Detection of KMT2A-PTDs in healthy donor and myeloid malignant samples using next generation sequencing

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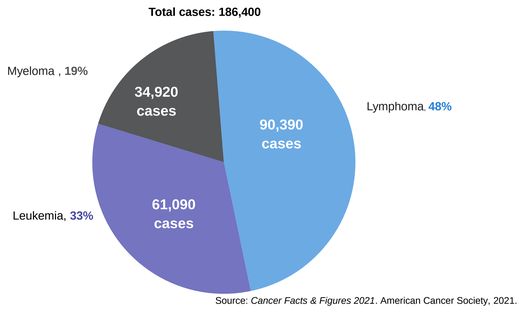
INTRODUCTION

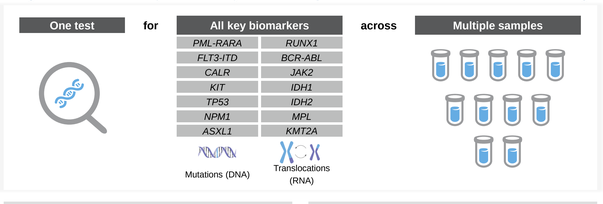
KMT2A (*MLL*) fusions and *KMT2A*-PTD (partial tandem duplication) are vital biomarkers in myeloid malignancies traditionally detected by RT-qPCR (quantitative real-time PCR). This study utilizes next generation sequencing (NGS) with Oncomine™ Myeloid Assay GX v2 to report the detection of *KMT2A*-PTDs in both healthy donors and myeloid malignancy samples. *KMT2A* fusions in myeloid malignant samples are also reported.

Fig 1. Estimated new cases (%) of Leukemia, Lymphoma and Myeloma in USA, 2021



Source: *Cancer Facts & Figures 2021*. American Cancer Society, 2021.

Fig 2. Oncomine™ Myeloid assay enables rapid and efficient multi-biomarker testing



- Full mutational profile in 2 days
- Consolidate testing of individual biomarkers
- Free up time for lab techs and bioinformaticians
- Reduce the need for NGS expertise

Fig 3. Genexus Instruments

MATERIALS AND METHODS

We sequenced 8483 research samples with known myeloid malignancies in both Sonora Quest Laboratories™ and at Thermo Fisher Scientific™ South San Francisco site. We also acquired 20 healthy donor whole blood samples (total 127 replicates) from Stanford™ Blood Center and Discovery Life Sciences™ and sequenced them at 3 different sites of Thermo Fisher Scientific™ (South San Francisco, CA; Guilford, CT; Carlsbad, CA). Samples were processed on the Ion Torrent™ Genexus™ Software 6.6 and analyzed using the Oncomine™ Myeloid Assay GX v2 for fusion profiling targeting 6 different *KMT2A*-PTD variants and 199 *KMT2A* fusion isoforms.

Table 1. Oncomine™ Myeloid Assay GX v2 Panel

DNA Panel				RNA Panel			
Hotspot genes (28)		Full genes (17)		Fusion Driver Genes (30)		Expression genes (5)	Expression control genes (5)
ANKRD26	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*MECOM*	*BAALC*	*EIF2B1*
ABL1	*MPL*	*BCOR*	*RB1*	*ABL2*	*MET*	*MECOM*	*FBXW2*
BRAF	*MYD88*	*CALR*	*RUNX1*	*BCL2*	*MLLT10*	*MYC*	*PSMB2*
CBL	*NPM1*	*CEBPA*	*SH2B3*	*BRAF*	*MRTFA*	*SMC1A*	*PUM1*
CSF3R	*NRAS*	*ETV6*	*STAG2*	*CCND1*	*(MKL1)*	*WT1*	*TRIM27*
DDX41	*PPM1D*	*EZH2*	*TET2*	*CREBBP*	*MYBL1*		
DNMT3A	*PTPN11*	*IKZF1*	*TP53*	*EGFR ETV6*	*MYH11*		
FLT3 (ITD + TKD)	*SMC1A*	*NF1*	*ZRSR2*	*FGFR1*	*NTRK2*		
GATA2	*SMC3*	*PHF6*		*FGFR2 FUS*	*NTRK3*		
HRAS	*SETBP1*			*HMGA2*	*NUP214*		
IDH1	*SF3B1*			*JAK2*	*NUP98*		
IDH2	*SRSF2*			*KAT6A*	*PAX5*		
JAK2	*U2AF1*			*(MOZ)*	*PDGFRA*		
KIT	*WT1*			*KAT6B*	*PDGFRB*		
				KMT2A (ML	*RARA*		
				L)	*RUNX1*		
				KMT2A-	*TCF3*		
				PTDs (MLL-	*TFE3*		
				PTDs)	*ZNF384*		

