Detection of KMT2A Partial Tandem Duplication (PTD) in AML by Whole Genome Sequencing (WGS): Addressing Limitations of Traditional Techniques in the Era of Revumenib Approval

Robert Huether[1], Derick Hoskinson[1], Pavana Anur[1], Raul Torres[1], Karl R. Beutner[1], Kristiyana Kaneva[1], Yan Yang[1], Kelly A. Potts[1], Andrew Frazier[1], Iris Braunstein[1], Brett Mahon[1], Michael A. Thompson[1], Kate Sasser[1], Halla Nimeiri[1], Lewis J. Kraft[1], Francisco M. De La Vega[1], and Alejandro Marinos Velarde[2]

[1]Tempus AI, Inc., Chicago, IL // [2]The University of Texas Southwestern Medical Center, Dallas, TX

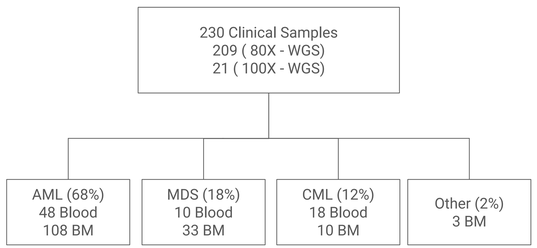
Abstract Presentation # 6532

INTRODUCTION

The menin inhibitor revumenib was recently FDA approved for treating patients with relapsed or refractory KMT2A-rearranged (rKMT2A) acute leukemias. Cytogenetics, FISH, and targeted next-generation sequencing (NGS) frequently miss KMT2A 11q23 partial tandem duplications (KMT2A-PTD). Although KMT2A-PTDs have expression signatures similar to rKMT2A, they were excluded from revumenib's registration trial. Preclinical models have shown that menin inhibitors may also be effective for KMT2A-PTD, highlighting the need for precise breakpoint and KMT2A fusion product detection. Here, we evaluated the effectiveness of high-resolution WGS to identify a diverse array of KMT2A-PTD.

METHODS

Using a WGS assay (Tempus xH) optimized for comprehensive profiling of myeloid neoplasms, we capture the entire KMT2A locus at base pair resolution. DNA was extracted from blood or bone marrow aspirates and was used to construct paired-end libraries via tagmentation. Sequencing was performed on the Illumina NovaSeq-X platform, achieving a mean coverage of 80X. Data were analyzed using the DRAGEN Platform with custom post-processing filters. Exon copy number calls from a targeted NGS assay and exon capture RNAseq NGS assay (Tempus xT and xR, respectively) were used for verification.



- 13 specimens (5.6% of all patients) were identified as harboring a KTM2A-PTD
  - 11 AML (7% of all AML patients)
  - 2 MDS (4.7% of all MDS patients)

Figure 1. Overview of study population with key clinical characteristics.

SUMMARY

- WGS is an effective tool for detecting clinically impactful KMT2A alterations that may be missed by traditional techniques such as NGS targeted capture, FISH or cytogenetics.
- 100% sensitivity was observed between WGS and RNA-seq for KMT2A-PTDs, supporting the reliability of WGS.
- The FDA approval of menin inhibitors for KMT2A-rearranged AML/ALL suggests potential clinical opportunities for broad tests (WGS) to identify diverse rearrangements, including KMT2A-PTDs.

RESULTS

Overview of detected KMT2A-PTDs

Sample	Duplicate Exons	Tempus xH (VAF %)	Detected in NGS Panel	Detected in RNAseq
1	Exon 2-Exon 8	16	Yes	N/A
2	Exon 2-Exon 8	23	Yes	N/A
3	Exon 2-Exon 8	36	Yes	Yes
4	Exon 2-Exon 8	43	Yes	Yes
5	Exon 2-Exon 8	49	Yes	Yes
6	Exon 2-Exon 8	67	Yes	Yes
7	Exon 2-Exon 10	10	No	Yes
8	Exon 2-Exon 10	13	Yes	Yes
9	Exon 2-Exon 10	21	No	Yes
10	Exon 2-Exon 10	21	No	Yes
11	Exon 2-Exon 10	26	Yes	Yes
12	Exon 2-Exon 10	26	Yes	Yes
13	Exon 2-Exon 10	28	Yes	Yes

