CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY

SOMATIC EXOME ANALYSIS REPORT

PATIENT

Patient ID: LK0302
Diagnosis: NUT midline carcinoma
Date of biopsy: 15.8.2023
Number of biopsy: 11979/23
Cancer cells content: 50%

METHODOLOGY*

Specimen type: FFPE tissue
Method used: Whole-exome sequencing
Target enrichment: KAPA HyperExome
Sequencing device: NextSeq 500
Date of sequencing: 25.10.2023
Report issued: 31.10.2023

*Details regarding the methodology are listed on page 4.

RESULTS

Gene	Variant	VAF (%)	Variant classification	Therapeutic option
KMT2A	c.10313C>T p.(Thr3438Met)	11	IVb	-
PCM1	c.5770G>T p.(Ala1924Ser)	18	IVb	-
FGFR2	c.819_824del p.(Asp273_Val274del)	4	IVc	(RTK inhibitors)

VAF = variant allele frequency

TUMOR MUTATIONAL BURDEN

1 mutation/Mb

FUSION GENE ANALYSIS RESULTS

The *BRD4*::*NUTM1* fusion gene was identified in the analyzed tumor sample.

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INTERPRETATION

The result interpretation part is divided into the following sections:

1) Variant found

i. Gene/protein function
ii. Diseases and/or cancer types associated with alterations in the respective gene/protein
iii. Variant description with supporting database and/or literature information regarding its effect on protein function (if available). Prediction algorithm results (in novel variants or variants of uncertain significance)
iv. Therapeutic options
v. Additional comments, alternative nomenclature (if relevant)

1) c.10313C>T p.(Thr3438Met) variant was found in the *KMT2A* gene.

i. The *KMT2A* gene encodes for a histone methyltransferase that functions as an epigenetic modulator that methylates lysine residue 4 on the tail of histone H3 (H3K4). Methylation of H3K4 leads to increased genome accessibility, recruitment of transcriptional complexes, and activation of gene expression (PMID: 25998713). KMT2A is crucial for embryogenesis and normal hematopoiesis by stimulating the expression of several important developmental genes, including the homeobox genes (PMID: 24213472) (provided by the **OncoKB database**).

ii. *KMT2A* is most commonly altered through translocations, which are frequently observed in specific leukemias, including acute myeloid, acute lymphoblastic, and mixed lineage (PMID: 17957188, PMID: 32243611) (provided by **The Clinical Knowledgebase**). In addition, loss-of-function mutations in *KMT2A* have been identified in solid tumors, including bladder, stomach, and endometrial cancers (PMID: 23636398) (provided by the **OncoKB database**).

iii. **c.10313C>T p.(Thr3438Met) in *KMT2A*** (NM_001197104.1, exon 27) has only been listed in the **ClinVar database** and classified as "Benign" by a single submitter when of germline origin. Besides this database entry, the variant hasn't been described in the scientific literature or functionally characterized. Therefore, its effect on protein function is not known.

Prediction algorithm results:

- Align GVGD (v2007): Class C0 (GV: 353.86 - GD: 0.00) – least likely deleterious
- PolyPhen2 (v): HVar: Benign (score: 0.428)
- SIFT (v6.2.0): Tolerated (score: 0.27, median: 4.32)
- MutationTaster (v2021): Benign, Tree vote: 11|89 (del|benign)

Varsome: Likely benign

iv. –

2) c.5770G>T p.(Ala1924Ser) variant was found in the *PCM1* gene.

i. The *PCM1* gene encodes for a centriolar satellite protein that recruits microtubule and dynactin-dependent proteins for appropriate microtubule organization (PMID: 12403812) (provided by **The Clinical Knowledgebase**).

ii. Reported cancer-associated alterations of *PCM1* include mostly gene fusion. For example, the *PCM1*::*RET* fusion has been identified in papillary thyroid carcinoma (PMID: 10980597), and *PCM1* fusions with other genes have been observed in hematological cancers (PMID: 29797824, PMID: 26522767, PMID: 31085913, PMID: 29169164). Both overexpression and amplification of *PCM1* have been linked to myeloid leukemias (PMID: 10980597, PMID: 23431203) (provided by **The Clinical Knowledgebase**).

iii. **c.5770G>T p.(Ala1924Ser) in *PCM1*** (NM_006197.3, exon 36) hasn't been described in the scientific literature or functionally characterized. Therefore, its effect on protein function is not known.

