NGS Report – Complete Solid Panel

Patient Information

Name-Surname: Az[redacted]ri	**Examined Material:** FFPE
Date of Birth, Gender: 47 Years, Female	**Type of Tissue Examined:** Rectal
Diagnosis: Rectal Adenocarcinoma	**Tumor Percentage:** 50 %
Material Code: 0034	**Test Conducted:** Complete Solid Tumor
Sample Receipt Date-Time: 09/10/2025	**Report Date-Time:** 09/23/2025

Case History: The **FFPE** tissue sample from a patient diagnosed with **Rectal Adenocarcinoma** was analyzed using the Complete Solid Tumor Panel to assess potential drug interactions.

Molecular Analysis: The **RNA** from the paraffin-embedded tissue **(FFPE)** that passed the quality control **(QC)** review was analyzed using a 430-gene next-generation sequencing **(NGS)** panel. The analysis covered SNV/indel, expression, fusion, Internal Tandem Duplication, and exon skipping in the following genes:

AKT1	BRAF	EGFR	ERBB2	FOXL2	GNA11	GNAQ
GNAS	HRAS	IDH1	IDH2	KIT	KRAS	MET
NRAS	PDGFRA	PIK3CA	RET	TERT	TP53	ALK
APC	AR	ATRX	CDK4	CDK6	CDKN2A	CTNNB1
DDR2	ERBB3	ERBB4	ESR1	FBXW7	FGFR1	FGFR2
FGFR3	H3F3A	HIST1H3B	JAK2	MAP2K1	MAP2K2	MTOR
MYC	NOTCH1	NOTCH2	NOTCH3	NOTCH4	NTRK1	NTRK2
NTRK3	POLD1	POLE	PTEN	RAF1	RB1	RICTOR
ROS1	SMAD4	SMO	VHL	ATM	BARD1	BRCA1
BRCA2	BRIP1	CDK12	CHEK1	CHEK2	FANCA	FANCL
PALB2	RAD51B	RAD51C	RAD51D	RAD54L	STK11	ABL1
ACVR1	AKT2	AKT3	ARID1A	ARID1B	ARID2	ATR
AURKA	B2M	BAP1	BCOR	BLM	BMPR1A	CCND1
CCND2	CCND3	CCNE1	CDH1	CDKN2B	CHD1	CIC
CSF1R	DAXX	DDX3X	DICER1	EIF1AX	EP300	EPCAM
ERCC1	ERCC2	EZH2	FANCI	FGF19	FGFR4	FH
FLCN	FLT1	FLT3	FLT4	FOXA1	FUBP1	H3F3B
HIST1H3C	HNF1A	JAK1	JAK3	KDM6A	KDR	KEAP1
KLF4	KMT2C	KMT2D	LZTR1	MAP3K1	MDM2	MDM4
MED12	MEN1	MLH1	MPL	MRE11A	MSH2	MSH3
MSH6	MUC16	MUTYH	MYCN	NBN	NF1	NF2
NKX2-1	NPM1	PBRM1	PIK3CB	PIK3R1	PLCB4	PMS2
PPP2R1A	PPP2R2A	PRKD1	PTCH1	PTPN11	RAD50	RAD51
RHOA	RNF43	SDHA	SDHB	SDHC	SDHD	SETD2
SF3B1	SMAD2	SMARCA4	SMARCB1	SRC	SRSF2	STAG2
SUFU	TGFBR2	TP63	TRAF7	TSC1	TSC2	TSHR
U2AF1	XRCC2	XRCC3	ABL2	ACVR2A	ARAF	ARHGAP35
ARID5B	ASXL1	ASXL2	AURKB	AXIN1	AXIN2	AXL
BCL2	BCL2L1	BCL2L11	BCL6	BCORL1	BIRC3	BTG1

