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POSTER H057

The Challenges of Developing Classification Guidelines for Fusions in AML; How and When to Use a *KMT2A* Fusion-Specific Workflow

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Background

- *KMT2A* fusions are a critical oncogenic driver in ~10% of acute myeloid leukemia (AML) patients and are associated with poor prognosis (Lenard et. al., 2020, Mercher and Schwaller, 2019). Wild-type *KMT2A*, along with various partners, form a complex that regulates the expression of a number of genes, including *HOXA9*, a gene critical for hematopoiesis. One of these partners is menin, a scaffolding protein, and its binding site is located within the first exon of *KMT2A*.
- Clinically significant *KMT2A* fusions retain the menin binding site as well as the CXXC zinc finger domain, located in exons 5 and 6 (Meyer et. al., 2023). Most often the breakpoint in *KMT2A* is between exon 7 and 13. *KMT2A* fusions are a targetable biomarker by menin inhibitors, a category of therapies currently in clinical trials (Hernandez-Sanchez et. al., 2024). *KMT2A* has >100 partner genes reported in the literature and the oncogenic effect of these fusions is dependent on the *KMT2A* breakpoint. The oncogenic impact of the breakpoint location in the partner gene remains unclear.
- Currently there are no published somatic guidelines for fusions and therefore developing methods to accurately classify *KMT2A* fusions is essential for patient care.

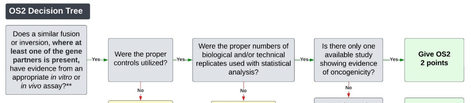
Methods

- We developed a LabPMM® General Workflow for classifying fusions and, additionally, a LabPMM *KMT2A* Fusions Workflow. These workflows were designed similarly to the somatic guidelines by Horak et al., 2022, where classification of oncogenicity is based on points awarded for varying types of evidence. *KMT2A* fusions previously detected by LabPMM's CAP/CLIA-certified MyAML® and MyMRD® gene panels were used to test our workflows.

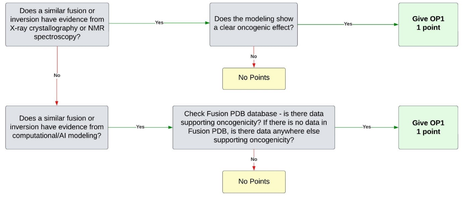
A LabPMM General Workflow for Fusions

Evidence for Oncogenicity	Max Points	Notes
Present in Guidelines	10	Is the fusion or inversion described in professional guidelines for AML and therefore fall under a genomic classification? For fusions, both partners must be described in guidelines unless a specific partner isn't required.
OS2	4	**Functional evidence: See decision tree below.**
OS3	4	**Previously reported fusions:** Give OS3 4 points if 5 or more patients with a similar fusion have been reported in an external database or publication(s) in patients with hematological neoplasms. **For counting patients for OS3/OM3/OP3, a similar fusion or inversion is defined as having similar breakpoints, having overlap of at least one exon or intron, or having overlap of at least one functional domain, especially a domain critical for oncogenicity. For fusions, both partners must match.**
OM3	2	**Previously reported fusions:** Give OM3 2 points if 2-4 patients with a similar fusion have been reported in an external database or publication(s) in patients with hematological neoplasms.
OP3	1	**Previously reported fusions:** Give OP3 1 point if 1 patient with a similar fusion has been reported in an external database or publication in patients with hematological neoplasms.
OM1	2	**Protein domains:** Give OM1 2 points if the fusion or inversion retains a functional domain important for oncogenicity. In the cases where each partner must retain an important domain, give 1 point for each partner if they do retain a critical domain.
OP1	1	**Computational evidence: See decision tree below.**
Evidence for Benign	**Max Points**	**Notes**
SBS1	-4	**Population data:** Give up to SBS1 -4 points if similar fusions or inversions have been identified in a healthy control population. Potential points schema should reflect OS3/OM3/OP3
SBS2	-4	**Functional evidence:** Give up to SBS2 -4 points if similar fusions or inversions have been identified as having no functional impact from functional studies. Potential points schema should reflect OS2.
SBP1	-1	**Computational evidence:** Give SBP1 -1 point if computational tools predict that a similar fusion or inversion will have no damaging impact.

