Distinct Molecular Subtypes in *DDX41*-Mutated MDS: Germline vs Somatic Landscapes

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INTRODUCTION

DDX41, a DEAD-box RNA helicase, is the most common germline mutation in myeloid neoplasms defining a unique myelodysplastic syndrome (MDS) subtype.

These cases display distinct clinical-pathologic features that are only partially captured by the current Molecular International Prognostic Scoring System (IPSS-M).

AIM

To deepen our understanding of the biological and clinical implications of *DDX41* mutations, we investigated the co-mutation landscape in patients harboring either putative germline (GL) and/or somatic (SM) mutations. Our goal was to provide a more refined molecular and clinical characterization of these peculiar subgroups of cases.

METHODS

We analyzed three independent datasets (Cleveland Clinic, Humanitas Research Hospital, and IPSS-M cohort) to identify *DDX41*-mutated MDS patients.

Cases were stratified by:

A) putative Germline (GL) vs. Somatic Mutation (SM),

B) Sex,

C) single-hit (SH) vs. multi-hit (MH) (GL+SM, SM+SM)

NB:

- GL were defined by VAF ≥45%.
- MH definition: patients carrying ≥2 *DDX41* mutations, irrespective of whether mutations occur on the same or different alleles.

RESULTS

- **Cohort:**
  - Among 4,373 MDS patients, 86 cases (2%) carried *DDX41* mutations.
  - Variants: 59 germline (GL), 74 somatic (SM)
  - Strong male predominance (78%)
- **Mutation burden:**
  - 63% of DDX41-mutated apatients had at least one co-mutation
  - Median: 3 mutations per case (range 0–7)
  - Double SM in 19% of cases
  - GL+SM in 32% and SH in 12%
- **Co-mutation profile (Fig. 1):**
  - Most frequent: *TET2, SF3B1, DNMT3A, ASXL1, TP53*
  - Both MH subgroups (GL+SM, SM+SM) enriched for *SRSF2* (20%)
- **Sex-specific patterns (Fig. 2):**
  - In females:
    - *SF3B1* (42% vs 0%, P=0.2451)
    - *DNMT3A* (17% vs 57%, P=0.3047)
  - In males:
    - *TET2* more frequent in SH vs MH (36% vs 2%, P=0.0091)
    - *SRSF2* mutations more frequent in MH vs SH (28% vs 12%, P=0.2216)
- **Prognostic impact (Fig. 3):**
  - No statistically differences among male MH subgroups (GL+SM, SM+SM) has been observed

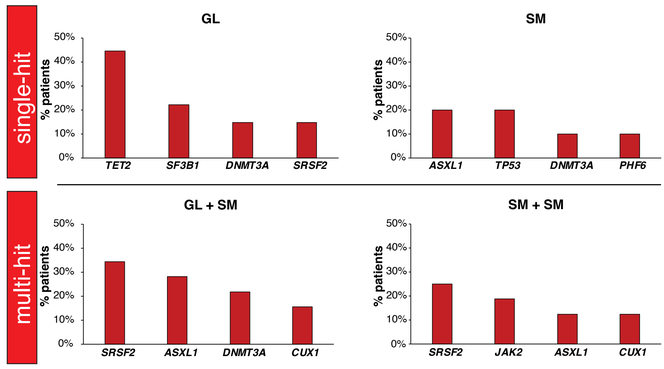


Figure 1: Co-mutation landscape by *DDX41* mutation type and status.
Distribution of the most frequent co-mutations in patients with germline (GL), somatic (SM), and multi-hit (GL+SM or SM+SM) *DDX41*-mutated MDS. Bars represent the proportion of patients carrying the indicated co-mutation within each subgroup.

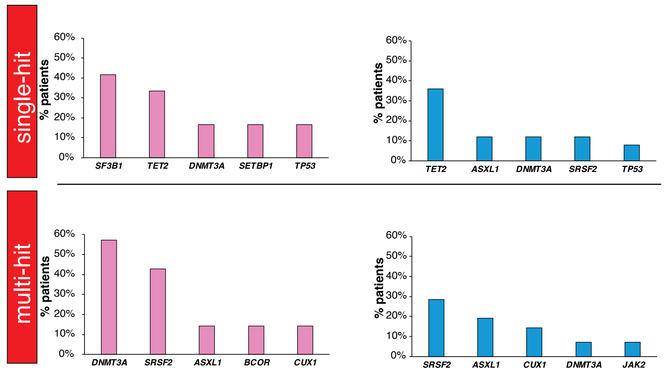


Figure 2: Sex-specific co-mutation patterns in *DDX41*-mutated MDS.
Most recurrent co-mutated genes stratified by sex in single-hit (SH) vs. multi-hit (MH) *DDX41*-mutated patients. Pink = female; blue = male.