Table 1. Overall, 100% (11/11) of the samples that underwent RNA-seq had evidence of a KMT2A-PTD and matched the exon structure of the WGS (Tempus xH) findings. Additionally, 69% (9/13) of the samples identified via WGS as harboring a KMT2A-PTD, which also underwent targeted panel DNA-seq (Tempus xT), had evidence of an observed exon gain in the targeted DNA-seq panel. Samples lacking RNA-seq are labeled as "N/A".

DNA WGS depth change and breakpoints of KMT2A-PTD (sample 6)

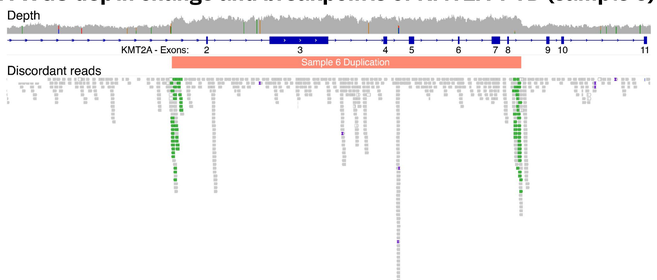


Figure 2. IGV pileup view of the exon 2-exon 8 region in *KMT2A*. DRAGEN identified the SV shown as a blue bar below gene diagram. A duplication manifests itself as an increase in coverage at the locus of the duplication (seen above the gene diagram). Softclip (discordant reads) are shown below the gene diagram in gray with paired reads colored green.

RNAseq supported KMT2A-PTD with softclip reads (sample 6)

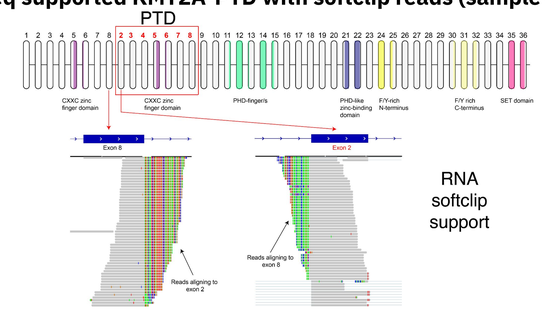


Figure 3. Gene diagram of an example of KMT2A-PTD with an exon 2 - exon 8 duplication (red box), including relevant exon names and domain locations (PFAM). RNA-seq soft clip reads that map to exon 2 (multi-colored reads in left pileup) and exon 8 (multi-colored reads in right pileup) are shown.

Genomic landscapes of KMT2A-PTD samples

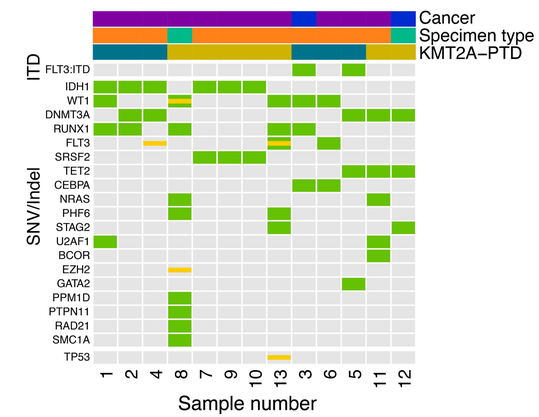


Figure 4. 100% overlap between targeted sequencing (Tempus xT) for SNV/indels. The threshold used to define low SNV/Indel VAF on xT was set to 10%. IDH1 co-mutations are the most recurrent. We searched for other SV co-mutations and found that KMT2A-PTDs were mutually exclusive with other SVs.

ACKNOWLEDGMENTS

We thank Cecile Rose T Vibat and Victoria L Chiou from the Tempus Clinical Development team for critical discussions of this work and Adam J Hockenberry for assistance with poster development.

Correspondence: robert.huether@tempus.com