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Poster # H011

Assessment of *FLT3* Somatic Variant Oncogenicity using *FLT3*-Gene-Specific Guidelines in Acute Myeloid Leukemia

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Background

- Comprehensive assessment of somatic variant oncogenicity is crucial to avoid discrepancies in clinical settings, especially for the *FLT3* gene, which is mutated in about 30% of Acute Myeloid Leukemia (AML) cases and is often associated with poor prognosis.
- *FLT3* internal tandem duplications (ITD) and tyrosine kinase domain (TKD) missense variants play a key role in the diagnosis, prognosis, and therapy of AML patients.
- There is a lack of clarity on the role of missense and indel variants detected in other protein domains, therefore, we developed *FLT3*-specific LabPMM® guidelines based on the somatic variant classification by Horak et al. (2022), and the latest available literature.

Methods

- *FLT3* SNVs (77) and indels (524) detected by LabPMM's CAP/CLIA certified MyAML® and MyMRD® NGS gene panels, which target genes specific for both MDS and AML, were assessed. These variants were previously classified as suspicious variants of uncertain significance (VUS-Suspicious), likely oncogenic (LO), or oncogenic (O).
- We also compared our interpretations with the preliminary guidelines for missense variants by the ClinGen *FLT3* Somatic Cancer Variant Curation Expert Panel (SC-VECP).

Results

❑ Reclassification of 77 missense variants using LabPMM somatic variant classification guidelines

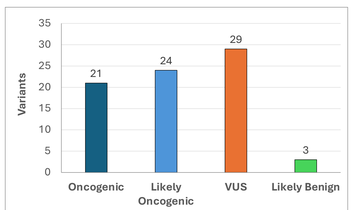

Figure 1: Reclassified *FLT3* missense variants; Using updated LabPMM somatic variant interpretation guidelines, we reassessed evidence for the previously classified O, LO and VUS-suspicious variants.

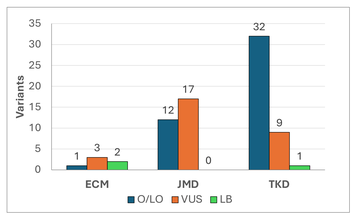

Figure 2: *FLT3* missense variants distribution by protein domains; Abbreviations: ECM-extracellular domain, JMD-juxtamembrane domain and TKD-tyrosine kinase domain.

❑ Which evidence impacts reclassification of missense variants?

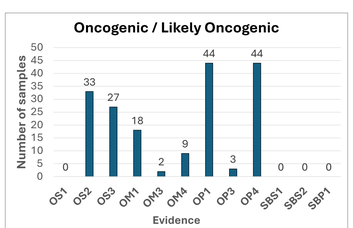

Figure 3A: Evidence used to classify variants as Oncogenic/Likely Oncogenic variants; Functional assays (OS2), sample counts (OS3/OM3/OP3), protein domain (OM1), and computational evidence (OP3) had the most impact on reclassification.

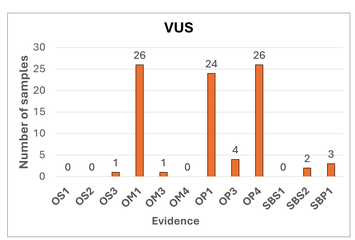

Figure 3B: Evidence used to reclassify variants as VUS; Benign functional impact (SBS2)/lack of functional studies, and/or other evidence were important factors for VUS classification.

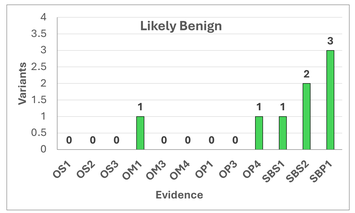

Figure 3C: Evidence used to classify variants as Likely benign; Population frequencies (SBS1) and benign functional impact (SBS2) had the most impact.

KEY POINTS

- Reclassification of *FLT3* missense variants resulted in 58% O/LO and 42% VUS/LB variants.
- Evidence like functional assays, sample counts, protein domain, and computational prediction evidence had the most impact on classifying the variants to O/LO.
- Benign protein effect or lack of functional data had the most significant impact on the variants reclassified as VUS/LB.

❑ Does confirmed somatic origin of a variant affect variant reclassification?

