XIX CONGRESSO NAZIONALE SIES2026
Firenze | 4-6 marzo 2026
Palazzo degli Affari

Myeloproliferative disorders

ASXL1 VARIANT ALLELE FREQUENCY AS A MODULATOR OF GENETIC HETEROGENEITY IN MYELOFIBROSIS

G. Iaquinta[1], M. Breccia[2], P. Chiusolo[3], A. Laganà[2], M. Santopietro[4], M. Ragazzo[1], C. Cerroni[1], E. Rossi[3], A. Tamburini[5], S. Crescenzi Leonetti[5], M. Rossi[3], C. Tatarelli[6], E. Abruzzese[7], M. Trawinska[7], R. Latagliata[8], A. Andriani[9], L. Maurillo[10], L. Guarnera[10], K. Paciaroni[11], P. Cercola[12], G. Pessina[12], V. De Stefano[3] and P. Grammatico[1]

[1]Medical Genetics Experimental Medicine Department Sapienza University San Camillo-Forlanini Hospital; [2]Hematology Department of Translational and Precision Medicine Policlinico Umberto I Sapienza University; [3]Institute of Hematology Catholic University; [4]Hematology Department San Camillo--Forlanini Hospital; [5]Hematology Department San Giovanni Hospital; [6]Unit of Hematology Sapienza University Sant'Andrea Hospital; [7]Sant'Eugenio Hospital Tor Vergata University; [8]Hematology Department Belcolle Hospital; [9]Hematology Department Nuovo Regina Margherita Hospital; [10]Hematology Department AOU Policlinico Tor Vergata; [11]Hematology Department Santo Spirito Hospital; [12]Laboratory of Medical Genetics Santa Rosa Hospital.

Introduction: Mutations in ASXL1 represent one of the most frequent additional lesions in myelofibrosis (MF). Although the prognostic significance of ASXL1 mutations is well recognized, the role of ASXL1 variant allele frequency (VAF) as a potential modulator of genetic heterogeneity and clonal complexity in MF has not yet been fully elucidated. This study aims to explore whether ASXL1 VAF correlates with overall mutational burden and to identify a potential cut-off with biological and clinical relevance.

Methods

We analyzed a cohort of 53 ASXL1-mutated myelofibrosis (MF) samples, including 6 early-PMF, 33 overt PMF, and 14 SMF cases. For each sample, targeted next-generation sequencing (NGS) was performed using a 73-gene myeloid panel with a minimum VAF detection threshold of 3%. For statistical analysis, only pathogenic and likely pathogenic mutations were considered. A comparison between ASXL1 VAF and the number of mutations or the number of pathways involved were performed using the Mann–Whitney test for quantitative variables and Fisher's exact test for qualitative variables. A receiver operating characteristic (ROC) curve was generated using the free Jamovi software to identify the optimal ASXL1 VAF cut-off predictive of higher genetic complexity.

Results: Samples with low ASXL1 VAF predominantly harbored two mutations, typically driver mutation in combination with ASXL1 (VAF median, 14.0%; VAF mean ± SD, 17.1 ± 13.8%). Conversely, samples with higher ASXL1 VAF exhibited a significantly greater mutational burden, generally >2 variants - mean 3.92 (VAF median, 34.0%; VAF mean ± SD, 28.1 ± 15.6%) [Figure 1 – Table 1], frequently involving high-molecular-risk (HMR) genes such as EZH2, U2AF1 and SRSF2, as well as non-HMR genes like *NRAS*. ROC curve analysis identified an ASXL1 VAF of 19.70% as the optimal cutoff for discriminating cases with more than two variants or more than two functional classes involved (AUC = 0.714; sensitivity, 69.4%; specificity, 66.7%) [Figure 2 – Table 2]. These findings suggest a quantitative relationship between ASXL1 VAF and genomic complexity in myelofibrosis.

Conclusions

Our data indicate that increasing ASXL1 VAF is associated with higher clonal complexity and the involvement of multiple functional pathways in myelofibrosis. This pattern may reflect an expansion of genetically heterogeneous subclones driven by chromatin dysregulation, leading to the loss of gene repression and contributing to clonal evolution, an effect that appears more pronounced as the size of the mutant ASXL1 clone increases. These findings support the concept that the allelic burden of ASXL1 could serve as a quantitative biomarker of genomic heterogeneity and disease evolution in myelofibrosis, potentially aiding future risk stratification and clinical decision-making.

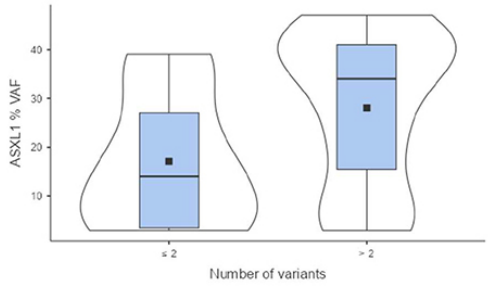
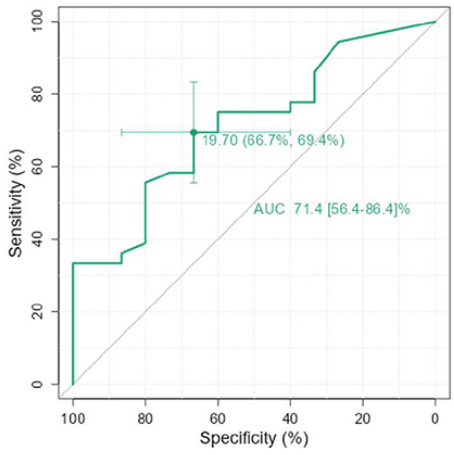


Figure 1. Distribution of ASXL1 variant allele frequency (VAF) according to mutational burden (≤2 vs >2 variants) in ASXL1-mutated myelofibrosis (MF) patients.

	Variants	N	Media	Mediana	SD	p
ASXL1 % VAF	**≤ 2**	15	17.1	14.0	13.8	
	> 2	36	28.1	34.0	15.6	
t di Student						0.022
U di Mann-Whitney						0.017

Note. H_a μ≤ 2 ≠ μ> 2

Table 1. Descriptive statistics of ASXL1 variant allele frequency (VAF) stratified by mutational burden (≤2 vs >2 variants) in ASXL1-mutated myelofibrosis (MF) patients. Mean, median, standard deviation (SD), and p-values were calculated using Student's t-test and Mann–Whitney test.



ROC Curve Summary

	AUC	Std. Error	95% Confidence Interval Lower	95% Confidence Interval Upper	p
ASXL1 % VAF	0.714	0.0763	0.564	0.864	0.005

Figure 2 and Table 2. Receiver operating characteristic (ROC) curve and corresponding summary statistics evaluating the predictive ability of ASXL1 variant allele frequency (VAF) for identifying cases with increased genetic complexity (>2 variants or >2 functional classes). The optimal cut-off value for ASXL1 VAF was 19.70% (based on optimal Youden's Index score), with an area under the curve (AUC) of 0.714 (95% CI, 0.564–0.864; p = 0.005), sensitivity of 69.4%, and specificity of 66.7%.