FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

CONVEGNO FISiM

Firenze, CSF Montedomini
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FISiM NGS study: impatto clinico del monitoraggio NGS longitudinale

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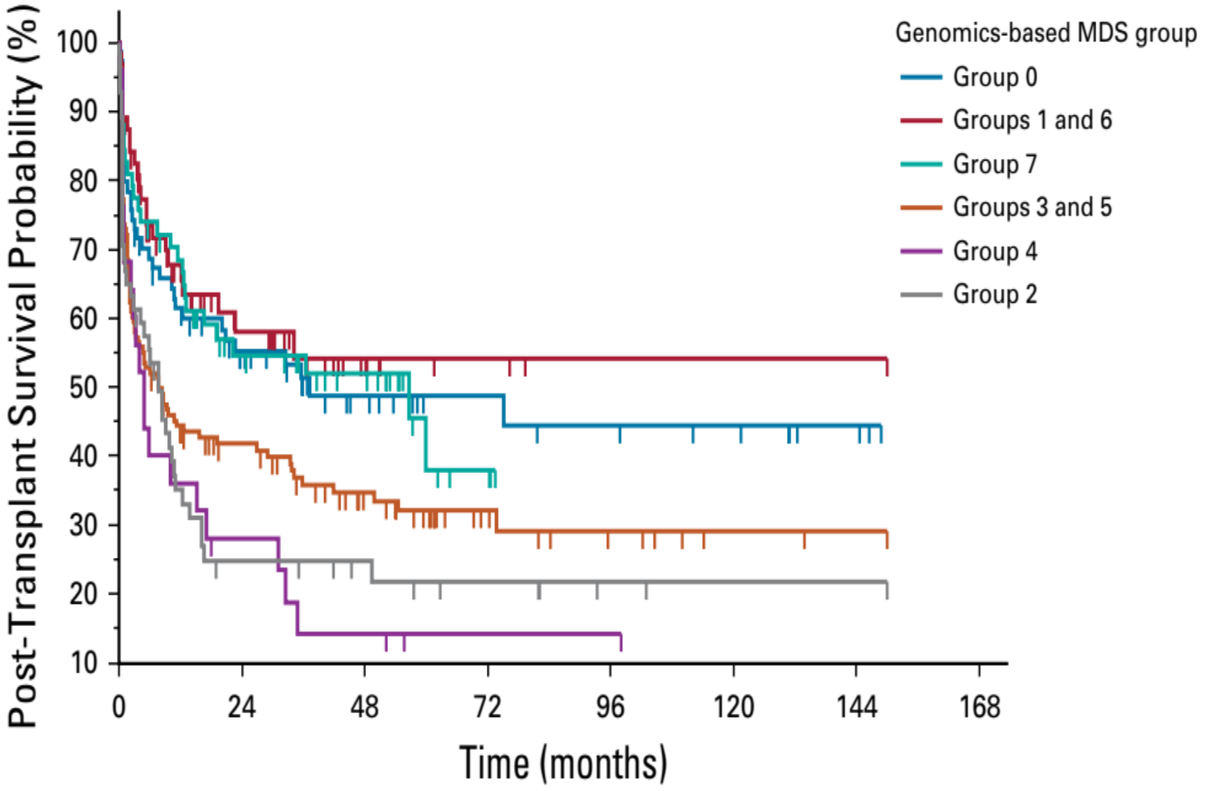
No disclosures to declare

BACKGROUND

- Myelodysplastic syndromes (MDS) represent a group of clonal disorders associated with an increased risk of progression to acute myeloid leukemia (AML)

- The mechanisms of disease evolution involve the sequential acquisition of somatic mutations and clonal selection over time; the heterogeneity of these molecular landscapes results in highly variable clinical courses

- Analyses of large patient populations are needed to correctly estimate the independent effect of somatic mutations on various clinical outcomes