Table 1: Evaluation of variant sample counts (OP3 evidence) using LabPMM and *FLT3* SC-VCEP guidelines

Guidelines	Evidence	Description
LabPMM	OS3 – 4 points	12 samples at same codon 3 samples with same missense change
	OM3 – 2 points	8 samples at same codon 3 samples with the same missense change
	OP3 – 1 point	<8 samples at same codon 3 samples with same missense change
***FLT3* SC-VECP**	OS3 – 4 points	15 heme samples at same codon 5 samples with same missense change 3 confirmed somatic
	OM3 – 2 points	<15 heme samples at same codon 5 samples with same missense change 3 confirmed somatic
	OP3 – 1 point	3 heme samples with same missense change

Table 2: Results of OP3 evidence evaluation; Somatic origin of a variant impacted about 8% of the variant classifications

Impact	Variant count	Comments
O to LO	3	Change in evidence/classification
LO - no change	4	Change in evidence but no change in classification
LO to VUS	4	Change in evidence and/or classification
Inconclusive	2	Not enough confirmed somatic samples
No change	63	No change in evidence/classification

❑ Does assessing drug* response data to determine oncogenicity of a variant affect its classification?

- In a subset of 27 missense variants for which drug response data was available, we did not observe a change in classification category.
- We currently include the drug response data in the variant description for O/LO variants instead of evaluating it as an additional evidence point towards oncogenicity.
- For VUS variants, which are otherwise excluded from the report, applying AMP/ASCO/CAP tier-based guidelines evidence along with variant oncogenicity may provide a better approach for clinical reporting.

* Drug response data was evaluated for *FLT3* kinase inhibitors

❑ Reclassification of *FLT3* insertion/deletion variants using LabPMM guidelines

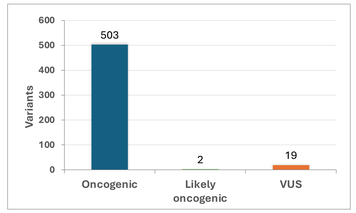

Figure 4: *FLT3* indel (N=524) variants were reclassified using LabPMM guidelines; Indel variants include insertions and deletions.

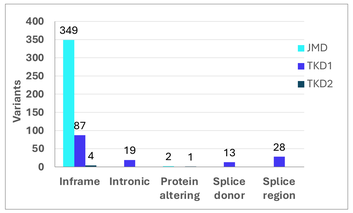

Figure 5: Location of O/LO *FLT3* insertions (N=503) based on variant ontology; The variant ontology included in-frame insertions, and out-of-frame insertions/deletions that involved exon14, intron 14 and exon 15 of the *FLT3* gene.

- Overall, 505 variants were reclassified as likely oncogenic and oncogenic.
- We also identified complex insertions in the TKD2 domain which affect the D835/I837 residues.
- Most of the variants were inframe insertions with an integration site in the JMD and TKD, while others had integration site in intron 14.

Table 3: Distribution of *FLT3* ITDs (N=499) in the JMD and TKD1; ITD count, size range and median are provided for the variants in the JMD and TKD1

Domains	JMD	TKD1
ITD count	351	148
ITD Size Range (bp)	3-267	24-297
Median ITD Size (bp)	46.4	80

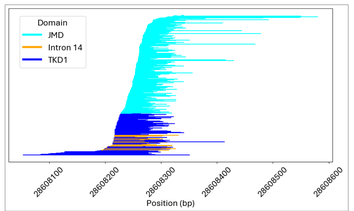

Figure 6: Graphical representation of *FLT3* ITDs; Integration site of an ITD is represented by the GRCh37 chromosome position on the X-axis; size of the bar represents length of individual ITDs.

- We identified 351 ITDs in the JMD and 148 ITDs in the TKD1 domains.
- Median insertion size for ITDs in the TKD1 domain was longer when compared to ITDs in JMD.
- Additionally, 32 ITDs had integration site within intron 14 and are expected to result in a longer, yet functional, protein.
- Nomenclature of intronic ITDs and additional functional studies are needed to better assess the impact on protein function and oncogenicity assessment of these variants.

Conclusions

- The *FLT3*-gene-specific guidelines provide a better framework to assess the oncogenicity of *FLT3* variants and their clinical significance.
- Applying AMP/ASCO/CAP tier-based guidelines along with variant oncogenicity may provide a precise approach for clinical reporting.
- *FLT3* insertions resulting in complex in-frame and out-of-frame events require further assessment to establish their oncogenicity.