Patient Name:	Cancer Type:	Patient ID:	Sample ID:	Patient Birthdate:	Validated on:
	Sarcoma		2Z8P3TVX		

OncoDEEP Analysis Report

GENERAL INFORMATION

Birthdate:		**Sex:**		**Medical Doctor:**	Constantinos Ferentinos
Pathologist:	TOPCIU Lucian	**Tumor Cell:**	True%	**Biopsy Date:**	Jan, 13 2022
Biopsy Site:	–	**Primary Tumor Site:**	Unmentioned	**Cancer Type:**	Sarcoma

Clinical Diagnosis: Atypical myofibroblastic tumor of the maxillary sinus

Histological Diagnosis: Atypical myofibroblastic tumor of the maxillary sinus

REPORT RESULT SUMMARY

Drugs

- **6** with potential clinical benefit
- **2** with lack of clinical benefit
- **0** associated with toxicity

Variants

- **0** Pathogenic
- **3** Likely Pathogenic
- **49** Variants of Uncertain Significance (VUS)

IDENTIFIED BIOMARKERS FOR THERAPIES RESPONSE

[1]Variants

Biomarker	Result	VAF/CNV	Biological Impact	Therapeutical Impact
[2] FANCE	INS	54 %	Likely Pathogenic	Tier III
KEAP1	LOH	1	Likely Pathogenic	Tier III
[2] STK11	LOH	1	Likely Pathogenic	Tier III

[1] This table shows all pathogenic and likely pathogenic variants. Details of variants of unknown significance can be accessed through OncoSHARE platform.
[2] Analysis has detected a mutation in STK11, FANCE. Mutations in those genes may be somatic (present only on cancer cells) or inherited. As tumour-only sequencing cannot distinguish between somatic and inherited mutations, consideration should be given to referral for testing for an inherited mutation.

Other Biomarkers

Biomarker	Result	Scoring
CD8	Positive expression	20%
HRD	Negative	–
MSI	Stable	–
pan-TRK	Negative expression	0%
PD-L1	High expression	98%

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Biomarker	Result	Scoring
Tumor Mutational Burden	Low	1.74 Mut/Mb

THERAPIES

Legend

Potential clinical benefit | Potential lack of clinical benefit | Potential toxicity | Unkown

Approved therapies for the cancer type

Name	Class	Approval				Linked Biomarker	Clinical Benefit
Nothing to report	*Nothing to report*	-	-	-	-	*Nothing to report*	*Nothing to report*

Non approved therapies for the cancer type

Name	Class	Status	Linked Biomarker	Clinical Benefit
Durvalumab And Oleclumab		In Development	PD-L1 High expression	
Durvalumab and Tremilimumab	PD-L1/CTLA-4 inhibitor	In Development	PD-L1 High expression	
Entrectinib	Receptor tyrosine kinase inhibitors	Approved For Other	pan-TRK Negative expression	
Larotrectinib	NTRK inhibitors	Approved For Other	pan-TRK Negative expression	
Lenvatinib And Pembrolizumab	PD-1 and Receptor tyrosine kinase inhibitors	Approved For Other	PD-L1 High expression	
Nivolumab	PD-1 inhibitor	Approved For Other	Tumor Mutational Burden Low PD-L1 High expression	
Pembrolizumab	PD-1 inhibitor	Approved For Other	Tumor Mutational Burden Low MSI Stable PD-L1 High expression	
Pembrolizumab and ALKS 4230	PD-1 and IL-2 inhibitors	In Development	PD-L1 High expression	

The clinical trials associated with the drugs in development are shown in the clinical trials table below

CLINICAL TRIALS

Clinical trial drug	Biomarker	NCT
Neoadjuvant Durvalumab and Tremelimumab Plus Radiation for High Risk Soft–Tissue Sarcoma	PD–L1 High expression	NCT03116529
Pembrolizumab and Radiation Therapy in Treating Patients With Intermediate or High–Grade Soft Tissue Sarcoma	Tumor Mutational Burden Low MSI Stable PD–L1 High expression	NCT03338959
A Dose Escalation and Cohort Expansion Study of Subcutaneously–Administered Cytokine ALKS 4230 (Nemvaleukin Alfa) as a Single Agent and in Combination With Anti–PD–1 Antibody (Pembrolizumab) in Subjects With Select Advanced or Metastatic Solid Tumors (ARTISTRY–2)	PD–L1 High expression	NCT03861793
Combination of Nivolumab Plus Relatlimab in Patients With Advanced or Metastatic Soft–tissue Sarcoma: a Proof–of–concept Randomized Phase II Study	Tumor Mutational Burden Low PD–L1 High expression	NCT04095208
Oleclumab and Durvalumab for the Treatment of Recurrent, Refractory, or Metastatic Sarcoma	PD–L1 High expression	NCT04668300
Lenvatinib and Pembrolizumab in People With Advanced Soft Tissue Sarcoma	PD–L1 High expression	NCT04784247