BTG2	BTK	CACNA1A	CARD11	CASP8	CBFB	CBL
CD274	CD70	CD79A	CD79B	CDC73	CDK8	CDKN1A
CDKN1B	CDKN2C	CEBPA	CHD3	CHD4	CHD8	COL5A1
CREBBP	CRKL	CSDE1	CSF3R	CTCF	CTLA4	CTNNA1
CUL3	CYSLTR2	DMD	DNMT3A	DOT1L	EEF1A1	EEF2
EGR3	ELF3	EPAS1	EPHA2	EPHA3	EPHA7	EPHB1
ERCC4	ERG	ERRFI1	EWSR1	FAM175A	FAM46C	FAM46D
FANCC	FANCD2	FANCE	FANCF	FANCG	FAT1	FGF1
FGF2	FGF3	FGF4	FGF7	FGF8	FGF9	FLNA
FOXA2	FOXO1	FOXP1	FOXQ1	GATA1	GATA2	GATA3
GEN1	GLI1	GNA13	GPS2	GRIN2D	GRM3	H3F3C
HGF	HIST1H1C	HIST1H1E	HIST1H2BD	HOXB13	HUWE1	IGF1R
IKBKE	IKZF1	IL6ST	IL7R	INPP4B	INPPL1	IRF2
IRF4	IRF6	IRS2	JUN	KANSL1	KDM5A	KDM5C
KEL	KIF1A	KLF5	KMT2A	KMT2B	KRT222	LAMP1
LATS1	LATS2	LEMD2	LRP1B	MACF1	MAP2K4	MAP3K13
MAP3K4	MAPK1	MAX	MCL1	MECOM	MEF2B	MGA
MGMT	MITF	MLLT3	MST1R	MUC6	MYCL	MYD88
MYH9	NCOR1	NFE2L2	NFKBIA	NIPBL	NSD1	NUP93
PARP1	PAX5	PAX8	PDCD1	PDCD1LG2	PDGFRB	PGR
PHF6	PIK3C2B	PIK3C2G	PIK3CD	PIK3CG	PIK3R2	PIK3R3
PIM1	PLCG1	PLXNB2	PMS1	POLQ	POLRMT	PPARG
PPM1D	PPP6C	PRDM1	PRKAR1A	PRKDC	PSIP1	PTMA
PTPDC1	PTPRC	PTPRD	PTPRT	RAC1	RAD21	RAD52
RARA	RASA1	RBM10	RECQL4	REL	RHEB	RIT1
RPL5	RPS6KB1	RPTOR	RRAS2	RUNX1	RUNX1T1	RXRA
SCAF4	SETBP1	SH2D1A	SLX4	SMAD3	SMC1A	SMC3
SOCS1	SOX17	SOX2	SOX9	SPEN	SPOP	SPTA1
SPTAN1	STAG1	STAT3	SYK	TAF1	TBL1XR1	TBX3
TCEB1	TCF3	TCF7L2	TET1	TET2	TFRC	TGFBR1
TGIF1	THRAP3	TLR4	TMSB4X	TNFAIP3	TNFRSF14	TRAF3
TXNIP	UNCX	USP9X	WT1	XPO1	ZBTB20	ZFHX3
ZMYM2	ZMYM3	ZNF750				

Potential drug interactions related to detected variants were investigated through databases such as OncoKB, cBioPortal, **CancerGenomeInterpreter (CGI)**, CIVIC, COSMIC, and OncoPortal. Findings are reported in the interpretation section.

Results:

In the analysis of the RNA sample from the patient's tumor tissue, the following molecular alterations were observed:

1. **ATM c.6108T>A p.(Tyr2036Ter)**: A stop gained in the **ATM** gene, with an allele fraction of **15 %** is associated with **pathogenic** .

2. **BRIP1 c.3039_3040inv p.(Gly1014Ser)**: A missense variant in the **BRIP1** gene, with an allele fraction of **4.3 %** is associated with **VUS**.This missense variant replaces glycine with serine at codon 1014 of the BRIP1 protein. To our knowledge, functional studies have not been reported for this variant. This variant has not been reported in individuals affected with BRIP1-related disorders in the literature. This variant has not been identified in the general population by the Genome Aggregation Database (gnomAD). The available evidence is insufficient to determine the role of this variant in disease conclusively. Therefore, this variant is classified as a Variant of Uncertain Significance.