OS2 Decision Tree

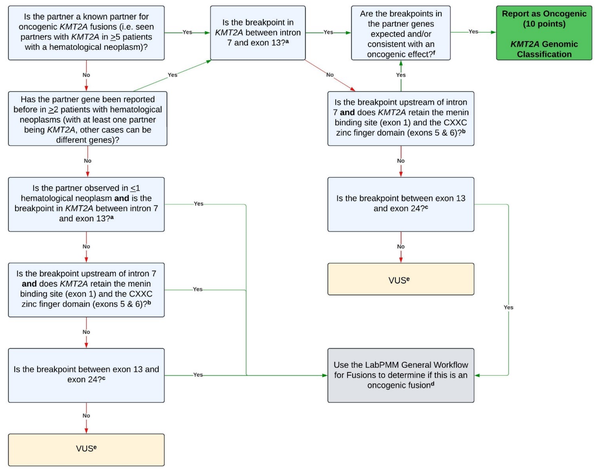


OP1 Decision Tree



**This applies for fusions (and in some cases inversions). The evidence must clearly show that if only one of the two gene partners in the fusion is present, it is the gene that shows supporting data that is driving the oncogenic effect. The mechanism must be understood to apply OS2.

B LabPMM *KMT2A* Fusions Workflow



a This is defined as the major breakpoint region (MBR); the region spanning intron 7 to exon 13 is the location of the *KMT2A* breakpoint in the majority of patients with AML and a *KMT2A* fusion (Meyer et. al., 2023).
b The CXXC zinc finger domain starts in exon 5 and bridges across to the first 4 amino acids of exon 6, aa 1148-1194.
c The region between intron 20 and exon 24 is defined as a minor breakpoint region however breakpoints in this region are rare for patients with AML (Meyer et. al., 2023). Therefore, any breakpoints between exon 13 and exon 24 are worth assessing with the LabPMM General Workflow for Fusions.
d It is good practice to assess novel fusions, where the partner has never been reported in hematological neoplasms, with our LabPMM General Workflow for Fusions.
e Breakpoints between exon 1 through intron 5 will disrupt or result in the loss of the CXXC domain, therefore negatively impacting DNA binding. Breakpoints downstream of exon 24 are not reported in PeCan and are not expected to impact function in the same way as known oncogenic fusions. Therefore, those *KMT2A* fusions upstream of exon 6 and downstream of exon 24 are most likely to be a VUS.
f *KMT2A* partners do have diverse functions and structures but there are common features among them, and many should retain certain functional domains to contribute to the oncogenic effect of that fusion.

Figure 1. (A) The LabPMM General Workflow for classifying fusions was designed for all fusions in AML that involve a driver that is an oncogene or has a mechanism other than loss of function. The 2022 European LeukemiaNet (ELN) guidelines for AML (Arber et. al., 2022) are the primary guidelines used for genomic classification for a clinical sample at LabPMM. Because functional studies and computational studies require specific information, the decision trees below the table were designed to provide specific guidance for assigning points. **(B)** The LabPMM *KMT2A* Fusions Workflow was designed specifically for *KMT2A* due to the specific mechanisms driving the oncogenicity of *KMT2A* fusions. The breakpoints in *KMT2A* must be assessed to ensure they retain the menin binding site and the CXXC zinc finger domain. A recurrent partner is given more points towards classification versus a novel partner. Because COSMIC has limited information on *KMT2A* fusions, our laboratory refers to the latest publication on the *KMT2A* recombinome (Meyer et. al., 2023) first, to search for previously reported *KMT2A* fusions. To determine the number of cases in which a specific *KMT2A* partner has been reported, one can run a general search that includes PubMed, Google Scholar, and ClinVAR, and any other available resources. To apply points for a similar fusion, it must have been detected in an individual with a hematological neoplasm.