Fig 4. *KMT2A*-PTDs: a relevant biomarker in myeloid malignancies

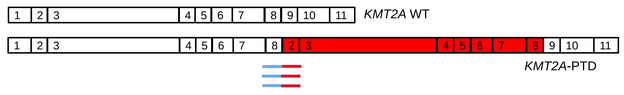


Table 2. Oncomine™ Myeloid assay is designed to detect six *KMT2A*-PTD variants

Variant	Length (bp)
KMT2A-KMT2A.nt51.K11K2	178
KMT2A-KMT2A.K9K2	172
KMT2A-KMT2A.K7K2	169
KMT2A-KMT2A.K11K2	116
KMT2A-KMT2A.K8K2	105
KMT2A-KMT2A.K10K2	105

Table 3. Report only the PTDs associated with myeloid malignancy with high confidence

Example: *KMT2A*-PTDs @ Genexus software 6.6 OMAv2 w4.3.2 (a past release)

Sample name	Isoforms detected	Read count
Healthy donor sample #A	KMT2A-KMT2A.K8K2	9
	KMT2A-KMT2A.K10K2	147
	KMT2A-KMT2A.K7K2	201
	KMT2A-KMT2A.K9K2	343
Myeloid malignant sample #B	KMT2A-KMT2A.K10K2	551
	KMT2A-KMT2A.K7K2	722
	KMT2A-KMT2A.K8K2	311
	KMT2A-KMT2A.K9K2	2466

Is there a differentiation in PTD read count in myeloid malignant vs healthy donor samples?

RESULTS

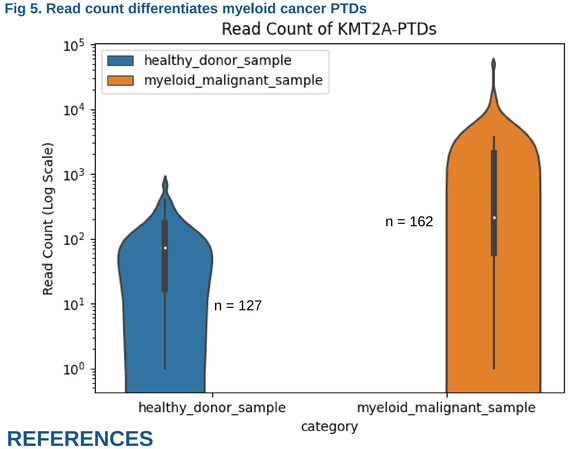
The mean read length of this data set is 90 – 120 bp and the mapped fusion reads is 20,000 – 30,000. *KMT2A*-PTDs were detected in both healthy donors and myeloid samples. Healthy donor PTD read counts were consistently <2000 and averaged 1/3 of myeloid samples. About 33% of myeloid samples had higher PTD read counts than any healthy donor sample. BLAT (BLAST™–like Alignment Tool) analysis confirmed specific exon matching on the *KMT2A* gene in both cohorts. Among the 8503 myeloid samples, 162 contained a total of 5 unique *KMT2A* PTDs, and 105 contained a total of 30 unique *KMT2A* fusion isoforms with *KMT2A-MLLT1* and *KMT2A-MLLT3* being the most prevalent *KMT2A* fusion gene pairs.