3. **BRIP1 c.2947del p.(Ile983LeufsTer2)**: A frameshift variant in the **BRIP1** gene, with an allele fraction of **54 %** is associated with **Pathogenic/Likely_pathogenic**. This sequence change creates a premature translational stop signal (p.Ile983Leufs*2) in the BRIP1 gene. While this is not anticipated to result in nonsense-mediated decay, it is expected to disrupt the last 267 amino acid(s) of the BRIP1 protein. This variant is present in population databases (no rsID available, gnomAD 0.0009%). This variant has not been reported in the literature in individuals affected with BRIP1-related conditions. ClinVar contains an entry for this variant (Variation ID: 461044). This variant disrupts a region of the BRIP1 protein in which other variant(s) (p.Thr997Argfs*61, p.Lys998Glufs*60, p.Lys998Glufs*3) have been determined to be pathogenic (PMID: 18628483; internal data). This suggests that this is a clinically significant region of the protein, and that variants that disrupt it are likely to be disease-causing. For these reasons, this variant has been classified as Pathogenic.

4. **CACNA1A c.6674_6675delinsCC p.(His2225Pro)**: A missense variant in the **CACNA1A** gene, with an allele fraction of **10 %** is associated with **VUS**.

5. **COL5A1 c.82_84del p.(Leu28del)**: An in-frame deletion in the **COL5A1** gene, with an allele fraction of **2.8 %** is associated with **VUS**.

6. **KMT2C c.2578C>T p.(Pro860Ser)**: A missense variant in the **KMT2C** gene, with an allele fraction of **7.3 %** is associated with **VUS**. Variant summary: KMT2C c.2578C>T (p.Pro860Ser) results in a non-conservative amino acid change in the encoded protein sequence. Three of five in-silico tools predict a damaging effect of the variant on protein function. The variant was absent in 247282 control chromosomes (gnomAD). The available data on variant occurrences in the general population are insufficient to allow any conclusion about variant significance. The variant, c.2578C>T, has been reported in the literature in multiple exome sequencing studies, and was found in individuals affected with varying phenotypes, including autism spectrum disorder (ASD), familiar medullary thyroid cancer (MTC), congenital insensitivity to pain with anhidrosis (CIPA) and seizures, cortical blindness, and microcephaly syndrome (SCBMS), however several other variants, including other KMT2C variants were also found in these patients (Sponziello_2017, Garcia-Ortiz_2020, Lopez-Cortes_2020, Kaustio_2021). These reports do not provide unequivocal conclusions about the association of the variant with disease. To our knowledge, no experimental evidence demonstrating an impact on protein function has been reported. No clinical diagnostic laboratories have submitted clinical significance assessments for this variant to ClinVar after 2014. Based on the evidence outlined above, the variant was classified as of uncertain significance.

7. **KMT2C c.2573G>T p.(Trp858Leu)**: A missense variant in the **KMT2C** gene, with an allele fraction of **6.9 %** is associated with **VUS**. The observed missense variant c.2573G>Tp.Trp858Leu in the KMT2C gene has been reported in the literature in multiple exome sequencing studies, and was found in individuals affected with varying phenotypes, including autism spectrum disorder ASD, congenital insensitivity to pain with anhidrosis CIPA, seizures, cortical blindness, and microcephaly syndrome SCBMS. However, several other variants, including other KMT2C variants, were also found in these patients García-Ortiz JE, et al., 2020; López-Cortés A, et al., 2020. Hence, these reports do not provide unequivocal conclusions about the association of the variant with disease. The p.Trp858Leu variant is absent in gnomAD Exomes. This variant has been reported to the ClinVar database as UncertainSignificance / Pathogenic. Computational evidence SIFT-Tolerated and Mutation Taster-disease causing predicts conflicting evidence on protein structure and function for this variant. The amino acid Trp at position 858 is changed to Leu, changing the protein sequence, and it might alter its composition and physicochemical properties. The reference amino acid p.Trp858Leu in KMT2C is predicted as conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Uncertain Significance.