COMPREHENSIVE SUMMARY

CONCLUSION

We did not find any pathogenic or likely pathogenic variant associated with treatments.

Based on the package plus analysis, this patient ***should be sensitive to*** PD–1/PD–L1 inhibitors.

On the other hand, this patient ***should not be sensitive to*** NTRKx inhibitors.

Rmk:

The immunogram shows a high potential response to immunotherapy. We showed a positive infiltration of CD8 + T cells in the tumor and also a high expression of PD–L1 (98% of tumor cells). Moreover, we didn't observe microsatellite instability (MSI), tumor mutational burden (TMB) or sensibility/resistance mutation. Therefore, based on the level of CD8+ lymphocytes and PD–L1 expression, treatment based on PD–1/PD–L1 inhibitors ***could be associated with potential clinical benefit*** for this patient.

Please note that the analyses were performed on the FFPE block labelled 407119 K.

OTHER BIOMARKERS - DETAILS

HRD: Negative - Scoring: –

Conclusion
We observed a negative HRD scoring for this patient. Deficiency in HRR (HRD) is a target for PARP inhibitors. PARP inhibitors have been approved by the FDA for ovarian cancer that is positive for a HRD scoring. Therefore for this patient, PARP inhibitors should be associated with potential lack of clinical benefit.

Description

Module to detect Loss of Heterozygosity events (LOH) and to predict homologous recombination deficiency (HRD).
The module is based on the analysis of highly polymorphic SNPs from dbSNP with MAF>0.3. These selected SNPs are distributed along the genome and on telomeric regions . LOH is computed on targeted genes and HRD score is computed on 3 ways:
a global score on all the targets:

- a score only on the telomeric regions (TAI)
- a score on all the regions except the telomeric ones (LOH)

HRD scoring is considered as positive if >= 40
Rmk: The genomic HRD score should be interpreted with caution and take into consideration with other information and data available. The HRD score is relevant for tumor types for which defective DNA repair is well documented (e.g. breast, ovarian, pancreatic and prostate cancer) since its significance with other cancer remains unknown and under investigation trough clinical trials. Caution should also be used when interpreting score associated with poor quality sample since this may cause bias.

PD-L1: High expression - Scoring: 98%

Conclusion
We showed by IHC analysis a high expression of PD–L1. Since the formation of the complex between PD–1 (on the surface of T cells) and PD–L1 (on the surface of tumor cells) transmits an inhibitory signal to T cells, treatments based on PD–1/PD–L1 inhibitors would be associated with potential clinical benefit for this patient. Nonetheless, this marker may not be enough by its own and it may need to be combined with other biomarkers like the ones present in the immunogram.

Description

Programmed death–ligand 1 (PD–L1) is a 40kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. Normally the immune system reacts to foreign antigens where there is some accumulation in the lymph nodes or spleen which triggers a proliferation of antigen–specific CD8+ T cell. The formation of PD–1 receptor / PD–L1 or B7.1 receptor /PD–L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph nodes and supplementary to that PD–1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl–2.

- Programmed Death Ligand (PD–L1) Clone 22C3 pharmDx™ kit for KEYTRUDA

Scoring Criteria

TPS: Tumor Proportion Score describing the % of viable tumor cells showing complete or membrane staining at >or = 1+
TPS <1% = No Expression (Negative, low expression).
TPS >or = 1% = High Expression

pan-TRK: Negative expression - Scoring: 0%

Conclusion

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Conclusion

We showed by IHC analysis a negative expression of TRK protein. Therefore, TRK inhibitors would be associated with a lack of clinical benefit for this patient.