Results

Distribution of *KMT2A* Breakpoints

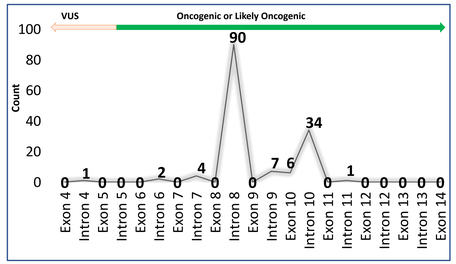
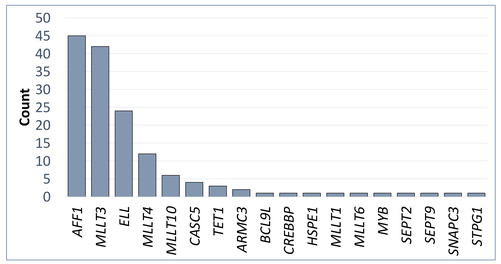


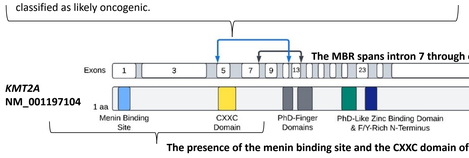
Figure 2. Overview of breakpoints in *KMT2A*. Of the 147 *KMT2A* fusions, 144 had the breakpoint in the MBR between intron 7 and exon 13. One had a breakpoint in intron 4, removing the CXXC domain, and therefore that fusion (*KMT2A*::*MLLT6*) was classified as a VUS. The two fusions with a breakpoint in intron 6, *KMT2A*::*SEPT9* and *KMT2A*::*MYB*, were classified as likely oncogenic.

KMT2A Fusion Partners



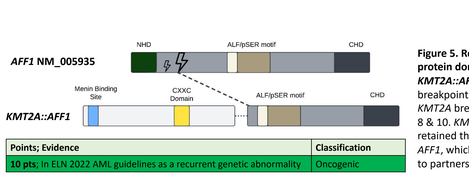
KMT2A Partner	Count	Percentage
AFF1	45	30.61%
MLLT3	42	28.57%
ELL	24	16.33%
MLLT4	12	8.16%
MLLT10	6	4.08%
CASC5	4	2.72%
TET1	3	2.04%
ARMC3	2	1.36%
BCL9L	1	0.68%
CREBBP	1	0.68%
HPSE2	1	0.68%
MLLT1	1	0.68%
MLLT6	1	0.68%
MYB	1	0.68%
SEPT2	1	0.68%
SEPT9	1	0.68%
SNAPC3	1	0.68%
STPG1	1	0.68%

Figure 3. Overview of the different *KMT2A* partners. Eighteen different partners were identified in *KMT2A* fusions with the most common partners being *AFF1*, *MLLT3*, *ELL*, *MLLT4*, and *MLLT10*. The genes *HPSE2*, *MYB*, *SNAPC3*, and *STPG1* were determined to be novel partners at time of classification.



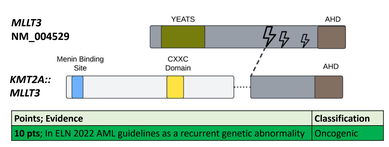
The presence of the menin binding site and the CXXC domain of *KMT2A* are critical for determining clinical significance for fusions.

Figure 4. *KMT2A* protein domains and exons. The MBR falls between intron 7 and exon 13 of *KMT2A* in transcript NM_001197104. This means that *KMT2A* fusions retain the menin binding site and the CXXC domain when *KMT2A* is the 5' partner.