8. **KRAS c.436G>A p.(Ala146Thr)**: A missense variant in the **KRAS** gene, with an allele fraction of **6.0 %** is **likely pathogenic**. This sequence change replaces alanine, which is neutral and non-polar, with threonine, which is neutral and polar, at codon 146 of the KRAS protein (p.Ala146Thr). This variant is not present in population databases (gnomAD no frequency). This variant has not been reported in the literature in individuals affected with KRAS-related conditions. ClinVar contains

an entry for this variant (Variation ID: 197243). Invitae Evidence Modeling incorporating data from in vitro experimental studies (internal data) indicates that this missense variant is expected to disrupt KRAS function with a positive predictive value of 95%. Experimental studies have shown that this missense change affects KRAS function (PMID: 20147967, 20570890, 26110767). This variant disrupts the p.Ala146 amino acid residue in KRAS. Other variant(s) that disrupt this residue have been determined to be pathogenic (internal data). This suggests that this residue is clinically significant, and that variants that disrupt this residue are likely to be disease-causing. In summary, the currently available evidence indicates that the variant is pathogenic, but additional data are needed to prove that. Therefore, this variant has been classified as Likely Pathogenic.

9. **PIK3CA c.1637A>C p.(Gln546Pro)**: A missense variant in the **PIK3CA** gene, with an allele fraction of **4.7 %** is **likely pathogenic**.
10. **PIK3CA c.1637A>C p.(Gln546Pro)**: A missense variant in the **PIK3CA** gene, with an allele fraction of **4.7 %** is **likely pathogenic**.
11. **RASA1 c.1583A>G p.(Tyr528Cys)**: A missense variant in the **RASA1** gene, with an allele fraction of **4.7 %** is associated with **Conflicting interpretations of pathogenicity**.
12. **SMARCA4 c.2275-3C>A**: A splice region variant, splice polypyrimidine tract variant, and intron variant in the **SMARCA4** gene, with an allele fraction of **46%** is associated with **Conflicting_interpretations_of_pathogenicity**.This variant was observed in the ICSL laboratory as part of a predisposition screen in an ostensibly healthy population. It had not been previously curated by ICSL or reported in the Human Gene Mutation Database (HGMD: prior to June 1st, 2018), and was therefore a candidate for classification through an automated scoring system.

Utilizing variant allele frequency, disease prevalence and penetrance estimates, and inheritance mode, an automated score was calculated to assess if this variant is too frequent to cause the disease. Based on the score and internal cut-off values, a variant classified as benign is not then subjected to further curation. The score for this variant resulted in a classification of benign for this disease.

13. **TGFBR2 c.383del p.(Lys128SerfsTer35)**: A frameshift variant in the **TGFBR2** gene, with an allele fraction of **9.8 %** is associated with **Conflicting interpretations of pathogenicity**. Frameshift variant predicted to result in protein truncation or nonsense-mediated decay in a gene or region of a gene for which loss of function is not a well-established mechanism of disease; This variant is associated with the following publications: (PMID: 26948038, 28847661, 29951173, 23578328, 33105726, 31779681, 23585368, 34102952)

Interpretation and Drug Interaction:

1. The patient's provided material detected that the **ATM c.6108T>A p.(Tyr2036Ter)** mutations are **pathogenic**. It has been reported that **NOS** patients carrying known **ATM** mutations may benefit from FDA-approved drugs such as **Olaparib, Talazoparib + Enzalutamide** [OncoKB™ Therapeutic Level of Evidence V2].
2. The patient's provided material detected that the **BRIP1 c.2947del p.(Ile983LeufsTer2)** mutations are **pathogenic**. It has been reported that **NOS** patients carrying known

BRIP1 mutations may benefit from FDA-approved drugs such as **Olaparib** [OncoKB™ Therapeutic Level of Evidence V2].

3. The patient's provided material detected that the **KRAS c.436G>A p.(Ala146Thr)** mutations are **likely pathogenic**. It has been reported that **Colorectal Cancer** patients carrying known **KRAS** mutations may benefit from FDA-approved drugs such as **Adagrasib + Cetuximab, Sotorasib + Panitumumab, Cetuximab, Cetuximab + Chemotherapy, Panitumumab, Panitumumab + Chemotherapy, Tucatinib + Trastuzumab, and Adagrasib + Panitumumab.** It has been reported that **all solid tumor** patients carrying known **KRAS** mutations may benefit from FDA-approved drugs such as **Daraxonrasib, Binimetinib, Cobimetinib, and Trametinib** [OncoKB™ Therapeutic Level of Evidence V2].

