Comprehensive Molecular Profiling
Final Report

theracision

SAMPLE,Patient

Date of Birth: **01/11/1987** Gender: **Female**
Specimen Source/ID: **FFPE / 7323B14809**
Ordering Physician: **Prof. Dr. Sample Clinician**

Report Number: **S517**

Report Date: **13-Feb-2024**

Diagnosis: **CERVICAL CANCER**

GENETIC RESULT: POSITIVE - CLINICALLY SIGNIFICANT MUTATIONS IDENTIFIED

TMB	MSI	PD-L1
3.9 muts/Mb	0.9% Unstable Sites	TPS: 0% CPS: 0%
Low	Stable	Negative

0 Clinical Trials		1 Clinical Trials	0 Clinical Trials
IA	IB	IIC	IID
PTEN	**No variants reported**	**EWSR1, FLI1**	**MED12**
Copy number loss in PTEN (1 copy)		EWSR1-FLI1 fusion transcript	p.? c.204+2T>G
Trametinib / Binimetinib		**Vincristine, Dactinomycin, Cyclophosphamide, Doxorubicin, Ifosfamide, Etoposide**	

REPORT SUMMARY

The comprehensive genomic analysis of the sample tissue containing 10% tumor cells from the patient, **SAMPLE Patient**, revealed genomic markers: Low Tumor Mutation Burden (**TMB low, 3.9 muts/mb**), and Microsatellite Stability (**MS stable, unstable sites 0.9%**). In the same sample of tumor tissue, a comprehensive genomic analysis was performed for 523 genes and 55 transcripts, investigating point mutations, deletions, amplifications, and translocations. Pathogenic and potentially driving mutations were identified, including **PTEN (1 copy)** copy number loss, **EWSR1-FLI1 fusion, MED12 p.? C. 204+2T>G (VAF 48.4%)** mutation.

In addition to the above mutations, clinical significance unknown or not yet pathogenic (VUS) copy number changes were detected in the sample tissue, including **MDM4 (3 copies)** and **FGF8 (1 copy)**, as well as **NTRK1 p.E551K c.1651G>A variation (VAF 66%)**.

The detected EWSR1-FLI1 fusion in the patient supports the presence of a sarcomatoid component, suggesting the **possibility of genital system primary Ewing sarcoma**. Although rare, literature reports include 19 cases of cervical primary Ewing sarcoma, and there is also a case report of uterine primary Ewing sarcoma (PMID: 26549660, PMID: 33494989).

EWSR1-FLI1 fusion is an alteration that activates several oncogenic genes and accelerates the cell proliferation. According to the NCCN guidelines, these patients have been reported to benefit from alternating treatment with **vincristine, dactinomycin, cyclophosphamide, doxorubicin, ifosfamide, and etoposide.**

Loss of PTEN and copy number alterations were detected in our patient's tumor tissue, suggesting the potential activation of the RAS/PI3K/MEK-MAPK pathways. Therefore, it is considered that **trametinib or binimetinib treatment**, which inhibits these pathways, may be beneficial.

A MED12 p.? c.204+2T>G (VAF % 48.4) mutation has been identified in our patient. It has been reported that MED12 mutations are frequently encountered in uterine leiomyomas and leiomyosarcomas (PMID: 25108465). The neuroendocrine differentiation observed in our patient's pathology report may be consistent with histopathological findings of Ewing sarcoma.

If clinically deemed necessary, the use of the liquid biopsy tests can be considered for monitoring our patient.