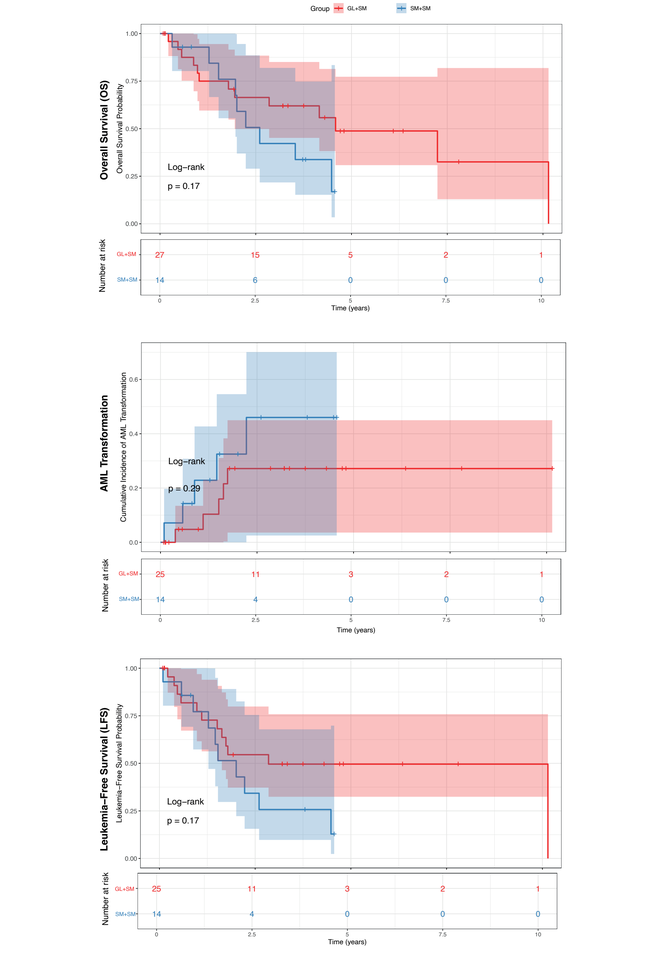


Figure 3: Prognostic impact of single-hit vs multi-hit DDX41 mutations in male MDS patients
Overall Survival (OS): Comparison of MH GL+SM vs. MH SM+SM *DDX41*-mutated males.
AML Transformation: Cumulative incidence of AML transformation according to MH GL+SM vs. MH SM+SM status. Leukemia-Free Survival (LFS): Event-free survival (progression to AML or death) in MH GL+SM vs. MH SM+SM patients. Shaded areas represent 95% confidence intervals; log-rank p-values are reported.

Table 1: Cohort characteristics

DDX41-patients		Count	
Total N°	-	86	100%
Sex	Male	67	78%
	Female	19	22%
Age	Median	69	-
	Range	41-97	-
Diagnosis	MDS-LB	17	20%
	MDS-SF3B1mut	5	6%
	MDS-IB1	28	32%
	MDS-IB2	34	40%
	MDS-U	2	2%
Cytogenetics	Normal	58	68%
	1 abnormality	9	10%
	Complex	2	2%
	NA	17	20%
IPSS-R	VL	5	6%
	L	17	20%
	I	38	44%
	H	14	16%
	VH	4	5%
	NA	8	9%
IPSS-M	VL	5	6%
	L	27	31%
	ML	14	16%
	MH	17	20%
	H	15	17%
	VH	4	5%
	NA	4	5%

Table 2: DDX41-mutated patients distribution

DDX41 status	mut_type	Patient Count (%)
single-hit (SH)	somatic (**SM**)	10 (12%)
	putative germline (**GL**)	16 (19%)
multi-hit (MH)	SM + SM	26 (32%)
	SM + GL	33 (37%)

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CONCLUSION

DDX41-mutated MDS account for ~2% of cases in our cohort and display a unique molecular profile.

- **Mutation burden:** Most patients carry additional mutations, with a distinct enrichment of *SRSF2* in multi-hit (MH) cases.
- **Co-mutation landscape:** Frequent associations with *TET2, SF3B1, DNMT3A, ASXL1, TP53,* with sex-specific patterns (*SF3B1/DNMT3A* in females, *TET2* in males).
- **Prognosis:** Despite clear molecular distinctions, although we did not observe statistically significant differences in OS, AML transformation, and LFS (possibly due to the limited cohort size), we noted a trend to better outcome for male patients carrying GL and acquired SM.