Description

The NTRK gene family encodes three tropomyosin–related kinase (TRK) receptors (PMID:29986850). NTRK rearrangements represent the molecular driver of a subset of solid tumors (PMID:28444624), and they lead to transcription of chimeric TRK proteins with constitutively activated or overexpressed kinase function that confer oncogenic potential (PMID:27843590).

The VENTANA pan–TRK (EPR17341) CE IVD/US Class I Assay is designed to detect C–terminal protein expression, which allows for the detection of TRK–fusion as well as wild–type protein expression. The epitope detected by the antibody is encoded downstream of the tyrosine kinase domain within the 3 prime coding region of the NTRK 1, 2 and 3 genes and is conserved across all three TRK proteins, A, B and C (https://diagnostics.roche.com/global/en/news\char"2013\relaxlisting/2018/TRK\char"2013\relaximmunohistochemistry\char"2013\relaxassay.html)

A staining in the nucleus and/or cytoplasm will be considered as positive.

CD8: Positive expression - Scoring: 20%

Conclusion

By IHC we showed a positive expression of CD8 indicating the presence of CD8+ T cells around the tumor. Since the formation of PD–1 receptor / PD–L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph node, treatment based on PD–1/PD–L1 inhibitors could be associated with potential clinical benefit for this patient.

Description

The CD8 co–receptor is expressed on the surface of cytotoxic T cells (CD8+ T cells) allowing the TCR to bind to the class I MHC molecule. The interaction keeps the TC cell and the target cell bound closely together during antigen–specific activation. CD8 exists as both a heterodimer (CD8αβ) and a homodimer (CD8αα) of which the former is expressed on the majority of CTLs. CD8 has been shown to be involved in CTL co–activation by increasing antigen sensitivity, and by stabilising the TCR/pMHC interaction. In order to carry out these roles, the CD8 co–receptor binds to a distinct invariant region of the pMHCI molecule, compared to the TCR, allowing the potential for tripartite (TCR/pMHC/CD8) complex formation. The main interface between CD8αα and pMHCI is between CD8 residues 51–55 and pMHC residues 223–229 in the α3–domain, which form a CD8 binding loop.

CD8 antibody: C8/144B clone (Dako)

Scoring criteria:

CD8+ infiltration was scored semi–quantitatively. Ideally, 8–10 representative fields of tumor would be assessed when possible. We avoid counting areas with necrosis to minimize false or non–specific reactions. The 0–1–2–3 score represente average infiltration into the pathologist selection

To provide a more quantitative assessment of these four levels of infiltration, we determine the approximate numbers of CD8+ cells per mm2 that correspond to the 0-3 scores.

(Although they vary between microscopes, a typical high-powered field (10x ocular and 40x objective) is 0.15 mm2 meaning there are 7 high power fields (HPF) per mm2)

Lymphocyte CD8+ infiltration is considered as positive for score of 2 or greater.

Scoring:

0 (absent/negative) = 0–14 Lymphocytes CD8+/HPF

1 (Low/negative) = 15-39 Lymphocytes CD8+/HPF

2 (Moderate/positive)= 40-69 Lymphocytes CD8+/HPF

3 (High/positive) = >70 Lymphocytes CD8+/HPF

MSI: Stable - Scoring: –

Conclusion

We did not observe a high level of microsatellite instability (MSI). MSI–High has been linked to increased sensitivity to immune checkpoint inhibitor drugs (PD–1/PD–L1 inhibitors) (PMID:28877075). Therefore, PD–1/PD–L1 inhibitors would be associated with a lack of clinical benefit for this patient. Nonetheless, this information may need to be combined with other biomarkers like the ones present in the personalized immunogram.

Description

Microsatellite instability (MSI) is a hypermutable phenotype caused by the loss of DNA mismatch repair (MMR) activity (PMID:20420947), which is associated with inactivation, loss or epigenetic silencing of MMR genes (MSH2, MLH1, MSH6 and PMS2).