Clinically Relevant Results

Tier I - Strong Clinical Significance

PTEN Copy number loss (1 copy)

A

Interpretation

Copy number loss of the PTEN gene was identified (equivalent to 1 copy and a 0.56-fold change). PTEN deletions result in a loss-of-function and are known to be oncogenic (PMID: 28481359). PTEN encodes a tumor suppressor that is one of the most frequently mutated genes in human cancer (PMID: 9072974, 9090379, 22473468). PTEN has several physiological functions, most notably operating as a phosphatase that converts phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol (4,5)-triphosphate (PIP2) at the cell membrane (PMID: 18767981, 28481359). PTEN deletions been reported in **prostate cancer**, 5.4% of large cell neuroendocrine cancers (PMID: 28481359). Loss of PTEN protein expression has been identified as a marker of poor prognosis in NSCLC (PMID: 21782507, 22982652). Loss of PTEN may also lead to greater genomic instability and provide a setting for the accumulation of other deleterious mutations (PMID: 28481359). Germline loss-of-function PTEN mutations occur in approximately 80% of patients with the cancer predisposition syndrome Cowden disease, which is associated with rare high-penetrance breast and thyroid cancer (PMID: 9467011, 24136893, 21430697, 28481359). This assay cannot distinguish between somatic (in tumor only) versus germline (inherited) changes. Clinical correlation is recommended.

CLINICAL IMPACT

Tier II - Potential Clinical Significance

EWSR1, FLI1 fusion transcript

C

CLINICAL IMPACT

Diagnostic of — Peripheral neuroectodermal tumor, Round cell sarcoma, Ewing's sarcoma of bone, or Ewing's sarcoma

Interpretation

A EWSR1-FLI1 fusion t(11;22)(q24.1-q24.3;q12.2)(chr22:g.29683123::chr11:g.128675261) has been identified in this tumour.

The genetics of EWS are characterized by a canonical fusion involving EWSR1 (Ewing's Sarcoma Region 1) gene and a member of the ETS family of transcription factors, such as FLI1 (Friend Leukaemia virus Integration 1) and ERG. If a gene fusion occurs in Ewing's sarcoma, most frequently it is the recurrent t(11;22)(q24;q12) translocation resulting in the fusion of EWSR1 and FLI1 genes, identified in 80-90% of cases (PMID: 3779625_Turc□Carel_1986; PMID: 1522903_Delattre_1992; PMID: 8022439_Delattre_1994). The second most common molecular abnormality is the t(21;22)(q22;q12), which accounts for approximately 5-10% of the cases, resulting in an EWSR1-ERG fusion (PMID: 10561219_Ginsberg_1999; PMID: 26690869_Chen_2015).

Evidence suggests that fusion of EWS to different members of the ETS family of transcription factor genes may result in the expression of similar disease phenotypes (PMID: 8162068_Sorenson_1994). One study has shown that EWSR1-FLI1 and EWSR1-ERG gene fusions are associated with similar clinical phenotypes in Ewing’s sarcoma, with no significant differences observed between the two groups in age of diagnosis, sex, metastasis at diagnosis, primary site, event-free survival, or overall survival (PMID: 10561219_Ginsberg_1999). Recent reports from the EURO-EWING 99 study and the Children’s Oncology Group study suggest that with currently available effective therapies, patients with Ewing sarcoma have similar outcomes, regardless of fusion subtype in contrast to some previous reports (see commentary by Barr & Meyer (PMID: 20308653_2010). On the other hand, chromosomal translocation itself could be a treatment target of trabectedin, and the specific translocation of Ewing sarcoma could be the target of trabectedin. In a preclinical study, trabectedin inhibited NR0B1 expression by suppressing the activity of EWSR1-FLI1 and a partial response to recurrent/metastatic Ewing sarcoma was observed (PMID: 2469622_Ueda_2014).

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Date of Birth: **01/11/1987** Gender: **Female** Report Number: **S517** Report Date: **13-Feb-2024**

Tier II - Potential Clinical Significance

CLINICAL IMPACT

MED12 p.? c.204+2T>G

NM_005120.2
VAF % 48.4
DEPTH 403

D

Interpretation

MED12 encodes for a component of CDK8, which is involved in transcription initiation, and is involved in a variety of estrogen dependent tumors. The splice site variant identified is expected to result in protein truncation / inactivation and is likely oncogenic.

