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Poster # H029

Impact of reclassification and subcategorization of variants of uncertain significance in Acute Myeloid Leukemia

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BACKGROUND

Next generation sequencing assays detect an abundance of variants of uncertain significance (VUSs). Although it is presumed that many VUSs will eventually be classified as benign, a small number of them may be clinically important. Reclassification rates of VUSs are not known in Acute Myeloid Leukemia (AML). Overall, there is a lack of information on when and how to reclassify VUSs in somatic cancers, including AML. Here, we reclassified VUSs to determine overall reclassification rates. Furthermore, VUSs were subcategorized to determine how often to perform variant reviews, how to track variant evidence, and how to incorporate VUS subcategories into clinical reporting.

METHODS

- A total of 635 variants, detected by LabPMM®'s CLIA validated MyAML® and MyMRD® NGS assays, which target genes specific for both Myelodysplastic syndromes (MDS) and AML, were assessed. These variants were previously classified as VUS using the LabPMM somatic variant interpretation guidelines.
- All the variants were reclassified based on latest research articles.
- VUSs were further subcategorized based on the evidence points following the chart (Figure 1).

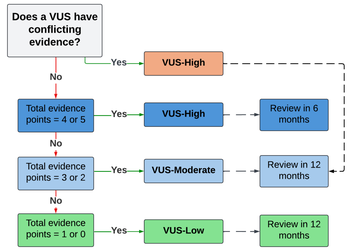

Figure 1: Chart for VUS subcategorization. Variants were labeled as VUS-High (VUS-H, 4-5 pts), VUS-Moderate (VUS-M, 2-3 pts), VUS-low (VUS-L, 0 or 1 pts). VUS-Conflicting (VUS-C) includes VUS with both pro-oncogenic and pro-benign evidence. The review period was approximately 2 years after their initial classification.

RESULTS

Impact of VUS reclassification

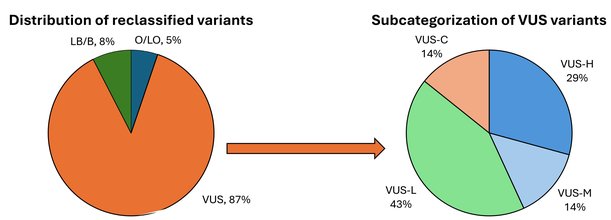

Figure 2: Distribution of reclassified variants. The first pie chart represents reclassified variants-O/LO, VUS, and LB, arrow indicates second pie chart, which represents subcategorization of the VUS variants into VUS-H, VUS-M, VUS-L, and VUS-C. Abbreviations: Oncogenic-O, Likely oncogenic-LO, Likely benign-LB, and Benign-B.

- A total of 33 variants were reclassified as O/LO and 48 as LB/B.
- VUS (n=554) remained unchanged and include varied evidence.
- Upon subcategorization we detected that 29% variants fall in VUS-H, while 43% in VUS-L, two groups with significantly high variants.

Distribution of evidence groups across all variant classifications

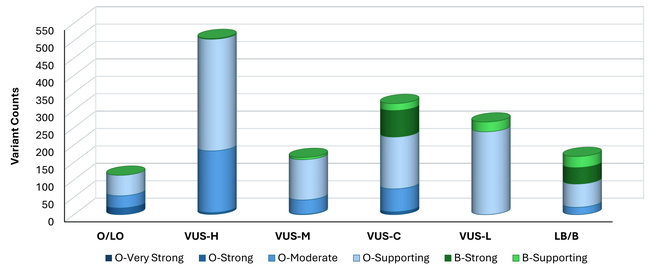

Figure 3: Distribution of evidence groups across all variant classifications. X-axis represents variant classifications; segment size represents variant counts within each evidence group. O-Very strong = OVS1, O-Strong = OS2 and OS3, O-Moderate = OM1 to OM4, Supporting = OP1, OP3, and OP4; B-Strong = SBS1 and SBS2, B-supporting = SBP1 and SBP2; Abbreviations: Oncogenic-O, and Benign-B.