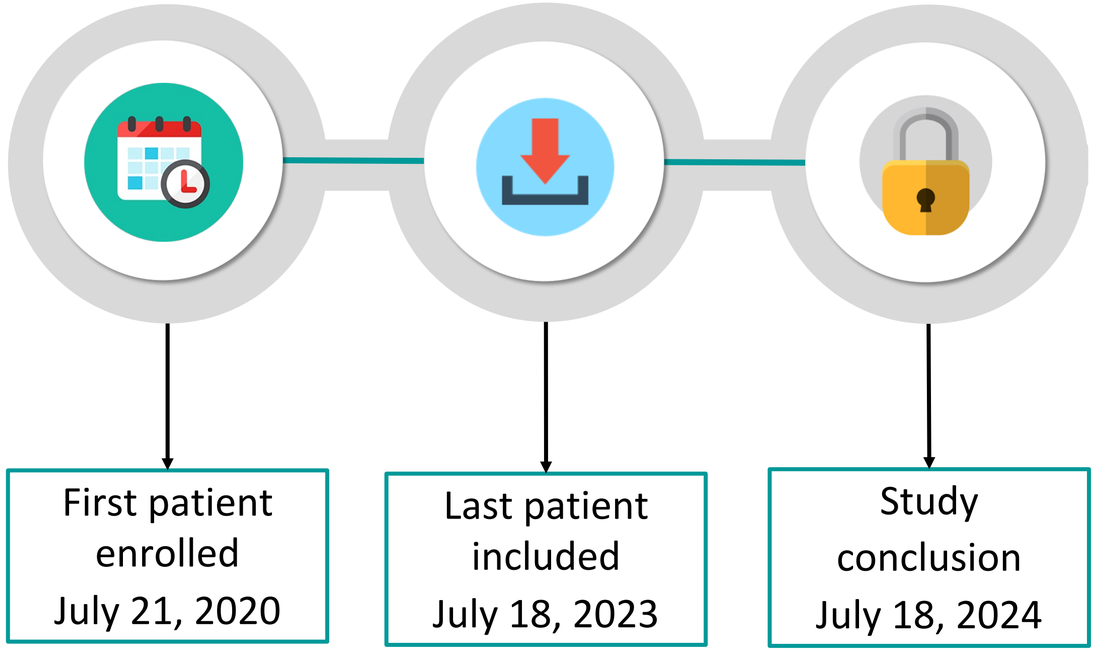


Bersanelli et al. Journal of Clinical Oncoloy (JCO), 2021

PURPOSE

In this context, the FISIM-NGS-MDS study (ClinicalTrials.gov Identifier: NCT04212390) was designed to prospectively collect longitudinal clinical and molecular data from peripheral blood (PB) in patients with MDS, with the aim to evaluate the clinical relevance of mutational screening in the diagnostic process and classification of MDS according to WHO criteria, to investigate clonal evolution and identify molecular patterns predictive of disease progression

KEY STUDY MILESTONES



METHODS

- A total number of 882 adult patients with a diagnosis of MDS according to the 2016 WHO classification were prospectively enrolled at diagnosis across 28 Italian hospitals affiliated with the Fondazione Italiana Sindromi Mielodisplastiche (FISiM)

- Peripheral blood samples were collected at diagnosis, annually during follow-up, before and after treatment, and at any time of disease progression, including transformation to AML

- In addition to collecting and sequencing samples, each participating center gathered clinical information from patients and regularly updated follow-up data to enable a comprehensive analysis of all cases