Table 4. *KMT2A* fusions & PTDs existed in ~3% samples

	Gene Pair	Found in # of unique samples	Found in % of all samples (N=8503)
KMT2A fusions	*KMT2A-AFF4*	2	0.02%
	KMT2A-CASC5	2	0.02%
	KMT2A-CBL	4	0.05%
	KMT2A-ELL	11	0.13%
	KMT2A-EPS15	4	0.05%
	KMT2A-MLLT1	30	0.35%
	KMT2A-MLLT10	9	0.11%
	KMT2A-MLLT3	29	0.34%
	KMT2A-MLLT4	14	0.16%
	Total	**105**	**1.23%**
KMT2A-PTDs	*KMT2A-KMT2A*	162	1.91%
	Total	**162**	**1.91%**
Samples w/ ≥ 1 *KMT2A* fusion or PTD	**Total**	**265**	**3.12%**

~2% samples had at least one KMT2A-PTD. ~1% samples had at least one KMT2A fusion.

Fig 5. Read count differentiates myeloid cancer PTDs



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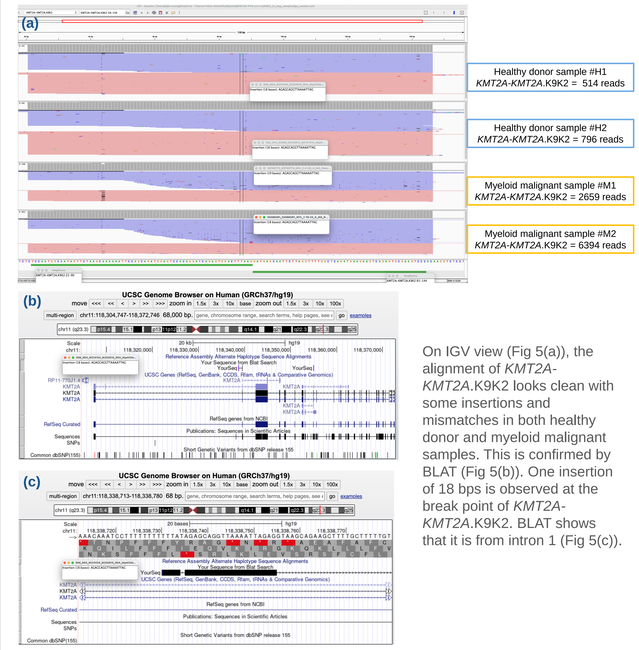
Dai B, Yu H, Ma T, Lei Y, Wang J, Zhang Y, Lu J, Yan H, Jiang L, Chen B. The Application of Targeted RNA Sequencing for KMT2A-Partial Tandem Duplication Identification and Integrated Analysis of Molecular Characterization in Acute Myeloid Leukemia. J Mol Diagn. 2021 Nov;23(11):1478-1490. doi: 10.1016/j.jmoldx.2021.07.019. Epub 2021 Aug 10. PMID: 34384895.

Fig 5. IGV view of *KMT2A-KMT2A*.K9K2 in healthy donor samples vs myeloid samples



On IGV view (Fig 5(a)), the alignment of *KMT2A-KMT2A*.K9K2 looks clean with some insertions and mismatches in both healthy donor and myeloid malignant samples. This is confirmed by BLAT (Fig 5(b)). One insertion of 18 bps is observed at the break point of *KMT2A-KMT2A*.K9K2. BLAT shows that it is from intron 1 (Fig 5(c)).

CONCLUSIONS

In this study, we describe the detection of *KMT2A* fusions in myeloid malignant samples. Our study also describes the detection of *KMT2A*-PTDs in both healthy donor and myeloid samples, with myeloid cases showing significantly higher PTD read counts. additional studies to understand the relevant expression level of PTD are in progress. This intriguing finding opens opportunities for prospective studies to monitor individuals with elevated PTD levels for myeloid malignancy development and retrospective studies to explore whether healthy donors identified with this alteration years ago after blood donation were subsequently recorded in the national health system with myeloid malignancies.

ACKNOWLEDGEMENTS

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DISCLAIMER