Released by: Dr [redacted]

NGS Report – TMB Panel

Patient Information

Name-Surname: [redacted]eri	**Examined Material:** FFPE
Date of Birth, Gender: 47 Years, Female	**Type of Tissue Examined:** Rectal
Diagnosis: Rectal Adenocarcinoma	**Tumor Percentage:** 50 %
Material Code: 0034	**Test Conducted:** TMB Panel
Sample Receipt Date-Time: 09/10/2025	**Report Date-Time:** 09/23/2025

History:

An **FFPE** tissue sample from a patient diagnosed with **Rectal Adenocarcinoma** has been taken for TMB analysis.

Result:

Additional Biomarkers

Biomarker	Additional Detail
Tumor Mutational Burden (TMB)	**1.9 Mutations/Mb**
Tumor Mutational Burden (TMB)	**TMB-Low**

Comment:

"TMB" refers to Tumor Mutational Burden, which measures the number of mutations per megabase (mut/Mb) in a tumor's DNA. A TMB-Low status with a **TMB score of 1.9 mutations/Mb** means the cancer has a very low mutation rate, essentially showing no detectable mutations per megabase in this assessment. Generally, a lower TMB indicates that the tumor may **not respond** as well to certain types of immunotherapy, as higher TMB levels are sometimes associated with better responses to these treatments.

When there are 10 or more mutations per megabase (Mb) of tumor DNA, it is classified as Tumor Mutational Burden-High (TMB-H). TMB-H status can help predict the response to cancer treatments, particularly the targeting of immune checkpoint inhibitors.

Released b[redacted]

NGS Report – MSI Panel

Patient Information

Name-Surname: Azita Naseri	**Examined Material:** FFPE
Date of Birth, Gender: 47 Years, Female	**Type of Tissue Examined:** Rectal
Diagnosis: Rectal Adenocarcinoma	**Tumor Percentage:** 50 %
Material Code: 0034	**Test Conducted:** MSI Panel
Sample Receipt Date-Time: 09/10/2025	**Report Date-Time:** 09/23/2025

History:

An FFPE tissue sample from a patient diagnosed with **Rectal Adenocarcinoma** has been taken for MSI analysis.

Method:

Fragment analysis was performed using a fluorescence-based MSI Analysis System Kit (Promega MD1641) with a capillary electrophoresis system. The data obtained from capillary electrophoresis were analyzed using the GeneMapper Software program to evaluate the MSI status.

Molecular Analysis:

Seven different regions were examined and evaluated using the **DNA** sample obtained from the patient's **FFPE** tissue block: two different pentanucleotide repeat markers **(Penta C, Penta D)** for control purposes and five different mononucleotide repeat markers **(BAT25, BAT26, MONO27, NR-21, NR-24)** to determine **MSI**. The microsatellite regions examined in the patient's **FFPE** tissue sample were stable (**MSS**).

Result:

The patient's **FFPE** tissue showed **-MSI-Stable (2.63%).**

Comment:

The finding of microsatellite stability (MSS) in this liquid biopsy analysis suggests a low level of microsatellite instability, which is consistent with proficient DNA mismatch repair (MMR) function. This result makes the presence of Lynch syndrome (HNPCC) less probable, though it cannot definitively exclude it, as tissue-based testing remains the gold standard for diagnostic confirmation. Liquid biopsy for MSI assessment has technical limitations, including lower sensitivity compared to traditional tissue testing, especially for tumors with lower shed burden. Therefore, clinical correlation with patient and family history is essential. If there is a strong suspicion for Lynch syndrome based on clinical criteria, follow-up with immunohistochemistry (IHC) for MMR proteins and/or germline genetic testing should be considered. Therapeutically, MSI-stable status is generally associated with a lower predicted response to immune checkpoint inhibitor **immunotherapy**.

Released by: Dr. [redacted]