CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY

SOMATIC EXOME ANALYSIS REPORT

PATIENT

Patient ID: VH0452
Diagnosis: Alveolar rhabdomyosarcoma
Date of biopsy: 3.4.2023
Number of biopsy: 592/23/5
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: 26.4.2023
Report issued: 3.5.2023

*Details regarding the methodology are listed on page 2.

RESULTS

No variants with known or potential clinical significance were found.

TUMOR MUTATIONAL BURDEN

2 mutations/Mb

FUSION GENE ANALYSIS RESULTS

***PAX3-FOXO1* positive**

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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 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).

RNA for RNA sequencing was extracted from FFPE tissue using MiRVana miRNA Isolation Kit and treated with a DNA-free DNA Removal Kit (ThermoFisher Scientific). Messenger RNA was purified using NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). Sequencing libraries from polyA selected mRNA were prepared using NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs). Sequencing was carried out on the NextSeq 500 device using NextSeq 500/550 Mid Output Kit v2,5 (75 cycles) (Illumina).

Patient-specific information regarding the analysis

DNA was extracted from FFPE tissue. DIN was 1,7. The sequencing was performed on the 26th of April, 2023. 89% of the target regions were covered at least 20 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