POTENTIAL CLINICAL TRIALS

Clinical Trials associated with this patient's genomic profile and tumor type are displayed below.

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
Lurbinectedin in FET-Fused Tumors	NCT05918640 https://clinicaltrials.gov/ show/NCT04848337	I/II	**EWSR1, FLI1** EWSR1-FLI1 fusion transcript

TIER III - VARIANTS OF UNCERTAIN SIGNIFICANCE

FGF8
Copy number loss in FGF8 (1 copy)

MDM4
Copy number gain in MDM4 (3 copies)

NTRK1
p.E551K NM_002529.3 c.1651G>A
VAF 66%
DEPTH 591

CLASSIFICATION AND LEVELS OF EVIDENCE

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)	Variant of strong clinical significance, Level B Evidence (consensus in the field based on well□powered studies in patient's tumor type)	Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)	Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

III Variant of uncertain clinical significance

III Benign or likely benign variant

REPORTED GENES

A total of 523 genes were subjected to targeted next generation sequencing analysis. Details available upon request.

CGW VERSION

CGW_v6.26

DATABASE DETAILS

The versions, releases, builds, dates of the following databases were used to generate this report.

— Genomic Build: GRCh37.p13
— Genomic Annotation Sources: NCBI RefSeq v105
— gnomAD: r2.1
— dbNSFP: 3.5c
— COSMIC: v92
— NHLBI ESP: v.0.0.30
— ClinVar: 20210328
— dbSNP: 149
— ExAC: v1.0

CODING EXON COVERAGE METRICS

Level 2: 100x coverage for > 50% of positions was not achieved for the targeted exon regions listed below:

Gene Transcript ID (Exon/Intron('))		
FANCA NM_000135.2 (1)	**SDHA** NM_004168.2 (1)	**FANCE** NM_021922.2 (1)
GPR124 NM_032777.9 (1)	**INSR** NM_000208.2 (1)	**TGFBR1** NM_004612.2 (1)
PTCH1 NM_000264.3 (1)	**CCNE1** NM_001238.2 (2)	**ARID1A** NM_006015.4 (1)
PTPRS NM_002850.3 (15)	**PTPRT** NM_007050.5 (1)	**FGF3** NM_005247.2 (1)
RAB35 NM_006861.6 (1)	**CDH1** NM_004360.3 (1)	**FGF4** NM_002007.2 (1)
PDPK1 NM_002613.4 (2)	**CEBPA** NM_004364.3 (1)	**CENPA** NM_001809.3 (1)
KMT2B NM_014727.1 (1)	**BBC3** NM_014417.4 (2)	**RB1** NM_000321.2 (1)
FLT3 NM_004119.2 (1)	**FLT4** NM_182925.4 (1)	**RECQL4** NM_004260.3 (2)
RET NM_020975.4 (1)	**PIK3R2** NM_005027.3 (6)	**STAT5A** NM_003152.3 (8)
MAGI2 NM_012301.3 (22)	**STAT5B** NM_012448.3 (7)	**MALT1** NM_006785.3 (1)
IDH2 NM_002168.2 (1)	**MAP2K4** NM_003010.3 (1)	**GATA4** NM_002052.3 (2)
MAP3K1 NM_005921.1 (1)	**NOTCH3** NM_000435.2 (24)	**NOTCH3** NM_000435.2 (1)

PERTINENT NEGATIVES

Pertinent negatives were not reported for this case

METHODOLOGY

Pathology Assessment: Pathologist reviews on H&E stained section of the tissue block or stained cytology slide were considered to assess adequacy and, as needed, guide enrichment of tumor for sequencing analysis. The in-house validation ensured that the samples passed all established laboratory QC metrics. This excludes exons, within the specified transcripts of the genes and listed in the Coding Exon Coverage Metrics section above, for which variants may not have been reliably detected.