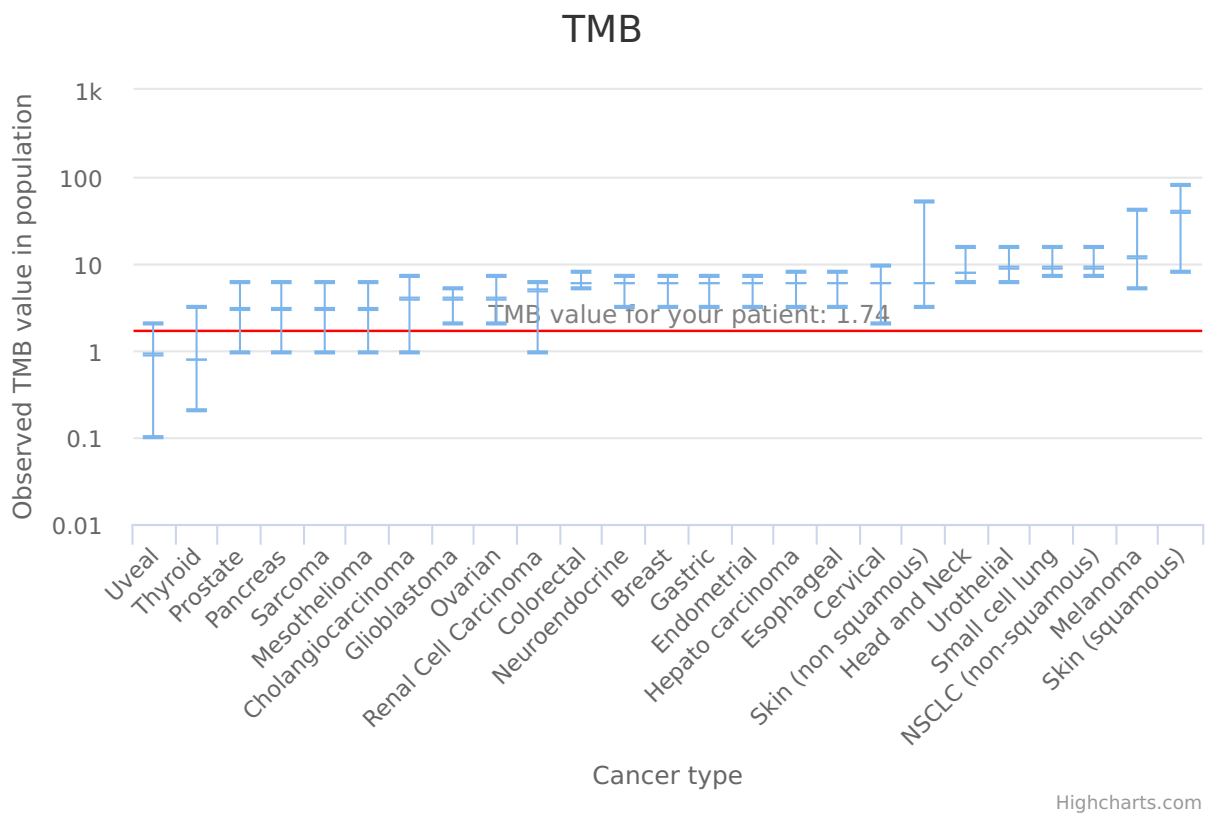
Tumor Mutational Burden: Low - Scoring: 1.74 Mut/Mb

Conclusion

We did not observe a high tumor mutational burden (TMB). In patients with high TMB, checkpoint inhibitors (PD–1/PD–L1 blockade) have been associated with clinical benefits across diverse tumors (PMID:28835386). Therefore, treatments based on PD–1/PD–L1 inhibitors would be associated with a lack of clinical benefit for this patient. Nonetheless, this information may need to be combined with other biomarkers like the ones present in the personalized immunogram.

Description

The TMB calculation is performed by the biologists as stated below:
TMB is defined as the number of mutations per megabase (Mb).
First, the number of covered bases during the sequencing of the patient's DNA is calculated. On average, 2Mb is sequenced but, this number may slightly vary in each run. Hence, this calculation is done for every patient.
Then, the number of mutations is assessed considering only the SNVs and insertions/deletions that are either damaging/potentially damaging or VUS (VAF <80%) and excluding specific germline mutations (50% +–10). Synonymous mutations, polymorphisms (MAF ≥ 1%), low coverage, and low–frequency variants (<5%) are excluded.
The TMB obtained is classified as the following (PMID:28835386):
High: ≥ 10 mut/Mb
Intermediate: >4 mutations/Mb
Low: < 4 mut/Mb
Remark 1: Our TMB calculation has been benchmarked against the Sample Seracare Tumor Mutation Load Assay, obtaining similar result:
Remark 2: For the moment, the cut–offs used are the same independently of the cancer type; however, we are working on a cancer type–specific TMB determination, as the number of mutations varies greatly across tumor types, and different cut–offs may be needed.



