Poster # H004 The Challenges of Classifying and Reporting *TP53* Variants in Acute Myeloid Leukemia (AML)

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INTRODUCTION

- The push for standardization in variant classification over the past few years has been a significant step towards providing consistency in data interpretation and reporting among clinical laboratories performing genetic testing. The ClinGen/CGC/VICC working group provided general guidelines for determining oncogenicity. However, one set of somatic standards is not suitable for all cancer types. Therefore, the guidelines need to be tailored for specific disease as well as the genes driving oncogenicity when necessary. *TP53*, a tumor suppressor that is mutated across many cancer types, is one gene for which the guidelines need to be tailored for classifications in myeloid malignancies. Here, we present how our laboratory has applied the ClinGen/CGC/VICC working group points system for addressing *TP53* variant classification in acute myeloid leukemia (AML).
- The presence of *TP53* variants in AML patients often confer poor prognosis, and therefore *TP53* variants in AML have been included in professional guidelines for clinical reporting for many years (Papaemmanuil et. al., 2016, Dohner et. al., 2017). Recent studies have provided further insight into how the variant allele frequency (VAF) of detected *TP53* variants plays a more significant role in prognosis than previously established. A *TP53* variant with a VAF of ≥10% is associated with inferior overall survival (OS) and progression-free survival whereas patients with a VAF of <10% had higher response rates to therapy and OS similar to patients with wild-type *TP53* (Daver et. al., 2022, Shah et. al., 2023). The recent guidelines published by European LeukemiaNet (ELN) utilize this data and present an updated genomic classification for *TP53* variants, in which a case must present with a *TP53* variant with a VAF of ≥10% (Dohner et. al., 2022). Using *TP53* variant data from previously reported clinical cases from our own laboratory, we assessed how updates to the 2022 ELN AML guidelines affect the reporting of cases with *TP53* variants.

METHODS

- Assessed variants included those detected via the CAP/CLIA-validated MyAML® and MyMRD® next generation sequencing (NGS) gene panels, which target genes specific for both myelodysplastic syndrome (MDS) and AML. Our analysis focused on suspicious variants of uncertain significance (VUS-Suspicious), which was a category used for genomic alterations that have not yet been identified as benign, but there is some evidence for oncogenicity. VUS-Suspicious classifications were reviewed using LabPMM® *TP53*-specific rules for AML and reclassified as benign (B), likely benign (LB), VUS, likely oncogenic (LO), or oncogenic (O).
- We compared the reclassified variants against submissions for the same variants in ClinVar. LabPMM previously utilized the AML classifications by Papaemmanuil et. al., 2016, where any case with a detected clinically significant *TP53* variant was classified as "AML with *TP53* mutations, chromosomal aneuploidy, or both." All MyAML and MyMRD cases from 2017-2022 with *TP53* variants were reviewed to see how the use of a ≥10% VAF threshold from ELN AML guidelines would impact reporting.

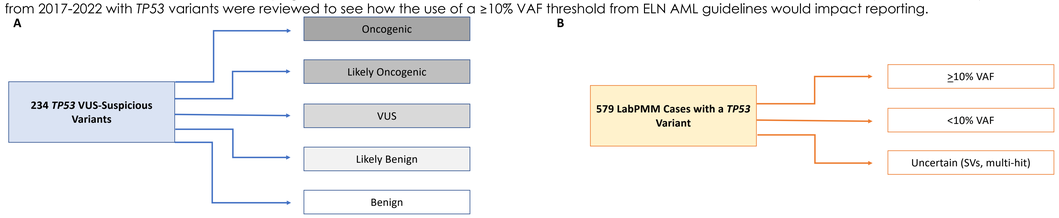

Figure 1. An overview of the methods. A total of 243 *TP53* variants previously classified as VUS-Suspicious (**Figure 1A**) were reclassified using LabPMM AML-specific decision trees based on the points system provided by Horak et. al., 2022 (**Table 1**). Subsequently, all cases from the LabPMM database with *TP53* variants were downloaded and assessed for how many cases had a *TP53* VAF of at least 10% (**Figure 1B**).