Prediction algorithm results:

- Align GVGD (v2007): Class C0 (GV: 209.54 - GD: 0.00) – least likely deleterious
- PolyPhen2 (v): HVarPred: Probably damaging (score: 0.987)
- SIFT (v6.2.0): Tolerated (score: 0.72, median: 3.59)
- MutationTaster (v2021): Benign, Tree vote: 4|96 (del|benign)

Varsome: Likely benign

iv. –

3) c.819_824del p.(Asp273_Val274del) variant was found in the *FGFR2* gene.

i. The *FGFR2* gene encodes for a receptor tyrosine kinase that is a member of the fibroblast growth factor receptor (FGFR) family. The binding of FGF ligands to FGFR2 results in the rapid dimerization and activation of downstream signaling pathways, including the PI3K/AKT and MAPK pathways (PMID: 28030802). FGFR2 is expressed in ectoderm-derived and endothelial tissues, and FGFR2 signaling contributes to a variety of cellular functions, including homeostasis, mitogenesis, proliferation, and differentiation (PMID: 20094046) (provided by the **OncoKB database**).

ii. Somatic mutations, fusions, and amplifications of *FGFR2* have been identified in several human tumors, including endometrial, gastric, and breast cancer, as well as ameloblastomas (PMID: 28430863) (provided by the **OncoKB database**).

iii. **c.819_824del p.(Asp273_Val274del) in *FGFR2*** (NM_000141.5, exon 7) is an in-frame deletion of 6 consecutive nucleotides encoding for residues Asp273 and Val274. The variant hasn't been described in the scientific literature or functionally characterized. Therefore, its effect on protein function is not known.

iv. Oncogenic alterations of *FGFR2* can be targeted by RTK inhibitors.

METHODOLOGY

Library preparation and sequencing

DNA for whole-exome sequencing was extracted from FFPE tissue using QIAmp DNA FFPE Tissue Kit (Qiagen, Germany) and treated with NEBNext FFPE DNA Repair Mix (New England Biolabs, MA, USA). Sequencing libraries were prepared using the KAPA HyperExome Kit (Roche, Switzerland). Sequencing was carried out on the NextSeq 500 device using NextSeq 500/550 Mid Output Kit v2,5 (150 cycles) (Illumina, CA, USA). Additionally, Sanger sequencing of the *H3-3A* K28 and G35 codons was performed, as these are not part of the HyperExome design. The target region was amplified using custom primers and Taq Recombinant Polymerase Kit and sequenced on the ABI 3131xl device (ThermoFisher Scientific, MA, USA).

RNA for targeted RNA sequencing was extracted from fresh frozen tissue using a MiRVana miRNA Isolation Kit and treated with a DNA-free DNA Removal Kit (ThermoFisher Scientific). Sequencing libraries were prepared using the TruSight RNA Pan-Cancer Panel (Illumina). Sequencing was carried out on the NextSeq 500 device using NextSeq 500/550 Mid Output Kit v2,5 (150 cycles) (Illumina).

Patient-specific information regarding the analysis

DNA was extracted from FFPE tissue. DIN was 5,7. The sequencing was performed on the 25th of October, 2023. 94% of the target regions were covered at least 40 times.

Bioinformatic workflow

Whole-exome sequencing

Data processing:	BWA (alignment to GRCh38), Picard (marking duplicates), Samtools (sorting/indexing)
Quality Control:	Qualimap, PicardTools CollectHsMetrics, FastQC (aggregated in MultiQC)
Variant Calling:	Strelka, VarDict, Mutect2, SomaticSniper, LoFreq, MuSE, VarScan (SomaticSeq pipeline, consensus approach – calls made by 3 or more callers out of 7)
Annotation:	Ensembl Variant Effect Predictor + in-house annotation scripts for Gene, Transcript assignment, Variant Consequence, Population databases (1000 Genomes, GnomAD, ExAC), Clinical databases (dbSNP, CancerGeneCensus, COSMIC, HGMD, NHLBI ESP, TruSight, ClinVar, MD Anderson, Foundation One CDx), Protein structure predictors (SIFT, PolyPhen2)
TMB computation:	Count only non-synonymous exonic SNVs with VAF > 5% TMB = variant count × 10^6 / exome size

Targeted RNA sequencing

Data processing:	STAR (alignment to GRCh38)
Quality Control:	Qualimap, PicardTools
Fusion calling:	Arriba, STARFusion
Manual verification:	Integrative Genomics Viewer

Limitations

Mutations outside of the coding regions, in other genes, copy number changes, and changes that cannot be detected at present technical possibilities, and the current level of knowledge cannot be excluded.

Not all detected variants are reported. Reported variants were pre-selected based on their known or potential significance in the disease. All variants found are listed in the protocol supplement.

VARIANT CLASSIFICATION SYSTEM

Level I – The variant being an established clinical utility
Ia – in the respective tumor type
Ib – in another tumor type

Level II – The variant's significance is supported by clinical evidence (case series, case reports)
IIa – in the respective tumor type
IIb – in another tumor type

Level III – The variant's significance is supported by preclinical studies (*in vitro*, *in vivo*)
IIIa – in the respective tumor type
IIIb – in another tumor type
Or the variant presumably leads to loss-of-function of a tumor suppressor gene (nonsense, frameshift, and splicing variants) that is altered
IIIc – in the respective tumor type
IIId – in another tumor type

Level IV – The variant's effect on protein function is unknown and solely based on prediction algorithms
IVa – the variant is predicted to be "damaging" / "pathogenic"
IVb – the variant is predicted to be "benign"
IVc – the variant is predicted with conflicting pathogenicity scores by prediction algorithms (i.e., PolyPhen-2 evaluates the variant as "damaging", whereas SIFT as "tolerated") or the role of the encoded protein in disease is unclear