DETECTED VARIANTS LIST

Likely Pathogenic

Gene	Cat.	VarFreq or Copy Number	cDNA	AA	Therapeutical impact	Incidental finding
FANCE	INS	54%	c.929dup	p.V311Sfs*2	Tier III	Yes
KEAP1	LOH	1			Tier III	No
STK11	LOH	1			Tier III	Yes

Variants of Uncertain Significance (VUS)

Gene	Cat.	VarFreq or Copy Number	cDNA	AA	Therapeutical impact	Incidental finding
APC	SNV	40%	c.2438A>G	p.N813S	Tier III	No
ASXL1	SNV	45%	NM_015338.5:c.1654A>G	p.I552V	Tier III	No
BRCA2	SNV	50%	c.5635G>A	p.E1879K	Tier III	No
CYSLTR2	SNV	52%	c.601A>G	p.M201V	Tier III	Yes
FAM58A	INS	94%	c.50dup	p.A19Gfs*37	Tier III	No
HLA-B	SNV	97%	c.41_49delinsGGGGGGCAG	p.S14_L17delinsWGAV	Tier III	No
HLA-B	SNV	35%	c.538_540delinsGAC	p.R180D	Tier III	No
HLA-B	SNV	60%	c.354_363delinsTTGGCAGACG	p.L119_S121delinsWQT	Tier III	No
HLA-B	SNV	34%	c.353_363delinsTCATCCAGAGG	p.T118_S121delinsIIQR	Tier III	No
HLA-B	SNV	86%	c.272_283delinsTCAAGACCAACA	p.Y91_A95delinsFKTNT	Tier III	No
HLA-B	SNV	14%	c.259_261delinsGAG	p.N87E	Tier III	No
HLA-B	SNV	80%	c.204_206delinsGAC	p.E69T	Tier III	No
HLA-B	SNV	18%	c.204_205delinsGA	p.E69K	Tier III	No
HLA-B	SNV	10%	c.165_167delinsGCT	p.Q56L	Tier III	No
HLA-B	SNV	99%	c.103_106delinsGCCA	p.S35_V36delinsAM	Tier III	No
HLA-B	SNV	96%	c.559_560delinsCT	p.E187L	Tier III	No
HLA-C	SNV	22%	c.538_539delinsTG	p.L180W	Tier III	No
HLA-C	SNV	95%	c.984_993delinsGGCTGTTGTT	p.T329_M331delinsAVV	Tier III	No
HLA-C	SNV	98%	c.872_873delinsCG	p.Q291P	Tier III	No
HLA-C	SNV	96%	c.648_652delinsTCTCG	p.P217_L218delinsLV	Tier III	No
HLA-C	SNV	25%	c.559_560delinsCT	p.T187L	Tier III	No
KAT6A	SNV	59%	NM_006766.5:c.5791A>G	p.M1931V	Tier III	No
MET	SNV	51%	c.3313+845G>T		Tier III	No
MET	DEL	8%	c.3083-721del		Tier III	No
MET	SNV	43%	NM_000245.3:c.3259+1107C>T		Tier III	No
NADK	SNV	60%	c.786C>A	p.N262K	Tier III	No