RESULTS

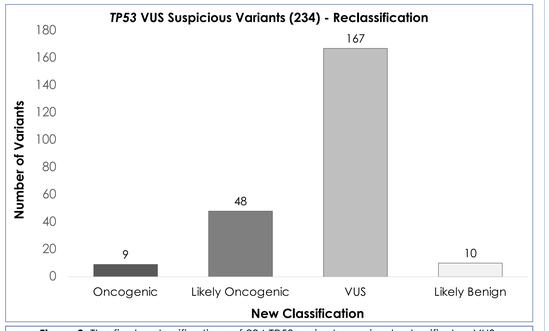

Figure 2. The final reclassifications of 234 *TP53* variants previously classified as VUS-Suspicious.

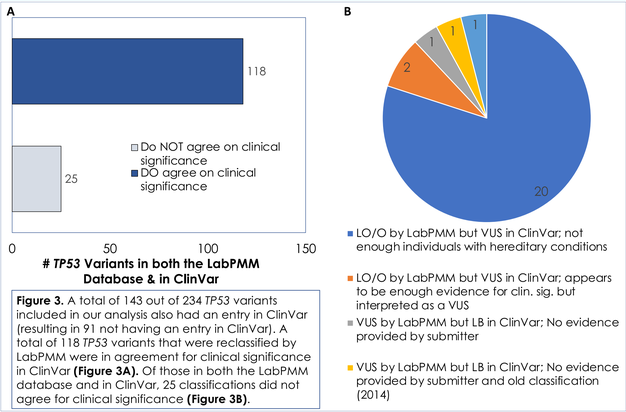

Figure 3. A total of 143 out of 234 *TP53* variants included in our analysis also had an entry in ClinVar (resulting in 91 not having an entry in ClinVar). A total of 118 *TP53* variants that were reclassified by LabPMM were in agreement for clinical significance in ClinVar **(Figure 3A).** Of those in both the LabPMM database and in ClinVar, 25 classifications did not agree for clinical significance **(Figure 3B).**

RESULTS

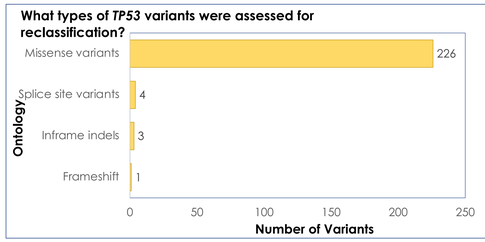

Figure 4. Of the 234 *TP53* VUS-Suspicious variants that were reclassified, 226 were missense variants. Additionally, 193 (85.4%) of the 234 variants were located in the P53 DNA binding domain.

What evidence was critical for reclassification of *TP53* VUS-Suspicious variants?

- **Oncogenic and likely oncogenic variants:**
  - Functional evidence from the *TP53* Database and other peer-reviewed studies, sample/hotspot data from the Catalogue of Somatic Mutations in Cancer (COSMIC), computational prediction scores, gnomAD minor allele frequency (MAF) (see **Table 1**)
- **VUS:**
  - Lack of functional evidence from the *TP53* Database or conflicting data across in vitro studies, lack or low number of samples with the variant in question from COSMIC, conflicting data in general
- **Likely benign variants:**
  - Functional evidence from the *TP53* Database and other peer-reviewed studies, computational prediction scores, gnomAD MAF

Table 1. How the guidelines from Horak et. al., 2022 were applied for LabPMM and *TP53* variants. The evidence types with the most significant impact on *TP53* variant reclassification is highlighted in green. The same points system was used as Horak et. al. 2022 with the exception of OVS1, which was awarded 10 points for null variants in *TP53*. SNV = single nucleotide variant; indel = insertion or deletion.