- VUS-H have the highest pro-oncogenic evidence and appear to have more potential to move up to LO/O classification.
- VUS-C had evidence both in favor of oncogenic and benign impact, largely due to conflicting functional assay results. VUS-C include only *TP53* gene variants.
- VUS-L represents variants with the least available evidence and most of the time the available evidence was either population frequencies or computational prediction results.
- Computational predictions were not considered as conflicts for the VUS-subcategories.

Which variant ontology is common in the reclassified variants?

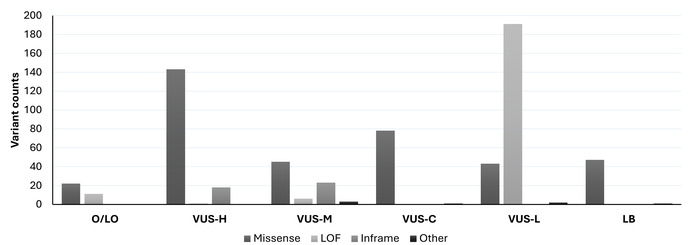

Figure 4: Summary of variant type across all variant classifications. LOF-loss of function variants (frameshift, nonsense, splice donor/acceptor). "Other" variant types includes intronic, synonymous, and splice region variants.

- Missense variants are the most frequently detected variants across all VUS subcategories.
- LOF variants were higher in VUS-L category. These variants were in genes where gain of function mechanism is a known disease mechanism for AML or the impact of SNV/indels is not known. This includes genes like *KMT2A*, *KMT2B*, and *MYC*, for which gene fusions and copy number variations are associated with AML, while the role of SNV/indels is unclear.
- Inframe indel variants were the next significant group (6.5%) followed by other variant types comprised only 1.1% of total variants. Inframe insertions were detected in the *ZRSR2* gene where the role of these variants is unknown.

Important AML genes and VUS subcategories

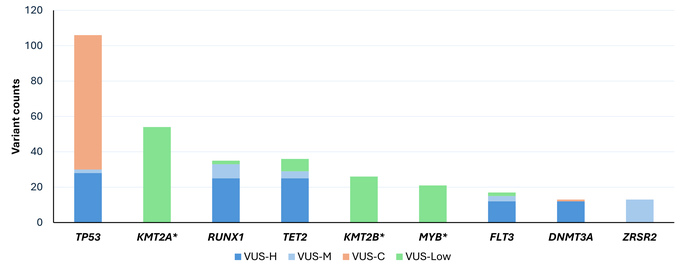

Figure 5A: Select AML genes and distribution of VUS variants in different subcategories. Asterix * represents genes with all variants classified as VUS-L

- *TP53*, *KMT2A*, *RUNX1*, *TET2*, *FLT3,* and *ZRSR2* represents the genes with most VUS variants. Other important genes had less than 5 variants and are not presented here.
- All the variants identified in the VUS-C subcategory are in *TP53* gene with conflicting functional results.
- VUS-L includes *KMT2A*, *KMT2B*, and *MYC*, for which gene fusions and copy number variants are frequently associated with AML, while the role of SNV/indels is unclear.
- VUS-subcategories provides an option to prioritize variants for review at different time intervals based on the available evidence (see figure 1) and minimize time spent during real time variant reviews.

AML genes associated with drug response

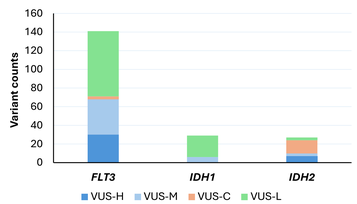

Figure 5B: Distribution of evidence type across AML genes associated with drug response. X-axis represent *FLT3*, *IDH1*, and *IDH2*, Y-axis represents number of variants in each variant category.

- Overall, 5 *FLT3*, 2 *IDH1*, and 3 *IDH2* variants associated with drug resistance were identified in VUS-H and VUS-C subcategories.
- These variants have an altered mechanism, currently not reported as oncogenic.
- By using VUS-subcategories, we can prioritize the review and reporting of these variants, which would otherwise not be reported.

CONCLUSIONS

- Overall, we observed a 12.8% reclassification rate for VUSs in AML.
- VUS subcategorization may help to prioritize VUSs for review, reduce overall review time, example is VUS-H which have more evidence compared to VUS-L subcategory.
- VUS reclassification also helps in reporting variants which are associated with drug response in AML but do not have enough evidence based on oncogenicity assessment.

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