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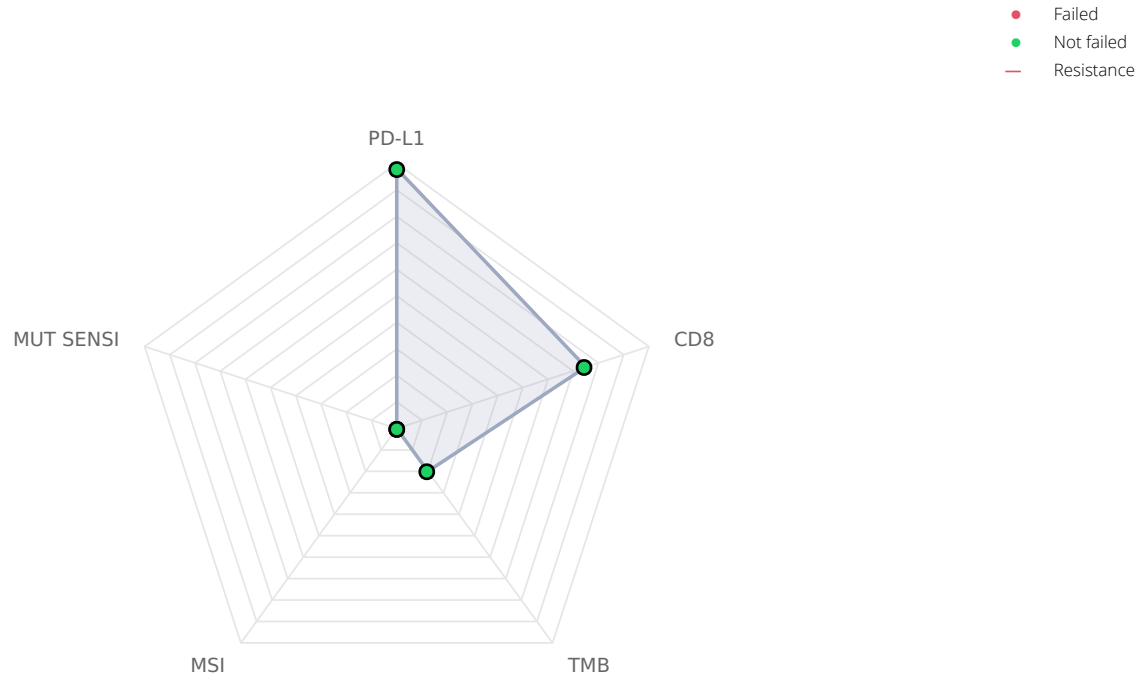
Gene	Cat.	VarFreq or Copy Number	cDNA	AA	Therapeutical impact	Incidental finding
NOTCH3	SNV	36%	c.1999G>A	p.G667S	Tier III	No
PARK2	SNV	55%	c.454G>C	p.G152R	Tier III	No
PARP3	SNV	100%	c.299A>G	p.H100R	Tier III	No
PDGFRB	SNV	8%	NM_002609.3:c.2548G>T	p.D850Y	Tier III	No
PGBD5	SNV	59%	c.253G>A	p.A85T	Tier III	No
PIK3C2B	SNV	53%	NM_002646.3:c.853C>T	p.P285S	Tier III	No
PPP4R2	SNV	46%	NM_174907.3:c.*1C>T		Tier III	No
RECQL4	SNV	55%	NM_004260.3:c.2966T>G	p.M989R	Tier III	No
ROS1	SNV	50%	c.6682_6686delinsCAGTG	p.K2228_S2229delinsQC	Tier III	No
RSPO2	SNV	100%	c.557T>C	p.L186P	Tier III	No
RTEL1	SNV	57%	NM_016434.3:c.1189C>G	p.Q397E	Tier III	No
SETD8	SNV	22%	c.362_364delinsGAC	p.K121_G122delinsRR	Tier III	No
SETD8	DEL	30%	c.59_64del	p.A20_A21del	Tier III	No
SETD8	DEL	8%	c.542_543del	p.L181Hfs*20	Tier III	No
SMARCA2	DEL	8%	c.705_707del	p.Q238del	Tier III	No
SMARCA4	SNV	35%	c.403C>G	p.P135A	Tier III	No
STK19	SNV	58%	NM_032454.1:c.494C>T	p.A165V	Tier III	No
TERT	SNV	61%	c.-58-910T>C		Tier III	No
TERT	INS	58%	c.-58-1094_-58-1093insC		Tier III	No
TERT	SNV	61%	c.-58-1597G>A		Tier III	No
TP53BP1	SNV	100%	c.1059C>G	p.D353E	Tier III	No
TP53BP1	SNV	100%	c.3406A>C	p.K1136Q	Tier III	No
USP8	SNV	6%	c.2292C>A	p.N764K	Tier III	No

IMMUNOGRAM



The immunogram shows a high potential response to immunotherapy. We showed a positive infiltration of CD8 + T cells in the tumor and also a high expression of PD–L1 (98% of tumor cells). Moreover, we didn't observe microsatellite instability (MSI), tumor mutational burden (TMB) or sensibility/resistance mutation. Therefore, based on the level of CD8+ lymphocytes and PD–L1 expression, treatment based on PD–1/PD–L1 inhibitors ***could be associated with potential clinical benefit*** for this patient.

