CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY

SOMATIC EXOME ANALYSIS REPORT

PATIENT

Patient ID: MR1507
Diagnosis: Peripheral T-cell lymphoma, NOS
Date of biopsy: 14.3.2023
Number of biopsy: 477/23/1
Cancer cells content: 90%

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 5.

RESULTS

Gene	Variant (cDNA/protein)	VAF (%)	Variant classification	Therapeutic option
PTEN	c.755A>G/p.D252G	16	IIb	PI3K inhibitors
ZFHX3	c.1114C>T/p.R372*	55	IIIc	-

VAF = variant allele frequency

TUMOR MUTATIONAL BURDEN

1 mutation/Mb

FUSION GENE ANALYSIS RESULTS

No clinically relevant fusion genes were found.

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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 algorithms results (in novel variants or variants of uncertain significance)
iv. Therapeutic options
v. Additional comments, alternative nomenclature (if relevant)

1) c.755A>G/p.D252G variant was found in the *PTEN* gene.

i. The *PTEN* gene encodes for tumor suppressor, which is one of the most frequently mutated genes in human cancer (PMID: 22473468). PTEN has several physiological functions, most notably operating as a phosphatase that converts phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol (4,5)-diphosphate (PIP2) at the cell membrane (PMID: 18767981). Impairment of PTEN function through multiple mechanisms, including through non-synonymous mutations, results in PIP3 accumulation and constitutive activation of catabolic downstream AKT/mTOR signaling. Therefore, PTEN inactivation promotes cell growth, proliferation, and survival (PMID: 12040186) (provided by the **OncoKB database**).

ii. *PTEN* somatic alterations resulting in loss of function have been found in many types of cancer, including, but not limited to, endometrial (PMID: 30142194), melanoma (PMID: 30148988), and prostate cancer (PMID: 18767981, PMID: 30153654) (provided by **The Clinical Knowledgebase**). The gene is altered in 7.24% of all cancers and in 3.05% of non-Hodgkin lymphoma patients (provided by **mycancergenome.org**).

iii. **c.755A>G/p.D252G in *PTEN*** (NM_000314.8, exon 7) has been listed in the **OncoKB database** as "likely oncogenic" with a "likely loss-of-function" effect and the following description: "This mutation has been found as a germline mutation in autism (PMID: 15805158, 25527629). Expression of this mutation in a glioblastoma cell line demonstrated that it is inactivating as measured by decreased protein stability compared to wildtype (PMID: 25527629). This mutation also leads to loss of PTEN function by yeast gene interaction assay, by an altered developmental rate in Drosophila, by changes in rat neural development, and by becoming a dominant negative regulator of the PI3-AKT signaling pathway (PMID: 32350270)."

This mutation is also listed in **The Clinical Knowledgebase** as a "loss of function" variant with the following description: "*PTEN* D252G lies within the C2 tensin-type domain of the Pten protein (UniProt.org). D252G demonstrates reduced lipid phosphatase activity in a yeast assay and in cell culture (PMID: 21828076, PMID: 25527629, PMID: 29373119), decreased Pten protein stability (PMID: 25527629, PMID: 29706633, PMID: 32350270), and results in impaired nuclear localization, and failure to regulate neuronal growth (PMID: 29373119), and loss of ability to suppress Akt phosphorylation in cell culture, and delays developmental growth in flies (PMID: 32350270)."

In the **COSMIC database**, the variant is described as a confirmed somatic mutation found mostly in CNS tumor patients.

iv. According to the **OncoKB database,** oncogenic mutations in *PTEN* can be targeted by PI3K inhibitors.

Level	Alterations	Level-associated cancer types	Drugs
4	Oncogenic Mutations	All Solid Tumors	GSK2636771, AZD8186

Figure 1: OncoKB database therapeutic implications for *PTEN* oncogenic mutations.

Level 4 – Compelling biological evidence supports the biomarker as being predictive of response to a drug.

2) c.1114C>T/p.R372* variant was found in the *ZFHX3* gene.

i. The *ZFHX3* gene encodes a transcription factor with multiple homeodomains and zinc finger motifs and regulates myogenic and neuronal differentiation. The encoded protein suppresses the expression of the alpha-fetoprotein gene by binding to an AT-rich enhancer motif. It has also been shown to negatively regulate c-Myb, and transactivate the cell cycle inhibitor cyclin-dependent kinase inhibitor 1A (also known as $p21^{CIP1}$) (provided by **RefSeq**).

ii. *ZFHX3* mutations and loss of heterozygosity have been identified in gastric (PMID: 17671116), prostate (PMID: 15750593), and endometrial cancer (PMID: 26330387) (provided by **The Clinical Knowledgebase**). The gene is altered in 5.37% of all cancers, with colon adenocarcinoma, prostate adenocarcinoma, lung adenocarcinoma, endometrial endometrioid adenocarcinoma, and cutaneous melanoma having the greatest prevalence of alterations (provided by **mycancergenome.org**).

Ohki *et al.* (2021, PMID: 34258755) identified *ZFHX3* as one of the recurrent mutational targets for pediatric peripheral T-cell lymphoma (PTCL). The authors suggested that the accumulation of some of the genetic alterations related to epigenetic regulation, such as those in *TET2*, and cell proliferation, including *TNS1*, *ZFHX3*, and *NCOA2*, participate in the onset and development of pediatric PTCL.

iii. **c.1114C>T/p.R372* in *ZFHX3*** (NM_006885.4, exon 2) is a substitution that generates 'nonsense' as a coding effect, which means that the reading frame is interrupted by a premature STOP codon, and the mRNA produced might therefore be targeted for nonsense-mediated decay. The variant itself has not been described in databases of genetic variants or literature. However, according to the **OncoKB database**, truncating mutations of *ZFHX3*, defined as nonsense, frameshift, or splice-site mutations that are predicted to shorten the coding sequence of the gene, are "likely oncogenic" with a "likely loss-of-function" effect.

iv. -

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 targeted RNA sequencing was extracted from fresh frozen tissue using 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 6. The sequencing was performed on the 26th of April, 2023. 95% 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