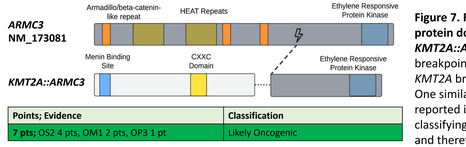
Points; Evidence	Classification
10 pts; In ELN 2022 AML guidelines as a recurrent genetic abnormality	Oncogenic

Figure 5. Representation of the protein domains retained by 45 *KMT2A*::*AFF1* fusions. The *AFF1* breakpoints included introns 3 & 4. *KMT2A* breakpoints included introns 8 & 10. *KMT2A*::*AFF1* fusions retained the ALF/pSER domain in *AFF1*, which is important for binding to partners.



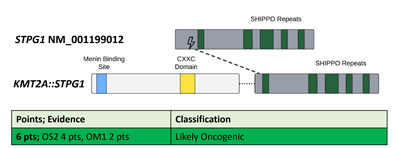
Points; Evidence	Classification
10 pts; In ELN 2022 AML guidelines as a recurrent genetic abnormality	Oncogenic

Figure 6. Representation of the protein domains retained by 42 *KMT2A*::*MLLT3* fusions. The *MLLT3* breakpoints included introns 5, 6 & 8. *KMT2A* breakpoints included introns 8, 9, 10, & exon 10. *KMT2A*::*MLLT3* fusions retained the AHD domain which is critical for binding to Dot1l and Aff1.



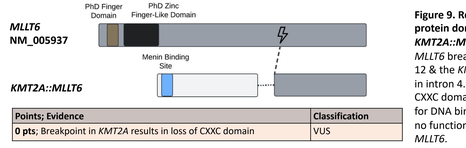
Points; Evidence	Classification
7 pts; OS2 4 pts, OM1 2 pts, OP3 1 pt	Likely Oncogenic

Figure 7. Representation of the protein domains retained by 2 *KMT2A*::*ARMC3* fusions. The *ARMC3* breakpoints were in intron 12 & the *KMT2A* breakpoints were in intron 7. One similar fusion was previously reported in a patient with ALL, classifying *ARMC3* as a rare partner, and therefore OP3 1 pt was given.



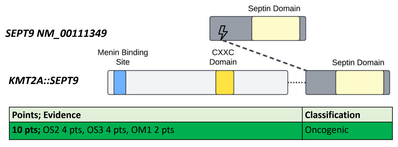
Points; Evidence	Classification
6 pts; OS2 4 pts, OM1 2 pts	Likely Oncogenic

Figure 8. Representation of the protein domains retained by 1 *KMT2A*::*STPG1* fusion. The *STPG1* breakpoint was in intron 1. The *KMT2A* breakpoint was in intron 9. This *KMT2A*::*STPG1* fusion retained the five SHIPPO repeats, which are found in eukaryotic sperm-tail proteins.



Points; Evidence	Classification
0 pts; Breakpoint in *KMT2A* results in loss of CXXC domain	VUS

Figure 9. Representation of the protein domains retained by 1 *KMT2A*::*MLLT6* fusion. The *MLLT6* breakpoint was in intron 12 & the *KMT2A* breakpoint was in intron 4. This fusion lacks the CXXC domain of *KMT2A*, critical for DNA binding, and contains no functional domains from *MLLT6*.



Points; Evidence	Classification
10 pts; OS2 4 pts, OS3 4 pts, OM1 2 pts	Oncogenic

Figure 10. Representation of the protein domains retained by 1 *KMT2A*::*SEPT9* fusion. The *SEPT9* breakpoint was in intron 1. The *KMT2A* breakpoint was in intron 6. This *KMT2A*::*SEPT9* fusion retained the Septin domain. Septin proteins are involved in the regulation of cell division.

Conclusions & References

The LabPMM *KMT2A* Fusions Workflow provides an efficient process for assigning classification. A total of 146/147 *KMT2A* fusions were classified as likely oncogenic or oncogenic with the one outlier classified as a VUS due to the *KMT2A*-BP being upstream of the CXXC domain. There was consistency in the location of breakpoints in *KMT2A* fusion partner genes and essential functional domains were retained in the majority of cases.

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