Assay Methods: The test was performed using the Illumina TruSight™ Oncology 500 (TSO500) targeted hybrid-capture based next generation sequencing assay. It employs Unique molecular identifiers (UMI) to enable detection of variants, present in formalin-fixed paraffin-embedded (FFPE) tumor samples, at low VAFs with a high degree of sensitivity and specificity. TSO500 is designed to detect multiple classes of mutations including single-nucleotide variants (SNVs), multi-nucleotide variants (<3bp), small Insertions (1-18bp)/Deletions (1-27bp) and Copy Number Variants (CNVs). The assay also detects, quantitatively, microsatellite instability (MSI) and tumor mutational burden (TMB). Fusions and splice variants are detected in RNA. DNA and RNA are extracted from the same FFPE tissue using the Allprep DNA/RNA FFPE Kit (Qiagen, Inc.). RNA is then reverse transcribed to cDNA. The genomic DNA and cDNA are sheared to prepare sequencing libraries. The regions of interest are hybridized to biotinylated probes, magnetically pulled down with streptavidin-coated beads, and eluted to enrich the library pool. Finally, libraries are normalized using a simple beadbased protocol, then pooled and sequenced on an Illumina NovaSeq 6000 instrument.

Secondary Analysis Methods: The DNA and RNA data is analyzed using the Illumina Software TSO500 v2.2 Local App and a customized analysis pipeline within the Clinical Genomics Workspace software platform from PierianDx.

Variant Calling: Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen) and classified as per the AMP classification system into tiers IA, IB, IIC, IID, III and IV. These tiers are stratified by clinical utility ('actionability' for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variations found in gnomAD (https://gnomad.broadinstitute.org/) that have ≥1% minor allele frequency (except those that are also in ClinVar denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication) are classified as known polymorphisms. Exons from some transcripts included in the RefSeq annotation release v105 found in genes reported in certain gene subsets of this test are not targeted by the assay. The untargeted exons are disclaimed and are identified as follows: HIST2H3A NM_001005464.2 exon 1, HIST2H3C NM_021059.2 exon 1, MYB NM_001130173.1 exon 1, PAX8 NM_003466.3 exon 8, PDPK1 NM_002613.4 exon 3, PDPK1 NM_002613.4 exon 8, PDPK1 NM_002613.4 exon 1, PDPK1 NM_002613.4 exon 6, PDPK1 NM_002613.4 exon 4, PDPK1 NM_002613.4 exon 5, PDPK1 NM_002613.4 exon 9, PDPK1 NM_002613.4 exon 10, RANBP2 NM_006267.4 exon 13, RANBP2 NM_006267.4 exon 8, REL NM_002908.2 exon 9, SUZ12 NM_015355.2 exon 3, FGF8 NM_033164.3 exon 1, RECQL4 NM_004260.3 exon 1, ICOSLG NM_015259.4 exon 1, NOTCH1 NM_017617.3 exon 1. Additionally, all small variant calls in the HLA-A, KMT2B, KMT2C, and KMT2D genes are filtered out due to potential mis-mapping as a result of sequence homology with other genomic regions.

Notes:

• This assay does not detect complex structural alterations or indels, with the exception of a subset of clinically relevant complex EGFR exon 19 indels that are specifically targeted. Variants located outside of targeted regions too will not be detected.

• It is possible that pathogenic variants may not be reported by one or more of the tools because of the parameters used. However, tool parameters were optimized to maximize specificity and sensitivity.

DISCLAIMER

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The test performance characteristics were determined by the PierianDx Molecular Laboratory. The Report was generated by the PierianDx Molecular Laboratory as required by the CLIA 1988 regulations. The Report, and the tests used to generate the Report, have not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test results have been shown to be clinically useful. This laboratory is CLIA certified to perform high complexity testing.

This **Authorized Signature**
pertains to this laboratory report:

Prof.Dr. M.Cengiz Yakıcıer
NPG Genetic Diseases
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Responsible Medical Dr.
Center Manager

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