CLINICAL FORM

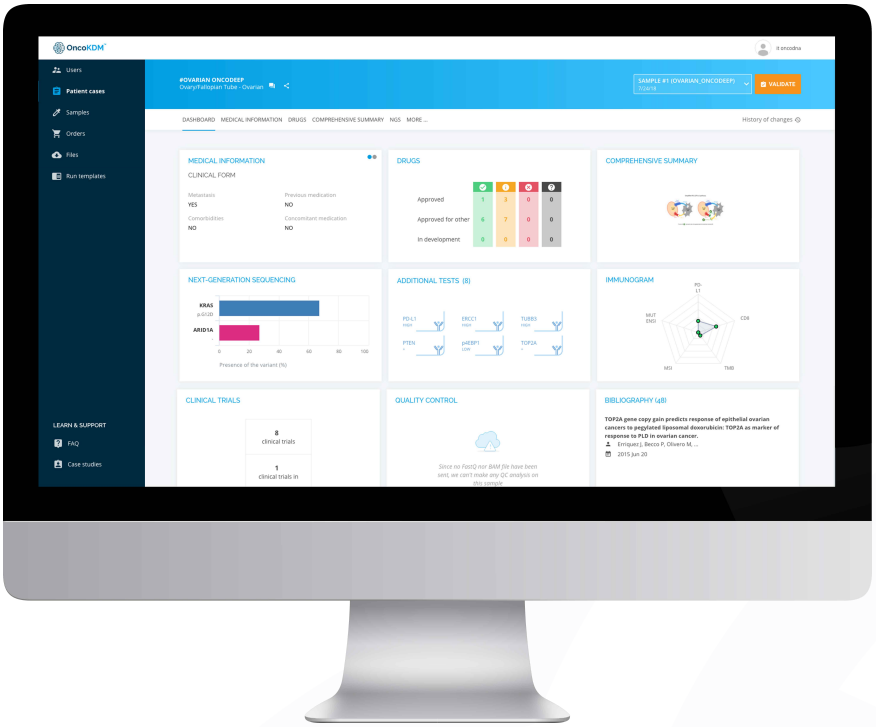
Date informed consent given/signed	31012022
Initial diagnosis date	01092021
Clinical diagnosis	Atypical myofibroblastic tumor of the maxillary sinus
Primary tumour site	Unmentioned
Known metastatic sites	No
Date of biopsy/surgery or blood withdrawal	13012022
Histological diagnosis	Atypical myofibroblastic tumor of the maxillary sinus
TNM known ?	Not applicable
Biomarkers tested	No
Is the tissue sample sent for molecular diagnostics the one used for the diagnosis (detailed above) ?	Yes
Sample site	Primary tumour
Does patient have comorbidities ?	No
Is patient currently receiving a cancer therapy ?	No
Known previous cancer therapies	Yes
Does the patient have a previous history of cancer?	No
ECOG	-
Smoking status	-
Alcohol consumption	-
Comments	Rare tumor, no TNM. Locally aggressive with infiltration of bones, muscles and invasion into the base of skull. Radiotherapy is planned.

REPORT

Please keep in mind that this summary is not the complete report and is to be printed only for archiving purposes.

For more information, please see the dynamic version of the report displayed on **oncoshare.oncokdm.com**

This report has been generated and validated on **February, 21 2022**



For more information or help about your report, contact the molecular team:

molecular@oncodna.com

PROCESS

IPG is the biggest Belgian anatomopathology laboratory and is among the biggest laboratories of its kind in Europe with headquarters in Gosselies and a large section in Brussels. It has a total workspace of 285 people, among whom medical specialists including 20 pathologists and 8 geneticists, 10 clinical biologists and highly skilled technicians. It was one of the first companies to implement a high degree of integration of anatomic pathology data and molecular genetics. The ability to integrate pathological data and molecular biology is not common and is an asset for the products provided by OncoDNA.

All the technical processes including the pathology QC check are performed by the Institute of Pathology and Genetics (IPG) which is ISO15189 accredited (ISO15189:2012 Medical Laboratories – Requirements for Quality and Competence) since the 6th October 2009 by BELAC, an ILAC MRA signatory. The quality of raw data is validated by OncoDNA before any further interpretation.

OncoDNA is compliant with the Guideline for Good Practices of the International Conference on Harmonization (ICH GCP E6 R2) and certified ISO/IEC 27001:2013 (Requirement for Information Security Management Systems) since the 23rd November 2018 by the European Certification Accredity Body ICTS – International Certifcation Trust Services.

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