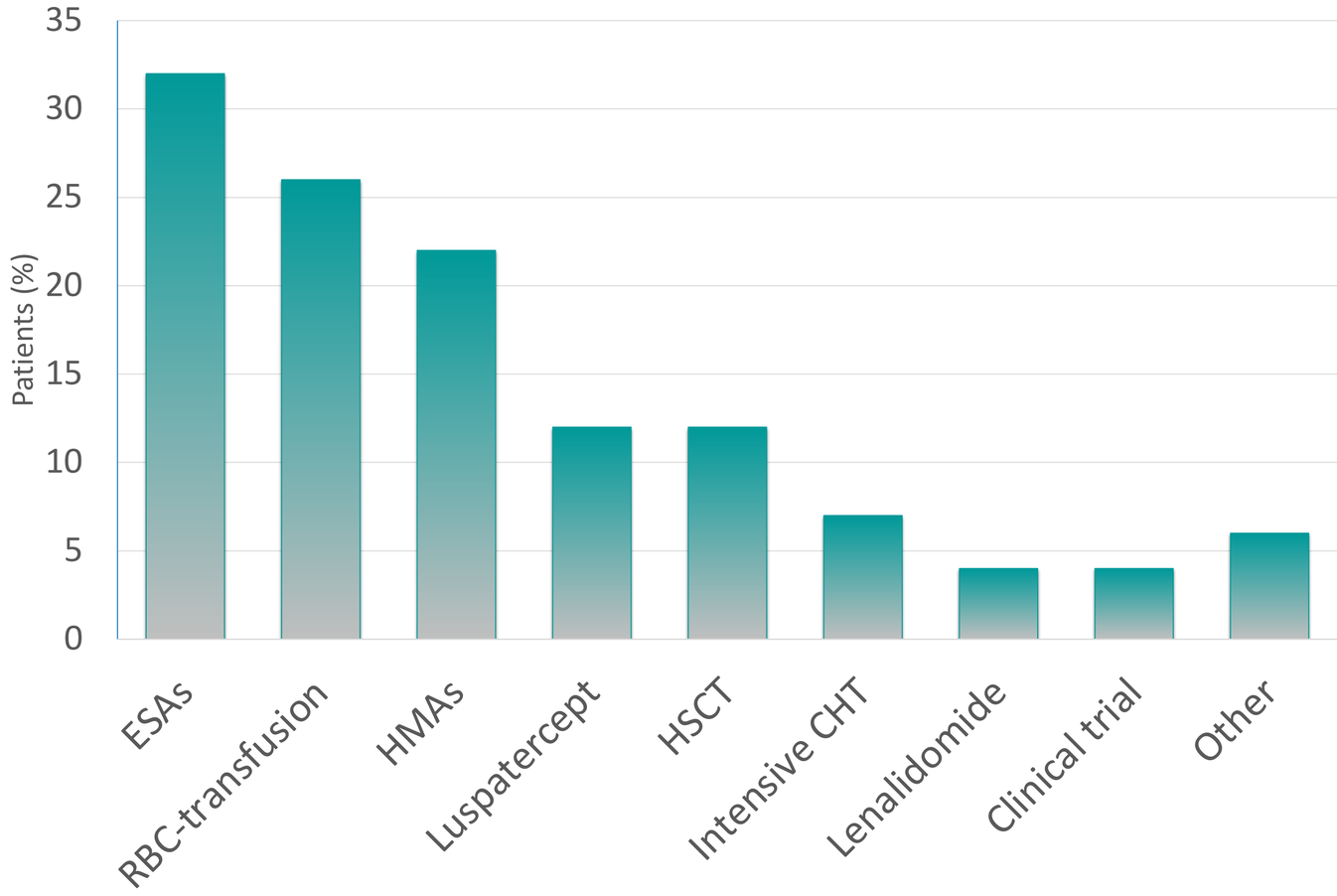
STUDY POPULATION

	Patients	1 timepoint	2 timepoint	3 timepoints	4 timepoints	5 timepoints	6 timepoints	7 timepoints	8 timepoints	Samples
IRCCS Istituto Clinico Humanitas-Rozzano	212	75	62	27	20	13	5	2	3	493
Ospedale CTO – Roma	92	28	29	14	9	10	2	0	0	231
AOU Careggi – Firenze	74	23	20	20	8	3	0	0	0	170
Ospedale Oncologico Businco – Cagliari	66	20	19	22	3	2	0	0	0	144
Ospedali riuniti – Ancona	64	41	12	8	1	0	0	0	0	95
IRCCS Ca' Granda Ospedale Maggiore Policlinico – Milano	39	21	17	2	0	0	0	0	0	61
AOU Maggiore della Carità - Novara	38	13	12	7	5	2	0	0	0	89
Policlinico S.Orsola Malpighi – Bologna	37	14	17	5	0	1	0	0	0	69
Ospedale San Francesco - Nuoro	28	15	8	2	2	0	0	0	0	50
Ospedale degli Infermi – Biella	23	4	5	4	3	5	2	0	0	75
IRCCS Casa Sollievo della Sofferenza – S. Giovanni Rotondo	21	16	5	0	0	0	0	0	0	26
AOU consorziale Policlinico – Bari	21	1	8	6	2	4	0	0	0	63
AO SS. Antonio e Biagio –Alessandria	13	8	4	0	1	0	0	0	0	25
IRCCS ospedale San Raffaele – Milano	18	9	7	2	0	0	0	0	0	29
IRCCS San Martino – Genova	17	2	10	2	3	0	0	0	0	39
AO Ordine Mauriziano – Torino	17	5	1	4	4	3	0	0	0	50
Cliniche San Pietro –Sassari	16	7	8	1	0	0	0	0	0	26
AO Città della Salute e della scienza - Torino	13	3	2	4	3	0	1	0	0	37
AOU San Giovanni di Dio e Ruggi D'Aragona – Salerno	13	7	4	2	0	0	0	0	0	22
ASST dei Sette Laghi – Varese	12	7	2	2	0	0	0	0	0	15
Ospedale A. Perrino – Brindisi	9	1	2	1	1	4	0	0	0	32
Centro di riferimento oncologico – Aviano	9	1	8	0	0	0	0	0	0	17
FPO-IRCCS Candiolo Cancer Institute – Candiolo	8	5	2	1	0	0	0	0	0	14
Azienda sanitaria locale 1 imperiese – Imperia	6	6	0	0	0	0	0	0	0	6
ASL TO4 Chivasso- Ivrea Cirié	6	1	1	4	0	0	0	0	0	15
Grande Ospedale Metropolitano - Reggio Calabria	5	1	3	1	0	0	0	0	0	10
Pia fondazione di culto e religione Card. G. Panico – Tricase	3	3	0	0	0	0	0	0	0	3
AO Sant'Anna e San Sebastiano - Caserta	2	2	0	0	0	0	0	0	0	2
	882	**339**	**268**	**141**	**65**	**47**	**10**	**2**	**3**	**1891**

STUDY POPULATION

Characteristic	N= 882[1]
Sex	
Female	305 (34.5%)
Male	577 (65.5%)
Age	75 (24-96.4)
WHO 2016 classification	
CMML	96 (11%)
MDS with isolated de (5q)	48 (5.5%)
MDS-EB-1	95 (10.8%)
MDS-EB-2	86 (9.7%)
MDS-MLD	311 (34.2%)
MDS-RS-MLD	73 (8.3%)
MDS-RS-SLD	74 (8.4%)
MDS SLD	91 (10.4%)
MDS-U	8 (0.9%)
Haemoglobin g/dL	9.8 (5-16.8)
WBC x10^3/mmc	4 (0.3- 96.3)
ANC x10^3/mmc	1.9 (0-38.2)
AMC x10^3/mmc	0.4 (0-12.9)
Platelets x10^3/mmc	136 (3-1073)
BM blasts %	2 (0-19)

[1]n (%); median (min-max)



DNA SEQUENCING

- Targeted Next Generation Sequencing (NGS) was performed at the Genomics Laboratory of Myeloid Neoplasms of the Humanitas Research Hospital