Evidence Code	Horak et. al., 2022; Description of Evidence	LabPMM; Description of How the Evidence Codes were Applied for *TP53*
OVS1	It is a null variant in a tumor suppressor gene	It is a null variant in *TP53* that results in a loss of function
OS1	The variant has a different nucleotide change but same amino acid change as a previously classified clinically significant variant	The variant has a different nucleotide change but same amino acid change as a previously classified clinically significant variant
OS2	The variant has functional evidence supporting an oncogenic effect	The variant has functional evidence showing an oncogenic effect either in the *TP53* Database at https://tp53.isb-cgc.org/ or from other published studies
OS3	A variant is located in a hotspots in cancerhotspots.org when the number of somatic variants at this codon is >50 **AND** there are ≥10 samples with exact same amino acid change	The variant is located in a hotspot where the number of somatic samples at that codon in COSMIC is ≥50 **AND** there are ≥10 samples with exact same amino acid change
OM1	The variant is located in a critical, well-established functional domain	The variant is located in the P53 transactivation motif, the P53 DNA binding domain, or in the P53 tetramerization motif
OM2	The variant results in a change in the length of the protein	The variant results in a protein length change that is not in a repetitive region **AND** there are well-established pathogenic SNVs or indels in that region
OM3	A variant is located in a hotspots in cancerhotspots.org when the number of somatic variants at this codon is <50 **AND** there are ≥10 samples with exact same amino acid change	The variant is located in a hotspot where the number of somatic samples at that codon in COSMIC is ≥20 **AND** there are ≥10 samples with exact same amino acid change
OM4	The variant is a missense change at an amino acid where a different missense change is oncogenic	The variant is a missense change at an amino acid where a different missense change is oncogenic **AND** the variant has a higher Grantham score than the previously classified oncogenic variant
OP1	Computational predictions support an oncogenic effect	Computational predictions from Capice, Mutation Assessor, etc. support an oncogenic effect (do not use PolyPhen2 or SIFT)
OP3	A variant is located in a hotspot; hotspots can be identified in cancerhotspots.org and OM3 is applied when the number of somatic variants at this codon is <10	The number of somatic samples with the same exact amino acid change in COSMIC is ≥3
OP4	The variant is absent from a healthy control cohort or at an extremely low frequency in gnomAD	The variant is absent or at an extremely low frequency in gnomAD

Table 2. For assessing updates to 2022 AML guidelines, of the 579 cases reported between 2017-2022, 294 had a *TP53* variant with a VAF of >10% , therefore meeting the threshold for "AML with mutated *TP53*" per the 2022 ELN guidelines. There were 267 cases with a *TP53* variant <10% VAF and 18 cases with *TP53* structural variants or with multiple hits to *TP53*. The MyMRD Assay detected more *TP53* variants than MyAML at VAFs <10%, likely due to the lower VAF reporting threshold. Of note, LabPMM does not receive pathology reports for subjects and the ELN guidelines combine *TP53* VAF with blast percentage to classify samples as "AML with mutated *TP53*" and therefore guidance on classification for receiving providers must be limited to *TP53* VAF only.

Clinical Cases (579 total)	MyAML Assay 194 Gene Panel, 1.0% VAF Threshold	MyMRD Assay 23 Gene Panel, 0.25% VAF Threshold	Total # Cases (% of Total)
Cases with a likely oncogenic/oncogenic *TP53* variant ≥10% VAF	185	109	**294 (50.78%)**
Cases with a likely oncogenic/oncogenic *TP53* variant <10% VAF	26	241	**267 (46.11%)**
Cases with a likely oncogenic/oncogenic structural variant involving *TP53* or cases with multiple *TP53* variants	9	9	**18 (3.1%)**

CONCLUSIONS

- The application of the ClinGen/CGC/VICC somatic guidelines to our LabPMM-specific *TP53* classification system provides a suitable set of rules to perform oncogenicity classification specific for AML.
- The future stratification in ClinVar for somatic vs. hereditary disease classifications will provide more appropriate interpretations based on disease.
- *TP53* variants at an allele fraction ≥10% have been associated with poor prognosis in AML subjects according to NCCN guidelines. Therefore, cases with LO/O *TP53* variants detected with a VAF ≥10% will be reported as meeting ELN guidelines for "AML with mutated *TP53*". Since our gene panels detect variants at a VAF down to 1% for MyAML and 0.25% for MyMRD, all *TP53* detected variants will still be listed on LabPMM clinical reports.

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