clin Oncol. 2025 May

E



No. at risk:				
TP53 biallelic	87	8	2	1
TP53 monoallelic	81	19	10	3
TP53wt	998	392	152	72

F



No. at risk:				
TP53 biallelic	85	4	1	1
TP53 monoallelic	75	13	7	1
TP53wt	951	316	111	44

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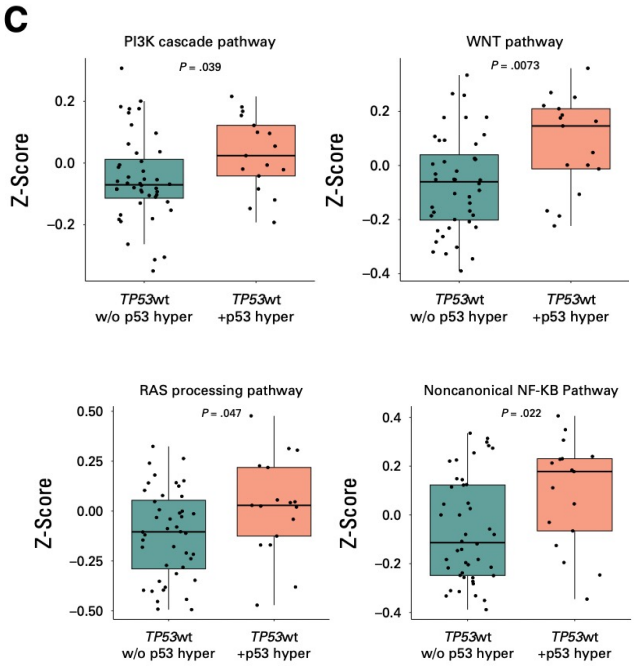
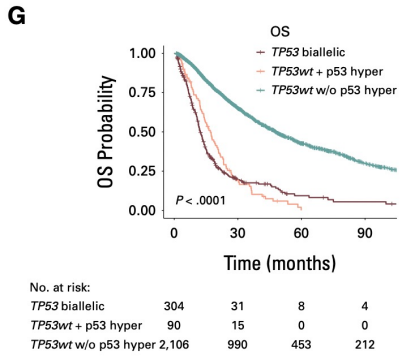
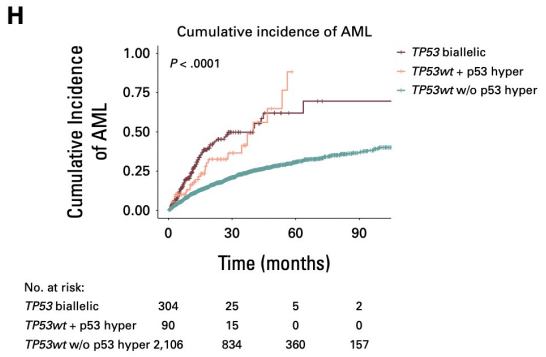
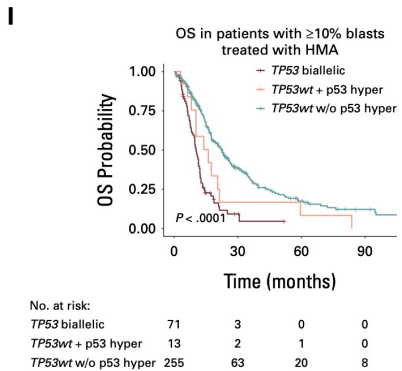
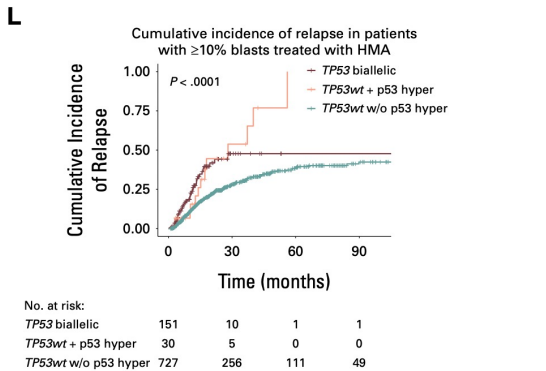
Dysfunction of p53 has a negative impact on prognosis











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Conclusions

Combining genomic profiling with hematologic and cytogenetic parameters, the IPSS-M improves the risk stratification of patients with MDS and represents a valuable tool for clinical decision-making

IPSS-M improves MDS prognostication and might result in a more effective selection of candidates to HSCT

TP53-multihit alterations were predictive of an increased risk of leukemic transformation.

The recognition of patients with p53 dysfunction is relevant to provide correct disease-risk assessment and interventions

Mauriziano Hospital, Turin

Clinical Trial Team

The Lab