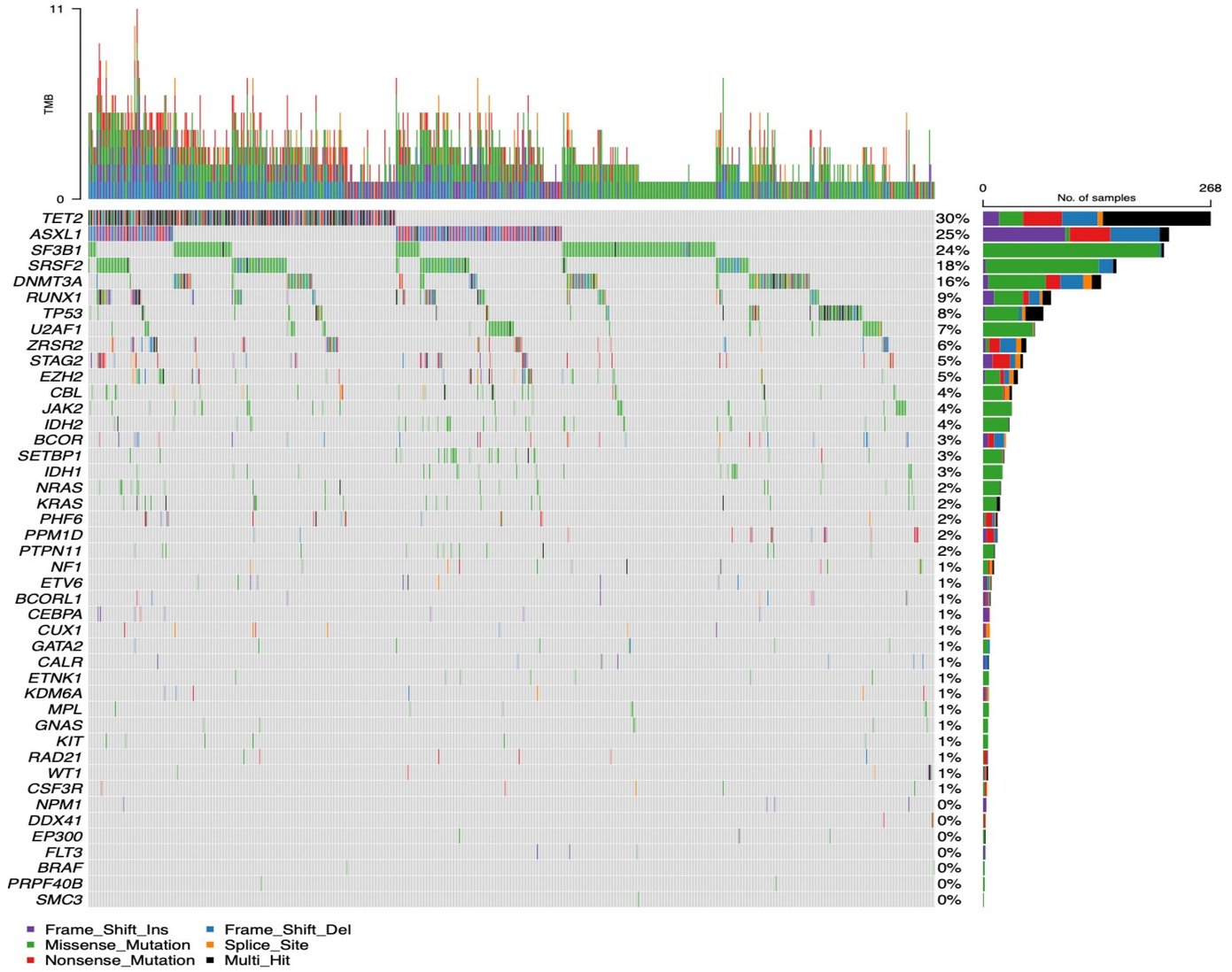
- Genomic DNA was extracted from 1891 peripheral blood samples and subsequently sequenced using the NextSeq 550 sequencer (Illumina) with a panel of RNA probes designed to isolate the coding exons of 48 genes frequently mutated in MDS (ABL1, ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CDKN2A, CEBPA, CSF3R, CUX1, DDX41, DNMT3A, EP300, ETNK1, ETV6, EZH2, FLT3, GATA2, GNAS, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KMT2A (MLL), KRAS, MPL, NF1, NPM1, NRAS, PHF6, PPM1D, PRPF40B, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2)

- Only variants with a sequencing depth > 500X and a Variant Allele Frequency (VAF) > 1% were further analyzed by comparing them with population databases (1000 Genomes project, the Exome Sequencing Project, Exome Aggregation Consortium, GnomAD) and pathogenicity databases (COSMIC) to identify pathogenic and likely pathogenic somatic variants and exclude artifacts and polymorphisms of unknown significance

RESULTS

Data analysis has shown concordance between the collected data and existing literature on somatic mutations in MDS patients:

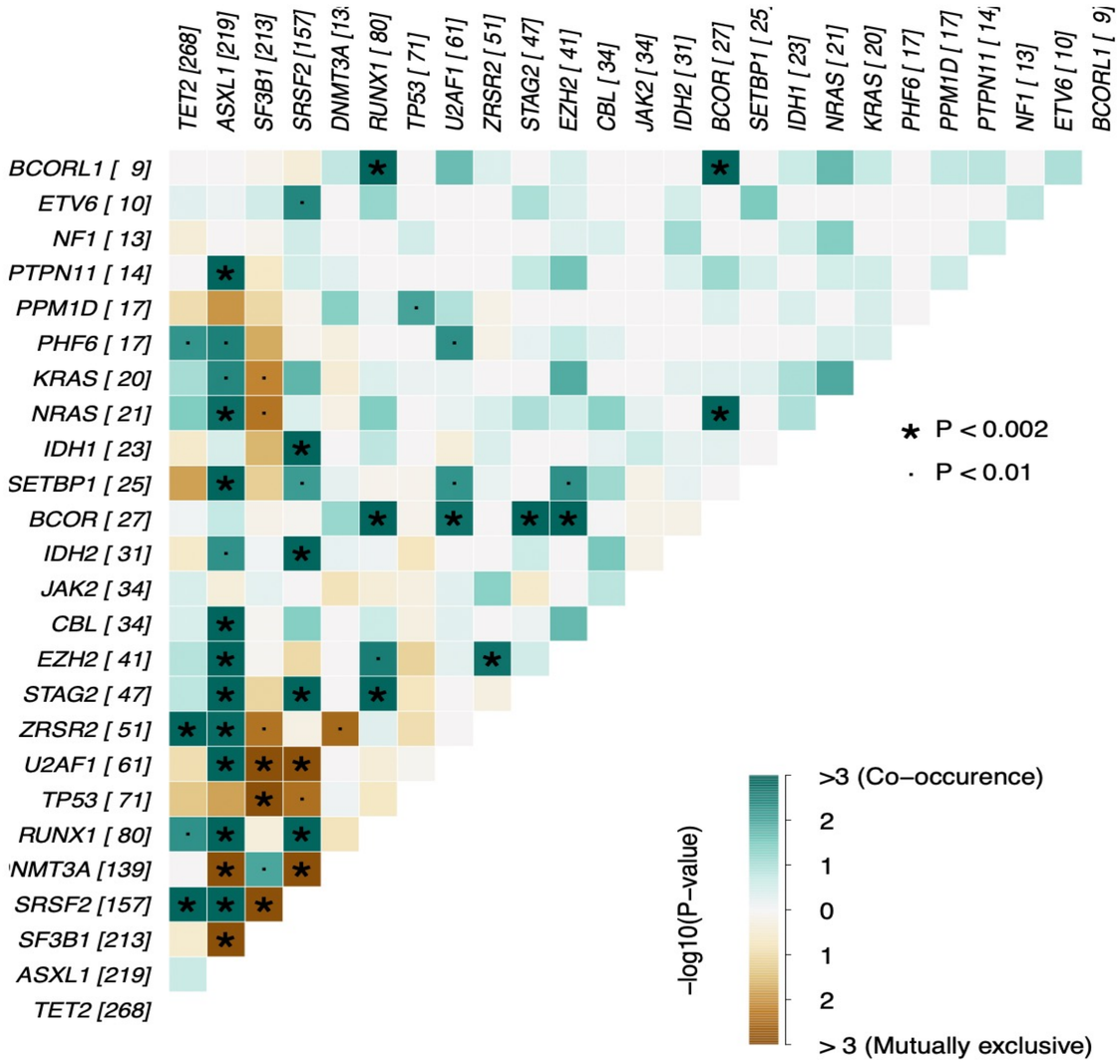
- 86% of patients harbored somatic mutations at time of diagnosis, with a median number of 2 mutations per patient (range 1-11)
- Identified mutations involved epigenetic regulators and splicing factor genes with higher frequency (TET2, ASXL1, SF3B1, SRSF2, DNMT3A), as well as genes involved in critical signaling pathways (TP53, RUNX1)
- The mutations primarily consisted of single nucleotide missense variants



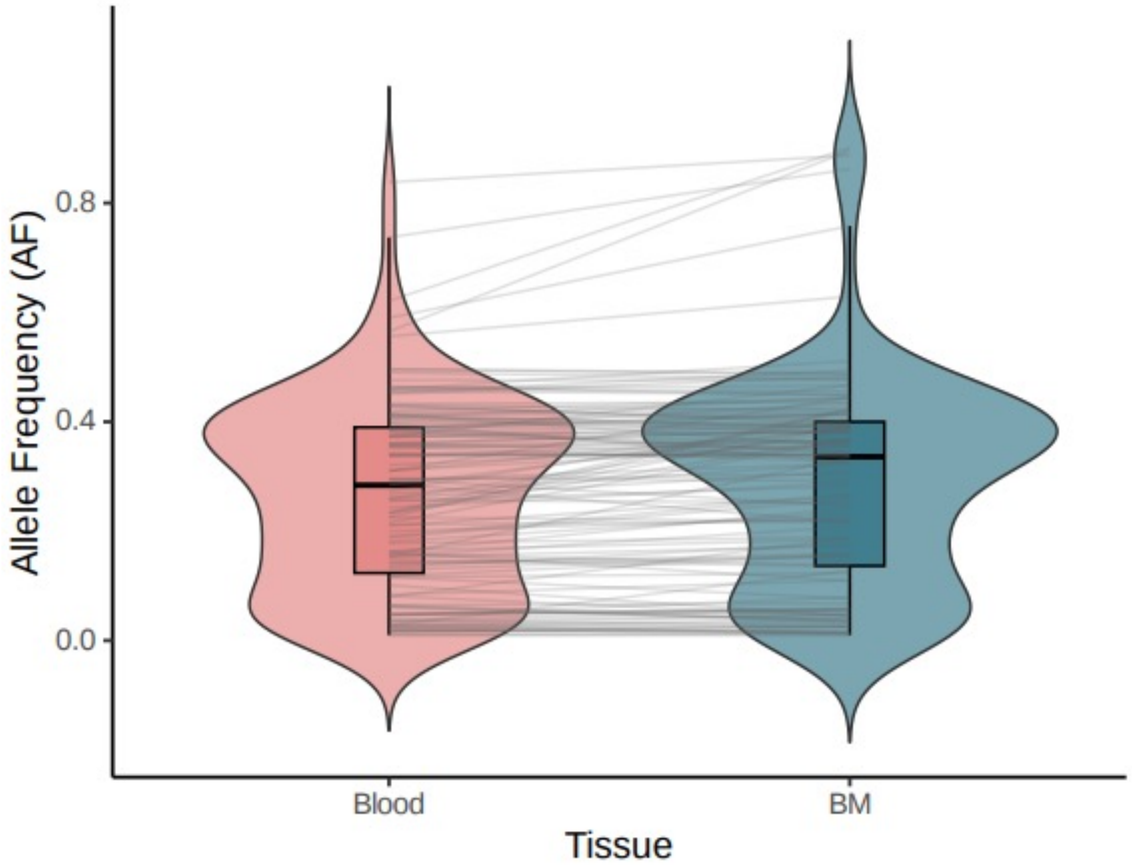
RESULTS

Correlation analyses revealed distinct patterns of co-occurrence and exclusivity among mutations:

- Mutations in SF3B1 were found to be mutually exclusive with mutations in other splicing factors (e.g., SRSF2, U2AF1, ZRSR2) and proliferative genes (e.g., KRAS, NRAS, TP53)
- In contrast, genes such as ASXL1, BCOR, and RUNX1 tend to co-mutate with a broader range of other genes



RESULTS

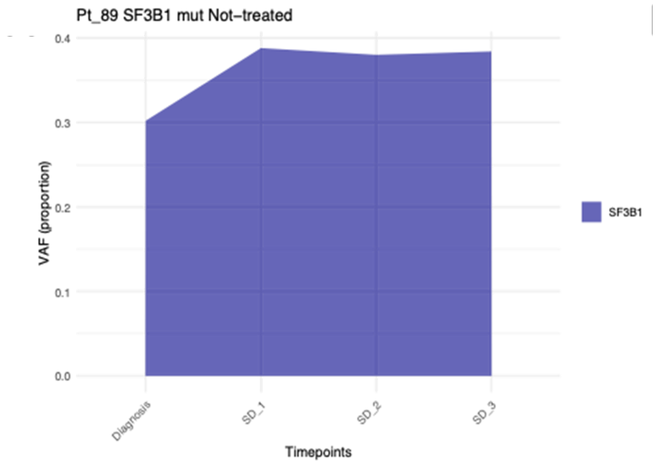


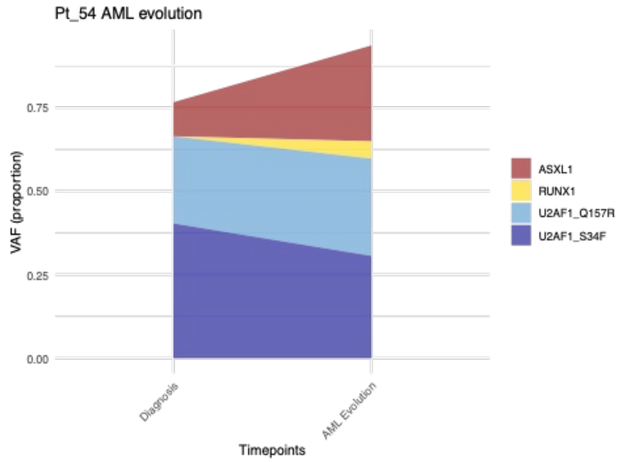
To validate mutation detection accuracy, a subset of PB samples was analyzed in parallel with paired bone marrow (BM) samples

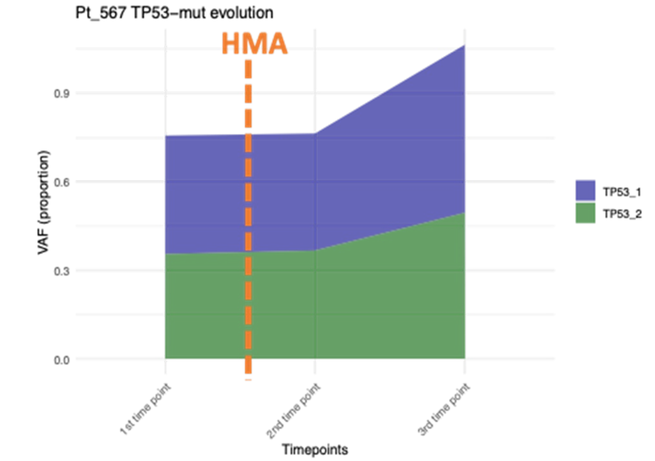
- Analysis of paired PB/BM samples from 115 patients revealed 97.1% concordant variants
- The few discordant variants had a variant allele frequency (VAF) <3%. The rate of discordant events remained low (3.8%) even when restricting the analysis to variants with VAF <5%
- Overall, VAF values from BM samples were slightly higher than those from paired PB, with a median difference of 2% ($p < 0.001$)

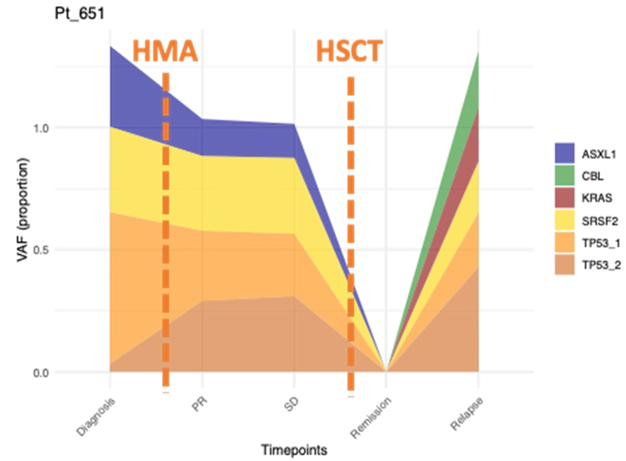
These data confirm that the mutational analysis performed on peripheral blood is as reliable and reproducible as that performed on bone marrow samples

RESULTS







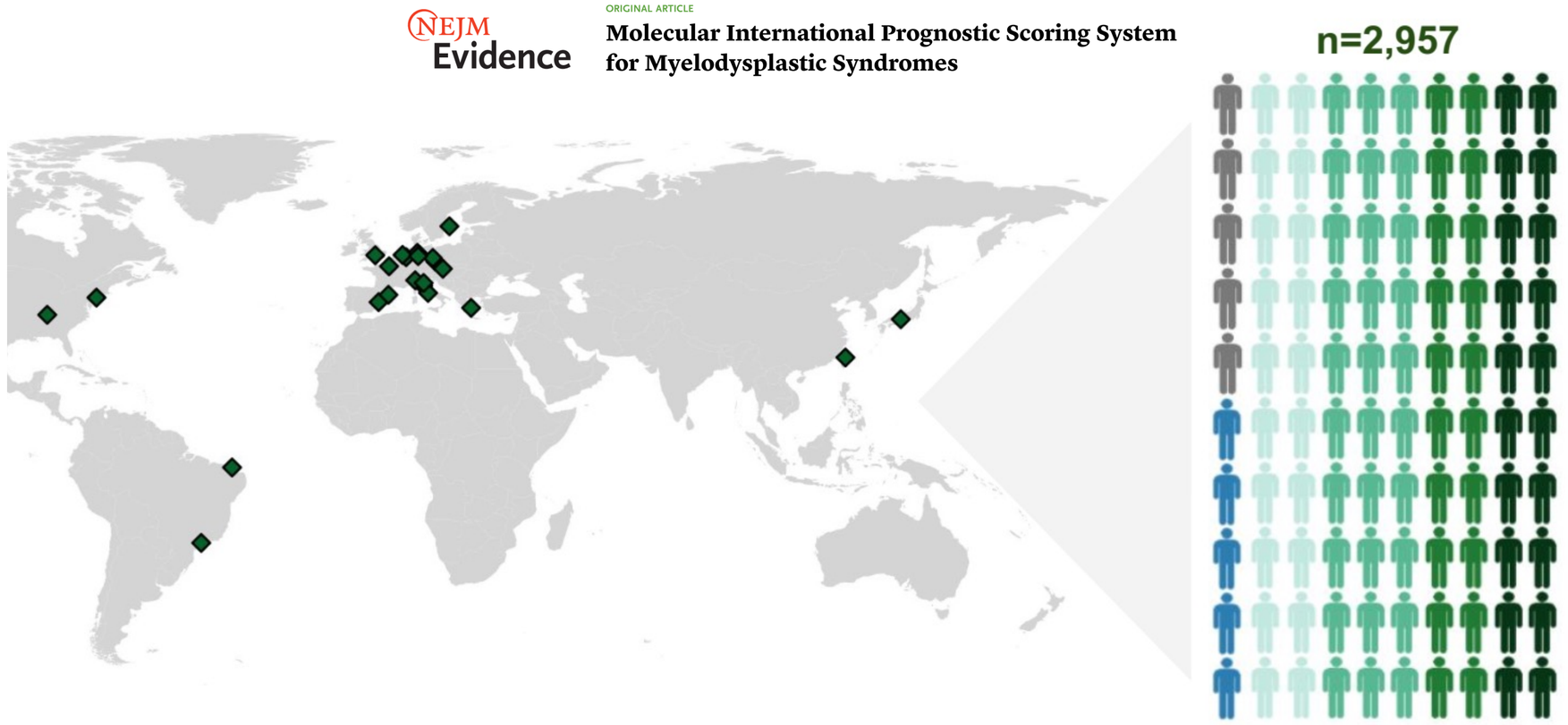


- Preliminary longitudinal analyses on several PB samples suggested that the variant allele frequency of mutational clones exhibits varying behaviors over time across all patients, reflecting different clinical course

- Thus, longitudinal analysis could improve the comprehension of both the natural evolution of the disease and the response to treatment

RESULTS

The Molecular International Prognostic Scoring System (IPSS-M) represents the state-of-the-art for risk stratification at diagnosis; however, its applicability in a dynamic, longitudinal context has not yet been validated.



RESULTS

Characteristic	n (%)
IPSS-R Cytogenetic Risk	46 (4.7%)
Very Good	680 (69%)
Good	178 (18%)
Intermediate	40 (4.1%)
Poor	38 (3.9%)
Very Poor	
IPSS-R Risk	
Very Low	199 (20%)
Low	421 (43%)
Intermediate	193 (20%)
High	108 (11%)
Very High	61 (6.2%)
IPSS-M Risk	
Very Low	154 (16%)
Low	389 (40%)
Moderate Low	141 (14%)
Moderate High	87 (8.9%)
High	129 (13%)
Very High	82 (8.4%)

- In our cohort 31% of patients were classified as IPSS-M moderate-high or higher at baseline

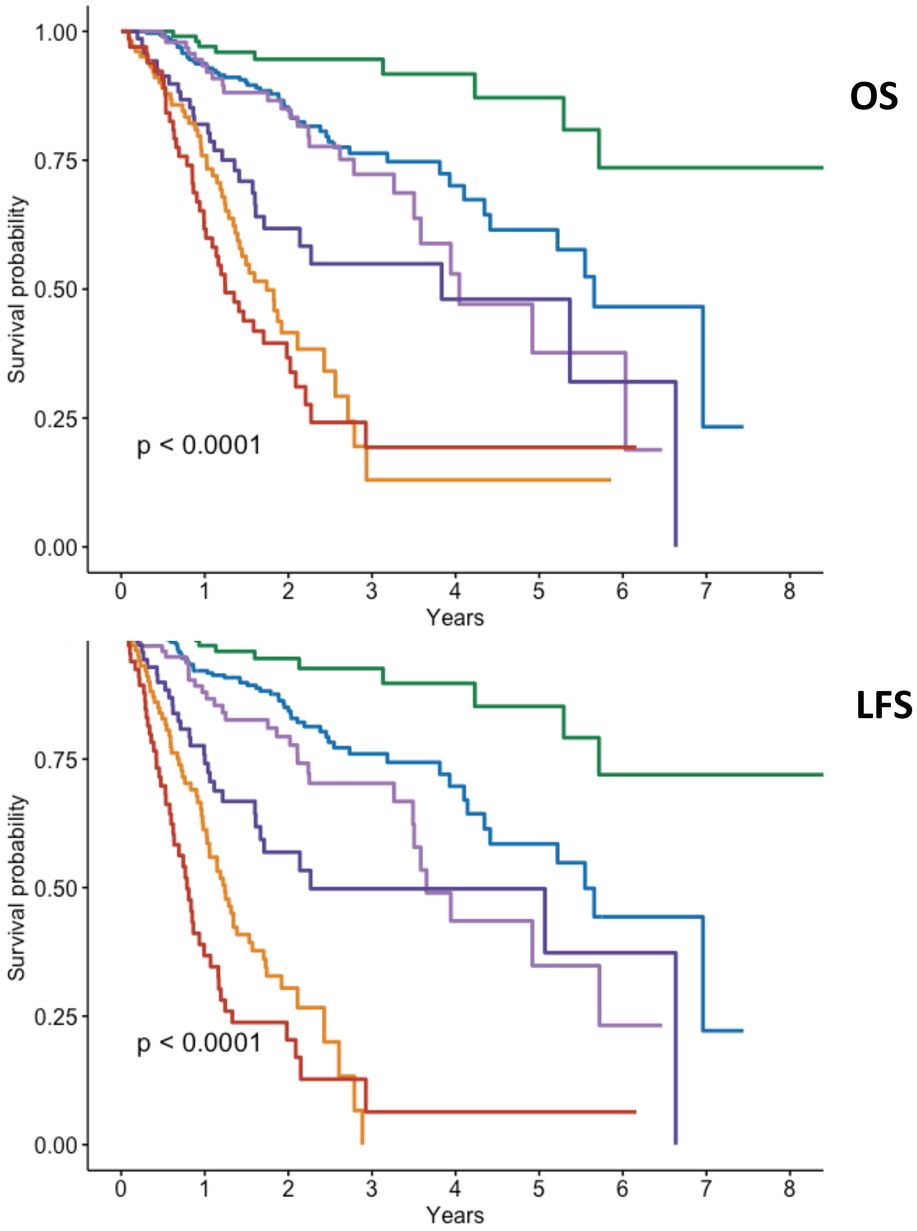
- Dynamic validation of the IPSS-M was performed using time-dependent Cox regression models, with model performance assessed by concordance index (c-index) metric

RESULTS

- Based on longitudinal clinical and genomic data, dynamic IPSS-M risk classification changed over time in 28.6% of patients

- Compared with conventional baseline assessment, dynamic IPSS-M showed improved predictive performance across all clinically relevant outcomes

Outcome	Static Model c-index	Dynamic Model c-index	Difference
Overall Survival	0.74	0.80	6%
Leukemia-Free Survival	0.77	0.81	4%



CONCLUSIONS

- Preliminary results from the FISIM-NGS-MDS study highlight that the evolving mutational landscape is intricately linked with clinical outcomes in MDS patients, in particular disease progression

- Dynamic validation of the IPSS-M demonstrated superior prognostic performance compared to baseline conventional assessment, and provides the rationale for its use in re-evaluating individual patient risk over time during the disease course

- The mutational screening performed on peripheral blood has been shown to be as reliable and reproducible as the evaluation on bone marrow blood; therefore, it could represent a useful tool for non-invasive monitoring of clonal evolution in MDS patients

Overall, these findings support the concept that Next-Generation prognostic tools for MDS should be grounded in the analysis of longitudinal data

Clonal Evolution and Risk Assessment in Myelodysplastic Syndromes (MDS): A Prospective Study of Dynamic IPSS-M Validation and Evolutionary Trajectory Modeling by the Italian MDS Foundation (FISIM)

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THANK YOU FOR YOUR ATTENTION!